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The cardiovascular effects of opioid analgesics: Studies on the role of opioid and non-opioid receptors in man

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Declaration

I hereby declare that the ideas and work presented in this thesis are my own, except where stated in the text. The work has not been submitted in any previous application for a degree.

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Abstract

In this thesis, a series of related studies on opioids are reported. In epidemiological studies of opioid overdose it is shown that opioid overdose has increased 14 times more than other overdoses in Edinburgh in the past 4 decades. I also discussed the predisposing factors for overdose. I developed and calculated a series of toxicity indices for opioids in Scotland, and used hospital discharge data, poisons information statistics (telephone enquiries/TOXBASE accesses) and prescription data to calculate fatality indices (FTI) and minimise the effects of confounders on the traditional FTI which uses only prescription volume as the denominator. I used an identical methodology to relate non-fatal consequences of overdose to prescriptions and proposed toxic morbidity indices (TMIs). I suggest an integrated approach by using both FTIs and TMIs as new methods for toxico-vigilance. Using this methodology I demonstrated that co-proxamol has a10 times excess risk of fatality in comparison to co-codamol and co-dydramol, while TMIs are similar. This demonstrates the inherent toxicity of the drug in overdose, and led, in part, to withdrawal of this drug in the UK. Further I showed in patients that QRS duration is prolonged in co-proxamol overdose, an effect which was dose dependent, suggesting sodium channel blockade as a potential cause of its excess mortality in overdose. I showed from mortality statistics that dihydrocodeine appears safer than methadone. I also estimated diamorphine illicit availability from overdose rates in Edinburgh. I introduced a comparison of mortality from single agent in comparison to multiple agent overdose (MSDPR) as a measure of risk from co-intoxications. I showed that diamorphine, morphine and codeine are significantly more dangerous in co-intoxication than other opioids.

Studies on the cardiovascular effects of opioids in overdose and in volunteers were then performed. It has been suggested previously that therapeutic doses morphine have no effects on the cardiovascular system in man in the supine position. I first showed acute depressor effects of dihydrocodeine and methadone overdose on peripheral systolic, diastolic, pulse, and aortic and end systolic pressures, and O_2 saturation in dihydrocodeine overdose in comparison to a parallel control group. I was able to exclude any effect on arterial stiffness. I showed that O_2 saturation under 95% is a marker of haemodynamic depressant effects of dihydrocodeine.

Later in a controlled trial in healthy volunteers, I verified the cardiovascular depressor effects of intravenous morphine in doses to a maximum of 16 mg. These effects were not dose dependent. There was also no relationship to change in reaction time, and no major change in plasma concentrations of histamine or catecholamines. Lower O_2 saturation, and higher end tidal volume CO_2 potentially contributed to the haemodynamic effects. I showed that intra venous morphine decreased aortic and peripheral systolic, diastolic, mean, pulse, end systolic, and sitting systolic pressures, while heart rate increased. A number of other indices, stroke index, systemic vascular resistance, ventricular ejection time, peak flow index, ejection ratio, end diastolic index, index of contractibility and acceleration index also decreased. Overall these findings indicate that at these doses morphine decreased afterload, was negatively inotropic, positively chronotropic, had no effect on cardiac work, while maintaining left ventricular performance. In a second study I found that these effects in general were not antagonised by naloxone. Using occlusion plethysmograph and intra arteriolar morphine infusion, I further showed the existence of a peripheral action of morphine on arteries, at higher concentrations 0.6 to 3 microgram/ml, which was dose dependent.

Weal, flare and itching also developed rapidly and were dose dependant. Tachyphylaxis to these effects did not develop. By using pre treatment with antihistamines and measurement of plasma histamine I showed that histamine was the prime mediator for both arteriolar and skin effects. The peripheral site of action is likely to be mediated via mast cell release of histamine from arteriolar surrounding supporting tissues, and this effect influences vascular tone in man. The arteriolar effects were antagonised by L-NMMA, indicating that nitric oxide release is probably caused by histamine.

High concentrations of morphine induce anaphylactoid reactions. The novel observations in this thesis explain this phenomenon and may clarify the pathophysiology of opioid-induced non-cardiac pulmonary oedema, and anaphylactoid reactions. If fluid shifts occur elsewhere in the body this may contribute to hypovolemia in shock, since endogenous opioids are thought to have a role in this situation. These findings suggest that H_1 and H_2 blockers should be studied in the management of patients with opioid-induced non-cardiac pulmonary oedema, and those receiving high doses of morphine such as in surgery and acute pain. The effects of H_1 and H_2 blockers in opioid overdose should also be investigated. afsharireza@yahoo.com

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"Praise Him for granting mercy and strength. My deepest gratitude if He preserves lives through the withdrawal of co-proxamol and if the use of H_1 & H_2 blockers in diamorphine overdose is beneficial."

This work was performed over 4 years; 30 months during which I was a postgraduate student in the Toxicology Ward and Scottish Poison Information Bureau, Royal Infirmary of Edinburgh, and 18 months during which I was a Clinical Research Fellow in the speciality of Clinical Toxicology in the Department of Medical Sciences at the University of Edinburgh.

The topic of cardiovascular toxicity of opioids was originally suggested by Professor D.N. Bateman and Dr. S.R.J. Maxwell. They guided me initially through the controversies in this field, and were both responsible for the supervision of this work. I am most grateful for the opportunity they gave me to perform this research and for their continued advice. Thanks especially to my primary supervisor, Professor Bateman, for his time spent reviewing this project.

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This project was not possible without the co-operation of the overdose patients from the Royal Infirmary of Edinburgh and healthy volunteers within the Clinical Pharmacology Unit.

Finally I would like to acknowledge the patience and support of my wife during the conduct of this work.

Dedication

With all my love

- To Yasamin, whose willingness to sacrifice playtime made this project possible
- To My mother, who will never retire from teaching
- In memory of my father, especially for the impact of his book *Principles of Pedagogy* has had on me
- To my brother, for his support and encouragement

I dedicate this thesis to my precious wife

List of Publications

1. Afshari R, Bhopal RS (2002) Changing pattern of use of 'ethnicity' and 'race' in scientific literature. Department of Community Health Sciences, Section of Public Health Sciences, University of Edinburgh, Edinburgh, UK. Int J Epidemiol 2002 Oct;31(5):1074
2. Afshari R, Bateman DN. ECG abnormalities in co-proxamol (paracetamol / dextropropoxyphene) poisoning. Journal of Toxicology Clinical Toxicology 2003; 41: 4: 560.
3. Bateman DN, Afshari R (2003) Co-proxamol and suicide: Licence needs to be changed. BMJ. Aug 2;327(7409):287
4. Bateman DN, Good AM, Afshari R, Kelly CA (2003) Effects of licence change on prescribing and poisons enquiries for antipsychotic agents in England and Scotland. Br J Clin Pharmacol. Jun;55(6):596-603.
5. Afshari R, Maxwell S, Kelly CA, Bateman DN. Arterial stiffness in dihydrocodeine overdose compared with minor paracetamol overdose, J Toxicol Clin Toxicol 2004; 42: 4: 540-541.
6. Afshari R, Maxwell S, Kelly CA, Bateman DN. ECG abnormalities in co-proxamol (paracetamol/dextropropoxyphene) poisoning compared with co-codamol and co-dydramol, Journal of Toxicology Clinical Toxicology; 2004; 42: 4: 476-477.
7. Reza Afshari, Simon Maxwell, Catherine Kelly and David Nicholas Bateman, Co-proxamol indices QRS prolongation, Cellular & Molecular Biology Letters; 2004, 9-2; 11.
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9. Afshari, R. Maxwell, S. Dawson, AH. Bateman, DN. ECG abnormalities in co-proxamol (paracetamol/dextropropoxyphene) poisoning. Journal of Toxicology Clinical Toxicology, 2005, 43 (4):255-259.
10. R Afshari, SRJ Maxwell, DN Bateman. Extensive description of fatal and non fatal consequences of drugs, applied for pure opioids, Journal of Toxicology Clinical Toxicology, In press (2005)
11. R Afshari, AM Good, SRJ Maxwell, DN Bateman. Co-proxamol overdose is associated with a 10-fold excess in mortality compared with other paracetamol combination analgesics, BJCP, In press (2005).

List of Presentations

3 oral presentations and 1 poster were presented to the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) in 2003 (Rome), 2004 (Strasbourg) and 2005 (Berlin), and 1 oral presentation was given to the British Pharmacology Society in 2004 (Newcastle).

Also 3 talks were presented in Western General Hospital during the weekly meeting of the Clinical Research Centre and 3 talks were given in the Royal infirmary of Edinburgh.

List of Abbreviations

Abbreviations	Descriptions	Abbreviations	Descriptions
AcI	Acceleration index	M & N	Morphine and naloxone
ADBP	Aortic diastolic blood pressure	M & NO	Morphine and nitric oxide
		M & P	Morphine and placebo
ADH	Anti diuretic hormone	MBP	Mean Blood pressure
AI	Augmentation index	MSDPR	Multiple over single death probability ratio
APP	Aortic Pulse Pressure		
ASBP	Aortic systolic Blood pressure	NHS	UK National Health Service
B.I.D	Twice a day		
BMI	Body mass index	NMN	N-Methylnaltrexone
BP	Blood pressure	NO	Nitric oxide
BPM/bpm	Beat per minute	NR	no response
CI	Cardiac index	O_2 Sat.	Oxygen saturation
CR	Correct response	OTC	Over the contour
CRC	Clinical Research Centre	PFI	Peak Flow Index
DBP	Diastolic plood pressure	PPP	Peripheral pulse pressure
ECG	Electrocardiogram		
ED	Ejection duration	RIE	Royal Infirmary of Edinburgh
EDI	End diastolic index		
EF	Ejection fraction	RR	Respiratory rate
ER	Ejection ratio	RT	Reaction time
ESP	End systolic pressure	S.C.	Subcoetaneous
$ETCO_2$	End tidal CO_2	SBP	Systolic Blood Pressure
F	F value (ANOVA)	sDBP	Sitting diastolic blood pressures
f	Frequency		
FTI	Fatal toxicity index	SI	Stroke index
FTI_{HD}	Hospital discharges-FTI	SNP	Sodium Nitroprusside
FTI_{Tel}	Telephone enquiries-FTI	SPIB	Scottish Poison Information Bureau
FTI_{Tel}	Telephone enquiries-FTI		
FTI_{TOX}	TOXBASE accesses-FTI	sSBP	Sitting systolic blood pressures
Gr.	Greek		
HR	Heart rate	STR	Systolic time ratio
HRP	Heart rate period	SVR	Systemic Vascular Resistance
IA	Intra-arteriolar		
IC	Index of contractibility	TFI	Thoracic fluid index
ICV	Intracerebroventricular	TMI	Toxic morbidity index
IL-6	Interleukin-6	TMI_{HD}	Hospital discharges-TMI
Inf	Infused arm	TMI_{Tel}	Telephone enquiries-TMI
IR	Incorrect response	TMI_{TOX}	TOXBASE accesses-TMI
IV	Intra-venous	TNF- ά	Tumour necrotising factor
L-NMMA	L-NG-Monomethyl-L-Arginine		
log	Log x to the base 10, i.e.. common logarithm	t-PA	Tissue Plasminogen Activator
LVF	Left ventricular function	VER	Ventricular ejection time
M	Morphine	vWF	von Willebrand Factor
M & H_1H_2	Morphine and cetirizine & cimetidine	WGH	Western General Hospital, Edinburgh

Glossary Table of cardiovascular variables

Acceleration Index (AcI); AcI is a measures of inotropic state. It is much less dependent on preload and after load. Normal range is 0.7 -1.5 sec -2 for males (0.9-1.7 for females).

Afterload; Afterload is a measure of the tension produced by a chamber of the heart (left ventricle) in order to contract.

Aortic pulse pressure (APP); APP is the difference between ASBP and ADBP. As the physiological pulse pressure is amplified between central and peripheral arteries (Nichols & O'Rourke, 1998; O'Rourke & Frohlich, 1999; Stergiopulos & Westerhof, 1998) brachial peripheral PP may not reflect aortic PP. APP influences left ventricular afterload and coronary perfusion.

Aortic systolic and diastolic blood pressure (ASBP & ADBP); They are the maximum and minimum pressures of the central waveform. Total arterial resistance and total arterial compliance are sufficient to accurately describe systolic and diastolic aortic pressure (Stergiopulos & Westerhof, 1998). Central systolic blood pressure should be considered for planning therapeutic strategies for prevention of left ventricular hypertrophy in hypertensive patients (Lekakis et al., 2004).

Cardiac index (CI); CI describes the level of pump perfusion capability (adequacy of perfusion). The electrical bioimpedance measurement is a reproducible and accurate technique (Northridge et al., 1990) and agrees with serial thermodilution methods (Appel et al., 1986). CI corrected for body surface area (BSA) is less subject to the effects of metabolic rate than CI corrected for weight. CI by weight is also not accurate in overweight subjects. The result is reported as l per min per square meter. CI = CO/BSA (l/min/m^2; normal values are 3.5 and 3.3 in young and old adults respectively).

Cardiac output (CO); CO is the volume of blood the heart pumps in one minute. In a poor ventricular performance with low SV the heart can increase HR and still perfuse the body adequately. CO = SV × HR; (l/min); normal value for a 50kg woman is 5.0 and for a 85 kg male body builder is 8.5.

Chronotropic effect; Chronotropic effects refer to the positive or negative changes in heart rate.

Diastolic blood pressure (DBP); DBP is the lowest pressure (at the resting phase of the cardiac cycle). It is largely determined by peripheral arterial resistance.

Ejection duration (ED); ED is the period of time from the start of the pulse for which the aortic valve is open to the closure of the aortic valve or end of systole. It was measured by the SphygmoCor equipment. It theorically provides the ability to distinguish primarily systolic from primarily diastolic dysfunction in heart failure patients, and to then manage the diastolic dysfunction patients more effectively.

Ejection Fraction (EF); EF is related to left ventricular performances and pump emptying efficacy. EF represents the volumetric emptying efficiency of the left ventricle (percentage of the total volume contained in the ventricle just before beginning of the systolic phase (end diastolic volume)). EF is calculated using the following formula; EF = [0.84 - (0.64 * STR)] *100 % or EF = SV/EDV %

Ejection ratio (ER); Ejection ratio can be calculated from Heart Rate Period (HRP) in seconds and ventricular ejection time (VET). ER = 100* VET/HRP, where HRP = 60/HR. This can eliminate the effects of heart rate. ER can be called a preload index.

End Diastolic Index (EDI); EDI is the body mass indexed form of end diastolic volume (EDV). Normal EDI values are 45-100 ml/m^2; mean 71 ml/m^2

Glossary Table of cardiovascular variables

End Diastolic Volume (EDV); The amount of blood in the ventricle immediately before a cardiac contraction begins. It's a measure of cardiac filling, and is related to diastolic function. EDV = 100 * SV/EF (ml)

End systolic pressure (ESP); ESP is the pressure at the end of systole. It is determined by the amount of blood in the ventricle at the end of the cardiac ejection period and immediately preceding the beginning of ventricular relaxation. ESP is the measurement of the adequacy of cardiac emptying, related to the systolic function. ESP measured by SphygmoCor.

End tidal CO_2 ($ETCO_2$); $ETCO_2$ reflects the CO_2 level in blood at the end of expiration.

Heart rate (HR); HR is the number of contractions of the heart in one minute. The results are reported as beat per minutes. Normal range is 65 for adult male (75 for female).

Index of Contractibility (IC); IC measures the inotropic state during the ejection phase. It is volume dependent, and is related to the left ventricular performance. Under normovolumic states it can be used to measure changes of contractibility in response to inotropic therapy. Its normal range is 0.033-0.065 sec-1.

Inotropic effect; Inotropic effects are the ones that change the force of heart muscle contractions, and are defined and positive or negative.

Mean blood pressure (MBP); MBP is the average or the mean pressure for the peripheral and aortic waveform. In these studies it is true mean, not the 1/3 method as commonly used.

Peak Flow (PF); PF represents the highest rate of left ventricular volumetric delivery during the ejection phase. Flow reaches its peak value in the first third of systole, typically 65 ml per sec after the opening of the aortic valve. PF time remains unchanged with variation of heart rate. PF is directly linked to the ejection phase contractibility and as such is dependent on volumetric status.

Peak Flow Index (PFI); PFI is related to left ventricular performance. To calculate PFI, peak flow (PF) should be corrected on body surface area. PFI = PF/BSA (ml/sec/m^2; normal ranges of PFI are 170-370).

Peripheral pulse pressure (PPP); PPP is the height of the peripheral pulse. This is determined by subtracting the minimum pressure from the maximum pressure. Large artery stiffening is associated with a widened pulse pressure and increased central systolic BP, which is augmented by early peripheral waveform reflection. Arterial stiffness can influence the value of PP as well as HR, cardiac contractility, and venous pressure (Nichols & O'Rourke, 1998; O'Rourke & Frohlich, 1999; Stergiopulos & Westerhof, 1998). Therefore, brachial PPP is a surrogate index of arterial stiffness.

Preload; Preload is the volume of blood present in a ventricle of the heart (left ventricle), after passive filling and atrial contraction. Increase in preload will result in increase of SV and hence an increase in pumping time.

Reaction time (RT); RT for a reflex is the interval of time between application of a stimulus and detection of a defined response.

Respiratory rate (RR); Respiratory rate (RR) is the number of breaths per minute.

Sitting systolic and diastolic blood pressures (sSBP & sDBP); sSBP and sDBP were measured after one minute sitting in 90 degree position.

Stroke index (SI); SI is stroke volume adjusted for body surface area (SI = SV/BSA ml/m^2; normal range 30-65). It describes the volumetric delivery of the pump per each contraction. SI values are affected by heart rate, preload, contractibility, afterload and ejection fraction. SI is the

Glossary Table of cardiovascular variables
most commonly used parameter to describe left ventricular performance. (SI can also be calculated from SV and weight.) **Systemic Vascular Resistance (SVR);** Systemic vascular resistance is the resistance to blood flow from all of the systemic vasculature (excluding the pulmonary vasculature). Mechanisms that cause vasodilatation or reduce viscosity decrease SVR. SVR = (mean arterial pressure - central venous pressure) / cardiac output. As the central venous pressure is normally near 0 mmHg, the simplified version of SVR is: SVR = MAP / CO. **Systolic blood pressure (SBP);** Blood pressure is the pressure exerted by the blood on the walls of the blood vessels. The peak pressure in the arteries during the cardiac cycle is the SBP. It is determined by the stiffness of large arteries, as well as peripheral pulse wave reflection and the pattern of left ventricular ejection. **Systolic Time Ratio (STR);** STR is related to the left ventricular performance and pump emptying efficacy. It can be used to estimate ejection fraction. **Thoracic Fluid Index (TFI);** TFI is the total bioimpedance of the thorax (measured between the root of the neck and the diaphragm). TFI is related to thoracic fluid, represents total impedance (resistance to the high frequency measurement AC current) of the thorax. As more fluid is present within the Thorax, the thorax becomes more conductive, hence its TFI will be lower. TFI is affected by the conductibility of the thorax, hence by thoracic cross-sectionand, therefore it is gender dependent. Normal values are 20-33 ohm for males (27-48 for females). A decline in TFI can occur as a result of redistribution of intravascular fluids due to gravity (changing position from standing to supine), and also as a result of replacement of non conductive air in the lungs by conductive fluids (pulmonary oedema).

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Chapter I; Introduction
The cardiovascular effects of opioids

1.1. Historical introduction

Picture 1- 1. Papaver somniferum

Picture 1- 2. Avicenna (AD 980-1030)

Opium, a poppy extract from the plant Papaver somniferum (Picture 1-1), has been used for centuries as an analgesic (Table 1-1). The use and extraction of opium were described in a Babylonian text dated from 4000 BC. Egyptians used it as a children's sedative and teething remedy in 2000 BC (Polson *et al.*, 1983). The papyrus Ebers from 1500 BC mentions opium as a poison (Gettler, 1956). The first reference in Greek literature to the opium poppy was written in Homer's Odyssey where it was described as a drug that "quiets all pains and quarrels". Nicander of Colophone who lived in western Asia Minor about 130 B.C. wrote about fatal opium overdose and described some antidotes; hot wine and the syrup made from grapes, the oil of roses, olive oil, the oil of iris, and even slapping the hapless victim on the cheeks, shaking him too, hoping that vomiting will follow (Scarborough, 1995). The Roman Theophrastus refers to it in the third century B.C. (Polson *et al.*, 1983). Razi (AD 864 – 930), the Persian surgeon used opium for anaesthesia (Al-ghazal, 2003). Another Persian scientist, Avicenna (AD

980-1030) wrote enthusiastically about opium, especially in diarrhoea and is believed to have died from an accidental overdose (Macht, 1915) (Picture 1-2). Paracelsus (1490-1540) owed much of his success to the bold way in which he administered opium to his patients. He carried opium in the pommel of his saddle and called it the stone of immortality. In 1680 Syddenham wrote: "among the remedies which it has pleased Almighty God to give to man to relieve his sufferings, non is not so universal and so efficacious as opium" (Gutstein & Akil, 2001).

Source	Discovery	Date
Babylonian texts	The use and extraction of opium were described	4000 BC
The papyrus Ebers	Mentions opium as a poison	1500 BC
The Roman Theophrastus	Refers to it in details	300 BC
Nicander of Colophone	Wrote about fatal opium overdose & described some antidotes	130 BC
Razi (Persian surgeon)	Used opium for anaesthesia for the fist time	864 – 930
Avicenna (Persian physician)	Recommended opium especially in diarrhoea & is believed to have died from an accidental overdose	980-1030
Paracelsus	Owed much of his success to opium. He called it the stone of immortality	1490-1540
Syddenham	among the remedies which it has pleased Almighty God to give to man to relieve his sufferings, non is not so universal and so efficacious as opium	1680
Robiguet	Isolated codeine	1832
Serturner	Isolated morphine (Gr. Morpheus, God of sleep)	1863
	Heroin (diacetylmorphine) was isolated. It was claimed to be non addictive.	1874

Table 1- 1. Opioids, historical perspective

The pharmacology of the opium alkaloids has been closely studied for nearly 200 years. Serturner isolated morphine (Gr. Morpheus, God of sleep) in 1863. Robiguet isolated codeine in 1832. Heroin (diacetylmorphine) was isolated in 1874 and introduced to clinical practice in 1898. It is interesting to reflect that it was claimed to be non-addictive (Polson *et al.*, 1983). Extensive study of the pharmacology of opioids in the 20th century has led to a more detailed understanding of the complexity of its receptor mechanisms. Opioids that have been studied for their haemodynamic effects in this thesis are summarised in Table 1-2.

Product	Comment	References
Agonist*		
Non specific agonists		
Morphine	Mu (+++) & K (+), K3(+)	(Gutstein & Akil, 2001)
beta-endorphin	Mu (+++) & Delta (+++)	(Gutstein & Akil, 2001)
Mu agonists		
Hydromorphone		(Preston & Bigelow, 1993)
Oxycodone	Morphine like mu agonist	(Mildh *et al.*, 2000)
DAGO; [D-Ala2, N-Me-Phe4, Gly5-ol]-enkephalin	Selective	(Shen & Ingenito, 1999a; Keay *et al.*, 1997)
H-Tyr-D- Arg-Phe-Lys-NH2	Highly selective opioid peptide	(Kett *et al.*, 1998)
[D-ala2, MePhe4,Gly-(ol)5] enkephalin		(Krumins *et al.*, 1985)
RX783016		(Petty & Reid, 1982)
Pentamorphone		(Afifi *et al.*, 1990)
Endomorphin 1 (Tyr-Pro-Trp-Phe-NH2; EM1)		(Czapla *et al.*, 2000)
Endomorphin 2 (Tyr-Pro-Phe-Phe-NH2; EM2)		(Czapla *et al.*, 2000)
Delta agonists		
Metkephamid	Relatively selective	(Pasanisi *et al.*, 1985)
DPDPE [D-Pen2,D-Pen5]enkephalin		(Marson *et al.*, 1989)
D-ala2, D- Leu3] enkephalin		(Krumins *et al.*, 1985)
[D-Ala2, D-Leu5] enkephalin		(Petty & Reid, 1982)
Kappa agonists		
U-62066E		(Rimoy *et al.*, 1994)
Niravoline		(Bellissant *et al.*, 1996)
U50488H		(Hall *et al.*, 1988)
Dynorphin A		(Gutstein & Akil, 2001)
Spiradoline (U-62,066E)		(Ur *et al.*, 1997)
Asimadoline		(Kramer *et al.*, 2000)
Orphanin FQ		(Zhang *et al.*, 1999)
spiradoline mesylate		(Shen & Ingenito, 1999a; Pugsley *et al.*, 1998)
((5,7,8)-(+)-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-y l]-benzeneacetamide		(Keay *et al.*, 1997)
Ketazocine		(Petty & Reid, 1982)
Antagonists		
Non specific antagonists		
Naloxone	Mu, Kappa & Delta	(Gutstein & Akil, 2001) (Kienbaum *et al.*, 2002a)
Naltrexone	Mu (more potent); kappa receptor	(Preston & Bigelow, 1993)
Specific antagonists		
beta-FNA	Mu selective antagonist	(Kai *et al.*, 2004)
Naloxonazine	Mu selective antagonist	(Sakamoto & Liang, 1989)
Binaltorphimine (nor-BNI)	Selective kappa	(Chen *et al.*, 2003)
Nor-binaltorphimine dihydrochloride	Selective kappa	(Shen & Ingenito, 1999a)
Mixed agonist –antagonist		
Pentazocine	Mixed agonist (kappa) -antagonist	(Preston & Bigelow, 1993)

Table 1- 2. Opioids that have been studied for their haemodynamic effects, *; Classification has been done based on references

1.2. Current Background

Opioid overdose is a common cause of drug-induced hospitalisation in the UK and Scotland, in particular, is facing a serious drug problem. Fifty six thousand individuals aged 15 to 54 years (2% of the Scottish population) were misusing opioids or benzodiazepines in 2000 (Simoens *et al.*, 2002). In the Royal Infirmary of Edinburgh from July 2000 to July 2002, there were 1331 overdose admissions that involved an opioid (alone or in combination). In contrast in a middle-income Middle Eastern country (Mashhad, Iran) opioid overdose is the most common intoxication in all age groups with the highest relative frequency of death (22.5 %) with an 8^{th} rank of case fatality rate (1.9%) (Afshari, 2001; Afshari *et al.*, 2004).

1.3. Epidemiology and importance of opioid overdose

Trends in hospital discharge for the diagnosis of poisoning over the past decade in Scotland demonstrate a clear increase in admissions for poisoning with or misuse of opioids. Overdose was more common in males (Bateman *et al.*, 2003). Official data on overdose deaths attributed to illicit drug addiction and abuse (ICD-9 codes 304 and 305) from 1984 to 2000 in Italy showed that in both genders the age group 35-44 was subject to the highest mortality rate increase over the study period, however, the highest overdose rates for both males and females were observed in the 25-34 age group (Preti *et al.*, 2002). In another study, trends in opiate overdose deaths in Australia from 1979 to 1995 have been studied. The average age at death for males increased from 24.5 years in 1979 to 30.6 years in 1995. The increase in overdose mortality was greatest among men and women aged 35 to 44 years and 25 to 34 years respectively

(Hall & Darke, 1998). Overall opioid overdose is a progressive health problem with more effects on males and young, and middle-aged subjects.

1.4. Clinical uses

The clinical usefulness of opiates has been established for centuries. Opioids, particularly morphine sulphate, are used widely for therapeutic purposes including pain control post operatively and during anaesthesia, and in the management of pulmonary oedema and myocardial infarction. This is despite the main concern about the compounds, particularly the prevalence of drug addiction and abuse (Table 1-3).

Medical usage of opioids

- Analgesia (acute, chronic, postoperative)
- Terminal/palliative care
- Premedication
- Myocardial infarction
- Acute pulmonary oedema
- Cough
- Antimotility (antidiarrhoeal)
- Adjunct in treatment of opioid dependence
- Euphoria

Table 1- 3. Medical usage of opioids

1.5. Opioid drugs and the cardiovascular system

The cardiovascular effects of opioids in man are not consistently reported in the literature and potential peripheral mechanisms of action have not been well studied. A systematic review of the international literature was carried out to evaluate the reported haemodynamic effects of opioids and, in particular, to examine influence of receptor type, mechanisms of action and sources of discrepancy which result from different experimental designs.

1.6. Review literature strategies

The systematic review of the literature (PubMed) was undertaken with the aim of evaluating the known haemodynamic effects of opioids and generating further hypotheses about their potential mechanism of actions. In particular the influence of receptor type, mechanisms of action and sources of discrepancy in different experimental design were considered. Additional information was sourced from individual theses, books in relevant scientific disciplines, Scottish Executive publications in the field of addiction and Scottish Poison Information Bureau files. The review focussed on studies published between 1967 and 2002 written in English or Persian were included. For human studies, preference was given to randomised control trials but quasi-randomised controlled trials, case-control studies, and patient case series were also included. Preference was given to the studies that had enrolled healthy participants above 18 years old of both sexes. The full text as far as possible or abstract of each potentially relevant article was obtained. The initial search strategy is summarised in Figure 1-1.

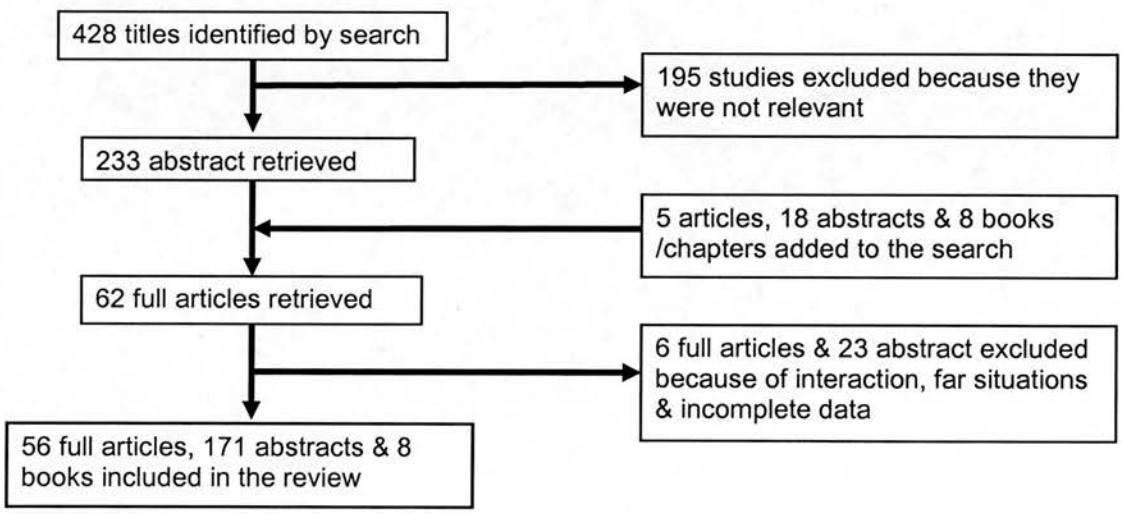


Figure 1- 1. Search Strategy; Number of articles identified, retrieved, and included in the review

1.7. Haemodynamic effects of opioids

In spite of a wide range of experimental evidence supporting the role of opioids in cardiovascular regulation particularly in pathophysiological conditions, the mechanisms involved are not well elucidated and, in some cases, disputed. The available evidence suggests strongly that opioids have significant effects on the cardiovascular system. In many experimental studies looking at different cardiovascular parameters opioids have shown to produce significant effects (Holaday, 1983). The presence of opioid peptides or opioid receptors in brain and spinal cord sites (e.g. hypothalamus, nucleus ambiguous, nucleus tractus solitarius, intermediolateral nucleus and peripheral blood vessels) suggests their involvement in cardiovascular control (Khachaturian *et al.*, 1985b; Martin-Schild *et al.*, 1999). This is supported by observed cardiovascular changes after microinjection of opioids into these sites, which are reversible by opioid receptor antagonists (Faden, 1993). Moreover, evidence of altered levels of endogenous opioids, or their receptors, in pathophysiological conditions such as hypertension also supports this hypothesis (Zamir *et al.*, 1980). Opioids might modulate the influence of psychosocial stress on blood pressure as a mechanism of participation of endogenous opioids in the tonic regulation of blood pressure and in pathogenesis and maintenance of essential hypertension (Kraft, 1994). Systemically injected opioids have cardiovascular effects (Faden, 1993), however they are different in effect, magnitude and time course from the effect seen after direct CNS administration, suggesting different mechanisms of action depending on site of effect.

Patients undergoing opiate withdrawal experience cardiovascular effects, which are usually the opposite of the effects of opioid agonism. The Fultz and Senay grading of

withdrawal includes hypertension as grade 3 in hospitalised patients undergoing opiate withdrawal (Fultz & Senay, 1975). Haemodynamic changes have also been shown in acute naloxone detoxification of addicted patients (Kienbaum *et al.*, 2002a). Antagonist studies also show cardiovascular effects (Foss *et al.*, 1997). Thus there might be benefits, in addition to pain relief, from morphine use in the treatment of angina pectoris and acute myocardial infarction, and this is probably because of decreases in preload, force of contraction and heart rate. The evidence of opioid effects in the literature that may support a role for opioids on the cardiovascular system is summarised in Table 1-4.

	Evidence	Reference
1	Reported experimental studies in which opioids have shown some actions on the cardiovascular system	(Holaday, 1983)
	Systematically injected opioids have shown cardiovascular effects	(Faden, 1993; Martin-Schild *et al.*, 1999)
	Cardiovascular changes after microinjection of opioids into central sites, which are reversible by opioid receptor antagonists	(Faden, 1993)
	Hypotension occurs in opioid overdose	(TOXBASE, 2001)
2	Evidence of altered levels of endogenous opioids or their receptors in pathophysiological such as hypertension conditions as compared with a normal status	(Zamir *et al.*, 1980)
3	The presence of opioid peptides or opioid receptors in brain and spinal cord sites suggests their involvement in cardiovascular control	(Khachaturian *et al.*, 1985a; Khachaturian *et al.*, 1985b)
4	Opioids might modulate the influence of psychosocial stress on tonic regulation of blood pressure and essential hypertension	(Kraft, 1994)
5	Patients undergoing withdrawal experience some cardiovascular effects, which are usually the opposite of the effects of opioid agonism.	(Fultz & Senay, 1975)
6	Haemodynamic changes in acute naloxone detoxification of addicted patients	(Kienbaum *et al.*, 2002a)
7	N-methylnaltrexone bromide (methylnaltrexone), an antagonist that has a limited ability to cross the blood-brain barrier induced orthostatic hypotension	(Foss *et al.*, 1997)

Table 1- 4. Evidence supporting the role of opioids in the cardiovascular system

Although, hypotension occurs in opioid overdose (TOXBASE, 2001), therapeutic doses of morphine-like opioids, do not cause major effects on blood pressure or cardiac rate and rhythm in supine patients, despite evidence of peripheral vasodilatation (Gutstein & Akil, 2001). Reduced peripheral resistance and an inhibition of baroreceptor reflex, which may lead to orthostatic hypotension in the head up position have been reported (Feldberg & Wei, 1986). These effects are not consistent for all opioids, for instance fentanyl has less cardiovascular effects (Rosow *et al.*, 1982).

It is also important to differentiate effects on the cardiovascular system due to effects on opioid receptors from those due to effects on other receptors. Thus, dextropropoxyphene causes arrhythmia, an effect not thought to be related to its opioid agonist properties (Whitcomb *et al.*, 1989; Stork *et al.*, 1995). In summary, the site and mechanism of action of opioids on the cardiovascular system in man are not well understood. The profile of response is likely to be due to actions at several sites, the effects seen being dependant on the receptor selectivity of the compound.

1.8. Opioid receptors

The existence of an opioid receptor was suggested in the pioneering work of Beckett and Casy in 1954 (Beckett & Casy, 1954a; Beckett & Casy, 1954b). Portoghese developed the theory that there were separate kinds of opioid receptors in 1965 (Pugsley, 2002). Opioid binding sites were originally believed to be a homogenous group (Simon *et al.*, 1973; Terenius, 1973). Later however, based on pharmacological evidence, multiple opioid receptors were suggested (Gilbert & Martin, 1976). Now, it is clear that at least three major classes of opioid receptors, so called μ (mu), κ (kappa),

and δ (delta) exist in the central nervous system. However a wide range of other opioid receptors have been suggested including: σ (sigma) (Gilbert & Martin, 1976) which Zukin later suggested might belong to another class (Zukin & Zukin, 1979); ε (epsilon) from rat vas deferan (Chang *et al.*, 1984; Garzon *et al.*, 1984); ι (Iota) or intestinal from rabbit ileum (Oka, 1980); λ (lambda) from rat brain (Grevel & Sadee, 1983); ζ (zeta) from the growth inhibitory effects of opioids in neuroblastomas (Zagon *et al.*, 1989). There is also evidence for the existence of subtypes of the major types of opioid

	Opioid receptor	References
1	Suggestion of the existence of opioid receptor by Beckett and Casy in 1954.	(Beckett & Casy, 1954a; Beckett & Casy, 1954b)
2	Theory of existence of separate opioid receptors developed by Portoghese in 1965	(Portoghese, 1965)
3	Opioid binding sites were originally believed to be a homogenous group in 1973	(Simon *et al.*, 1973; Terenius, 1973)
4	Pharmacological evidence suggest the existence of multiple opioid receptors in 1976	(Gilbert & Martin, 1976)
5	Existence of endogenous encephalin has been shown in rat and rabbit atria in 1977	(Hughes *et al.*, 1977)
6	Iota (intestinal) receptors reported from rabbit ileum in 1980	(Oka, 1980)
7	Evidence for the existence of subtypes of the major types of opioid receptors, such as μ_1 & μ_2 reported in 1981	(Wolozin & Pasternak, 1981)
8	Evidence for the existence of κ_1 & κ_2 reported in 1982	(Attali *et al.*, 1982)
9	lambda receptors reported from rat brain in 1983	(Grevel & Sadee, 1983)
10	ε receptor reported from rat vas deferan reported in 1984	(Chang *et al.*, 1984; Garzon *et al.*, 1984)
11	[3H] diprenorphine binding sites in the heart suggested as physiologically active receptors, involved in regulation of peripheral cardiovascular system in 1985	(Krumins *et al.*, 1985)
12	Myocardial infarction, hypertension, and cardiomyopathy reported to lead to increased enkephalin or proenkephalin mRNA in the heart in 1988 & 1992.	(Paradis *et al.*, 1992) (Dumont & Lemaire, 1988) (Ouellette & Brakier-Gingras, 1988)
13	Zeta receptors reported from the growth inhibitory effects of opioids in neuroblastomas in 1989	(Zagon *et al.*, 1989)
14	Cardiac tissue and isolated cardiac myocytes were shown to contain dynorphin and prodynorphin mRNA in 1991	(Spampinato *et al.*, 1991) (Canossa *et al.*, 1993)
15	An endothelial opioid receptor, μ_3, claimed in 1995	(Stefano *et al.*, 1995a; Cadet *et al.*, 2000).

Table 1- 5. Opioid receptors, historical presentation

receptors, such as μ_1 and μ_2 (Wolozin & Pasternak, 1981) and κ_1 and κ_2 (Attali *et al.*, 1982). The endogenous opioid encephalin has been shown in rat and rabbit atria (Hughes *et al.*, 1977). Recently, an endothelial opioid receptor, μ_3, has been hypothesised (Stefano *et al.*, 1995a; Cadet *et al.*, 2000). Based on an experiment in rat hearts using the opioid ligand [3H] diprenorphine it has been suggested that binding sites in the heart may be physiologically active receptors, involved in regulation of peripheral cardiovascular processes (Krumins *et al.*, 1985). Table 1-5 describes the progressive understanding of opioid receptors over the last 50 years.

1.9. Endogenous opioid peptides

The opioid peptide families first identified were enkephalins, dynorphines and endorphins. These are a large group of small proteins that interact with cell membrane receptors in a similar way to opioid alkaloids. Endogenous opioid peptides may be either secreted from nerves that innervate the heart or be produced in myocardial tissue (Pugsley, 2002). There is some evidence that endogenous opioids play a role in the in development of hypertension. A range of different opioid receptors is present in the brain nuclei involved in cardiovascular regulation (Khachaturian *et al.*, 1985b). Injection of endogenous opioid peptides into certain areas of the brain (cerebral ventricles and brain nuclei) in experimental animals elicit cardiovascular changes (Feuerstein, 1985). Systemic injection of these compounds in anaesthetised rats elicited dose-dependent hypotensive responses concomitant with decreases in peripheral vascular resistance (Czapla *et al.*, 1998; Champion *et al.*, 1997). Experimentally hypertensive rats have a 45% higher level of opioid activity in the spinal cord than controls measured with the radioreceptor assay in several brain regions and the pituitary gland compared to control

(Zamir *et al.*, 1980). Experimentally hypertensive rats have reduced nociceptive responses compared with normotensives (Zamir & Segal, 1979). Under *in vitro* conditions, stimulation of presynaptic opioid kappa, but not mu or delta, receptors inhibits the release of noradrenaline from sympathetic neurones innervating the sinus node in the rabbit isolated heart (Starke *et al.*, 1985). In perfused rat hearts delta and mu opioid receptor agonists directly depress cardiac function (Vargish & Beamer, 1989; McIntosh & Faden, 1986; Feuerstein & Siren, 1987; Barron, 2000; Pugsley, 2002).

1.10. The cardiac effects of opioids

Opioid peptides (enkephalins, dynorphins, and endorphins) have long been considered as neuropeptides or neurotransmitters. There is some evidence that cardiac myocytes produce enkephalins and they may therefore have functions in the heart. Enkephalins have also been localised to many autonomic ganglia and nerves (e.g. stellate ganglia and vagus) (Lundberg *et al.*, 1978; Tang *et al.*, 1982) and non-neuronal tissues. Cardiac tissue and isolated cardiac myocytes contain dynorphins and prodynorphin mRNA (Spampinato *et al.*, 1991; Canossa *et al.*, 1993). It has also been reported that myocardial infarction (Paradis *et al.*, 1992), hypertension (Dumont & Lemaire, 1988), and cardiomyopathy (Ouellette & Brakier-Gingras, 1988) lead to increased levels of enkephalins or proenkephalin mRNA in the heart. The documented effects of opioids in man are summarised in table 1-6.

1.11. Haemodynamic effects of opioids

The haemodynamic effects of opioids, categorised by the type of receptor and experimental model are summarised in Table 1-7 (A & B). As can be seen the effects of opioid agonism are overwhelmingly reported as haemodynamic depressor. In general,

- **Central nervous system**

Analgesia, Drowsiness, Changes in mood, Mental clouding, Euphoria, anaesthesia (Large doses of Morphine), Hypothalamus; body temperature ↓, chronic high doses ↑

- **Neuroendocrine effects**

GnRH ↓ & CRF ↓, LF↓, FSH↓; & ACTH↓, β-endorphin↓ =>Testosterone↓, Cortisol ↓, Thyrotropin ↔, PRL↑, GH ↔ & ↑, ADH ↓(μ),↑(κ)

- **Myosis**

Excitatory action on the parasympathetic intervention of the pupils

- **Convulsions**

Induced by high doses in animals, ? by ↓GABA by interneurones

- **Respiration**

↓ respiration (rate, minute volume, & tidal volume) Reduction of the responsiveness of the brainstem respiratory centres to the carbon dioxide), Irregular, periodic breathing, ↓ the cough reflex (direct effect of the cough centre in the medulla)

- **Gastrointestinal tract**

Nauseant & emetic effect (direct stimulation of the chemoreceptor trigger zone for emesis , in the area postrema of the medulla)
Stomach; Hydrochloric acid ↓, Somatostatin ↑ from pancreas, Acetylcholine ↓, Gastric motility ↓, gastric emptying time ↓, =>oesophageal reflux↑, tone antral & first part of duodenum ↑, delayed passage and delayed absorption of drugs.
Small intestine; intestinal secretion↓, delays digestion of foods, resting tone↑ & periodic spasm, Amplitude of non propulsive rhythm↑, propulsive ↓,
Large intestine; propulsive peristaltic waves in the colon ↓, tone↑ & spasm, delayed passage, desiccation of the faeces, tone of anal sphincter↑
Biliary tract; sphincter Oddi constriction, rise in pressure up to 10 fold

- **Other smooth muscles**

Ureter; tone & amplitude ↑, inhibit the urinary voiding reflux
Bladder; tone of external sphincter & volume ↑

- Uterus; prolong labour
- Skin; dilation of cutaneous blood vessels, facial flushing, urticaria (histamine release, not blocked by Naloxone)
- Immune system; inhibition of formation of rosetts by human lymphocytes
- **Tolerance & physical dependence**
- **Cardiovascular system**

BP; S↓,↓, ↔, ↑, S↑
HR; S↓,↓, ↔, ↑, S↑
Peripheral vasodilatation, Dilation of cutaneous blood vessels & facial flashing
Peripheral resistance S↓,↓
Inhibition of baroreceptor reflexes
Cardiac indexes ↓, ↔, Cardiac work ↓
Oxygen consumption↓
Left ventricular end diastolic pressure ↓
Hypovolaemic shock ↑
Cerebral circulation (vasodilatation) (Indirect by opioid induced respiratory depression and CO_2 retention)
Arrhythmia

Table 1- 6. Overview of the effects of opioids on man, S↓; Significant decrease, ↓; Non significant decrease, ↔; No changes, ↑; Non significant increase, S↑; Significant increase, ?; Probably

A) Agonist				
Non-specific				
		Human		Animal
Effect	H. Volunteers	Dependent	Patients	
↑	(Mildh *et al.*, 2000)	(Rubio *et al.*, 1997)		(Gomes *et al.*, 1976; Kayaalp & Kaymakcalan, 1966; Sitsen *et al.*, 1982; Vatner *et al.*, 1975)
↓	(Petry *et al.*, 1998)	(Rubio *et al.*, 1992)	(Cathelin *et al.*, 1980b; Cathelin *et al.*, 1980a; Rosow *et al.*, 1982)	(Gomes *et al.*, 1976; Sitsen *et al.*, 1982)
↔	(Lowenstein *et al.*, 1969)			
μ specific				
↑		(Preston & Bigelow, 1993)		(Kiritsy-Roy *et al.*, 1989; Bachelard & Pitre, 1995; Keay *et al.*, 1997; Bachelard *et al.*, 1997; Widy-Tyszkiewicz & Czlonkowski, 1991)
↓			(Patschke *et al.*, 1976; Sebel *et al.*, 1995; Lyons *et al.*, 1995)	(Marson *et al.*, 1989; Randich *et al.*, 1993; Czapla *et al.*, 2000; Laubie *et al.*, 1977; Widy-Tyszkiewicz & Czlonkowski, 1991; Petty & Reid, 1982)
↔	(Mildh *et al.*, 2000)		(Crosby *et al.*, 1994; Murat *et al.*, 1988; Rosow *et al.*, 1982; Flacke *et al.*, 1987)	(Shen & Ingenito, 1999a; Keay *et al.*, 1997; Ogutman *et al.*, 1995; Laubie *et al.*, 1977; Petty & Reid, 1981)
δ specific				
↑				(Kiritsy-Roy *et al.*, 1989; Rochford & Henry, 1990; Bhargava & Rahmani, 1993; Widy-Tyszkiewicz & Czlonkowski, 1991; Petty & Reid, 1982).
↓	(Pasanisi *et al.*, 1985)			(Marson *et al.*, 1989; Widy-Tyszkiewicz & Czlonkowski, 1991)
↔	(Pasanisi *et al.*, 1985)			(Randich *et al.*, 1993; Bachelard & Pitre, 1995; Keay *et al.*, 1997)
κ specific				
↑	(Bellissant *et al.*, 1996)			(Rochford *et al.*, 1991; Glatt *et al.*, 1987; Widy-Tyszkiewicz & Czlonkowski, 1991; Petty & Reid, 1982).
↓				(Shen & Ingenito, 1999a; Zhai & Ingenito, 1998; Hall *et al.*, 1988; Pugsley *et al.*, 1998) (Keay *et al.*, 1997; Ogutman *et al.*, 1995; Wright & Ingenito, 2001; Shen & Ingenito, 1999c) (Wright & Ingenito, 2000; Zhai & Ingenito, 1997; Zhai & Ingenito, 1998; Wang & Ingenito, 1994a) (Zhang *et al.*, 1999; Randich *et al.*, 1993; Wang & Ingenito, 1994b; Widy-Tyszkiewicz & Czlonkowski, 1991; Glatt *et al.*, 1987; Shen & Ingenito, 1999b).
↔	(Rimoy *et al.*, 1994; Kramer *et al.*, 2000)			(Bachelard & Pitre, 1995)
B) Mixed antagonist-agonist				
Non-specific				
		Human		*Animal*
Effect	*H. Volunteers*	*Dependent*	*Patients*	
↑		(Preston & Bigelow, 1993) (Lamas *et al.*, 1994)		
↓				
↔				(Petty & Reid, 1982)

C) Antagonist

Non-specific		Human		Animal
Effect	H. Volunteers	Dependent	Patients	
↑		(Kienbaum *et al.*, 1998; Kienbaum *et al.*, 2002a; Preston & Bigelow, 1993)		
↓				
↔	(Coppola *et al.*, 1994; Fuenmayor & Cubeddu, 1986; Staessen *et al.*, 1989).	(Coppola *et al.*, 1994)		(Petty & Reid, 1982)
μ specific				
↑				
↓				
↔				
δ specific				
↑				
↓				
↔				(Rochford & Henry, 1990; Marson *et al.*, 1989; Randich *et al.*, 1993)
κ specific				
↑				(Shen & Ingenito, 2000; Wright *et al.*, 1999)
↓				
↔				(Shen & Ingenito, 2000; Wright *et al.*, 1999)

Table 1- 7. Haemodynamic effects of opioids agonists (A), mixed agonist-antagonist (B) and antagonists (C) (↑; Pressor (e.g. increase in blood pressure), ↓; Depressor, ↔; No effect) and antagonism

opioid antagonism results in no effect or, more rarely, a pressor effect. The type of receptor involved and experimental conditions are other determinants of the results.

1.12. Electrophysiological effects

Although opioids are generally assumed to have no effect on the electrocardiogram, there is evidence suggesting that structurally similar opioids, dextropropoxyphene and methadone, particularly in overdose, are electrophysiologically active. In anaesthetized rats has been shown that the kappa agonist spiradoline dose-dependently reduces blood pressure and heart rate and prolongs the PR interval and QRS width. These effects suggest of sodium channel blockade in the rat were dose-dependently

increased. Spiradoline produced its antiarrhythmic actions via sodium channel blockade in myocardial tissue, although higher doses also block potassium currents (Pugsley *et al.*, 1998). Dose-dependent QT prolongation and occurrence of Torsades des pointes in patients treated with methadone has been reported (Krook *et al.*, 2004; Krantz *et al.*, 2003). High dosages of the long-acting methadone derivative, levomethadyl acetate HCl (LAAM; ORLAAM) has also induced a prolonged QTc interval and polymorphic QRS complexes (Deamer *et al.*, 2001).

Dextropropoxyphene in particular causes prolongation of PR interval and QRS duration in high doses in animals (Bredgaard *et al.*, 1984; Holland & Steinberg, 1979). In man, dextropropoxyphene overdose has also been shown to cause QRS complex widening, and other arrhythmias (Stork *et al.*, 1995; Whitcomb *et al.*, 1989; Heaney, 1983). This effect has been attributed to its membrane stabilising effect through blockade of fast

	Electrophysiological effects of opioids	**References**
1	Prolongation of PQ and QRS duration has been reported in intoxicated pigs with dextropropoxyphene	(Bredgaard *et al.*, 1984)
2	Prolongation of PR interval in conscious dogs has been shown to be significant ($p<0.05$) with dextropropoxyphene but not significant with norpropoxyphene	(Holland & Steinberg, 1979)
3	Death has frequently been reported from dextropropoxyphene	(Jonasson *et al.*, 2000a; Obafunwa *et al.*, 1994)
4	Death from dextropropoxyphene overdose is rapid	(Whittington, 1984).
5	In a case report, dextropropoxyphene intoxication induced QRS complex widening	(Stork *et al.*, 1995)
6	This is claimed to be attributed to its membrane stabilising effect through blockade of fast sodium channel, as quinidine cause similar wide complex dysrrhythmia	(Henry & Cassidy, 1986; Stork *et al.*, 1995)
7	In another case report marked QRS widening was reversed by lidocaine	(Whitcomb *et al.*, 1989)
8	One case of left bundle branch block following acute dextropropoxyphene hydrochloride overdose has also been reported, which was transient and associated with no permanent sequelae.	(Heaney, 1983)

Table 1- 8. Electrophysiological effects of opioids

sodium channel, as quinidine cause similar wide complex dysrrhythmia (Henry & Cassidy, 1986; Stork *et al.*, 1995). Evidence related to dextropropoxyphene induced ECG changes are summarised in table 1-8.

1.13. Potential sources of discrepancy in literature

The inconsistent results of experimental studies on the haemodynamic effects of opioids make it difficult to produce a single hypothesis as to their mechanism of action. This is partly because opioid effects on the cardiovascular system may be a result of

	Confounders	References
Related to opioid properties		
1	Type of opioid	(Rosow *et al.*, 1982; Flacke *et al.*, 1987; Fuenmayor & Cubeddu, 1986; Khalid *et al.*, 1987; Wang & Ingenito, 1994b)
2	Anaesthetic state	(Gomes *et al.*, 1976; Sitsen *et al.*, 1982)
3	Type of anaesthetic	(Sitsen *et al.*, 1982)
4	Background condition	(Feuerstein *et al.*, 1989)
5	Route of administration	(Glatt *et al.*, 1987; Widy-Tyszkiewicz & Czlonkowski, 1991)
6	Concentration of opioid receptors at administration site	(Feuerstein & Faden, 1982)
7	Dose	(Bellet *et al.*, 1980; Faden & Feuerstein, 1983)
8	Acute or chronic administration	(Tress & El Sobky, 1980)
9	Subject position	(Pasanisi *et al.*, 1985)
10	Agonist – antagonist pharmacokinetics	(Ngai *et al.*, 1976)
11	Type of experimental species	(Nickander *et al.*, 1984)
12	Exercise	(Carter *et al.*, 2002)
Related to opioid induced interaction with other pathways		
1	Opioid induced hypoxia	(Leino *et al.*, 1999; Moody *et al.*, 2001; Ishimura *et al.*, 1996)
2	Opioid induced hypercapnia	(Thompson *et al.*, 1995; Ganong, 2001)
Related to opioid induced secondary mechanism		
1	Opioid induced histamine release	(Flacke *et al.*, 1987)
2	Nitric oxide pathway activation	(Stefano *et al.*, 1995a)
3	Sodium channel blockade	(Stork *et al.*, 1995; Heaney, 1983; Whitcomb *et al.*, 1989)

Table 1- 9. Major confounders affecting experimental studies on the role of opioids in the cardiovascular system.

action on central and peripheral sites and partly because of different experimental study designs employed in the literature (Table 1-9). The source of discrepancy may be related to opioid properties, to opioid-induced interaction with other pathways, or related to opioid-induced secondary mechanism.

1.14. Mechanistic studies (Human studies, whole animal models, isolated tissues)

Morphine is traditionally regarded as a hypotensive agent. Several mechanisms of action have been hypothesised for this effect including histamine release, changes in vagal tone, and venous and arterial vasodilatation (Lowenstein *et al.*, 1969), decreases in cardiac and renal sympathetic nerve activity (Feldberg & Wei, 1986; Mori *et al.*, 1998), actions on ion channels, and concurrent hypoxia and hypercapnia. On the other hand, morphine has also reported as a pressor compound. Central mechanisms, activation of the sympathetic system (Hoar *et al.*, 1981), and renin-angiotensin system (Bailey *et al.*, 1975) have also been proposed. The potential mechanisms of haemodynamic effects man are discussed below.

1.14.1. Mu receptor

The peripherally active antagonist N-methylnaltrexone bromide has a limited ability to cross the blood-brain barrier and induced orthostatic hypotension with no release of histamine (Foss *et al.*, 1997). Moreover, a μ_3 opiate alkaloid-specific receptor has been claimed to be present in the vasculature based on cell culture (Stefano *et al.*, 1995a). This has been extensively studied by the same group (Cadet *et al.*, 2000; Stefano *et al.*, 2002; Stefano, 1998; Stefano *et al.*, 1998; Cadet *et al.*, 2004). They also showed this receptor is identical with the neuronal human mu_1 receptor (Cadet *et al.*, 2000). However, its presence has not been confirmed independently.

1.14.2. Histamine

Morphine stimulates the release of histamine from mast cells directly and without prior sensitization (Brown & Reberts, 2001). Moreover, the majority of normal subjects receiving intravenous bolus doses of morphine or nalbuphine have been reported to show significant elevations in plasma histamine (Fahmy *et al.*, 1983; Doenicke *et al.*, 1995). The degree of haemodynamic compromise was related to the plasma histamine concentration (Flacke *et al.*, 1987). This effect is probably due to relaxation of arteriolar smooth muscles, precapillary sphincters and muscular venules mediated via H_1 and H_2 receptors, which may stem from the activation of adenylate cyclase (Brody *et al.*, 1998).

Histamine causes dilatation of small blood vessels and reduces total peripheral resistance causing a fall in systemic blood pressure. Vasodilatation involves both H_1 and H_2 receptors distributed throughout the resistance vessels in most vascular beds (Brown & Reberts, 2001). This action appeared to be independent of the action of the endothelium (Ganong, 2001). Histamine has been shown to induce a concentration-dependent coronary vasodilatation, with increase in basal cGMP and nitric oxide release in the isolated pig hearts. This is mediated either by H_1-receptor mediated nitric oxide release from the endothelium (Kelm *et al.*, 1993) or an cAMP-initiated action through the histamine H_2-receptor (Kishi *et al.*, 1998). Histamine mediated vasodilatation was partially blocked by H_1 antagonists, however, it was also effectively reversed by naloxone (Gutstein & Akil, 2001).

Involvement of histamine is also supported by the fact that the selective μ agonist, fentanyl, and its derivatives, which do not release histamine (Flacke *et al.*, 1987), are less likely to cause haemodynamic instability (Gutstein & Akil, 2001). Remifentanil induced a slight reduction in systolic blood pressure, which was not associated with alterations in histamine concentration (Sebel *et al.*, 1995). Therefore, it is likely that differences in the release of histamine might account for some, if not all, of the different effects of morphine and fentanyl on the peripheral vasculature (Rosow *et al.*, 1982). Moreover, in man, antagonism of the haemodynamic effects of morphine can be obtained by the use of the combination of H_1 (diphenhydramine) and H_2 (cimetidine) antagonists (Philbin *et al.*, 1981). A combination of H_1 and H_2 antagonists is optimal as shown by the protective effects of preoperative terfenadine and ranitidine on tubocurarine and morphine-induced blood pressure changes (Treuren *et al.*, 1993).

1.14.3. Nitric oxide (NO)

It is accepted that the flow induced arteriolar dilation is due to local release of NO. Also a prompt rise in blood pressure occurs when an inhibitor of NO is administered to experimental animals and man (Ganong, 2001; Haynes *et al.*, 1993). Morphine and NO are related in many biological circumstances. For instance, they have been linked in gastrointestinal regulation in which It is suggested that endogenous nitric oxide is likely to be involved in the gastroprotective action of morphine (Gyires, 1994). In mice endogenous nitric oxide modulates morphine-induced constipation (Calignano *et al.*, 1991). Peripheral morphine analgesia probably involves NO-stimulation of cGMP (Ferreira *et al.*, 1991). Endothelial cells might therefore be under the direct control of opioids.

It has been shown that morphine, but not the mu agonist DAGO, resulted in a dose-dependent release of nitric oxide from endothelial cells, which was blocked by naloxone. This has led to the suggestion of alleged mu_3 opioid specific receptor (Stefano *et al.*, 1995a). These authors described a specific binding site for morphine on endothelial cells coupled to nitric oxide release in human endothelial cells. This suggests a direct modulatory control over the activities of endothelial cells leading to vasodilatation.

This production of nitric oxide has been shown to be sensitive to naloxone antagonism, as well as nitric oxide syntheses inhibition (Stefano *et al.*, 1995a). However, it has been reported that unlike morphine, fentanyl, a mu specific agonist which has less ability to release histamine (Flacke *et al.*, 1987), does not possess the ability to bind to this alleged mu_3 receptor, and therefore does not increase nitric oxide release (Bilfinger *et al.*, 1998a). These facts together raise the possibility of the existence of a morphine induced non-opioid receptor pathway for NO release, possibly due to histamine release. This hypotheses is explored later in this thesis.

1.14.4. Hypoxia

Morphine causes hypoxia. Intravenous morphine and oxycodone decrease respiratory rate, minute ventilation and respiratory cycle (Leino *et al.*, 1999). Pentamorphone which has a rapid onset and short duration of action, produced dose-related reductions in the ventilatory response to hypoxia (Afifi *et al.*, 1990). In volunteers intrathecal administration of morphine led to a dose-related decrease the SpO_2. heart rate, systolic blood pressure, and respiratory rate changes were not dose related, but low doses

were used (Bailey *et al.*, 1993). Also intravenous morphine has shown to decrease breathlessness during exercise in healthy man (Masood *et al.*, 1995).

A fall in O_2 tension in most tissues produces vasodilatation by local autoregulatory mechanisms (Ganong, 2001). Hypoxia also stimulates the vasomotor centre directly (Ganong, 2001) and leads to tachycardia and increased cardiac output by reflex activation of the sympathetic nervous system. Therefore unless hypoxia is prolonged or severe, blood pressure will be maintained (Moody *et al.*, 2001). In cats hypoxia-induced arteriodilation was related to changes in the nitric oxide pathway (Ishimura *et al.*, 1996). In newborn pigs norbinaltorphimine, a kappa-opioid antagonist, potentiated hypoxia-induced pial dilation. An increase in CSF methionine encephalin, a mu-opioid agonist was seen. N omega-nitro-L-arginine (L-NMMA), an NO synthase inhibitor also blunted hypoxia-induced vasodilatation (Armstead, 1995).

Overall, respiration is inhibited by opioids and hypoxia has cardiovascular effects. Therefore cardiovascular changes due to opioids may in part be secondary to hypoxia. In overdose, where respiratory effects are predominant and more serious, hypoxia might be more important.

1.14.5. Hypercapnia

The main adverse reaction limiting the therapeutic potential of opioids is dose-dependent respiratory depression (Florez & Hurle, 1993). Intravenous morphine produces a significant increase in arterial PCO_2 (Thompson *et al.*, 1995). In healthy volunteers pentamorphone reduced the ventilatory responses to hypercapnia in a dose-related manner (Afifi *et al.*, 1990). Coronary blood flow increases at arterial PCO_2

values above 85 mmHg. Carbon dioxide in high concentration is a vasodilator and is a negative inotrope (Van den bos *et al.*, 1979). Increase in CO_2 level increases the plasma concentration of adrenaline and noradrenaline by activation of sympathetic nervous system (Moody *et al.*, 2001). The vasodilator effect of CO_2 is most pronounced in the skin and brain (Ganong, 2001). However, rise in arterial PCO_2 stimulates the vasomotor area. Therefore, the central and peripheral effects tend to cancel each other. Exposure to high concentration of CO_2 is associated with marked cutaneous and cerebral vasodilatation, but there is vasoconstriction elsewhere and usually a slow rise in blood pressure (Ganong, 2001). In a study using colour Doppler imaging in 12 volunteers showed CO_2 altered flow velocity predominantly in the middle cerebral artery and less so in other vessels studied. Peak systolic and end-diastolic velocities rose (Harris *et al.*, 1996). Positron scanning also show regional differences in cerebral vascular response to $PaCO_2$ changes (Ito *et al.*, 2000).

Overall, the effects of hypercapnia, both central and peripheral, should be considered as a possible mechanism for the haemodynamic effects of opioids. Moreover, in opioid overdose in which the respiratory effects are predominant and more serious this mechanism might be particularly important.

1.14.6. Sympathetic activity

1.14.6.1. Human studies

Although opioids are normally considered as cardiovascular depressor agents, a short duration increase in arterial blood pressure caused by morphine has been reported in man (Mildh *et al.*, 2000). These authors reported no change in plasma adrenaline levels, however, some experiments show that plasma adrenaline increases in man after

morphine injection (Flacke *et al.*, 1987). This may suggest an activation of the adrenal medulla by histamine (Fahmy *et al.*, 1983). Concurrent vasodilatation and sympathoadrenal activation have been shown in patients with coronary artery disease that received morphine sulphate for surgery (Pant *et al.*, 1983; Hoar *et al.*, 1981; Yoshimoto *et al.*, 2005).

Despite evidence suggesting an association between opioids and muscle sympathetic nerve activity in man at rest, opioids do not alter cardiovascular and muscle sympathetic nerve activity responses to isometric handgrip or post-exercise muscle ischemia (Carter *et al.*, 2002). Overall these studies suggest that mu agonism has effects on the sympathetic system, which probably coincides with opioid depressor effects.

Other opioid receptors might also be involved. A selective kappa agonist, niravoline, significantly increased plasma levels of noradrenaline accompanied by a slight and transient increase in blood pressure (Bellissant *et al.*, 1996). However, another selective kappa agonist, spiradoline (U-62066E) failed to change the plasma catecholamines, blood pressure, or pulse rate (Ur *et al.*, 1997). The delta receptor agonist, deltorphin, attenuated stress-induced activation of the sympathetic nervous system (an inhibitory effect on noradrenaline release) induced by both insulin-induced hypoglycemia and the cold pressor test (degli Uberti *et al.*, 1993). Opioid involvement in muscle sympathetic activity in man is also supported by the studies in which subjects on chronic mu-opioid receptor agonist thrapy have been shown to havbe a decrease in

the resting muscle sympathetic activity, despite similar arterial blood pressure and heart rate to matched healthy subjects (Kienbaum *et al.*, 2001; Kienbaum *et al.*, 2002b).

The contribution of catecholamines to the cardiovascular effect of opioids is also supported by antagonist studies. Opioid receptor blockade in chronic users markedly increased resting muscle sympathetic activity and noradrenaline and adrenaline arterial plasma concentrations as well as mean arterial pressure and heart rate (Kienbaum *et al.*, 2001). However, this result was not consistent with some other studies (Farrell *et al.*, 1991). During acute detoxification with naloxone and under anaesthesia a 30-fold increase in adrenaline and a three-fold increase in noradrenalin plasma concentrations were seen. These were associated with increased oxygen consumption and marked cardiovascular stimulation. This suggests that opioid receptor agonists may act via the sympathetic nervous system particularly by effects on the adrenal system (Kienbaum *et al.*, 2000). However, increase in catecholamines might be in part due to concurrent but independent withdrawal syndrome.

In general naloxone has been shown to be incapable of changing muscle sympathetic activity at rest (Estilo & Cottrell, 1982; Rubin *et al.*, 1983; McMurray *et al.*, 1991); however, in some other studies naloxone potentiated an increase in MSA during exercise and in response to lower body negative pressure (Hara & Floras, 1992; Farrell *et al.*, 1991). Also it is shown that dynamic or static exercise induced cardiovascular effects were not association with an opioid antagonist (Cook *et al.*, 2000; Floras, 1991; Kirno *et al.*, 1993). As Carter argued, this is probably because, the exercise stimulus is

not sufficient enough to activate the opioid system, particularly as the naloxone is an receptor antagonist, rather than activator (Carter *et al.*, 2002).

It is also suggested that catecholamine secretion from pathological chromaffin tissue is modulated by endogenous opioids. This was particularly evident in patients with pheochromocytoma (Mannelli *et al.*, 1986). The main evidence supporting an association of opioids and sympathoadrenal system in man is summarised in table 1-10.

	Comments	Reference
1	Plasma adrenaline increases in man after morphine injection	(Flacke *et al.*, 1987)
2	Morphine induced ↑ BP in man.	(Mildh *et al.*, 2000)
3	Morphine activates the adrenal medulla via histamine release	(Fahmy *et al.*, 1983)
4	Concurrent vasodilatation and sympathoadrenal activation in cases receiving morphine prior to surgery	(Hoar *et al.*, 1981)
5	Selective kappa agonist, niravoline, significantly increased plasma levels of noradrenalin accompanied by a slight and transient increase in blood pressure.	(Bellissant *et al.*, 1996)
6	Delta receptor agonist, deltorphin, attenuated stress-induced activation of sympathetic nervous system.	(degli Uberti *et al.*, 1993)
7	Dependent subjects have decreased resting muscle sympathetic tone.	(Kienbaum *et al.*, 2001; Kienbaum *et al.*, 2002b)
8	Opioid receptor blockade in chronic users markedly increased the resting muscle sympathetic activity and noradrenalin and adrenaline arterial plasma concentrations as well as mean arterial pressure and heart rate	(Kienbaum *et al.*, 2001)
9	The cardiovascular pattern in acute detoxification with naloxone and under anaesthesia has revealed a 30-fold increase in adrenaline and three-fold increase in noradrenalin plasma concentrations.	(Kienbaum *et al.*, 2000)
10	Catecholamine secretion from normal and pathological chromaffin tissue is modulated by endogenous opioids.	(Mannelli *et al.*, 1986)

Table 1- 10. Evidence supporting the effects of opioid on sympathetic system in man

1.14.6.2. Animal studies

Based on animal studies, there is considerable evidence to support a centrally mediated role for endogenous opioid peptides in the brain as a regulator of cardiovascular system activities (Feuerstein, 1985) (Table 1-11). Opioid receptors and peptides have been found in the brain (Mansour *et al.*, 1988). Central administration of opioid peptide result in cardiovascular effects (Kiritsy-Roy *et al.*, 1986). It has been shown that the hypertensive response to central opioid stimulation is mediated by an increase in the sympathetic outflow to the adrenal medulla and sympathetic nerve terminals (Bachelard *et al.*, 1997). Experimentally hypertensive rats have a 45% higher level of opioid activity in the spinal cord compared to control (Zamir *et al.*, 1980). *In vivo* morphine has a dose- dependent depolarizing effect on the resting membrane potential of most of the neurons in the stellate ganglion (Bosnjak *et al.*, 1986).

Opioid receptors in the brain regulate autonomic outflow. Injections of either the mu-selective agonist, [D-Ala2, MePhe4, Gly-ol5]encephalin (DAMGO), [D-Ala2,N-Me-Phe4,Gly5-ol]encephalin (DAGO) or the delta-selective agonist, [D-Pen2, D-Pen5]encephalin (DPDPE), morphine, fentanyl and [D-Ala2]-met-enkephalinamide have been shown to increase plasma catecholamine levels and blood pressure in a dose-related manner (Kiritsy-Roy *et al.*, 1989; Bellet *et al.*, 1980; Feldberg & Wei, 1986; Kiritsy-Roy *et al.*, 1986; Appel *et al.*, 1986a; Bachelard *et al.*, 1997; Paakkari *et al.*, 1992; Marson *et al.*, 1989).

From a cell biological point of view, opioid signalling plays an extensive role in the medullospinal network that controls the sympathetic tone and arterial pressure. Mu-

opioid receptors are found post-synaptically, whereas presynaptic receptors probably include both mu and delta subtypes (Guyenet *et al.*, 2002; Ang *et al.*, 1999; Khachaturian *et al.*, 1985a). A counteracting and masked or biphasic effect is also suggested, as remifentanil decreases HR and MAP by its central vagotonic effect and by stimulating peripheral mu-opioid receptors (Shinohara *et al.*, 2000; Vatner *et al.*, 1975; Randich *et al.*, 1993; Wallenstein, 1979).

	Comments		Reference
1	Opioid receptors & peptides have been found in the brain		(Mansour *et al.*, 1988)
2	Mu-opioid receptors are found post-synaptically, Both mu and delta subtypes are found presynaptic		(Guyenet *et al.*, 2002; Ang *et al.*, 1999; Khachaturian *et al.*, 1985a)
3	In vivo morphine has a dose-dependent depolarizing effect on the resting membrane potential of most of the neurons in the stellate ganglion		(Bosnjak *et al.*, 1986)
4	Central administration of opioid peptide result in cardiovascular effects		(Kiritsy-Roy *et al.*, 1986)
5	Hypertensive response to central opioidergic stimulation is mediated by an increase in the sympathetic outflow to the adrenal medulla and sympathetic nerve terminals		(Bachelard *et al.*, 1997)
6	Experimentally hypertensive rats have a higher level of opioid activity in the spinal cord		(Zamir *et al.*, 1980)
7	Injections of DAMGO, DAGO, DPDPE, morphine, fentanyl and [D-Ala2]-met-enkephalinamide have been shown to increase plasma catecholamine levels and blood pressure in a dose-related manner		(Kiritsy-Roy *et al.*, 1989; Bellet *et al.*, 1980; Feldberg & Wei, 1986; Kiritsy-Roy *et al.*, 1986; Appel *et al.*, 1986a; Bachelard *et al.*, 1997; Paakkari *et al.*, 1992; Marson *et al.*, 1989)
8	Concurrent or biphasic haemodynamic effects are also suggested, such as remifentanil is centrally haemodynamically depressor, and pressor via peripheral mu-opioid.		(Shinohara *et al.*, 2000; Vatner *et al.*, 1975; Randich *et al.*, 1993; Wallenstein, 1979).
9	I.v. morphine induced HR ↑, reversed by beta blockade, Alpha receptor blockade abolished the late coronary vasoconstriction		(Vatner *et al.*, 1975)
10	DAGO & DPDPE Restraint stress: catecholamines ↑; BP↑, DAGO & DPDPE: catecholamines ↑; BP↑, DAGO & DPDPE during restraint, HR ↓; BP ↓, Effects blocked by naloxone but not by the delta-selective antagonist ICI 174864		(Marson *et al.*, 1989)
11	I.c.v. DAMGO or DPDPE induced plasma catecholamine levels↑, BP ↑(dose-related), & HR↑ in highest dose. Antagonized by naloxone		(Kiritsy-Roy *et al.*, 1989)
12	After haemorrhage Intrathecal & intracisternal naloxone methiodide abolished the fall in blood pressure		(Ang *et al.*, 1999)
13	I.v. DAGO & morphine	↓HR	(Randich *et al.*, 1993)
	Either with bilateral cervical vagotomy or pre-treatment with the mu_2 opioid receptor antagonist beta-FNA.	↓HR of both significantly attenuated	
	Pre-treatment with the mu 1 opioid receptor antagonist naloxonazine	Affected DAGO, but not morphine induced ↓HR	
14	Remifentanil in intact rabbits	HR ↓; MAP↓, Renal sympathetic nerve activity, DD	(Shinohara *et al.*, 2000)
	Baro-denervated rabbits	HR ↓; MAP↓, Increased RSNA had returned to baseline	
	Baro-denervated & remifentanil pre-treated with naloxone	Abolished these changes	

Table 1- 11. Evidence supporting the central role of opioids in animals, A; anaesthetised, C; conscious, D; dog, DD; dose dependent, I; intact, N; normal, R; rabbit,

1.14.7. Other potential mechanisms

Morphine infusion in conscious newborn piglets results in elevated mean arterial blood pressure, and caused significant elevations in plasma ET-1(Modanlou & Beharry, 1998). In man also morphine significantly increased plasma ET-1 levels through activation of neutral endopeptidase 24.11 (Wang & Chang, 2001; Wang & Hung, 2003). Sodium channels may mediate some non opioid effects of opioids, although this has shown to be controversial (Grudt & Williams, 1993; Pugsley, 2002; Pugsley *et al.*, 1998; Ingram & Williams, 1994; Tai *et al.*, 1992; Laurent *et al.*, 1986).

1.15. Conclusion

Public exposure to opioids is high, and for illegal opioids such as diamorphine, precise usage is unknown. In Edinburgh more than 10% of patients admitted to the toxicology ward have taken an opioid as a part of their ingestion and opioids are also a significant cause of death in young people. For these reasons improved understanding of the immediate cardiovascular effects of opioids would be desirable. Chapter I has summarised the evidence supporting haemodynamic activity of mu and kappa opioid receptor agonists in particular. However, the findings are somewhat inconsistent in animal and man (Gomes *et al.*, 1976; Kayaalp & Kaymakcalan, 1966; Sitsen *et al.*, 1982; Gomes *et al.*, 1976; Kayaalp & Kaymakcalan, 1966; Vatner *et al.*, 1975), (Fahmy *et al.*, 1983; Rosow *et al.*, 1982; Philbin *et al.*, 1981; Lowenstein *et al.*, 1969; Mildh *et al.*, 2000). Overall opioids seem to be haemodynamically depressor, although a transient pressor effect has been seen from i.v. doses in man (Mildh *et al.*, 2000).

In general, it seems that the haemodynamic effects of opioids are probably due to a combination of depressor effects of direct opioid receptor agonism and some addition vasodilatation effects caused by histamine release. In addition, opioids induce sympathetic and parasympathetic activation while also interact on the haemodynamic responses (Marson *et al.*, 1989; Randich *et al.*, 1993; Czapla *et al.*, 2000). Direct mu agonism and histamine both seem to act on the vasculature via nitric oxide (Stefano *et al.*, 1995a; Cadet *et al.*, 2000). Some opioids block sodium channels and this is another potential mechanism of action of some opioids on the cardiovascular system. Indeed, prolonged QRS duration is seen in dextropropoxyphene overdose. The complexity of the potential influence of opioids on cardiovascular regulation is shown in Figure 1-2.

1.16. Scope of this thesis

The primary aims of this thesis were:

- To investigate the epidemiology and outcome of opioid overdose as it presents to NHS Scotland using a series of approaches including information on supply (measured by number of prescriptions), morbidity (telephone enquiries, TOXBASE accesses, hospital discharges for overdose), mortality (number of deaths). This information is used to compare the inherent toxicity of individual compounds using a series of new epidemiological approaches.
- To investigate the cardiovascular effects (haemodynamic and electrophysiological) of opioids following ingestion in overdose amongst patients admitted to the Poison Centre of the Royal Infirmary of Edinburgh. These studies focused in particular on dihydrocodeine, methadone and dextropropoxyphene (as a compound of co-proxamol) as these drugs were taken frequently in the period of these studies.

- To investigate the cardiovascular effects of opioids in healthy volunteers following systemic (intravenous) administration.
- To investigate the local effects of opioids in the forearm circulation following intra-arterial administration into the brachial artery and the mechanisms that underlie these effects.

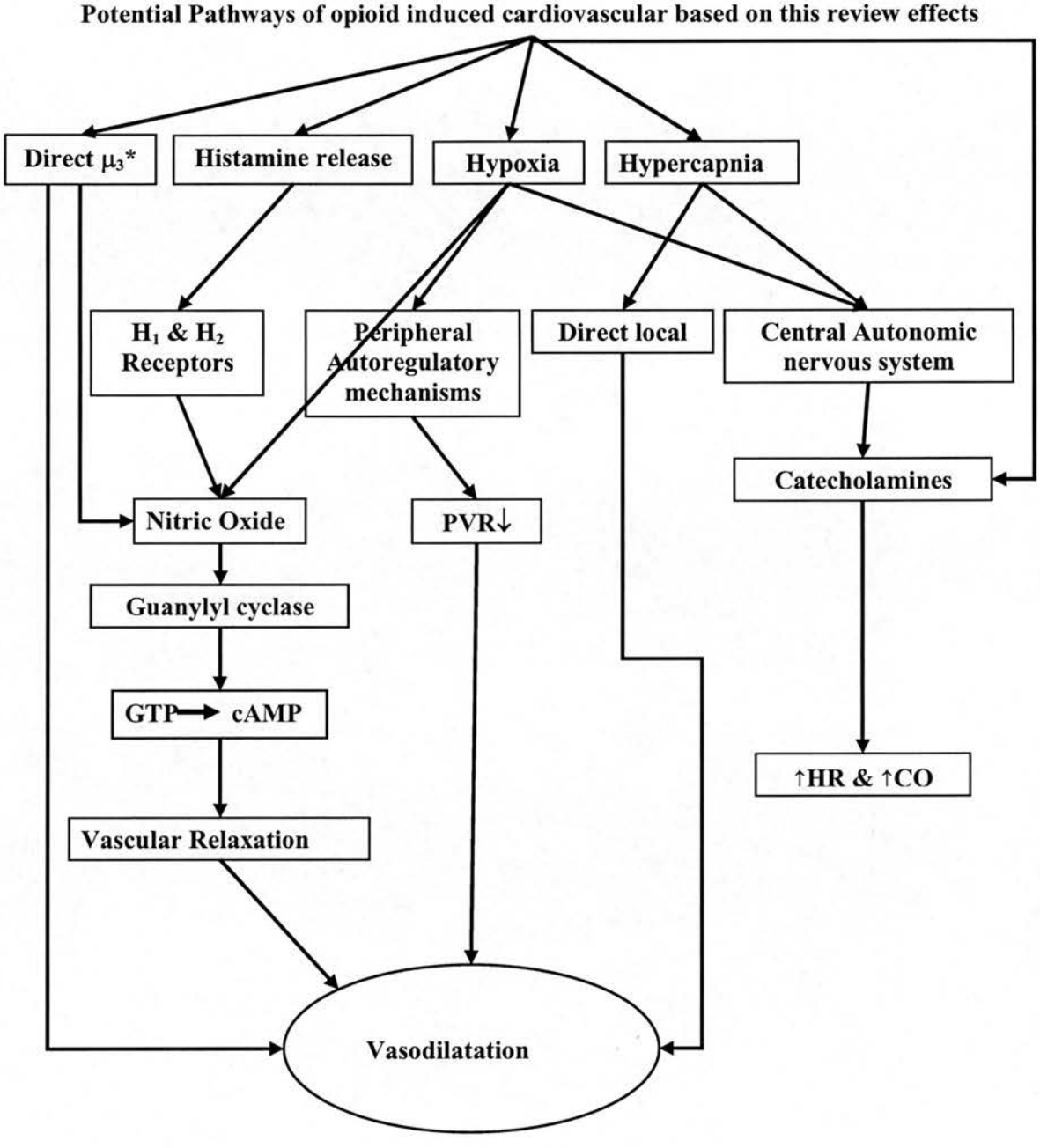


Figure 1- 2. Potential mechanisms of opioid induced vasodilatation, *; Existence of this receptor will be challenged

Chapter II, Materials and Methods

2.1. Study designs, planned samples and target populations

The studies described in this thesis use a variety of methodologies to suit specific goals, required powers and practical limitations. This chapter describes the overall profile of techniques used, both epidemiological and clinical, but the details are described in the individual chapters. The individual studies are listed in Table 2-1.

Studies	Study Designs	Planned samples	Target population
P-P1-1	Retrospective case series study	Young (18-50y)[1] co-proxamol overdoses	Admissions to the RIE from July 2001 to July 2002.
P-P1-2	Observational prospective parallel groups	Young (18-50y)[1] combined opioid and paracetamol overdoses	Admissions to the RIE from September 2002- to April 2003.
P-P1-3	Retrospective case series	Young (18-50y)[1] co-proxamol overdoses	Admissions to the RIE from September 2002- to April 2003 admissions to the Clinical Toxicology Unit of the Mater Hospital in Newcastle, Australia.
P-P1-4	Observational prospective case series study	Young (18-50y)[1] co-proxamol overdoses	Admissions to the RIE from June 2003- to December 2004.
P-P2	Observational prospective parallel groups (case control) study	Young (18-50y)[1] pure opioids & paracetamol overdoses	Admissions to the RIE from September 2002- to April 2003.
HV-P1 & HV-P2	Single blind[2] two ways crossover randomized clinical trial	Young (18-50y) non smoker, normotensive, total cholesterol level <6.0 mmol/lit) & naïve to opioids	Healthy volunteers from the database of the CRC-WGH-UE, who replied to the letter of information sheet on a first come first served manner.
FBF-P0	Single blind in terms of order of morphine saline clinical trial	As for HV-P1	As for HV-P1
FBF-P1	As for FBF-P0	As for HV-P1	As for HV-P1
FBF-P2	As for FBF-P0	As for HV-P1	As for HV-P1
FBF-P3	Opened labelled, single blind were possible as for FBF-P0, four ways crossover randomized clinical trial	As for HV-P1	As for HV-P1

Table 2- 1. List of studies' designs, RIE; Royal Infirmary of Edinburgh, CRC-WGH-UE; Clinical Research Centre of the Western General Hospital- The University of Edinburgh, P-P1-1; Co-proxamol overdose induced electrophysiological changes study. P-P1-2; Combined opioid overdose induced electrophysiological changes study. P-P1-3; Co-proxamol overdose induced electrophysiological changes study (Edinburgh and Newcastle, Australia study). P-P1-4; Co-proxamol overdose induced electrophysiological changes study (actual plasma measurements). P-P2; Pure opioid overdose induced haemodynamic changes study. HV-P1; Morphine induced haemodynamic changes study in comparison to saline (M & S, n=8)). HV-P2; Naloxone antagonist effects on morphine induced haemodynamic changes study. (M/S & M/N, n=8). FBF-P0; Local arteriolar morphine dose ranging study (pre-trial). FBF-P1; Local arteriolar morphine dose ranging study (n=6). FBF-P2; Tachyphylaxis study (n=8). FBF-P3; Mechanism of action of local arteriolar morphine study (n=8), 1; [A]ll patients with history of cardiologic diseases and co-ingestion of drugs with known ECG effects in overdose were excluded, 2; Because of the probability of occurrence of side effects of these high doses of morphine, and also as morphine is a controlled drug (two people were needed to document its use), these studies did not design as double blinded.

For epidemiological studies, the populations studied were either the whole of Scotland or residents of Edinburgh in the catchment of the Royal Infirmary of Edinburgh (prescriptions and patients admitted to the hospital with the poisoning). For volunteer studies, healthy subjects were recruited in three different groups; 1) morphine versus saline (coded HV-P_1), 2) morphine versus naloxone (coded HV-P_2), and 3) forearm blood flow studies (coded FBF-P_{0-3}).

2.2. Eligibility Criteria

2.2.1. Ethics approval

Ethical approval was obtained from the relevant ethics committees before commencing these studies. These were either the Multi-Centre Research Ethics Committee for Scotland (MREC), the Lothian Healthy Volunteers/Student Research Ethics Committee (LREC), and Lothian Research (Ethics) Committee (LREC)). The studies were all carried out in line with the principles outlined in the Declaration of Helsinki. The Royal Infirmary Research and Development Office also gave Trust Management approval to allow the project to be performed in the Royal Infirmary of Edinburgh or Western General Hospital. The Health and Safety Department of the University of Edinburgh confirmed that volunteer studies came within the liability insurance cover held by the University. Written informed consent was obtained from all subjects prior starting the studies. The details of these letters of approval and their amendment are summarised in Appendix I (Table I-I).

2.2.2. Inclusion criteria

A. For epidemiologic studies, no inclusion, or exclusion criteria were set. Data sets were anonymised for analyses.

B. For patient studies, patients treated in the Royal infirmary of Edinburgh without a history of cardiac disease and co-ingestion of drugs known to cause ECG changes or haemodynamic manifestation in overdose were included. Data were extracted from patients' notes in retrospective studies. In prospective studies case records were used and verbal confirmation from the patients was sought.

C. For volunteer studies, male healthy subjects between 18 and 55 years old participated. Their ages were determined by verbal ascertainment of their date of birth. Volunteers weighed between 60 and 100 kilograms weight. They were weighed using an electrical scale before starting the first visit by the principal researcher. Volunteers had no history and physical characteristics of opioid abuse. They were required to undergo screening for drugs of abuse via TRIAGE^{TM}8 (see later). The test procedure was followed as per the package insert. All had no history of cardiovascular disease, high plasma cholesterol, excess alcohol intake, and clinically significant hepatic, renal or respiratory diseases. The subject's primary care physician was notified of subject's involvements. Subjects were required not to have taken part in any study for three months prior to starting the study. All subjects were asked to stop vasoactive medications in the two weeks before each study, and abstain from alcohol, caffeine-containing drinks, and tobacco from at least 12 h before each study. Each subject fasted for at least 3 h before any measurements are taken.

2.2.3. Exclusion criteria

1. Subjects with systemic blood pressure outside the normal reference range (systolic blood pressure 90-150 mmHg and diastolic BP 50 to 90 mmHg).
2. Subjects with a positive screening urine test.
3. Subjects who revealed history of drug abuse, attempted suicide or any clinical symptoms or signs of volume depletion or dehydration.
4. Intolerance to the study procedures.

2.2.4. Early withdrawal and discontinuation

1. For patient studies, subjects who decided not to sign consent form retrospectively.
2. For volunteer studies, adverse events (severe nausea or vomiting, clinically important CNS depression, orthostatic hypotension (SBP less than 90 mmHg) and or hypersensitivity).
3. Subjects who withdrew consent.

2.2.5. Recruitment

For epidemiologic studies patients were in the NHS in Scotland. For volunteer studies, subjects were recruited by adverts approved by the ethics committee, and sent to subjects on the list of healthy volunteers held by the clinical research centre.

2.3. Power of the studies

Power calculations were performed for patients (electrophysiological and haemodynamic) and volunteer studies (whole man and forearm blood flow). Standard deviation of QRS duration in similar overdoses, systolic blood pressure in opioid overdoses, augmentation index and forearm blood flow in previous vasodilator studies

were used to estimate sample sizes which an acceptable shift in the overall response at first measurement following baseline. A sample of 12 cases in the patient electrophysiological study, 10 cases in the patient haemodynamic study, 8 cases for the whole man studies and forearm studies would have at least 80% power to detect a consensually agreed mean difference of 5% change in QRS duration, systolic blood pressure, augmentation index and forearm blood flow in these studies ($n_1 = n_2$). Significance levels were set at 5% level.

2.4. Screening for drugs of abuse

Before participation in the clinical trials, volunteers were required by protocol to undergo screening for drugs of abuse. This test was done once for each volunteer who joined the study. The test kit used was TRIAGE^{TM}8 produced by Biosite Incorporated, 11030 Roselle Street San Diego, California 92121, USA. Briefly, freshly voided urine samples were collected in a clean, previously unused plastic container. The reaction cap was opened. The urine sample (140 microlitters) pipetted into the reaction cap and incubated 10 minutes at 15 to 25 degree C. Pipette tips have discarded after use. A new tip was used to transfer the reaction mixture from the reaction cup to the detection area. Three drops of Wash Solution added to the centre of the Detection Area and allowed to soak completely. The results were read within 5 minutes after completion of the incubation. The test procedure was followed as per package insert. The result was recorded in the drug test recording sheet.

2.5. Sources of epidemiological data

The numbers of deaths from opioid single overdose in Scotland from 1st July 2000 to 1st July 2002 were obtained from the General Register Office (GRO). Hospital discharges

from the Royal Infirmary of Edinburgh during the same period were gathered from data held in the Scottish Poison information Bureau. Prescription data for primary care prescriptions dispensed for NHS patients in Scotland and Lothian Health Board were provided by the Information & Statistics Division of the Scottish Executive Health Department for the years 1998-2002. The number of telephone enquiry data to the Scottish Poisons Information Bureau from 1st July 2000 to 1st July 2002 was extracted for the drugs of interest. The number of accesses to the Internet database run by Scottish Poisons Information Bureau, TOXBASE, was extracted for the drugs of interest for the same period.

2.6. Applied techniques

Instrumentation used to determine physiological variables in this thesis are summarised in Table 2-2. Picture 2-1 shows the instruments used to measure cardiovascular variables and deliver drugs.

2.6.1. Bioimpedance

Electrical bioimpedance has been shown to be a simple, reproducible and accurate technique allowing continuous monitoring of cardiac output (Northridge *et al.*, 1990). It produces similar results to cardiac output measured by thermodilution and Doppler echocardiography (Northridge *et al.*, 1990; Appel *et al.*, 1986b). The device evaluates the transthoracic electrical bioimpedance (TEB) wave-form beat-by-beat (making a separate measurement of the waveform for every individual cardiac cycle). BOMED automatically ignores beats where the signal fails to meet certain predefined criteria - e.g. a pre-ejection period that is too short, an inappropriate heart rate. Values for 16

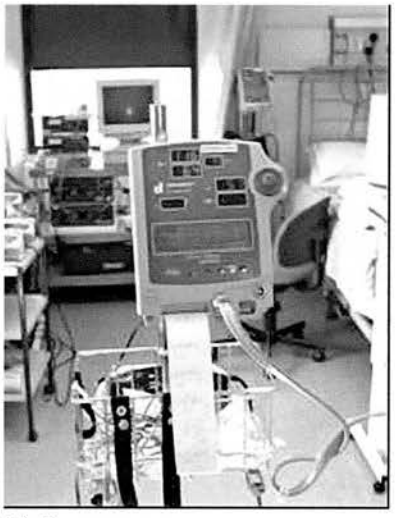
consecutive heart-beats are averaged for variables such as cardiac output and these appear on the printer or screen. The TEB waveform has been shown to relate to certain periods of the cardiac cycle (systolic time interval) such as pre-ejection period and

Instrument	Model	Manufacturer
Bomed	NCCOM3® R7	BoMed® Medical Manufacturing Ltd, USA
Centrifuge	SIGMA 3-16K	SCIQUIP
Digital timer	SMITHS	--
Dinamap		
Processor	Compact TS, 1998	CRTICON Vital Answer™, FL, USA
Cuff	DURA-CUF™, REF 2774	Johanson-Johanson
Interpretive cardiograph recorder	M1700A 3350A06976	Hewlett-Packard, USA
Normocap	DATEX NORMCAP 200	
Airflow Sensor Cannula	P/N: 1257, Qty:1,	Pro-Tech, USA
PenScreen	APPLE MessagePad 2000	
Plethysmograph		ADInstruments, Australia
Data Recording System	MacLab/2e, Version 1.0	
RAPID CULF INFLATOR	E20	
CUFF INFLATOR ATR SOURCE	HOKINSON AG 101	
PLETHY SMOGRAPH	HOKINSON EC 4	
Amplifier	Bridge, bio and LVDT	
Mac Computer, Monitor & Key board		
Pumps	IVAC® P7000 MK 11	ALARIS™ MEDICAL SYSTEM, Hampshire
Scale	Model 8241890	GEC AVERY, UK
SphygmoCor*		West Ryde, NSW, Australia
Notebook	Toshiba Satellite 2140CDS	
Software	SphygmoCor 2000, version 7 (SCOR-2000)	Copyright Atcor Medical Pty Ltd, 1999-2002
Tonometer	Micro-Tip®, Pulse transducer SPT-301	Millar instruments INC. Huston, Texas, USA

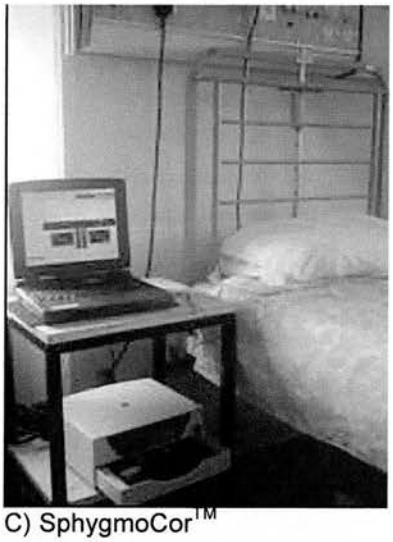
Table 2- 2. Instruments that have been used in these studies, *SphygmoCor™ Model BPAS-1/ mm- PIO (PWV MEDICAL PTY LTD, 1998) used for patients study.

2.6.2. Blood pressures and heart rate

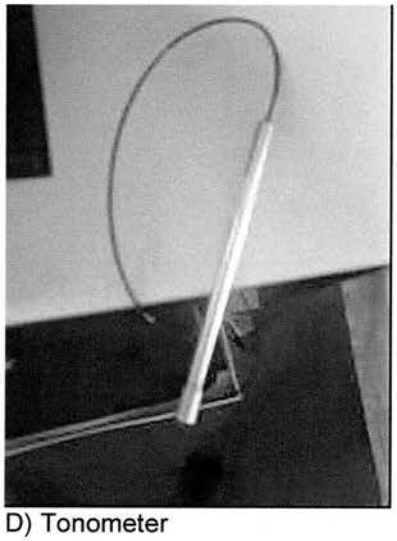
A) BOMED



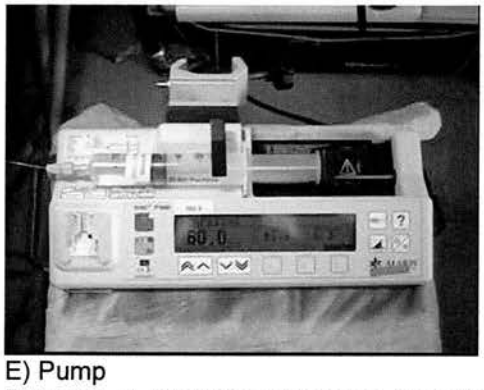
B) Dynamap



C) SphygmoCor™



D) Tonometer



E) Pump

Picture 2- 1. Instruments used to measure cardiovascular variables and deliver drugs. A) BOMED, B) Dynamap, C) SphygmoCor™, D) Tonometer, E) Pump.

ventricular ejection time (Lababidi *et al.*, 1970; Bernstein, 1986b; Bernstein, 1986a; Northridge *et al.*, 1990; White *et al.*, 1990). A BOMED set up was used as shown in Picture 2-1-A.

Peripheral blood pressure and oxygen saturation were determined indirectly in patient and healthy volunteer in the supine position and after sitting at 90 degrees for one minute. Appropriate inflatable cuffs (at least 80% of circumference of the arm and at least 40% of the width) were used to achieve adequate occlusion.

Patients were comfortable in the supine position for at least 10 minutes prior to measurement. The arm was held horizontally and supported at mid sternal level. Tight or restrict clothing was removed from the arm. The midpoint of an appropriate sized cuff was placed over the position of maximal pulsation of the brachial artery. For sitting systolic and diastolic blood pressure, patients were comfortably seated for 1 minute prior to measurement of the blood pressure. The results are reported in mmHg. A Dynamap was also used to determine the oxygen saturation (Picture 2-1-B).

2.6.3. Capnography

Capnography is the measurement and display of carbon dioxide (CO_2) on a digital or analogue monitor. Maximum inspiratory and expiratory CO_2 concentrations during a respiratory cycle were displayed, end tidal CO_2 calculated by the instrument and partial pressure of CO_2 at the end of expiration was reported as percentage. Capnography is widely used in clinical practice. It provides a rapid and non-invasive method for estimating carbon dioxide tension in different situations, including emergency

departments, intensive care units, and during anaesthetic procedures (Soubani, 2001). The measurements were recorded for one minute and the median value was used as the end tidal CO_2. Respiratory rate was measured using the same device. The results are reported as breath per minute (bpm). All measurements were done after at least 10 minutes resting in the supine position.

2.6.4. Central nervous system assessments

Changes in central nervous system function due to drugs can be objectively measured by using performance testing. Impaired performance is seen with sedative drugs (Tiplady, 1991; Tiplady *et al.*, 2003). The pen-computer system accepts information using a special pen or stylus on a computer screen. The equipment is simple and portable (Frewer & Lader, 1993). The device weighs approximately 700g and has screen dimension of 12.5 × 8.5 cm, which makes it suitable for taking to a patient's bedside in a hospital setting (Tseng *et al.*, 1998). Such devices have been used for patient questionnaires and psychomotor and cognitive tests (Drummond *et al.*, 1995; Swift *et al.*, 1999). Validity and sensitivity of these tests have been previously shown (Cameron *et al.*, 2001).

Volunteers sat in a comfortable position for two minutes, and were asked to tap the targets on the screen with a pen as fast as possible. Based on consensus the "arrows test" was used. The length of this test was agreed to be three blocks (75 questions), which is smaller than the existing conventional computer tasks for the purposes of detection of drug-induced impairments. It takes around two minutes to be performed. The arrows "light up" in random sequence, and the subjects respond by pressing the

appropriate button as quickly as possible. The mean response time for three blocks of repetitive and random sequences and number of correct, incorrect and no responses were recorded. The results of reaction time are reported in milliseconds (Figure 2-1).

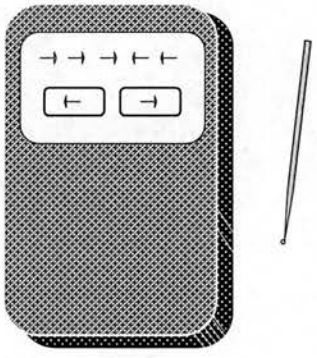


Figure 2- 1. PenSceen, and arrows test

Figure 2- 2. Contractor for Forearm blood flow measurements

2.6.5. Electrocardiography

ECG recordings were obtained after 10 minutes lying in the supine position. The computer software within the machine automatically calculated electrocardiographic indices, including heart rate, PR interval, QRS duration and axis, QT duration, and QTc using the Bazett correction i.e. QTc= QT/square root of RR (Sagie *et al.*, 1992).

2.6.6. Pulse Oximetry

Pulse oximetry monitors the percentage of haemoglobin that is saturated with oxygen and can detect hypoxemia (Pedersen *et al.*, 2003). It provides a rapid non-invasive method for estimating arterial oxygen saturation (Soubani, 2001). A probe was attached to the patient's or volunteer's finger and linked to a computerised unit of Dynamap. The unit displays the percentage of haemoglobin saturated with oxygen. An

audible alarm was also in use for detection of hypoxia. In volumteer experiments this was set at 90%. Dynamap uses a source of light which originates from the probe at two wavelengths. The amount of light, which is partly absorbed by haemoglobin, depends on its saturation fraction of oxygen. The processor then computes the proportion of haemoglobin which is oxygenated by calculating the absorption at the two wavelengths. It also distinguishes pulsatile flow from other more static signals such as venous signals, and picks up only the arterial flow, thus calculating pulse rate.

2.6.7. Pulse wave analysis

Pulse wave analysis was undertaken using the SphygmoCor system. The augmentation index (AI) is an indicator of arterial stiffness and was determined from the radial artery using the technique previously described (Nichols & O'Rourke, 1998). A high-fidelity micro-manometer was used to acquire accurate recordings of the peripheral pressure waveforms by flattening, but not occluding the artery (applanation tonometry). Blood pressure and recordings of peripheral pulse waveforms are used to estimate the central aortic pulse waveform is derived using a generalized transfer function (Nichols & O'Rourke, 1998). Augmentation index (AI) is the difference between the second and first systolic peaks of the central pressure waveform expressed as a percentage of the pulse pressure, and is a measure of systemic arterial stiffness (Wilkinson *et al.*, 2002b). Pulse wave analysis is a simple and reproducible technique (Wilkinson *et al.*, 1998) that provides an assessment of arterial stiffness (Wilkinson *et al.*, 2002a). Data are collected directly into a microcomputer and after 20 sequential waveforms an averaged peripheral waveform is generated. A corresponding averaged central pressure waveform is then estimated by using a validated transfer function (Karamanoglu *et al.*,

1993; Pauca *et al.*, 2001). AI, ascending aortic pressure and heart rate are then determined using the integral software.

To conduct these studies, subjects lay on a bed with their arm along side their body and their palm facing upwards for at least 10 minutes. Their forearm rested on the bed. The wrist was dorsifelexed to push the artery towards the surface and therefore make for easier access. The wrist was supported using a small cushion. The strongest pulse at the radial artery was located by placing using the index and middle finger, and the Tonometer was placed between the two fingers. The Tonometer was then adjusted to get maximal response. As a standard method at least eleven seconds of high quality waveform (consistent, large-at least 3 cm on the screen and in a steady vertical position) was used. The results are reported as percentage.

2.6.8. Venous occlusion plethysmography

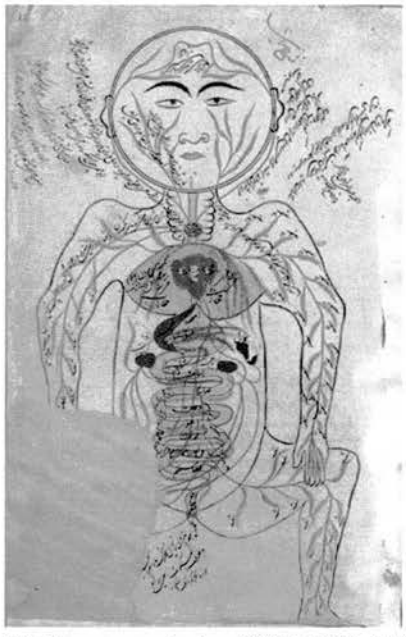
The use of venous occlusion plethysmography to measure blood flow in human was first described around 100 years ago by Hewlett and van Zwaluwenburg. It has become an accepted method with which to assess the effect of vasoactive drugs and hormones in human in man. The underlying principle of this technique is simple; when venous drainage from the arm is briefly interrupted, arterial inflow is unaltered and blood can enter the forearm but cannot escape. This results in a linear increase in forearm volume over time, which is proportional to arterial blood flow, until venous pressure rises towards the occluding pressure. It is standard practice to exclude the hands from the circulation during measurement of forearm blood flow, as the hands contain a high proportion of arterio-venous shunts. Venous return from the forearm is briefly

interrupted by inflation a cuff, placed around the upper arm, to well above venous pressure but below diastolic pressure, typically around 40 mmHg for intervals of 10 seconds followed by 5 second of deflation. The hands are excluded by rapid inflation of another cuff, placed around the wrist to well above systolic pressure (220 mmHg for normotensive subjects). The wrist cuffs must be inflated at least 60 s before starting measurements of flow in order to allow FBF to stabilise. Changes in forearm volume are measured by a plethysmograph. Strain gauges are placed around the widest part of the forearm. Venous occlusion plethysmography is usually expressed as ml per 100 ml of forearm volume per minute (Wilkinson & Webb, 2001). Picture 2-2 shows the forearm blood flow in a historical point of view, examining the brachial artery for arterial bloodletting, forearm blood flow set up and arteriolar and concurrent arteriolar and venous brachial cannulation.

2.6.9. Data acquisition and statistical analysis

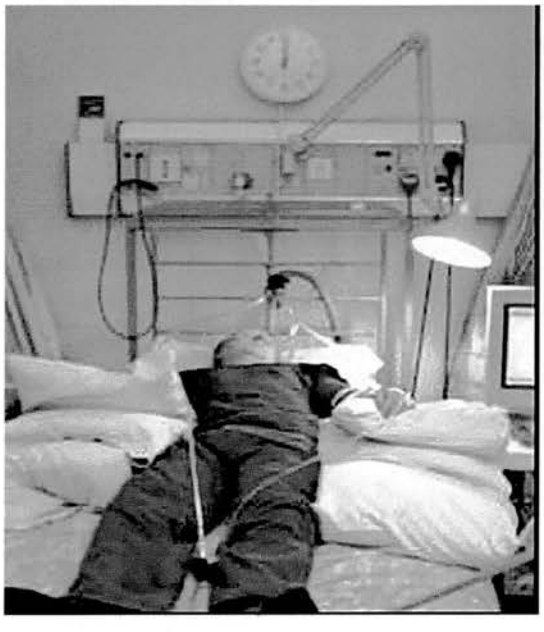
Voltage output from a dual channel Vasculab SPG 16 strain gauge plethysmograph was transferred to a Macintosh personal computer using a MacLab analogue-digital converter and Chart software. Plethysmographic data converted to windows compatible chart, and were extracted from data files and forearm blood flows calculated for individual venous occlusion cuff inflations using a template spreadsheet (Excel 5.0; Microsoft).

FBF was obtained from the mean of the last five consecutive recordings of each period. Curves manually rejected if portrated unsuitable for analysis where necessary. The

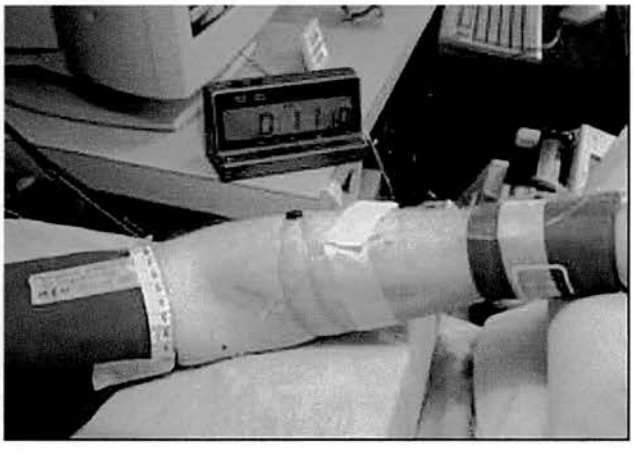


A) Forearm arteries (980-1037 AD)

B) Brachial artery pulse (1700-1800 AD)



C) Venous occlusion plethysmography set up



D) Insertion of the IA cannula

Picture 2- 2. A) Forearm arteries according to Avicenna (980-1030), from Avicenna's al-qanun Fi-T-Tibb (Canon of Medicine) in 1632, Isfahan, Persia (Iran), courtesy of Welcome Trust. B) "Method of putting hand on the [brachial] artery pulse [blood letting]" according to Persian Medicine, courtesy of the Clendening History of Medicine Library, University of Kansas Medical Centre, USA for their permission to reproduce a copy of their original miniature painting from Ghajar dynasty (1700-1800) during the government of Fath-ali-Shah, Shiraz (?), Persia (Iran). C) Venous occlusion plethysmography experimental set up. The volunteer lies supine with his arms

steep linear part of each slope of the response curve was taken to be recorded. By measuring the slope a mean value for FBF was finally produced. Baseline blood flow was taken as the last measurement during the saline infusion (0 min), before the start of the active drug infusion. As morphine appeared to be arterio-dilator, forearm blood flow results are expressed as absolute number of change in infused arm in ml/min/100ml of forearm volume

(Wilkinson & Webb, 2001). For a statistical & assumption checking purposes, the data of infused arm were also compared to the non infused arm.

2.7. Equipment

Equipment that was used in the volunteer studies is summarised in Appendix (Table II-I)

2.8. Definition determining the haemodynamic variables

Some of the indices such as blood pressure, index of contractility and heart rate were measured non-invasively and directly, whereas the others such as systemic vascular resistance index were derived. Variables were measured 10 minutes after resting in the supine position unless stated otherwise. All measurements, except reaction time and peripheral blood pressures were done twice and the mean calculated. See Glossary of the variables for the details.

2.9. Plasma assays

A) In volunteer studies with systemic intravenous morphine, the brachial vein of the right dominant arm was cannulated. Before each blood sampling, 3 ml of blood taken and throw away and then 10 ml blood taken. After each sampling the canullas flushed with 5 ml saline.

B) In tachyphylaxis study, both right and left arms were cannulated in the direction of flow. Before each blood sampling, 3 ml of blood taken and throw away and then 30 ml samples taken from each arm. Specimen collection, sample handling and storage procedure are summarised in table 2-3.

Test	Technique	Size	Tube	Ice	Centrifuge	Storage
t-PA	Coaliza t-PA(antigen) , Chromogenix for activity	4.5ml	Stabylite (black)	+	2000g for 30 minutes at 4°C	-80 for 6 months
PAI-1	As for t-PA	3 ml	Trisodium citrate (green)	+	As for t-PA	As for t-PA
vWF	Both for antigen and activity	3 ml	Trisodium citrate	+	1000g for 10 min at 4°C	As for t-PA
TNF-α		3 ml	EDTA (red)	+	As for vWF	As for t-PA
IL-6		3 ml	EDTA	+	As for vWF	As for t-PA
Histamine		3 ml	EDTA	+	2000g for 10 min at 4°C	As for t-PA
Tryptase		3 ml	Clotting, no anticoagulation	-	As for vWF	As for t-PA
Spare	HLA Typing	6 ml	EDTA	+	As for vWF	As for t-PA
Spare		6 ml	Heparin Lithium	+	As for vWF	As for t-PA

Table 2- 3. Specimen collection, sample handling and storage procedure.

2.10. Drugs

Manufacturing process and pharmacokinetics of the drugs administered in this thesis are summarised in table 2-4. Summary of the pharmacokinetics of the drugs used are shown in table 2-5.

Products	Strength	Type	Factory & Country	Expired	Route
Cetirizine	Zertek, 10 mg	Tablet	UCB Pharma Ltd, UK	10.2008	Oral
Cimetidine	400 mg	Tablet	DEXCEL® Pharm Ltd, England	09.2007	Oral
Morphine	10 mg/ml	1 ml vial	Martindale Pharmaceuticals, UK	07.04.2007	i.a.
Naloxone	NARCAN ® 400 mcg/ml	1 ml amp	Myers Squible Pharmaceutical Ltd, Bristol, UK	08.2007	i.v.
L-NMMA	270 mg	Dry powder	(Clinalfa) Merck Biosciences AG, Laufelfingen, Switzerland	Manufactured on 16.08.04	i. a.
Saline	Sodium chloride .09%	500 ml	MacoPharma, UK	12.2006	i.v. & i.a.
SNP	50 mg Powder for Infusion	Dry powder	Mayne Pharma Plc, Australia	09.2006	i.a.
Lignocaine	1% W/V	5 ml	B. Braun Melsungen AG, Melsungen	08.2005	s.c

Table 2- 4. Summary of the manufacturing process of the drugs used.
Cetirizine (hydrochloride); H_1 receptor antagonist, Cimetidine (hydrochloride); H_2 receptor antagonist, Morphine (Sulphate) non-specific, but dominantly μ opioid receptor agonist, 4. Naloxone (hydrochloride) non-specific opioid antagonist, L-NMMA or N^G-Monomethyl-L-Arginine Acetate; is an inhibitor of the synthesis of nitric oxide (NO) which acts in a dose-dependent fashion. SNP; sodium nitro nitroprusside, *; references for this table are specific drug pamphlets and (Dollery, 1999; Dollery C, 1991; ClinAlfa, 2005; Dux *et al.*, 2002; Grossmann *et al.*, 1999; Clough *et al.*, 1998).

Products*	Bioavailability	Peak plasma concentration	Plasma half-life mean (range)	Volume of distribution	Protein binding	plasma clearance
Cetirizine[1]		257 mg.l-1, 1 h of 10 mg oral	7.4 (6.7-10.9) h	33.4	93%	
Cimetidine[2]	50% oral	60-90 min 28.8 mmol.l-1 after 800 mg	2 (1-3) h	0.8-1.2 l.kg-1	13-25%	500 ml.min-1
Morphine[3]	30 (10-50)%	30-120 min after oral in man	3 (1-5) h	1.5-4.0 l.kg-1	25-35%	99, ml.min-1
Naloxone[4]	very low	0.5 - 2 h 200 mg i.m	60-90 min elimination	5 l.kg-1	50%	

Table 2- 5. Summary of the pharmacokinetics of the drugs used

2.11. Drug administration

Drug administration is summarised in Table 2-6. All dilutions were prepared in saline from sterile stock solutions under standard aseptic conditions within the Clinical Research Centre, Western General Hospital on the day of the study. All the dilutions

Product	Administration
Saline	Continuously i.v. infused at a rate of 60 ml/h throughout the study for both visits in HV-P1 and HV-P2. Continuously i.a. infused at a rate of 1ml per minutes for 30 minutes before and after administering morphine in FBF-P0, FBF-P1, and FBF-P3. 30 min before and 60 min after in FBF-P2. Also co-infused 40 and 20 ml/h with morphine in FBF P3-4. All syringes were coded.
Morphine	Non selective (mainly (90%) μ, less κ) agonist (Gutstein & Akil, 2001) Systemic intravenous-active compound. I.v. infusion in HV-P1 visit 1 and HV-P2 visits 1 & 2; 0.25, 0.5, 1, 2, 4, 8 mg each one over 5 min infused, and repeated every 20 minutes for two hours. Concentration in the serum quantified. Local arteriolar-active compound. I.a. infusions; P0 incremental doses of 1, 3, 10, 30, 100, and 300 mcg/ml/min each one for 10 minutes including 3 minute measurements. The doses were 1, 3, 10, 30 and 100 mcg/ml/min in P1 in the same way. 50 mcg/ml/min were infused in P2 and 80 mcg/ml/min in P3 for 30 minutes. All rates were 60 ml/h, but FBFP3-4 20ml/h.
Naloxone	Non-selective morphine receptor antagonist Continuously i.v. infused at a systemically active dose of 200 mcg/h throughout the HV-P2 & FBF P3-2, and just after 400 mcg bolus via dominant brachial arm (BNF, 2003).
Cetirizine	H_1 receptor selective antagonist Administered at a dose of 10 mg/d for 2 days prior the study day, and followed by a single 10 mg dose 1 hour before starting the study. Cetirizine administered orally with maximum 200 ml water. Although concentration did not quantify in the serum, but it is expected to be at the peak plasma level.
Cimetidine	H_2 receptor selective antagonist Administered at a dose of 400 mg/BID for 2 days prior the study day and followed by a single 400 mg dose 1 hour before starting the study. Cimetidine administered orally with maximum 200 ml water. Although concentration did not quantify in the serum, but it is expected to be at the peak plasma level.
L-NMMA	L-NMMA is a specific substrate analogue inhibitor of nitric oxide synthesised in humans (Vallance *et al.*, 1989b; Vallance *et al.*, 1989a). It has been shown that 100 nmol/min has no effect on basal hand vein size in contrast to forearm resistance vessels (Vallance *et al.*, 1989b). L-NMMA continuously i.a. infused at a dose of 4 mcg/min at a rate of 20 ml/h for 8 to 20 minutes to achieve maximal inhibition of local vascular endogenous NOS activity. Thereafter, Sodium nitroprusside co-infused. Once a stable baseline FBF obtained, the "NO clamp" continued with these doses of L-NMMA and SNP for the reminder of the study to allow stimulation of basal NO activity during continuous inhibition of endogenous NO synthesis.
SNP	When maximal inhibition of local vascular endogenous NOS activity achieved by L-NMMA, SNP co-infused at titrated doses (80 to 600 ng/min) (Helmy *et al.*, 2003) until FBF had been restored to within 10% of baseline flow and become sustain for at least two consecutive FBF measurements. Once a stable baseline FBF obtained, the "NO clamp" continued with these doses of L-NMMA and SNP for the reminder of the study.
Lignocaine	0.5 ml subcutaneously injected.

Table 2- 6. Administrative methods of different drugs in this thesis, see table 2-1 for the list of abbreviations.

(except for L-NMMA which was used up to 4 days later) were discarded at the end of the study. For locally active intra-arterial (IA) drug administration, the brachial artery of the (left) non-dominant arm was cannulated under local anaesthesia (lignocaine 0.5%) with a 27 SWG steel needle attached to a 16G epidural catheter (Figure 2.2). Potency was maintained by infusion of 0.9% physiologic saline via a syringe pump. In all studies, saline was infused at least for 30 min prior and at least 30 min after stopping morphine to the infusion of the study agent. The total rate of intra-arterial infusion was maintained constant throughout all intra-arterial studies at 60 ml/h. Measurements were done every 10 minutes. For systemic drug administration the brachial vein of the left (non-dominant) arm was cannulated in whole man studies. Both arms were cannulated in tachyphylaxis study.

2.12. Dermal effects

Cannulation site and local effects were examined and measured by meter (precision 1mm) for any potential redness (flare), weal, and any other adverse effects every 10 minute. This inspection continued for at least 30 minutes after stopping morphine infusion. Volunteers were usually contacted 24 hours after the study to monitor for any symptoms resulting from the cannulation procedure. Regular pictures were taken.

2.13. Itching

Itching measured on a subjective scale from 0 (no itching) to 9 irresistible itching.

2.14. Measurement of restoring forearm blood flow in NO clamp visit

L-NMMA was infused to achieve maximal inhibition of local vascular endogenous NOS activity. Thereafter, sodium nitroprusside was co-infused at titrated doses until FBF had been restored to within 10% of baseline flow (Helmy *et al.*, 2003). The flow output was displayed on a monitor screen and an overlay transparency used to estimate the 10% of baseline flow level (Figure 2-2). This technique is explained in chapter 8.

2.15. Statistics

2.15.1. Data analysis methods

The majority of the haemodynamic variables are reported as percentage of change from baseline. In most cases, the statistical significance of the observed difference of mean values for subgroups was determined using two-tailed distribution t-test at each time point. ANOVA was used for the result of all measurements in one group such as morphine versus placebo arm. A p-value of less than 0.05 was considered to be statistically significant. Power was determined to be above 80% for all studies. All statistics were analysed using SPSS (Statistical Package for the Social Sciences) 11.5 and Microsoft Excel 2000. Raw data are attached in appendix III.

Chapter III, Pharmaco-epidemiology and toxico-epidemiology of opioids in Scotland

The difficulty many intelligent people have with "sums" are infinite.
Greenwood, 1948

3.1. Introductory remarks

3.1.1. Aims and scope of this chapter

The aim of this chapter is to develop indices for evaluating the consequences of overdose, in particular for opioids. To do this I will use prescription data, poison enquiries, and mortality data for Scotland, and prescription data and hospital discharge data for Edinburgh. Fatal and non-fatal consequences of drug overdose will be considered as independent variables.

This chapter deals with a wide rage of different issues in toxico-epidemiology, and includes the results of a variety of different studies. It firstly discusses the pattern of opioid overdoses in the Royal Infirmary in the past four decades. This is followed by a detailed study of opioid overdose cases over two years. The main part of this chapter is focused on describing the current methodology of adjusting national data on overdose and death. It also outlines weaknesses of current approaches, describes predisposing factors, and introduces new indices which may address some of the current problems in toxico-epidemiology. Prescription data, telephone enquiries, TOXBASE accesses, hospital discharges, and deaths from opioid overdoses in Scotland over two years are discussed in detail. Mortality risk in relation to opioid ingestion from opioid drugs is examined using death certificate data. Finally risks from co-intoxication are estimated from this mortality data.

3.1.2. Risk determinants in this thesis

Absolute availability of drugs, a major determinant of frequency of overdose, is not clear, as there are illicit sources of supply and over the counter supply of some drugs.

However, prescription volumes have been used frequently as a surrogate of the availability of drugs (Buckley & McManus, 2004). The approach ignores the fact that the number of prescriptions that reach suicidal people from pharmacies, and the amount of drugs which remain in houses as a potential source for overdose by others are not considered separately. In this chapter, volume of prescriptions to Health Boards in Scotland is nevertheless used as a measure of "exposure risk".

An important source of information on poisoning is the number of enquiries to poisons information services. In Scotland two such routes of enquiry exist, telephone enquiry to the local centre of the National Poisons Information Service (NPIS), and, increasingly, accesses to the NPIS internet database, TOXBASE (Bateman *et al.*, 2002). These are not the actual volumes of overdoses, however, they are a nationally available surrogate of potential of overdose numbers by product. Their volumes are influenced by the individual professionals seeing cases, and the frequency and severity of individual overdoses, inherent toxicity of the products and local health policy. For example overdoses with very minor clinical manifestation or the ones which kill rapidly before reaching the hospital are under reported. Overdoses involving more than one component, such as ethanol, may not be accurately reflected in centrally collected datasets. A better estimate is probably the number of hospital discharges, but at the moment this is not nationally available by product. Therefore, discharges from the Royal Infirmary of Edinburgh were used as a surrogate for Scotland. This hospital serves approximately 10% of the Scottish population. Three data sets, two of poisoning enquiries, and one of discharges were used to estimate "poisoning risks".

Overdose fatality data are also available nationally by product. However, case finding is not 100%. The reports may not be completely accurate as only some of them are based on laboratory findings. In cases of co-intoxication, it may be unclear which agent is the primary cause of death. These data have been used to estimate "fatality risk".

Definition; In this chapter, buprenorphine, codeine phosphate, dextropropoxyphene, diamorphine hydrochloride, dextromoramide, fentanyl, meptazinol, methadone hydrochloride, morphine, nalbuphine hydrochloride, pentazocine, pethidine hydrochloride, and tramadol hydrochloride are considered as pure opioids. Data on dipipanone with cyclizine was also included in this category.

Co-codamol (codeine & paracetamol), co-dydramol (dihydrocodeine & paracetamol) and co-proxamol (dextropropoxyphene & paracetamol) are referred as compound opioids.

3.1.3. Epidemiology

Epidemiology (epi = among; demos = people, logos = discourse, Gr.) is thus defined literally as the study of epidemics in humans. John M. Last defined epidemiology as "the study of the distribution and determinants of health-related states or events in specified populations and the application of this study to control health problems". Epidemiology describes health and diseases in population rather than individuals, and relates measurement outcomes to population at risk, and allows conclusion based on comparison (Detels *et al.*, 2002; Rathman & Greenland, 1998). This approach my also be used clinical toxicology. In an epidemiological approach, the disease and population should be quantified. Well defined populations, that of Scotland (approximately 5 000

000 population) and Edinburgh (500 000) are considered in this chapter as the target populations.

In epidemiology, events are described in terms of crude and specific rates (Coggon *et al.*, 1997). In clinical toxicology, event-related data can be sought from a variety of sources, and population at risk can be replaced to a more reliable subpopulation that is exposed to drugs (volume of prescriptions). As a result, classical mortality and morbidity may be replaced with case fatality or case morbidity rates. In clinical toxicology identifying more dangerous drugs in overdose is a priority in national surveillance. The aim of an epidemiological approach should, therefore, be standardizing the statistics (population based describing the events) to elucidate the spectrum of different risks. I have attempted to develop indices to describe these risks. Any new index or system should be clear and simple, and adaptable to current surveillance systems (Klaucke *et al.*, 1988; Birmingham *et al.*, 1997). To focus on drugs with a higher burden of disease co-proxamol, diamorphine and methadone were selected.

3.2. Pure opioid overdose discharges of Royal infirmary of Edinburgh from 1967 to 2002

Drug overdose is a common cause of hospital discharges. An increasing frequency of opioid overdose was reported in the 1990s in Scotland (Bateman *et al.*, 2003). National statistics, however, do not provide information on the precise nature of the opioid product consumed.

Methods

This study was designed to determine of the frequency of opioid, and all other overdoses in the Royal Infirmary of Edinburgh from 1967 to 2002. The frequency of pure opioid discharges was gathered from different sources (Table 3-2). The target

Period	Source of data
01.01 1967 - 30.12.1976.	from the files of 6 monthly reports of the Toxicology department
01.01.1977 - 30.12.1986	from the files of a research (Medical Research Council) project in the Royal Infirmary of Edinburgh
01.01.1987 - 30.12.2000	from hospital discharge records of the Information and Statistics Division (ISD) of the NHS in Scotland through the Scottish Morbidity Record 01 (SMR01)[2].
2001-2002	from the data set of the Scottish Poison Information Bureau[3]

Table 3- 1. Source of data for opioid overdose in Scotland, 1967 to 2002, 1) it was not possible to access the frequency of compound opioids during this 36 years period, 2) the diagnostic codes used in this analysis were ICD9 until the end of March 1996, and ICD10 codes later, when they were introduced. The relevant codes were: opioid poisoning ICD9 (965.0) and ICD10 (T40.0–T40.4), opioid misuse ICD9 (304.0, 305.5) and ICD10 (F11), 3) these data start from 01.07.2000 to 01.07.2002, as the whole year data, at the time of study, was not accessible.

population, Lothian Health Board, was assumed stable and homogenous, for all overdoses admitted to the Royal Infirmary of Edinburgh. No inclusion and exclusion criteria have been set. The results are reported descriptively, and Chi-square used to define the difference.

Results

Overdose discharges overall have increased from around 1000 per year in 1967 to around 2500 in 2002. In the same period, opioid overdoses have increased from less than 10 cases a year to over 270 in 2002 Figure 3-1.

To describe the ratios of these changes, odds ratio of the probability of opioid overdose discharges to all toxicological discharges were calculated in the Royal infirmary of Edinburgh Figure 3-2. This ratio (95% CI) has increased 14.36 fold (7.17, 29.88) during this period (Chi-square $P < 0.001$). In conclusion, opioid overdose has been an increasing toxicological issue over the past 4 decades in Edinburgh.

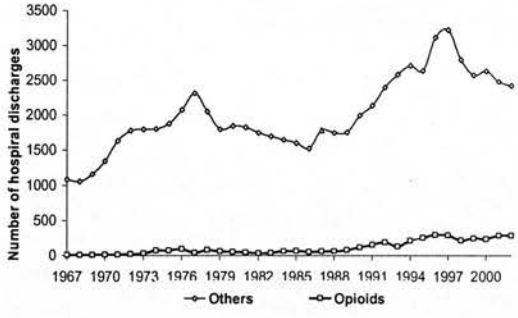


Figure 3- 1. Frequency of opioid overdose in comparison to total cases from 1967 to 2002 in the Royal Infirmary of Edinburgh.

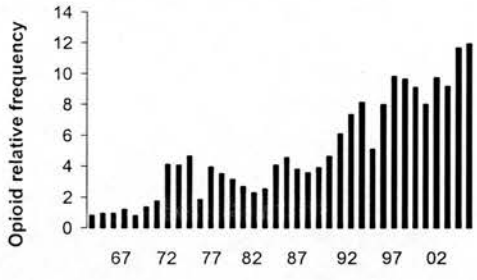


Figure 3- 2. Opioid relative frequency rate (RFR) in Royal Infirmary of Edinburgh, 36 years.

3.3. Demographics of opioid overdose patients from 2000 to 2002

To understand the demographics of opioid overdose, hospital discharges from 01.07.2000 to 30.06.2002 were examined with respect to agents involved, patient age and gender.

Results

1331 opioid overdose patients were discharged in this period. Co-proxamol (286, 21.5%), co-codamol (16.7%), dihydrocodeine (15.9%), diamorphine (14.7%), methadone (10.7%) and co-dydramol (8.9%) were the most common agents (Table 3-2). Compound opioid discharges (57.8%) were more frequent than pure opioids.

Diamorphine (27.4y) and methadone (29.1y) had the lowest mean age, while migraleve (38.6y), co-proxamol (38.1y) and tramadol (38.0y) had the highest mean age (Table 3-2). Second and third decades were the dominant age groups of patients. Mean (95%CI) age of compound opioids 36.06 (35.07, 37.05) is significantly higher than pure opioids 30.64 (29.81, 31.47). Mean age for whole opioid overdoses was around 34 year with a range of 74 years (13 to 87).

Among the cases 685 (51.5%) were female. All cases of feminax, paracodol, and pethidine were female. Diamorphine and methadone were dominantly male. 63.3% of compound opioids, but only 36.0% of pure opioids were in females (Table 3-2).

Duration of admission in the hospital for compound opioids 0.76 (0.70, 0.82) days was not significantly different from pure opioids 0.66 (0.60, 0.71). Destination of discharge was home in 79.9% of cases. Patients on buprenorphine, morphine and diamorphine

had the highest tendency for police custody. Major association with self discharge were diamorphine (25.0%), dihydrocodeine (21.2%) and methadone (15.2%). 41% of 29 cases referred back to Nursing Homes were intoxicated with just co-proxamol. 35 cases (2.5%) were homeless (Table 3-2).

A) Products					
	N	%	F%	Mean age (95% CI)	Duration of admission*
CO-PROXAMOL	286	21.5	60	38.1 (35.9, 39.4)	0.9 (0.7, 0.9)
CO-CODAMOL	248	16.7	62	34.8 (32.3, 36.3)	0.7 (0.7, 0.9)
DIHYDROCODEINE	212	15.9	48	33.1 (31.3, 35.0)	0.6 (0.5, 0.7)
DIAMORPHINE	196	14.7	24	27.4 (26.3, 28.5)	0.6 (0.5, 0.7)
METHADONE	143	10.7	32	29.1 (27.7, 30.4)	0.8 (0.7, 0.9)
CO-DYDRAMOL	118	8.9	57	36.2 (33.6 38.6)	0.8 (0.6, 1.0)
TRAMADOL	42	3.2	57	38.0 (34.4, 41.7)	0.8 (0.5, 1.0)
CODEINE	21	1.6	66	33.5 (28.1, 38.7)	0.7 (0.4, 1.0)
SOLPADEINE	21	1.6	81	33.4 (27.9, 38.9)	0.7 (0.5, 0.9)
MIGRALEVE	13	1	84	38.6 (26.4, 50.8)	1.3 (-0.1, 2.7)
FEMINAX	11	0.8	100	30.6 (21.8, 39.4)	0.6 (0.1, 1.0)
MORPHINE	10	0.8	50	31.8 (15.4, 48.1)	1.0 (0.1, 1.9)
BUPRENORPHINE	2	0.2	0	33.7 (-68.1, 135.6)	0.5
PETHIDIN	2	0.2	100	34.0 (-6.8, 74.8)	1
CO-CODAPRIL	1	0.1	**		
CODAFEN	1	0.1			
CODIS	1	0.1			
DICONAL	1	0.1			
MEDOCODEN	1	0.1			
SYNDOL	1	0.1			
TOTAL	1331	100			

B) Referral		
Destination	N	%
Home	1024	76.9
Self Discharge	132	9.9
REH	51	3.8
Police c	43	3.2
Nursing Homes	29	2.2
Others	52	3.9

Table 3- 2. A) Socio-demographics of discharged cases of opioid overdose from RIE, 01. 07. 2000-01. 07. 2002. B) Referral of opioid overdose discharges. N; frequency, %; percentage, F%; female percentage, (*) Any admission is assumed to be a minimum of 24 hours, (**) Percentage and mean for the products with frequency of "one" have not shown.

In conclusion opioid overdose presentations in general have short duration of admission. For pure opioids; male dominance, low mean age, tendency to self discharge and discharge to police custody of illegal (diamorphine) or highly supervised

drugs (methadone) are noted. The high percentage of co-proxamol overdoses, which were referred from nursing homes, is a health policy concern. Based on these demographic factors, pure and compound opioids represent different public health challenges.

Implications from this work are that preventive programs should target particularly the young male homeless population to minimise diamorphine abuse. Older females were at most risk of co-proxamol use.

3.4. Adjusting raw data in overdose events

3.4.1. Current approaches and pitfalls in toxo-epidemiology

In the past, fatality risk related to prescription volume has been calculated with a wide range of drugs. An index, standardized fatal toxicity index (FTI) as deaths per million prescriptions was derived (King & Moffat, 1981; Henry *et al.*, 1995; Buckley & McManus, 1998; Buckley & McManus, 2002; Serfaty & Masterton, 1993). In this way, the number of drug poisoning deaths is divided by a measure of drug exposure (prescriptions). FTI is currently considered the best means for comparing fatal toxicity in human overdose (Buckley & McManus, 2004). However, all studies that used FTI fail to take into account any predisposing factors in drug overdose. This separation of "frequency of being taken for overdose" from drug "availability" is important. When a drug appears more frequently in overdose deaths, it maybe because: a) it is frequently prescribed (higher availability such as paracetamol), b) it is frequently taken for overdose (higher overdose tendency such as use in depression, c) it could be because the drug is highly toxic relative to other drugs prescribed for the same indication (more severe inherent toxicity), or d) because there is an additional source of supply (such as

illicit use of diamorphine). The relationships between different categories of these epidemiological determinants in fatal toxicity are illustrated in Figure 3-3. This figure clarifies the magnifying effect of predisposing factors. Later in this chapter, these relationships have been descriptively scrutinised to develop particular indices.

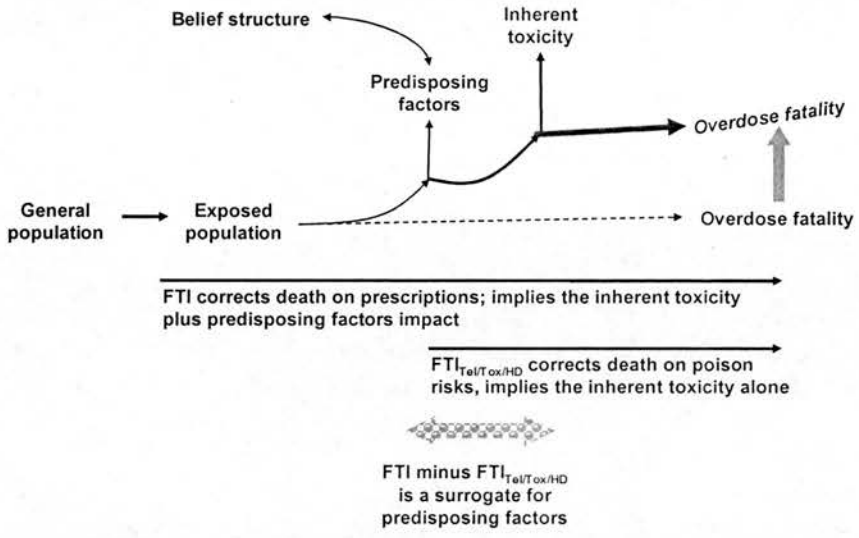


Figure 3- 3. Predisposing factors' contribution in overdose induced fatality. Exposed population; number of prescriptions, FTI; fatal toxicity index, which relates deaths to volume of prescription. $FTI_{Tel/TOX/HD}$; corrected FTIs based on telephone enquiries, TOXBASE accesses and hospital discharges newly introduce indices, true model (⟶), hypothetical frequency-exclusive-based model (····►).

These relationships can be summarised in the equations 3-1 for fatal consequences of overdose. As can be seen, volume of death in overdose for a certain drug is directly associated with prescriptions, predisposing factors and inherent toxicity.

Equation 3 - 1. $D = e \times p_K \times i_K$, where
D is the number of deaths, e is exposure, p_K is the constant co-efficient for predisposing, and i_K is the constant co-efficient for inherent toxicity.

In Edinburgh, all cases of overdose are admitted; therefore, hospital discharges would be interdependent from inherent toxicity (Equation 3-2).

Equation 3 - 2. $HD = e \times p_K$, where
HD is the volume of discharges.

Inherent toxicity and exposure of a particular drug can be considered constant. In equation 3-1 and 3-2; therefore, death (D) and hospital discharges (HD) may change just by p_K. This means that the predisposing factors are the only determinants capable of decreasing volume of deaths and hospital discharges. For example widely available cimetidine hardly ever is taken in overdose. This is partly because; it mainly reaches to a sub-population who is not prone to suicide or weak perceived susceptibility leading to a low P_K. To my knowledge, predisposing factors has never been taken into account in toxicological monitoring of drugs, and might be of interest of the pharmaceutical industry.

One of the goals of analytic epidemiology is to identify the factors that predispose individuals to the development of diseases and to quantify the risks. In this way preventive measures can be more easily demonstrated. For health policy makers, p_K could also is the most important determinant in planning preventive measures. In this regard reforming of the belief structure of individuals seems the only educate-able determinants in overdose. Health behaviors can be explained by using Health Belief Model (HBM), in which attitudes and beliefs of individuals are the core determinants (Janz & Becker, 1984; Roden, 2004; Yarbrough & Braden, 2001); although, belief structure is only one part of multifactor strategies for actions. As HBM can be more easily manipulated, it was separated from other predisposing factors.

Consider a particular drug in overdose is in part consciously selected from a potential variety of accessible drugs. I have adapted classical HBM and its revised versions to suit "choice of preference" in overdose. Potentially this can be used as another source of information for planning preventive measures and toxicological licensing of drugs.

Concept	Definition	Decisive determinants
Perceived Susceptibility	likelihood of experiencing overdose of a drug	Determined; effective killer
		Attempted; well known but safe
Perceived Severity	Likelihood of seriousness of overdose and its consequences of a drug	Personal; drugs with less pain, discomfort, financial burdens, duration and long side effects.
		Familial-social; Drugs with high loss of work time, difficulties with family, and relationships.
Perceived Benefits	Likelihood of benefits from taking particular overdose to reduce current impact	Clarity of process, and familiarity of particular drugs in overdose; need for a long antidote therapy.
Perceived Barriers	Likelihood of costs of a particular drug, which drive them away.	Known drugs for inconvenience, expensive, and painful overdose or completely unknown drugs
Cues to Action	Strategies to activate "readiness", incentive s or reminder messages	Internet messages; Path to successful suicide
Self-Efficacy	Likelihood of ability to find or buy a particular drug	OTC, or stigmatized drugs (e.g. female hormones)

Table 3- 3. Belief structure for choice of preference in clinical Toxicology (Definition and determinants). The idea of expressing result in this way obtained from ReCAPP (HBM & In Resource centre for adolescence pregnancy prevention, 2005)

In summary, health belief of individuals is affected by the feeling of capability to avoid a negative condition, positive expectation towards recommended action, and belief in their capability of taking this recommendation. Definitions and determinants in this model for choice of preference in clinical toxicology are developed and summarized in Table 3-3.

The overall purpose of this discussion is to direct focus of attention to the dynamic variables of choice of preference in taking overdose rather than static variables of exposure and final impact (death).

Traditional FTI, in which frequency of deaths is adjusted on prescriptions, assumes that drugs are proportionately taken in suicide. However, some drugs may be prescribed more frequently in high risk group patients, particularly those with psychiatric illnesses Age, gender, social class, other medical conditions, dependency, psychiatric illness, drugs that are indicated for counteracting suicidal behavior, books and internet confounders of disproportionate use of drugs in suicide attempts (Buckley & McManus, 2004). Concerns about using FTI have previously been identified (Buckley & McManus, 1998).

These problems can possibly be overcome by adjusting the number of deaths over a more relevant target population, people who actually took overdose, instead of prescription volume. In this way predisposing factors for taking overdose are no longer determinants. This should lead to a more reliable index for quantifying the severity of inherent toxicity of a particular drug.

Focusing on FTIs of different drugs can address fatal consequences of overdose but does not necessarily relate to non-fatal consequences of toxicity. In this thesis overdose rates have been studied using hospital discharges, telephone enquiries and TOXBASE accesses, and comparative ratios between different drugs derived. When opioids are taken in overdose as the sole agent they are rarely fatal, which is reflected in FTI; however, overdoses of these products result in admission for decreased level of consciousness, bradypnea, nausea, vomiting, hypotension and for patient observation.

These events are not reflected in FTI. Measurements that are relevant to mortality assessments, indices based on non-fatal consequences of overdose, are thus needed.

In conclusion, an integrated method, in which FTI of a drug is studied concurrently with indices of its non-fatal consequences of overdose, should be developed. These indices should be calculated in a similar way to be comparable.

3.4.2. Current approach for quantifying non fatal consequences of drug overdose

Poisoning risks (telephone enquiries, TOXBASE accesses, frequency of hospital discharges) can be studied and adjusted for prescription volumes. However, in an attempt to compare different products, statistical derivatives (based on likelihood; odds ratios or risk ratios) have been calculated in which the rate of one drug is expressed in comparison to the rate of the average of the group or one particular drug as the baseline index (Buckley *et al.*, 1995; Isbister *et al.*, 2003; Kelly *et al.*, 2004). This approach seems problematic Table 3-4, as particularly strong effects (an extremely toxic agent), which will change the risk ratio of that drug, can also change the average of the group and eventually, inversely, the ratios of other drugs (via changes in denominator). The proportions remain stable in a particular study, but this may make some drugs appear safer than they are when compared with other studies.

Moreover, odds ratios are comparisons of two numbers with the same units, as a result the ratio itself posses no unit, and therefore is a difficult concept to be understood. In general, however, ratios are good indicators for comparison of two drugs in the same subgroup such as for studying the risk of mirtazepam in comparison to other antidepressants, but they have no absolute significance. The result cannot be extended to another drug group, in which for example, odds ratio of diamorphine in comparison to

codeine is reported. For similar reasons these ratios cannot be compared with fatal toxicity index (FTI) of the same, or different drugs, as the methodologies are different. The aim, therefore, should be shifted to presenting a "rate", expressing the value of one quantity (telephone enquiries) in terms of another scale (prescriptions) for a particular drug. The result would be a fixed number with an understandable unit (e.g. number of telephone enquiries per 1 000 000 prescriptions). The absolute number of the rate of any drug in a particular study can then easily be compared with those of any other study.

3.4.3. Poisoning risks and deaths

Health professionals in Scotland call the Scottish Poisons Information Bureau (SPIB) to consult about overdose patients. They also access TOXBASE for poison information. These two resources are nationally available, and have previously used to describe and compare different drug overdoses (Bateman D.N. *et al.*, 2003).

However, there is a lack of objective evidence to show that to what extend these measure overdose rate. The possibility of over reporting for complicated overdoses and underreporting of frequent (familiar) or low toxicity overdoses is also not clear. TOXBASE accesses can also potentially be used as an education source, and health professional may access repeatedly for one case.

The Royal Infirmary of Edinburgh has a policy of admitting all overdoses. This provides the best estimate of overdose. This type of measure has previously been used to describe the pattern of poisoning risks of drugs (Isbister *et al.*, 2004; Kelly *et al.*, 2004; Bateman D.N. *et al.*, 2004; Bateman D.N. *et al.*, 2003; Wynne *et al.*, 1987). However,

currently national data for hospital discharges by product are not available, limiting the scope of studies using national studies. None of these data sets are ideal, but they may represent an advance on previous approaches.

Overall, in view of a lack of availability of national data for hospital discharges by product, and possibility of multiple use of TOXBASE for one patient, telephone inquiries are arguably the best surrogates of poisoning risk index.

3.4.4. Modelling of overdose

The epidemiological factors underlying overdose are complex. A range of independent variables which potentially determine the rate and impact of overdose (death) can be proposed. These are illustrated in Figure 3-3. Overall, exposure determinants, predisposing determinants, and errors suggested as the major influential factors on overdose outcomes.

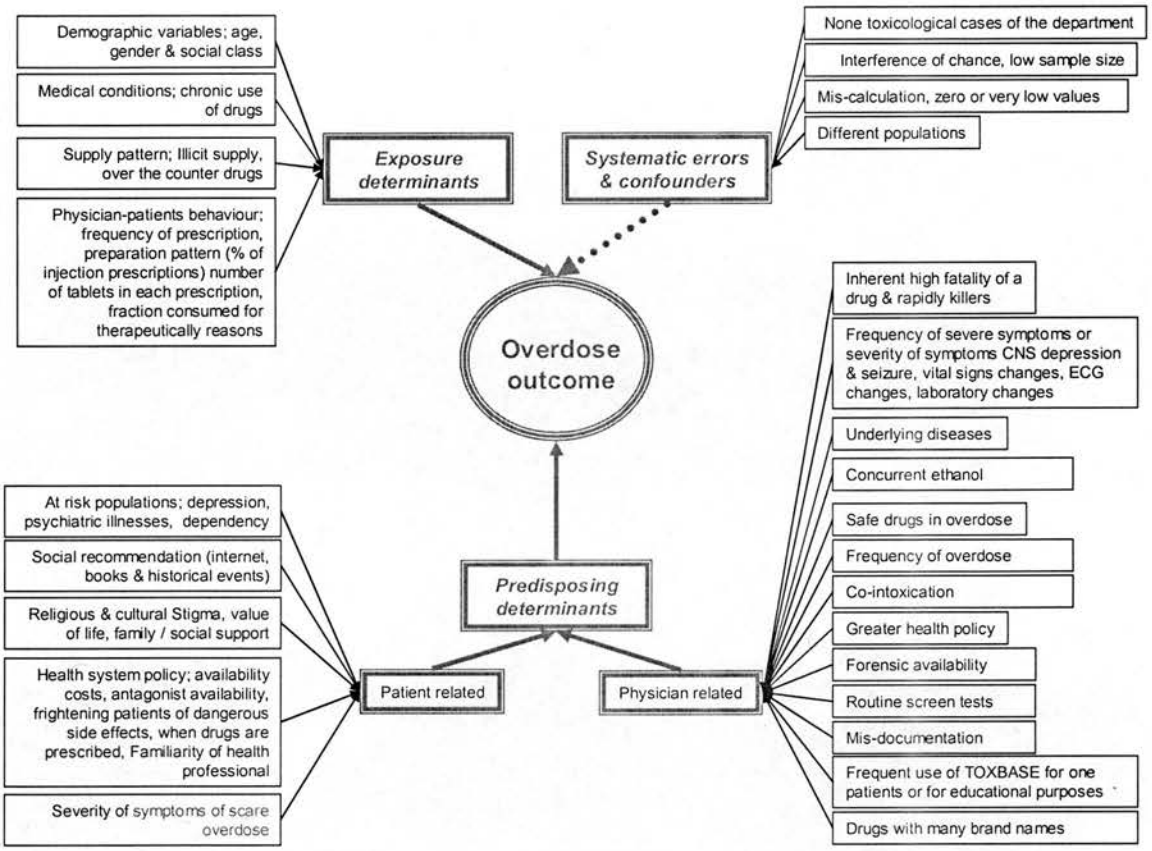


Figure 3- 4. Epidemiological variables, which potentially can influence overdose outcomes.

3.4.5. Net Inherent Toxicity

As discussed before the number of deaths can be related to the number of prescriptions, omitting the impact of exposure as a determinant, and a "case fatality rate" calculated. This was called FTI, and used as an indicator for comparing inherent toxicity of drugs in overdose, which is rather problematic. By combining equations 3-1 and 3-2, however, it is possible to estimate the net inherent toxicity co-efficient.

Equation 3 - 3. $i_K = D \div HD$

As can be seen the net co-efficient of inherent toxicity is actually derived by dividing number of deaths by an index of overdose rate, such as the number of hospital discharges.

To explore the relationship between "FTI" and "inherent toxicity", both sides of equation 3-3 were divided by "exposure (E) $\times 10^6$" and equation 3-4 derived.

$$D \div (E \times 10^6) = [E \times P_K \times i_K] \div (E \times 10^6) \text{ or}$$

Equation 3 - 4. $FTI = I_K \times P_K \times 10^{-6}$

Equation 3-4 suggests a relationship between FTI and the net inherent toxicity of drugs. As can be seen FTI is in fact (predisposing constant $\times 10^{-6}$) times different from the net inherit toxicity (I_K), and therefore should not be considered as equal as inherent toxicity of a particular drug. Despite the differences, equation 3-5 shows that FTI and net inherit toxicity are directly related, so FTI can be considered as a surrogate of net inherit toxicity.

Inherent toxicity is actually derived by dividing number of deaths by an index of overdose rate, such as the number of hospital discharges. As hospital discharges by products are not nationally available, telephone enquiries and TOXBASE accesses may be used as a surrogate for overdose rate.

3.4.6. Quantifying the indices

Non-fatal consequences

Indices relating poison risks and prescriptions toxic morbidity indices are called (TMIs). They include telephone enquiries (TMI_{Tel}), TOXBASE accesses (TMI_{TOX}) and hospital

discharges (TMI_{HD}). The methodology for calculating these indices is the same as the calculation of FTI. The upper and lower 95% confidence intervals for the index are calculated as described before (Buckley & McManus, 1998). Briefly, I assumed that prescriptions (i.e. the denominator) were fixed and that the telephone enquiries, TOXBASE accesses, and hospital discharges followed a Poisson distribution. The so-called "exact" 95% confidence limits were obtained for the rates. These indices are expressed as number of events per million prescriptions.

Fatal consequences

In line with FTI, fatal toxicity index for telephone enquiries (FTI_{Tel}), TOXBASE accesses (FTI_{TOX}) and hospital discharges (FTI_{HD}) were similarly calculated. For these calculations, however, I assumed that telephone enquiries, TOXBASE accesses, and hospital discharges (i.e. the denominator) were fixed and that the deaths followed a Poisson distribution. The results are expressed as number of events per 1000 poison risks, and show the probability of death reports to overdose reports for a particular drug. In these new FTIs the denominator of the rate (prescriptions) is replaced by a different measure of the target population (poison risks; i.e. telephone enquiries (FTI_{Tel})). In this way, the effects of predisposing factors, OTC, and illegal supply can be included in the index, as it reflects case presentation rates. Table 3-4 summarises these indices, and the way that they are calculated. The use of identical methodology for calculating FTI and TMIs thus creates an opportunity to compare fatal and non-fatal consequences of drug overdose.

The FTI, FTI_{HD} and TMI_{HD} seem to be more realistic measurements as both their numerator and denominators (prescriptions, hospital discharges and deaths) are true

values, while FTI_{Tel}, FTI_{TOX}, TMI_{Tel}, and TMI_{TOX} contain one surrogate event. These limitations inevitably restrict the applicability of these indices. However, as actual numbers of overdoses "by product" are not nationally available, TMI_{Tel}, TMI_{TOX}, FTI_{Tel}, and FTI_{TOX} posses some advantages. Target populations of the numerator (Scotland) and denominator (Edinburgh) of FTI_{HD} are also different.

Abbreviation	Explanation	Calculation
A) Current index		
FTI	Fatal toxicity index	Number of deaths/ volume of prescription $*10^6$
B) Introduced indices		
FTI_{HD}	Hospital discharge-FTI	Number of deaths/ volume of admission $*10^3$
FTI_{Tel}	Telephone enquiries-FTI	Number of deaths/ volume of Telephone enquiries $*10^3$
FTI_{TOX}	TOXBASE accesses-FTI	Number of deaths/ volume of TOXBASE accesses $*10^3$
TMI	Toxic Morbidity Index	In this chapter "morbidity" & "case fatality" are used interchangeably
TMI_{HD}	Hospital discharge-TMI	Number of hospital discharges/ volume of prescription $*10^6$
TMI_{TOX}	TOXBASE accesses-TMI	Number of TOXBASE accesses / volume of prescription $*10^6$
TMI_{Tel}	Telephone enquiries- TMI	Number of telephone enquiries/ volume of prescription $*10^6$
MSDR	Multi/single death ratio	Number of multi drug overdose deaths/ number of single drug overdose deaths$*10^2$

Table 3- 4. Glossary table of the current available index (A), and new indices introduced in this chapter (B)

In summary fatal and non fatal consequences of overdose are different, thus to have a valid judgement about drug-induced consequences of overdose both fatal (FTIs) and non fatal indices (TMIs) of all drugs should be calculated. For comparability they should be calculated in a similar way.

Associations of poison risks and indices

In order to investigate the relationships between the measurements used correlations are shown in Table 3-5. In the table, strength of correlation is used as an indicator of

validity of an index. If an index is not associated to other indices, it is assumed to be less representative.

	P L	TE	TA	HD	SD	TMI_{Tel}	TMI_{TOX}	TMI_{HD}	FTI	[n=14] FTI_{Tel}	FTI_{TOX}	[n=10] FTI_{HD}
P S	.975	.872	.939	.808	.493	-.184	-.229	-.119	-.161	-.195	-.139	-.310
	.000	.000	.000	.000	.038	.465	.360	.638	.524	.504	.583	.384
P L		.878	.917	.838	.585	-.189	-.236	-.122	-.165	-.198	-.142	-.310
		.000	.000	.000	.011	.453	.346	.628	.513	.497	.575	.383
TE			.942	.923	.68	.148	-.182	.223	-.141	-.258	-.105	-.336
			.000	.000	.002	.558	.469	.375	.577	.373	.680	.343
TA				.853	.453	-.046	-.202	.022	-.152	-.233	-.194	-.422
				.000	.059	.855	.420	.931	.546	.422	.440	.225
HD					.704	.244	-.139	.331	-.120	-.240	-.099	-.486
					.001	.329	.582	.180	.636	.409	.697	.154
SD						.278	-.068	.294	.117	.073	.300	.079
						.263	.790	.236	.645	.803	.226	.828
TMI_{Tel}							.253	.967	.328	.137	.210	-.095
							.310	.000	.184	.639	.403	.795
TMI_{TOX}								.232	.235	.616	.106	-.134
								.354	.349	.019	.676	.711
TMI_{HD}									.115	-.070	.029	-.129
									.649	.811	.910	.723
FTI										.980	.861	-.047
										.000	.000	.897
FTI_{Tel}											.899	.590
											.000[14]	.073
FTI_{TOX}												.794
												.006

Table 3- 5. Correlation of availability, poison risks, fatality and derived indices for opioids in Scotland & Edinburgh 01.07.2000-01.07.2002, In each cell Pearson Correlation (P value) [number of cases] are reported, wherever [number of cases] is not reported, n is equal to 18. PS; prescription in Scotland, PL; prescription in Lothian Health Board. TE; telephone enquiries, TMI_{Tel}; telephone toxic morbidity index, TA; TOXBASE accesses, TMI_{TOX}; TOXBASE toxic morbidity index, HD; hospital discharges, TMI_{HD}; hospital discharge toxic morbidity index, SD; single deaths, SD-FTI; single death fatal toxicity index, (1) products sorted by descending TMI_{Tel}.

I have used these indices in a variety of ways: firstly to explore the toxicity of co-proxamol in comparison to other compound analgesics; secondly to examine the relative toxicity of drugs in overdose.

To assess the validity of these indices the effects of mis-documentation, illicit supply, and rarely prescribed drugs were studied by comparing the result of diamorphine, dextropropoxyphene and rarely prescribed drugs. TMI_{Tel}, TMI_{TOX}, TMI_{HD} and FTI are sensitive to these problems, while FTI_{Tel}, FTI_{TOX}, and FTI_{HD} are relatively robust to these confounders.

3.5. Prescription of opioids in Scotland

In a retrospective study of prospectively gathered data, prescriptions for opioids in Scotland from 01.07.2000 to 01.07.2002 were obtained. Prescriptions dispensed within the NHS in primary care (ie excluding use in hospitals) in Scotland (population 5.1 million) and for the Lothian catchment area (population approximately 500,000) were obtained.

Results

In Scotland compound opioid prescriptions (74.4%) are more common than pure opioids. Some of the opioids are prescribed very rarely (e.g. nalbuphine 575 prescription in two years). Diamorphine is prescribed rarely. The most commonly prescribed opioids in Scotland and Edinburgh are shown in Table 3-6.

Prescription items of co-codamol and co-proxamol were approximately three times higher than for co-dydramol. Dihydrocodeine and methadone are prescribed almost equally in Scotland. In Edinburgh with a tenth of population, methadone is prescribed at a similar rate to the whole of Scotland, but dihydrocodeine is prescribed twice as frequently.

As compound opioids are prescribed even more frequently than pure opioids, they should also be studied when evaluating opioid overdose. Illegal supply of some opioids makes systematic comparison of the frequency of opioid use difficult.

Drug	Prescription Scotland	Prescription Lothian	Telephone enquiries[1]	TOXBASE accesses[1]	Hospital discharges[2]	Single deaths[1]	Multiple deaths[1]
Pure opioids							
Diamorphine	24845	2842	46	238	196	20	371
Dextropropoxyphene	2285	420	1	19	0	10	42
Morphine	235718	30062	20	59	10	15	352
Pethidine	30380	3349	1	16	2	1	2
Meptazinol	29975	1931	0	29	0	1	2
Methadone	847416	80063	25	105	143	19	144
Dihydrocodeine	865460	145347	48	558	212	13	95
Tramadol	632763	48643	47	489	42	3	11
Codeine	219581	28721	5	92	21	1	30
Dipipanone with cyclizine	13296	612	0	18	0	0	2
Dextromoramide	6829	333	1	7	0	0	1
Nalbuphine	575	200	0	16	0	0	0
Pentazocine	5141	555	1	5	0	0	0
Fentanyl	32221	3597	0	8	0	0	0
Buprenorphine	11932	1707	1	8	0	0	0
Compound opioids							
Co-proxamol	3453604	448166	92	907	286	43	86
Co-dydramol	1217376	184592	28	370	118	1	3
Co-codamol	3932153	363403	79	1155	245	3	8

Table 3- 6. Reference table, 1) prescriptions, telephone enquiries, TOXBASE accesses, and single and multi-agent deaths of pure and compound opioids in Scotland from 1st July 2000 to 30th June 2002 , 2) hospital discharges in Royal Infirmary of Edinburgh and prescription from its catchments area, Lothian Health Board in the same period.

3.6. Non fatal consequences of opioid overdose in Scotland

The aim of this study was to calculate toxic morbidity indices of different opioids in Scotland and compared then to the traditional FTI. In this study, an overdose death is a single agent-cause-death with or without alcohol documented by death certificate or forensic laboratories, which is captured by General Registry Office.

Results

To illustrate the overall profile of opioid exposure and non fatal consequences of overdose, TMI_{Tel}, TMI_{TOX}, and TMI_{HD}, are compared with traditional FTI, and shown in Table 3-7.

Products	TMI_{Tel}	TMI_{TOX}	TMI_{HD}	FTI
Pure opioids				
Diamorphine	1851.5 (1347.1, 2454.3)	9579.4 (6189.3, 14373.2)	68965.5 (42985.6, 108685.4)	805.0 (488.7, 1235.5)
Dextropropoxyphene	437.6 (1.1, 242.2)	8315.1 (4974.6, 12901.0)	0.0 (0.0, 9222.2)	4376.4 (2098.6, 8048.3)
Pethidine	32.9 (0.8, 185.7)	526.6 (304.8, 866.1)	597.2 (73.4, 2189.3)	32.9 (0.8, 185.7)
Dihydrocodeine	55.5 (40.9, 73.6)	645.1 (301.0, 855.3)	1458.6 (894.4, 2208.6)	15.0 (8.0, 25.7)
Meptazinol	0.0 (0.0, 123)	967.5 (647.3, 1388.3)	0.0 (0.0, 1941.5)	33.4 (0.8, 185.7)
Tramadol	74.3 (54.6, 98.7)	772.8 (56.6, 217.0)	863.4 (622.8, 1168.1)	4.7 (1.0, 13.9)
Methadone	29.5 (18.5, 42.2)	123.9 (98.3, 145.4)	1786.1 (956.0, 2933.9)	22.4 (13.1, 33.9)
Codeine	22.8 (7.4, 53.3)	419.0 (338.7, 515.2)	731.2 (452.9, 1118.5)	4.6 (0.1, 25.4)
Morphine	84.8 (52.0, 131.4)	250.3 (191.1, 323.9)	332.6 (159.3, 611.0)	63.6 (35.7, 105.3)
Nalbuphine	0.0 (0.0, 6148.1)	27826.1 (15242.3, 43305.0)	0.0 (0.0, 18444.4)	0.0 (0.0, 6148.1)
Pentazocine	194.5 (5.1, 1114.3)	972.6 (324.7, 2333.7)	0.0 (0.0, 6148.1)	0.0 (0.0, 737.8)
Dextromoramide	146.4 (3.6, 795.9)	1025.0 (402.1, 2060.4)	0.0 (0.0, 12296.3)	0.0 (0.0, 527.0)
Fentanyl	0.0 (0.0, 115.3)	250.0 (107.9, 492.6)	0.0 (0.0, 1024.7)	0.0 (0.0, 115.3)
Buprenorphine	83.3 (.0, 464.0)	670.5 (287.8, 1313.6)	0.0 (0.0, 2169.9)	0.0 (0.0, 307.4)
Dipipanone with cyclizine	0.0 (0.0, 283.8)	1353.8 (820.6, 2189.3)	0.0 (0.0, 6148.1)	0.0 (0.0, 283.8)
Compound opioids				
Co-proxamol	26.6 (21.4, 32.7)	262.6 (212.1, 2323.5)	638.2 (335.9, 1135.6)	12.5 (9.0, 16.8)
Co-dydramol	23.0 (14.6, 31.9)	303.0 (214.0, 418.99.0)	639.2 (529.4, 765.9)	0.8 (0.0, 4.4)
Co-codamol	20.1 (15.9, 25.0)	293.7 (243.8, 352.4)	674.2 (445.2, 1015.5)	0.8 (0.1, 2.2)

Table 3- 7. Toxic morbidity indices (TMIs) and fatal toxicity index (FTI) (with 95% CI) of pure and compound opioids extracted from prescription, poison risk values in Scotland from 1st July 2000 to 30th June 2002, and hospital discharges in Royal Infirmary of Edinburgh and prescription from its catchments area, Lothian Health Board, in the same period.

3.7. Fatal consequences of opioid overdose in Scotland

The aim of this study was to calculate the fatality rates of different opioids in Scotland and to examine the range of Fatal Toxicity Indices I have described. In this study, death is an overdose death due to a single agent with or without alcohol, as documented by death certificate, and captured by the General Registry Office.

Results

To illustrate the overall profile of opioid exposure and fatal consequences of overdose, FTI_{Tel}, FTI_{TOX}, and FTI_{HD}, are compared with traditional FTI, and shown in Table 3-8.

In Scotland overdose deaths are due both to pure opioids (63.8) and to a lesser extend compound opioids. Among the compound opioids co-proxamol is by far the most common cause of deaths (Table 3-5) followed by diamorphine (15.4%), methadone (14.6%) and morphine (11.5%) and dihydrocodeine (10.0%).

Product	Single deaths / million prescriptions (95% CI) FTI	Single deaths /thousand Telephone enquiries (95% CI) FTI_{Tel}	Single deaths /thousand TOXBASE accesses (95% CI) FTI_{TOX}	Single deaths /thousands discharges (95% CI) FTI_{HD}
Pure opioids				
Diamorphine	805.0 (488.7, 1235.5)	434.8 (265.6, 671.5)	84.0 (51.3, 129.8)	102.0 (62.3, 157.6)
Dextropropoxyphene	4376.4 (2098.6, 8048.3)	10000.0 (4795.4, 18390.4)	526.3 (252.4, 967.9)	∞
Morphine	63.6 (35.7, 105.3)	750.0 (419.8, 1237.0)	254.2 (142.3, 419.3)	1500.0 (839.5, 2474.0)
Pethidine	33.0 (0.8, 185.7)	1000.0 (25.3, 5571.6)	62.5 (1.6, 348.2)	500.0 (12.7, 2785.8)
Meptazinol	33.4 (0.8, 185.7)	∞	34.5 (0.9, 192.1)	∞
Methadone	22.4 (13.1, 33.9)	760.0 (457.6, 1186.8)	181.0 (108.9, 282.6)	132.9 (80.0, 207.5)
Dihydrocodeine	15.0 (8.0, 25.7)	270.8 (144.2, 463.1)	23.3 (12.4, 39.8)	61.3 (32.7, 104.9)
Tramadol	4.7 (1.0, 13.9)	63.8 (13.2, 186.5)	6.1 (1.3, 17.9)	71.4 (14.7, 208.7)
Codeine	4.6 (0.1, 25.4)	200.0 (0.1, 1114.3)	10.9 (0.3, 60.6)	47.6 (1.2, 265.3)
Dipipanone with cyclizine	0.0 (0.0, 283.8)	∞	0.0 (0.0, 204.9)	∞
Dextromoramide	0.0 (0.0, 527.0)	0.0 (0.0, 3688.9)	0.0 (0.0, 527.0)	∞
Nalbuphine	0.0 (0.0, 6148.1)	∞	0.0 (0.0, 230.6)	∞
Pentazocine	0.0 (0.0, 737.8)	0.0 (0.0, 3688.9)	0.0 (0.0, 737.8)	∞
Fentanyl	0.0 (0.0, 115.3)	∞	0.0 (0.0, 461.1)	∞
Buprenorphine	0.0 (0.0, 307.4)	0.0 (0.0, 3688.9)	0.0 (0.0, 461.1)	∞
Compound opioids				
Co-proxamol	12.5 (9.0, 16.8)	467.4 (338.3, 629.6)	47.4 (34.3, 63.9)	150.4 (108.8, 202.5)
Co-dydramol	0.8 (0.0, 4.4)	35.7 (1.0, 199.0)	2.7 (0.1, 15.1)	8.5 (0.2, 47.2)
Co-codamol	0.8 (0.1, 2.2)	38.0 (7.8, 111.0)	2.6 (0.5, 7.6)	12.2 (2.5, 35.8)

Table 3- 8. Fatal toxicity indices, indexed based on overdose volumes for pure and compound opioids in Scotland from 1st July 2000 to 30th June 2002, hospital discharges in Royal Infirmary of Edinburgh and prescription from its catchments area, Lothian Health Board, in the same period, ∞; infinity; denominator is zero.

Dextropropoxyphene (4376.4), which is probably a result of mis-documentation, and diamorphine, which is due to illicit supply, have the highest FTI, followed by morphine.

Commonly prescribed compound opioids have very low FTI. The rank order of FTI_{Tel}, FTI_{TOX} and FTI_{HD} are different to traditional FTI (Table 3-8).

Diamorphine has an illicit source of supply that cannot be detected by FTI. This problem can be overcome by using FTI_{Tel}, FTI_{TOX}, and FTI_{HD}. Diamorphine-FTI is 805 and over 50 times higher than dihydrocodeine. By calculating FTI_{HD} this difference decreases to 1.6 times. This shows the benefit of using indices that reflect net inherent toxicity to the traditional one based on prescriptions only.

3.8. Estimation of co-proxamol excess risk

Co-proxamol is a prescription-only analgesic that combines paracetamol (325mg) with the opioid analgesic dextropropoxyphene (32.5mg). The data for this study was used to assess its toxicity in overdose.

Prescription of co-codamol and co-proxamol were rather higher than co-dydramol in both Scotland and Edinburgh (Table 3-9). A similar pattern was also found in the telephone enquiries and TOXBASE accesses for these medicines. Frequency of death related to co-proxamol was predominantly higher than with the other combinations Table 3-10 and Figure 3-5 illustrates the relationship between exposure to these medicines based on numbers of prescriptions (FTI) and (TMIs).

Products	Prescription Scotland	Prescription Lothian[1]	Telephone enquiries	TOXBASE accesses	Hospital discharges[1]	Single deaths
Co-proxamol	3453604	448166	92	907	286	43
Co-codamol	3932153	363403	79	1155	245	3
Co-dydramol	1217376	184592	28	370	118	1

Table 3- 9. Prescriptions, TOXBASE accesses, telephone enquiries, single deaths, and admissions of different combinations of opioids and paracetamol in Scotland from 1st July 2000 to 30th June 2002, 1; Data from admission in Royal Infirmary of Edinburgh and prescriptions from its catchments area, Lothian

Products	Telephone enquiries/million prescriptions (95% CI)	TOXBASE accesses/million prescriptions (95% CI)	Hospital discharges/million prescriptions (95% CI)[1]	Deaths single/million prescriptions (95% CI)
Co-proxamol	26.6 (21.5, 32.7)	262.6 (212.1, 2323.5)	638.2 (433.4, 929.3)	12.5 (9.0, 16.8)
Co-codamol	20.1 (15.9, 25.0)	293.7 (243.8, 352.4)	674.2 (445.2, 1015.5)	0.8 (0.16, 2.2)
Co-dydramol	23.0 (15.3, 33.2)	303.0 (214.0, 418.99.0)	639.2 (529.1, 765.5)	0.8 (0.0, 4.6)

Table 3- 10. Telephone, TOXBASE, hospital discharges and deaths indices (per million prescription) in Scotland from 1st July 2000 to 30th June 2002, 1; Data from admission in Royal Infirmary of Edinburgh, and prescriptions from its catchments area, Lothian

The national rate of TOXBASE accesses and telephone enquiries were similar for each medicine when expressed per million prescriptions. Local hospital admissions were also similar when expressed in this way. Similar TMIs of telephone enquiries range from 20.1 to 26.6 per million prescriptions, of TOXBASE accesses from 263.0 to 304, and of admissions from 638.2 to 647.0. When the prescribing data were combined with fatalities to give the FTI it was apparent that this index was significantly higher for co-proxamol (12.5 deaths per million prescriptions) than co-codamol (0.8) and co-dydramol (0.8) Figure 3-5.

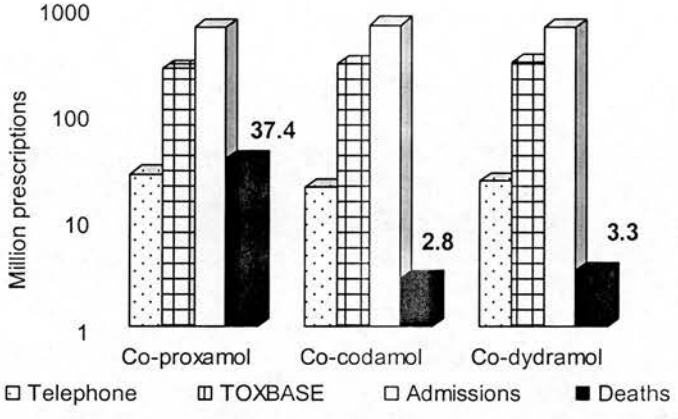


Figure 3- 5. Telephone enquiries, TOXBASE accesses, hospital discharges and deaths adjusted on the volume of prescriptions /10^6 of combination opioids and paracetamol, July 2000 to June 2002 in Scotland

Discussion

The main findings of this study are that while the rates of overdose for three common paracetamol/opioid combination analgesics are very similar based on the number of prescriptions within the Scottish population the proportion of these episodes, which result in fatality is very significantly higher for co-proxamol. The 16 times higher FTI and 13 times higher FTI_{Tel} of co-proxamol than co-codamol and co-dydramol are

sufficient evidence to suggest a complete withdrawn of co-proxamol from the market. These data suggests that toxicologically, co-proxamol should be replaced by other available combination analgesics. This study was done in early August 2004. The Committee on Safety of Medicines advised that co-proxamol should be withdrawn from the market on January 2005 (MHRA, 2005).

3.9. Estimation of methadone excess risk

This approach to toxicity assessment can also be applied to the comparison of methadone and dihydrocodeine. Both methadone and dihydrocodeine are used for replacement therapy (Krausz *et al.*, 1998; Puigdollers *et al.*, 2003; Puigdollers *et al.*, 2004; Backmund *et al.*, 2003).

The results are summarised in Table 3-11. As can be seen, prescription of methadone and dihydrocodeine were similar in Scotland, while in Edinburgh dihydrocodeine was used more. Deaths with methadone were more frequent than dihydrocodeine.

TMIs of dihydrocodeine in general is non-significantly higher than methadone except for TMI_{TOX} in which dihydrocodeine is significantly higher than methadone (123.9 (64.8, 232.3).

However the FTI_{TOX} of methadone (137.1 (72.9, 223.7)) is significantly higher than dihydrocodeine (17.0 (13.8, 20.8)). This is consistent with the significant higher FTI_{Tel} of methadone (576.0 (306.2, 939.6)) compared to dihydrocodeine (197.9 (160.1, 241.9).

Thus, although prescribed to a similar extent methadone and dihydrocodeine seem different in overdose. Methadone has a higher mortality as measured by FTIs (FTI_{Tel} & FTI_{TOX} significantly, and FTI and FTI_{HD} non-significantly). This pattern may suggest a social trend in which dihydrocodeine is taken more frequently in overdose, while methadone is more toxic. This suggests a strategy in which methadone was replaced by dihydrocodeine in maintenance therapy of addicts might reduce overdose mortality. This ought to be explored further.

A) Raw data

Products	Prescription Scotland	Prescription Lothian	Telephone enquiries	TOXBASE accesses	Hospital discharges[1]	Deaths Single
Methadone	847416	80063	25	105	143	19
Dihydrocodeine	865460	145347	48	558	212	13

B) TMIs

	Telephone enquiries /million prescription	TOXBASE accesses /million prescription	Hospital discharges/million prescription[1]
Methadone	29.5 (19.1, 43.5)	123.9 (64.8, 232.3)	1786.1 (956.0, 2933.9)
Dihydrocodeine	55.5 (40.9, 73.5)	644.7 (488.8, 840.3)	1458.6 (894.4, 2208.6)

C) FTIs

	Deaths/million prescription	Deaths/thousand telephone enquiries	Deaths/thousand TOXBASE accesses	Deaths/thousands hospital discharges[1]
Methadone	169.9 (90.3, 277.2)	576.0 (306.2, 939.6)	137.1 (72.9, 223.7)	100.7(53.5, 164.3)
Dihydrocodeine	109.8 (88.8, 134.2)	197.9 (160.1, 241.9)	17.0 (13.8, 20.8)	44.8 (36.3, 54.8)

Table 3- 11. Prescriptions, TOXBASE accesses, telephone enquiries, and deaths for Scotland and prescriptions and admissions for Lothian and Edinburgh Royal Infirmary for methadone and dihydrocodeine from 1st July 2000 to 30th June 2002, TMIs. B) Estimated poisoning risks of telephone and TOXBASE accesses (Scotland), or admissions (Edinburgh/ Lothian) per million prescriptions (95%CI). C) Death in relation to risks of exposure (95%CI), FTIs. Data from deaths in Scotland and hospital discharges from Royal Infirmary of Edinburgh.

3.10. Estimation of illicit supply of diamorphine using toxicological data

Illicit supply of drugs can be calculated from the demand side via the data from the Health Research Unit's National Drug Survey (Wilkins *et al.*, 2002) as well as drugs seized on the illicit drug market by police (Kaa & Bowmann, 1998). Calculating FTI for the drugs that have illicit supply is not appropriate. The aim of this study is to estimate the illicit supply of diamorphine via overdose related data. As methadone prescriptions are for a similar population as diamorphine abusers, and methadone has no other clinical indication than for management of drug addiction, poison risks and fatality of diamorphine were compared with methadone (as an index) from 1st July 2000 to 30th June 2002 in Edinburgh and Scotland.

Within Lothian 80,063 prescriptions of methadone resulted in 143 hospital discharges. In the period of the study there were 196 admissions for diamorphine poisoning. If diamorphine were used in a similar way to methadone, which seems at least plausible, I estimate that over 100,000 prescription items for diamorphine would have been required to produce the rate of presentation we observed in our catchment area of around half a million. Since most prescriptions are for 28 days supply, the street availability of diamorphine may equate to over a million doses a year.

Police data reflect availability, this data may be a better indication of usage. This approach provides a potentially useful marker of illicit drug availability.

3.11. Risk factors in multi-agent intoxication

3.11.1. Introduction

The death rate for opioid intoxication has increased over the past 20 years, and these rates of change have been different among various opioids (Hickman *et al.*, 2003; Rossler *et al.*, 1993). Opioid overdose has emerged as a major international public health issue. Observational forensic studies have shown that opiate multi-drug intoxications are common (Soja *et al.*, 2003) and are a major risk factor for death after acute opioid overdose (Coffin *et al.*, 2003; Darke, 2003).

Some mechanisms for this risk factor have been suggested including enhancement of acute toxicity (with ethanol) (Ruttenber *et al.*, 1990), severe central nervous system and respiratory depression (with benzodiazepines) (Burrows *et al.*, 2003), cardiotoxic death (with amphetamine and benzodiazepines) (Klys *et al.*, 2001). Higher levels of methadone may occur in acute and chronic mixed intoxication of methadone and benzodiazepines (Mikolaenko *et al.*, 2002).

Recently fatality has been shown to be increased in co-intoxication of benzodiazepines and a variety of opioids in rats (Borron *et al.*, 2001). This has raised the possibility of different inherit toxicity of various opioids in this regard. Moreover, although benzodiazepines were shown to alter neuro-respiratory toxicity of buprenorphine in a patient study (Megarbane B *et al.*, 2004), pharmacokinetic interactions have been ruled out as a cause of death in rats by the same group (Megarbane B *et al.*, 2001) suggesting a pharmacodynamic interaction. Another possibility might be the existence of a particular metabolite, which is toxic in co-intoxication. Co-intoxication in opioid overdoses and deaths related to opioids is common (Sporer, 1999), and opioid

overdoses are associated with longer periods of heroin use (Darke *et al.*, 1996) suggesting that overdose deaths are not exclusively related to opioid effects.

Fatality rates for various opioids are different in man, however, it is not clear what role co-intoxication plays. To explore the effects of co-intoxication in fatality of different opioids, I have compared the pattern of deaths in single and mixed opioid overdoses in Scotland. To my knowledge, no methodology is currently available to quantify the effects of co-intoxication on fatality in overdose.

3.11.2. Methods

The number of deaths in Scotland due to acute poisoning by a single opioid agent or opioid co-ingestions from 1st July 2000 to 30th June 2002 (population 5.2 million) was obtained. Single overdose of commonly used tablets of combination of opioids and paracetamol (co-codamol, co-dyddramol & co-proxamol), in which the ratio of ingredients are fixed and pharmaceutically accepted, were considered as single agent overdose.

I used a similar methodology to FTI (Buckley & McManus, 1998) to calculated a multiple agent to –single agent death probability ratio (MSDPR) as a quantifier of co-intoxication risk. Opioids were subsequently categorised into two main groups, with or without ability to biotransform to morphine, and MSDPR was calculated for these two groups.

3.11.3. Results

In the period of study 1149 (89.8%) overdose deaths were documented as co-intoxication and 130 as single opioid deaths in the Scotland. MSDPR for all opioids was 7.3 (8.8, 10.6) suggesting that co-intoxication is an important risk factor for opioid death. Table 3-12 shows the values of MSDPR in descending order by product. Rank order of the frequency of opioids in single agent and co-intoxication groups was similar except for co-proxamol, which with 33.1% is the most common cause of single agent death. No report of deaths due to nalbuphine, pentazocine, fentanyl and buprenorphine were documented.

Products	Multiple deaths	Single deaths	MSDPR
Diamorphine	371	20	18.6 (10.0, 25.5)
Dextropropoxyphene	42	10	4.2 (3.0, 5.7)
Morphine	352	15	23.5 (16.3, 32.5)
Pethidine	2	1	2.0 (0.2, 7.2)
Meptazinol	2	1	2.0 (0.2, 7.2)
Methadone	144	19	7.6 (4.0, 12.4)
Dihydrocodeine	95	13	7.3 (5.9, 8.9)
Tramadol	11	3	3.7 (1.8, 6.6)
Codeine	30	1	30.0 (20.2, 42.8)
Dipipanone & cyclizine	2	0	∞ (∞, ∞)
Dextromoramide	1	0	∞ (∞, ∞)
Nalbuphine	0	0	NaN (NaN, ∞)
Pentazocine	0	0	NaN (NaN, ∞)
Fentanyl	0	0	NaN (NaN, ∞)
Buprenorphine	0	0	NaN (NaN, ∞)
Co-proxamol	86	43	2.0 (1.6, 2.4)
Co-dydramol	3	1	3.0 (0.6, 8.8)
Co-codamol	8	3	2.7 (1.2 (5.3)

Table 3- 12. Reference table; single and multi-agent deaths of pure and compound opioids in Scotland from 1st July 2000 to 30th June 2002, and multiple over single death probability ratios, MSDPR; multiple over single death probability ratio, NaN; not a number, ∞; infinity

The literature to categorise opioids into different subgroups based on their major metabolites. The results are summarised in descending order of MSDPR (95% CI) in Table 3-13. As can be seen opioids may be categorised into two subgroups; morphine, codeine, and diamorphine for all of which the main active agent is morphine and its metabolites; and the opioids which are not morphine related (methadone, dihydrocodeine, pethidine, meptazinol, tramadol, dextropropoxyphene). Figure 3-6 shows the values of MSDPR in descending order by product. As can be seen, codeine,

morphine and diamorphine have significantly had higher probability of death in co-intoxication than other opioids. MSDPR for two categories of opioids based on their

Drug	MSDPR	Opioid metabolites
Codeine	*30*	Morphine, glucronate and sulphate of both codeine and its metabolites, conjugated codeine, norcodeine , conjugated norcodeine, conjugated morphine, hydrocodone
Morphine	*23.5*	6-glucronade, 3-glucronade, morphine conjugates, normorphine, conjugated normorphine, normorphine-3-glucronade, morphine-3-ethereal sulphate, morphine-3, 6-diglucuronide, codeine
Diamorphine	*18.6*	6-monoacetylmorphine, morphine, morphine-6-glucuronide, morphine-3-glucuronide, Conjugated morphine, normorphine, normorphine-3-glucronade
Methadone	7.6	2-ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine (EDDP), 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP), p-hydroxylated derivatives, glucuronide conjugated derivatives, methadol, normethadol
Dihydrocodeine	7.3	Nordihydrocodeine, dihydromorphine, dihydromorphine-6-glucronide, Dihydrocodeine-6-glucuronide
Co-dydramol	3	Nordihydrocodeine, dihydromorphine, dihydromorphine-6-glucronide, Dihydrocodeine-6-glucuronide (& paracetamol)
Pethidine	2	Pethidine acids & its conjugates, norpethidine, norpethidinic acid & its conjugates, pethidine N-oxide, 4 hydroxy pethidine
Meptazinol	2	O-glucronide, azepin-[2H]-2-??
Tramadol	3.7	O-demethyl tramadol, and -demethylation
Dextropropoxyphene	4.2	Norpropoxyphene, dinorpropoxyphene, p-hydroxypropoxyphene, p- hydroxynorpropoxyphene, cyclic dinorpropoxyphene
Co-codamol[1]	2.7	Morphine, glucronate and sulphate of both codeine and its metabolites, conjugated codeine, norcodeine , conjugated norcodeine, conjugated morphine, hydrocodone (& paracetamol)
Co-proxamol	2.0	Norpropoxyphene, dinorpropoxyphene, p-hydroxypropoxyphene, p- hydroxynorpropoxyphene, cyclic dinorpropoxyphene (& paracetamol)

Table 3- 13. Different opioids and their metabolites in their MSDPR order, Grey areas; are opioids which biotransform to morphine & its metabolites, Italic numbers; are significantly higher than others, (1) total number of co-codamol deaths was 11 and each tablet contains a very small amount of codeine, perhaps explaining this finding

ability to biotransform to morphine are shown in Figure 3-7. The opioid subgroup, which bio-transforms to morphine has a ratio of multiple to single death probability of 19.5 (15.4, 24.4) indicating that they are extremely fatal in co-intoxication. The ratio for opioids, which are not related to morphine, is significantly lower, and only 4.3 (3, 5.9) times more fatal in co-intoxication.

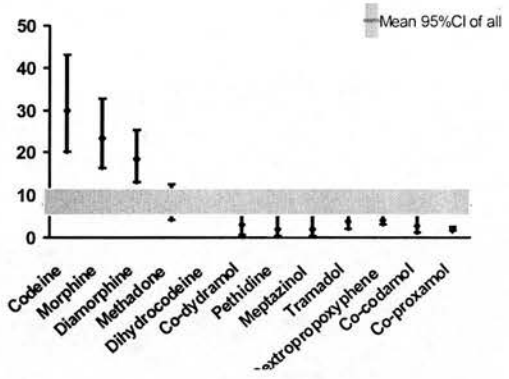


Figure 3- 6. Probability of multiple agent deaths as a ratio of single agent deaths for individual opioids

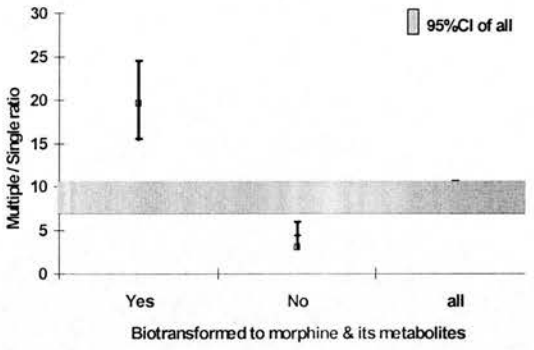


Figure 3- 7. Multiple single drug probability ratio overdose ratio in different opioid, categorised base on their ability to biotransform to morphine

MSDPR of the highest opioids (codeine) was 15.0 (10.1, 21.4) times higher than the lowest (co-proxamol).

Association between single agent (death equal to opioids) and co-ingestion (death contain opioids) is shown in Figure 3-10. Diamorphine and morphine are clearly differently from other agents, including co-proxamol. They appear safer when are taken as a sole agent than in combination.

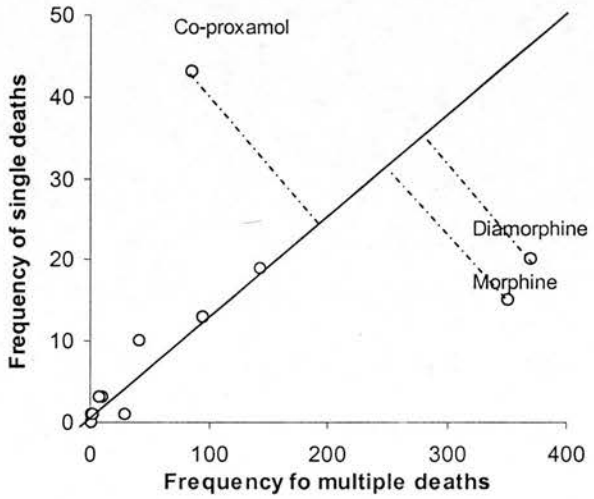


Figure 3- 8. Association between single agent and co-ingestion opioids-induced deaths

3.11.4. Discussion

The main findings of this study are that opioids in general are significantly more fatal in co-intoxication, suggesting that co-intoxication maybe a risk factor for opioid overdose death, which is consistent with the previous reports (Coffin *et al.*, 2003; Darke, 2003). Mirakbari et al 2003 showed that 79.8% of surviving patients with acute opioid overdose were co-intoxicated. This raises the possibility that the high prevalence forensic reports of co-intoxication may at least in part reflects a high rate of multi-drug administration as well as a higher mortality rate (Mirakbari *et al.*, 2003). In this study, two metabolically different subgroups of opioids emerged. Opioids which biotransform to morphine appear to be far more fatal in co-intoxication in man. This suggests that morphine itself, or one of its metabolites (eg morphine-6-glucuronide,) contribute to this problem. Opioids are an important problem, as the death rate associated with them is increasing (Hickman *et al.*, 2003; Rossler *et al.*, 1993). Understanding the risk factors in overdose is therefore valuable.

This study uses a methodology to quantify the risk of dying from different opioids in co-intoxication in man. It has compared the effects of co-intoxications on overdose mortality. The confounders for both multiple and single agent deaths are likely to be the same.

It should also be considered that diamorphine and morphine are in general illicitly supplied and different doses, and methods of administration are used. Therefore, an interaction of one of their illicit additives with other drugs might make them more fatal. This hypothesis is not consistent with findings related to codeine in this subgroup, or previous reports in which diamorphine fatalities were associated with its average purity

(Darke *et al.*, 1999). Co-codamol contains codeine which is also metabolised to morphine, however, as amounts of codeine in each tablet is very little (8- 30 mg). The lower co-codamol MSDPR might be because there is not enough codeine to be metabolised to the required level of morphine.

3.11.5. Conclusion

Mixed overdose including opioids are more toxic than single opioid ingestion. Morphine may be the main factor that increases mortality risk in mixed overdose.

3.12. Limitations of these indices

Applying the indices described in toxico-epidemiology would not be reliable if the sample size is small, period of study is short, or samples limited to certain fractions of population that have different level of self harm, or use drugs that are rarely prescribed.

Only a proportion of people who overdose are captured by the health system, and only for a minority of these is an electronic or telephone consultation made. Therefore, these indices do not show the prevalence of overdose, however, they are surrogates of relative product use and toxicity. Potential systematic errors and confounders in this approach are summarised in Table 3-14.

The indices cannot be used as means of comparing the incidence of death and overdose. However, these indices are a method for describing characteristics of drugs in overdose. They compare rates of drug use in self harm and suicide and offer practical rates for public health policy makers.

Probable bias	Example of disproportionate result ⇒ suggestion
Illicit supply, or over the counter drugs.	Diamorphine illicit supply or OTC paracetamol leads to a bigger FTI ⇒ FTI_{Tel}, FTI_{TOX}, or FTI_{HD}
Mis-documentation of one ingredient instead of combination product	Co-proxamol has a high FTI of dextropropoxyphene ⇒ FTI_{Tel}, FTI_{TOX}, or FTI_{HD}
High tendency for taking some drugs more frequently in overdose	Favourable drugs give high FTI and also TMI ⇒ FTI_{Tel}, FTI_{TOX}, or FTI_{HD}.
Familiarity of medical personnel with more common overdoses.	Morphine overdose give low TMI_{Tel} & TMI_{TOX} ⇒ TMI_{HD}, FTI or FTI_{HD}
High prevalence of dangerous consequences of overdose or likelihood of medico-legal involvement	Chloroquine overdose give high TMI_{Tel} & TMI_{TOX} ⇒ TMI_{HD}, FTI or FTI_{HD}
Use of TOXBASE for non overdose related reasons such as education purposes	Nalbuphine gives a high TMI_{TOX} ⇒ TMI_{Tel}, TMI_{HD}, FTI, FTI_{Tel}, or FTI_{HD}
Repeated use of TOXBASE for one patients such as prolonged toxicity	Nalbuphine gives a high TMI_{TOX} ⇒ TMI_{Tel}, TMI_{HD}, FTI, FTI_{Tel}, or FTI_{HD}
Rapid mortality in overdose	Co-proxamol gives low TMIs. ⇒ FTIs.
Drugs that have many brand names might result in more enquiries as a result of self confidence	Dihydrocodeine (DF118 forte, DHC Continuous) gives high TMI_{Tel} & TMI_{TOX} ⇒ TMI_{HD} or FTIs
Effect of chance by one case of death for rare products	Dextromeromide deaths gives high FTIs or TMIs ⇒ no suggestion
Safe drugs in overdose, which are not fatal	Codeine gives low FTIs ⇒ TMIs

Table 3- 14. The potential source of biases in the use of TMIs & FTIs, ⇒; implies to "confounding effect will be adjusted by concurrent using of the following indices".

3.13. Conclusion

From an epidemiological point of view, any approach in toxico-epidemiology should be systematically developed to assist professional decisions about appropriate management for specific circumstances. Validity, reliability, clinical applicability, flexibility and clarity are essential for this approach and any indices applied. This

chapter deals with examples of epidemiological methodologies in the field of clinical toxicology. Since the introduction of fatal toxicity index (FTI) in the early 1980s, there have been significant developments in recognising problems of this approach. I have attempted to develop more valid and reliable methodologies capable of evaluating new resources which improve the current approach.

Using a non-homogenous group of drugs, I have demonstrated practical applications of the approach. In this chapter the risk posed by the opioid overdoses in the past four decades in Edinburgh is quantified for individual products. The data suggested a withdrawal of co-proxamol and this happened on 31.01.05.

Evidence that methadone is more toxic in overdose than dihydrocodeine is presented here, and replacement of methadone with dihydrocodeine for maintenance therapy suggested. Diamorphine supply was estimated from toxicology data. Diamorphine and morphine are identified as dangerous products in co-intoxication, and a more careful monitoring is suggested.

I would propose this integrated method of concurrent measuring of TMIs and FTIs for future toxico-epidemiological studies. Interpretation of just one part of available data may lead to inappropriate conclusions. Applying this integrated method can promote toxicological appraisal in licensing of drugs.

Toxicological appraisal should be shifted towards, predisposing factors, which are dynamic determinants of overdose fatality. In this way, individual belief structure can be altered to improve preventive measures in overdose.

I have shown that extra supply, mis-documentation, zero values of prescription are potential confounders of these indices, therefore, in these situations the result should be interpreted conservatively.

Morphine (or one of its metabolites), but not other opioids or their metabolites, induce higher fatality in co-intoxication. Diamorphine and morphine overdoses should be considered at high risk when they co-intoxicated with other drugs. Pure diamorphine and morphine overdoses seem relatively safer.

Use of codeine, morphine and diamorphine in patients who are at risk of suicide and have access to other drugs should if possible be replaced by alternatives. MSDPR can be used as a practical index to describe differences of fatal interactions (co-intoxication) of a particular group of drugs in man.

Overall, this suggested integrated method should improve evidence in the field of toxico-vigilance, it should be considered as a *scale* for assessment in toxicological hazard appraisals.

Chapter IV, Electrophysiological effects of co-proxamol overdose

"One must see the musical character of the pulse. For the art of music, sounds are juxtaposed in orderly relation of loudness and softness, which keep on repeating at regular intervals; rates of utterance vary-some sounds coming close to one another, and others being further apart; the attack may be abrupt or gentle, sharp or dull. The notes may be sounded clearly or be indefinite; they may be strong or weak; the volume may be full or thin, the rhythm of the sequence of the sounds may be regular or irregular...Irregularity of the pulse applies to a succession of beats or to any individual beat."

Avicenna (980-1030 A.D.), The Canon of Medicine

4.1. Introductory remarks

Co-proxamol is widely used as an analgesic in the United Kingdom, this drug was the most common product ingested among 1331 hospitalised patients in Royal Infirmary of Edinburgh (from July 2000 to July 2002) who took an opioid as part of their ingestion (co-proxamol cases 270, 23.3% of total) (chapter 1). Co-proxamol is a common cause of drug-induced death in the UK (Whittington, 1984; Shah *et al.*, 2002), and it is also recognized to be associated with a greater risk of successful suicide than paracetamol or tricyclic antidepressants (Hawton *et al.*, 2003).

Co-proxamol comprises two ingredients, paracetamol 325 mg, and dextropropoxyphene 32.5 mg. In post mortem reports in the UK, the presence of dextropropoxyphene is generally taken to indicate co-proxamol ingestion, as so few dextropropoxyphene prescriptions are dispensed. Overdose with co-proxamol is well known to cause liver damage because of the paracetamol content. Although naloxone is an effective antidote to the opioid effects of dextropropoxyphene, death in overdose may occur very rapidly as early as 1 hour after overdose (Whittington, 1984), and subjects frequently succumb before treatment can be made available (Young, 1983). Co-proxamol overdose frequently causes death. This is more common in middle-aged, habitual or social-drinking men, on medication for pain (Sloth *et al.*, 1984; Jonasson *et al.*, 2000a; Jonasson *et al.*, 2000b). It has also been suggested that suicide may be over reported and accidents underreported among fatalities in which dextropropoxyphene is implicated (Jonasson *et al.*, 1999). In the Lothian and Borders of Scotland a large proportion of the dextropropoxyphene associated deaths were related to suicide

(81.3%), which was more common in the urban areas, and equally common in men and women (Obafunwa *et al.*, 1994).

Death with co-proxamol overdose is highly likely to be due to the dextropropoxyphene content because in addition to its opioid receptor agonist properties, dextropropoxyphene has cardiovascular effects (Holland & Steinberg, 1979; Stork *et al.*, 1995).

It has previously been shown that dextropropoxyphene induces a decrease in heart rate and contractility of dog and cat heart muscles *in vitro* (Holland & Steinberg, 1979; Amsterdam *et al.*, 1981; Nickander *et al.*, 1984). Dextropropoxyphene-induced ECG changes are similar to a variety of potent anti-arrhythmic agents that act at least partly by inhibiting the rapid sodium channel in the cardiac membrane (Roth & Seeman, 1971; Hondeghem & Katzung, 1977; Lund-Jacobsen, 1978; Stork *et al.*, 1995; Henry & Cassidy, 1986). The dextropropoxyphene metabolite, norpropoxyphene, also induces cardiotoxicity and has been shown to be associated with changes in ion-selectivity and gating of HERG currents (Ulens *et al.*, 1999). In the dog, which like man mainly converts dextropropoxyphene into norpropoxyphene, and pigs, oral and intravenous dextropropoxyphene caused an increase in the PR interval, QTc and QRS, and arrhythmias such as intermittent A-V block and ventricular extrasystole occurred (Holland & Steinberg, 1979; Bredgaard *et al.*, 1984; Bredgaard *et al.*, 1985). PR prolongation was maximal 2 hour after dosing and gradually subsided over the next 6 hours (Holland & Steinberg, 1979; Lund-Jacobsen, 1978). QRS prolongation was correlated with plasma concentrations during and after dextropropoxyphene and norpropoxyphene infusion (Lund-Jacobsen, 1978). In contrast, in some animal and

human studies no significant ECG effects are reported (Warren *et al.*, 1974; Mauer *et al.*, 1975; Nickander *et al.*, 1984; Page *et al.*, 1979).

Although the acute toxic effect of dextropropoxyphene in animals may be prevented by naloxone (Nickander *et al.*, 1977) and it is also recommended in man overdose (Kersh, 1973), cardiac depressor effects were not reversed by naloxone (Amsterdam *et al.*, 1981; Nickander *et al.*, 1984). Therefore, cardiac changes can be considered as non specific effects of dextropropoxyphene. These effects may partly be due to norpropoxyphene in man (Holland & Steinberg, 1979; Amsterdam *et al.*, 1981). In some cases of dextropropoxyphene overdose in man, electrocardiographic alterations have been reported (Stork *et al.*, 1995), which were concentration dependent (Gustafson & Gustafsson, 1976). Cardiac conduction abnormalities, dysrhythmia, cardiac haemodynamic impairment and death have been widely reported from overdose (Gary *et al.*, 1968; Starkey & Lawson, 1978; Barraclough & Lowe, 1982; Heaney, 1983; Staikowsky *et al.*, 1995; Hantson *et al.*, 1995; Sloth *et al.*, 1984). Some patients do, however, respond well to naloxone (Elonen & Neuvonen, 1984; Hantson *et al.*, 1995).

Sodium bicarbonate, lignocaine, dopamine, or even plasma exchange have been used as treatment in some case reports(Whitcomb *et al.*, 1989; Strom *et al.*, 1985; Thamdrup *et al.*, 1986; Stork *et al.*, 1995; Krantz *et al.*, 1985).

4.2. Pharmacokinetics

The paracetamol half life ($t_{1/2}$) at therapeutic doses in man is considered to be around 2 hours (Dollery C, 1991; Sahajwalla & Ayres, 1991); however, prolongation of the half life with increasing dose has been reported in hamster, mouse, and rats (Miller &

Jollow, 1986; Siegers *et al.*, 1978; Davis *et al.*, 1976). Following over dosage of paracetamol in man, significant increase in plasma $t_{1/2}$ has been reported (Prescott *et al.*, 1971; Prescott & Wright, 1973; Prescott, 1980; Forrest *et al.*, 1979; Schiodt *et al.*, 2002). The volume of distribution (Vd) of paracetamol is less likely to be related to dose-effect and is regarded to be 0.8 L/kg. The half life of paracetamol used in the treatment monogram is 4 hours (Dollery C, 1991; BNF, 2003; Prescott LF, 2004; Baselt & Cravey, 1995; BNF, 2003).

Dextropropoxyphene is rapidly absorbed from the gastrointestinal tract and detectable in plasma 5 minutes after administration by mouth (Rodda *et al.*, 1971). The peak concentration in plasma is reached between 1 and 2 hour after oral administration on an empty stomach (Verebely & Inturrisi, 1974). Reported plasma half lives vary widely (8 to 35 h) for dextropropoxyphene and (6 to 53 h) for its metabolite, norpropoxyphene (Pearson, 1984; Gram *et al.*, 1979; Crome *et al.*, 1984), The half life was longer in poisoned patients, the elderly and after repeated doses (Schou *et al.*, 1978; Crome *et al.*, 1984; Pearson, 1984).

In clinical practice, plasma concentrations of dextropropoxyphene are rarely measured (Proudfoot, 1984). The interpretation of plasma levels in poisoning is difficult as dextropropoxyphene and norpropoxyphene both persist and may have different dynamic effects (Buckley & Vale, 1984). Dextropropoxyphene is redistributed post-mortem (heart blood/femoral blood concentration ratio averaged 3.5) (Anderson & Prouty, 1989) which may make concentration relationships inaccurate in fatal cases.

In summary, available data regarding ECG changes in man is limited to some case reports, and the extent to which respiratory depression from the opioid, and electrophysiological changes from the sodium channel blockade, manifest in clinical cases of poisoning has not been well described. In their recent paper Hawton and colleagues concentrated on the opioid properties as the likely reason for mortality (Hawton *et al.*, 2003).

In this chapter ECG changes in co-proxamol overdose cases were studied, and the following hypotheses addressed.

1. Dextropropoxyphene (present in co-proxamol) overdose in man, unlike dihydrocodeine and codeine (present in co-dydramol and co-codamol) will lead to widening of QRSD and elongation of QTc.
2. The electrophysiological (QRSD) effect of dextropropoxyphene in co-proxamol overdose is dose dependent after an acute overdose.

4.3. Methods

Hospital discharge records for patients admitted to the Royal Infirmary of Edinburgh from July 2001- to July 2002 were retrospectively examined as a preliminary case series. Patients were included if they had an ECG in the first 24 hours following exposure to co-proxamol. Patients who had co-ingested paracetamol or drugs known to cause cardiac conduction abnormalities, and patients with plasma paracetamol level of zero were excluded. Nine eligible cases were identified in a preliminary analysis. This study suggested that QRS duration prolongation was occurring.

A prospective case control parallel group study was then conducted. ECG changes in 15 consecutive patients ingesting co-proxamol admitted to the Royal Infirmary of Edinburgh from September 2002- to April 2003 were compared with age and sex matched controls who had ingested co-dydramol (paracetamol and dihydrocodeine) or co-codamol (paracetamol and codeine). However, due to late presentation of some subjects, uncertain timing of some ECGs, and early discharge of some patients the power of the study falls towards the end of the patient observation period. Overall 4 cases were excluded and 11 subject pairs were entered into the final analysis. Routine paracetamol levels and ECG readings were obtained four hours after drug ingestion or as soon after four hours as possible. ECGs were performed again, 8-10 hours after the stated time of ingestion. Further ECGs were performed at 6-8 hour intervals until hospital discharge. In all patients paracetamol poisoning was managed routinely, using the normal regimen of intravenous n-acetylcysteine in patients whose paracetamol concentration at four hours or beyond was above the treatment line (British National Formulary 2003). In eligible cases, alleged number of co-proxamol tablets ingested, 4 hour paracetamol levels and time interval to sample, time interval to ECG, heart rate, blood pressure, PR, QRS and QT interval were documented on a data collection form.

Electrocardiographic indices were automatically calculated by the Hewlett-Packard machine in Edinburgh, (see chapter II for details related to the machine). The maximum perturbation on the ECG observed was also recorded. Cases and controls were considered independent groups for analysis.

4.3.1. Estimation of dextropropoxyphene dose

Plasma concentration of paracetamol and time from ingestion were used to estimate the dose of paracetamol by back extrapolation (measurements before 4 hours and beyond 12 hours were omitted). The half life of paracetamol assumed to be 4 hours (Forrest *et al.*, 1979; Prescott LF, 2004), and the volume of distribution of 0.8 L/kg (Dollery C, 1991). Estimated paracetamol dose was used as a surrogate to approximate ingested dextropropoxyphene dose, since the ratio of dextropropoxyphene to paracetamol is fixed in co-proxamol tablets. No plasma concentration measurements of dextropropoxyphene or its major metabolite norpropoxyphene were available at that stage. Certain assumptions have made to apply this methodology (Table 2-8). The following equations were used:

Equation 2-1.	$C = C_0 * e^{-ke X t}$
Equation 2-2.	ke = total plasma clearance / volume of distribution (CL/Vd) (first order rate constant)
Equation 2-3.	$\ln C = \ln C_0 - CL/V * t$
Equation 2-4.	$\text{Log } C = (\text{Log } C_0) - CL/Vd*t/ 2.303$
Equation 2-5.	$Vd = \text{dose} / C_0$
Equation 2-6.	dextropropoxyphene dose = paracetamol dose * 10^{-1}

where t = any particular time, C = plasma concentration at time t, C_0 = estimated concentration at time zero (the ordinate intercept), e = base for natural logarithm (ln), ke = the negative of the slop of the curve. A co-proxamol tablet contains 325 mg paracetamol and 32.5 mg dextropropoxyphene).

List of assumptions that are necessary for relating concentrations of two drugs by back extrapolation, and using drug response curve are summarised in Table 4-1.

Assumptions
Back extrapolation
1 The body is a single homogeneous and theoretical divided into one part, compartment (simplest model).
2 Their absorption and distribution is rapid, first order (rate of handling drug is proportional to concentration) and identical, and half-lives are constant
Drug response curve
1 The plasma concentration is related to therapeutic efficacy, which is linear (a sigmoid curve)
2 Drug's actions are reversible
3 Drug's concentration in plasma and at the receptor site are related
4 Active metabolites play no role in drug action
5 Tolerance to drug does not develop
6 Plots of logarithm of both drug concentrations in plasma against time assumed to be linear.

Table 4- 1. List of assumptions necessary for relating concentrations of two drugs by back extrapolation and using drug response curve

4.4. Results

Firstly (study 1), ECG effects of co-proxamol were prospectively compared to co-codamol and co-dydramol. Data on the two groups are shown in Table 4-2.

As can be seen, the groups were similar in respect to age, blood pressure and heart rate. However, QRS duration was significantly longer in patients who ingested co-proxamol (mean (95%CI)) 99 (96, 103) in comparison to the subjects ingesting the other opioid combinations 83 (81, 85)). There was no significant difference in other ECG parameters Table 4-2.

QRS durations in 6 hour time points are shown in Figure 4-1. As can be seen, QRS intervals in co-proxamol overdoses in comparison to controls become significantly prolonged soon after exposure (during the first 6 hours after overdose) and remained prolonged in those patients still in hospital 24 hours after drug ingestion. In no patient was QRS prolongation to a level at which arrhythmias would be expected in a well oxygenated patient, and no arrhythmias were observed.

Variables	Cases mean (95% confidence interval)	Controls mean (95% confidence interval)
Male %	45.5%	45.5%
Age	38.1 (30.1, 46.0)	37.6 (28.9, 46.4)
Plasma Paracetamol level	71 (38, 103)	115 (27, 202)[4]
Systolic blood pressure	116 (111, 121)	119 (109, 128)
Diastolic blood pressure	69 (65, 73)	69 (54, 84)
Hear rate	75 (72, 79)	71 (63, 78)
PR interval	166 (162, 171)	163 (155, 171)
QRS duration*	99 (96, 103)	83 (81, 85)
QT interval	379 (369, 389)	389 (377, 401)
QTc interval	421 (413, 428)	417 (410, 425)
P axis	40 (34, 46)	47 (41, 53)
Q axis	27 (17, 38)	41 (33, 49)
T axis	40 (35, 45)	33 (27, 40)

Table 4- 2. Demographic and cardiovascular variables of co-proxamol overdose in comparison to co-codamol and co-dydramol, *; Significant different, [4]; plasma paracetamol level of one of the cases was very high. n=11 in each group.

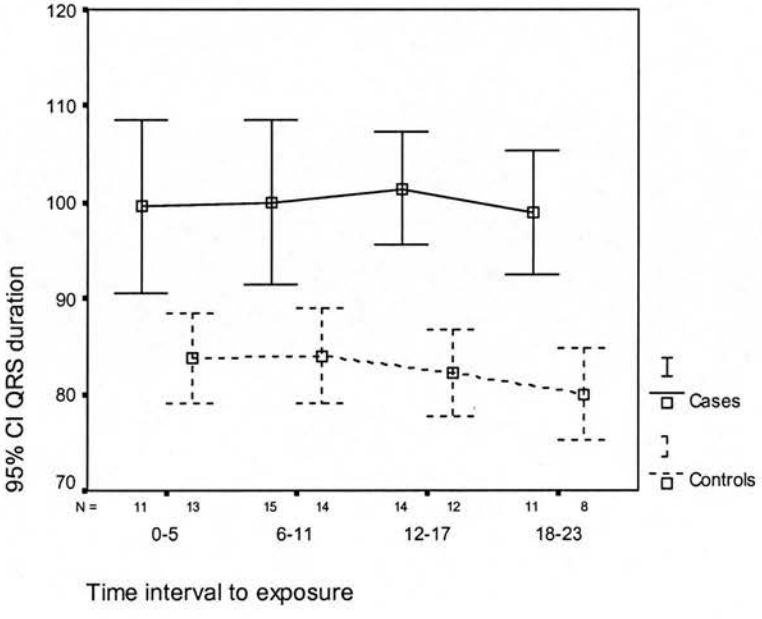


Figure 4- 1. QRS duration (mean +- 95%CI) in millisecond measured during recovery from co-proxamol overdose (▬) or other combination of opioids and paracetamol (• •), n=11.

In order to compare the relationship between ingested dextropropoxyphene dose and ECG changes in a larger sample, data from a retrospective study and prospectively gathered data in Edinburgh were amalgamated with data from a cohort of patients from the Clinical Toxicology Unit of the Mater Hospital in Newcastle, Australia (Curtsey of Professor Dawson). In these studies, dextropropoxyphene dose was estimated using paracetamol plasma concentrations as a means of estimating dextropropoxyphene dose. Back extrapolation was used to estimate plasma paracetamol level 4 hours after exposure (see chapter 2 for details). A study of the pooled cohorts of co-proxamol overdose (study 2) was then performed. Figure 4-2 shows the relationship between actual or estimated 4 hour plasma paracetamol concentrations as a surrogate of dextropropoxyphene dose, and QRS duration (measured manually in Newcastle Australia) obtained from data in a group of 159 overdose cases. Measurements of paracetamol less than 4 h and more than 12 h were omitted as these were less likely to produce accurate back extrapolation. Data for 74 cases were complete.

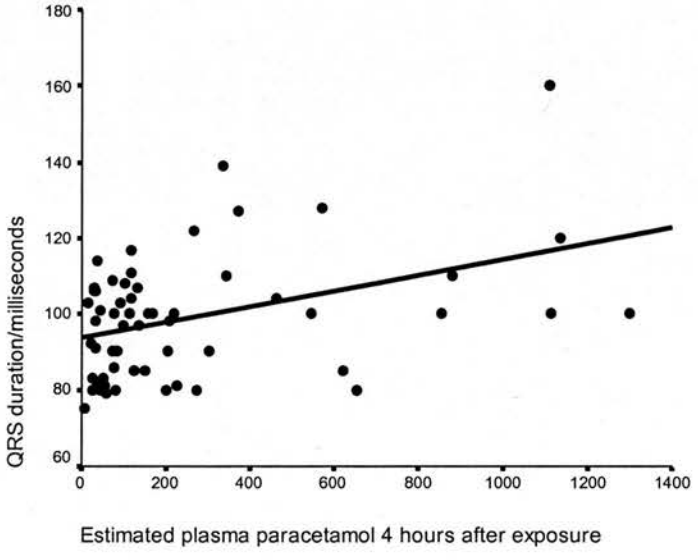


Figure 4- 2. Actual or estimated plasma paracetamol level 4 hours after exposure in mg per dL was statistically significantly correlated with QRSD in seconds. Pearson Correlation was 0.338, P value (2-tailed) 0.003, n=74.

As can be seen from Figure 4-2 there was a weak, but statistically significant positive correlation between the estimated paracetamol 4 hours after exposure and QRS duration (r=0.338, P (2-tailed) 0.003, n=74).

There was no significant correlation between maximum perturbations in other cardiac indices, including blood pressure, heat rate, PR interval and QTc Table 4-3.

Variable	Pearson Correlation	P value	Number
Systolic blood pressure	-0.046	0.696	76
Diastolic blood pressure	0.068	0.561	76
Heart rate	0.030	0.783	85
PR interval	0.144	0.218	75
QRS duration	0.338**	0.003	74
QTc interval	0.114	0.333	74

Table 4- 3. Correlation of cardiac indices with actual or estimated plasma paracetamol level in mg 4 hours after exposure.

4.5. Discussion

In chapter 1, I have shown that co-proxamol was a common cause of hospital discharges in Edinburgh. Major confounders such as age and sex were not different for these three combinations of paracetamol and opioids. This increases the validity of any comparison between these treatments. In chapter 3 I have shown that this combination opioid is extremely toxic in overdose in comparison with other combinations. In this chapter, I attempted to demonstrate a reason for these findings.

Dose dependent ECG abnormalities in animals and case reports of arrhythmias in man were reported previously (Holland & Steinberg, 1979; Amsterdam *et al.*, 1981; Nickander *et al.*, 1984; Bredgaard *et al.*, 1984; Bredgaard *et al.*, 1985). Data presented here expanded the current information to show firstly QRSD is prolonged in a

preliminary retrospective case series in man. Secondly in a prospective study it is shown that the effects on QRS are not shared by other combinations of paracetamol and opioids. In addition these effects appeared to occur rapidly (within 6 hours) after exposure and remained for at least 24 hours. These effects therefore are at least in part, related to the parent drug, and tolerance does not appear to happen in the first 24 hours after exposure. They may also be related to a long pharmacologic effect. In the large combined study is was demonstrated these effects are dose dependent, therefore, they will probably happen in all patients if the dose is high enough. However, these studies cannot rule out the possibility of an idiosyncratic effect in some patients at lower quantities.

In clinical practice, plasma concentrations of dextropropoxyphene are rarely measured (Proudfoot, 1984; Buckley & Vale, 1984). In the current study plasma paracetamol concentrations have been used to estimate the dextropropoxyphene dose to explore the dose response effect.

These findings, taken with the previous data on rapidity of death in co-proxamol overdose add to the weight of evidence supporting a role for sodium channel blockade as a factor in causing death in co-proxamol poisoning, particularly in the context of a hypoxic patient. All cases reported here were in hospital and adequately oxygenated. In-hospital deaths are extremely unusual, and we did not document arrhythmias. The high mortality seen in the community raises the possibility that other, at present unknown factors may be operating, including a sub-group of patients who are particularly sensitive to the cardiac effects of this drug or the possibility of interaction with other cardio active drugs such as antidepressants in mixed overdoses.

There are difficulties for licensing authorities in limiting availability of prescription medicines that are only hazardous in overdose. The present data suggest that the electrocardiologic effects of dextropropoxyphene warrant further evaluation of its risk-benefit as a prescription analgesic. In January 2005, the license for co-proxamol was changed, in part due to data presented here.

4.6. Limitations

The power of the prospective study was affected by some patients taking early discharge. Other potential confounders in the results such as age, sex, inaccurate time interval to exposure, gastric emptying half life, dose dependency of half life, and involvement of the metabolites in the effects were not assessed. The combined Edinburgh and Australian data showed a relatively weak correlation between paracetamol concentration and effects on QRS, nevertheless these findings might be potential explanation for the high mortality of co-proxamol in overdose.

4.7. Conclusion

The dextropropoxyphene ingredient of co-proxamol is electrophysiologically active in a manner which is consistent with sodium channel blockade. These effects appear within 6 hours of overdose, are dose dependent and last for at least 24 hours. The ECG effects seem likely to contribute to the mortality of co-proxamol overdose.

Chapter V, Haemodynamic effects of methadone, dihydrocodeine and diamorphine overdose in comparison with low dose paracetamol overdose.

5.1. Introductory remarks

5.1.1. Scope of the problem and justification for the study

I have previously shown that in a middle income country (Mashhad, Iran) opium was the most common cause of overdose hospital discharges overall in almost all age groups. In that study opium overdose had the highest mortality rate (22.5 %) but was the 8th rank cause of hospital fatality (1.9%) (Afshari, 2001; Afshari *et al.*, 2004). Scotland, a high income country, is also facing a serious drug problem. Fifty six thousand individuals aged between 15 and 54 years, that is 2% of the Scottish population, were misusing opioids or benzodiazepines in 2000 (Simoens *et al.*, 2002). In chapter 3 of this thesis it is shown that opioid overdose is a common cause of drug related hospital discharges and death in Edinburgh and Scotland.

As discussed in chapter 1, the haemodynamic effects of opioids reported in animal and human studies are contradictory (Gomes *et al.*, 1976; Kayaalp & Kaymakcalan, 1966; Sitsen *et al.*, 1982; Vatner *et al.*, 1975), (Mildh *et al.*, 2000; Fahmy *et al.*, 1983; Lowenstein *et al.*, 1969; Rubio *et al.*, 1997; Rubio *et al.*, 1992; Cathelin *et al.*, 1980a; Rosow *et al.*, 1982). In overdose, these effects are also inconsistent (Whipple *et al.*, 1994) but can be antagonised by the opioid antagonist naloxone (Lenton & Hargreaves, 2000). In chapter 4, I have suggested that electrophysiological effects of dextropropoxyphene contribute to morbidity and mortality of some opioid overdoses. Haemodynamic effects of opioid overdose also might contribute in their morbidity and mortality and warrant investigation.

5.1.2. Cardiovascular variables

The pattern of left ventricular ejection, vasodilatation and the changes in the stiffness of the large arteries such as the aorta and its major branches largely account for the changes in systolic BP and pulse pressure (PP); however, diastolic BP is more dependant on peripheral arterial resistance. The contribution of drug effects to arterial stiffness is an important factor in SBP, DBP and PP. It is not clear if and how opioids affect systolic and diastolic blood pressure in man. A range of opioid-induced effects on the cardiovascular system will be studied in chapters 6, 7 and 8 in healthy volunteers. In this chapter I will describe studies in overdose patients.

Pulse wave analysis (PWA) is a noninvasive method that allows large artery stiffness to be quantified in vivo (Mackenzie *et al.*, 2002). The haemodynamic effects of opioid overdose is usually thought to be due vasodilatation (el Sharkawy *et al.*, 1991; Patschke *et al.*, 1977); however, the role of arterial stiffness has not been studied. The SphygmoCor equipment used in these studies also allows anumber of other cardiovascular indices to be calculated.

5.1.3. Arterial stiffness; definition, description and mechanisms

The heart pumps blood into the aorta, which then travels to the tissues through relatively non-elastic vessels. Systole initiates an arterial pressure wave from the heart to the periphery. Wave reflection occurs at impedance mismatch points, mainly at the high-resistance arterioles (Nichols & O'Rourke, 1998). The augmentation index (AI) quantifies the extent to which central BP is augmented during systole by pressure waveforms reflected from the peripheries.

The circulation is a central elastic reservoir (the large arteries with high elastin to collagen ratio in their walls) (Windkessel theory). The elasticity is negatively associated with its distending pressure. This pressure in the circulation is determined by mean arterial pressure. If this changes, it will alter the elasticity of the arterial wall and measurements of arterial stiffness. In addition, the endothelium and arterial wall smooth muscle bulk and tone (partly controlled by endothelium) also influence elasticity. Therefore, potential opioid-overdose-induced changes in arterial stiffness warrant measurement.

Arterial stiffness is also determined by a number of genetic influences such as fibrillin-1, angiotensin II type-1 receptor, and endothelin receptor genes, and angiotensin-converting enzyme (ACE) I/D polymorphism (Medley *et al.*, 2002; Lajemi *et al.*, 2001b; Lajemi *et al.*, 2001a).

If opioid-induced peripheral haemodynamic effects were to be AI independent, these effects should also be reflected in aortic indices, as in a healthy young population high-pressure amplification and low AI at baseline are expected findings (Wilkinson *et al.*, 2001).

5.1.4. Oxygen saturation and haemodynamic effects

Decreases in O_2 tension in most tissues produces vasodilatation (Ganong, 2001). These effects are at least partly nitric oxide related (Ishimura *et al.*, 1996; Armstead, 1995; Wilderman & Armstead, 1996).

In man, oral morphine or pentamorphone produced significant dose-dependent effects on blood oxygen saturation and the respiratory system (Afifi *et al.*, 1990; Petry *et al.*,

1998; Bailey *et al.*, 1993; Leino *et al.*, 1999). Therefore low oxygen saturation may contribute to opioid-induced haemodynamic effects.

In summary despite the high frequency of opiate overdose the profile of effects and mechanisms in opioid overdose are not clear. In addition, AI (an index of arteriolar stiffness), aortic systolic and diastolic blood pressures (ASBP & ADBP), peripheral and aortic pulse pressure (PPP & APP), end systolic pressure (ESP) and diastolic duration (DD) have not been previously studied non-invasively in overdose.

CO_2 level is also an influence on haemodynamic variables, which might also be affected by opioids. The effects of morphine on CO_2 are discussed in chapters 6 and 7.

5.1.5. Objectives

To prospectively describe the pattern of change in haemodynamic indices in overdose due to methadone, dihydrocodeine and diamorphine in comparison with minor paracetamol overdose, not requiring antidotal therapy, as control.

This study will address the following hypotheses:

1. Methadone, diamorphine and dihydrocodeine overdoses lead to decrease in augmentation index measured by SphigmoCor (primary endpoint).
2. Methadone, diamorphine and dihydrocodeine overdoses lead to decrease in peripheral and central systolic and diastolic blood pressure, mean pressure and pulse pressures, end systolic pressure, heart rate and diastolic duration.

Secondary hypotheses

3. Methadone, diamorphine and dihydrocodeine overdoses lead to decrease in oxygen saturation.

4. The cardiovascular effects of opioid overdoses and decreased oxygen saturation are associated.

5.2. Methods

5.2.1. Study design

Observational, prospective, parallel group study using low dose paracetamol overdose cases as a control group.

5.2.2. Inclusion criteria

It was intended to study 10 concurrent patients with alleged single overdose of methadone, diamorphine, dihydrocodeine, or low dose paracetamol admitted to the Royal Infirmary of Edinburgh from January 1st 2003 to December 30th 2003. History of ingestion and evidence of prescription of methadone and dihydrocodeine were taken into account for diagnosis of the cases.

5.2.3. Exclusion criteria

Patients clinically in withdrawal state (normal or wide pupils, shaking, sweating, or craving), patients, in whom an additional diagnoses were made, or those who retrospectively refused to give consent, were excluded.

5.2.4. Consent taking

Ethical permission, in which consent could be sought retrospectively, was obtained for this study. The details of eligibility criteria and consent processes were accepted by the Multi Centre Research Ethics Committee (MREC). Although this was not a multi-centre study, this design was deemed sufficiently novel to warrant this referral.

5.2.5. Baseline definition

Patients were studied every 6 hours for up to 18-23 hours after exposure or until hospital discharge. Values of variables obtained at 18-23 hours after exposure were used as the baseline of the variables in analysis, as at this time drug concentrations and their related effects are expected to be lowest. Absolute change in each variable was used in the analysis.

Techniques, measurements, and statistical methodology are described in Chapter 2. The following variables were measured; systolic blood pressure; SBP, diastolic blood pressure; DBP, mean blood pressure; MBP, aortic systolic blood pressure; ASBP, aortic diastolic blood pressure; ADBP, peripheral pulse pressure; PPP, aortic pulse pressure; APP, augmentation index; AI, end systolic pressure; ESP, diastolic duration; DD, and oxygen saturation; O_2 Sat. in supine position.

5.3. Results

All diamorphine cases, but two, were excluded from the study, as by the time of measurements they were not in state of overdose, but in withdrawal. Therefore, no diamorphine cases were included in this analysis.

Demographic variables (sample size, gender, age, weight, dependence, and withdrawal clinical features at admission) of patients are summarized in Table 5-1. As can be seen 40% of patients in dihydrocodeine and 60% in methadone groups and 30% in control group were male. All methadone (M) and 90% of dihydrocodeine (D) subjects, but none of paracetamol (P) overdoses were drug dependent on history. Mean (95% CI) ages (M; 36.0 (11.0), D; 33.6 (10.0), and P; 33.1 (13.4) years) and weight (M; 67.70 (6.5), D; 66.8 (8.7), and P; 72.9 (14.2) kg) were similar Table 5-1.

	Dihydrocodeine		Methadone		Paracetamol	
	Min-Max	**Mean (SD)**	**Min-Max**	**Mean (SD)**	**Min-Max**	**Mean (SD)**
AGE	22-55	36.0 (11.0)	23-53	33.6 (10.0)	19-55	33.1 (13.4)
WEIGHT	58-79	67.70 (6.5)	50-79	66.8 (8.7)	59-108	72.9 (14.2)
Male (%)	40%		60%		30%	
Dependence (%)	90%		100%		0%	
Withdrawal clinical features (%)	0%		0%		0%	

Table 5- 1. Demographic variables of patients in 3 different groups (n=10), two eligible cases of diamorphine overdose are not reported.

Not all the patients were admitted soon after overdose and not all of them stayed in the hospital for a full 24 hours. The number of cases in each group is reported on the horizontal axis of each time point in the figures. Time is the interval from stated time of ingestion of the drugs.

Haemodynamic variables (SBP; systolic blood pressure, DBP; diastolic blood pressure, MBP; mean blood pressure, ASBP; aortic systolic blood pressure, ADBP; aortic diastolic blood, PPP; peripheral pulse pressure, APP; aortic pulse pressure, AI; augmentation index, ESP; end systolic pressure, HR; heart rate, DD; diastolic duration) and O_2 Sat.; oxygen saturation of patients are summarized in Table 5-2.

Variables	Drugs n(P;D;M)	0-5 h[1] (8; 6; 7) Mean	SEM	6-11 h (10; 10;10) Mean	SEM	12-17 h (10; 9; 10) Mean	SEM	18-23 h (9; 9; 9) Mean	SEM
SBP[2]	P	140	4.7	134	4.1	135	4.9	135	5.2
	D	105	6.9	113	5.2	113	5.4	121	7.1
	M	112	3.2	112	3.9	116	4.9	125	5.3
DBP	P	76	3.8	72	2.6	71	4.1	70	2.8
	D	52	3.2	59	3.1	66	3.9	65	4.5
	M	67	5.4	68	5.7	69	3.1	73	3.4
MBP	P	99	3.2	94	3.1	93	3.6	92	3.5
	D	72	3.9	78	3.2	82	3.4	84	4.9
	M	85	4.7	83	4.4	88	3.3	92	3.1
ASBP	P	124	4.9	116	4.6	116	5.1	115	5.5
	D	94	5.7	100	4.5	102	4.8	106	6.7
	M	99	3.7	100	3.9	104	3.3	111	3.6
ADBP	P	79	3.7	73	2.7	74	3.9	72	2.7
	D	57	3.1	61	2.9	66	3.3	66	4.6
	M	71	4.3	66	2.8	72	3.0	75	3.1
PPP	P	64	5.6	62	4.0	64	4.8	65	3.7
	D	52	4.5	55	4.7	47	4.4	56	5.9
	M	46	4.1	44	3.8	47	3.3	53	2.9
APP	P	45	5.0	43	3.8	42	4.3	42	3.7
	D	38	3.9	39	3.3	36	4.1	40	5.2
	M	28	3.0	35	2.9	32	2.4	37	3.0
AI (%)	P	12	3.2	18	3.0	14	3.0	10	3.0
	D	20	4.6	14	3.2	21	4.5	27	5.2
	M	4	8.2	14	5.8	17	4.7	14	7.6
ESP	P	106	4.6	103	4.1	102	4.5	100	4.7
	D	81	4.1	87	3.6	89	4.2	90	6.7
	M	88	3.9	89	2.5	91	3.6	92	3.4
DD ($S*10^{-2}$)	P	52	4.9	51	5.1	50	4.5	49	4.5
	D	54	4.7	53	4.6	53	4.2	52	4.1
	M	42	3.0	49	4.1	50	4.3	51	4.6
O_2 Sat. (%)	P	98	0.3	98	0.3	99	0.3	99	0.3
	D	95	2.0	95	1.3	96	0.6	97	0.7
	M	97	0.6	95	1.7	95	1.4	95	1.4

Table 5- 2. Mean SEM of haemodynamic variables and oxygen saturation of patients in the first 24 hours after exposure, (1) hours after exposure, (2) in this table mmHg is the unit unless clarified, SBP; systolic blood pressure, DBP; diastolic blood pressure, MBP; mean blood pressure, ASBP; aortic systolic blood pressure, ADBP; aortic diastolic blood, PPP; peripheral pulse pressure, APP; aortic pulse pressure, AI; augmentation index, ESP; end systolic pressure, HR; heart rate, DD; diastolic duration, O_2 Sat.; oxygen saturation. P; paracetamol, M; methadone, D dihydrocodeine.

In paracetamol overdose all variables were relatively stable in the first 24 hours after exposure.

5.3.1. Systolic Blood Pressure (SBP)

Overall absolute values of SBP were significantly different in dihydrocodeine, methadone and paracetamol overdose ($F=22.1$, $p<0.001$) Table 5-3, Figure 5-1-A (mean $\pm$ 95% CI). Bonferroni multiple comparisons show that dihydrocodeine and methadone groups were similar, but significantly different from paracetamol ($p<0.001$) Table 5-3. These differences are greatest at 0-5 hours after exposure (mean decrease around 30 mmHg), and less clear cut at 6-11, and 12-17 hours after exposure Figure 5-1. Dihydrocodeine and methadone decreased SBP to a similar extent.

5.3.2. Diastolic Blood Pressure (DBP)

Overall DBP was significantly different in dihydrocodeine, methadone and paracetamol overdose ($F=9.295$, $p<0.001$) Table 5-3, Figure 5-1. Bonferroni multiple comparisons show that DBP was significantly lower with dihydrocodeine than paracetamol ($p<0.001$) and methadone ($p=0.007$) Table5-3, Figure 5-1-B. These differences are significant at 0-5 and 6-11 hours after exposure (mean decrease around 25 and 20 mmHg), and not different at 12-23 hours after exposure. DBP of methadone and paracetamol are not different. Dihydrocodeine, but not methadone, deceased DBP in this study.

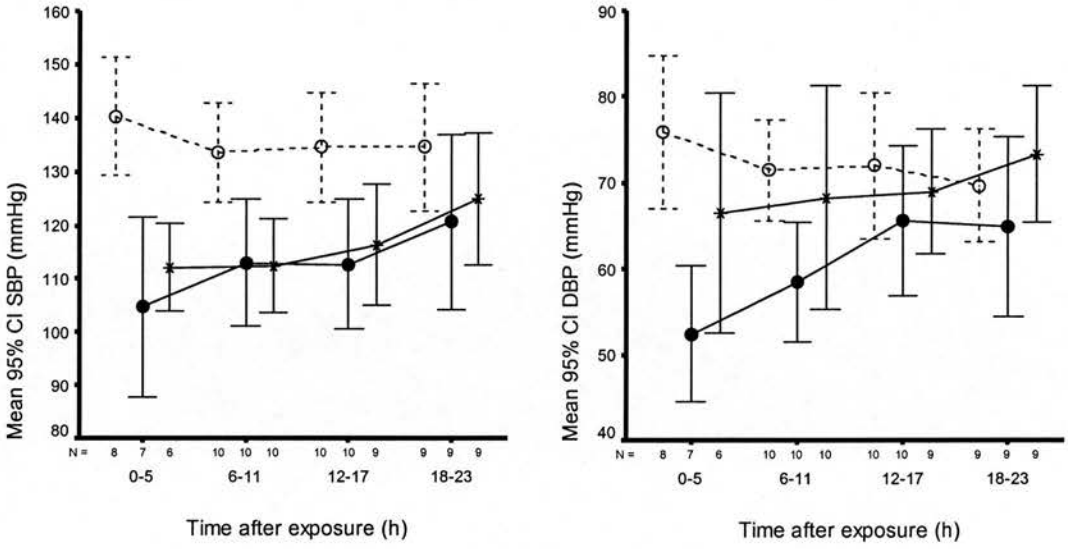
5.3.3. Mean Blood Pressure (MBP)

Since there were differences in systolic and diastolic BP effects on MBP were also examined. Overall MBP values were significantly different in dihydrocodeine, methadone and paracetamol overdose ($F=16.6$, $p<0.001$) Table 5-3, Figure 5-1-C.

A) ANOVA for different groups				
Variable	Homogeneity of Variances		ANOVA	
	Levene Statistic	P value	F	P value
SBP	2.810	.065	22.073	.000
DBP	.999	.372	9.295	.000
MBP	.499	.608	16.606	.000
ASBP	2.638	.076	14.021	.000
ADBP	.659	.520	13.033	.000
PPP	1.997	.141	15.101	.000
APP	2.669	.074	6.173	.003
AIx	6.554	.002	2.858	.062
ESP	3.998	.021	16.529	.000
HR	.411	.664	.805	.450
DD	1.794	.171	1.113	.332
O_2 sat	5.851	.004	10.622	.000

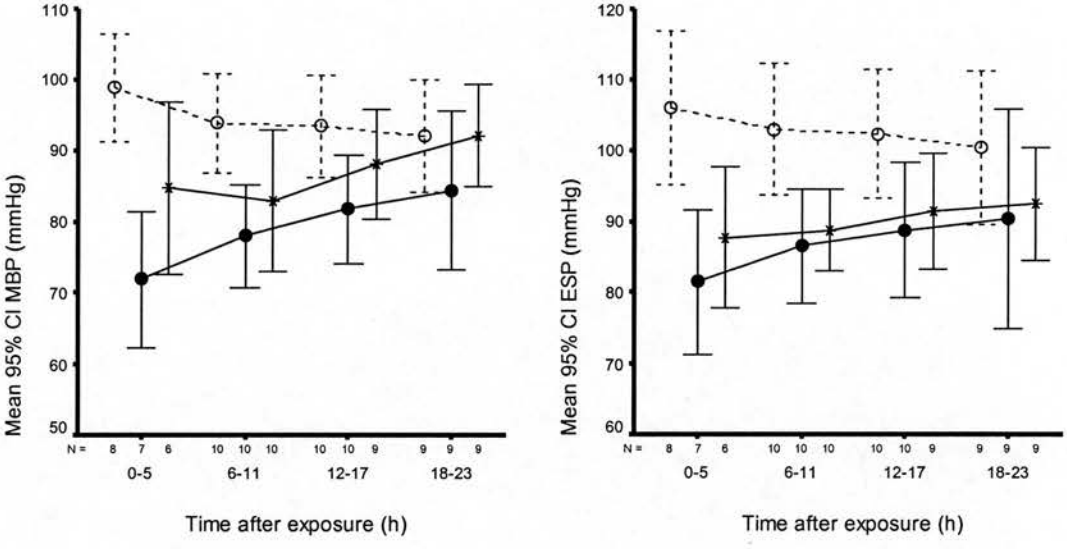
B) Post Hoc multiple comparison with Bonferroni correction				
Variables	Drug (1)	Drug (2)	Mean Difference (1 & 2)	P value
SBP[1]	P (n=37)	D (n=34)	22.29(*)	.000
		M (n=36)	18.81(*)	.000
	D	M	-3.48	1.000
DBP	P	D	11.25(*)	.000
		M	2.64	1.000
	D	M	-8.61(*)	.007
MBP	P	D	14.93(*)	.000
		M	7.32(*)	.019
	D	M	-7.61(*)	.015
ASBP	P	D	16.51(*)	.000
		M	13.63(*)	.000
	D	M	-2.88	1.000
ADBP	P	D	11.84(*)	.000
		M	4.10	.265
	D	M	-7.73(*)	.005
PPP	P	D	11.47(*)	.001
		M	16.31(*)	.000
	D	M	4.83	.352
APP	P	D	4.68	.222
		M	9.23(*)	.002
	D	M	4.56	.264
AI (%)	P	D	-6.54	.153
		M	.74	1.000
	D	M	7.29	.101
ESP	P	D	15.70(*)	.000
		M	12.58(*)	.000
	D	M	-3.12	.878
HR (bpm)	P	D	.09	1.000
		M	-2.90	.832
	D	M	-2.98	.798
DD ($S*10^{-2}$)	P	D	-1.64	1.000
		M	2.96	1.000
	D	M	4.60	.429
O_2 sat. (%)	P	D	2.82(*)	.001
		M	2.96(*)	.000
	D	M	.14	1.000

Table 5- 3. Summary of analytical statistics for differences in haemodynamic variables & O_2 sat. A) ANOVA for difference in all groups (*df=2) B) Post Hoc Multiple* Comparisons with Bonferroni correction for difference in individual drugs, (*); the mean difference is significant at the 0.05 level, (1) units in this table are mmHg unless clarified, SBP; systolic blood pressure, DBP; diastolic blood pressure, MBP; mean blood pressure, ASBP; aortic systolic blood pressure, ADBP; aortic diastolic blood, PPP; peripheral pulse pressure, APP; aortic pulse pressure, AI; augmentation index, ESP; end systolic pressure, HR; heart rate, DD; diastolic duration, O_2 Sat.; oxygen saturation, P; paracetamol, M; methadone, D dihydrocodeine.



A) Systolic blood pressure

B) Diastolic blood pressure



C) Mean blood pressure

D) End systolic pressure

Figure 5- 1. Mean (95% CI) of A) systolic blood pressure in mmHg, B) Diastolic blood pressure in mmHg, C) Mean blood pressure in mmHg, D) End systolic pressure in mmHg, paracetamol group (···○···), methadone group (–✳–) dihydrocodeine group (–●–), (n in each time point is reported in horizontal axis for paracetamol, methadone and dihydrocodeine consecutively.

Bonferroni multiple comparisons showed statistical difference between dihydrocodeine and methadone (p=0.015) and that both were significantly different from paracetamol

(p<0.001, p=0.019) Table 5-3. The differences between dihydrocodeine and paracetamol were highest and significant in pair wise comparisons at 0-5 and 6-11 hours after exposure (mean decrease around 25 and 20 mmHg), and not significantly lower at 6-11, and 12-18 hours after exposure. Thus dihydrocodeine decreased MBP to a greater extent than methadone in this study.

5.3.4. Augmentation index (AI)

Overall absolute values of AI were not significantly different in dihydrocodeine, methadone and paracetamol overdoses (F=2.9, p<0.062) Table 5-2 and 5-3, Figure 5-2. Table 3-4.

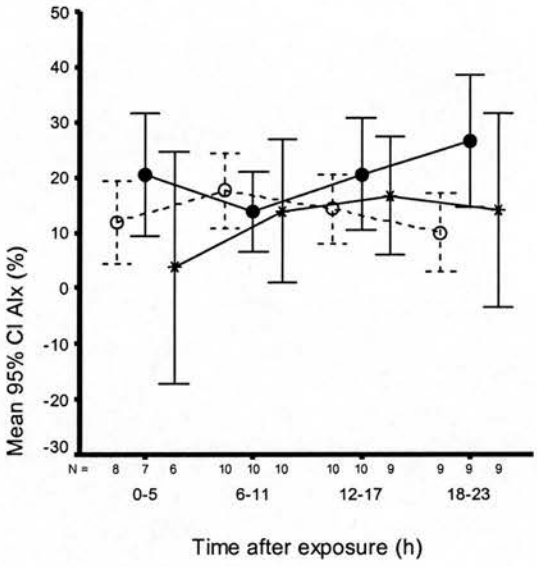
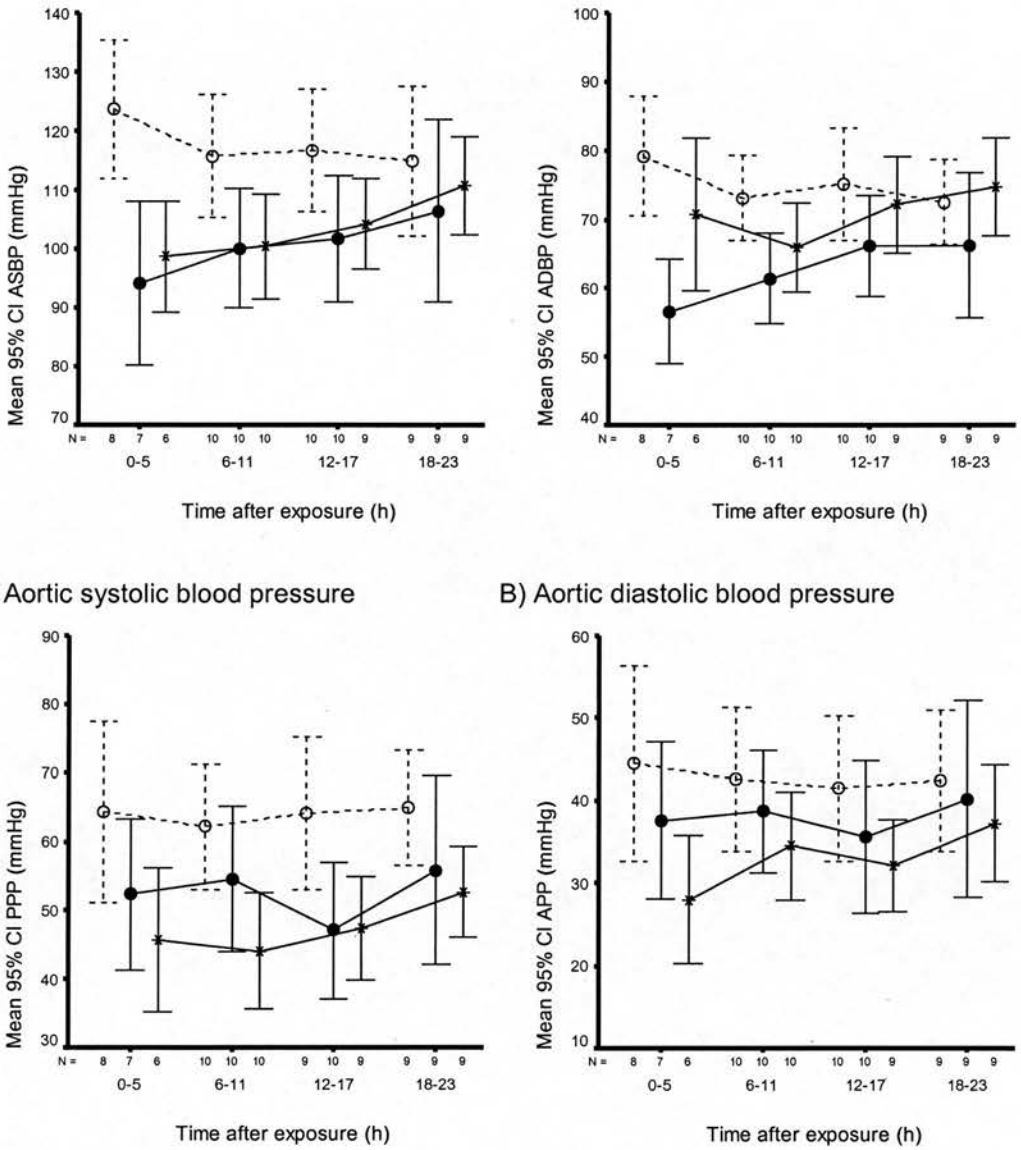


Figure 5- 2. Mean (95% CI) of augmentation index in (%), paracetamol group (···○···), methadone group (–✳–) dihydrocodeine group (–●–), (n in each time point is reported in horizontal axis for paracetamol, methadone and dihydrocodeine consequently.

5.3.5. Derived variables

Overall Aortic Systolic Blood Pressure (ASBP) (F=14.0, p<0.001), Aortic Diastolic Blood Pressure (ADBP) (F=13.0, p<0.001), Peripheral Pulse Pressure (PPP) (F=15.1,

p<0.001), Aortic Pulse Pressure (APP) (F=6.2, p<0.003) and End Systolic Pressure (ESP) (F=16.5, p<0.001) were significantly different. Post hoc multiple comparisons with Bonferroni showed that dihydrocodeine and methadone ASBP, PPP and ESP, and



A) Aortic systolic blood pressure

B) Aortic diastolic blood pressure

C) Peripheral pulse pressure

D) Aortic pulse pressure

Figure 5- 3. Mean (95% CI) of A) aortic systolic blood pressure in mmHg, B) aortic diastolic blood pressure in mmHg, C) peripheral pulse pressure in mmHg, D) aortic pulse pressure in mmHg, paracetamol group (···○···), methadone group (–✳–) dihydrocodeine group (–●–), (n in each time point is reported in horizontal axis for paracetamol, methadone and dihydrocodeine consequently.

dihydrocodeine ADBP were significantly different from paracetamol ($p<0.001$). Methadone APP was significantly different from paracetamol ($P=0.002$).

Dihydrocodeine and methadone decreased ASBP, PPP and ESP to similar extents but dihydrocodeine alone decreased ADBP, and methadone alone decreased APP. Dihydrocodeine and methadone overdose had no effect on HR and DD.

For detail of absolute values of pairwise differences, level of significance and graphical description see Tables 5-2 and 5-3, and Figure 5-4.

Overall HR ($F=0.8$, $p<0.450$) and DD ($F=1.1$, $p<0.332$) were not significantly different among these three groups.

5.3.6. Oxygen Saturation (O_2 Sat.)

Overall absolute values of O_2 Sat were significantly different in dihydrocodeine,

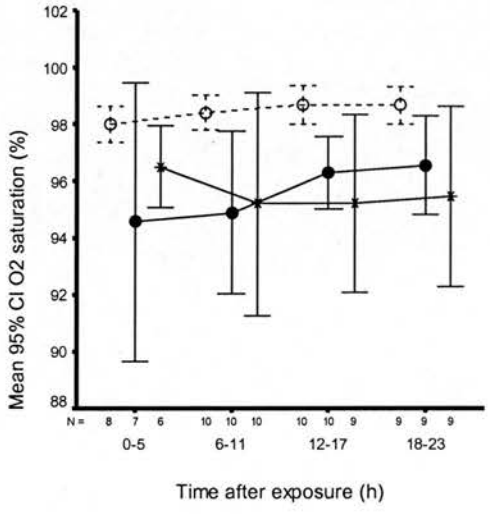


Figure 5- 4. Mean (95% CI) of oxygen saturation in (%), paracetamol group (···○···), methadone group (–✳–) dihydrocodeine group (–●–), (n in each time point is reported in horizontal axis for paracetamol, dihydrocodeine and methadone consequently,

methadone and paracetamol overdoses (F=10.6, p<0.000) Table 5-3, Figure 5-4. Bonferroni multiple comparisons showed no difference between dihydrocodeine and methadone, but that both were significantly different from paracetamol group (DHC; p=0.001, M; p<0.001) Table 5-3.

The lower O_2 Sat of dihydrocodeine and methadone is stable throughout the first 24 hours after exposure. Close examination of the values of both dihydrocodeine and methadone revealed wide ranges of SD (see Figure 5-4 for CI). This implies that hypoxia happens in just a subpopulation.

5.3.7. Relationship of haemodynamic changes and oxygen saturation

The relationships between dihydrocodeine, methadone and paracetamol overdose-induced haemodynamic effects and oxygen saturation are shown in Table 5-4. As can be seen, in general haemodynamic variables are not correlated with oxygen saturation in paracetamol and dihydrocodeine groups. SBP (r=-0.349, P=0.043), DBP

	Paracetamol (n=37)		**Dihydrocodeine (n=34)**		**Methadone (n=36)**	
	r	**P Value[1]**	**r**	**P Value**	**r**	**P Value**
SBP	-.110	.518	.198	.247	-.349(*)	.043
DBP	.203	.229	-.020	.908	-.531(*)	.001
MBP	.082	.630	.019	.914	-.519(*)	.002
ASBP	-.003	.987	.129	.454	-.469(*)	.005
ADBP	.184	.276	-.046	.791	-.299	.086
PPP	-.251	.139	.263	.121	.211	.232
APP	-.151	.372	.212	.215	-.297	.088
AI (%)	-.018	.917	-.036	.834	-.073	.683
ESP	.111	.512	-.108	.532	-.058	.745
HR (bpm)	-.165	.330	-.442(*)	.007	-.359(*)	.037
DD	.410(*)	.012	.029	.868	-.432(*)	.011

Table 5- 4. Association of dihydrocodeine, methadone and paracetamol overdose induced haemodynamic changes with oxygen saturation, 1) *df1=2,* 2) in this table mmHg is the unit unless clarified, SBP; systolic blood pressure, DBP; diastolic blood pressure, MBP; mean blood pressure, ASBP; aortic systolic blood pressure, ADBP; aortic diastolic blood, PPP; peripheral pulse pressure, APP; aortic pulse pressure, AI; augmentation index, ESP; end systolic pressure, HR; heart rate, DD; diastolic duration, *; correlation is significant at the 0.05 level (2-tailed).

(r=-0.531, P=0.001), MBP (r=-0.519, P=0.002), ASBP (r=-0.469, P=0.005), HR (r=-0.359, P=0.037) and DD (r=-0.432, P=0.011) were significantly correlated with oxygen saturation in methadone overdose.

These associations were examined in detail graphically, and as an example the result for SBP in dihydrocodeine suggest the possibility of two distinctive subpopulations separated below oxygen saturation of 95%. In this group for the small sample with oxygen saturation less than 95%, SBP appeared to be lower. This suggests that if oxygen saturation drops to less than 95% it may contribute to opioid-induced cardiovascular effects.

5.4. Discussion

The two opioids studied lowered blood pressure in overdose, and there was no reflex tachycardia. This study has shown that dihydrocodeine and methadone overdoses significantly depress haemodynamic function. This effect was seen on SBP, MBP, and the derived measures of ASBP, PPP, and ESP. Dihydrocodeine reduced DBP and ADBP, an effect not induced by methadone. As a result, peripheral and aortic pulse pressures were also significantly decreased. Methadone induced a significant depressor effect on APP. Both opioids decreased oxygen saturation.

A decrease in SBP might be attributed to vasodilatation and decrease in systemic vascular resistance, and decrease in arterial stiffness or to negative inotropism and /or chronotropism.

The pattern of observed effects suggests that dihydrocodeine and methadone induce decrease in afterload, which may provide a pharmacologic explanation for their widespread use in congestive heart failure and acute myocardial infarction. The significant decrease in the derived measurement of end systolic pressure in opioid overdose suggests that dihydrocodeine and methadone may have effects on cardiac emptying, and in turn energy consumption of the heart. In the context of heart failure treatment this may also maybe of benefit.

There was no significant effect on AI, HR and DD. Augmentation index results suggest that a change in arterial stiffness is not the mechanism of haemodynamic effects. As AI did not decrease, large artery stiffness is probably not the mechanism of depressor effects of opioids. HR also did not change, suggesting that vagal tone is also not affected, nor did baroreceptor mediated tachycardia occur. As all subjects were supine, this may have masked postural effects.

Both dihydrocodeine and methadone overdoses significantly decreased oxygen saturation. The strength of these haemodynamic effects, however, was not clearly associated with oxygen saturation in general. An oxygen saturation of less than 95% might contribute to haemodynamic effects of opioids in overdose. A concurrent increase in CO_2 may also be a factor. CO_2 is studied in volunteers in chapters 6 and 7.

The observed effects happen early after exposure (0-5 hours), which is consistent with peak plasma concentration of 1.8-1.9 and 4 hours after oral doses of dihydrocodeine and methadone respectively after therapeutic dosing (Dollery C, 1991).

The effects were maximal early after exposure and decreased over the next 24 hours, suggesting these effects are probably concentration dependent in overdose, however, tolerance to the effects cannot by fully excluded. In the case of dihydrocodeine, which has a partial agonist metabolite (nordihydromorphine and dihydromorphine-6-glucuronide) (Rowell *et al.*, 1983), the potential interpretations are more complex.

In this study methadone and dihydrocodeine in overdose appeared to be haemodynamic depressant. It has been shown previously that chronic methadone administration in rats did not change blood pressure (Lewanowitsch *et al.*, 2004). In patients on maintenance methadone significant positive associations with heart rate have reported (Mitchell *et al.*, 2004).

As might be expected haemodynamic indices were most similar between groups at "baseline" (18-23 hours after exposure).

In terms of cardiovascular risks, opioid overdose induced reduction in SBP, PPP, and to a lesser extend DBP suggests an effect of opioids which might offer benefits in hypertension. These results were in overdose and might explain why opioid induced haemodynamic effects have not been seen in some studies in which lower doses were used (Lowenstein *et al.*, 1969; Crosby *et al.*, 1994; Murat *et al.*, 1988).

5.5. Limitations

As in any patient study, the homogeneity of the subjects and reliability of control group make interpretation of data more conservative. Some of the techniques used in this study were also indirect measurements.

Moreover, opioid doses were different and not precise from a design perspective. Most patients were hospitalised and habituated to opioids, and these findings might not apply to the general population.

Some opioid overdose patients in this study received naloxone in low doses some hours prior to these investigations. Any contribution of naloxone in these effects is therefore ignored. Sample size was limited, and all diamorphine cases were excluded due to strict eligibility criteria. The power of the study was affected as it was not possible to keep all cases admitted soon after exposure, and not all of them remained in the hospital for a full 24 hours. The potential contribution of dependency raises the possibility that naive subjects might produce different results.

The last clinical examination was approximately 18-23 hours after exposure, and as the half life of methadone is longer than dihydrocodeine, it is probably not be enough for effects to have completely worn off.

5.6. Conclusion

In conclusion, dihydrocodeine and methadone overdose result in haemodynamic depression. A fall in oxygen saturation to less than 95%, may also contribute to these changes. These findings have clinical relevance to management of opioid overdoses in terms of continuous blood pressure monitoring and oxygenation.

An opioid-induced decrease in afterload, as measured by fall in MAP, and negative inotropism, as measured by reduced ESP are consistent with the use of opioids such as morphine in congestive heart failure and acute myocardial infarction pain. It seems

likely these cardiovascular effects might also be seen following use of opioids in pain management.

If these effects are seen with lower doses of opioids in healthy volunteers, and if tolerance does not develop, these findings might suggest that opioid related haemodynamic depression could occur, particularly in end stage patients with pain who are receiving long term morphine.

In addition, these findings raise the possibility that suitable peripheral acting opioids could have a role in the management of cardiovascular diseases such as hypertension and heart failure.

These results are of interest since high doses cannot be ethically tested in volunteers, and also overdose cases are not able to give consent in advance for research purposes; therefore, little experimental data are available in this patient group.

Chapter VI, Haemodynamic effects of morphine; a randomised control trail of incremental doses of morphine and placebo

6.1. Introductory remarks

Opioids may act at a range of opioid receptors, and the affinity to them may vary. Haemodynamic effects of morphine are controversial; depressor (Fahmy *et al.*, 1983; Petry *et al.*, 1998) pressor (Mildh *et al.*, 2000) and lack of effect (Lowenstein *et al.*, 1969) have been reported. Histamine and catecholamines have been claimed as potential secondary mechanisms of morphine induced cardiovascular effects (see introduction).

6.1.1. Cardiac mechanic

Mechanisms involved in cardiac mechanics are summarised in Figure 6-1.

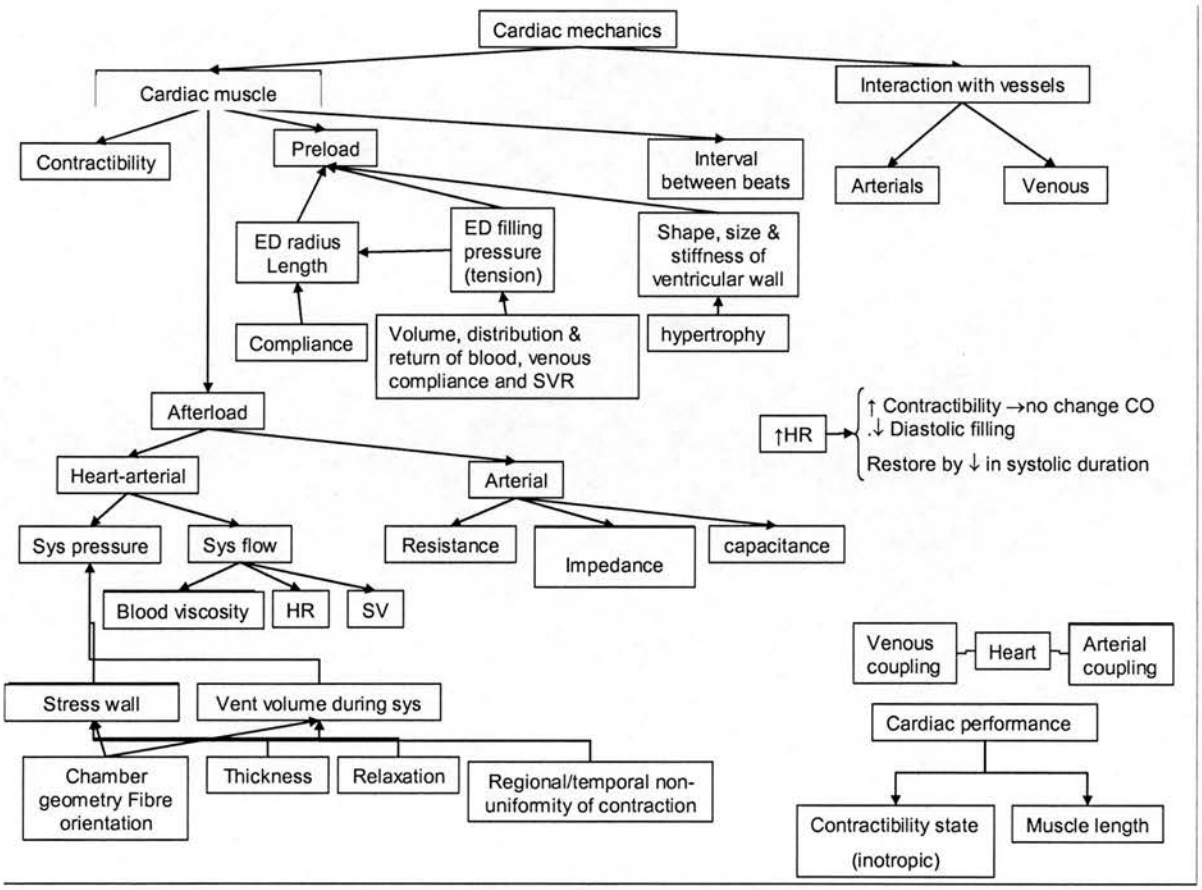


Figure 6- 1. Schematic diagram of mechanisms involved in cardiac function. ED; end diastolic, Sys; systolic, SVR; systemic vascular resistance, ↓; decrease,

6.1.2. Alteration in cardiovascular performance

Cardiac performance can alter in two major ways, changing the initial ventricular muscle length (Frank-Starling phenomenon) and changing the contractile state through inotropic intervention, or changing cardiac output, chronotropism. However, passive ventricular wall stress or tension at the end of diastole (preload) and total myocardial wall stress or tension during systolic ejection (afterload) (Norton, 2001) also influence cardiovascular performance.

6.1.3. Outline of the mechanisms of afterload

Afterload is determined by arterial and cardiac factors. Arterial factors include resistance, impedance and capacitance. A second compound is systolic pressure and flow which in turn are determined by ventricular wall stress, ventricular volume during systole, blood viscosity, heart rate and stroke volume.

6.1.4. Outline of the mechanisms of arterial stiffness

The circulation has a central elastic reservoir (Windkessel theory) (Nichols & O'Rourke, 1998). Blood is pumped into the aorta and its major branches by the heart, and travels to the tissues through relatively non-elastic vessels (peripheral arteries). These include more muscular conduit arteries, such as the radial, and the smaller, predominantly muscular, peripheral arteries. The elasticity associated with its distending pressure. The pressure in the circulation is determined by mean arterial pressure, which affects the elasticity of the arterial wall in measurements of arterial stiffness. Systole initiates an arterial pressure wave from the central circulation to the periphery. Wave reflection occurs at impedance-mismatch points, mainly at the high-resistance arterioles (Nichols

& O'Rourke, 1998). Arterial stiffness is also determined by a number of genetic influences such as fibrillin-1, the angiotensin II type-1 receptor, endothelin receptor genes, and angiotensin-converting enzyme (ACE) I/D polymorphism (Medley *et al.*, 2002; Lajemi *et al.*, 2001b; Lajemi *et al.*, 2001a). Drugs affect arterial stiffness by acting on endothelium, arterial wall smooth muscle tone or both. The endothelium and arterial wall smooth muscle tone also influence elasticity.

6.1.5. Therapeutic uses of morphine in cardiovascular diseases

Morphine is recommended in the treatment of the patients with acute left ventricular failure and ischaemic-type chest pain and without ST segment elevation to control the pain and anxiety. Morphine decreases pain, and therefore diminishes the sympathetic nervous system activity and catecholamine secretion. Pain related catecholamines increase blood pressure, cardiac chronotropic and inotropic responses, and oxygen consumption and therefore intensifying ischemia. Although morphine has been reported to have no significant cardiac depressor effect (Fuster, 2001), repeated doses of morphine, may decrease cardiac work, and oxygen consumption, by causing venodilatation and slightly decreasing heart rate and blood pressure (Topol, 1998).

6.1.6. Primary Hypothesis

This study addresses the following hypotheses; Morphine causes cardiovascular changes in man which are dose dependent. These effects are in part related to concurrent CNS and respiratory effects of morphine, and histamine and catecholamines play some role it them.

To test this hypothesis, a range of haemodynamic, respiratory and CNS variables were estimated, following a stepped-dose of morphine given by IV infusion. This chapter will

discuss key findings, but the full range of indices measured and derived is shown in Table 6-1.

6.2. Materials and methods

Ethical approval was obtained from the Lothian Research Ethics Committee (LREC), Research and Development Office (R&D), and liability insurance cover held by the

Abbreviations	Primary end points	Abbreviations	
AI	augmentation index		
ASBP	aortic systolic blood pressure		
SBP	systolic blood pressure		
	Secondary endpoints		
	Cardiovascular		**Respiratory**
AcI	acceleration index	**$ETCO_2$**	end tidal carbon dioxide
APP	aortic pulse pressure	**O_2 Sat.**	oxygen saturation
CI	cardiac index	**RR**	respiratory rate
DBP	aortic diastolic blood pressure		
DBP	diastolic blood pressure		**Central nervous system**
ED	ejection duration	**RT**	reaction time
EDI	end diastolic index	**CR**	correct response
EF	ejection fraction	**IR**	incorrect response
ER	ejection ratio	**NR**	no response
ESP	end systolic pressure		
HR	heart rate		
HRP	heart rate period		
IC	index of contractibility		
MBP	mean blood pressure		
PFI	peak flow index		
PPP	peripheral pulse pressure		
SI	stroke index		
sSBP	sitting systolic blood pressure		
sSDP	sitting diastolic blood pressure		
STR	systolic time ratio		
SVR	systemic vascular resistance index		
TFI	thoracic fluid index		
VER	ventricular ejection time		

Table 6- 1. Primary and secondary end points and list of abbreviations in alphabetical order. Morphine only altered reaction time values, other CNS responses are not reported.

Health and Safety Department of The University of Edinburgh. Written informed consent of each subject was obtained.

6.2.1. Study design

The study was a single-blind two-way crossover randomized clinical trial. Potential adverse effects of high doses of morphine prevented us ethically designing a fully double blind study.

6.2.2. Subjects

Subjects were healthy male volunteers aged 18 to 50 years, weighing 60 to 100 kg. See Chapter II for study population.

6.2.3. Inclusion criteria

Volunteers with no history or physical characteristics of opioid abuse were selected. All subjects were, however, required to undergo screening for drugs of abuse using TRIAGE^{TM}8. The test procedure was followed as per package insert. Volunteers had no history of cardiovascular disease, known high plasma cholesterol, excess alcohol intake, clinically significant hepatic, renal and respiratory diseases. All subjects were required to sign a written informed consent. All subjects were asked to desist from vasoactive medications in the two weeks before each study and from alcohol, caffeine-containing drinks, and tobacco for at least 12 h before each study. Each subject was fasted for at least 3 h before any measurements were taken.

6.2.4. Exclusion criteria

Subjects were excluded if their systolic blood pressure was lower than 90 mmHg or higher than 160 mmHg, and diastolic BP 50 or 90 mmHg, women and anyone positive for illegal drugs, subjects with any history of drug toxicity, subjects with any clinically symptoms or signs of volume depletion or dehydration were also excluded.

6.2.5. Specific criteria for early withdrawal and discontinuation

The study was planned to be discontinued if moderate adverse events (severe nausea, vomiting, clinically important drowsiness, respiratory depression, orthostatic hypotension and hypersensitivity) occured. In addition, if any severe abnormal test results develop, considered dangerous by the principle researcher, subjects would be withdrawn. Subjects could withdraw their consent at any time.

6.2.6. Outline of the study

The subjects were supine and cannulated in both arms at 10 a.m. They remained in a supine position for at least 20 minutes before starting the study. Patients were connected to all measurement equipment. A rehearsal of the study explained, and each subject tried a reaction time test to be familiar with this prior to starting the study. Sitting blood pressure was measured 1 minute after sitting in 90 degrees.

Incremental does of morphine sulphate (0.25, 0.5, 1, 2, 4, 8 mg) or saline control were infused over a 5 minute period, using an automated pump at 20 minutes intervals.

There was a 15 minutes gap between each infusion in which measurements were done. Another pump continuously infused saline in the same cannula at a rate of 60 ml per hour. All solutions were prepared sterile in the morning of each study day.

Blood samples were taken at baseline, 5 minutes prior to starting the experiment, and 5 minutes after 0.25 (+25 mins), 1 (+65 mins), 8 (+125 mins) mg of morphine infusion to measure morphine, histamine, adrenaline and noradrenaline concentrations. The period of the study in total was about two hours followed by 4 hours observation of the subjects for their own safety and for documenting side effects. The patients were accompanied home by principle researcher in a taxi, as the ethics committee required. SPSS (Statistical Package for the Social Sciences) 11.5 and Microsoft Excel 2000 were used to describe and analyse the data.

Chapter II, the method section, discusses drugs, drug administration, sample handling, plasma assays, applied techniques, and tools for assessing end points, power of the study and data analysis method.

6.3. Results

The demographic variables (age, height, weight, and body surface area (BSA)) of the 8 volunteers are summarized in Table 6-2.

Variable	Min	Max	Mean	SD
Age (year)	20	50	34.13	10.79
Height (cm)	165	189	173.25	7.13
Weight (kg)	61	99	80.75	11.82
Body Surface Area (m^2)	1.67	2.28	1.97	0.17

Table 6- 2. Descriptive Statistics of volunteers, BSA; is calculated from the square root of ([Height (cm) x Weight (kg)]/ 3600), Mosteller formula (Mosteller, 1987).

The difference between values for placebo and active drug were then compared first by ANOVA TABLE 6-3. Secondly when ANOVA was significant, their differences at individual time points were compared and P value of the significance were calculated, and are summarized in Table 6-3.

6.3.1. Systolic blood pressure

Variables	F	P value
Haemodynamic variables		
Acceleration index (%)	11.471	0.001
Aortic diastolic blood pressure (%)	4.996	0.027
Aortic systolic blood pressure (%)	14.726	< 0.001
Aortic pulse Pressure (%)	10.309	0.002
Augmentation index (%)	2.942	0.089
Cardiac index (%)	0.149	0.700
Diastolic blood pressure (%)	3.576	0.620
Ejection fraction (%)	0.276	0.601
Ejection duration (%)	0.564,	0.454
Ejection ratio (%)	8.087	0.005
End diastolic index (%)	19.800	< 0.001
End systolic pressure (%)	9.270	0.003
Heart rate (%)	12.909	< 0.001
Heart rate period (%)	2.507	0.116
Index of contractibility (%)	16.238	< 0.001
Mean blood pressure (%)	15.117	< 0.001
Peak flow index (%)	9.818	0.002
Peripheral pulse pressure (%)	14.294	< 0.001
Sitting diastolic blood pressure (%)	0.051	0.821
Sitting systolic blood pressure (%)	11.563	0.001
Stroke index (%)	13.787	< 0.001
Systemic vascular resistance index (%)	5.202	0.024
Systolic blood pressure (%)	19.583	< 0.001
Systolic time ratio (%)	1.081	0.301
Thoracic fluid index (%)	1.853	0.176
Ventricular ejection time (%)	14.538	< 0.001
Respiratory and CNS variables		
End tidal carbon dioxide (%)	36.291	< 0.001
O_2 Saturation (%)	6.938	0.010
Respiratory rate (%)	19.096	< 0.001
Reaction time (%)	5.025	0.027
Plasma assays		
Plasma histamine concentration	0.457	0.501
Plasma adrenaline concentration	2.119	0.162
Plasma noradrenalin concentration	0.637	0.437
Plasma morphine concentration	N/A	N/A

Table 6- 3. Analysis of variance of percentages of change from baseline between morphine and placebo groups for homodynamic, respiratory and central nervous system variables, NA; did not applied as the technique used was sensitive enough to detect morphine in just two samples of placebo group, (%); percentage of change from baseline.

Overall morphine induced significant decrease in SBP (F=19.583, P< 0.001) Table 6-3, Figure 5-1-A (mean ± SEM). Mean difference (SEM) between percentage of change from baseline in morphine and placebo was -5.64 (1.35) %. Visual comparison of each time point from the two visits showed that this depressor effect started with 0.250 mg morphine (P=0.047). To explore dose response an association analysis was done and this showed that the effect did not appear to intensify with higher doses (r= 0.003, P=0.983) Tables 6-4, and 6-5.

Overall, morphine significantly decreased systolic blood pressure in a non dose-dependent manner, an effect which appeared to start at very low doses.

6.3.2. Mean, sitting systolic and Diastolic blood pressures, and pulse pressure

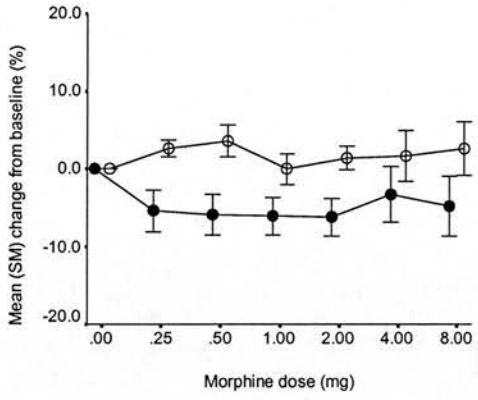
Overall morphine induced significant decrease in MBP (F=15.117, P<0.001) Table 6-3, Figure 6-1-C, significant decrease in sitting systolic blood pressure (F=11.563, P=0.001) Figure 6-1-D, and significant decrease in peripheral pulse pressure (F=14.294, P<0.001) Figure 6-1-C. Again these effects were not clearly dose-dependent.

Tables 6-4 and 6-5 and Figure 6-1-B. Morphine had no effect on diastolic blood pressure at these doses.

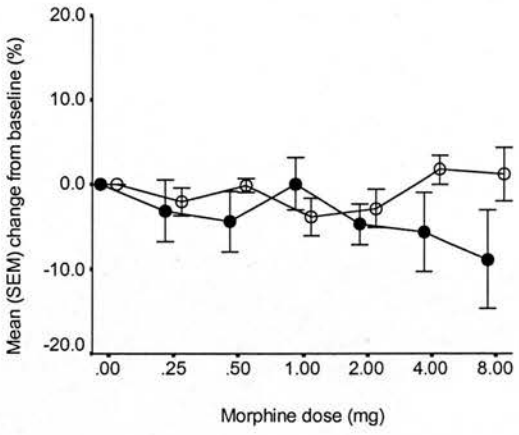
Morphine	Baseline		0.250mg		0.500mg		1mg		2mg		4mg		8 mg	
Abb.	M[1]	P[2]	M	P	M	P	M	P	M	P	M	P	M	P
Haemodynamic variables														
AcI	0.00	N/A	-6.1	0.11	-2.8	0.36	-5.48	0.21	-3.8	0.24	-3.2	0.54	-3.9	0.10
ADBP	0.00		-1.7	0.67	-4.3	0.18	1.06	0.74	-1.5	0.56	-6.5	0.11	-5.3	0.29
ASBP	0.00		-6.2	0.03	-8.4	0.02	-4.85	0.07	-4.5	0.12	-3.8	0.46	-6.3	0.32
APP	0.00		-15.8	0.05	-18.3	0.06	-12.17	0.14	-16.8	0.02	-2.3	0.83	-12.6	0.28
EDI	0.00		-7.1	0.05	-7.2	0.08	-9.34	0.05	-4.3	0.27	-2.9	0.60	-8.2	0.02
ER	0.00		4.3	0.08	4.3	0.12	2.94	0.30	1.8	0.64	0.9	0.85	5.3	0.07
ESP	0.00		-5.9	0.05	-6.8	0.06	-3.01	0.20	-1.5	0.35	-3.3	0.46	-4.4	0.50
HRB	0.00		6.4	0.07	4.9	0.10	6.40	0.02	4.2	0.28	1.2	0.81	5.4	0.05
IC	0.00		-6.6	0.07	-3.7	0.18	-6.11	0.10	-4.2	0.17	-3.6	0.46	-4.6	0.05
MBP	0.00		-5.6	0.06	-7.2	0.03	-2.99	0.16	-3.3	0.16	-5.3	0.23	-6.0	0.26
PFI	0.00		-5.6	0.13	-2.2	0.42	-4.74	0.18	-5.2	0.19	-2.5	0.61	-3.5	0.17
PPP	0.00		-15.5	0.04	-20.1	0.05	-14.05	0.03	-16.2	0.02	-2.5	0.81	-12.0	0.26
sSBP	0.00		-5.3	0.20	-8.4	0.00	-3.54	0.40	-5.8	0.10	-5.2	0.07	-7.4	0.13
SI	0.00		-8.1	0.13	-4.4	0.33	-8.90	0.07	-10.1	0.08	-4.5	0.53	-6.6	0.11
SVR	0.00		-5.0	0.38	-10.7	0.08	-2.92	0.55	-3.3	0.61	-6.3	0.51	-7.1	0.38
SBP	0.00		-7.5	0.05	-9.2	0.06	-3.90	0.24	-7.0	0.01	-4.8	0.36	-7.2	0.22
VET	0.00		6.1	0.08	4.9	0.17	5.75	0.18	5.5	0.33	3.6	0.51	10.6	0.05
Respiratory and CNS variables														
$ETCO_2$	0.00		1.5	0.41	1.4	0.63	2.72	0.05	11.6	0.01	11.5	0.00	21.0	0.00
O_2 Sat.	0.00		0.1	0.84	0.1	0.74	0.14	0.77	-1.4	0.11	-1.2	0.27	-2.3	0.05
RR	0.00		-4.0	0.44	-3.8	0.57	2.24	0.68	-12.0	0.21	-17.8	0.01	-32.0	0.00
RT	0.00		-3.9	0.23	-6.4	0.03	-3.86	0.23	-4.2	0.21	0.4	0.88	3.3	0.25

Table 6- 4. Mean of paired differences of percentages of changes from baseline at each time points of morphine and placebo visits for haemodynamic, respiratory and CNS variables with the P value of significance of their differences at each time point, df; 7, Abb.; abbreviations, M; mean of paired differences of percentages of changes from baseline at each time points of morphine and placebo visits, P; P value, N/A; could not be computed because the standard error of the difference was 0.

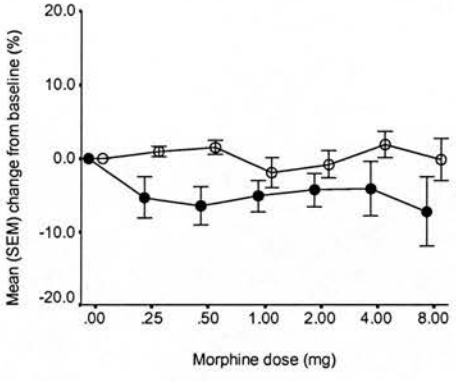
Haemodynamic variables; AcI; acceleration index, AI; augmentation index, ADBP; aortic diastolic blood pressure, APP; aortic pulse pressure, ASBP; aortic systolic blood pressure, CI; cardiac index, DBP; diastolic blood pressure, ED; ejection duration, EDI; end diastolic index, EF; ejection fraction, ER; ejection ratio, ESP; end systolic pressure, HR; heart rate, HRP; heart rate period, IC; index of contractibility, MBP; mean blood pressure, PFI; peak flow index, PPP; peripheral pulse pressure, SI; stroke index, sSBP; sitting systolic blood pressure, sDBP; sitting diastolic blood pressure, SBP; systolic blood pressure, STR; systolic time ratio, SVR; systemic vascular resistance index, TFI; thoracic fluid index, VER; ventricular ejection time. *Respiratory variables*; $ETCO_2$; end tidal CO_2, O_2 Sat.; oxygen saturation, RR; respiratory rate, *CNS variable*; RT; reaction time.



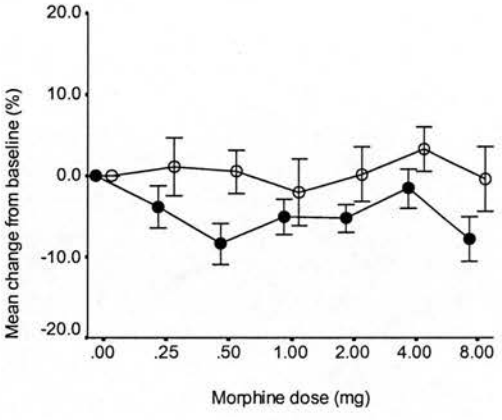
A) Supine systolic blood pressure



B) Supine diastolic blood pressure



C) Supine mean blood pressure



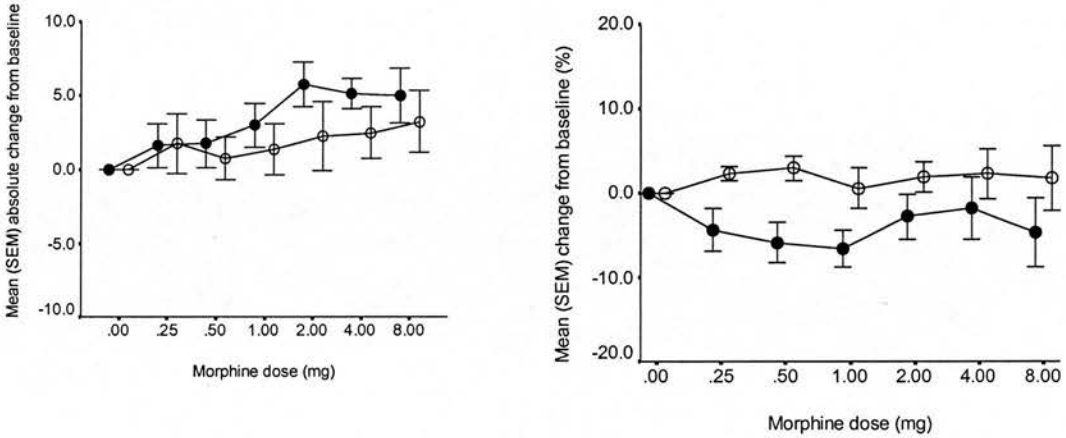
D) Sitting systolic blood pressure

Figure 6- 2. Mean (SEM) of percentage of change from baseline of A) supine systolic blood pressure, B) supine diastolic blood pressure, C) supine mean blood pressure Sitting, D) sitting systolic blood pressure in morphine group (—●—) and placebo group (—○—) (n=8).

6.3.3. Augmentation index, systemic vascular resistance and aortic derived variables

Overall, morphine had no effect on arterial stiffness (AI) (F=2.942, P=0.089) Table 6-4, Figure 6-3-A. Despite this, systemic vascular resistance (F=5.202, P=0.024) and aortic derived variables significantly decreased; aortic systolic blood pressure (F=14.727, P<0.001) Figure 6-3-B, aortic diastolic blood pressure (F=4.996, P=0.027), and aortic

pulse pressure (F=10.309, P=0.002). These effects were not dose-dependent Tables 6-3, 6-4 and 6-5.

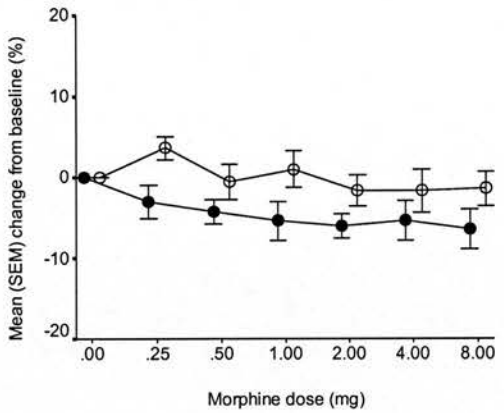


A) Augmentation index B) Aortic systolic blood pressure

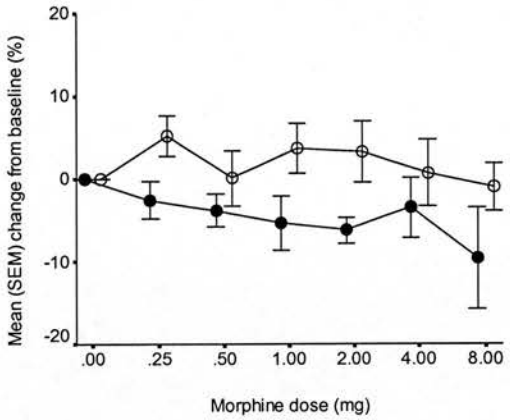
Figure 6- 3. Mean (SEM) of percentage of change from baseline of A) augmentation index, B) aortic systolic blood pressure, C) Sitting Systolic blood pressure, D) Mean blood pressure in morphine group (–●–) and placebo group (–○–) (n=8).

6.3.4. Heart related variables

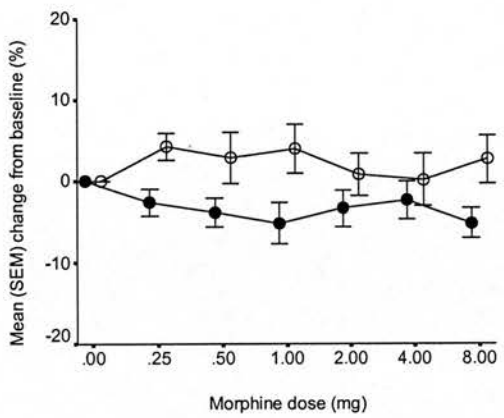
Overall morphine induced significant decrease in the index of contractibility (F=16.238, P<0.001), stroke index (F=13.787, P<0.001), end diastolic index (F=19.800, P<0.001), end systolic pressure (F=9.270, P=0.003), and peak flow index (F=9.818, P=0.002) (Figure 6-4 A-E), and Tables 6-3 to 6-5. Heart rate in the placebo group, was significantly lower than morphine group (F=12.909, P<0.001) (Figure 6-4-F). Morphine had no effect on the other variables Table 6-4.



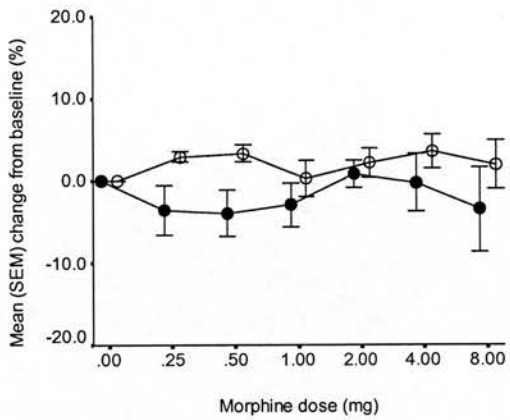
A) Index of contractibility



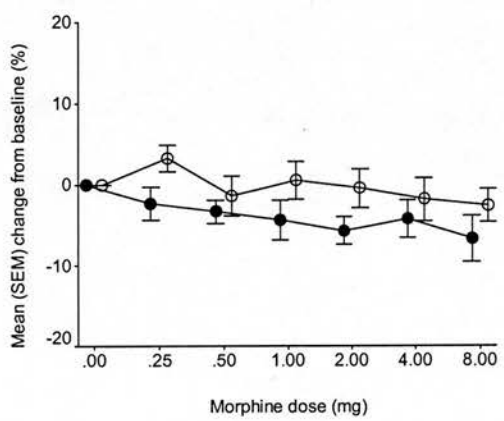
B) Stroke index



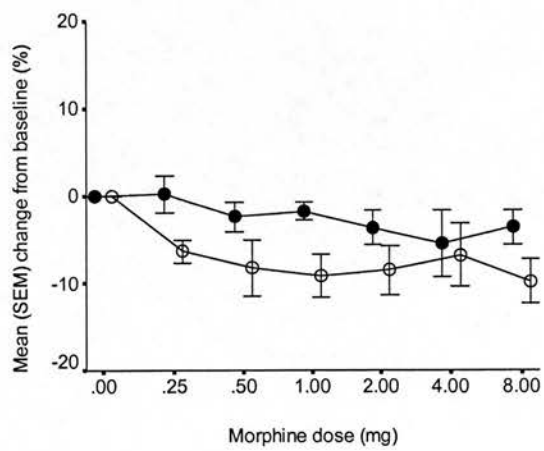
C) End diastolic index



D) End systolic pressure



E) Peak flow index

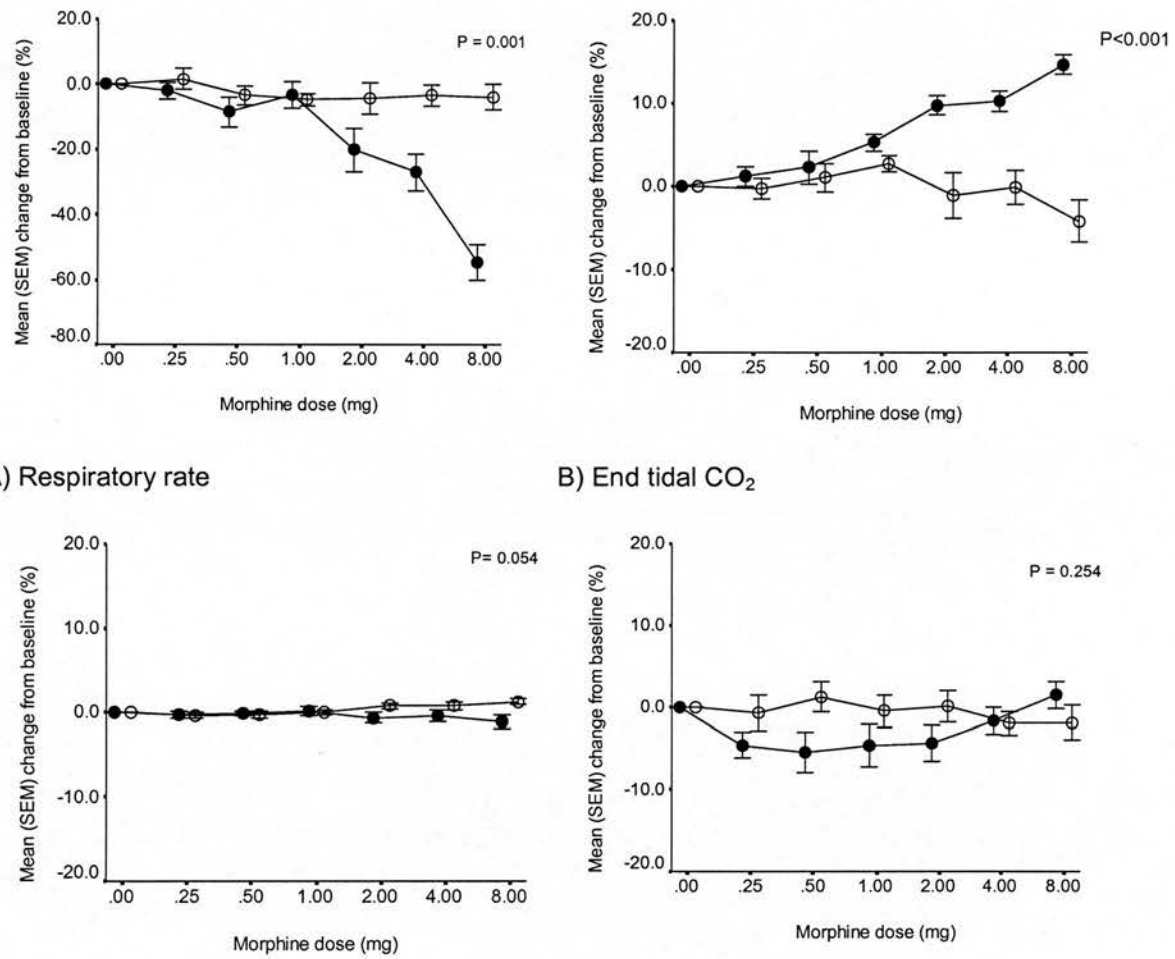


F) Heart rate

Figure 6- 4. Mean (95% CI) of percentage of change from baseline of A) systolic blood pressure, B) diastolic blood pressure, C) Sitting Systolic blood pressure, D) Stroke index, E) End diastolic index in morphine group(—●—) and placebo group (—○—) (n in each time point is reported in horizontal axis for paracetamol, methadone and dihydrocodeine consequently.

6.3.5. Non-haemodynamic variables

Overall morphine produced a significant decrease in respiratory rate (F=19.096, P<0.001), end tidal CO_2 (F=32.296, P<0.001), oxygen saturation (F=6.938, P<0.010), and reaction time (F=5.025, P=0.027) (Figure 6-5 A-D, Tables 6-3, and 6-4).



A) Respiratory rate

B) End tidal CO_2

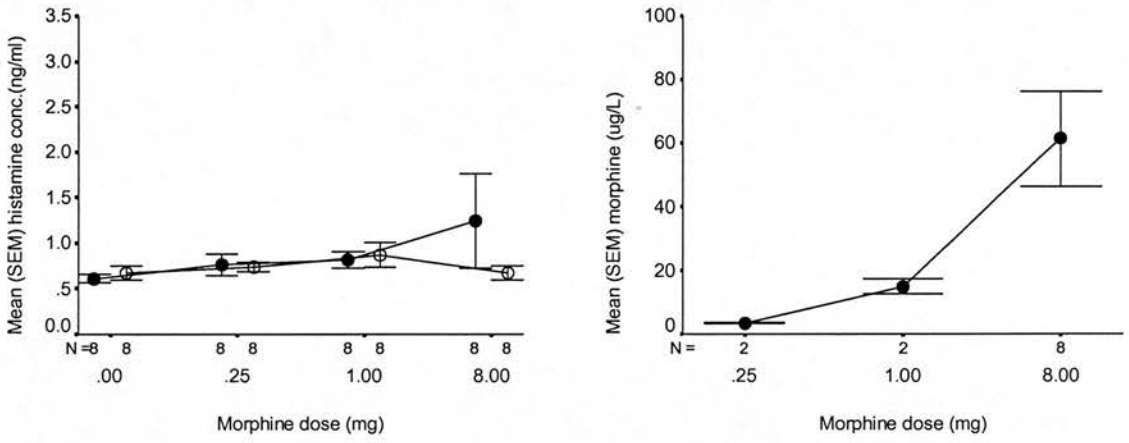
C) Oxygen saturation

D) Reaction time

Figure 6- 5. Mean (SEM) of percentage of change from baseline of A) respiratory rate, B) end tidal CO_2, C) oxygen saturation, and D) reaction time in morphine group, P; P value of the difference at the last dose (df; 7)(—●—) and placebo group (—○—) (n=8).

6.3.6. Plasma assays

Overall morphine induced no significant change in plasma histamine, adrenaline and noradrenaline concentrations Figure 6-6, Tables 6-3, and 6-4. Morphine plasma concentrations are also shown in Figure 6-6



A) Histamine B) Morphine (1 visit)

Figure 6- 6. Mean (SEM) of absolute values of plasma concentrations of A) histamine in ng/ml, B) morphine mcg/ml in morphine group (—●—) and placebo group (—○—) (n=8).
The techniques were not sensitive for values less than 2.5 mcg/ml for morphine levels. These values were omitted, and the actual N in reported below the horizontal axis for each time point for morphine and saline visits respectively.

The used techniques were not sensitive for values less than 30 pg/ml and 150 pg/ml for adrenaline and noradrenaline respectively. When catecholamines were detected, they were in a small amounts with no significant different to placebo arm, and there were no dose response.

6.3.7. Dose, age and body mass index dependency

In general in the morphine group, haemodynamic variables were not dose dependent, but non-haemodynamic variables of RR ($r=-0.813$, $P<0.001$), $ETCO_2$ ($r=0.775$, $P<$

0.001) and RT (r=0.303, P=0.023) were dose dependent. This probably suggests an independent mechanism of action.

As it is expected augmentation index (r=0.793, P<0.001), and also ED (r=0.445, P=0.001)) and VET (r=0.276, P=0.040) were significantly age related. Reaction time was negatively age related (r=-0.301, P=0.024).

SBP, DBP, sSBP, ASBP, ADBP, ESP, STR, MBP, SVR, and O_2 Saturation were positively, and PF, EF, IC, AcI, and ED were negatively body mass index related. Age and particularly BMI should be considered as potential confounders.

6.3.8. Reported adverse effects

In eight cases and one control light-headedness were reported. Three cases and two controls reported sleepiness. Four cases vomited. One case reported itching. Light-headedness started during the higher doses of morphine and lasted a maximum of 8 hours. The first episode of vomiting started with a median of 3 hours (range 3 to 7 h) after discontinuing morphine. In two cases it lasts for 4 and 15 hours. These two subjects received cyclizine. Nausea was not reported for more than a minute before vomiting. These effects are illustrated in the Figure 6-8.

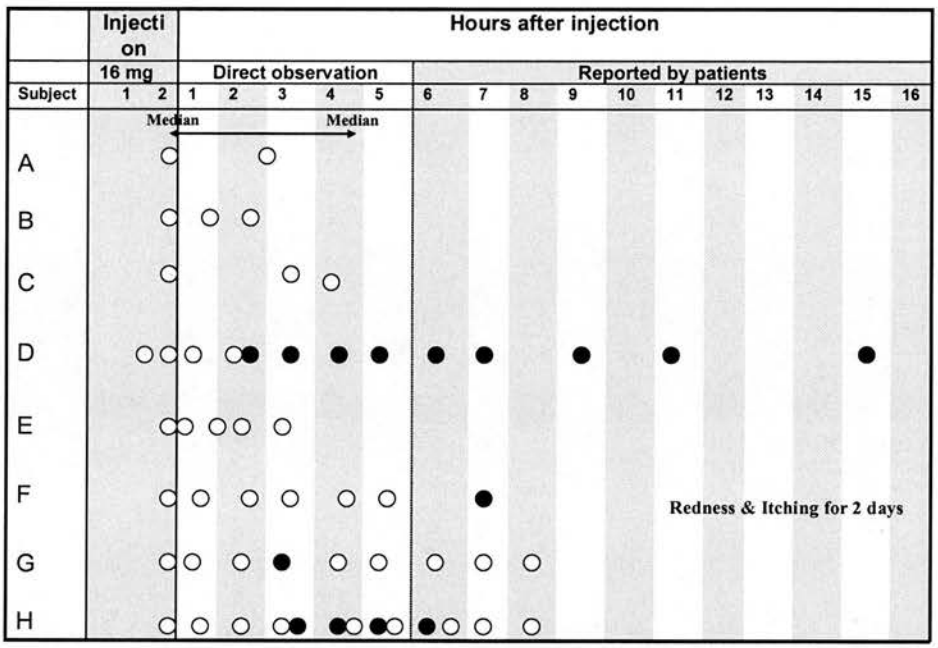


Figure 6- 7. Morphine-induced side effects, Light headedness (o), vomiting (●) (n=8)

6.4. Discussion

This study has investigated a wide variety of haemodynamic indices, together with respiratory and CNS variables in subjects receiving morphine into a slightly higher than therapeutic range (total dose 16 mg). Overdose cannot be ethically tested in volunteers, but ethical approval for this supra-therapeutic dose of morphine was obtained.

The findings can be summarised as; SBP, sSBP, O_2 saturation, SI, EDI, PFI, HRB, IC, ER, ACI, ESP, ASBP, ADBP, MBP, PPP, APP, VET, SVR, and RR, O_2 Sat., ET CO_2 and RT were significantly changed by morphine. In general haemodynamic changes appeared to start after very low doses (0.25mg), but did not appear to be dose dependent. On the contrary respiratory and CNS effects were dose dependent. In these studies there was no evidence of an effect on histamine release catecholamine level in the circulation.

To interpret these findings, an integrated model of morphine-induced changes on veins-heart-arteries may need to be considered, but the effects of morphine described on each part separately.

6.4.1. Preload

In this study morphine did not change total bioempedance of the thorax (thoracic fluid index), which probably suggests that it has no effect on preload at these doses.

6.4.2. Heart

Cardiac rate; Heart rate fell in both placebo and morphine groups, but morphine treatment was associated with higher heart rate in comparison to the saline visit. Morphine appeared to be is positively chronotropic. This is consistent with previous finding that morphine is positively chronotropic (Mildh *et al.*, 2000), but since there was a fall in blood pressure this effect might also reflect autoregulation.

Cardiac work; in this study morphine appeared to be negatively inotropic, which is indicated by a significant decrease in index of contractibility and peak flow index. The later indicates a decrease in the highest rate of left ventricular volumetric delivery during the ejection phase. As the patients were normovolaemic these changes are likely to be a true effect. Negative inotropism of morphine was also confirmed by a significant decrease in acceleration index, which is much less dependent on preload and afterload (Anonymous2005; Bernstein, 1986a; Scherhag *et al.*, 1999).

Morphine also significantly decreased end diastolic index (cardiac filling in diastole). Morphine induced a significant decrease in end systolic pressure (cardiac emptying in systole).

Stroke index in this study significantly decreased, which is probably a result of a negative inotropic effect of morphine. Other reasons for a decrease in stroke index including decrease in preload, and ejection fraction have been ruled out in this study. In summary morphine decreased cardiac work.

Morphine-induced changes in stroke index and heart rate together resulted to a stable cardiac index (CI=HR×SI), which indicates maintenance of left ventricular performance and overall perfusion. Significant increase in ventricular ejection time and ejection ratio, with no change in systolic time ratio and ejection fraction, is also consistent with this hypothesis. Thus morphine did not appear to impare left ventricular performance.

6.4.3. Afterload

Morphine significantly decreased systolic blood pressure in supine and 90 degrees sitting positions, and mean blood pressure and pulse pressure. These changes were also mirrored in aortic systolic and diastolic blood pressure, and pulse pressure. Diastolic blood pressure in supine and sitting positions, however, were not changed. These findings suggest morphine decreased afterload.

Overall, morphine did not change preload, is probably positively chronotropic, negatively inotropic, and decreased afterload. Morphine reduces heart work, but did not impair the adequacy of the cardiac performance. The effects of morphine on the cardiovascular system in this study were surprisingly not dose dependent.

6.4.4. Non-haemodynamic variables

Morphine significantly decreased respiratory rate, and as a result increased end tidal Co_2 and reduced O_2 saturation. Reaction time surprisingly initially appeared to decrease suggesting an initial improvement effect of morphine on central nervous system.

However, scrutiny of the results in separate time points revealed that reaction time has decreased with 0.250 (-3.89%), 0.500 (-6.37%), 1 (-3.86%), 2 (-4.23%) mg infusions, but increased with 4 (0.36%) and 8 (3.34%) mg. This suggests that as expected morphine induces CNS depression in higher doses. Improved CNS function in lower doses remains unexplained. The well recognised respiratory and also CNS effects of morphine were dose dependent.

6.4.5. Potential pathways

The fall in SBP is not caused by a decrease in stiffness of large arteries or peripheral pulse wave reflection, as augmentation index did not altered.

Morphine also significantly decreased systemic vascular resistance. Assuming that viscosity of blood was stable in this study, this is probably mediated via morphine induced vaso (arterio) dilatation. The increase in the radius of arteries should be profound, as despite a minor increase in AI which potentially may increase afterload, aortic variables significantly decreased.

Velocity was not measured in this study, but based on Bernoulli's equation, ($Q = A_1 \times V_1 = A_2 \times V_2$, where A is cross-section of the vessels, v is velocity, and Q is volume of

liquid entering per unit time) (Nichols & O'Rourke, 1998), as the radius of arteries were increased the velocity should have decreased.

Increased CO_2 and decreased O_2 may play some contribution to the cardiovascular effects of morphine, as CO_2 is a vasodilator (Van den bos *et al.*, 1979; Ganong, 2001). Opioids have been shown to induce histamine release or increase plasma catecholamine concentrations, which may affect haemodynamics (Flacke *et al.*, 1987; Fahmy *et al.*, 1983; Doenicke *et al.*, 1995).

Histamine, adrenaline, and noradrenaline concentrations at the sensitivity of the techniques, and power of the study were not significantly different, which imply that observed effects were independent of these neurotransmitters in the doses of morphine used in this study. As plasma level of these mediators were at the limit of the sensitivity of the assay, it remains possible that an effect of low mediator concentration could be a factor.

Vomiting happened with a three hours delay in comparison with other changes, suggesting that it might have been induced by an active metabolite rather than the parent drug. Alternatively, a late redistribution of morphine in the medulla cannot be ruled out.

6.5. Implication

As patients live longer, the prevalence of congestive heart failure is increasing. Currently vasodilators and diuretics are used in inpatient treatment to reduce mainly

afterload, and preload to a lesser extend. Inotropic support is also needed in some (Moazemi *et al.*, 2003; Mosesso *et al.*, 2003).

Acute left ventricular dysfunction in myocardial infarction should be treated to maximise preload (Bates, 1992), and decrease heart work and oxygen consumption.
Based on these findings, morphine appears to have specific cardiovascular effects and its indications in these situations should not be restricted to its current analgesic and relaxant effects which previously stated (Topol, 1998), but also a decrease on cardiac work and oxygen consumption, probably via negative inotropism and decrease in afterload.

If tolerance to these effects does not develop, these finding might support an argument for a heart-arteriolar indication of morphine in congestive heart failure and MI as well as its current painkiller and relaxant indication. Also end stage patients with chronic pain that are receiving long term morphine, might also develop hypotension from morphine.

6.6. Conclusion

Morphine causes a significant reduction in afterload and left ventricular cardiac work, with maintained cardiac performance. Morphine had little or no effect on preload in doses less than 16 mg. The cardiac effects were not dose dependent in this study.
Morphine induces a dose dependent increase in end tidal CO_2, and a decrease in end tidal O_2 saturation. Morphine had no effect on plasma concentration of histamine and catecholamines.

Arterial stiffness, plasma concentration of histamine and catecholamines appeared to play no role in the haemodynamic effects of morphine with this experiment protocol. An increase in end tidal CO_2 might be responsible in part in these effects.

Based on these studies, morphine induced haemodynamic effects are caused by arteriolar vasodilatation. This suggests that morphine, either via a receptor on endothelium or arteriolar smooth muscles, or peri-arteriolar tissues, increases the radius of the arteries. It can be postulated that this is an opioid receptor; however, a morphine induced non-opioid pathway cannot be excluded.

Chapter VII, A randomised control trial of the effect of naloxone on the effect of intra-venous morphine

7.1. Introductory remarks

In chapter V and VI, I have explored the haemodynamic effects of morphine and other opioids in overdose and healthy volunteers. In this chapter I conducted studies to clarify the role of opioid receptors in the haemodynamic effects of morphine in a single blind, prospective, two way crossover clinical trial. In this study the haemodynamic effects of morphine and placebo (saline) were compared with morphine and the opioid antagonist, naloxone, in healthy male volunteers.

7.1.1. Outline of morphine antagonism

A semi-synthetic derivative of thebaine, naloxone (C19H21NO4.HCl 4, 5-Epoxy-3,14-dihydroxy-17-(2-propenyl)morphinan-6-one hydrochloride) is a pure opioid antagonist. Its free base molecular weight is 399.9. It can cross the blood brain barrier. Naloxone is a competitive antagonist particularly at μ and κ receptors (Zhu *et al.*, 1995; Gutstein & Akil, 2001; Kienbaum *et al.*, 2002a). Naloxone is more potent as an antagonist at μ - receptors than at others.

In therapeutic doses naloxone has no direct effect on healthy volunteers, and all its effects are thought to be related to antagonism of endogenous or exogenous opioids. Haemodynamics of naive subjects, respiratory rate, oral temperature and plasma catecholamines are not affected by naloxone (Fuenmayor & Cubeddu, 1986). In isolated rat hearts, however, naloxone antagonised the hypotensive effect induced by morphine on the coronary pressure, but was ineffective in counteracting the negative inotropic and chronotropic effects (Ventura *et al.*, 1987). Haemodynamic changes, however, have been shown in acute naloxone detoxification of addicted patients (Kienbaum *et al.*, 2002a), with increase in blood pressure and pulse rate.

The aims of this study were to determine if naloxone had any significant effect on the morphine-induced haemodynamic, respiratory and CNS effects, and if histamine, adrenaline and noradrenaline are involved in these effects.

7.1.2. Hypotheses

1. Naloxone can reverse morphine-induced haemodynamic, respiratory and CNS effects.

2. Histamine, adrenaline and noradrenaline are the mediators by which naloxone antagonises these effects.

7.2. Materials and methods

Study design, inclusion and exclusion criteria, drug preparation, blood sampling, safety measurements, ethical approval, and the techniques that were used are identical to chapter VI (see also chapter II and the Table of Glossary for the details).

All subjects received morphine as described in chapter VI, a stepwise infusion with measurement after each dose. Naloxone was injected as an initial bolus of 400 microgram intravenously (1 ml), and by continuous infusion throughout the study at a rate of 200 micrograms per hour in 60 ml saline.

Saline was injected as a bolus of 1 ml followed by a continuous infusion at a rate of 60 ml per hour throughout the study for the other visit as placebo control. The order was randomized. Analysis was as described in chapter VI, with the groups being compared as morphine alone and morphine plus naloxone.

7.3. Results

Demographic variables (age, height, weight, and body surface area (BSA)) of 8 volunteers are summarized in Table 7-2

Variable	Min	Max	Mean	SD
Age (year)	23	50	38.9	10.8
Height (cm)	161	185	171.3	8.0
Weight (kg)	60	96	74.5	13.5
Body Surface Area (m^2)	22.2	30.0	25.2	2.7

Table 7- 1. Descriptive Statistics of volunteers, BSA; is calculated from the square root of ([Height (cm) x Weight (kg)]/ 3600), Mosteller formula (Mosteller, 1987).

The difference between morphine-saline and morphine-naloxone visits for each haemodynamic, respiratory and central nervous system variable was compared by using ANOVA (Table 7-2). When ANOVA was significant, mean and SEM of all variables at each time point for both visits was shown in Table 7-3 to emphasize the direction of the effects.

7.3.1. Cardiovascular effects

Overall morphine-induced decreases in supine DBP (F=4.728, P= 0.032), ESP (F=10.365, P=0.002), SI (F=5.978, P=0.016), and CI (F=10.038, P=0.002) were significantly reversed by naloxone Figure 7-1-B and Table 7-3. Mean (SEM) value at each time points are shown in Table 7-2.

Naloxone significantly decreased sSBP (F=4.366, P=0.039), as compared to the morphine alone. Naloxone significantly intensified morphine-induced decrease in supine HR (F=17.443, P<0.001) Figure 7-1-D and Table 7-3. Mean (SEM) value at each time points are shown in Table 7-2.

Supine systolic blood pressure fell in both morphine-saline and morphine-naloxone groups Figure 7-1-A (mean ± SEM). However, the difference between these two groups was not significant (F=0.027, P=0.869) Figure 7-1 and Table 7-3.

Morphine-saline and morphine-naloxone groups were not significantly different for mean blood pressure (F=2.386, P=0.125), sitting diastolic blood pressure (F=0.005, P=0.946) and pulse pressure (F=3.008, P=0.086) Table 7-3.

Variables	F	P value
Haemodynamic variables		
Systolic blood pressure (%)	.027	.869
Diastolic blood pressure (%)	4.728	.032
Mean blood pressure (%)	2.386	.125
Peripheral pulse pressure (%)	3.008	.086
Sitting systolic blood pressure (%)	4.366	.039
Sitting diastolic blood pressure (%)	.005	.946
End systolic pressure (%)	10.365	.002
Heart rate (%)	17.443	< 0.001
Augmentation index (%)	.013	.910
Stroke index (%)	5.978	.016
Cardiac index (%)	10.038	.002
Respiratory and CNS variables		
Respiratory rate (%)	17.295	< 0.001
O_2 Saturation (%)	1.425	.235
End tidal carbon dioxide (%)	.124	.726
Reaction time (%)	7.800	.006
Plasma assays		
Plasma histamine concentration	.016	0.901
Plasma adrenaline concentration	.062	.806
Plasma noradrenalin concentration	.792	.377
Plasma morphine concentration	.343	.561

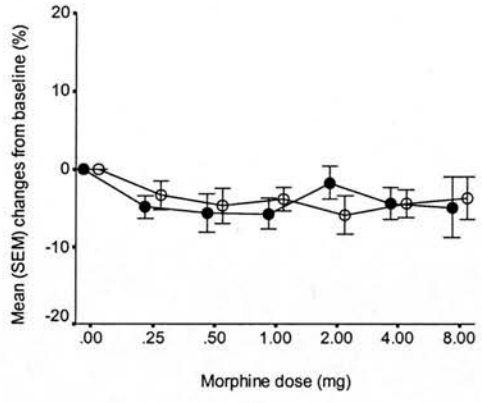
Table 7- 2. Analysis of variance of percentages of change from baseline between morphine and naloxone groups for homodynamic, respiratory and central nervous system variables

7.3.2. Respiratory variables

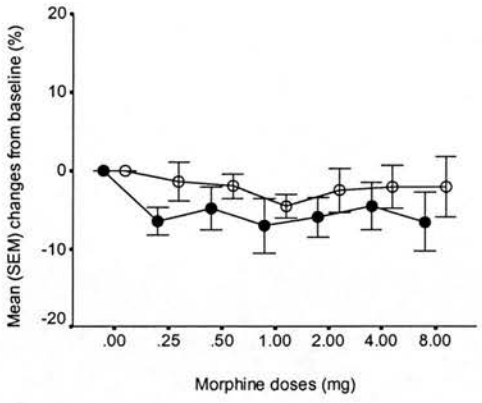
Overall morphine-induced decrease in supine respiratory rate was significantly reversed by naloxone (F=17.295, P<0.001) Figure 7-2-A and Table 7-3. Mean (SEM) values at each time point is shown in Table 7-2. Naloxone, however, failed to influence morphine's effect on oxygen saturation (F=1.425, P=0.235) or end tidal CO_2 (F=0.124, P=0.726) (Figure 7-2-B & C and Tables 7-3 and 7-4).

Dose	Baseline		0.25 mg		0.5 mg		1mg		2mg		4 mg		8 mg	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Morphine-Saline visit														
SBP*	132	4.2	126	4.0	125	3.6	125	4.0	130	5.1	127	4.9	121	6.4
DBP	77	3.9	71	2.9	72	2.6	71	3.0	72	3.9	73	4.0	70	4.7
MBP	95	3.4	89	2.8	90	2.2	89	2.7	91	3.6	91	3.7	87	5.0
PP	56	4.7	55	3.7	52	4.2	54	4.1	58	5.2	54	4.6	51	4.3
SSBP	127	4.0	127	5.2	127	5.6	126	5.2	125	6.7	126	7.5	115	9.8
SDBP	75	3.0	75	3.4	76	3.4	75	4.1	70	4.5	74	4.7	72	6.6
ESP	106	5.6	98	5.4	98	3.8	97	4.1	98	6.2	100	6.4	94	7.3
HR	62	2.7	60	2.5	59	2.2	57	1.9	59	1.8	57	2.0	57.6	1.7
AI	6.3	7.3	12.4	5.8	10.6	6.6	11.8	6.4	8.3	6.8	12.5	7.5	11.3	8.1
SI	57.9	5.9	60.0	4.8	55.4	4.7	58.0	4.3	55.5	3.8	55.5	4.2	56.7	5.4
CI	3.7	0.4	3.6	0.3	3.3	0.3	3.5	0.3	3.4	0.3	3.3	0.3	3.5	0.4
RT	0.86	0.05	0.83	0.04	0.85	0.06	0.86	0.05	0.86	0.05	0.87	0.06	0.88	0.06
RR	18	0.4	17	0.8	16	0.6	15	0.4	16	1.1	13	0.7	12	0.7
O2	98	0.5	98	0.4	98	0.5	98	0.5	98	0.4	98	0.5	98	0.6
CO2	5.3	0.1	5.4	0.1	5.4	0.1	5.4	0.2	5.5	0.1	5.5	0.2	6.0	0.2
Morphine-Naloxone visit **400 microgram Naloxone stat before baseline followed by 200 microgram per hour**														
SBP	129	4.7	125	5.3	123	3.6	124	4.3	122	5.0	123	4.4	124	6.0
DBP	71	3.8	70	3.7	70	3.5	68	3.9	72	5.4	70	3.7	70	5.3
MBP	91	3.4	88	3.8	87	3.1	87	3.2	88	4.9	88	3.6	88	5.1
PPP	58	4.9	55	4.3	53	3.5	56	5.2	50	4.3	53	3.6	54	4.4
SSBP	131	5.4	124	6.2	126	5.8	119	4.9	122	5.9	126	5.3	126	6.3
SDBP	76	4.2	75	4.7	73	4.5	76	3.3	74	5.2	74	5.3	77	5.2
ESP	97	6.1	94	6.2	94	5.6	94	5.5	96	6.4	97	5.3	97	6.8
HR	62	1.9	57	1.8	57	2.2	54	1.7	55	1.9	53	1.9	56	1.8
AI	9.5	7.0	8.9	8.1	10.5	6.6	11.0	6.3	14.7	6.6	12.4	6.8	11.9	7.0
SI	63.9	4.0	60.0	4.5	61.5	4.5	61.3	4.4	59.1	5.0	59.5	4.4	58.8	4.9
CI	3.9	0.3	3.6	0.3	3.5	0.3	3.5	0.3	3.3	0.3	3.2	0.3	3.3	0.3
RT	0.86	0.05	0.87	0.06	0.83	0.07	0.80	0.07	0.81	0.06	0.82	0.06	0.79	0.06
RR	16	0.5	16	0.6	16	0.9	15	0.8	16	0.7	15	0.7	15	0.7
O2	98	0.5	97	0.5	98	0.4	98	0.4	98	0.4	98	0.6	98	0.5
CO2	5.4	0.1	5.4	0.1	5.6	0.1	5.6	0.1	5.5	0.1	5.5	0.1	5.6	0.1

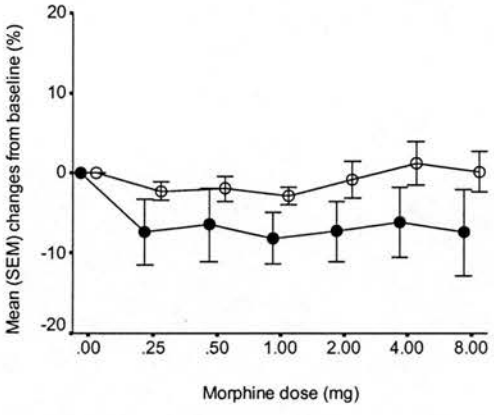
Table 7- 3. Mean and SEM of haemodynamic, respiratory and central nervous system variables in healthy volunteers who received incremental doses of morphine with saline or naloxone, Haemodynamic variables; AI; augmentation index, CI; cardiac index, DBP; diastolic blood pressure, ESP; end systolic pressure, HR; heart rate, MBP; mean blood pressure, PPP; peripheral pulse pressure, SI; stroke index, sSBP; sitting systolic blood pressure, sDBP; sitting diastolic blood pressure, SBP; systolic blood pressure,
Respiratory variables; $ETCO_2$; end tidal CO_2, O_2 Sat.; oxygen saturation, RR; respiratory rate, CNS variable; RT; reaction time (n=8).



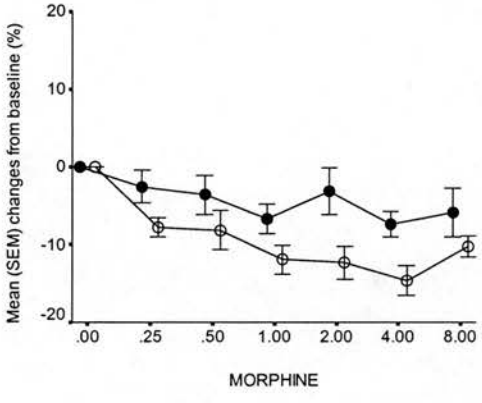
A) Supine systolic blood pressure



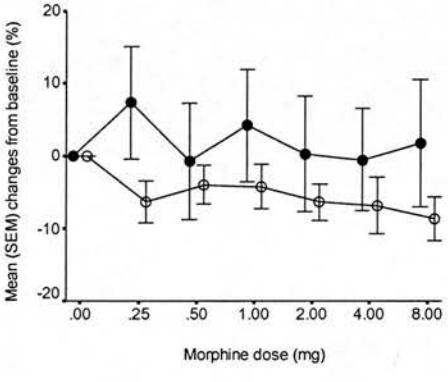
B) Supine diastolic blood pressure



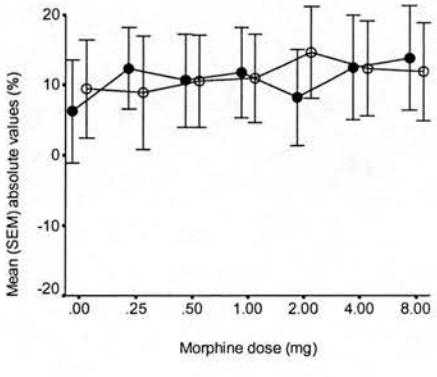
C) Supine end systolic pressure



D) Supine heart rate



E) Stroke index

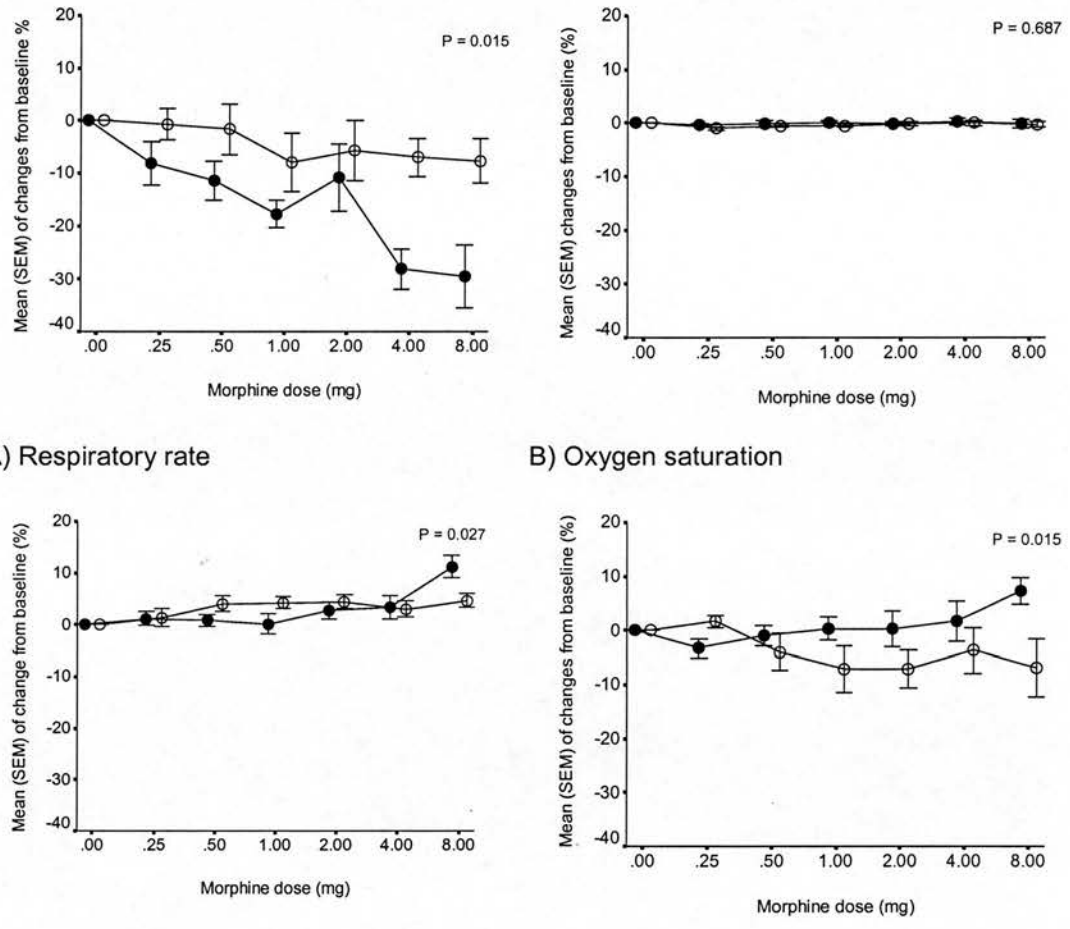


F) Augmentation index

Figure 7- 1. Mean (SEM) of percentage of change from baseline of A) systolic blood pressure, B) diastolic blood pressure, C) end systolic pressure, D) heart rate, E) stroke index and F) Augmentation index in morphine-saline group (–●–) and morphine-naloxone group (–○–) (n=8).

7.3.3. Reaction time (RT)

Overall morphine-induced change in reaction time was significantly decreased by naloxone (F=7.800, P=0.006) (Figure 7-2-D and Tables 7-2 and 7-3). At the highest dose of morphine, the pair wise difference between two groups was also significant (p=0.015).



A) Respiratory rate

B) Oxygen saturation

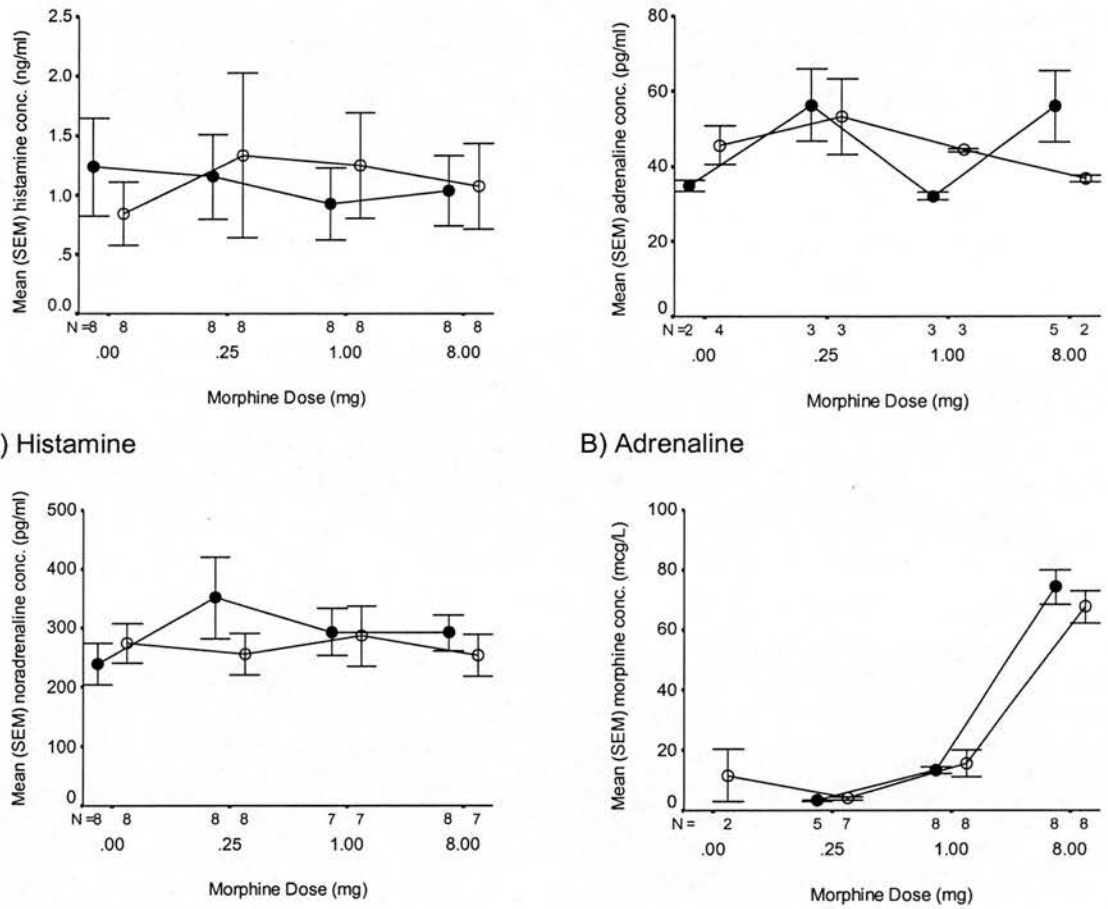
C) End tidal CO_2

D) Reaction time

Figure 7- 2. Mean (SEM) of percentage of change from baseline of A) respiratory rate, B) oxygen saturation, C) end tidal CO_2, D) reaction time, in morphine-saline group (–●–) and morphine-naloxone group (–○–) (n=8).

7.3.4. Plasma assays

Concentration (mean (SEM)) of plasma histamine (ng/ml), adrenaline (pg/ml), noradrenalin (pg/ml) and morphine (mcg/ml) at baseline, and after 0.25, 1 and 8 mg morphine are shown in Figure 7-3.



A) Histamine

B) Adrenaline

C) Noradrenaline

D) Morphine

Figure 7- 3. Mean (SEM) of absolute values of plasma concentrations of A) histamine in ng/ml, B) adrenaline in pg/ml, C) noradrenalin in pg/ml, D) morphine mcg/ml in morphine group (—●—) and placebo group (···○···) (n=8). Techniques, which have been used, were not sensitive for values less than 30 pg/ml, 150 pg/ml and 2.5 µg/ml for adrenaline, noradrenalin and morphine levels respectively. These values were omitted, and the actual N in reported below the horizontal axis for each time point for morphine and saline visits respectively.

Overall, morphine-saline and morphine-naloxone groups were not significantly different for plasma assays of histamine (F=0.16, P=0.901), adrenaline (F=0.062, P=0.806) and

noradrenalin (F=0.792, P=0.377) Table 7-2. Morphine plasma concentrations are shown in Figure 7-3-D. It seems that naloxone has no effect on pharmacokinetics of morphine.

7.3.5. Association of haemodynamic variables with central nervous system and respiratory variables

	RT%	P	RR%	P	O_2%	P	CO_2%	P
Morphine-Saline visit								
SBP* **	.105	.441	.065	.636	.127	.350	.106	.436
DBP	-.231	.087	.096	.484	.145	.287	.161	.235
MBP	-.091	.503	.094	.490	.163	.229	.159	.241
PP	.226	.093	-.008	.952	-.035	.796	-.058	.670
sSBP	.002	.989	.202	.138	-.052	.705	-.245	.072
sDBP	-.164	.232	.249	.067	-.085	.536	-.340*	.011
ESP	-.191	.159	.240	.075	-.132	.331	-.105	.441
HR	-.057	.677	.214	.113	-.025	.854	-.206	.128
SI	-.258	.055	-.284*	.034	-.510*	.000	.240	.075
CI	-.205	.130	-.189	.163	-.364*	.006	.196	.147
AI	.014	.917	-.044	.748	-.088	.517	-.047	.732
RT	1	.	-.053	.701	.404*	.002	-.041	.763
RR	-.053	.701	1	.	.282*	.035	-.390*	.003
O_2	.404*	.002	.282*	.035	1	.	-.164	.228
CO_2	-.041	.763	-.390*	.003	-.164	.228	1	.003
Morphine-Naloxone visit								
SBP	.462*	.000	.547*	.000	.260	.053	-.240	.075
DBP	.291*	.029	.039	.777	.044	.750	-.044	.747
MBP	.431*	.001	.317*	.017	.167	.217	-.171	.207
PP	.269*	.045	.500*	.000	.210	.120	-.158	.244
sSBP	.115	.401	-.133	.328	.088	.517	.351*	.008
sDBP	.172	.204	-.015	.911	.050	.716	-.092	.501
ESP	.263	.051	-.175	.196	.095	.485	.445*	.001
HR	.016	.905	-.033	.811	-.182	.179	-.405*	.002
SI	-.424*	.001	.279*	.037	.120	.379	-.459*	.000
CI	-.254	.058	.208	.124	-.040	.772	-.547*	.000
AI	.246	.070	.032	.816	-.125	.364	-.178	.193
RT	1	.	.164	.226	.193	.154	-.013	.925
RR	.164	.226	1	.	.339*	.011	-.367*	.005
O_2	.193	.154	.339*	.011	1	.	.015	.913
CO_2	-.013	.925	-.367*	.005	.015	.913	1	.

Table 7- 4. Association of percentage of change from baseline of haemodynamic variables with central nervous system and respiratory variables (df=55), *; correlation is significant at the 0.05 level (2-tailed), **; correlation is significant at the 0.01 level (2-tailed),
Haemodynamic variables; CI; cardiac index, DBP; diastolic blood pressure, ESP; end systolic pressure, HR; heart rate, MBP; mean blood pressure, PPP; peripheral pulse pressure, SI; stroke index, sSBP; sitting systolic blood pressure, sDBP; sitting diastolic blood pressure, SBP; systolic blood pressure, Respiratory variables; $ETCO_2$; end tidal CO_2, O_2 Sat.; oxygen saturation, RR; respiratory rate, CNS variable; RT; reaction time (n=8).

Results of correlation of haemodynamic variables with non-haemodynamic variables are shown in Table 7-4. As can be seen, haemodynamic variables are in general not correlated with central nervous system and respiratory variables. This suggests lack of a common mechanism of action for these different effects. the The correlatation of reaction time and SBP, DBP, MBP, and PP following naloxone and morphine was, surprisingly, stronger than for morphine alone. Among non haemodynamic variables, respiratory rate was, as expected, associated with both O_2 and CO_2 measurements.

7.4. Discussion

Chapter 6 suggests that morphine in incremental IV doses of 0.250 to 8 mg tends to depress both haemodynamic and respiratory systems in man. Afterload, (systolic blood pressure, pulse pressure, systemic vascular resistance) and cardiac work (index of contractibility and stroke index) were decreased but left ventricular performance maintained (cardiac index).

In this study naloxone did not antagonise morphine-induced changes in SBP, MBP, PP or sDBP. Morphine induced depressor effects on DBP, ESP, SI and CI, however, were revered by naloxone. SSBP and HR were also significantly different between treatments, but it seemed that naloxone increased the effect of morphine rather than reversing it. Thus naloxone at these doses reversed some of the morphine-induced effects on afterload and cardiac variables.

DBP is largely determined by peripheral arterial resistance, in contrast, SBP and PP are influenced more by the stiffness of large arteries, as well as peripheral pulse wave

reflection and the pattern of left ventricular ejection (Oliver & Webb, 2003). As the determinants of these variables are different, and naloxone can antagonise some but not the others, it can be postulated that haemodynamic effects of morphine might be mediated via more than one pathway. Based on this study, haemodynamic variables which are more related to afterload (SBP, MBP, and PP) may be opioid receptor independent. Heart related variables such as stroke and cardiac indices, however, appeared to be opioid receptor related. As majority of measured variables were indirect or derived, the detailed relationships between them were not scrutinised.

Naloxone's failure to reverse some of the haemodynamic variables might also be also due to the fact that the power of this study was not enough to detect some effects as statistically significant, or that its dose was not adequate. Potential reasons for this discrepancy in result are shown in Figure 7-4. Details of the potential mechanism s are discussed in chapter 1.

This study indicates that changes in reaction time and respiratory rate are reversible by naloxone and therefore mu receptor mediated. These effects were not time-linked to haemodynamic effects of morphine. This suggests that it is less likely that a common pathway causes CNS, respiratory, and cardiovascular effects. The effects on the cardiovascular system, therefore, are compatible with a peripheral cardiovascular site of action reversed by naloxone. This might be consistent with the work of Stefano et al who have reported opioid effects on the cardiovascular system *in vitro*, and in animal

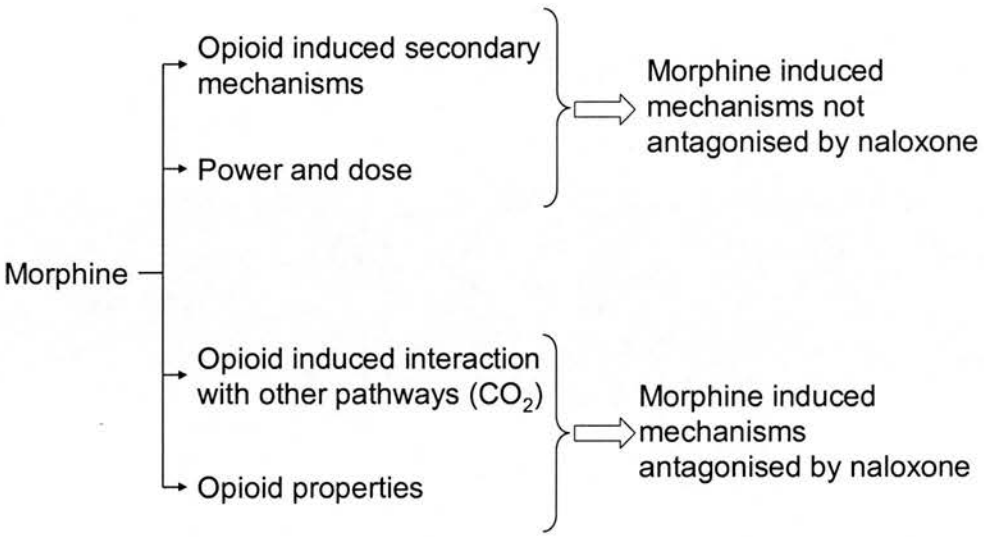


Figure 7- 4. Morphine induced opioid receptor dependent and independent haemodynamic effects

studies in relation to a probable μ_3 receptor and nitric oxide pathway (Bilfinger *et al.*, 1998b; Bilfinger *et al.*, 2002; Cadet *et al.*, 2000; Fimiani *et al.*, 1999b; Fimiani *et al.*, 1999a; Magazine *et al.*, 1996; Solenkova *et al.*, 2002; Stefano *et al.*, 1995a; Stefano *et al.*, 2000; Stefano, 1998; Stefano *et al.*, 1998; Stefano *et al.*, 2002). As discussed in the introduction of this thesis, these effects are likely to be due to opioid induced secondary mechanisms such as histamine release. Stefano et al also showed that morphine's cardiovascular effects are not shared by fentanyl (Bilfinger *et al.*, 1998a; Stefano, 2002). There is some evidence that fentanyl, unlike morphine, did not release histamine and thus has less cardiovascular effects (Cathelin *et al.*, 1980a; Philbin *et al.*, 1981; Rosow *et al.*, 1982; Flacke *et al.*, 1987; Gutstein & Akil, 2001). In the present study, histamine concentration did not increase, and this may explain differences in results from different studies.

In this study naloxone significantly intensified the effects of morphine on heart rate. The interpretation of this finding is difficult, since heart rate will rise if blood pressure falls, and subjects may feel relaxed under the assumption that they are receiving morphine, and as the study goes on. The fact that adrenaline and noradrenaline did not change adds to the difficulties in interpreting this finding.

Overall, haemodynamic effects of morphine (up to 16 mg in 2 hours intravenously) appear, at least in part, not mediated via naloxone sensitive receptors. In addition, systemic histamine and catecholamine release probably play no role in the effects at these doses of morphine.

The presence of a potential concurrent involvement of central nervous system or respiratory mediated effects on cardiovascular responses related effects to morphine induced were examined in both this chapter and chapter VI, but the magnitude of their contribution could not be determined in these experiments. Based on these studies the presence of a peripheral site of action for morphine can be postulated but not confirmed. Further studies such as a forearm blood flow study are needed to explore these effects and the mechanisms of action.

7.5. Limitation

The effect of naloxone in the absence of morphine was not studied, although other workers suggest that it has no haemodynamic effects (Fuenmayor & Cubeddu, 1986). The doses of morphine used were relatively low, in comparison to the many published studies in anesthetic patients. The study was not placebo controlled, and this make the conclusion less clear cut. The design was similar to that in the study described in

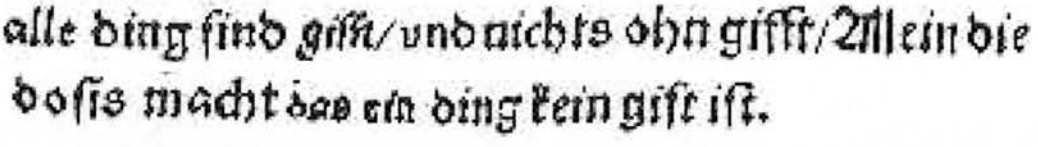
chapter VI. The effects of morphine in this study appeared to be in general similar to those seen in the subjects of that experiment.

7.6. Conclusion

In conclusion, at the doses studied naloxone did not antagonize some of haemodynamic caused by morphine demonstrated in Chapter VI, specifically those on SBP, MBP and PP. This suggests the presence of receptor pathways in man which are affected by morphine but not antagonized by naloxone. These may be non-opioid effects. Naloxone, however, was able to antagonize some haemodynamic changes induced by morphine. This supports the presence of an opioid receptor pathway for some of the effects.

Histamine and catecholamine release, oxygen saturation, and effects on end tidal CO_2 seemed to play little role in the naloxone-morphine interaction. Both morphine and naloxone have no effect on arterial stiffness.

Chapter VIII, Effects of intra-arterial morphine



Everything is a poison, and nothing is not-poisonous, this is just the dose which makes a poison

Philip Theophrastus Bombast von Hohenheim (Paracelsus) 1564 A.D.

Courtesy of the National Library of Medicine, National Institutes of Health, Bethesda, which gave their permission to reproduce a copy of the original print of Paracelsus metaphor in this thesis from Paracelsus' Drey Bucher printed in Cologne by the Heirs of Arnold Byrckmann in 1564.

8.1. Introductory remarks

8.1.1. The maintenance of vascular tone

Many physiological mechanisms, including cardiac output and peripheral vascular resistance contribute to blood pressure control. Peripheral vascular resistance and blood pressure are measurable indicators of vascular tone. Vasodilatation decreases peripheral resistance, as the radius of the lumen is the most important factor in resistance (Nichols & O'Rourke, 1998). Systems effecting basal vascular tone in the human forearm include the sympathetic nervous system, renin-angiotensin system, L-arginine nitric oxide pathway, and endothelin system (Wilkinson & Webb, 2001).

8.1.2. Mast cell degranulation and anaphylactoid reactions

Anaphylactoid (anaphylaxis-like) reactions should be distinguished from anaphylaxis, as they are not mediated by IgE antibodies. Similar pharmacologic mediators, including histamine, are responsible for the clinical features of both of them; however, the stimuli for their release differ. In anaphylactoid reactions substances act directly on mast cells or the alternative pathway of complement activation. This is not immunologically specific and does not need to have been previously sensitised (Chapel *et al.*, 1999). Urticaria (oedema in the superficial portion of the dermis) may be related to IgE or IgE-receptor dependent reactions, complement system activation of cellular arachidonic acid, be idiopathic and also occur after direct mast cell degranulation. The common causes of acute urticaria are upper respiratory tract infections and drugs, it is often idiopathic. Urticaria is also associated with dilation of the venules (Soter, 1999). Mast cells are probably the major effector cell in most forms of urticaria. They reside in tissue adjacent to blood vessels Surrounding supporting tissues (Steven & Lowe, 2005). Skin

mast cells contain secretary granules of tryptase, chymase, carboxypeptidase and cathespin G (Soter, 1999; Abbas & Lichtman, 2004).

Various therapeutic and diagnostic agents have been associated with urticaria, including opiates, which directly release histamine from mast cells and basophils. However, the molecular mechanism is unknown (Soter, 1999).

Mast cell degradation releases a variety of mediators including histamine and tryptase. Histamine induces vasodilatation via action on H_2 receptors on the smooth muscle of the arteries. It also increases vascular permeability. Dermal injection of histamine induces urticaria (weal-and-erythema (flare) reaction), which develops in 1 to 2 minutes and reaches to maximum at 10 minutes (Davies *et al.*, 2001). As histamine has been found in the weal fluid, it probably induces it, but histamine was not found in the erythema suggesting a different pathway (Soter, 1999). This supports the theory that the histamine related flare reaction is a neurogenic reflex not involving histamine release at its effector site. The H_1 antagonist cetirizine blocks both weal and flare effects (Clough *et al.*, 1998). In man a particular class of neurons is selectively excited by iontophoretic histamine (Andrew & Craig, 2001). Vagus nerve stimulation reduces histamine induced itching in man (Kirchner *et al.*, 2002).

Tryptase is the most abundant mediator stored in the mast cell granules. Increased beta-tryptase levels are suggestive of an immunologically mediated reaction or may also occur following direct mast cell activation (Payne & Kam, 2004).

8.1.3. Permeability, extravasation and inflammation

Morphine has been shown to release histamine (Flacke *et al.*, 1987; Brown & Reberts, 2001). Histamine is one of the endogenous mediators of the acute inflammatory response, which causes immediate transient increase of vascular permeability. Extravasation of plasma increases colloid osmotic pressure and in turn increases the fluid loss from vessels. Any increase in hydrostatic pressure in arterial end capillaries will intensify this process (Underwood, 2004). Vascular permeability in skin is produced by the interactions of both H_1 and H_2 histamine receptors (Soter, 1999). It has also been shown that in mice, stimulation of mu opioid receptors results in an increase in BBB permeability (Baba *et al.*, 1988). During prolonged venous occlusion plethysmography there is a small but measurable continued increase in forearm volume, which is due to extravasation of fluid from the capillaries (Wilkinson & Webb, 2001).

Opiates including diamorphine and morphine cause non-cardiac pulmonary edema (NCPE) (Frishman *et al.*, 2003; Lusk & Maloley, 1988). NCPE is a common respiratory complication in opioid agonist addiction, heroin overdose and methadone causes death (Ben Noun, 2000; Sporer, 1999; Sporer & Dorn, 2001; Corkery *et al.*, 2004). Common features of NCPE includes damage to and increased permeability of vascular endothelium (Overland & Severinghaus, 1978). The mechanism, by which opioids particularly in overdose, cause non-cardiac pulmonary oedema, however, is not clear.

Heroin overdose has been shown to induce profound circulatory shock (Remskar *et al.*, 1998). Morphine exacerbates but naloxone prevents fatal histamine shock in mice. The mechanism appear to be histamine release (Amir, 1984). Opioids play a role in response to shock or stress (Smith & Lee, 1988). Morphine degranulate mast cells. They are the major effector of the immediate hypersensitivity (allergic) reactions (Abbas

& Lichtman, 2004). An extreme systemic form of immediate hypersensitivity results in anaphylaxis (anaphylactic shock).

8.1.4. Endothelium

The endothelium lines the entire circulatory system by a continuous, single cell layer. It is a selective permeability barrier, and source and target of biologically active agents (Gerritsen & Bloor, 1993; Gimbrone MA & Topper JN, 1999). Nitric oxide is released continuously by the endothelium to regulate basal vascular tone (Vallance *et al.*, 1989a; Haynes & Webb, 1998) and contributes in blood flow and blood pressure in normal subjects and cardiovascular diseases (Casino *et al.*, 1995; Luscher, 1992; Panza *et al.*, 1990). The effects of morphine on endothelial action and nitric oxide have not yet been studied in man.

The aims of the studies described in this Chapter are to describe the effects of intra arteriolar morphine in the forearm and investigate its mechanisms of action.

8.2. Exploratory dose ranging study

8.2.1. Planning the study protocols

Morphine has never been used intra-arteriolarly in man. The first step, therefore, was to define a dose at which morphine induces changes in vascular tone in man. The aim was to find a dose that was locally effective but with no systemic effects, and that did not induce significant adverse effects. The lowest effective dose and the shortest period of administration were sought for further studies. Thus, it was necessary to establish a dose response curve. Any adverse consequences of these injections were carefully monitored and used to inform future protocols.

8.2.2. Methods

In an observational clinical trial two healthy men without history of opioid abuse were recruited to the study, after obtaining the approval of the Research Ethics Committee and with the written informed consent of each subject. Subjects were asked to rest recumbent throughout each study in a quiet temperature-controlled room (23-25 °C). Strain gauges and arm cuffs were applied and a cannula sited in the brachial artery of the non-dominant arm. Blood pressure and heart rate were measured in the non-infused arm using a semi automated non-invasive method, Dinamap. Blood pressure was measured immediately after forearm blood flow to avoid any effect on these measurements from the venous congestion caused by this procedure. FBF was measured as described previously (Helmy *et al.*, 2003). Briefly, the response to intra-arterial infusion was assessed by measurement of forearm blood flow in both the infused and non-infused forearms by venous occlusion plethysmography using mercury-in-silastic strain gauges securely applied around the widest part of the forearm. The hands were placed above the level of the heart throughout the study period and

were excluded from the circulation during measurements through inflation of wrist cuffs to 220 mmHg. Upper arm cuffs were intermittently inflated to 40 mmHg for the first 10 s in every 15 s to temporarily prevent venous outflow from the forearm and thus obtain plethysmographic recordings. Recordings of forearm blood flow were made over 3 min periods at 10 min intervals. Venous occlusion plethysmography (dual channel strain gauge plethysmograph) was used and calibration was performed prior to the study. The infusion rates were kept constant at 1 ml/min for all dose levels. All dilutions were prepared in 0.9% saline from sterile stock solutions on the day of the study. In this study subjects received incremental doses every 6 minutes of 1, 3, 10, 30, 100, and 300 mcg/min/ml morphine sulphate with ten minutes wash out of saline applied between each dose at a rate 1 ml per min.

Blood pressure and heart rate were measured in the non-infused arm just after each FBF measurement to avoid any effect on measurements from the venous congestion caused by this procedure (Patterson *et al.*, 1954).

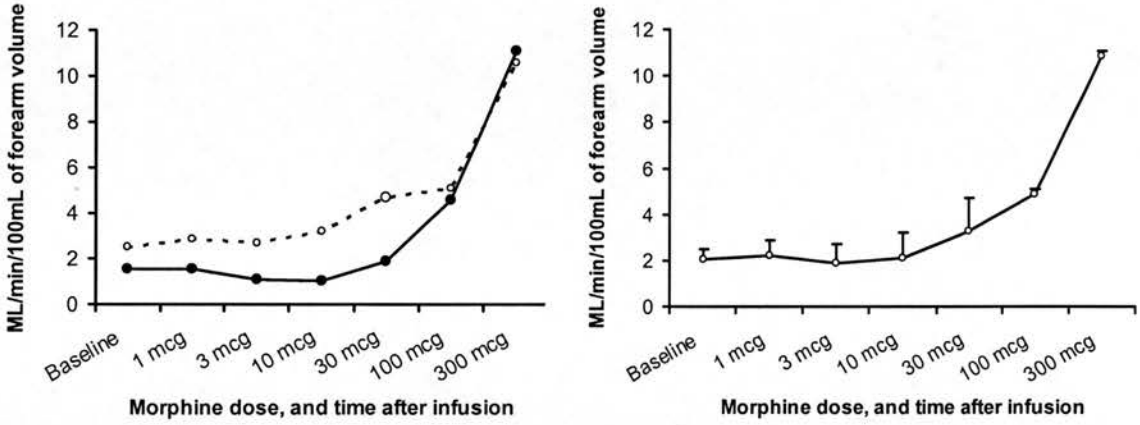
8.2.3. Results

Two male subjects were studied with no history of smoking, high BP, and hypercholesterolemia or positive result for drugs of abuse. Their age (mean (SD)) was 33.0 (14.1)y , BMI (mean (SD)) 26.2 (3.1)kg/m^2 and the ratio of flow in the Infused/non-infused arm at baseline 1.1 (0.2).

8.2.3.1. Forearm blood flow

Forearm blood flow in the infused arm for incremental doses of 1, 3, 10, 30, 100, 300 mcg/min/ml (6 min each) for two individual volunteers are shown in Figure 8-1 A), mean

values are in Fig 1 B. As can be seen, FBFs in the infused arm increased from 2.0 (0.5) ml/100 ml forearm at baseline, to 3.3 (1.4) at 30 mcg/ml/min morphine, 4.8 (0.3) at 100 mcg/ml/min morphine and 10.8 (0.2) at 300 mcg/ml/min morphine. Overall forearm blood flow increased from around 30 mcg/ml/min. Baseline measurements of forearm



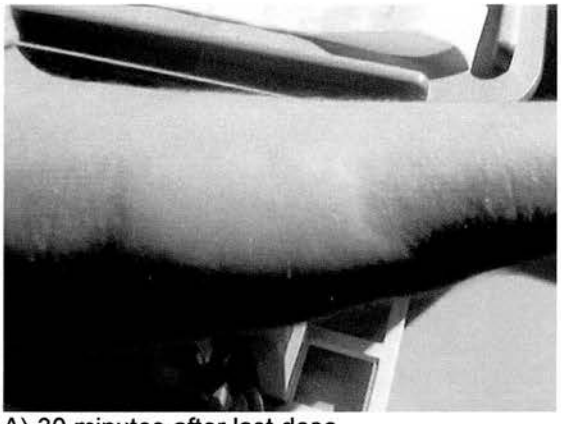
A) Individual results B) Mean (SEM)

Figure 8- 1. Individual results (A) and mean (SEM) (B) of forearm blood flow in two subjects that received incremental dose of 1, 3, 10, 30, 100, 300 mcg/min/ml (6 min each) in pre trial of dose ranging study.

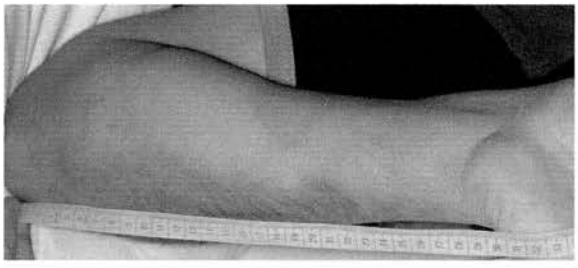
blood flow in the infused and non-infused arms were similar. There was no change in FBF of the non-infused arm. No further statistical analytical calculation has been made, as the sample was small.

8.2.3.2. Skin effects

Local skin effects were considerable in these subjects. These were local itching, which was severe. The intensity of itching was not quantified. Both cases developed local redness and weal, which in one case, was around 300 cm^2 Picture 8-1 (A & B). This giant weal developed quickly in a few minutes after starting the highest morphine dose. Pitting existed in the area of the weal.



A) 30 minutes after last dose



B) 30 minutes after last dose

Picture 8- 1. Local effects of IA morphine in one subject who received incremental doses of 1, 3, 10, 30, 100, 300 mcg/ml/min every 6 minutes, A) 30 minutes after the last dose, B) 60 minutes after the last dose.

8.2.3.3. Systemic effects

In total, the subjects received less than 3 mg morphine sulphate. They developed no systemic effects in terms of systolic and diastolic blood pressure, heart rate, generalised itching, dyspnoea, cough and CNS related symptoms and signs. This lack of systemic effects together with the fact that forearm blood flow was not changed in the non-infused arm confirms that any drug effects were confined to the infused arm.

8.2.4. Discussion

Mast cells reside in tissue adjacent to blood vessels (Steven & Lowe, 2005). They contain numerous pharmacologically active substances and express high affinity Fc receptors for IgE (Soter, 1999; Abbas & Lichtman, 2004).

This preliminary study shows that morphine sulphate is an arteriolar dilator agent, and at these doses induces massive weal, flare and itching. Figures 1 and 2 indicate that vasodilatation started at doses around 30 mcg/min/ml (concentration of 0.6 mcg/ml) and

increased up to 300 mcg/min/ml (concentration of 6 mcg/ml). This novel observation is dose dependent.

The protocol was revised and ethics approval obtained for continuing the study with a lower maximal dose following the observation of this large increase in forearm blood flow and the skin effects.

8.3. Dose response study

8.3.1. Methods

Venous occlusion plethysmography was used to evaluate intra-arteriolar morphine-induced effects as described in section 8.1. Six healthy men without history of opioid abuse were recruited to the study. Subjects received incremental doses at 6 minutes of 1, 3, 10, 30, and 100 mcg/min/ml morphine. The rate of infusion was constant at 1 ml per min. A measuring tape was used to estimate the area of the flare and weal just after each forearm blood flow measurement. Maximum length and breath were used to estimate the size of each lesion. To quantify the intensity of the itching, subjects were asked to express subjectively the intensity of itching from a range of scores from zero (no itching) to nine (irresistible itching). All skin related measurements were done every 6 minutes during baseline, morphine infusion, saline washout and up to 60 minutes after last dose.

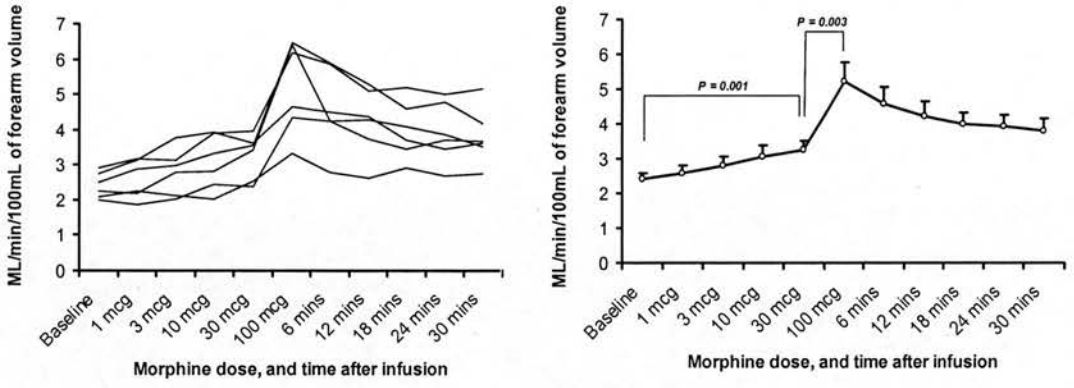
8.3.2. Results

Six male subjects were studied with no history of smoking, hypertension, and hypercholestrolaemia or positive result for drugs of abuse. Their age (mean (SEM)) was 31.2 (1.3) y, body mass index 25.8 (0.8) kg/m^2.

8.3.2.1. Forearm blood flow

Mean (SEM) FBF in infused arms are shown in Figure 8-2 (A & B). The ratio of infused/non-infused arm FBF at baseline was 1.0 (0.1). As can be seen, overall forearm blood flow increased gradually from baseline to 30 mcg/ml/min (P=0.001) and then increased sharply from 30 to 100 mcg/ml/min (P=0.003).

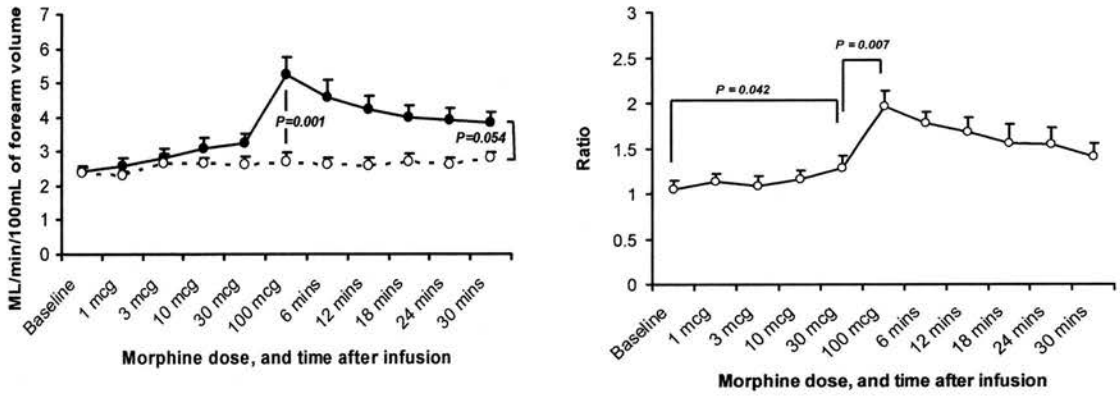
Baseline FBF measurements in the infused and non-infused arms were similar. There



A) Individual values B) Mean (SEM)

Figure 8- 2. Forearm blood flow in infused arm, A) individual results, B) mean (SEM) in six subjects that received incremental dose of 1, 3, 10, 30, 100 mcg/min/ml (6 min each) in modified dose ranging study.

were no changes in FBF of the non-infused arms. The baseline FBF was different among individual subjects. FBF increased with morphine in all six subjects, and the highest rate of FBF consistently achieved at 100 mcg/ml/min in all of them. The rate and magnitude of the peak of response, however, were not similar in different individuals Figure 8-2-A. During the washout period, FBF gradually decreased, and after 12 minutes the difference from the highest measurement was significant (P=0.028). Mean FBF did not return to baseline even after a 30 minute washout. Mean (SEM) of forearm blood flow in infused and non-infused arms and the ratio of forearm blood flow in infused over non-infused arms are shown in Figure 8-3. Incremental doses of IA morphine increased FBF significantly in the infused arm in comparison to the non-infused arm (P=0.001). This is also reflected in the ratio of flare in the infused to non-infused arms.



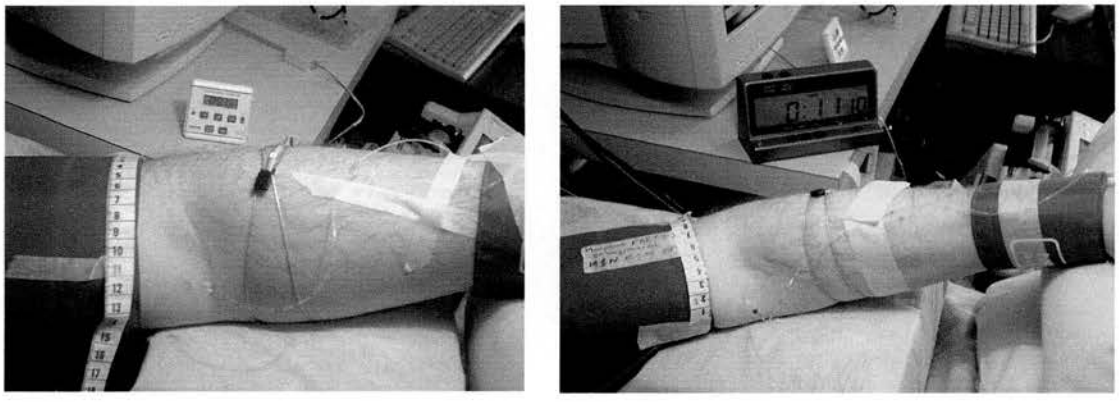
A) FBF in both arms B) Ratio

Figure 8- 3. A) Mean (SEM) of forearm blood flow in infused arm (—●—) and non-infused arm (··○··) in ml/100ml forearm volume, B) Ratio of forearm blood flow in infused arm over non-infused arm in six subjects that received incremental dose of 1, 3, 10, 30, 100 mcg/min/ml (6 min each) in modified dose ranging study (P_1).

8.3.2.2. Skin effects

This study caused measurable skin effects, which were maximal at 100 mcg/ml/min

Picture 8-2.



A) Subject 1 B) Subject 2

Picture 8- 2. Local effects of IA morphine in one subject who received incremental doses of 1, 3, 10, 30, 100 mcg/ml/min every 6 minutes (1 minute after replacing 100 mcg/ml/min with saline.

Flare

Area of the flare (cm^2) in the infused arm is shown in Figure 8-4-A. Overall area of the flare was significantly increased from 30 to 100 mcg/ml/min (P=0.022). The dose at

which flare occurred was different among individual subjects, but all six subjects finally developed local redness.

During washout local redness gradually decreased and after 18 minutes the difference from the highest value of red area was significant (P=0.028). Redness disappeared in all cases after 90 minutes.

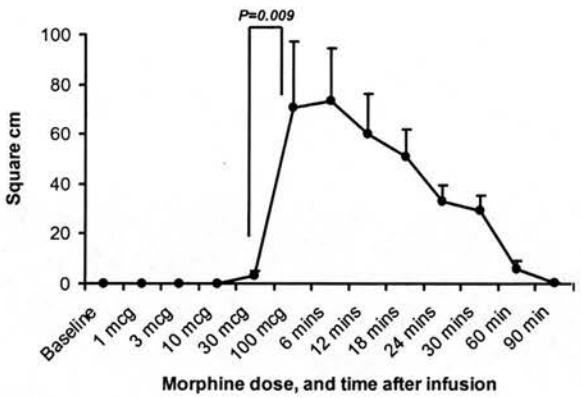
Weal

Area of the weal (cm^2) in the infused arm is shown in Figure 8-4-B. The area of the weal was significantly increased from 30 to at 100 mcg/ml/min (P=0.009). The dose at which weal was occurred was different among individual subjects, bur all six subjects developed a local weal. Pitting of the weal was also present.

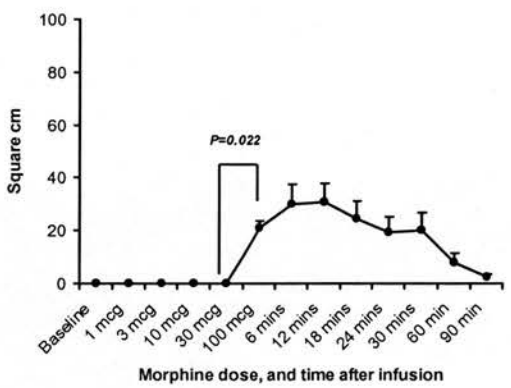
During the washout period, local erythema gradually decreased and after 24 minutes the difference to the highest value of erythema area was significant (P=0.011). Weal disappeared in all cases after 90 minutes.

Presence of itching

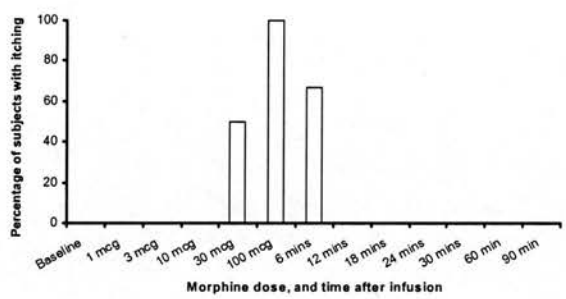
Presence of itching in the infused arm is shown in Figure 8-4-C as a percentage of subjects. Itching occured in 50% of subjects who received 30 mcg/ml/min and affected all subjects when they are receiving 100 mcg/ml/min. Itching was reported up to the first washout measurement (6 mins). Intensity of itching followed the same pattern Figure 8-4-D. In the majority of cases itching was reported during the FBF measurements (at 30 at 100 mcg/ml/min). Itching decreased or disappeared between measurements during the morphine infusion.



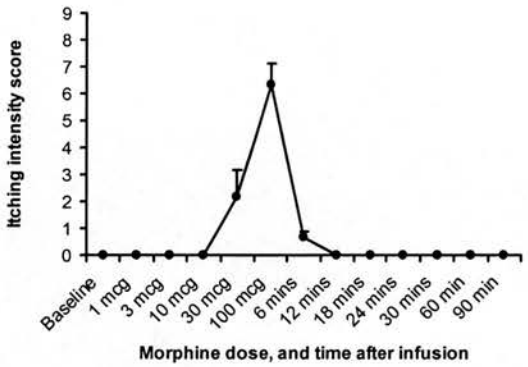
A) Area of flare



B) Area of weal



C) Percentage of subjects with itching

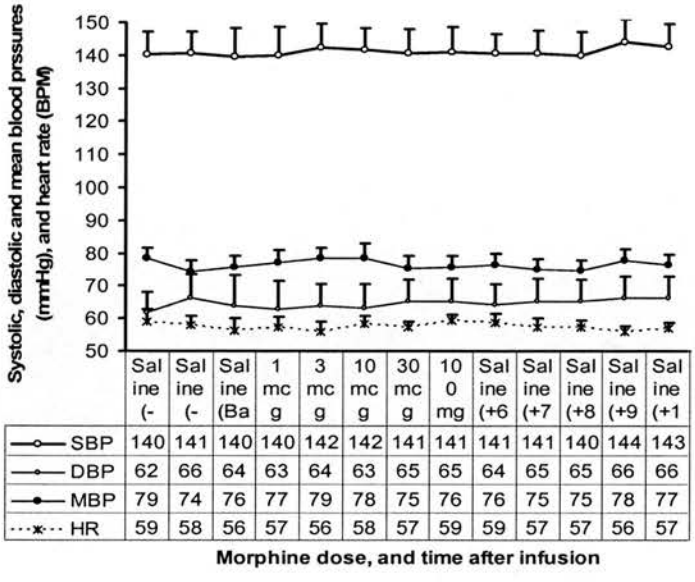


D) Intensity of itching

Figure 8- 4. Mean (SEM) of A) area of flare in square centimetre, B) area of weal in square centimetre, C) percentage of subjects with itching (%), D) intensity of itching (on a subjective scale of 0 (no itching) to 9 irresistible itching) in six subjects that received incremental dose of 1, 3, 10, 30, 100 mcg/min/ml (6 min each) in modified dose ranging study.

8.3.2.3. Systemic effects

Systemic haemodynamics were stable throughout the study, and are summarized in Figure 8-5. As can be seen none of the variables changed during the study and all values were within the normal ranges.



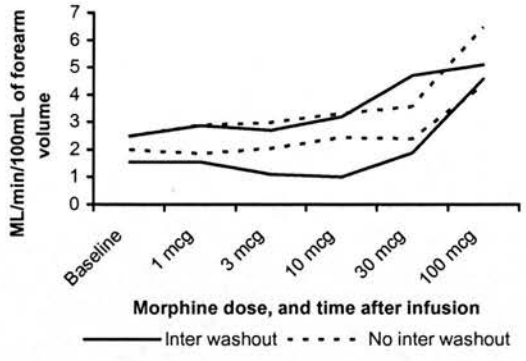
	Saline (-	Saline (-	Saline (Ba	1 mcg	3 mcg	10 mcg	30 mcg	100 mg	Saline (+6	Saline (+7	Saline (+8	Saline (+9	Saline (+1
SBP	140	141	140	140	142	142	141	141	141	141	140	144	143
DBP	62	66	64	63	64	63	65	65	64	65	65	66	66
MBP	79	74	76	77	79	78	75	76	76	75	75	78	77
HR	59	58	56	57	56	58	57	59	59	57	57	56	57

Figure 8- 5. Mean (SEM) of systemic haemodynamic variables in the modified dose ranging study; dose ranging study of IA incremental doses of 1, 3, 10, 30, 100 mcg/ml/min of morphine (n=6).

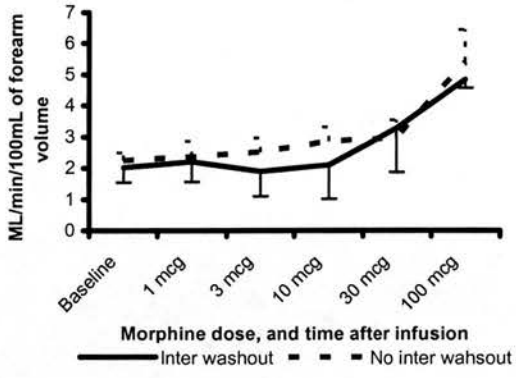
8.3.2.4. Reproducibility

By comparing the results of two subjects who participate in the pilot and main studies reproducibility of morphine-induced increases in FBF were assessed. A common range of doses of baseline, 1, 3, 10, 30 and 100 mcg/ml/min were studied (Figure 8-6 A and B). Unlike the second study, there were 10 minutes inter-treatment washout in the pilot. Comparing the effects, however, showed that there was no major difference in the pattern of FBF changes.

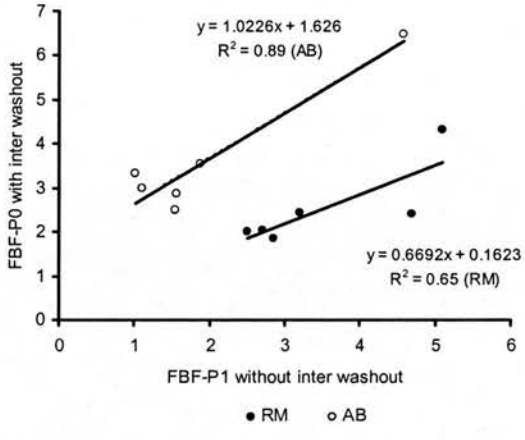
Figure 8-6-C shows the correlation between values from two visits of subject (1) (r^2=0.89, P<0.001), and subject (2) (r^2=0.64, P=0.000). Figure 8-6-D compares the forearm blood flow in ml/min/100ml in the two visits. As can be seen the results are reasonably reproducible.



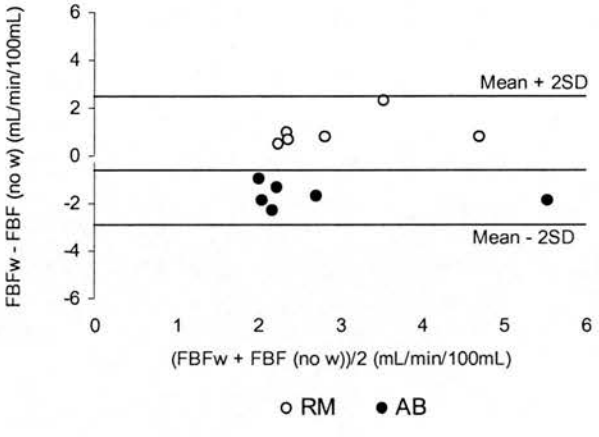
A) Individual cases



B) Mean



C) Association of two visits



D) Comparison of two visits

Figure 8- 6. Reproducibility. A) Individual values with and without inter saline washout of FBF in morphine intra-arteriolar infusion, B) Mean values for two subjects, C) Association of FBF in morphine intra-arteriolar infusion with or without inter saline washout D) Compares the forearm blood flow in mL/min/100mL with washout (w) versus no washout (no w) (RM and AB are initials for the two subjects) (n=2).

8.3.3. Discussion

The principle finding of this study is that IA morphine sulphate causes dose-dependent arteriolar dilatation, and induces weal, flare and itching. The highest dose of morphine in this study was 100 mcg/min/ml and with a forearm flow of 50 ml per minute would produce a concentration of 2 mcg/ml. The concentration of morphine in this study is

thus higher than concentrations in studies (peak 80 ng/ml, mcg/l) reported in chapters VI and VII.

Development of the weal implies extravasation of fluid from the vasculature. Opioid-induce non carcinogenic pulmonary edema and anaphylactoid reactions, and these findings suggest a mechanism.

8.4. Tachyphylaxis study

Dorland's 28th Medical Dictionary defines tolerance as diminution of response to a stimulus after prolonged exposure, or repeated constant doses of a drug. It may also be the need for an increasing dose to maintain a constant response. Acquired tolerance is divided into pharmacokinetic (i.e. increased metabolism), or pharmacodynamic (i.e. receptor density).

Tolerance to opioids (loss of efficacy in vitro and in vivo) is a well known consequence of opioid administration. Tolerance to opioids happens because of altered receptor sensitivity, desensitization of the opioid receptor signaling pathways and complex adaptative changes that occur at different levels in the nervous system (Angers *et al.*, 2002; Winstanley & Walley, 1996). Opioids increase dopamine release by inhibiting GABAergic input onto the dopaminergic neurons (Neal, 1997). Tolerance develops more rapidly to euphoria, than to gastrointestinal effects (O'Brien, 2001) and no tolerance is seen for pupillary effects. Sensitization or reverse tolerance should also be considered, which shifts the dose response curve to the left.

Dorland's defines tachyphylaxis (rapid protection) as rapidly decreasing response to a drug after administration of a few doses, or a rapidly decreasing response to a drug following administration of the initial doses. Tachyphylaxis is less common and more selective than tolerance. It represents the adaptive response in the tissues, or exhaustion of the stores of the responsible agent, or slow dissociation of drug from receptor-drug complex (Lewis, 1980). Histamine releasing agents and sympathomimetic amines that act indirectly by releasing noradrenaline cause depletion of available mediators and may result in tachyphylaxis (Nies & Spielberg, 1996).

The mechanism of acute tolerance, however, can also partially be explained by receptor internalisation.

A tachyphylaxis study was performed to examine changes in the magnitude of response after short term continuous IA infusion of morphine.

8.4.1. Methods

On revue of effective doses from the previous study 50 mcg/ml/min IA morphine in saline at 1ml/min for 30 minutes was used to establish the pattern of change in response over a short period. Potential mediators were also investigated via concurrent blood sampling from both brachial veins. Venous occlusion plethysmography was used to measure effects. After obtaining the approval of the Research Ethics Committee and with the written informed consent, eight healthy men without history of opioid abuse were recruited to the study.

8.4.1.2. Plasma assays

Overall 240 ml blood was taken from each volunteer, 60 ml at baseline, at 10, and 30 minutes after starting morphine, and 60 min after discontinuing morphine. Samples of 30 ml were taken concurrently from both arms. Samples were prepared and labeled to be suitable for serum tryptase, plasma histamine, t-PA, PAI-1, vWF, TNF-alpha, IL-6, and assays. Sample collection, handling, centrifuging, storing and analysis are summarized in chapter 2.

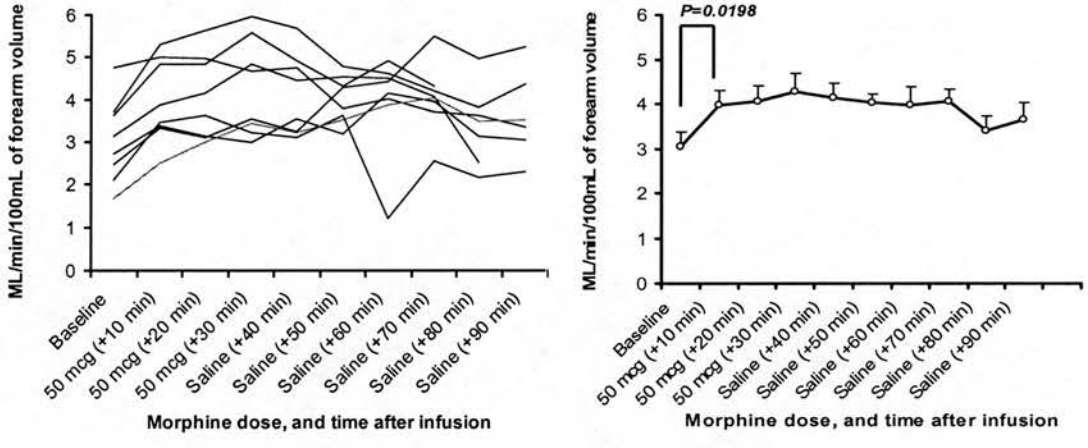
8.4.2. Results

Eight male subjects were studied with no history of smoking, hypertension,

hypercholestrolaemia or positive result for drugs of abuse. Their age (mean (SD)) was 34.4 (5.2), body mass index 25.7 (1.1).

8.4.2.1. Forearm blood flow

Forearm blood flow results are shown in Figure 8-7 (A & B). The ratio of flow in infused/non-infused arm at baseline was 1.2 (0.1). Baseline measurements of forearm blood flow in the infused and non-infused arms were not significantly different. There was no change in FBF of the non-infused arm. The baseline FBF was different among individual subjects but all of the eight subjects responded 10 minutes after starting 50 mcg/ml/min morphine by an increase in FBF (p=0.020). The magnitude of response was different in different individuals.



A) Individual results B) Mean (SEM)

Figure 8- 7. Forearm blood flow in A) individual subjects and B) mean (SEM) of eight subjects that received continuous IA 50 mcg/min/ml for 30 minutes

In this study, the forearm blood flow response to a continuous infusion of 50 mcg/min of morphine did not change over the 30-minute infusion period, consistent with no

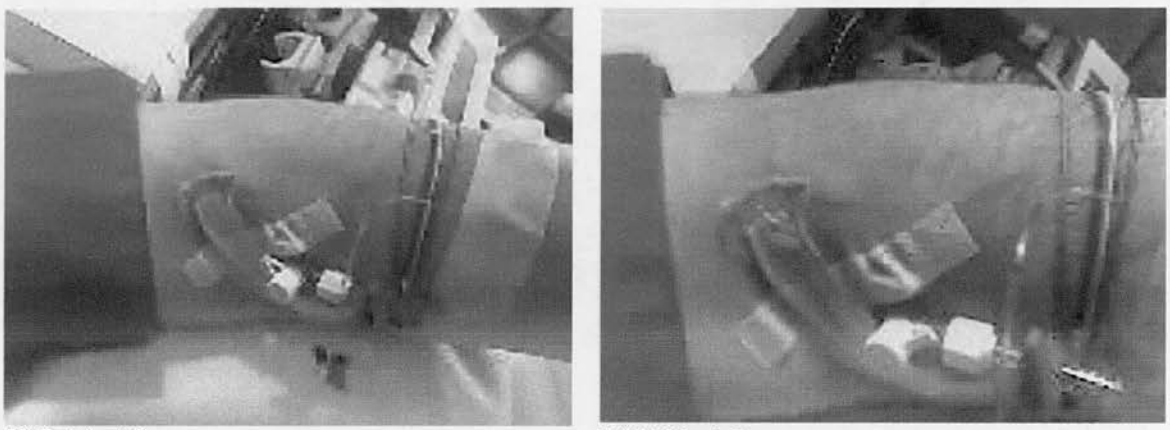
development of tachyphylaxis. Therefore, morphine induced receptor down-regulation in the short term is unlikely.

Washout

During 30-minutes washout forearm blood flow did not change significantly (P=0.542), which shows a short term persistent effect. This may suggest one of the following possibilities; continued release of mediators such as histamine from the weal area; continued effects of morphine in the area; irreversible binding of either morphine or one of the mediators with half life of action of more than 30-minutes.

8.4.2.2. Skin effects

Continuous infusion of 50 mcg/ml/min caused measurable skin effects Picture 8-4.



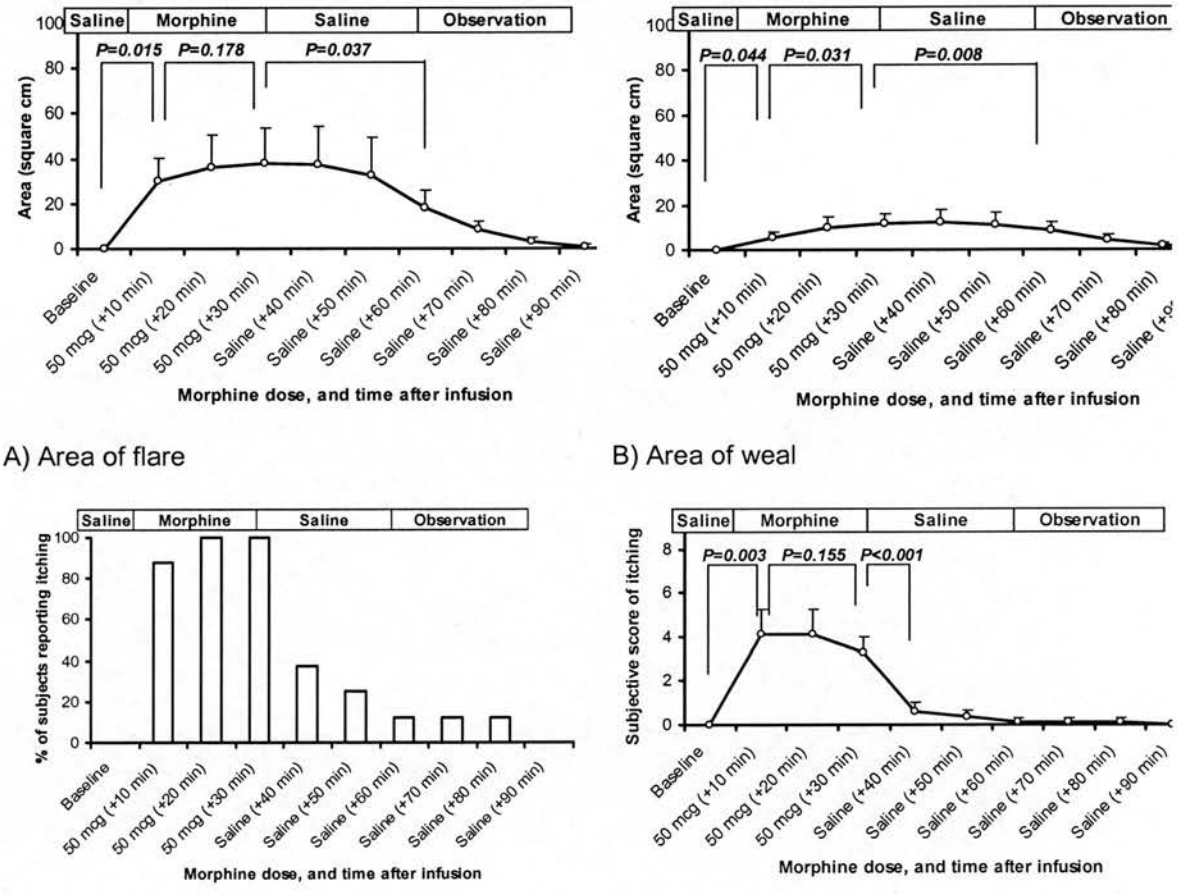
A) Subject 3 B) Subject 6

Picture 8- 3. Local effects of IA morphine in two subjects who received 50 mcg/ml/min for 10 minutes.

Tachyphylaxis to 30 minutes continuous infusion of 50 mcg/min/ml morphine for area of the flare (p=0.178; 10 min and 30 min) and intensity of itching (P=0.155) did not develop However, the weal developed slowly Figure 8-8 (A to D). Itching duration was shorter than flare and weal. This might be due to slow recovery from weal once it was

established. All subjects reported an intensified feeling of itching during 10, 20 and 30 minutes measurements, when the cuffs were up.

Sixty minutes after stopping morphine, the skin response to morphine had almost returned to baseline; this suggests a relatively slow recovery from the effects.



A) Area of flare

B) Area of weal

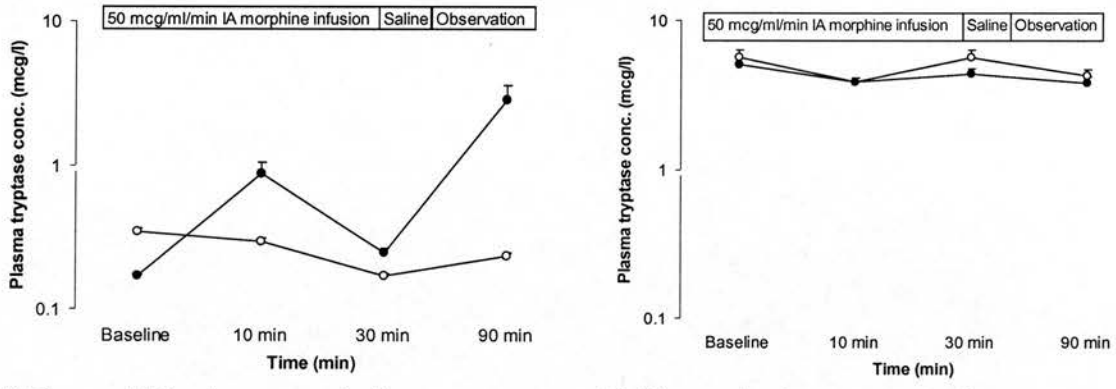
C) Percentage of subjects with itching

D) Intensity of itching

Figure 8- 8. Mean (SEM) of A) area of flare in square centimeter, B) area of weal in square centimeter, C) percentage of subjects with itching (%), D) intensity of itching (on a subjective scale of 0 (no itching) to 9 irresistible itching) in eight subjects that received continuous IA 50 mcg/min/ml for 30 minutes in tachyphylaxis study

8.4.2.3. Plasma assays

Morphine did not cause increase in venous histamine during the 30 minutes continuous infusion of 50 mcg/min/ml. However, at 90 minutes the ratio of plasma concentration of histamine in infused arm over non-infused arm significantly increased mean (95% CI) 7.2 (4.1, 10.4). This delayed response is probably due to diffusion of histamine from the weal to the vessel lumen. Plasma concentrations of tryptase were not changed Figure 8-9 (A & B).



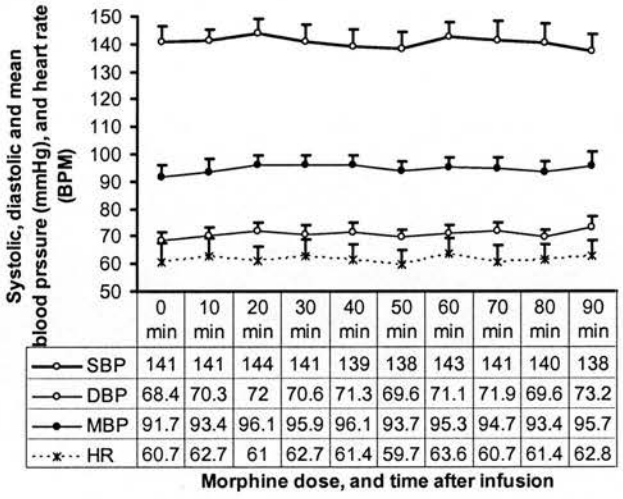
A) Plasma histamine concentration

B) Plasma tryptase concentration

Figure 8- 9. Mean (SEM) of plasma concentration of histamine (A) and tryptase (B) in mcg/l in the tachyphylaxis study; IA continuous infusion of 50 mcg/ml/min morphine for 30 minutes (n=8)

8.4.2.4. Systemic effects

Systemic haemodynamic effects in this study are summarized in Figure 8-10. As can be seen, none of the systolic, diastolic, mean blood pressure and heart rate variables were changed during the study and all values were within normal ranges.



	0 min	10 min	20 min	30 min	40 min	50 min	60 min	70 min	80 min	90 min
SBP	141	141	144	141	139	138	143	141	140	138
DBP	68.4	70.3	72	70.6	71.3	69.6	71.1	71.9	69.6	73.2
MBP	91.7	93.4	96.1	95.9	96.1	93.7	95.3	94.7	93.4	95.7
HR	60.7	62.7	61	62.7	61.4	59.7	63.6	60.7	61.4	62.8

Figure 8- 10. Mean (SEM) of systemic haemodynamic variables in the tachyphylaxis study; IA continuous infusion of 50 mcg/ml/min morphine for 30 minutes (n=8)

8.4.3. Discussion

This study shows that morphine-induced effects on FBF, area of the flare and weal and itching were not subject to tachyphylaxis over a 30 minutes period. As histamine increased at 90 minutes, the delayed response seen might be due to histamine diffusion into the lumen of the vessels from weal fluid.

8.5. Mechanism of action of morphine

8.5.1. Introductory remarks

Previously in this chapter it has been shown that morphine causes peripheral arterio-dilatation, with no tachyphylaxis over a 30 minutes infusion. However, the mechanisms involved in the arteriolar effects of morphine are not clear. Based on *in vitro* data, animal and a small number of human studies a range of mechanisms has been suggested or can be postulated. They include: direct mu receptor stimulation (Stefano *et al.*, 1995a), histamine release (Doenicke *et al.*, 1995; Flacke *et al.*, 1987; Rosow *et al.*, 1982; Philbin *et al.*, 1981; Fahmy *et al.*, 1983), and increased nitric oxide release (Stefano *et al.*, 1995a; Cadet *et al.*, 2000). In this study the potential mechanisms of this peripherally mediated action of morphine were evaluated pharmacodynamically. Involvement of opioid receptors, morphine-induced histamine release and nitric oxide mediated pathways were tested.

8.5.2. Methods

Venous occlusion plethysmography was used to evaluate these effects. An open label, randomised, four ways crossover trial was used. Approval of the local Research Ethics Committee and the written informed consent of each subject were obtained. Eight healthy men without history of opioid abuse were recruited to the study. Four study protocols were compared, i) morphine alone, ii) morphine and the nitric oxide clamp (L-NMMA) (see below), iii) morphine and naloxone, iv) morphine following pre-treatment with cetirizine and cimetidine (H_1 & H_2 blockers). The order of these visits was randomised. The NO-clamp was used as described previously (Helmy *et al.*, 2003; Verhaar *et al.*, 1998; Stroes *et al.*,). Briefly, L-NMMA was continuously infused at a dose of 4 micromol/min for 12 to 20 minutes to achieve maximal inhibition of local

vascular endogenous NOS activity as measured by forearm blood flow. Thereafter, sodium nitroprusside (SNP) was co-infused at titrated doses (80 to 600 ng/min) until FBF had been restored to within 10% of baseline flow and sustained for at least two consecutive FBF measurements. Once a stable baseline FBF was obtained, the "NO clamp" was continued at these doses of L-NMMA and SNP for the reminder of the study to allow simulation of basal NO activity during continuous inhibition of endogenous NO synthesis. Due to light sensitivity of SNP it was prepared and infused in syringes covered by opaque foil. Morphine was co-infused for 30 minutes in all visits.

Histamine antagonism was achieved by maximum therapeutic doses of the non-sedative H_1 cetirizine, which has been used for a similar purposes previously (Dux *et al.*, 2002; Grossmann *et al.*, 1999; Clough *et al.*, 1998), 10 mg per day for 2 days and 10 mg 1 hour before the study, and the H_2 blocker cimetidine, 400 mg bid for 2 days and 400 mg 1 hour before the study. For naloxone the therapeutic dose of a bolus of 400 mcg intravenously, followed by an intravenous infusion of 200 mcg/hour was co-infused with morphine.

In all visits a 30 min infusion of normal saline was given before starting the study and again for a 30 min washout period after discontinuation of morphine. Forearm blood flow, flare, weal, itching, intensity of itching, blood pressure and heart rate were measured every 10 minutes.

8.5.3. Results

The subjects age (mean (SEM)) was 34.4 (4.5) y and body mass index was 25.7 (1.0) kg/m^2.

8.5.3.1. Forearm blood Flow

FBF in the infused arm during 4 visits are shown in Figure 8-11. The ratio of infused/non-infused arm FBF at baseline was 1.2 (0.0). Overall forearm blood flow was significantly different in all four visits (F= 8.6, P < 0.001) (Table 8-1). Post hoc comparisons revealed that pre-treatment with histamine antagonists (mean difference (md)=0.87 ml/min/100 ml of forearm volume, P=0.008), and the nitric oxide clamp (md=1.23 ml/min/100 ml of forearm volume, P<0.001), significantly antagonised the vasodilator effects of morphine. In contrast naloxone failed to reverse the effects.

However, there was no significant difference between morphine alone and other arms with paired comparisons at individual time points.

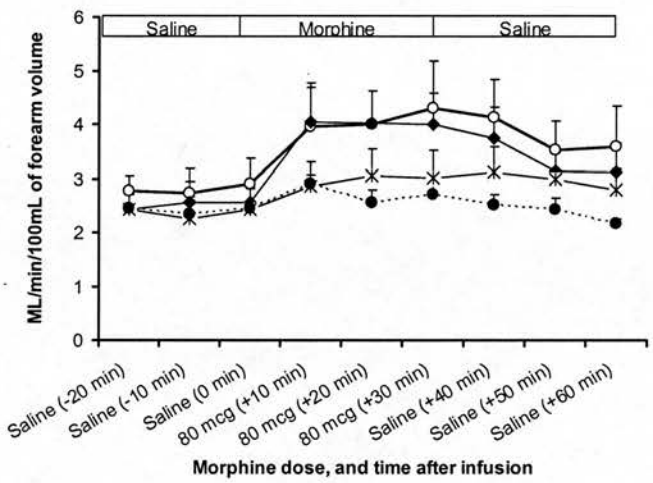


Figure 8-11. Mean (SEM) of forearm blood flow in infused arm in Ml/min/100 ml of forearm volume, morphine alone visit (—o—), morphine and naloxone visit (—●—), morphine and pre-treatment with cetirizine and cimetidine (—✳—), morphine and nitric oxide clamp visit (···●···) (n=8).

A) ANOVA for different visits		
Variable	***F***	***P value***
Infused	*8.621*	*<.001*
Inf/Cont ratio %	*1.994*	*.116*
Washout %	*2.619*	*.054*
Flare	*13.261*	*<.001*
Weal	*11.116*	*<.001*
Itching	*2.508*	*.060*
Intensity of itching	*2.944*	*.034*

B) Post hoc Multiple Comparisons with Bonferroni correction

Variable	VISIT (1)	VISIT (2)	Mean Difference (1 & 2)	SEM	*P value*
Infused	Morphine	M & Naloxone	*.236*	*.2715*	*1.000*
		M & H_1 H_2	*.873(*)*	*.2690*	*.008*
		M & NO Clamp	*1.226(*)*	*.2715*	*.000*
Flare	Morphine	M & Naloxone	*-19.071(*)*	*6.7519*	*.031*
		M & H_1 H_2	*22.201(*)*	*6.5580*	*.005*
		M & NO Clamp	*-6.342*	*6.6502*	*1.000*
Weal	Morphine	M & Naloxone	*-9.908(*)*	*2.5442*	*.001*
		M & H_1 H_2	*4.324*	*2.5442*	*.544*
		M & NO Clamp	*-.440*	*2.5800*	*1.000*
Itching	Morphine	M & Naloxone	*.161*	*.0630*	*.068*
		M & H_1 H_2	*.125*	*.0630*	*.290*
		M & NO Clamp	*.125*	*.0630*	*.290*
Intensity of itching	Morphine	M & Naloxone	*.732*	*.2865*	*.068*
		M & H_1 H_2	*.732*	*.2865*	*.068*
		M & NO Clamp	*.571*	*.2865*	*.284*

Table 8- 1. Summary of analytical statistics for mechanism of action study. A) ANOVA for difference in all visits B) Post hoc Multiple Comparisons with Bonferroni correction for differences in individual visits, Infused; forearm blood flow (FBF) in infused arm, Inf/Cont ratio %; percentage of change from baseline of FBF of infused/control arms, Flare; area of flare (cm^2), weal; area of weal (cm^2), Itching; percentage of subjects with itching, Intensity of itching; intensity of itching on a subjective scale of 0 (no itching) to 9 irresistible itching, Morphine; morphine alone visit, M & Naloxone; morphine and naloxone visit, M & H_1 H_2; morphine and pre-treatment with cetirizine and cimetidine, M & NO Clamp; morphine and nitric oxide clamp visit, (*); The mean difference is significant at the 0.05 level, Mean Difference of (1 & 2); the overall mean difference between two individual visits (positive values show antagonist effects, negative values show synergistic effect, * show the difference is significant at the 0.05 level (n=8).

8.5.3.2. Skin effects

Flare

Area of the flare (cm^2) in the infused arm in four visits is shown in Figure 8-12-A. Overall area of the flare was significantly different in all four visits ($F=13.3$, $P<0.001$). Post hoc comparisons revealed that pre-treatment with histamine antagonists significantly

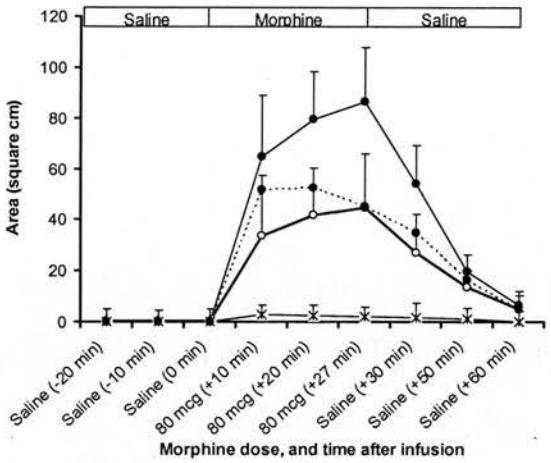
reduced the effects of morphine (md=22.2 cm^2, P=0.005). In contrast nitric oxide clamp failed to reverse the morphine-induced effects, and naloxone significantly increased the flare area (md=-19.1 cm^2, P= .031) (Table 8-1). There was no statistically significant difference in pair-wise comparisons of individual time points between the morphine visit and other arms.

Weal

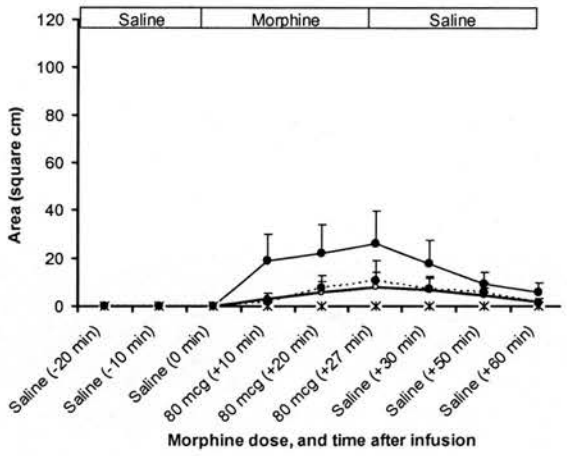
Area of the weal (cm^2) in the infused arm in the four visits is shown in Figure 8-12-B. The area of the weal was significantly different in all four visits (F=11.1, P<0.001). Post hoc comparisons revealed that pre-treatment with histamine antagonists or nitric oxide clamp failed to reverse the morphine-induced effects, and naloxone significantly intensified the weal area (md=-9.9 cm^2, P=0.001) Table 8-1.

Presence of itching

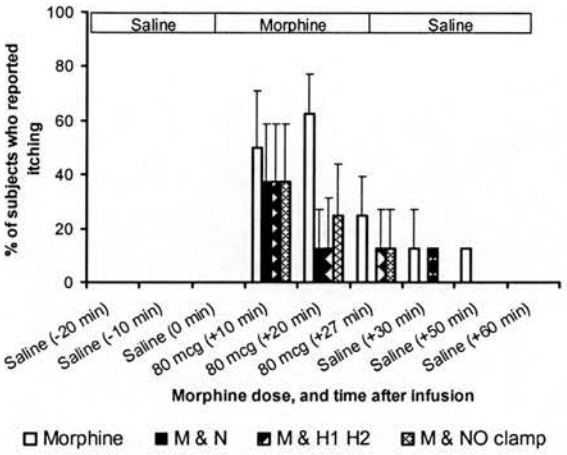
Presence of itching (%) in the infused arm in four visits is shown in Figure 8-12-C. The percentage of subjects who report itching was not significantly different in all four visits (F=2.5, P=0.06). Post hoc comparisons revealed that pre-treatment with histamine antagonists, nitric oxide clamp and naloxone failed to reverse the morphine-induced effects Table 8-1.



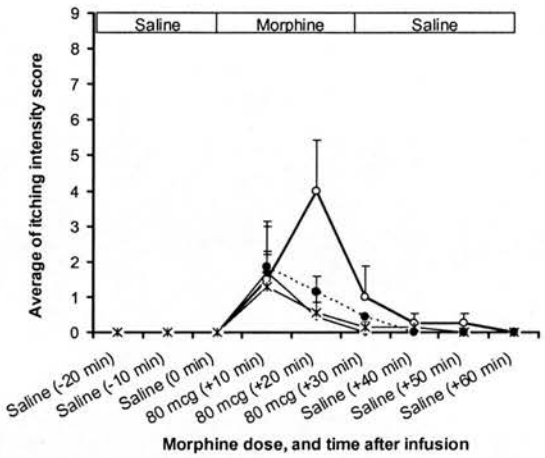
A) Area of flare



B) Area of weal



C) Percentage of subjects with itching



D) Intensity of itching

Figure 8- 12. Mean (SEM) of A) area of flare in square centimetre, B) area of weal in square centimetre, C) percentage of subjects with itching (%), D) intensity of itching (on a subjective scale of 0 (no itching) to 9 irresistible itching). morphine alone visit (–o–), morphine and naloxone visit (–◆–), morphine and pre-treatment with cetirizine and cimetidine (–✳–), morphine and nitric oxide clamp visit (·····●·····) (n=8).

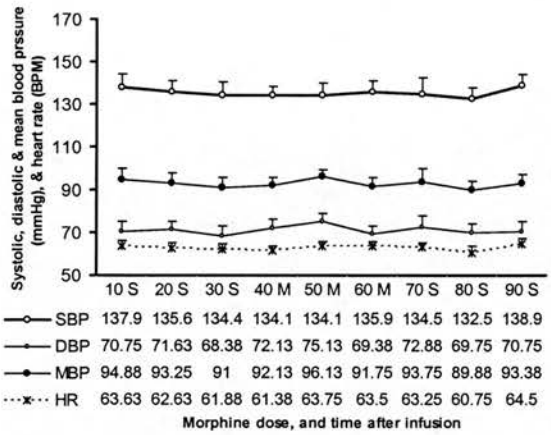
Intensity of itching

Intensity of itching in the infused arm in four visits is shown in Figure 8-12-D. The intensity of itching was significantly different in four visits (F=2.9, P=0.034). Post hoc comparisons revealed that pre-treatment with histamine antagonists (md=0.7 score, P=0.068) and naloxone (md=0.7 score, P=0.068) showed a non-significant tendency to

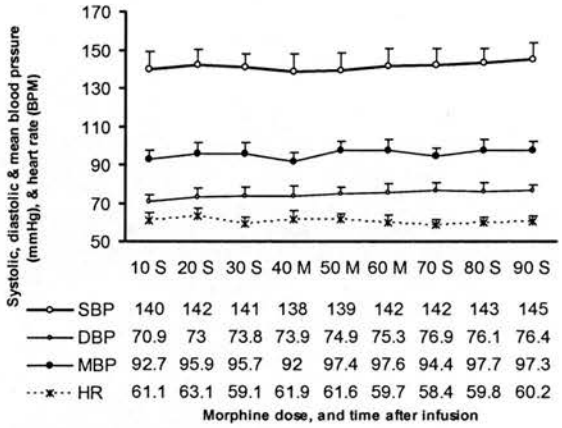
antagonise morphine-induced effects. Nitric oxide clamp has no effect on these effects Table 8-3. The overall significant difference was due to the magnitude of differences in visits with different antagonists.

8.5.3.3. Systemic effects

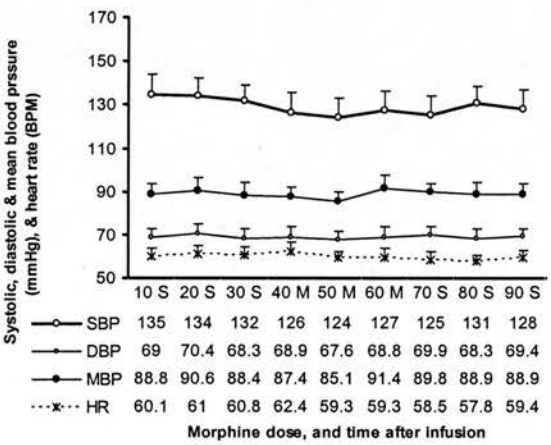
Systemic haemodynamic effects in this study are summarized in Figure 8-13. As can be seen, none of the systolic, diastolic, mean blood pressure and heart rate variables were changed during the study and all values were within normal ranges.



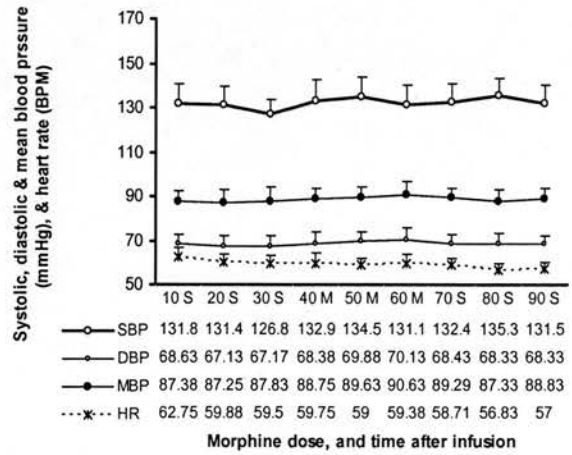
A) Morphine alone visit



B) Morphine & Naloxone



C) Morphine & Cetirizine & Cimetidine



D) Morphine and Nitric Oxide Clamp

Figure 8- 13. Systemic haemodynamic change in 4 visits of mechanism of action study, numbers in horizontal axes show the time interval from starting the study, S; saline (1ml/min), M; morphine (80mcg/ml/min) (n=8).

8.5.3.4. Summary of the results

To simplify these findings, the overall effects of the 3 potential antagonists on IA morphine-induced-changes are summarised in Table 8-2.

	FBF in infused arm (ml/min/100 ml of forearm volume)	Flare (mean) (cm^2)	Weal (mean) (cm^2)	Presence of itching (%)	Intensity of itching (mean)
Antagonised[1] by					
H_1 H_2 blockers	0.88 (*)	22.20 (*)	4.324	0.13	0.73
NO clamp	1.23 (*)	-6.34	-0.44	0.13	0.57
Naloxone	0.24	-19.07 (*)	-0.91 (*)	0.16	0.73

Table 8- 2. Summary of antagonists' effects. The overall mean difference between two individual visits are shown (positive values show antagonist effects, negative values show synergistic effect), *; significant effect.

In summary, H_1 and H_2 blockers significantly antagonised the morphine-induced increases in forearm blood flow and area of the flare, showed a non significant tendency to antagonise morphine-induced intensity of itching but had no effect on the other variables measured. Nitric oxide clamp significantly antagonised morphine-induced increase in forearm blood flow, but failed to antagonise skin variables. Naloxone, in contrast, significantly intensified the local effects of morphine on flare and weal, but had no effect on blood flow. Forearm blood flow in the non-infused arm, blood pressure and heart rate were stable throughout the four visits Figure 8-4.

8.5.4. Discussion

This study showed that naloxone at the dose administered failed to antagonise morphine-induced effects on FBF, flare, weal and itching. This suggests that these effects of morphine are not mediated via opioid receptors. This finding is in contrast with the previously claim of the existence of μ_3 receptors in the vasculature (Stefano *et al.*, 1995b). Reports about the existence of a μ_3 receptor in vasculature by this group have not been independently confirmed.

The present findings, however, are consistent with some parts of this groups work on remifentanil in which it failed to act as a vasodilator in the vasculature (Bilfinger *et al.*, 1998a). Remifentanil is a selective mu agonist, which does not induce histamine release and only induces minor haemodynamic effects systematically (Sebel *et al.*, 1995). Taken together, it can be postulated that histamine release, the difference between morphine and remifentanil, is the cause of the alleged μ_3 receptor action on the vasculature. This is consistent with this study.

Naloxone intensified the effect of morphine in causing flare and weal. This compound is a competitive antagonist of morphine with a similar chemical structure. The intensifying effect of naloxone might be due to an action on non-opioid receptors in inducing of histamine release or to opiod counter-regulatory pathways. Histamine release is not a function of therapeutic effects of naloxone (BNF, 2003).

Nitric oxide did not antagonise morphine-induced skin effects (flare, weal, and itching), therefore, these effects of morphine are nitric oxide pathway independent. However, it reversed arteriolar effects, and therefore, morphine directly or indirectly acts on arteries via the nitric oxide pathway.

In this study, histamine receptor blockers successfully reversed morphine induced FBF changes and area of the flare, suggesting these effects are mediated via histamine. Histamine receptor antagonists did not significantly prevent the development of morphine-induced weal or presence of itching. This might be because their dose was too low, or the power of the study was limited. It might also indicate a different mode of action of morphine in preventing weal and itching.

Overall, this study suggests that morphine-induced arteriodilatation is related to opioid induced secondary mechanisms related to histamine release. Either morphine itself, or the secondary release of histamine activates nitric oxide mechanisms to cause arteriodilatation. This experiment was not designed to explore this hypothesis. It also shows that morphine-induced flare and probably to a lesser extend weal are related to histamine release.

Figure 8-14 summarises the potential mechanisms of action of morphine on the vasculature.

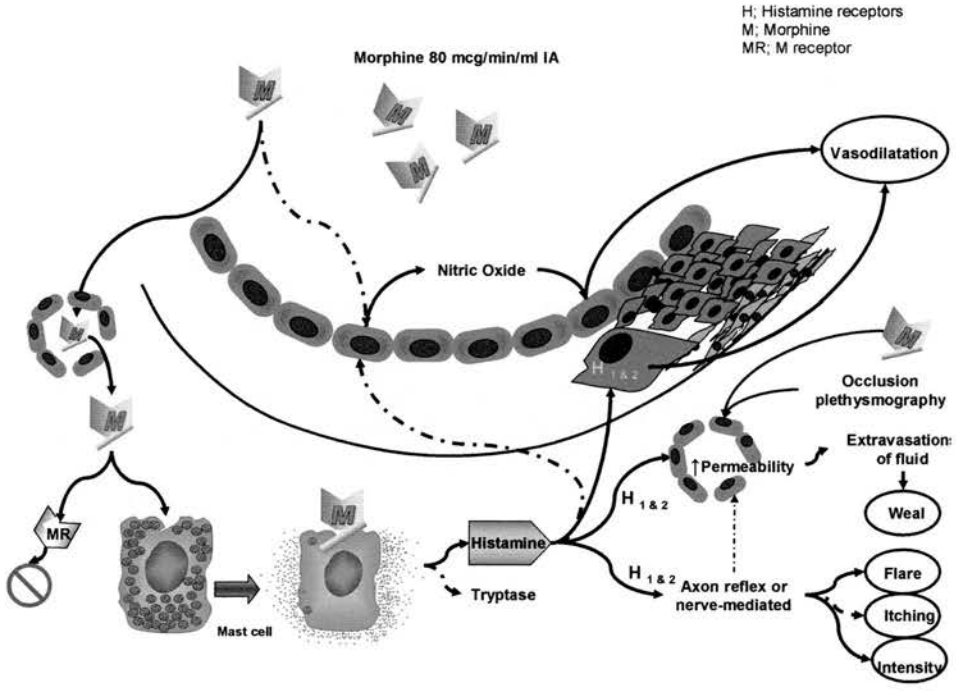


Figure 8- 14. Pathways of IA morphine-induced arteriolar and dermal effects, definite pathway (→) probable pathway (⇢), no pathway (⊘).

8.6. Overall discussion of FBF studies

In dose ranging studies I have shown that a range of 30 to 300 mcg/ml/min morphine sulphate intra-arteriolarly causes dose dependent increases in FBF and induces flare, weal and itching. Basal forearm blood flow is about 50ml/min, which is almost 100 fold lower than cardiac output (Wilkinson & Webb, 2001), thus the resulting concentrations of these doses would be 0.6 to 3 mcg/ml.

In systemic studies, a total dose of 16 mg IV morphine produced a maximal venous concentration of 0.08 mcg/ml, which is similar to other observed therapeutic studies (Hagen *et al.*, 2005). The effective concentrations of morphine in FBF studies reported in this chapter are 7.5 to 37.5 times higher than morphine concentrations in the whole man studies. Arteriolar and skin effects are mediated via histamine as shown by measuring plasma histamine level and also antagonising its effects. The likely source of histamine is mast cells, which are located in surrounding supporting tissues of arteries (Soter, 1999; Abbas & Lichtman, 2004; Steven & Lowe, 2005). This is also consistent with the fact that various drugs including morphine can directly degranulate mast cells and release histamine from them (Soter, 1999), and previous findings that morphine and diamorphine release histamine from mast cells rather than basophiles (Withington *et al.*, 1993). These findings suggest that surrounding supporting tissues of arteries may influence vascular tone.

In anaphylactoid reactions mediators such as histamine are involved and released by compounds acting directly on mast cells in a non-immunological way in which previous sensitization is not needed (Chapel *et al.*, 1999). Morphine is known to induce anaphylactoid reactions (Fahmy, 1981). Findings of this chapter suggest that morphine

concentration might be responsible for anaphylactic responses, and drug induced acute urticaria and itching caused by opioids.

Opioids, including morphine, methadone and diamorphine, in overdose and during high dose treatment for acute pain have also been shown to cause non-cardiac pulmonary oedema (NCPE) (Frishman *et al.*, 2003; Lusk & Maloley, 1988; Sporer, 1999; Sporer & Dorn, 2001; Corkery *et al.*, 2004; Bruera & Miller, 1989). Common features of NCPE includes damage to and increased permeability of vascular endothelium (Overland & Severinghaus, 1978). The mechanism, by which opioids, particularly in overdose, cause non-cardiac pulmonary oedema, may in part be explained by this study. Increased permeability causes weal and NCPE. Taken together these findings also indicate that opioid-induced noncardic pulmonary oedema might be a dose response effect.

The effects were dose dependent at lower doses, and accelerated at doses of 100-300 mcg/ml/min, and the curve did not become S shape; therefore, a quantal response (all-or-none) might be plausible at these doses. If such a phenomenon happens in whole man a large amount of fluid will extravasate, and it is plausible that a intravascular hypovolemic state may develop.

Diamorphine overdose has been shown to induce profound circulatory shock (Remskar *et al.*, 1998). Morphine exacerbated anaphylactic shock in mice by stimulating central opiate receptors (Amir, 1983). In this study high concentration of morphine caused extravasation. If extravasation systematically occurs opioid might facilitate development of shock by inducing intravascular hypovolemia.

In some studies release of endogenous opioids was provoked by induction of anaphylaxis (Amir, 1988). Morphine exacerbates histamine shock in mice (Amir, 1984). An opioid-dependent pathway has been previously suggested to be involved in the recovery from endotoxin shock (D'Amato & Holaday, 1984). In anaphylaxis mast cell or basophil mediators cause bronchial constriction, massive tissue oedema, and cardiovascular collapse. Overall, these findings suggest opioids may be involved in anaphylactoid reactions, drug induced non-cardiac pulmonary oedema and anaphyphylaxis, and endotoxin shock. Opioids by inducing systemic extravasation and hypovolemia might also contribute to the pathophysiology of shock.

Nitric oxide is well recognized as a target for cardiovascular therapies. Enhanced formation of NO contributes to the pathophysiology of experimental anaphylactic shock (Szabo & Thiemermann, 1994). The current studies indicate that nitric oxide plays a role in morphine-induced arteriodilatation. A schematic illustrating of the potential mechanisms of morphine induced effects is shown in Figure 8-15.

In all these studies, response and antagonism of FBF changes were different among different individuals, raising the possibility of genetic contribution to these effects. HLA DR4, HLA DRB4 53, and HLA DQ8 and DQA 3011/12 have been seen more frequently with patients with chronic idiopathic urticaria (Soter, 1999).

In this chapter a dose response in the vasculature and at the skin were reported. As the time period between drug administration and effects is short (less than 10 minutes) receptor activation is presumably mediated via parent drug. Morphine-induced release of histamine from mast cells was not antagonised by naloxone. This raises the

possibility of the existence of an opioid receptor for morphine on mast cells (extra circulatory opioid receptors) independent of naloxone antagonismin vivo. Activation of this receptor will release histamine from mast cells. However, as only therapeutic doses of naloxone were used further studies are indicated.

As a similar pathophysiology for drug induced anaphylactoid reaction and non-cardiac pulmonary oedema is suggested further studies on H_1 and H_2 blockers may be indicated. In a similar way, pretreated with H_1 and H_2 blockers might be relevant to

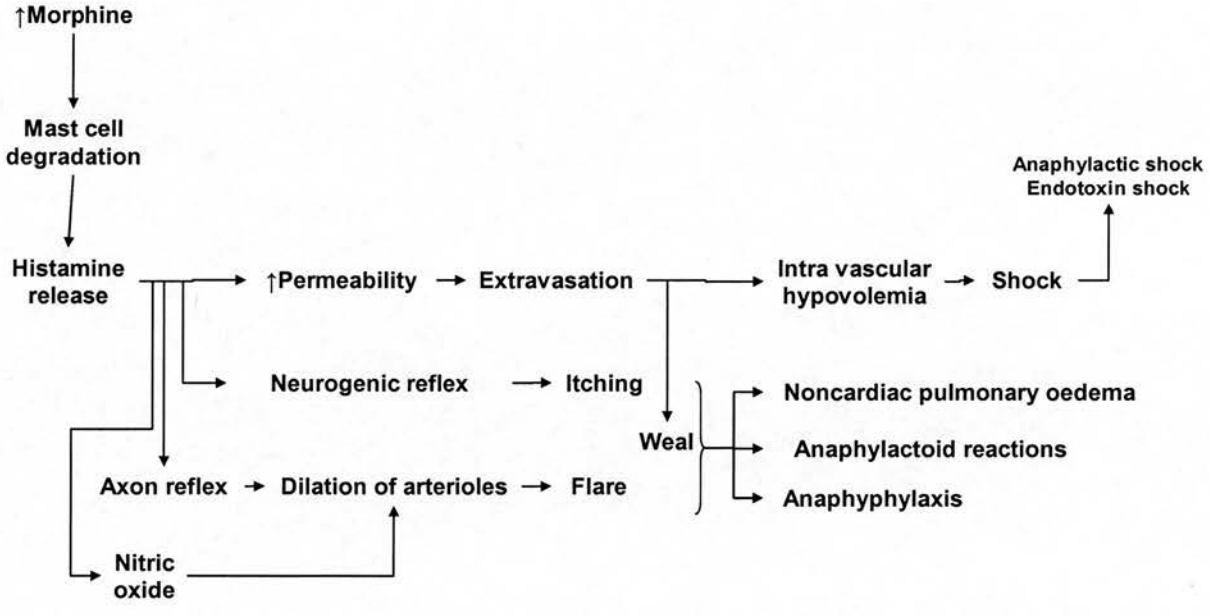


Figure 8- 15. Schematic of the pathophysiology of opioid induced effects based on the findings of the forearm studies.

treatment of acute pain and surgery with high doses of morphine. This is consistent with previous observed benefits of histamine blockers in haemodynamic stabilizing in anesthesia for the patients who were receiving high doses of morphine (Philbin *et al.*, 1981; Sanchez *et al.*, 2000).

Schematic of the pathophysiology of opioid induced effects based on the findings of the forearm studies is shown in Figure 8-15. . In this experiment a skin triple response was induced intra-arterially, suggesting a model for experimental studies on drug-induced anaphylactoid reactions in man.

8.7. Conclusion

A peripheral site of action on vasculature exists for morphine, which is mediated, at least in part, via histamine, is dose dependent, and is activated in high concentrations of morphine (0.6 to 3 mcg/ml). Involvement of peri-arteriolar mast cell in this process indicates that arteriolar surrounding supporting tissues may contribute to the control of vascular tone. These effects can be antagonized by anti-histamines. The observed arteriolar and skin effects are mediated via histamine, but arteriolar effects are both histamine and nitric oxide related.

High plasma concentrations of opioids maybe the pathophysiology of opioid induced non-cardiac pulmonary edema and anaphylactoid reactions. H_1 and H_2 blockers should be studied in the management of non-cardiac pulmonary oedema. Patients receiving high doses of morphine in surgery and severe pain may be at risk of the effects reported here.

Chapter IX, Discussion

9.1. Summary of the field of study

The history of opioids started 6000 years ago in Mesopatamia. Egyptians recognised their toxicity in 1500 B.C (Gettler, 1956). In the Greek and Islamic-Persian "golden ages" they were used as a "Teryak" or a universal remedy, and in surgery (Al-ghazal, 2003). In late medieval and early renaissance Europe, opium became "the stone of immortality". While in the 20th century a massive international body of regulation repeatedly failed to control opioid-induced addiction.

Currently, 19 pure opioids, 4 combination products, and 16 OTC opioid- containing products are available in the UK (BNF, 2003) within association with opioid illicit supply and excess risk of dependency, opioid overdose has become a common health problem in Scotland. Among the opioids, morphine is extensively used in acute medicine, and for end stage patients has a long term indication. This product is indicated for situations where haemodynamic effects, such as pain control in myocardial infarction and management of acute heart failure, may be important (Fuster, 2001; Dollery, 1999).

Despite this long history, heavy regulation of use, impurity of illegal supplies, and possibility of inducing dependence little work has been conducted on vascular effects of opioids in healthy volunteers. In addition, major central nervous system, respiratory and gastrointestinal depressor effects of opioids have overshadowed potential cardiovascular effects, and gradually a concept that opioids have little effect on the cardiovascular system in man in the supine position has developed (Gutstein & Akil, 2001). This is despite a body of evidence from animal and patient studies that show opioid-induced cardiovascular effects. In addition, opioids and endorphins have been

shown to be involved in many physiological and pathological processes.

9.2. Summary of the findings of this thesis

In my project, I have firstly describe the current situation in terms of determining opioid prescriptions, non-fatal overdose events and overdose deaths in Scotland and Edinburgh and introduced a series of indices for epidemiological use. Secondly, I studied the concept that opioids have effects on the cardiovascular system, and clarified the extent to which CNS and respiratory variables are involved in haemodynamic effects of morphine. In particular I have attempted to demonstrate a separation between non-opioid effects of opioids, such as dextropropoxyphene-induced sodium channel blocker effects and morphine-induced histamine release, and effects due to a direct action at opioid receptors. I conducted a series of studies aimed at defining an independent peripheral vascular site of action for morphine in vivo in man, and identifying the mechanism of action of direct effects of morphine on arterioles. The theme of this thesis has been on both opioid effects, and effects associated with opioids that are due to secondary mechanisms of opioids on the cardiovascular system.

In this thesis, a range of both epidemiological, patient and volunteer studies are reported. In chapter 3, opioid overdose are shown to have increased 14 times more than other overdoses in Edinburgh in the past 4 decades.

The predisposing factors for overdose are outlined. Fatal Toxicity Indices (FTI) for individual opioids in Scotland were calculated using prescription data, and telephone enquiries, TOXBASE accesses, and hospital discharge data used to develop a series of fatality ratios, FTIs, designed to reduce the effects of confounders that influence the

assessment of drug toxicity in overdose. A similar methodology was used to relate non-fatal overdose consequences to prescriptions, and toxic morbidity indices (TMIs) calculated. I suggested an integrated approach by calculating FTIs and TMIs as the gold standard for toxico-vigilance. Non fatal and fatal consequences of overdose are suggested as independent variables.

Using these approaches I calculated that co-proxamol has a more than 10 times excess fatal hazard in comparison to co-codamol and co-dydramol, while their TMIs are similar. This led, in part, to withdrawal of this drug in UK. I showed that dihydrocodeine appears safer than methadone, and may therefore be a better drug for substitution purposes. I also estimated the availability of diamorphine from illicit supply using overdose data. I used national mortality data to study the probability ratio of death from single agent or multiple agent ingestions (MSDPR). Using this I showed that diamorphine, morphine and codeine, which are all metabolised to morphine, seem far more dangerous in co-intoxication than other opioids. This data is in keeping with animal work suggesting an interaction with benzodiazepines (Burrows *et al.*, 2003; Klys *et al.*, 2001). Later, in healthy volunteers I showed that morphine-induced vomiting happens 3 hours after injection, raising the possibility of involvement of a morphine metabolite. Taken together, morphine 6 glucronide or one of the other morphine metabolites might be responsible for side effects and toxic interactions.

Currently it is believed that opioids in general do not induce electrophysiological effects in overdose. In chapter 4, I have explored the effects of dextropropoxyphene in acute overdose in man, and shown that QRS duration is prolonged in co-proxamol overdose, an effect not found following co-codamol or co-dydramol poisoning. These

electrophysiological effects were similar to other sodium channel blocking agents. In a larger study including retrospective data from Australia I showed a dose dependency of this phenomenon, using paracetamol levels as a surrogate for dextropropoxyphene dose. This study may explain the cause of the excess mortality risk I have described in overdose with co-proxamol, and may suggest why some of the individuals die so quickly after co-proxamol overdose. These findings are compatible with dextropropoxyphene being a sodium channel blocker (Roth & Seeman, 1971; Hondeghem & Katzung, 1977; Lund-Jacobsen, 1978; Stork *et al.*, 1995; Henry & Cassidy, 1986), and death perhaps being due to a combination of respiratory depression from its opioid effects and the sodium channel blocking effects my studies indicate.

It is known that in anaesthetised patients where high doses of opioids are used the cardiovascular system is affected (Pant *et al.*, 1983; Hoar *et al.*, 1981; Yoshimoto *et al.*, 2005). In chapter 5, I illustrated the haemodynamic profile of dihydrocodeine, and methadone in overdose. Depressor actions on aortic and peripheral systolic, diastolic, pulse, and end systolic pressures, and O_2 saturation were shown in comparison to a non-opioid poisoned control group. I also applied SphygmoCor techniques to measure cardiovascular effects for the first time in overdose patients and was thus able to exclude an action on arterial stiffness. My findings suggest that O_2 saturation under 95% is probably a feature of cardiovascular depression in these patients. These effects have a clinical impact on decisions for admission, duration of hospital admission, use of antidotes and the possible occurrence of complications.

Further work was required to clarify the potential mechanisms through which opioids influence the cardiovascular system, including contributions to effects on inotropism, systemic vascular resistance, central nervous system depression and respiratory function. In addition, the dose-response-effect relationship of opioids for any changes on the cardiovascular system needed to be estimated. In chapter 6, I studied the effects of therapeutic doses of morphine on the cardiovascular system in healthy volunteers in the supine position and expanded the initial findings in patients. I demonstrated cardiovascular depressor effects of 16 mg intravenous morphine, which were not clearly dose-dependent. These effects did not seem to be mediated via histamine or catecholamines. Reaction time, an index of central nervous system depression, was also unrelated to cardiovascular effects. Lower O_2 saturation, and higher end tidal volume CO_2, potentially contributed to the haemodynamic effects. Overall the findings suggest that morphine decreased afterload, was negatively inotropic, positively chronotropic, had no effect on cardiac work, but seemed to maintain left ventricular performance. These changes were also not related to arterial stiffness. I concluded vasodilatation is the likely mechanism of action of morphine. These findings also suggest that morphine may have beneficial effects in congestive heart failure and myocardial infarction due to its haemodynamic properties.

In chapter 7, the influence of morphine on the cardiovascular system was further clarified. An antagonist study was conducted, in which naloxone antagonized a range of morphine-induced haemodynamic effects. As the hypotensive effects of morphine in part were not antagonised by the dose of naloxone used, they might be due effects of morphine on a receptor other than a mu opioid one and therefore tolerance to these

effects might not develop. The mechanism of observed effects, however, was not related to arterial stiffness, histamine release, oxygen saturation, or end tidal CO_2.

Further work was required to clarify the potential vascular mechanisms through which opioids influence haemodynamics in healthy volunteers. Using occlusion plethysmograph and intra arteriolar infusion in chapter 8, I showed the existence of a peripheral site of action for morphine on arteries, at high concentrations 0.6 to 3 mcg/ml. These effects were dose dependent. Weal, flare and itching also developed rapidly and were dose dependant. Tachyphylaxis to the vascular effects did not develop over 30 minutes. By plasma histamine measurement, and by using pre treatment with anti-histamines, histamine was shown to have an important role for both arteriolar and skin effects. The peripheral site of action is probably mediated via mast cell release of histamine, and thus under these circumstances vascular tone is affected by transmitter release from arteriolar surrounding supporting tissues. Arteriolar effects were also mediated via nitric oxide, as L-NMMA also blocked this response.

Morphine is known to induce anaphylactoid reactions (Soter, 1999). The novel observations in this thesis may explain the pathophysiology of opioid induced non-cardiac pulmonary oedema, and anaphylactoid reactions. They may also be relevant to the suggested role of opioids in hypovolemia shock. These finding raise the possibility of the existence of a morphine effect on mast cells which at higher doses–causes histamine release which may lead to hypovolemia due to tissue redistribution of fluid as seen in the weal reaction in these studies. If further work confirms this H_1 and H_2 blockade should be considered in the management of patients with opioid induced non-cardiac pulmonary oedema, and those receiving high doses of morphine such as

surgery and acute pain, and opioid overdoses will benefit from pre-treated by H_1 and H_2 blocker.

9.3. The message of this thesis

In overdose, methadone and dihydrocodeine are haemodynamic depressors, due to vasodilatation rather than an action on arterial stiffness.

In comparison to other combinations of opioids and paracetamol, co-proxamol is more than ten times more likely to be fatal in overdose. This difference is probably caused by sodium channel blocking effects which my studies indicate are likely to be dose dependant. It should be withdrawn from the market on toxicological grounds.

In healthy volunteers, morphine is a haemodynamic depressor at doses around the therapeutic range, but this effect was not dose-dependent in my experimental model in man. Morphine decreases afterload and was negatively inotropic. These effects were partly antagonised by naloxone.

High concentrations of morphine directly affect mast cells, and release histamine. This transmitter caused weal, flare and itching. Arteriodilatation was also seen and as this was antagonised by L-NMMA, nitric oxide is one of the mediators. Antihistamines also block this affect, suggesting release of histamine. Histamine release was also observed by plasma measurement.

These findings together suggest that the haemodynamic effects of morphine can be divided into those directly related to an action on opioid receptors, probably μ, those

caused by morphine that are opioid receptor independent (histamine and nitric oxide), and those due to interaction with secondary mechanisms (O_2 and CO_2). An important effect is arterial dilatation, but arterial stiffness and catecholamines played no role in its mechanism in this work.

9.4. Future plans

I have shown the excess risk of co-proxamol in overdose, and this tablet is now gradually being withdrawn from the market. The next stage should be evaluating the impact of this withdrawal to see if mortality rate is Scotland has actually been affected.

I have shown that morphine is peripherally active. N-methylnaltrexone is a non selective opioid receptor antagonist, which has limited ability to cross blood brain barrier, (Yuan *et al.*, 1996; Yuan *et al.*, 1999; Yuan *et al.*, 2000; Yuan *et al.*, 2002; Yuan, 2003). This chemical should be tested as an antagonist as it would allow a differential of effects due to respiratory depression and those due to peripheral actions of morphine.

In opioid overdose, oxygen saturation of less than 95% was co-inside with low blood pressure. Concurrent effect of high CO_2 level should be investigated in overdose cases.

I have shown that morphine induced changes were different among individuals, and blood samples have been taken for HLA typing. Prime suspects would be HLA DR4, HLA DRB4 53, and HLA DQ8 and DQA 3011/12, which have been seen more frequently with patients with chronic idiopathic urticaria (Soter, 1999). The observed effects may activate, or be a result of activation of many pathways. Blood samples for IL-6, tPA, and PAI-1, vWF and TNF-α have also been taken, but not yet analysed.

Morphine-induced forearm blood flow changes should be tested in dependent subjects to explore the effects of dependency on these effects. I have shown that combination of H_1 and H_2 blockers antagonise the effects of morphine. The next stage would be examining their effects separately. Future studies with larger doses of naloxone, and lower doses of morphine are also suggested.

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Appendices

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Appendix I. List of ethics approval forms

Table I-I. List of ethics approval forms, *; Liability of patients undergoing research in RIE is covered by the NHS Trust.

Date	Signed	Number	Organisation
Epidemiological studies		--	--
Patient protocols			
08.10.02	Walter Hunter	MREC/02/0/85	Multi-Centre Research Ethic committee for Scotland
01.11.02	Liz Jamieson	MREC/02/0/85	Multi-Centre Research Ethic committee for Scotland
01.11.02	Walter Hunter	MREC/02/0/85	Multi-Centre Research Ethic committee for Scotland
13.01.03	Liz Jamieson	MREC/02/0/85	Multi-Centre Research Ethic committee for Scotland
03.06.03	Heather Cubie	MREC/02/0/85	Research & Development Office
Whole man healthy volunteer studies			
23.09.03	Heather Cubie	LREC/2003/3/57	Research & Development Office
25.09.03	Heather Cubie	LREC/2003/3/57	Research & Development Office
21.10.03	Liz Harden	LREC/2003/3/57	Healthy Volunteer/Student Research Ethics Committee
23.12.03	Heather Cubie	LREC/2003/3/57	Research & Development Office
25.11.03	Alastair G Reid	LREC/2003/3/57	Health and Safety Department
26.01.04	Liz Harden	LREC/2003/3/89	Lothian Research Committee 03
10.02.04	Alastair G Reid	LREC/2003/3/89	Health and Safety Department
13.03.04	Alastair G Reid	LREC/2003/3/89	Health and Safety Department
04.04.04	Liz Harden	LREC/2003/3/57	Lothian Research Committee 03
01.06.04	Liz Harden	LREC/2003/3/57	Lothian Research Committee 03
08.06.04	Heather Cubie	LREC/2003/3/57	Research & Development Office
16.06.04	Alastair G Reid	LREC/2003/3/57	Health and Safety Department
Forearm healthy volunteer studies			
26.06.04	Liz Harden	LREC/2003/3/89	Healthy Volunteers/Student Research Ethics Committee
31.08.04	Liz Harden	LREC/2003/3/89	Lothian Local Research Committee 03
19.11.04	Joyce Clearie	LREC/2003/3/89	Lothian Research Committee
29.11.04	Heather Cubie	LREC/2003/3/89	Research & Development Office
29.11.04	Alastair G Reid	LREC/2003/3/89	Health and Safety Department
22.12.04	Joyce Clearie	LREC/2003/3/89	Lothian Research Committee
24.02.05	Lyndsay Baird	LREC/2003/3/89	Lothian Research Ethics Committee

Appendix II. Equipment

Table II-I. Equipment that were used in these studies

Instruments	Model	Manufactured by	Country	Expiry date
Intra-arteriolar set				
Epidural catheter 16G	CT21 65L	PORTEX	UK	12.2008
Needle	27 SWG steel	COOPER'S NEEDLE WORK Ltd	Birmingham, UK	--
Rocialle Steriler		Rocialle Medical Limited	Cambridge, UK	--
COTTRELL Sticky Wax		COTTRELL Company	England	--
LATEX MEDICAL GLOVES		SHERMOND SURGICAL supply limited	UK	06.2005
I.V. Cannula B.D		BECTON DICKINSON Venflon™		
18 GA 1.2*45 mm Green				12.2005
17 GA 1.4*45 mm White				12.2005
20 GA1.0*32 mm Pink				12.2005
30 GA ½ 0.3*13 mm				08.2006
Sterile Needles		BD Microlance™ 3		
0.8*40 mm Green				12.2005
0.6*30 mm Blue				12.2005
Sterile Pre-injection Swabs		Isopropyl Alcohol	UK	08.2009
Transparent Dressing with Security Tab	7*8.8 cm	Tegaderm™	USA	01.2006
Topper 8 Swabs	5*5 cm	Johnson & Johnson Medical Limited	UK	03.2009
Blue line Manometer Connecting Tube		Portex Ltd	UK	02.07
Airflow Sensor Cannula	Adult size	PRO-TEK	WA, USA	--
DP 3500 Dispensing Pin			USA	09.2008

Appendix III. Raw data

Electrophysiological changes in opioid overdose

Table III-I. Electrophysiological effects of co-proxamol

Study	Age	Sex	time	Number	T.I. ECG	T.I P	P	SBP	DBP	HR	PR	QRSD	QT	QTc
1	24	1	17	20	5		70	131	64	72	152	102	384	423
1	45	0	6	14	5.5		25	137	87	70	185	77	402	434
1	31	1	20	19	4		82	135	85	80				
1	31	1	7.5	30	5.5		0			57	193	89	402	391
1	37	0	12	50	4		116			65	238	118	404	420
1	22	0	19	50	7		87	152	75	84	174	94	339	401
1	25	1	3	30	28		88			72	207	107	408	446
1	37	1					11			75	130	100	368	411
1	48	0	23		2.5		0			71	175	81	361	392
1	36	1	20		1.75		75			95	177	92	327	411
1	37	0	20	70	15	15	107	114	75	84	200	115	393	465
1	38	0	18	14	5		89			75	197	91	377	421
1	37	0	0	50	6	6	83			93	158	117	352	438
1		0	18	40	5	4	55	112	65	89	158	81	361	439
1	14	0	15	40	1.5	4.5	125	146	72	145	124	97	256	397
1	36	0		6			0	139	89	127	124	78	292	424
1	32	0	19	25	5.5	5.5	159			72	167	98	399	437
1	52	1	2		5	5	280	150	70	103	165	139	374	454
1	36	1	23		2	4	34	130	80	140	86	98	285	435
1	42	1	20	30	3	4	97	130	75	74	220	97	374	415
1	46	0	20	35	1	7	221	109	60	90	178	127	366	448
1	64	0		20			43	112	64	71	157	85	442	480
1	38	0	17	10	4.5	6	23	106	64	65	224	106	416	432
1	18	0	1	30	3	4	101			93	212	108	356	443
1	44	1	10	50	10	7.5	18	130	83	65	156	91	388	403
1	45	1	3.5	16	6	4	44	139	80	82	191	101	338	395
1	78	0	19	8	13		17	116	75	78	178	103	385	438
1	54	1	17	10	2	4	0	174	94	136	105	124	298	448
1	13	0	11	50	8	7	340			96	191	128	354	447
1					56					102	180	97	326	425
1	56	1	20	60	2	4	24	108	62	69	170	83	373	399
1	36	1	20	70	3	4	118	150	80	124	112	111	311	447
1	16	0	21	24	2	4	78	125	94	100	197	86	342	441
1	23	1	17	10	3	4	35			83	168	81	336	395
2	37	0	20	70	11	15	107	114	75	84	200	115	393	465
2	38	0	18	14	5		89			75	197	91	377	421
2	37	0	0		6	6	83			76	163	104	392	441
2		0	18	40	5	4	55	112	65	89	158	81	361	439
2	34	1	3.3	48	2	4	130	122	82	91	160	107	354	435
2	24	1	9.3	55	2	4	73	120	70	77	143	109	349	395
2	30	0	6	99	3	4		124	70	78	172	87	365	418
2	46	0	20		7	7	221	109	60	90	178	127	366	448
2	59	1	17	20	2.5	4	13			54	155	103	405	384
2	64	1	5.5	70	12.5	11	79	119	74	81	196	122	402	467
2	20	1	15		9	4	30	139	71	88	137	107	333	403
2	40	1	2	30	7	12	0	125	75	70	158	111	403	435
2	28	0	22	50	1.5	4	50	112	57	75	170	83	391	437
2	55	1	18	17	2	6	21	102	68	58	181	106	422	414
2	21	1	1.5	98	5	4	36	117	60	56	171	114	379	366
2	37	0	17	70	26	26	0	128	74	83	191	87	374	439
2	55	1			15		29	150	82	76	169	101	390	438
2	33	1		73	2	4	91			88	140	103	356	431
2	14	0		14	4	4	21	129	69	70	152	92	361	389
2		1			7	4	226			68	164	81	424	451

Study	Age	Sex	time	Number	T.I. ECG	T.I P	P	SBP	DBP	HR	PR	QRSD	QT	QTc
2		0			8	5	50	123	64	79	169	79	353	405
2	38	1	20	40	8	7	43	115	72	66	159	90	381	399
2	32	0	20	30	8	4	78	113	60	83	188	100	370	435
3						6	906							
3						2	0							
3						6	306							
3						10	505							
3						4	312							
3						4	0							
3						2	411							
3						3	84	100	60	86				
3					3.75	4	84	130	65	106	160	90		436
3						4	127							
3						7	270							
3					5	5	0	120	70	77	143	83		389
3						5	699							
3						5	0	130	70	96				
3					4.08	5	68	140	70	96	160	80		415
3						6	0	100	70	90				
3					2	4	622	110	70	80	130	85		426
3						12	609	140	80	92				
3						3	797	100	80	72				
3						66	0	120	70	72				
3					4.33	4	301	110	80	88	160	90		380
3					7.83	4	200	200	90	84	160	80		426
3						4	157	140	80	90				
3					1.5	2	298	88	50	56	200	80		388
3					9	15	0	110	70	90	240	80		500
3					13.9	15	277	124	70	96	160	80		400
3						5	432	102	72	72				
3						1.4	845	90	60	76				
3					2.58	3.7	615	115	70	72	240	80		447
3					19.5	20	302	90		60	200	140		612
3					3.5	3.8	338	138	70	72	160	90		447
3					24	26	136	130	80	88	200	140		447
3						11	352	130	90	72				
3					1.77	3.3	450	115	90	90	160	100		424
3					5.23	5.3	893	110	60	75	200	160		417
3						4.5	286	130	90	84				
3					4	4	460	110	74	80	156	104		433
3					2.17	4	0	140	80	90	160	110		471
3					6.42	7.6	593	100	70	100	180	100		481
3					3.58	4.3	0	130	88	100	200	80		313
3					0.6	4	199	118	80	96	160	80		400
3						15	66	120	60	92				
3					2.17	2	0	120	70	60	180	80		400
3					4.87	4.5	796	130	80	120	120	60		46
3					3.5	6	81	115	61	68	140	100		398
3					7	6.7	715	114	75	100	150	120		343
3					5.17	5.2	179	104	64	94	160	100		491
3					3.5	7	509	112	75	86	170	100		404
3					1.75			107	62	103	140	90		445
3					5	5	1092	121	84	76	200	100		472
3					19.2	19	0	109	72	62	180	100		457
3					8.5	5.3	132	125	85	50	160	100		377
3					10.8	11	111	127	70	70	150	110		428
3					4.13	4.3	626	134	72	78	200	80		449
3					8.3	16	0	113	73	78	160	110		479
3					2.08	4	545	130	80	100	180	100		437
3					3	4.5	186	145	87	102	140	90		443
3					2.23			133	70	80	125	80		487
3					3	4	0	80	50	62	140	90		456
3					7.17	7.3	502	182	99	80	160	110		458
3					4.98	4	123	155	101	94	160	85		473
3					3.67	4	69	110	70	80	130	100		478

Study	Age	Sex	time	Number	T.I. ECG	T.I P	P	SBP	DBP	HR	PR	QRSD	QT	QTc
3					145						140	100		383
3					1.28	3.8	76	123	74	79	180	80		426
3					7.25	7	94	184	123	114	180	100		421
3					13.7	13	222	150	80	80	180	90		427
3					1.25	3.8	451	143	77	95	160	80		425
3							15	150	100	62				
3					4.92	4.7	0				160	100		462
3					1.32	2.3	40	148	97	128	160	100		463
3					3.43	4	0	161	107	104	140	80		427
3					17.6	18	0	126	70	75	140	90		444
3					2.18	4.3	259	108	54	71	160	80		507
3							164	144	83	60	170	85		440
3						3.5	131	134	78	71				
3					9	10	0	156	110	70	180	90		403
3					2.82	3	465	117	67	83	160	80		453
3					7.08	2	79	124	74	86	180	90		447
3					1.6	1.5	0	131	85	128	160	85		434
3							7	129	93	72	170	80		437
3					6.75	7.3	24	152	83	102	180	80		464
3							84	137	87	61	160	100		394
3					2.87	4	332	152	100	55	140	80		432
3					2.55	2.3	388	115	82	97	180	80		438
3						2.8	535	145	87	81				
3					5.1	5	124	134	81	70	160	85		425
3					4.28	4	71	138	91	82	200	80		447
3					1.58			140	27	106	160	80		417
3							5	110	65	110	180	80		528
3					1.07	4.6	6	130	88	110	160	75		475
3					5.25			109	72	90	180	80		447
3					1.2	4.7	22	134	88	80	140	80		436

Study; 1 retrospective Edinburgh, 2 prospective Edinburgh, 3 retrospective, Australia, Sex; 1 male, P; paracetamol level, T.I; Time Interval

Haemodynamic changes in opioid overdose study

Table III-II. Haemodynamic effects of dihydrocodeine, methadone and paracetamol overdoses

	Drug	Age	Sex	TS	HR	SBP	DBP	MBP	ASBP	ADBP	PPP	APP	AI	DD	ESP	O_2	Time interval
1	D	36	1	3	87	98	51	67	83	51	47	32	35	63	67	98	0-5
2	D	.	.	10	78	102	52	72	89	54	50	35	5	61	76	96	6-11
3	D	.	.	12	75	123	60	79	105	61	63	44	15	63	72	98	12-17
4	D	.	.	19	72	127	62	84	102	63	65	39	19	60	69	99	18-23
5	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	D	37	0	10	55	123	48	76	114	62	75	52	23	66	79	97	6-11
7	D	.	.	17	55	94	60	73	88	61	34	27	29	66	84	96	12-17
8	D	.	.	24	57	139	59	85	115	60	80	55	43	63	87	98	18-23
9	D	27	1	5	76	102	48	66	95	64	54	31	7	65	83	96	0-5
10	D	.	.	8	76	116	65	80	97	67	51	30	-3	67	89	96	6-11
11	D	.	.	12	79	123	81	92	106	82	42	24	-8	65	96	95	12-17
12	D	.	.	19	84	122	82	93	105	83	40	22	-9	63	96	95	18-23
13	D	48	0	5	80	70	40	58	72	46	30	26	11	61	75	91	0-5
14	D	.	.	11	86	84	47	62	76	49	37	27	25	64	73	91	6-11
15	D	.	.	17	86	84	47	64	78	50	37	28	28	55	71	93	12-17
16	D	.	.	19	88	79	44	57	71	45	35	26	26	56	62	92	18-23
17	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
18	D	29	0	10	91	130	79	98	118	81	51	37	27	57	108	95	6-11
19	D	.	.	16	87	117	64	80	104	65	53	39	17	60	91	95	12-17
20	D	.	.	23	93	132	77	97	119	79	55	40	38	53	104	95	18-23
21	D	22	1	5	58	110	52	69	92	53	58	39	7	67	74	97	0-5
22	D	.	.	10	61	108	53	71	93	54	55	39	2	65	75	97	6-11
23	D	.	.	14	59	104	59	73	89	60	45	29	3	67	76	98	12-17
24	D	.	.	23	67	101	47	64	84	48	54	36	22	63	70	98	18-23
25	D	38	0	5	82	121	68	88	109	70	53	39	33	41	98	84	0-5
26	D	.	.	11	81	123	66	89	110	69	57	41	21	41	99	85	6-11
27	D	.	.	16	82	135	89	102	124	82	46	42	30	42	114	95	12-17
28	D	.	.	20	72	143	75	100	132	77	68	55	27	36	123	96	18-23
29	D	23	0	5	77	106	51	72	91	53	55	38	23	41	80	98	0-5
30	D	.	.	11	59	112	59	77	101	59	53	42	13	32	91	98	6-11
31	D	.	.	16	66	109	64	82	100	66	45	34	20	36	91	98	12-17
32	D	45	1	2	75	126	57	83	117	59	69	58	27	41	93	98	0-5
33	D	.	.	11	76	138	56	82	116	58	82	58	14	42	91	98	6-11
34	D	.	.	14	77	135	59	87	126	60	76	66	37	42	98	97	12-17
35	D	.	.	19	70	137	62	91	130	64	75	66	39	39	105	98	18-23
36	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
37	D	55	0	11	72	94	60	73	87	61	34	26	11	31	84	96	6-11
38	D	.	.	14	75	103	73	85	97	74	30	23	34	33	94	98	12-17
39	D	.	.	21	76	106	76	88	100	77	30	23	34	34	97	98	18-23
40	P	19	0	5	69	130	84	102	121	85	46	36	2	68	96	99	0-5
41	P	.	.	10	67	123	80	96	114	81	43	33	24	71	102	99	6-11

	Drug	Age	Sex	TS	HR	SBP	DBP	MBP	ASBP	ADBP	PPP	APP	AI	DD	ESP	O_2	Time interval
42	P	.	.	13	72	126	78	94	119	84	.	35	17	72	103	99	12-17
43	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
44	P	34	1	4	93	137	94	108	122	97	43	25	2	61	113	98	0-5
45	P	.	.	11	90	137	79	99	116	83	58	33	22	59	103	98	6-11
46	P	.	.	15	90	143	92	109	124	95	51	29	15	60	114	98	12-17
47	P	.	.	21	88	138	76	99	117	80	62	37	10	60	106	99	18-23
48	P	52	1	4	89	139	81	100	125	87	58	38	16	59	106	98	0-5
49	P	.	.	11	84	136	83	99	125	83	53	42	23	59	108	99	6-11
50	P	.	.	17	87	134	85	100	124	87	49	37	21	57	108	99	12-17
51	P	.	.	20	90	149	82	102	131	84	67	47	8	60	114	99	18-23
52	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
53	P	23	1	11	68	134	75	94	110	76	59	34	5	71	103	99	6-11
54	P	.	.	13	65	134	75	90	110	76	59	34	-5	67	97	98	12-17
55	P	.	.	19	83	122	68	83	100	69	54	31	-9	60	88	99	18-23
56	P	19	1	5	60	123	66	84	104	67	57	37	10	69	93	98	0-5
57	P	.	.	10	57	119	68	84	99	69	51	30	3	70	91	99	6-11
58	P	.	.	15	58	116	70	84	99	71	46	28	7	70	92	100	12-17
59	P	.	.	22	56	116	70	84	99	71	46	28	7	71	91	100	18-23
60	P	40	1	2	83	130	61	89	107	69	69	38	7	41	91	98	0-5
61	P	.	.	11	66	135	63	91	108	66	72	42	11	35	94	97	6-11
62	P	.	.	15	70	132	56	90	107	66	76	41	9	37	89	98	12-17
63	P	.	.	19	65	128	59	88	106	65	69	41	9	35	90	98	18-23
64	P	55	0	2	63	158	78	105	142	80	80	62	24	34	120	97	0-5
65	P	.	.	11	65	152	72	104	141	76	80	65	33	33	130	98	6-11
66	P	.	.	14	64	157	72	101	139	74	85	65	24	35	119	99	12-17
67	P	.	.	19	65	151	74	102	137	76	77	61	24	35	119	99	18-23
68	P	39	1	5	79	159	74	109	142	78	85	64	25	40	127	99	0-5
69	P	.	.	9	81	155	72	108	134	75	83	59	20	42	115	99	6-11
70	P	.	.	14	80	155	75	104	137	78	80	59	22	41	121	100	12-17
71	P	.	.	19	80	157	77	107	137	80	80	57	19	41	119	99	18-23
72	P	31	1	3	83	146	69	94	126	70	77	56	9	42	102	97	0-5
73	P	.	.	7	72	130	66	88	116	64	64	52	24	37	100	97	6-11
74	P	.	.	17	84	133	62	85	111	63	71	48	14	43	95	97	12-17
75	P	.	.	23	80	135	64	84	111	67	71	44	9	40	91	97	18-23
76	P	19	0	9	67	115	57	75	94	58	58	36	11	36	83	99	6-11
77	P	.	.	13	75	115	55	77	97	58	60	39	18	40	85	99	12-17
78	P	.	.	19	80	115	57	79	96	60	58	36	13	42	85	98	18-23
79	M	43	0	11	84	128	104	114	124	73	24	51	32	62	92	80	6-11
80	M	.	.	17	83	109	72	89	105	74	37	31	35	64	91	85	12-17
81	M	.	.	22	80	125	79	94	119	77	46	52	37	68	92	94	18-23
82	M	29	1	4	115	108	57	75	90	62	51	28	3	47	83	98	0-5
83	M	.	.	7	71	93	53	67	81	55	40	26	19	48	81	98	6-11
84	M	38	1	4	88	121	92	103	109	91	29	18	7	54	95	95	0-5
85	M	.	.	11	89	124	96	76	110	80	28	30	13	53	97	97	6-11
86	M	.	.	16	94	141	86	106	122	89	55	33	15	54	95	95	12-17
87	M	.	.	20	98	155	91	109	128	94	64	34	-26	57	93	85	18-23
88	M	36	1	11	80	112	53	72	92	56	59	36	5	65	78	95	6-11
89	M	.	.	16	73	118	67	85	105	69	51	36	13	64	77	95	12-17
90	M	.	.	19	83	135	74	97	121	76	61	45	31	65	75	98	18-23
91	M	23	1	10	68	121	66	81	98	67	55	31	-9	55	83	98	6-11

	Drug	Age	Sex	TS	HR	SBP	DBP	MBP	ASBP	ADBP	PPP	APP	AI	DD	ESP	O_2	Time interval
92	M	.	.	17	56	106	75	85	96	76	31	20	10	56	85	98	12-17
93	M	.	.	20	54	116	74	88	101	79	42	22	6	59	87	98	18-23
94	M	.	0	7	71	92	52	69	88	54	40	34	30	63	79	95	6-11
95	M	.	.	14	74	92	52	69	88	54	40	34	38	62	79	96	12-17
96	M	.	.	20	75	102	56	76	97	59	46	38	38	61	89	96	18-23
97	M	31	1	5	87	110	65	77	92	68	45	24	-29	38	78	96	0-5
98	M	.	.	11	83	115	72	86	99	75	43	24	-16	37	89	96	6-11
99	M	.	.	15	87	128	72	90	105	75	56	30	-6	40	96	95	12-17
100	M	.	.	23	84	130	78	95	103	70	52	33	-11	39	97	96	18-23
101	M	26	1	4	76	115	68	81	96	69	47	27	-3	37	86	97	0-5
102	M	.	.	11	75	115	68	85	99	70	47	29	5	37	91	97	6-11
103	M	.	.	13	73	122	70	88	102	72	52	30	10	36	94	98	12-17
104	M	.	.	22	79	128	78	95	108	71	60	37	6	35	90	97	18-23
105	M	23	0	3	58	100	57	77	94	63	43	31	12	35	81	98	0-5
106	M	.	.	11	66	105	59	85	101	67	46	34	18	30	98	99	6-11
107	M	.	.	14	68	106	62	84	100	72	44	28	8	36	92	98	12-17
109	M	.	.	20	64	105	62	83	103	74	43	29	10	34	95	98	18-23
109	M	53	0	3	83	119	60	95	111	71	59	40	32	39	103	95	0-5
110	M	.	.	9	69	119	60	94	112	62	59	50	42	36	99	97	6-11
111	M	.	.	17	78	125	65	97	115	68	60	47	27	35	113	97	12-17
112	3	.	.	19	76	128	68	92	117	72	60	45	35	38	114	97	18-23

DR; Drug, HR; heart rate, SBP; systolic blood pressure, DBP; diastolic blood pressure, ASBP, aortic systolic blood pressure, ADBP; aortic diastolic blood pressure, MBP, measnm blood pressure, ESP, end systolic pressure, AI; augmentation index, DD; diastolic duration, TS; time interval to sphygmogarphy, O_2 saturation, T; time interval after exposure (h), D; dihydrocodeine, P; paracetamol, M; methadone.

Morphine versus saline study

Table III-III-I. Haemodynamic effects of morphine versus saline

	Initials	Age	Height	Weight	Visit	Dose	SBP	SBP %	DBP	DBP %	SSBP	SSBP%	SDBP	SDBP%
1	PD	41	172	91	M	.00	127	0	86	0	136	0	88	0
2	PD	41	172	91	M	.25	121	-5	80	-8	123	-11	85	-4
3	PD	41	172	91	M	.50	127	0	80	-8	127	-7	89	1
4	PD	41	172	91	M	1.00	128	1	85	-1	129	-5	91	3
5	PD	41	172	91	M	2.00	127	0	80	-8	134	-1	89	1
6	PD	41	172	91	M	4.00	133	5	85	-1	135	-1	95	7
7	PD	41	172	91	M	8.00	133	5	90	4	135	-1	93	5
8	PD	41	172	91		.00	110	0	73	0	115	0	80	0
9	PD	41	172	91	P	.25	112	2	70	-4	132	13	89	10
10	PD	41	172	91	P	.50	114	4	75	3	120	4	79	-1
11	PD	41	172	91	P	1.00	111	1	71	-3	117	2	75	-7
12	PD	41	172	91	P	2.00	117	6	74	1	127	9	72	-11
13	PD	41	172	91	P	4.00	121	9	74	1	136	15	80	0
14	PD	41	172	91	P	8.00	120	8	72	-1	110	-5	78	-3
15	RK	49	166	76	M	.00	122	0	76	0	117	0	81	0
16	RK	49	166	76	M	.25	120	-2	79	4	117	0	82	1
17	RK	49	166	76	M	.50	116	-5	78	3	115	-2	83	2
18	RK	49	166	76	M	1.00	115	-6	75	-1	111	-5	78	-4
19	RK	49	166	76	M	2.00	114	-7	69	-10	105	-11	76	-7
20	RK	49	166	76	M	4.00	106	-15	62	-23	108	-8	74	-9
21	RK	49	166	76	M	8.00	103	-18	61	-25	108	-8	71	-14
22	RK	49	166	76	P	.00	114	0	78	0	113	0	79	0
23	RK	49	166	76	P	.25	116	2	76	-3	133	15	75	-5
24	RK	49	166	76	P	.50	123	7	78	0	122	7	76	-4
25	RK	49	166	76	P	1.00	126	10	78	0	116	3	77	-3
26	RK	49	166	76	P	2.00	123	7	73	-7	115	2	76	-4
27	RK	49	166	76	P	4.00	133	14	75	-4	117	3	79	0
28	RK	49	166	76	P	8.00	131	13	79	1	121	7	77	-3
29	AB1	20	174	78	M	.00	133	0	56	0	133	0	61	0
30	AB1	20	174	78	M	.25	121	-10	62	10	122	-9	65	6
31	AB1	20	174	78	M	.50	120	-11	59	5	112	-19	58	-5
32	AB1	20	174	78	M	1.00	113	-18	61	8	117	-14	57	-7
33	AB1	20	174	78	M	2.00	127	-5	62	10	127	-5	59	-3
34	AB1	20	174	78	M	4.00	117	-14	57	2	121	-10	55	-11
35	AB1	20	174	78	M	8.00	128	-4	58	3	126	-6	65	6
36	AB1	20	174	78	P	.00	131	0	61	0	137	0	68	0
37	AB1	20	174	78	P	.25	143	8	55	-11	122	-12	77	12
38	AB1	20	174	78	P	.50	135	3	62	2	131	-5	72	6
39	AB1	20	174	78	P	1.00	131	0	59	-3	131	-5	68	0
40	AB1	20	174	78	P	2.00	131	0	66	8	122	-12	57	-19
41	AB1	20	174	78	P	4.00	113	-16	66	8	131	-5	71	4
42	AB1	20	174	78	P	8.00	120	-9	73	16	111	-23	55	-24
43	WP	25	177	76	M	.00	113	0	68	0	122	0	71	0
44	WP	25	177	76	M	.25	121	7	71	4	123	1	73	3
45	WP	25	177	76	M	.50	121	7	69	1	115	-6	67	-6
46	WP	25	177	76	M	1.00	108	-5	65	-5	109	-12	69	-3
47	WP	25	177	76	M	2.00	117	3	68	0	121	-1	70	-1
48	WP	25	177	76	M	4.00	133	15	77	12	129	5	74	4
49	WP	25	177	76	M	8.00	121	7	69	1	110	-11	66	-8
50	WP	25	177	76	P	.00	114	0	72	0	116	0	79	0
51	WP	25	177	76	P	.25	116	2	72	0	121	4	79	0
52	WP	25	177	76	P	.50	110	-4	73	1	122	5	77	-3
53	WP	25	177	76	P	1.00	114	0	71	-1	126	8	78	-1
54	WP	25	177	76	P	2.00	114	0	66	-9	124	6	77	-3
55	WP	25	177	76	P	4.00	116	2	74	3	129	10	79	0
56	WP	25	177	76	P	8.00	129	12	70	-3	128	9	80	1
57	KN	34	165	61	M	.00	113	0	63	0	111	0	61	0

	Initials	Age	Height	Weight	Visit	Dose	SBP	SBP%	DBP	DBP%	SSBP	SSBP%	SDBP	SDBP%
58	KN	34	165	61	M	.25	101	-12	54	-17	105	-6	62	2
59	KN	34	165	61	M	.50	100	-13	56	-13	97	-14	62	2
60	KN	34	165	61	M	1.00	115	2	68	7	110	-1	66	8
61	KN	34	165	61	M	2.00	107	-6	60	-5	108	-3	58	-5
62	KN	34	165	61	M	4.00	112	-1	54	-17	102	-9	53	-15
63	KN	34	165	61	M	8.00	97	-16	46	-37	90	-23	54	-13
64	KN	34	165	61	P	.00	106	0	63	0	107	0	68	0
65	KN	34	165	61	P	.25	109	3	66	5	104	-3	63	-8
66	KN	34	165	61	P	.50	120	12	61	-3	94	-14	48	-42
67	KN	34	165	61	P	1.00	96	-10	55	-15	84	-27	55	-24
68	KN	34	165	61	P	2.00	103	-3	56	-13	96	-11	56	-21
69	KN	34	165	61	P	4.00	101	-5	59	-7	98	-9	53	-28
70	KN	34	165	61	P	8.00	101	-5	55	-15	106	-1	57	-19
71	AB2	30	189	99	M	.00	136	0	62	0	135	0	70	0
72	AB2	30	189	99	M	.25	138	1	66	6	147	8	71	1
73	AB2	30	189	99	M	.50	133	-2	63	2	135	0	60	-17
74	AB2	30	189	99	M	1.00	134	-1	67	7	142	5	79	11
75	AB2	30	189	99	M	2.00	116	-17	58	-7	122	-11	68	-3
76	AB2	30	189	99	M	4.00	136	0	69	10	144	6	76	8
77	AB2	30	189	99	M	8.00	151	10	66	6	138	2	62	-13
78	AB2	30	189	99	P	.00	139	0	59	0	129	0	66	0
79	AB2	30	189	99	P	.25	136	-2	60	2	132	2	68	3
80	AB2	30	189	99	P	.50	134	-4	57	-4	136	5	66	0
81	AB2	30	189	99	P	1.00	135	-3	54	-9	142	9	70	6
82	AB2	30	189	99	P	2.00	134	-4	58	-2	129	0	75	12
83	AB2	30	189	99	P	4.00	137	-1	62	5	135	4	62	-6
84	AB2	30	189	99	P	8.00	127	-9	59	0	134	4	63	-5
85	DC	24	174	72	M	.00	122	0	66	0	122	0	68	0
86	DC	24	174	72	M	.25	105	-16	57	-16	107	-14	59	-15
87	DC	24	174	72	M	.50	106	-15	53	-25	105	-16	54	-26
88	DC	24	174	72	M	1.00	108	-13	56	-18	113	-8	64	-6
89	DC	24	174	72	M	2.00	107	-14	59	-12	110	-11	60	-13
90	DC	24	174	72	M	4.00	110	-11	56	-18	121	-1	72	6
91	DC	24	174	72	M	8.00	109	-12	54	-22	111	-10	67	-1
92	DC	24	174	72	P	.00	112	0	63	0	122	0	67	0
93	DC	24	174	72	P	.25	114	2	60	-5	109	-12	66	-2
94	DC	24	174	72	P	.50	113	1	63	0	116	-5	66	-2
95	DC	24	174	72	P	1.00	111	-1	60	-5	114	-7	69	3
96	DC	24	174	72	P	2.00	111	-1	62	-2	115	-6	72	7
97	DC	24	174	72	P	4.00	114	2	66	5	122	0	69	3
98	DC	24	174	72	P	8.00	112	0	65	3	116	-5	76	12
99	DS	50	169	93	M	.00	143	0	88	0	146	0	91	0
100	DS	50	169	93	M	.25	134	-7	81	-9	146	0	93	2
101	DS	50	169	93	M	.50	133	-8	87	-1	142	-3	91	0
102	DS	50	169	93	M	1.00	132	-8	90	2	146	0	95	4
103	DS	50	169	93	M	2.00	137	-4	83	-6	147	1	93	2
104	DS	50	169	93	M	4.00	136	-5	80	-10	153	5	98	7
105	DS	50	169	93	M	8.00	132	-8	86	-2	138	-6	94	3
105	DS	50	169	93	P	.00	116	0	79	0	126	0	83	0
107	DS	50	169	93	P	.25	122	5	79	0	128	2	87	5
108	DS	50	169	93	P	.50	128	9	79	0	134	6	84	1
109	DS	50	169	93	P	1.00	120	3	84	6	128	2	85	2
110	DS	50	169	93	P	2.00	122	5	79	0	146	14	91	9
111	DS	50	169	93	P	4.00	127	9	82	4	135	7	88	6
112	DS	50	169	93	P	8.00	131	11	86	8	142	11	93	11

SBP; systolic blood pressure, SBP%; percentage of change from baseline of systolic blood pressure, DBP; diastolic blood pressure, DBP%; percentage of change from baseline of diastolic blood pressure, sSBP; sitting systolic blood pressure sSBP%; percentage of change from baseline of sitting systolic blood pressure, sDBP; sitting diastolic blood pressure, sDBP%; percentage of change from baseline of sitting diastolic blood pressure.

Table III-III-II. Haemodynamic effects of morphine versus saline (continued)

	Visit	dose	O_2	O_2 %	CI	CI%	SI	SI%	EDI	EDI %	PFI	PFI %	EF	EF%
1	M	.00	97	0	3.3	0	49.3	0	87.1	0	332	0	57	0
2	M	.25	98	1	3.3	0	48.5	-2	84.9	-3	319	-4	58	2
3	M	.50	96	-1	3.1	-6	45.5	-8	80.6	-8	310	-7	57	0
4	M	1.00	97	0	3.2	-3	45.9	-7	82.3	-6	314	-6	57	0
5	M	2.00	98	1	3.1	-6	47.2	-4	87.9	1	319	-4	54	-6
6	M	4.00	100	3	2.9	-14	41.3	-19	85.8	-2	301	-10	49	-16
7	M	8.00	99	2	2.6	-27	34.0	-45	79.1	-10	287	-16	53	-8
8	P	.00	98	0	2.9	0	48.0	0	90.0	0	304	0	53	0
9	P	.25	97	-1	3.1	6	51.0	6	95.0	5	318	4	53	0
10	P	.50	96	-2	2.9	0	47.0	-2	90.0	0	302	-1	51	-4
11	P	1.00	97	-1	2.9	0	49.0	2	90.0	0	301	-1	54	2
12	P	2.00	97	-1	2.8	-4	55.0	13	87.0	-3	287	-6	52	-2
13	P	4.00	96	-2	2.8	-4	49.0	2	88.0	-2	298	-2	55	4
14	P	8.00	98	0	2.4	-21	42.0	-14	85.0	-6	275	-11	50	-6
15	M	.00	97	0	3.5	0	53.0	0	82.0	0	315	0	65	0
16	M	.25	97	0	3.3	-6	48.0	-10	74.0	-11	285	-11	63	-3
17	M	.50	97	0	3.1	-13	47.0	-13	74.0	-11	284	-11	63	-3
18	M	1.00	96	-1	3.0	-17	47.0	-13	72.0	-14	279	-13	65	0
19	M	2.00	97	0	3.1	-13	48.0	-10	74.0	-11	281	-12	64	-2
20	M	4.00	95	-2	3.1	-13	56.0	5	86.0	5	315	0	66	2
21	M	8.00	96	-1	3.1	-13	53.0	0	84.0	2	306	-3	63	-3
22	P	.00	98	0	3.9	0	48.0	0	81.0	0	314	0	60	0
23	P	.25	98	0	3.9	0	53.0	9	83.0	2	332	5	63	5
24	P	.50	99	1	3.6	-8	45.0	-7	73.0	-11	292	-8	61	2
25	P	1.00	97	-1	3.9	0	54.0	11	86.0	6	336	7	62	3
26	P	2.00	98	0	3.4	-15	47.0	-2	75.0	-8	348	10	63	5
27	P	4.00	99	1	3.9	0	50.0	4	77.0	-5	310	-1	64	6
28	P	8.00	100	2	4.0	3	54.0	11	85.0	5	328	4	64	6
29	M	.00	98	0	3.7	0	64.0	0	104.0	0	392	0	61	0
30	M	.25	97	-1	3.9	5	69.0	7	107.0	3	415	6	64	5
31	M	.50	98	0	3.5	-6	61.0	-5	99.0	-5	381	-3	61	0
32	M	1.00	98	0	3.4	-9	63.0	-2	101.0	-3	374	-5	62	2
33	M	2.00	97	-1	3.3	-12	59.0	-8	92.0	-13	357	-10	63	3
34	M	4.00	98	0	3.0	-23	61.0	-5	97.0	-7	355	-10	63	3
35	M	8.00	97	-1	3.3	-12	62.0	-3	97.0	-7	369	-6	63	3
36	P	.00	97	0	3.5	0	55.0	0	98.0	0	359	0	57	0
37	P	.25	98	1	3.5	0	58.0	5	99.0	1	371	3	58	2
38	P	.50	98	1	3.3	-6	60.0	8	105.0	7	373	4	57	0
39	P	1.00	97	0	3.3	-6	61.0	10	104.0	6	363	1	58	2
40	P	2.00	98	1	3.2	-9	62.0	11	96.0	-2	348	-3	59	3
41	P	4.00	98	1	3.0	-17	59.0	7	98.0	0	355	-1	60	5
42	P	8.00	97	0	3.0	-17	56.0	2	98.0	0	347	-3	60	5
43	M	.00	97	0	3.4	0	59.0	0	92.0	0	342	0	63	0
44	M	.25	95	-2	3.1	-10	52.0	-13	86.0	-7	316	-8	60	-5
45	M	.50	97	0	3.2	-6	56.0	-5	92.0	0	338	-1	60	-5
46	M	1.00	98	1	3.3	-3	57.0	-4	93.0	1	343	0	61	-3
47	M	2.00	95	-2	3.0	-13	54.0	-9	89.0	-3	328	-4	59	-7
48	M	4.00	95	-2	3.0	-13	50.0	-18	80.0	-15	299	-14	62	-2
49	M	8.00	96	-1	3.1	-10	51.0	-16	83.0	-11	308	-11	61	-3
50	P	.00	97	0	3.1	0	46.0	0	78.0	0	299	0	59	0
51	P	.25	97	0	3.4	9	57.0	19	91.0	14	342	13	62	5
52	P	.50	97	0	3.2	3	55.0	16	92.0	15	338	12	59	0
53	P	1.00	97	0	3.2	3	50.0	8	83.0	6	321	7	60	2
54	P	2.00	97	0	3.4	9	57.0	19	88.0	11	329	9	64	8
55	P	4.00	97	0	3.5	11	59.0	22	93.0	16	352	15	62	5
56	P	8.00	98	1	3.2	3	48.0	4	82.0	5	311	4	58	-2
57	M	.00	99	0	3.1	0	54.0	0	87.0	0	318	0	61	0
58	M	.25	99	0	3.1	0	54.0	0	86.0	-1	329	3	62	2
59	M	.50	98	-1	3.1	0	52.0	-4	86.0	-1	328	3	61	0
60	M	1.00	98	-1	2.6	-19	45.0	-20	75.0	-16	287	-11	60	-2

	Visit	dose	O_2	O_2%	CI	CI%	SI	SI%	EDI	EDI %	PFI	PFI %	EF	EF%
61	M	2.00	96	-3	2.8	-11	51.0	-6	88.0	1	323	2	58	-5
62	M	4.00	97	-2	3.0	-3	52.0	-4	83.0	-5	323	2	63	3
63	M	8.00	94	-5	3.2	3	57.0	5	88.0	1	338	6	64	5
64	P	.00	97	0	2.7	0	48.0	0	79.0	0	306	0	60	0
65	P	.25	97	0	2.5	-8	49.0	2	83.0	5	312	2	58	-3
66	P	.50	97	0	2.5	-8	46.0	-4	83.0	5	295	-4	56	-7
67	P	1.00	98	1	2.5	-8	47.0	-2	80.0	1	295	-4	58	-3
68	P	2.00	99	2	2.5	-8	45.0	-7	79.0	0	286	-7	56	-7
69	P	4.00	99	2	2.6	-4	42.0	-14	72.0	-10	277	-10	57	-5
70	P	8.00	99	2	2.5	-8	49.0	2	96.0	18	316	3	51	-18
71	M	.00	97	0	4.1	0	61.0	0	102.0	0	382	0	60	0
72	M	.25	99	2	3.9	-5	61.0	0	105.0	3	385	1	58	-3
73	M	.50	97	0	3.9	-5	64.0	5	108.0	6	375	-2	59	-2
74	M	1.00	96	-1	3.9	-5	58.0	-5	99.0	-3	361	-6	58	-3
75	M	2.00	97	0	3.4	-21	57.0	-7	100.0	-2	344	-11	57	-5
76	M	4.00	96	-1	3.8	-8	63.0	3	107.0	5	377	-1	59	-2
77	M	8.00	97	0	3.3	-24	51.0	-20	94.0	-9	324	-18	54	-11
78	P	.00	97	0	4.7	0	82.0	0	133.0	0	474	0	61	0
79	P	.25	96	-1	4.3	-9	79.0	-4	131.0	-2	462	-3	60	-2
80	P	.50	96	-1	4.0	-18	73.0	-12	123.0	-8	429	-10	59	-3
81	P	1.00	96	-1	3.9	-21	72.0	-14	119.0	-12	423	-12	60	-2
82	P	2.00	98	1	4.2	-12	82.0	0	136.0	2	472	0	60	-2
83	P	4.00	98	1	3.9	-21	73.0	-12	121.0	-10	436	-9	60	-2
84	P	8.00	98	1	3.9	-21	74.0	-11	123.0	-8	438	-8	60	-2
85	M	.00	97	0	3.4	0	50.0	0	92.0	0	341	0	54	0
86	M	.25	96	-1	3.7	8	49.0	-2	91.0	-1	343	1	52	-4
87	M	.50	97	0	3.4	0	49.0	-2	86.0	-7	332	-3	53	-2
88	M	1.00	97	0	3.8	11	57.0	12	97.0	5	376	9	56	4
89	M	2.00	95	-2	3.5	3	52.0	4	98.0	6	339	-1	58	7
90	M	4.00	97	0	3.8	11	54.0	7	94.0	2	352	3	58	7
91	M	8.00	94	-3	3.8	11	55.0	9	91.0	-1	348	2	57	5
92	P	.00	96	0	4.0	0	61.0	0	104.0	0	403	0	58	0
93	P	.25	96	0	3.9	-3	63.0	3	108.0	4	414	3	57	-2
94	P	.50	96	0	3.7	-8	58.0	-5	109.0	5	387	-4	53	-9
95	P	1.00	97	1	3.8	-5	63.0	3	111.0	6	404	0	56	-4
96	P	2.00	97	1	3.5	-14	55.0	-11	99.0	-5	375	-7	55	-5
97	P	4.00	97	1	3.7	-8	61.0	0	107.0	3	390	-3	56	-4
98	P	8.00	98	2	3.6	-11	60.0	-2	104.0	0	382	-5	57	-2
99	M	.00	93	0	2.6	0	42.0	0	75.0	0	256	0	56	0
100	M	.25	92	-1	2.4	-8	42.0	0	72.0	-4	242	-6	58	3
101	M	.50	94	1	2.5	-4	43.0	2	72.0	-4	248	-3	58	3
102	M	1.00	96	3	2.5	-4	40.0	-5	71.0	-6	245	-4	56	0
103	M	2.00	95	2	2.5	-4	39.0	-8	71.0	-6	243	-5	55	-2
104	M	4.00	94	1	2.7	4	43.0	2	74.0	-1	251	-2	59	5
105	M	8.00	94	1	2.4	-8	39.0	-8	70.0	-7	237	-8	56	0
105	P	.00	93	0	2.9	0	40.0	0	67.0	0	254	0	59	0
107	P	.25	91	-2	2.7	-7	40.0	0	70.0	4	251	-1	57	-4
108	P	.50	92	-1	2.4	-21	43.0	7	75.0	11	255	0	56	-5
109	P	1.00	94	1	2.6	-12	45.0	11	82.0	18	272	7	55	-7
110	P	2.00	95	2	2.4	-21	41.0	2	76.0	12	258	2	55	-7
111	P	4.00	95	2	2.3	-26	39.0	-3	74.0	9	247	-3	53	-11
112	P	8.00	95	2	2.4	-21	40.0	0	73.0	8	243	-5	55	-7

O_2;oxygen saturation, O_2%;percentage of change from baseline of oxygen saturation, CI; cardiac index, CI%; percentage of change from baseline of cardiac index, SI; stroke index, SI%; percentage of change from baseline of cardiac index, stroke index EDI; end diastolic index, EDI; percentage of change from baseline of end diastolic index, EF; ejection fraction, EF%; percentage of change from baseline of ejection fraction, PFI; peak flow index, PFI%; percentage of change from baseline of peak flow index.

Table III-III-III. Haemodynamic effects of morphine versus saline (continued)

	Visit	Dose	HR	HR %	TFI	TFI %	IC	IC%	ER	ER %	STR	STR %	ESP	ESP %
1	M	.00	66	0	26.2	0	38	0	33	0	39	0	110	0
2	M	.25	65	-2	26.8	2	36	-6	34	3	39	0	106	-4
3	M	.50	66	0	26.9	3	35	-9	33	0	40	3	110	0
4	M	1.00	67	1	27.1	3	36	-6	34	3	41	5	111	1
5	M	2.00	62	-6	27.1	3	36	-6	32	-3	45	13	110	0
6	M	4.00	66	0	27.4	4	34	-12	32	-3	57	32	117	6
7	M	8.00	66	0	27.6	5	35	-9	32	-3	47	17	117	6
8	P	.00	61	0	25.4	0	42	0	32	0	40	0	96	0
9	P	.25	60	-2	24.8	-2	44	5	32	0	39	-3	97	1
10	P	.50	62	2	24.8	-2	42	0	32	0	43	7	99	3
11	P	1.00	59	-3	24.8	-2	42	0	32	0	39	-3	96	0
12	P	2.00	62	2	24.9	-2	40	-5	32	0	42	5	101	5
13	P	4.00	57	-7	24.8	-2	42	0	31	-3	38	-5	104	8
14	P	8.00	58	-5	25.0	-2	38	-11	29	-10	46	13	102	6
15	M	.00	66	0	28.5	0	49	0	37	0	28	0	103	0
16	M	.25	69	4	28.0	-2	45	-9	38	3	28	0	104	1
17	M	.50	66	0	28.0	-2	44	-11	37	0	30	7	102	-1
18	M	1.00	64	-3	28.2	-1	43	-14	36	-3	27	-4	101	-2
19	M	2.00	66	0	28.0	-2	44	-11	37	0	28	0	104	1
20	M	4.00	55	-20	28.6	0	49	0	33	-12	26	-8	90	-14
21	M	8.00	59	-12	28.6	0	47	-4	34	-9	30	7	89	-16
22	P	.00	81	0	28.6	0	49	0	42	0	34	0	97	0
23	P	.25	73	-11	28.8	1	52	6	39	-8	30	-13	100	3
24	P	.50	80	-1	29.2	2	46	-7	41	-2	33	-3	102	5
25	P	1.00	73	-11	28.8	1	52	6	39	-8	30	-13	106	8
26	P	2.00	73	-11	28.9	1	46	-7	38	-11	30	-13	101	4
27	P	4.00	77	-5	29.3	2	48	-2	42	0	28	-21	109	11
28	P	8.00	74	-9	29.0	1	51	4	40	-5	29	-17	110	12
29	M	.00	58	0	27.2	0	58	0	31	0	35	0	82	0
30	M	.25	57	-2	26.6	-2	62	6	31	0	31	-13	82	0
31	M	.50	56	-4	27.3	0	56	-4	30	-3	35	0	86	5
32	M	1.00	54	-7	26.4	-3	55	-5	30	-3	34	-3	84	2
33	M	2.00	57	-2	26.8	-1	53	-9	30	-3	32	-9	90	9
34	M	4.00	48	-21	27.1	0	52	-12	28	-11	33	-6	82	0
35	M	8.00	52	-12	27.2	0	55	-5	29	-7	32	-9	87	6
36	P	.00	63	0	26.1	0	52	0	32	0	41	0	84	0
37	P	.25	61	-3	25.0	-4	54	4	31	-3	40	-3	88	5
38	P	.50	54	-17	27.0	3	54	4	29	-10	41	0	88	5
39	P	1.00	54	-17	25.9	-1	54	4	30	-7	39	-5	83	-1
40	P	2.00	56	-13	27.0	3	52	0	30	-7	38	-8	94	11
41	P	4.00	52	-21	27.0	3	52	0	29	-10	38	-8	82	-2
42	P	8.00	54	-17	25.9	-1	52	0	29	-10	37	-11	78	-8
43	M	.00	58	0	30.2	0	50	0	33	0	31	0	90	0
44	M	.25	61	5	29.8	-1	46	-9	33	0	37	16	97	7
45	M	.50	58	0	29.6	-2	49	-2	31	-6	37	16	90	0
46	M	1.00	57	-2	29.8	-1	49	-2	31	-6	35	11	89	-1
47	M	2.00	56	-4	29.8	-1	47	-6	30	-10	38	18	94	4
48	M	4.00	61	5	29.8	-1	43	-16	33	0	34	9	105	14
49	M	8.00	59	2	30.3	0	45	-11	33	0	36	14	93	3
50	P	.00	65	0	29.6	0	44	0	34	0	38	0	92	0
51	P	.25	60	-8	29.9	1	50	12	33	-3	33	-15	97	5
52	P	.50	59	-10	30.6	3	49	10	32	-6	39	3	92	0
53	P	1.00	63	-3	29.8	1	47	6	33	-3	38	0	93	1
54	P	2.00	60	-8	30.0	1	48	8	34	0	31	-23	92	0
55	P	4.00	60	-8	29.8	1	51	14	33	-3	34	-12	97	5
56	P	8.00	66	2	29.8	1	45	2	34	0	40	5	97	5
57	M	.00	58	0	32.1	0	56	0	33	0	35	0	91	0
58	M	.25	57	-2	32.2	0	57	2	30	-10	34	-3	82	-11
59	M	.50	58	0	32.0	0	57	2	31	-6	36	3	80	-14
60	M	1.00	57	-2	33.2	3	50	-12	30	-10	37	5	80	-14
61	M	2.00	55	-5	33.2	3	56	0	29	-14	40	13	90	-1

	Visit	Dose	HR	HR %	TFI	TFI %	IC	IC%	ER	ER %	STR	STR %	ESP	ESP %
62	M	4.00	57	-2	32.6	2	56	0	30	-10	32	-9	90	-1
63	M	8.00	56	-4	32.6	2	58	3	31	-6	31	-13	71	-28
64	P	.00	56	0	33.4	0	53	0	29	0	36	0	88	0
65	P	.25	52	-8	33.1	-1	54	2	27	-7	39	8	92	4
66	P	.50	53	-6	33.2	-1	52	-2	27	-7	43	16	94	6
67	P	1.00	54	-4	33.2	-1	51	-4	29	0	40	10	79	-11
68	P	2.00	56	0	33.2	-1	50	-6	29	0	42	14	85	-4
69	P	4.00	66	15	32.8	-2	47	-13	32	9	42	14	82	-7
70	P	8.00	51	-10	32.5	-3	55	4	26	-12	50	28	82	-7
71	M	.00	68	0	30.9	0	46	0	36	0	30	0	86	0
72	M	.25	63	-8	31.2	1	46	0	34	-6	31	3	91	5
73	M	.50	61	-11	30.9	0	45	-2	34	-6	31	3	88	2
74	M	1.00	66	-3	30.5	-1	43	-7	35	-3	32	6	93	8
75	M	2.00	59	-15	30.7	-1	42	-10	32	-13	34	12	88	2
76	M	4.00	60	-13	30.5	-1	45	-2	33	-9	32	6	95	9
77	M	8.00	65	-5	30.9	0	39	-18	33	-9	38	21	104	17
78	P	.00	56	0	28.6	0	57	0	32	0	27	0	84	0
79	P	.25	55	-2	28.5	0	56	-2	31	-3	30	10	86	2
80	P	.50	55	-2	28.8	1	52	-10	31	-3	32	16	83	-1
81	P	1.00	54	-4	29.0	1	51	-12	31	-3	28	4	87	3
82	P	2.00	51	-10	28.7	0	57	0	29	-10	30	10	84	0
83	P	4.00	53	-6	28.9	1	53	-8	30	-7	30	10	89	6
84	P	8.00	53	-6	28.8	1	53	-8	29	-10	31	13	80	-5
85	M	.00	68	0	28.6	0	52	0	33	0	47	0	81	0
86	M	.25	76	11	28.3	-1	52	0	36	8	48	2	69	-17
87	M	.50	70	3	27.9	-3	51	-2	34	3	44	-7	69	-17
88	M	1.00	68	0	28.3	-1	57	9	34	3	40	-18	71	-14
89	M	2.00	68	0	28.3	-1	52	0	34	3	39	-21	76	-7
90	M	4.00	72	6	28.1	-2	53	2	36	8	41	-15	73	-11
91	M	8.00	71	4	28.0	-2	53	2	36	8	38	-24	73	-11
92	P	.00	67	0	27.9	0	61	0	33	0	40	0	80	0
93	P	.25	62	-8	26.2	-6	63	3	31	-6	41	2	80	0
94	P	.50	63	-6	26.5	-5	59	-3	31	-6	48	17	82	2
95	P	1.00	61	-10	26.8	-4	61	0	31	-6	43	7	77	-4
96	P	2.00	64	-5	26.7	-4	57	-7	31	-6	44	9	77	-4
97	P	4.00	62	-8	27.2	-3	60	-2	31	-6	43	7	83	4
98	P	8.00	60	-12	26.0	-7	60	-2	30	-10	42	5	80	0
99	M	.00	62	0	28.1	0	36	0	34	0	32	0	121	0
100	M	.25	59	-5	27.9	-1	33	-9	34	0	28	-14	110	-10
101	M	.50	58	-7	27.8	-1	34	-6	34	0	29	-10	114	-6
102	M	1.00	63	2	28.0	0	34	-6	34	0	33	3	117	-3
103	M	2.00	64	3	28.0	0	34	-6	35	3	34	6	118	-3
104	M	4.00	63	2	27.5	-2	35	-3	37	8	27	-19	115	-5
105	M	8.00	60	-3	27.7	-1	33	-9	34	0	32	0	115	-5
105	P	.00	73	0	30.9	0	35	0	38	0	27	0	100	0
107	P	.25	67	-9	30.9	0	35	0	36	-6	30	10	103	3
108	P	.50	58	-26	30.2	-2	36	3	31	-23	32	16	107	7
109	P	1.00	60	-22	30.1	-3	38	8	32	-19	34	21	106	6
110	P	2.00	59	-24	30.4	-2	36	3	32	-19	34	21	106	6
111	P	4.00	64	-14	30.3	-2	34	-3	33	-15	38	29	105	5
112	P	8.00	60	-22	30.6	-1	.	.	33	-15	34	21	115	13

HR; heart rate, HR%; percentage of change from baseline of heart rate , TFI; thoracic fluid index, TFI%; percentage of change from baseline of thoracic fluid index, IC; index of contractibility, IC%; percentage of change from baseline of index of contractibility, ER; ejection ratio, ER%; percentage of change from baseline of ejection ratio, STR; systolic time ratio, STR%; percentage of change from baseline of systolic time ratio, AI; absolute change from baseline of augmentation index.

Table III-III-IV. Haemodynamic effects of morphine versus saline (continued)

	Visit	Dose	AI	ASBP	ASBP%	ADBP	ADBP%	MBP	MBP%	PPP	PPP%	APP	APP%	ED	ED%
1	M	.00	0	119	0	87	0	102	0	41	0	32	0	321	0
2	M	.25	-3	115	-3	81	-7	96	-6	41	0	34	6	322	0
3	M	.50	-7	120	1	81	-7	99	-3	47	13	39	18	323	1
4	M	1.00	-6	121	2	86	-1	101	-1	43	5	35	9	325	1
5	M	2.00	-4	121	2	81	-7	98	-4	47	13	40	20	327	2
6	M	4.00	-12	128	7	86	-1	105	3	48	15	42	24	330	3
7	M	8.00	-8	127	6	91	4	106	4	43	5	36	11	325	1
8	P	.00	0	103	0	74	0	87	0	37	0	29	0	320	0
9	P	.25	9	104	1	73	-1	87	0	42	12	33	12	317	-1
10	P	.50	0	104	1	76	3	89	2	39	5	30	3	321	0
11	P	1.00	2	103	0	72	-3	85	-2	40	8	32	9	324	1
12	P	2.00	-2	109	6	75	1	89	2	43	14	35	17	325	2
13	P	4.00	-1	113	9	75	1	91	4	47	21	38	24	325	2
14	P	8.00	-5	111	7	73	-1	89	2	48	23	38	24	321	0
15	M	.00	0	113	0	77	0	94	0	46	0	36	0	319	0
16	M	.25	-10	113	0	80	4	94	0	41	-12	33	-9	332	4
17	M	.50	-10	109	-4	79	3	92	-2	38	-21	30	-20	331	4
18	M	1.00	-13	108	-5	76	-1	91	-3	40	-15	31	-16	334	4
19	M	2.00	-11	111	-2	76	-1	92	-2	40	-15	35	-3	341	6
20	M	4.00	1	99	-14	63	-22	78	-21	44	-5	36	0	348	8
21	M	8.00	-2	96	-18	62	-24	76	-24	42	-10	35	-3	349	9
22	P	.00	0	105	0	79	0	92	0	36	0	27	0	318	0
23	P	.25	7	108	3	77	-3	92	0	40	10	31	13	318	0
24	P	.50	-5	113	7	79	0	94	2	45	20	34	21	327	3
25	P	1.00	10	117	10	79	0	96	4	48	25	38	29	331	4
26	P	2.00	-3	114	8	74	-7	91	-1	50	28	40	33	332	4
27	P	4.00	0	123	15	77	-3	98	6	58	38	46	41	331	4
28	P	8.00	2	123	15	81	2	100	8	52	31	41	34	335	5
29	M	.00	0	103	0	58	0	79	0	75	0	45	0	341	0
30	M	.25	5	98	-5	63	8	78	-1	59	-27	35	-29	347	2
31	M	.50	-7	98	-5	60	3	78	-1	61	-23	38	-18	344	1
32	M	1.00	-5	92	-12	64	9	76	-4	52	-44	32	-41	344	1
33	M	2.00	-11	103	0	63	8	82	4	65	-15	40	-13	345	1
34	M	4.00	-12	94	-10	58	0	74	-7	60	-25	36	-25	339	-1
35	M	8.00	-7	102	-1	59	2	78	-1	70	-7	43	-5	341	0
36	P	.00	0	103	0	62	0	79	0	70	0	41	0	331	0
37	P	.25	2	109	6	57	-9	79	0	88	20	53	23	334	1
38	P	.50	3	106	3	63	2	81	2	73	4	43	5	336	1
39	P	1.00	1	110	6	60	-3	75	-5	60	-10	40	0	336	1
40	P	2.00	-5	110	6	67	7	86	8	65	-8	43	5	339	2
41	P	4.00	-2	93	-11	66	6	77	-3	45	-56	27	-52	329	-1
42	P	8.00	-2	90	-14	56	-11	71	-11	56	-25	34	-21	338	2
43	M	.00	0	99	0	69	0	83	0	45	0	30	0	340	0
44	M	.25	-9	106	7	72	4	88	6	50	10	34	12	338	-1
45	M	.50	-1	98	-1	70	1	83	0	42	-7	28	-7	337	-1
46	M	1.00	-1	95	-4	66	-5	80	-4	43	-5	29	-3	342	1
47	M	2.00	-7	109	9	69	0	85	2	49	8	33	9	352	3
48	M	4.00	-18	119	17	79	13	96	14	56	20	37	19	360	6
49	M	8.00	-13	103	4	70	1	86	3	52	13	33	9	354	4
50	P	.00	0	100	0	73	0	86	0	42	0	27	0	338	0
51	P	.25	12	105	5	74	1	89	3	44	5	32	16	339	0
52	P	.50	10	100	0	74	1	85	-1	37	-14	26	-4	338	0
53	P	1.00	2	101	1	72	-1	86	0	43	2	21	-29	350	3
54	P	2.00	8	101	1	67	-9	83	-4	48	13	34	21	343	1
55	P	4.00	14	106	6	75	3	89	3	42	0	31	13	343	1
56	P	8.00	0	110	9	71	-3	89	3	57	26	38	29	346	2
57	M	.00	0	100	0	64	0	80	0	50	0	36	0	322	0
58	M	.25	3	91	-10	55	-16	70	-14	47	-6	36	0	317	-2
59	M	.50	3	89	-12	57	-12	70	-14	44	-14	34	-6	325	1
60	M	1.00	-12	88	-14	57	-12	71	-13	44	-14	31	-16	320	-1
61	M	2.00	1	98	-2	61	-5	78	-3	47	-6	37	3	324	1

62	M	4.00	1	101	1	55	-16	76	-5	58	14	46	22	337	4
63	M	8.00	4	83	-20	47	-36	63	-27	51	2	37	3	327	2
64	P	.00	0	95	0	64	0	78	0	43	0	31	0	313	0
65	P	.25	4	97	2	67	4	81	4	43	0	30	-3	305	-3
66	P	.50	-3	103	8	62	-3	81	4	59	27	41	24	318	2
67	P	1.00	-5	85	-12	56	-14	69	-13	41	-5	29	-7	317	1
68	P	2.00	-6	90	-6	58	-10	72	-8	47	9	35	11	326	4
69	P	4.00	-11	89	-7	59	-8	72	-8	42	-2	30	-3	325	4
70	P	8.00	5	89	-7	56	-14	70	-11	46	7	33	6	325	4
71	M	.00	0	109	0	64	0	85	0	74	0	46	0	365	0
72	M	.25	0	110	1	67	4	85	0	72	-3	43	-7	343	-6
73	M	.50	-2	108	-1	64	0	82	-4	68	-9	42	-10	349	-5
74	M	1.00	-8	109	0	68	6	86	1	67	-10	41	-12	360	-1
75	M	2.00	-10	95	-15	59	-8	74	-15	58	-28	36	-28	358	-2
76	M	4.00	-1	110	1	69	7	88	3	67	-10	40	-15	352	-4
77	M	8.00	-19	122	11	67	4	92	8	85	13	55	16	360	-1
78	P	.00	0	110	0	59	0	80	0	80	0	51	0	362	0
79	P	.25	-3	108	-2	61	3	80	0	76	-5	45	-13	354	-2
80	P	.50	-12	106	-4	58	-2	77	-4	77	-4	48	-6	359	-1
81	P	1.00	-13	107	-3	55	-7	78	-3	81	1	52	2	355	-2
82	P	2.00	1	106	-4	59	0	77	-4	76	-5	47	-9	357	-1
83	P	4.00	-10	109	-1	63	6	83	4	75	-7	46	-11	364	1
84	P	8.00	-8	101	-9	60	2	77	-4	68	-18	42	-21	365	1
85	M	.00	0	100	0	67	0	81	0	56	0	34	0	332	0
86	M	.25	-3	86	-16	57	-18	69	-17	48	-17	29	-17	326	-2
87	M	.50	-2	84	-19	53	-26	67	-21	53	-6	31	-10	326	-2
88	M	1.00	11	87	-15	56	-20	70	-16	52	-8	31	-10	332	0
89	M	2.00	0	89	-12	60	-12	73	-11	48	-17	29	-17	335	1
90	M	4.00	4	89	-12	57	-18	72	-13	54	-4	32	-6	340	2
91	M	8.00	3	88	-14	55	-22	70	-16	55	-2	33	-3	349	5
92	P	.00	0	93	0	64	0	77	0	49	0	29	0	330	0
93	P	.25	-4	94	1	61	-5	76	-1	54	9	33	12	328	-1
94	P	.50	-6	94	1	64	0	78	1	50	2	30	3	325	-2
95	P	1.00	-2	90	-3	61	-5	75	-3	50	2	29	0	318	-4
96	P	2.00	-10	92	-1	63	-2	75	-3	49	0	29	0	317	-4
97	P	4.00	-3	96	3	67	4	80	4	48	-2	29	0	330	0
98	P	8.00	-6	93	0	65	2	78	1	47	-4	28	-4	325	-2
99	M	.00	0	132	0	89	0	109	0	55	0	43	0	338	0
100	M	.25	-7	123	-7	83	-7	100	-9	53	-4	41	-5	339	0
101	M	.50	-7	125	-6	88	-1	103	-6	46	-20	37	-16	344	2
102	M	1.00	-9	126	-5	91	2	107	-2	42	-31	35	-23	349	3
103	M	2.00	-6	129	-2	84	-6	103	-6	54	-2	45	4	359	6
104	M	4.00	-5	127	-4	81	-10	101	-8	56	2	46	7	352	4
105	M	8.00	-12	125	-6	87	-2	104	-5	46	-20	38	-13	356	5
105	P	.00	0	108	0	80	0	92	0	37	0	28	0	322	0
107	P	.25	-2	112	4	80	0	94	2	43	14	32	13	322	0
108	P	.50	2	117	8	81	1	97	5	49	24	37	24	326	1
109	P	1.00	7	114	5	86	7	98	6	36	-3	29	3	331	3
110	P	2.00	1	114	5	80	0	95	3	43	14	34	18	337	4
111	P	4.00	-1	113	4	84	5	97	5	37	0	28	0	331	3
112	P	8.00	-3	124	13	87	8	103	11	45	18	37	24	333	3

ESP; end systolic pressure, ESP%; percentage of change from baseline of end systolic pressure, ASBP; aortic systolic blood pressure, ASBP%; percentage of change from baseline of aortic systolic blood pressure, ADBP; aortic diastolic blood pressure, ADBP%; percentage of change from baseline of aortic diastolic blood pressure, MBP, mean blood pressure, MBP%, percentage of change from baseline of mean blood pressure, PPP; peripheral blood pressure, PPP%; percentage of change from baseline of peripheral blood pressure, APP; aortic pulse pressure, APP%; percentage of change from baseline of aortic pulse pressure, ED; ejection duration, ED%; percentage of change from baseline of ejection duration

Morphine versus morphine and naloxone study

Table III-IV-I. Haemodynamic effects of morphine versus morphine and naloxone

	No	VISIT	Dose	COR	INC	RT	NoR	O_2	CO_2	RR	CO	SV
1	1	1	0	54	0	.983	0	97	5.1	18	2.7	51
2	1	1	0.25	52	0	.913	2	97	5.1	12	3.2	61
3	1	1	0.5	54	0	.992	0	98	5.2	17	1.9	36
4	1	1	1	53	0	1.030	1	97	4.5	13	2.5	51
5	1	1	2	54	0	.950	0	98	5.3	22	2.6	50
6	1	1	4	54	0	1.184	0	100	4.8	11	2.8	49
7	1	1	8	54	0	1.189	0	100	5.6	19	3.5	56
8	1	2	0	54	0	.813	0	97	5.1	14	3.4	57
9	1	2	0.25	54	0	.868	0	97	5.2	16	2.6	51
10	1	2	0.5	54	0	.895	0	98	5.4	15	2.5	48
11	1	2	1	54	0	.873	0	98	5.5	16	2.5	48
12	1	2	2	54	0	.884	0	98	5.4	17	2.4	47
13	1	2	4	54	0	.959	0	99	5.4	14	2.7	55
14	1	2	8	54	0	1.012	0	99	5.4	15	2.5	48
15	2	2	0	54	0	1.181	0	99	5.2	18	4.3	69
16	2	2	0.25	54	0	1.253	0	97	5.5	19	3.2	55
17	2	2	0.5	54	0	1.242	0	98	5.7	16	3.4	60
18	2	2	1	54	0	1.234	0	98	5.5	12	3.4	61
19	2	2	2	54	0	1.134	0	99	5.3	13	3.8	65
20	2	2	4	54	0	1.078	0	99	4.9	16	3.5	63
21	2	2	8	54	0	1.049	0	96	5.2	16	3.6	60
22	2	1	0	53	0	1.079	1	96	5.3	18	4.0	57
23	2	1	0.25	54	0	1.057	0	96	5.6	18	3.9	62
24	2	1	0.5	53	0	1.190	1	99	5.4	16	3.6	61
25	2	1	1	54	0	1.069	0	98	5.2	16	3.6	63
26	2	1	2	54	0	1.145	0	97	5.6	19	3.7	61
27	2	1	4	54	0	1.015	0	98	5.8	15	3.4	56
28	2	1	8	54	0	1.158	0	97	6.1	12	3.7	57
29	3	2	0	54	0	.890	0	97	5.3	17	3.4	49
30	3	2	0.25	54	0	.891	0	97	5.2	17	3.1	48
31	3	2	0.5	54	0	.863	0	96	5.3	19	3.2	50
32	3	2	1	54	0	.712	0	97	5.6	18	3.0	51
33	3	2	2	54	0	.730	0	98	5.6	16	3.0	48
34	3	2	4	54	0	.712	0	96	5.6	16	2.8	47
35	3	2	8	54	0	.720	0	96	5.5	16	2.7	44
36	4	1	0	46	0	.776	0	99	5.1	17	2.6	50
37	4	1	0.25	54	0	.793	0	98	4.8	18	2.1	42
38	4	1	0.5	54	0	.769	0	99	4.8	16	2.4	47
39	4	1	1	54	0	.786	0	100	5.2	14	2.2	45
40	4	1	2	54	0	.822	0	99	4.7	15	2.3	43
41	4	1	4	54	0	.800	0	98	4.8	13	2.1	44
42	4	1	8	54	0	.820	0	97	5.6	12	2.0	39
43	3	1	0	54	0	.708	0	98	5.2	18	3.4	49
44	3	1	0.25	54	0	.709	0	98	5.2	17	3.0	48
45	3	1	0.5	54	0	.698	0	97	5.2	17	3.0	50
46	3	1	1	54	0	.717	0	97	5.3	16	3.1	48
47	3	1	2	54	0	.685	0	97	5.5	16	3.0	48
48	3	1	4	54	0	.658	0	99	5.2	14	2.9	45
49	3	1	8	54	0	.797	0	98	5.3	14	3.0	43
50	4	2	0	46	0	.776	8	98	5.1	16	2.7	51
51	4	2	0.25	54	0	.793	0	97	4.8	16	2.3	45
52	4	2	0.5	54	0	.769	0	97	5.1	14	2.3	47
53	4	2	1	54	0	.786	0	97	5.2	12	2.2	45
54	4	2	2	54	0	.822	0	98	5.3	14	2.2	44
55	4	2	4	54	0	.800	0	96	5.3	14	2.0	40
56	4	2	8	54	0	.820	0	98	5.3	15	2.0	42
57	5	1	0	54	0	.999	0	97	5.8	18	4.3	74
58	5	1	0.25	54	0	.875	0	97	5.7	17	4.1	70
59	5	1	0.5	54	0	.910	0	96	5.9	17	4.2	71

	No	VISIT	Dose	COR	INC	RT	NoR	O_2	CO_2	RR	CO	SV
60	5	1	1	54	0	.968	0	96	6.2	15	3.9	66
61	5	1	2	54	0	.829	0	98	6.1	15	3.9	63
62	5	1	4	54	0	.888	0	96	6.3	13	3.8	69
63	5	1	8	54	0	1.000	0	96	6.5	12	3.9	71
64	6	1	0	53	0	.811	1	100	5.1	20	2.3	29
65	6	1	0.25	54	0	.756	0	98	5.3	19	3.2	45
66	6	1	0.5	54	0	.772	0	97	5.1	13	2.9	43
67	6	1	1	54	0	.721	0	98	5.2	14	3.1	45
68	6	1	2	54	0	.771	1	97	5.4	13	3.1	44
69	6	1	4	54	0	.784	0	98	5.6	11	3.0	43
70	6	1	8	54	0	.810	0	96	6.2	10	2.9	46
71	7	2	0	54	0	.809	0	99	5.4	15	4.5	75
72	7	2	0.25	54	0	.796	0	97	6.0	13	3.9	68
73	7	2	0.5	54	0	.773	0	98	6.0	11	4.1	71
74	7	2	1	54	0	.687	0	98	5.9	13	3.6	69
75	7	2	2	54	0	.699	0	98	6.1	11	3.6	66
76	7	2	4	54	0	.782	0	98	5.9	11	2.8	57
77	7	2	8	53	0	.719	1	99	6.1	10	3.2	63
78	7	1	0	54	0	.673	0	97	5.6	17	4.6	76
79	7	1	0.25	54	0	.685	0	97	5.8	16	4.4	76
80	7	1	0.5	54	0	.659	0	97	5.9	14	4.0	64
81	7	1	1	54	0	.683	0	98	5.6	15	4.0	69
82	7	1	2	54	0	.750	0	97	5.8	12	3.3	62
83	7	1	4	54	0	.725	0	97	6.0	11	3.9	67
84	7	1	8	54	0	.692	0	99	6.4	9	4.5	66
85	6	2	0	54	0	.723	0	100	5.5	16	4.0	59
86	6	2	0.25	54	0	.734	0	99	5.5	15	4.1	58
87	6	2	0.5	54	0	.693	0	99	5.6	17	3.9	61
88	6	2	1	54	0	.675	0	99	5.5	15	4.5	63
89	6	2	2	54	0	.662	0	98	5.5	16	3.7	60
90	6	2	4	54	0	.660	0	100	5.6	16	3.8	64
91	6	2	8	54	0	.649	0	99	5.7	16	3.8	58
92	5	2	0	54	0	.734	0	97	5.6	17	4.1	75
93	5	2	0.25	54	0	.717	0	95	5.6	16	3.9	77
94	5	2	0.5	54	0	.607	0	97	5.7	17	3.8	77
95	5	2	1	54	0	.717	0	96	5.7	16	3.9	78
96	5	2	2	54	0	.616	0	97	5.7	17	3.6	75
97	5	2	4	54	0	.630	0	99	5.7	16	3.6	76
98	5	2	8	54	0	.659	0	98	5.8	16	3.9	75
99	8	1	0	54	0	.833	0	100	5.5	19	5.5	77
100	8	1	0.25	54	0	.816	0	100	5.7	16	5.0	76
101	8	1	0.5	54	0	.826	0	100	5.6	18	4.6	71
102	8	1	1	54	0	.909	0	100	5.6	16	5.2	77
103	8	1	2	54	0	.891	0	100	5.5	17	4.9	73
104	8	1	4	54	0	.913	0	100	5.7	16	4.4	71
105	8	1	8	54	0	.911	0	100	5.8	14	4.6	75
106	8	2	0	54	0	.922	0	100	5.6	17	5.0	76
107	8	2	0.25	54	0	.924	0	100	5.6	17	5.4	78
108	8	2	0.5	54	0	.763	0	100	5.7	19	4.7	78
109	8	2	1	54	0	.677	0	100	5.7	17	4.6	75
110	8	2	2	54	0	.819	0	100	5.8	18	4.7	75
111	8	2	4	54	0	.961	0	100	5.7	18	4.4	74
112	8	2	8	54	0	.703	0	100	5.8	16	4.9	80

No; randomisation number, COR; correct response, INC; incorrect response, NoR; no response, O"; oxygen saturation, CO"; end tidal carbon dioxide, RR; respiratory rate, CO; cardiac output, SV; stroke volume.

Table III-IV-II. Haemodynamic effects of morphine versus morphine and naloxone (continued)

	No	VISIT	Dose	SBP	DBP	SSBP	SDBP	HR	AI	ESP	MBP
1	1	1	0	144	81	136	78	52	18	116	102
2	1	1	0.25	138	74	139	61	51	18	109	95
3	1	1	0.5	131	78	142	84	51	23	110	96
4	1	1	1	123	68	128	83	52	26	103	86
5	1	1	2	147	82	147	73	55	21	120	104
6	1	1	4	151	83	158	88	51	25	125	106
7	1	1	8	161	82	.	.	56	32	131	108
8	1	2	0	128	73	138	82	62	16	102	91
9	1	2	0.25	132	71	133	81	53	20	105	91
10	1	2	0.5	133	72	133	86	48	19	106	92
11	1	2	1	132	73	127	79	48	12	102	93
12	1	2	2	128	72	132	81	49	15	101	91
13	1	2	4	128	72	135	80	48	20	105	91
14	1	2	8	143	79	135	86	60	18	112	100
15	2	2	0	149	89	159	89	61	22	122	109
16	2	2	0.25	147	83	154	99	55	22	117	104
17	2	2	0.5	139	81	153	88	54	31	117	100
18	2	2	1	137	81	142	88	54	27	114	100
19	2	2	2	144	89	146	88	53	25	118	107
20	2	2	4	144	83	142	93	53	26	117	103
21	2	2	8	142	87	146	87	55	23	118	105
22	2	1	0	148	88	143	89	68	18	120	108
23	2	1	0.25	142	81	143	87	58	21	115	101
24	2	1	0.5	137	82	143	83	57	21	111	100
25	2	1	1	142	81	139	88	57	20	112	101
26	2	1	2	137	77	138	85	56	16	107	97
27	2	1	4	136	82	138	83	57	23	112	100
28	2	1	8	135	78	138	88	55	20	109	97
29	3	2	0	132	79	136	92	68	28	113	97
30	3	2	0.25	139	78	133	82	65	25	110	98
31	3	2	0.5	127	78	135	81	66	27	107	94
32	3	2	1	129	79	117	83	59	26	108	96
33	3	2	2	126	83	129	87	63	31	110	97
34	3	2	4	127	81	132	90	62	33	111	96
35	3	2	8	136	89	134	93	62	34	118	105
36	4	1	0	123	88	117	73	52	20	98	100
37	4	1	0.25	117	73	115	82	52	20	97	88
38	4	1	0.5	122	70	120	76	50	21	99	87
39	4	1	1	114	67	120	78	49	15	89	83
40	4	1	2	114	76	120	76	51	18	90	89
41	4	1	4	114	74	121	84	48	15	93	87
42	4	1	8	126	81	133	89	51	27	107	96
43	3	1	0	144	85	.	.	67	25	121	105
44	3	1	0.25	135	79	131	83	62	30	117	98
45	3	1	0.5	117	81	127	90	62	30	104	93
46	3	1	1	128	85	127	89	60	29	112	99
47	3	1	2	145	87	120	86	65	30	123	106
48	3	1	4	128	88	128	90	60	36	117	101
49	3	1	8	137	87	139	91	64	32	120	104
50	4	2	0	128	74	129	79	52	24	106	92
51	4	2	0.25	121	84	114	80	50	25	107	96
52	4	2	0.5	117	76	117	83	49	20	97	90
53	4	2	1	117	73	116	83	49	26	100	88
54	4	2	2	123	82	120	85	49	21	105	96
55	4	2	4	126	79	129	86	48	26	106	95
56	4	2	8	121	78	126	92	48	27	108	92
57	5	1	0	132	63	120	60	61	-22	83	86
58	5	1	0.25	114	61	121	80	60	-7	78	79
59	5	1	0.5	128	64	132	70	61	-7	95	85

60	5	1	1	129	63	126	63	57	-7	90	85
61	5	1	2	131	65	122	58	62	-13	81	87
62	5	1	4	128	64	114	61	58	-6	87	85
63	5	1	8	109	60	123	54	60	-9	76	76
64	6	1	0	116	77	111	76	69	24	101	90
65	6	1	0.25	116	75	114	75	71	30	100	89
66	6	1	0.5	115	73	108	73	66	24	99	87
67	6	1	1	111	73	105	74	66	28	97	86
68	6	1	2	120	73	104	67	66	24	102	89
69	6	1	4	115	74	106	62	63	32	103	88
70	6	1	8	106	66	108	75	59	33	94	79
71	7	2	0	142	61	.	.	60	-23	77	88
72	7	2	0.25	129	62	132	71	56	-21	77	84
73	7	2	0.5	120	63	127	63	60	-7	78	82
74	7	2	1	134	55	127	76	51	-11	77	81
75	7	2	2	117	58	142	49	49	.	88	78
76	7	2	4	131	58	131	69	47	-8	91	82
77	7	2	8	135	61	142	72	54	-10	84	86
78	7	1	0	131	60	138	72	54	-20	124	84
79	7	1	0.25	129	58	147	64	56	-5	80	82
80	7	1	0.5	136	64	143	64	59	-8	86	88
81	7	1	1	136	64	151	67	54	4	93	88
82	7	1	2	139	54	152	58	58	-11	89	82
83	7	1	4	133	61	149	65	53	0	89	85
84	7	1	8	139	65	67	53	54	-7	85	90
85	6	2	0	110	76	108	70	68	26	95	87
86	6	2	0.25	105	68	101	69	62	34	91	80
87	6	2	0.5	114	76	101	62	60	21	98	89
88	6	2	1	110	74	97	68	61	25	96	86
89	6	2	2	102	70	108	60	59	30	91	81
90	6	2	4	106	65	98	63	57	25	91	79
91	6	2	8	102	64	97	62	58	27	89	77
92	5	2	0	131	60	133	63	58	-6	78	84
93	5	2	0.25	120	58	115	60	55	-18	74	79
94	5	2	0.5	122	55	133	57	57	-16	76	77
95	5	2	1	128	54	117	62	54	-3	77	79
96	5	2	2	113	51	122	64	55	-10	72	72
97	5	2	4	111	56	133	61	50	-11	74	74
98	5	2	8	114	50	123	57	49	-13	72	71
99	8	1	0	121	70	122	71	69	-13	88	87
100	8	1	0.25	116	69	109	68	68	-8	86	85
101	8	1	0.5	110	66	103	64	66	-19	81	81
102	8	1	1	114	63	110	58	62	-21	79	80
103	8	1	2	108	61	100	53	60	-19	75	77
104	8	1	4	108	57	96	59	64	-25	70	74
105	8	1	8	97	52	100	56	60	-17	66	67
106	8	2	0	112	59	115	59	66	-11	79	77
107	8	2	0.25	105	58	107	58	60	-16	74	74
108	8	2	0.5	108	58	109	62	60	-11	76	75
109	8	2	1	104	57	106	66	59	-14	74	73
110	8	2	2	116	55	99	56	56	-9	78	75
111	8	2	4	112	64	107	52	57	-12	82	80
112	8	2	8	102	54	102	63	58	-11	74	70

SBP; systolic blood pressure, DBP; diastolic blood pressure, sSBP; sitting systolic blood pressure, sDBP; sitting diastolic blood pressure, HR; heart rate, AI; augmentation index, ESP; end systolic pressure, MBP; mean blood pressure.

Table III-V. Histamine, adrenaline, noradrenaline, and morphine in whole man studies

Code	Visit	Volunteer	Dose	Visit No.	Histamine	Lease	Noradrenaline	Adrenaline	Morphine
1	M & M-N	1	0	1	0.68		231.57	33.5333	<2.5
2	M & M-N	1	0	2	0.44		180.63	<30	<2.5
3	M & M-N	1	0.25	1	0.60		317.23	75.4667	2.6
4	M & M-N	1	0.25	2	0.45		271.67	<30	3.1
5	M & M-N	1	1	1	0.51		275.93	34.1667	13.8
6	M & M-N	1	1	2	0.69		188.47	<30	13.3
7	M & M-N	1	8	1	0.64		299.43	43.3333	97.6
8	M & M-N	1	8	2	0.89		182.6	<30	63.9
9	M & M-N	2	0	1	0.99		208.03	<30	<2.5
10	M & M-N	2	0	2	0.80		283.73	41.1667	<2.5
11	M & M-N	2	0.25	1	1.10	h++	330.43	49.4333	2.8
12	M & M-N	2	0.25	2	0.61		467.17	33.4333	2.8
13	M & M-N	2	1	1	0.81		370.97	30.6667	10.8
14	M & M-N	2	1	2	1.02	h++	504	45.4	11.7
15	M & M-N	2	8	1	0.85		271.43	40.8	69.9
16	M & M-N	2	8	2	0.56		392.8	35.9333	56.6
17	M & M-N	3	0	1	1.16		232.03	36.3333	<2.5
18	M & M-N	3	0	2	0.54		178.9	37.1333	2.8
19	M & M-N	3	0.25	1	0.96		186.7	44.2667	3.3
20	M & M-N	3	0.25	2	0.97		155.77	65.8	5
21	M & M-N	3	1	1	0.67		184.17	<30	16.7
22	M & M-N	3	1	2	0.95		156.03	43.8667	20.6
23	M & M-N	3	8	1	0.96		175.63	70.9	68.1
24	M & M-N	3	8	2	0.82		248.17	<30	67.1
25	M & M-N	4	0	1	0.48		211.37	<30	<2.5
26	M & M-N	4	0	2	0.62		245.47	44.4667	20.5
27	M & M-N	4	0.25	1	0.95		417.4	<30	4.3
28	M & M-N	4	0.25	2	0.46		179.67	61.1	5.3
29	M & M-N	4	1	1	0.48		202.63	31.5333	16.2
30	M & M-N	4	1	2	0.69		232.67	43.6333	3.6
31	M & M-N	4	8	1	0.74		283	39.2	93.5
32	M & M-N	4	8	2	0.60		149.63	37.6333	78.2
33	M & M-N	5	0	1	1.05		201.17	<30	<2.5
34	M & M-N	5	0	2	0.62		290.8	<30	<2.5
35	M & M-N	5	0.25	1	0.75		307.93	<30	<2.5
36	M & M-N	5	0.25	2	0.51		258.03	<30	<2.5
37	M & M-N	5	1	1	0.61		357.6	<30	10.4
38	M & M-N	5	1	2	0.80		311.67	<30	8.7
39	M & M-N	5	8	1	0.65		311.23	85.5333	50.4
40	M & M-N	5	8	2	0.68		281.8	<30	50
41	M & M-N	6	0	1	0.58		189.07	<30	<2.5
42	M & M-N	6	0	2	0.56		208.23	<30	<2.5
43	M & M-N	6	0.25	1	0.47		180.27	<30	<2.5
44	M & M-N	6	0.25	2	0.69		205.07	<30	3.6
45	M & M-N	6	1	1	0.66		205.9	<30	12.6
46	M & M-N	6	1	2	0.83		177.6	<30	11
47	M & M-N	6	8	1	0.76		414.17	<30	70.7
48	M & M-N	6	8	2	0.80		<150	<30	69.9
49	M & M-N	7	0	1	0.92		474.7	<30	<2.5
50	M & M-N	7	0	2	0.47		469.17	60.2667	<2.5
51	M & M-N	7	0.25	1	0.81		799.8	<30	<2.5
52	M & M-N	7	0.25	2	0.79		306.7	<30	2.7
53	M & M-N	7	1	1	0.63		457.1	<30	10
54	M & M-N	7	1	2	0.66		432.37	<30	12.1
55	M & M-N	7	8	1	0.62		402.27	<30	59.4
56	M & M-N	7	8	2	0.66		353.3	<30	57.7
57	M & M-N	8	0	1	4.06		164.97	<30	<2.5
58	M & M-N	8	0	2	2.70		334.5	<30	<2.5
59	M & M-N	8	0.25	1	3.60		269.4	<30	3.1

Code	Visit	Volunteer	Dose	Visit No.	Histamine	Lease	Noradrenaline	Adrenaline	Morphine
60	M & M-N	8	0.25	2	6.19		196.57	<30	5.2
61	M & M-N	8	1	1	3.04		<150	<30	16.8
62	M & M-N	8	1	2	4.35		<150	<30	43.8
63	M & M-N	8	8	1	3.10		178.27	<30	85.1
64	M & M-N	8	8	2	3.59		172.83	<30	98.2
65	M & P	3	0	4	0.65		<150	<30	<2.5
66	M & P	3	0	5	1.07		<150	<30	<2.5
67	M & P	3	0.25	4	0.65		<150	<30	3.8
68	M & P	3	0.25	5	1.01		<150	<30	<2.5
69	M & P	3	1	4	1.09		<150	<30	12.5
70	M & P	3	1	5	0.70		<150	<30	<2.5
71	M & P	3	8	4	0.82		<150	<30	71
72	M & P	3	8	5	0.83		<150	<30	<2.5
73	M & P	6	0	4	0.63		183.8	<30	<2.5
74	M & P	6	0	5	0.75		324.4	<30	<2.5
75	M & P	6	0.25	4	0.92		231.87	<30	3.2
76	M & P	6	0.25	5	0.50		277.6	<30	<2.5
77	M & P	6	1	4	0.68		229.73	<30	17.3
78	M & P	6	1	5	0.87		358.6	<30	2.8
79	M & P	6	8	4	0.87		194.73	<30	105.4
80	M & P	6	8	5	0.90		173.3	<30	3.1
81	M & P	7	0	4	0.59		<150	<30	<2.5
82	M & P	7	0	4	0.49		<150	<30	<2.5
83	M & P	7	0.25	4	0.58		<150	<30	<2.5
84	M & P	7	0.25	5	0.62		<150	<30	<2.5
85	M & P	7	1	4	0.76		<150	32.77	<2.5
86	M & P	7	1	4	0.58		<150	<30	<2.5
87	M & P	7	8	4	4.86		<150	<30	48.4
88	M & P	7	8	4	0.99		<150	<30	<2.5
89	M & P	9	0	4	0.56		263.5	<30	<2.5
90	M & P	9	0	5	0.75		<150	<30	<2.5
91	M & P	9	0.25	4	1.37		<150	34.17	<2.5
92	M & P	9	0.25	5	0.75		153.83	<30	<2.5
93	M & P	9	1	4	1.08		185.2	<30	<2.5
94	M & P	9	1	5	1.73		<150	<30	<2.5
95	M & P	9	8	4	0.50		181.33	<30	40.6
96	M & P	9	8	5	0.50		<150	<30	<2.5
97	M & P	10	0	4	0.43		<150	<30	<2.5
98	M & P	10	0	5	0.45		<150	<30	<2.5
99	M & P	10	0.25	4	0.53		206.67	30.4667	<2.5
100	M & P	10	0.25	5	0.69		<150	<30	<2.5
101	M & P	10	1	4	1.15		301.03	<30	<2.5
102	M & P	10	1	5	0.78		<150	<30	<2.5
103	M & P	10	8	4	0.75		188.23	40.1667	34.6
104	M & P	10	8	5	0.48		<150	<30	<2.5
105	M & P	11	0	4	0.48		<150	<30	<2.5
106	M & P	11	0	5	0.70		<150	46.8	<2.5
107	M & P	11	0.25	4	0.64		<150	35.1667	<2.5
108	M & P	11	0.25	5	0.81		<150	<30	<2.5
109	M & P	11	1	4	0.75		<150	<30	<2.5
110	M & P	11	1	5	0.94		<150	<30	<2.5
111	M & P	11	8	4	0.82		<150	49.8	28.7
112	M & P	11	8	5	0.38		<150	<30	<2.5
113	M & P	12	0	4	0.73		<150	65.9	<2.5
114	M & P	12	0	5	0.37		<150	42.3	<2.5
115	M & P	12	0.25	4	0.38		300.53	78.5667	<2.5
116	M & P	12	0.25	5	0.77		<150	<30	<2.5
117	M & P	12	1	4	0.46		<150	88.2	<2.5
118	M & P	12	1	5	0.39		241.07	40.9	<2.5
119	M & P	12	8	4	0.43		<150	104.5	19.9
120	M & P	12	8	5	0.63		201.3	48.6	<2.5
121	M & P	14	0	4	0.82		<150	59.8	<2.5
122	M & P	14	0	5	0.83		<150	31.5	<2.5
123	M & P	14	0.25	4	1.04		<150	56.5	<2.5

Code	Visit	Volunteer	Dose	Visit No.	Histamine	Lease	Noradrenaline	Adrenaline	Morphine
124	M & P	14	0.25	5	0.79		<150	<30	<2.5
125	M & P	14	1	4	0.59		<150	53.9	<2.5
126	M & P	14	1	5	1.00		<150	37.9333	<2.5
127	M & P	14	8	4	0.91		<150	33.4	142.2
128	M & P	14	8	5	0.69		<150	42.5667	<2.5

Histamine concentration (ng/ml), Adrenaline concentration (pg/ml), Noradrenaline concentration (pg/ml), Morphine Concentration in ug/L, M & P; morphine versus saline study, M & M-N; morphine and saline versus morphine and naloxone study.

Forearm blood flow studies

Table III-VI. Exploratory dose ranging study

III-VI-I. FBF-P_0: Forearm blood flow in infused arm (ml/100ml forearm blood flow)

	A	B	Mean	SD	SEM
Baseline	2.5	1.55	**2.03**	**0.67**	**0.48**
1 mcg	2.86	1.56	**2.21**	**0.92**	**0.65**
3 mcg	2.7	1.1	**1.90**	**1.13**	**0.80**
10 mcg	3.2	1.01	**2.11**	**1.55**	**1.10**
30 mcg	4.69	1.87	**3.28**	**1.99**	**1.41**
100 mcg	5.1	4.59	**4.85**	**0.36**	**0.26**
300 mcg	10.6	11.08	**10.84**	**0.34**	**0.24**

Table III-VII. Dose ranging study

III-VII-I. FBF-P_1: Forearm blood flow in infused arm (ml/100ml forearm blood flow)

	A	B	C	D	E	F	Mean	SD	SEM
Baseline	2.00	2.49	2.90	2.26	2.77	2.09	**2.42**	**0.37**	**0.15**
1 mcg (0-6 min)	1.86	2.88	3.17	2.20	3.14	2.24	**2.58**	**0.55**	**0.23**
3 mcg (7-12 min)	2.04	2.98	3.14	2.79	3.78	2.11	**2.81**	**0.66**	**0.27**
10 mcg (13-18 min)	2.44	3.32	3.91	2.81	3.94	2.04	**3.08**	**0.78**	**0.32**
30 mcg (19-24 min)	2.39	3.56	3.95	3.42	3.62	2.53	**3.25**	**0.63**	**0.26**
100 mcg (25-30 min)	4.33	6.47	6.17	6.41	4.65	3.34	**5.23**	**1.31**	**0.53**
Saline (+ 6 min)	4.23	5.90	5.87	4.24	4.50	2.79	**4.59**	**1.17**	**0.48**
Saline (+12 min)	4.29	5.28	5.11	3.73	4.37	2.63	**4.24**	**0.97**	**0.40**
Saline (+18 min)	4.09	4.58	5.21	3.44	3.72	2.92	**3.99**	**0.82**	**0.33**
Saline (+24 min)	3.88	4.79	5.01	3.72	3.44	2.68	**3.92**	**0.87**	**0.35**
Saline (+30 min)	3.53	4.17	5.16	3.69	3.62	2.76	**3.82**	**0.80**	**0.33**

III-VII-II. FBF-P_1: Area of redness (cm^2)

	A	B	C	D	E	F	Mean	SD	SEM
Baseline	0	0	0	0	0	1	**0.1**	**0.2**	**0.1**
1 mcg (0-6 min)	0	0	0	0	0	0	**0.0**	**0.1**	**0.0**
3 mcg (7-12 min)	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
10 mcg (13-18 min)	0	0	0	0	0	0	**0.0**	**0.1**	**0.0**
30 mcg (19-24 min)	0	9	0	9	0	0	**3.0**	**4.6**	**1.9**
100 mcg (25-30 min)	62	196	56	18	70	22	**70.7**	**65.0**	**26.6**
Saline (+ 6 min)	84	168	64	35	70	20	**73.5**	**51.9**	**21.2**
Saline (+12 min)	74	131	56	35	48	17	**60.1**	**39.8**	**16.2**
Saline (+18 min)	50	100	46	32	55	22	**50.7**	**27.1**	**11.1**
Saline (+24 min)	60	24	42	33	25	14	**33.0**	**16.3**	**6.6**
Saline (+30 min)	53	24	39	26	20	15	**29.5**	**14.0**	**5.7**
Observed (+60 min)	18	0	5	14	0	0	**6.0**	**7.9**	**3.2**
Observed (+90 min)	0	0	0	2	0	0	**0.3**	**0.8**	**0.3**

III-VII-III. FBF-P_1: Area of weal (cm^2)

	A	B	C	D	E	F	Mean	SD	SEM
Baseline	0	0	0	0	0	0	**0**	**0**	**0**
1 mcg (0-6 min)	0	0	0	0	0	0	**0**	**0**	**0**
3 mcg (7-12 min)	0	0	0	0	0	0	**0**	**0**	**0**
10 mcg (13-18 min)	0	0	0	0	0	0	**0**	**0**	**0**
30 mcg (19-24 min)	0	0	0.5	0	0	0	**0.08**	**0.2**	**0.08**
100 mcg (25-30 min)	19	15	30	12	22	27	**20.8**	**6.91**	**2.82**
Saline (+ 6 min)	62	21	36	12	33	16	**30**	**18.3**	**7.46**
Saline (+12 min)	62	21	36	12	30	24	**30.8**	**17.3**	**7.06**
Saline (+18 min)	54	18	33	10	12	20	**24.5**	**16.6**	**6.76**
Saline (+24 min)	43	11	33	7.5	11	12	**19.4**	**14.6**	**5.96**
Saline (+30 min)	47	11	33	7.5	11	12	**20.2**	**16.1**	**6.56**
Observed (+60 min)	23	0	4.5	4	0	15	**7.75**	**9.27**	**3.79**
Observed (+90 min)	7	0	0	1	0	6	**2.33**	**3.27**	**1.33**

III-VII-IV. FBF-P_1: Presence of itching (%)

	A	B	C	D	E	F	Sum	Percentage
Baseline	0	0	0	0	0	0	**0**	**0**
1 mcg (0-6 min)	0	0	0	0	0	0	**0**	**0**
3 mcg (7-12 min)	0	0	0	0	0	0	**0**	**0**
10 mcg (13-18 min)	0	0	0	0	0	0	**0**	**0**
30 mcg (19-24 min)	0	1	1	1	0	0	**3**	**50**
100 mcg (25-30 min)	1	1	1	1	1	1	**6**	**100**
Saline (+ 6 min)	0	1	1	1	0	1	**4**	**66.7**
Saline (+12 min)	0	0	0	0	0	0	**0**	**0**
Saline (+18 min)	0	0	0	0	0	0	**0**	**0**
Saline (+24 min)	0	0	0	0	0	0	**0**	**0**
Saline (+30 min)	0	0	0	0	0	0	**0**	**0**
Observed (+60 min)	0	0	0	0	0	0	**0**	**0**
Observed (+90 min)	0	0	0	0	0	0	**0**	**0**

III-VII-V. FBF-P_1: Intensity of itching (subjective scale)

	A	B	C	D	E	F	Mean	SD	SEM
Baseline	0	0	0	0	0	0	0	0	0
1 mcg (0-6 min)	0	0	0	0	0	0	0	0	0
3 mcg (7-12 min)	0	0	0	0	0	0	0	0	0
10 mcg (13-18 min)	0	0	0	0	0	0	0	0	0
30 mcg (19-24 min)	0	5	3	5	0	0	2.17	2.48	1.01
100 mcg (25-30 min)	7	8	8	7	5	3	6.33	1.97	0.80
Saline (+ 6 min)	0	1	1	1	0	1	0.67	0.52	0.21
Saline (+12 min)	0	0	0	0	0	0	0	0	0
Saline (+18 min)	0	0	0	0	0	0	0	0	0
Saline (+24 min)	0	0	0	0	0	0	0	0	0
Saline (+30 min)	0	0	0	0	0	0	0	0	0
Observed (+60 min)	0	0	0	0	0	0	0	0	0
Observed (+90 min)	0	0	0	0	0	0	0	0	0

Table III-VIII. Tachyphylaxis study ($FBF\text{-}P_2$)

III-VIII-I. $FBF\text{-}P_2$; Forearm blood flow in infused arm (ml/100ml forearm blood flow)

	A	B	C	D	E	F	G	H	Mean	SD	SEM
Saline (0 min)	3.6	2.7	3.1	2.1	3.7	4.8	1.7	2.5	**3.03**	**0.99**	**0.35**
50 mcg (+10 min)	3.7	3.4	3.9	3.5	5.3	5	2.5	3.3	**3.82**	**0.92**	**0.326**
50 mcg (+20 min)	3.6	3.2	4.2	3.6	5.6	5	3	3.1	**3.91**	**0.96**	**0.34**
50 mcg (+30 min)	4.8	3	4.8	3.2	6	4.7	3.4	3.5	**4.19**	**1.04**	**0.368**
Saline (+40 min)	4.8	3.6	4.5	3.1	5.7	4.8	3.2	3.3	**4.11**	**0.96**	**0.338**
Saline (+50 min)	5.6	3.2	4.5	3.6	4.8	3.8	3.5	4.3	**4.17**	**0.79**	**0.279**
Saline (+60 min)	4.9	4.2	4.5	1.2	4.6	4	3.9	4.4	**3.97**	**1.16**	**0.411**
Saline (+70 min)	4.3	4	4.1	2.5	4.2	3.7	4	5.5	**4.05**	**0.81**	**0.287**
Saline (+80 min)		3.1	2.5	2.2	3.8	3.6	3.5	5	**3.4**	**0.92**	**0.326**
Saline (+90 min)		3.1		2.3	4.4	3.4	3.5	5.3	**3.65**	**1.04**	**0.366**

III- VIII II. $FBF\text{-}P_2$; Forearm blood flow in non-Infused arm (ml/100ml forearm blood flow)

	A	B	C	D	E	F	G	H	Mean	SD	SEM
Saline (0 min)	4.8	2.3	2.2	1.6	2.2	3.9	1.8	2.0	**2.60**	**1.13**	**0.40**
50 mcg (+10 min)	2.8	2.1	2.4	2.1	2.7	3.2	1.8	1.4	**2.30**	**0.58**	**0.21**
50 mcg (+20 min)	3.2	1.7	2.8	2.7	2.6	3.0	2.1	1.4	**2.44**	**0.65**	**0.23**
50 mcg (+30 min)	4.0	1.8	2.8	2.3	2.8	2.7	2.2	1.0	**2.45**	**0.86**	**0.31**
Saline (+40 min)	3.9	3.0	2.8	2.0	2.4	2.8	1.8	1.1	**2.48**	**0.84**	**0.30**
Saline (+50 min)	5.5	2.5	3.0	2.3	2.7	2.2	1.7	1.3	**2.63**	**1.26**	**0.45**
Saline (+60 min)	4.2	2.4	2.3	1.0	3.2	2.4	1.9	1.3	**2.35**	**0.99**	**0.35**
Saline (+70 min)	4.1	2.9	3.2	1.9	2.7	2.2	2.2	2.8	**2.76**	**0.68**	**0.24**
Saline (+80 min)		2.4	1.7	1.6	2.8	2.1	2.3	2.1	**2.16**	**0.41**	**0.15**
Saline (+90 min)		2.5		1.7	3.4	2.0	2.3	1.9	**2.29**	**0.61**	**0.21**

III-VIII-III. $FBF\text{-}P_2$; Area of the flare (cm^2)

	A	B	C	D	E	F	G	H	Mean	SD	SEM
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
50 mcg (+10 min)	12	8	54	40	18	80	5	22	**29.9**	**26.2**	**10.7**
50 mcg (+20 min)	18	8	60	48	10	110	8	26	**36.0**	**35.6**	**14.5**
50 mcg (+30 min)	20	6	52	60	10	120	10	26	**38.0**	**38.7**	**15.8**
Saline (+40 min)	18	6	42	60	10	128	10	24	**37.3**	**41.0**	**16.8**
Saline (+50 min)	12	6	25	50	10	128	10	20	**32.6**	**41.0**	**16.7**
Saline (+60 min)	12	4	18	20	10	62	10	10	**18.3**	**18.4**	**7.5**
Saline (+70 min)	10	2	12	20	2	20	2	0	**8.5**	**8.3**	**3.4**
Saline (+80 min)	0	0	8	10	1	6	1	0	**3.3**	**4.1**	**1.7**
Saline (+90 min)	0	0	0	0	0	6	0	0	**0.8**	**2.1**	**0.9**

III- VIII-IV. $FBF\text{-}P_2$; Area of the weal (cm^2)

	A	B	C	D	E	F	G	H	Mean	SD	SEM
Saline (0 min)	0	0	0	0	0	0	0	0	**0.00**	**0.00**	**0.00**
50 mcg (+10 min)	1	3	10	4	0	20	3	4	**5.63**	**6.52**	**2.66**
50 mcg (+20 min)	8	4	18	8	0	36	3	3	**10.00**	**11.84**	**4.84**
50 mcg (+30 min)	12	4	20	12	0	36	4	4	**11.50**	**11.80**	**4.82**
Saline (+40 min)	12	4	20	12	0	42	4	3	**12.13**	**13.72**	**5.60**
Saline (+50 min)	12	4	14	12	0	42	3	2	**11.13**	**13.56**	**5.54**
Saline (+60 min)	8	2	14	10	0	30	3	2	**8.63**	**9.87**	**4.03**
Saline (+70 min)	0	0	10	6	0	18	1	0	**4.38**	**6.63**	**2.71**
Saline (+80 min)	0	0	6	1	0	6	1	0	**1.75**	**2.66**	**1.09**
Saline (+90 min)	0	0	0	0	0	6	0	0	**0.75**	**2.12**	**0.87**

III- VIII-V. FBF-P_2; Presence of itching (%)

	A	B	C	D	E	F	G	H	Sum	Percentage
Saline (0 min)	0	0	0	0	0	0	0	0	**0**	**0.00**
50 mcg (+10 min)	1	1	1	1	1	1	0	1	**7**	**87.50**
50 mcg (+20 min)	1	1	1	1	1	1	1	1	**8**	**100.00**
50 mcg (+30 min)	1	1	1	1	1	1	1	1	**8**	**100.00**
Saline (+40 min)	0	1	0	1	0	1	0	0	**3**	**37.50**
Saline (+50 min)	0	1	0	1	0	0	0	0	**2**	**25.00**
Saline (+60 min)	0	0	0	1	0	0	0	0	**1**	**12.50**
Saline (+70 min)	0	0	0	1	0	0	0	0	**1**	**12.50**
Saline (+80 min)	0	0	0	1	0	0	0	0	**1**	**12.50**
Saline (+90 min)	0	0	0	0	0	0	0	0	**0**	**0.00**

III- VIII-VI. FBF-P_2; Intensity of itching (subjective scale)

	A	B	C	D	E	F	G	H	Mean	SD	SEM
Saline (0 min)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.00**	**0.00**	**0.00**
50 mcg (+10 min)	5.0	6.0	3.0	9.0	2.0	4.0	0.0	4.0	**4.13**	**2.70**	**1.10**
50 mcg (+20 min)	3.0	7.0	3.0	9.0	1.0	5.0	2.0	3.0	**4.13**	**2.70**	**1.10**
50 mcg (+30 min)	3.0	6.0	3.0	5.0	1.0	4.0	1.0	3.0	**3.25**	**1.75**	**0.72**
Saline (+40 min)	0.0	2.0	0.0	2.0	0.0	1.0	0.0	0.0	**0.63**	**0.92**	**0.37**
Saline (+50 min)	0.0	2.0	0.0	1.0	0.0	0.0	0.0	0.0	**0.38**	**0.74**	**0.30**
Saline (+60 min)	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	**0.13**	**0.35**	**0.14**
Saline (+70 min)	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	**0.13**	**0.35**	**0.14**
Saline (+80 min)	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	**0.13**	**0.35**	**0.14**
Saline (+90 min)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.00**	**0.00**	**0.00**

III- VIII-VII. Tryptase

		Patient										
Arm	**Sample**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**	**SD**	**SEM**
Control	Baseline	5.92	1.74	2.62	8.86	3.63	1.92	15.2		**5.7**	**4.9**	**0.6**
Control	10 min	5.58	2.33	2.5	6.98	4.17		1.65		**3.9**	**2.1**	**0.3**
Control	30 min	5.12	2.41	1.97	8.86	4.51	1.18	15.8		**5.7**	**5.1**	**0.6**
Control	90 min	5.13		2.64	10.9	4.43	2.26	2.21	2.28	**4.3**	**3.2**	**0.4**
	Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Mean	SD	SEM
Infused	Baseline	4.29	1.87	2.89	9.89	4.34	3.81	11.8	1.72	**5.1**	**3.7**	**0.5**
Infused	10 min	4.6	2.01		8.01	4.16	1.06	5.54	1.59	**3.9**	**2.5**	**0.3**
Infused	30 min	3.03	1.99	2.42	9.47	4.08		5.64		**4.4**	**2.8**	**0.3**
Infused	90 min	5.08	1.77	2.36	10	3.2	2.44	1.86		**3.8**	**3**	**0.4**

III- VIII-VIII. Histamine

		Patient										
Arm	**Sample**	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**	**6.00**	**7.00**	**8.00**	**Mean**	**SD**	**SEM**
Control	Baseline	0.36	0.31	0.25	0.26	0.27	0.28	0.50	0.60	4.50	2.45	
Control	10 min	0.16	0.49	0.24	0.20	0.26	0.26	0.29	0.49	0.35	0.13	0.31
Control	30 min	0.11	0.12	0.18	0.12	0.21	0.16	0.25	0.21	0.30	0.13	0.02
Control	90 min	0.11	0.17	0.22	0.39	0.20	0.13	0.19	0.47	0.17	0.05	0.02
	Sample	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	Mean	SD	SEM
Infused	Baseline	0.08	0.17	0.18		0.19	0.19		0.21	4.50	2.45	
Infused	10 min	0.12	0.60	0.18	4.17	0.36	0.13	0.90	0.57	0.35	0.13	0.31
Infused	30 min	0.16	0.20	0.19	0.21	0.29	0.23	0.54	0.17	0.30	0.13	0.02
Infused	90 min	0.27	0.12	0.23	2.88	0.30	0.26	0.89	17.82	0.17	0.08	0.02

Table III-IX. Mechanism of action study (FBF-P_3)

III-IX-I. FBF-P_3; Forearm blood flow in infused arm (ml/100ml forearm blood flow)

Morphine visit											
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Mean**	**SD**	**SEM**
Saline (-20 min)	3.1	2.5	2.6	4.3	1.9	2.2	3.4	2.3	**2.77**	**0.79**	**0.28**
Saline (-10 min)	2.7	2.4	2.0	5.8	1.8	2.2	2.9	2.1	**2.73**	**1.28**	**0.45**
Saline (0 min)	3.0	2.7	2.9	6.1	1.7	2.2	2.8	1.9	**2.90**	**1.38**	**0.49**
80 mcg (+10 min)	3.7	3.0	3.9	9.0	3.2	2.8	2.9	3.2	**3.95**	**2.06**	**0.73**
80 mcg (+20 min)	4.3	3.0	3.7	8.2	3.0	2.5	3.9	3.3	**3.99**	**1.79**	**0.63**
80 mcg (+30 min)	4.5	3.4	4.2	10.3	3.0	2.7	3.5	2.8	**4.30**	**2.51**	**0.89**
Saline (+40 min)	4.0	3.3	5.2	8.7	3.2	2.6	3.3	2.8	**4.13**	**2.03**	**0.72**
Saline (+50 min)	4.1	2.7	3.6	6.9	2.9	2.7	3.3	2.2	**3.54**	**1.48**	**0.52**
Saline (+60 min)	2.7	3.1	3.3	8.7	3.2	2.3	3.2	2.3	**3.60**	**2.08**	**0.74**
Morphine and naloxone visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	2.7	1.9	3.2	3.5	1.9	1.7	3.1	1.5	**2.43**	**0.79**	**0.28**
Saline (-10 min)	2.6	2.2	3.1	4.8	1.9	1.7	2.8	1.3	**2.56**	**1.08**	**0.38**
Saline (0 min)	2.8	1.7	3.7	4.1	2.2	1.6	3.0	1.4	**2.56**	**0.99**	**0.35**
80 mcg (+10 min)	3.3	3.5	5.4	8.6	3.3	1.9	3.2	2.9	**4.03**	**2.10**	**0.74**
80 mcg (+20 min)	4.2	3.5	6.0	7.0	3.1	1.6	3.2	3.5	**4.02**	**1.70**	**0.60**
80 mcg (+30 min)	4.1	3.8	5.3	7.2	2.1	2.4	3.3	3.7	**3.99**	**1.64**	**0.58**
Saline (+40 min)	4.1	2.8	5.6	6.4	2.6	1.5	3.8	3.2	**3.74**	**1.62**	**0.57**
Saline (+50 min)	3.8	3.0		4.5	1.9	1.8	3.9	3.0	**3.13**	**1.02**	**0.36**
Saline (+60 min)	3.5	2.8		5.6	1.5	1.7	3.6		**3.12**	**1.48**	**0.52**
Morphine and H_1 and H_2 blocker visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	2.9	2.2	4.1	2.2	2.8	1.6	2.3	1.4	**2.42**	**0.85**	**0.30**
Saline (-10 min)	2.4	1.8	4.4	1.9	2.7	1.2	2.3	1.3	**2.25**	**1.02**	**0.36**
Saline (0 min)	2.6	2.4	4.8	2.2	2.6	1.0	2.8	1.0	**2.43**	**1.20**	**0.42**
80 mcg (+10 min)	3.3	2.5	5.1	2.8	3.1	1.2	3.6	1.3	**2.87**	**1.27**	**0.45**
80 mcg (+20 min)	3.2	2.9	5.5	3.2	3.8	1.2	3.2	1.4	**3.06**	**1.36**	**0.48**
80 mcg (+30 min)	3.0	2.5	6.0	3.2	3.3	1.0	3.3	1.9	**3.02**	**1.46**	**0.52**
Saline (+40 min)	2.8	3.0	5.3	4.2	3.7	1.4	2.9	1.6	**3.13**	**1.31**	**0.46**
Saline (+50 min)	2.4	3.1	5.4	3.9	3.2	1.2	3.3	1.3	**2.99**	**1.37**	**0.49**
Saline (+60 min)		3.1	4.1	3.7	2.9	1.3	3.2	1.2	**2.79**	**1.12**	**0.40**
Morphine and nitric oxide clamp visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	2.1	2.6	1.6	4.3	1.9	1.7	3.5	2.1	**2.46**	**0.94**	**0.33**
Saline (-10 min)	2.1	2.2	1.4	3.8	1.7	2.5	3.3	1.8	**2.34**	**0.84**	**0.30**
Saline (0 min)	2.2	2.1	1.2	4.0	1.9	3.0	3.6	1.7	**2.46**	**0.96**	**0.34**
80 mcg (+10 min)	2.4	2.6	2.4	3.6	2.9	2.7	3.6	2.9	**2.90**	**0.48**	**0.17**
80 mcg (+20 min)	2.4	2.8	1.6	3.8	2.4	2.3	2.8	2.4	**2.57**	**0.63**	**0.22**
80 mcg (+30 min)	2.3	3.0	2.0	4.0	2.3	2.2	2.8	2.9	**2.71**	**0.63**	**0.22**
Saline (+40 min)	2.4	2.9	1.5		2.3	2.6	2.9	3.0	**2.52**	**0.52**	**0.18**
Saline (+50 min)	2.0	2.8	1.8		2.1	2.2	3.0	3.1	**2.44**	**0.55**	**0.19**
Saline (+60 min)	2.0	2.6	1.9		1.9	2.2	2.6	1.9	**2.17**	**0.32**	**0.11**

III-IX-II. FBF-P_3; Forearm blood flow in non-infused arm (ml/100ml forearm blood flow)

Morphine visit											
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Mean**	**SD**	**SEM**
Saline (-20 min)	2.3	2.4	2.7	4.9	1.9	3.4	2.8	1.9	**2.78**	**1.00**	**0.36**
Saline (-10 min)	1.9	2.1	2.6	5.4	1.9	3.4	2.8	1.6	**2.73**	**1.24**	**0.44**
Saline (0 min)	2.0	2.6	3.1	4.9	1.8	2.7	2.8	1.8	**2.72**	**1.03**	**0.36**
80 mcg (+10 min)	1.8	2.4	2.6	4.6	1.4	2.7	2.5	2.3	**2.52**	**0.92**	**0.33**
80 mcg (+20 min)	2.1	2.7	2.5	4.5	1.7	2.1	2.8	2.1	**2.56**	**0.84**	**0.30**
80 mcg (+30 min)	1.8	2.9	2.5	4.6	1.9	2.5	3.0	2.1	**2.66**	**0.91**	**0.32**
Saline (+40 min)	1.8	3.0	2.5	4.0	2.3	2.6	2.8	2.3	**2.68**	**0.65**	**0.23**
Saline (+50 min)	2.0	2.7	2.4	3.7	2.1	2.6	2.7	2.2	**2.54**	**0.54**	**0.19**
Saline (+60 min)	1.8	3.1	2.2	5.5	1.9	2.6	3.2	1.9	**2.77**	**1.24**	**0.44**
Morphine and naloxone visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	2.9	2.4	2.7	3.7	2.2	1.3	3.0	0.9	**2.39**	**0.94**	**0.33**
Saline (-10 min)	2.6	2.6	2.7	4.9	1.9	1.2	2.3	0.6	**2.37**	**1.27**	**0.45**
Saline (0 min)	2.8	2.3	2.8	4.3	2.4	1.4	2.5	0.7	**2.41**	**1.04**	**0.37**
80 mcg (+10 min)	2.4	2.3	3.0	4.7	2.3	1.2	2.3	1.1	**2.40**	**1.13**	**0.40**
80 mcg (+20 min)	2.9	2.1	2.5	4.1	2.0	1.1	2.6	1.6	**2.37**	**0.91**	**0.32**
80 mcg (+30 min)	3.1	2.2	3.1	4.0	1.6	1.3	2.4	1.8	**2.44**	**0.91**	**0.32**
Saline (+40 min)	3.3	3.0	3.8	3.9	2.2	0.9	2.5	1.7	**2.65**	**1.04**	**0.37**
Saline (+50 min)	2.8	2.6		3.2	1.7	1.7	2.7	1.5	**2.32**	**0.66**	**0.23**
Saline (+60 min)	2.6	2.9		3.8	1.5	1.5	2.1		**2.42**	**0.90**	**0.32**
Morphine and H_1 and H_2 blocker visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	1.8	1.9	3.6	3.3	3.8	1.5	3.2	0.9	**2.50**	**1.09**	**0.38**
Saline (-10 min)	1.8	1.5	3.6	2.5	3.4	1.5	2.1	1.0	**2.16**	**0.94**	**0.33**
Saline (0 min)	1.9	1.8	4.2	3.3	3.1	1.5	2.3	0.8	**2.37**	**1.09**	**0.39**
80 mcg (+10 min)	2.0	1.2	3.4	3.3	2.8	1.9	2.8	0.9	**2.29**	**0.94**	**0.33**
80 mcg (+20 min)	2.0	1.8	3.4	3.1	3.3	2.5	2.4	1.0	**2.44**	**0.81**	**0.29**
80 mcg (+30 min)	1.8	1.3	3.8	2.9	2.8	2.0	2.4	1.2	**2.26**	**0.88**	**0.31**
Saline (+40 min)	1.9	1.5	3.2	3.2	3.4	2.5	2.2	1.0	**2.37**	**0.87**	**0.31**
Saline (+50 min)	1.5	1.3	3.3	3.2	3.1	2.3	2.4	1.1	**2.27**	**0.88**	**0.31**
Saline (+60 min)		1.9	2.7	3.5	2.8	2.3	2.4	1.0	**2.36**	**0.79**	**0.28**
Morphine and nitric oxide clamp visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	1.5	2.9	1.8	4.6	2.1	2.8	3.5	1.5	**2.57**	**1.07**	**0.38**
Saline (-10 min)	2.0	2.3	1.3	3.7	1.9	3.3	3.0	1.2	**2.34**	**0.91**	**0.32**
Saline (0 min)	1.7	2.3	1.0	3.8	1.9	3.7	2.8	1.7	**2.37**	**1.01**	**0.36**
80 mcg (+10 min)	1.5	2.4	1.6	4.2	1.8	2.1	2.4	2.0	**2.24**	**0.88**	**0.31**
80 mcg (+20 min)	1.6	2.2	0.7	3.8	1.6	1.9	1.9	1.5	**1.90**	**0.88**	**0.31**
80 mcg (+30 min)	1.4	2.1	1.1	4.2	1.5	1.7	1.9	2.2	**2.00**	**0.95**	**0.34**
Saline (+40 min)	1.7	2.2	1.1	4.0	1.6	2.4	1.9	2.0	**2.10**	**0.86**	**0.30**
Saline (+50 min)	1.3	2.4	1.3	4.0	1.7	1.9	2.2	2.5	**2.17**	**0.86**	**0.30**
Saline (+60 min)	1.6	2.3	0.9	4.0	1.6	2.1	1.9	1.8	**2.02**	**0.90**	**0.32**

III-IX-III. FBF-P_3; Area of the flare (cm^2)

Morphine visit											
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	3	0	0	0	**0.4**	**1.1**	**0.4**
Saline (-10 min)	0	0	0	0	3	0	0	0	**0.4**	**1.1**	**0.4**
Saline (0 min)	0	0	0	0	3	0	0	0	**0.4**	**1.1**	**0.4**
80 mcg (+10 min)	6	15	14	56	20	120	2	36	**33.6**	**39.0**	**15.9**
80 mcg (+20 min)	6	5	60	80	5	121	11	45	**41.6**	**43.1**	**17.6**
80 mcg (+30 min)	32	2	42	80	4	144	8	45	**44.6**	**48.0**	**19.6**
Saline (+40 min)	32	0	25	63	2	80	0	15	**27.1**	**30.2**	**12.3**
Saline (+50 min)	18	0	12	40	1	36	0	0	**13.4**	**16.6**	**6.8**
Saline (+60 min)	15	0	8	6	1	9	0	0	**4.9**	**5.6**	**2.3**
Morphine and naloxone visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	70	27	0	45	40	196	77	64	**64.9**	**58.6**	**23.9**
80 mcg (+20 min)	100	27	0	65	104	131	89	120	**79.5**	**45.8**	**18.7**
80 mcg (+30 min)	86	25	0	75	128	156	101	120	**86.4**	**52.5**	**21.4**
Saline (+40 min)	83	16	0	48	104	91	55	36	**54.1**	**36.7**	**15.0**
Saline (+50 min)	50	9	0	15	24	9	36	15	**19.8**	**16.3**	**6.7**
Saline (+60 min)	0	9	0	0	0	6	38	0	**6.6**	**13.1**	**5.4**
Morphine and H_1 and H_2 blocker visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	24	0	0	0	0	0	0	**3.0**	**8.5**	**3.5**
80 mcg (+20 min)	0	20	0	0	0	0	0	0	**2.5**	**7.1**	**2.9**
80 mcg (+30 min)	0	15	0	0	0	0	0	0	**1.9**	**5.3**	**2.2**
Saline (+40 min)	0	12	0	0	0	0	0	0	**1.5**	**4.2**	**1.7**
Saline (+50 min)	0	9	0	0	0	0	0	0	**1.1**	**3.2**	**1.3**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Morphine and nitric oxide clamp visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	0	156	44	57	59	50	48	**51.8**	**48.4**	**19.7**
80 mcg (+20 min)	0	0	131	20	100	50	90	30	**52.6**	**49.1**	**20.1**
80 mcg (+30 min)	0	0	88	12	100	30	71	60	**45.1**	**39.8**	**16.3**
Saline (+40 min)	0	0	45		63	18	64	54	**34.9**	**28.4**	**11.6**
Saline (+50 min)	0	0	21		24	12	30	28	**16.4**	**12.6**	**5.2**
Saline (+60 min)	0	0	0		4	0	30	0	**4.9**	**11.2**	**4.6**

III-IX-IV. FBF-P_3; Area of the weal (cm^2)

Morphine visit											
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	0	0	8	0	15	2	0	**3.1**	**5.6**	**2.3**
80 mcg (+20 min)	1	0	1	18	0	28	1	0	**6.0**	**10.7**	**4.4**
80 mcg (+30 min)	2	0	2	18	0	42	1	0	**8.1**	**15.0**	**6.1**
Saline (+40 min)	2	0	1	11	0	39	1	0	**6.8**	**13.5**	**5.5**
Saline (+50 min)	1	0	1	11	0	21	1	0	**4.4**	**7.7**	**3.1**
Saline (+60 min)	1	0	1	3	0	9	1	0	**1.9**	**3.0**	**1.2**
Morphine and naloxone visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	23	0	3	65	56	3	2	**19.0**	**26.8**	**10.9**
80 mcg (+20 min)	0	23	0	4	73	64	6	5	**21.8**	**29.8**	**12.2**
80 mcg (+30 min)	0	24	0	2	77	80	19	5	**25.8**	**33.7**	**13.8**
Saline (+40 min)	0	18	0	1	66	36	18	3	**17.8**	**23.2**	**9.5**
Saline (+50 min)	0	13	0	1	34	6	18	3	**9.4**	**11.9**	**4.8**
Saline (+60 min)	0	3	0	1	28	4	9	1	**5.8**	**9.5**	**3.9**
Morphine and H_1 and H_2 blocker visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+30 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+40 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+50 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Morphine and nitric oxide clamp visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	0	0	1	10	0	2	0	**1.6**	**3.5**	**1.4**
80 mcg (+20 min)	0	0	28	1	28	0	3	1	**7.5**	**12.7**	**5.2**
80 mcg (+30 min)	0	0	18	0	56	0	9	1	**10.5**	**19.5**	**8.0**
Saline (+40 min)	0	0	15		28	0	4	1	**6.9**	**10.8**	**4.4**
Saline (+50 min)	0	0	12		24	0	3	1	**5.7**	**9.1**	**3.7**
Saline (+60 min)	0	0	2		3	0	3	0	**1.1**	**1.5**	**0.6**

III-IX-V. FBF-P_3; Presence of itching (%)

Morphine visit										
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Sum**	**Percentage**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
80 mcg (+10 min)	1	1	0	1	1	0	0	0	**4**	**50.0**
80 mcg (+20 min)	1	1	1	1	0	0	0	1	**5**	**62.5**
80 mcg (+30 min)	0	1	1	0	0	0	0	0	**2**	**25.0**
Saline (+40 min)	0	1	0	0	0	0	0	0	**1**	**12.5**
Saline (+50 min)	0	1	0	0	0	0	0	0	**1**	**12.5**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Morphine and naloxone visit										
	A	B	C	D	E	F	G	H	**Sum**	**Percentage**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
80 mcg (+10 min)	0	1	0	1	0	0	0	1	**3**	**37.5**
80 mcg (+20 min)	0	1	0	0	0	0	0	0	**1**	**12.5**
80 mcg (+30 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (+40 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (+50 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Morphine and H_1 and H_2 blocker visit										
	A	B	C	D	E	F	G	H	**Sum**	**Percentage**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
80 mcg (+10 min)	0	1	0	0	0	0	1	1	**3**	**37.5**
80 mcg (+20 min)	0	1	0	0	0	0	0	0	**1**	**12.5**
80 mcg (+30 min)	0	1	0	0	0	0	0	0	**1**	**12.5**
Saline (+40 min)	0	1	0	0	0	0	0	0	**1**	**12.5**
Saline (+50 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Morphine and nitric oxide clamp visit										
	A	B	C	D	E	F	G	H	**Sum**	**Percentage**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
80 mcg (+10 min)	0	0	0	1	0	0	1	1	**3**	**37.5**
80 mcg (+20 min)	0	0	0	0	0	0	1	1	**2**	**25.0**
80 mcg (+30 min)	0	0	0	0	0	0	0	1	**1**	**12.5**
Saline (+40 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (+50 min)	0	0	0	0	0	0	0	0	**0**	**0.0**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0**	**0.0**

III-IX-VI. FBF-P_3; Intensity of itching (subjective scale)

Morphine visit											
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	4	3	0	2	3	0	0	0	**1.5**	**1.7**	**0.7**
80 mcg (+20 min)	5	7	8	5	0	0	0	7	**4.0**	**3.5**	**1.4**
80 mcg (+30 min)	0	2	6	0	0	0	0	0	**1.0**	**2.1**	**0.9**
Saline (+40 min)	0	2	0	0	0	0	0	0	**0.3**	**0.7**	**0.3**
Saline (+50 min)	0	2	0	0	0	0	0	0	**0.3**	**0.7**	**0.3**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Morphine and naloxone visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	2	0	9	0	0	0	1	**1.7**	**3.1**	**1.3**
80 mcg (+20 min)	0	3	0	0	0	0	0	0	**0.4**	**1.1**	**0.4**
80 mcg (+30 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+40 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+50 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Morphine and H_1 and H_2 blocker visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	7	0	0	0	0	0	2	**1.3**	**2.5**	**1.0**
80 mcg (+20 min)	0	4	0	0	0	0	0	0	**0.6**	**1.4**	**0.6**
80 mcg (+30 min)	0	1	0	0	0	0	0	0	**0.1**	**0.4**	**0.1**
Saline (+40 min)	0	1	0	0	0	0	0	0	**0.1**	**0.4**	**0.1**
Saline (+50 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Morphine and nitric oxide clamp visit											
	A	B	C	D	E	F	G	H	**Mean**	**SD**	**SEM**
Saline (-20 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (-10 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (0 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
80 mcg (+10 min)	0	0	6	1	0	0	2	4	**1.9**	**2.3**	**0.9**
80 mcg (+20 min)	0	0	0	0	0	0	1	7	**1.1**	**2.4**	**1.0**
80 mcg (+30 min)	0	0	0	0	0	0	0	3	**0.4**	**1.1**	**0.4**
Saline (+40 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+50 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**
Saline (+60 min)	0	0	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**