Immune, microvascular and haemodynamic effects of dopexamine in rodent models of laparotomy & endotoxaemia
Bangash, Mansoor Nawaz

For additional information about this publication click this link.
http://qmro.qmul.ac.uk/jspui/handle/123456789/8574

Immune, microvascular and haemodynamic effects of dopexamine in rodent models of laparotomy & endotoxaemia

Mansoor Nawaz Bangash

Submitted in partial fulfilment of the requirements of the Degree of Doctor of Philosophy

Declaration

I, Mansoor Nawaz Bangash, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university.

The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without the prior written consent of the author.

Signature:

Date: 15 - 02 - 2015

Details of collaboration and previous publications:

Bangash MN, Patel NS, Benetti E, Collino M, Hinds CJ, Thiemermann C, Pearse RM. Dopexamine can attenuate the inflammatory response and protect against organ injury in the absence of significant effects on hemodynamics or regional microvascular flow. *Crit Care*. 2013 Mar 28;17(2):R57

Written by Bangash (editorial advice from C Hinds, C Thiemermann & RM Pearse; manuscript approved by all authors). All experimental work performed by Bangash except MPO analysis (E Benetti & M Collino). Signficant training and support in use of laboratory techniques by NS Patel. Significant training and support in the use of the Luminex machine for the analysis of cytokines by Elizabeth G Wood (laboratory assistant at Charterhouse Square, QMUL).

Bangash MN, Kong ML, Pearse RM. Use of inotropes and vasopressor agents in critically ill patients. *Br J Pharmacol*. 2012 Apr;165(7):2015-33.

Pages 2015-24 written by Bangash with editorial advice from RM Pearse & ML Kong. Section on clinical trials of inotropes and vasopressors (p2024-6) jointly written with RM Pearse. Editing and proof-reading by ML Kong. Final manuscript approved by all authors.

Bangash MN, Pearse RM. Microcirculation, In: Vincent J-L, Hall JB (Eds.) *Encyclopaedia of Intensive Care Medicine*. London. Springer Reference. 2012. P1395-9

Written by Bangash with editorial advice from RM Pearse.

The myography chapter in this thesis was the result of collaboration with Dr Rebecca Lever (School of Pharmacy, London). Dr Lever provided tutoring in wire myography techniques and significant support and advice during the conduct of experiments and writing of the myography chapter.

Acknowledgements

On a personal note, I am grateful to my parents for their support, and eternally indebted to my wife Sara for both her tolerance and love – without them I could not have achieved what I have.

This thesis is dedicated to Sara and my beautiful children, Baryalai and Zarak.

Abstract

A growing body of evidence suggests that the potential exists to reduce morbidity and high mortality rates associated with major surgery in high-risk patients. Dopexamine is a dopamine analogue with agonist activity at β_2-adrenoceptors and dopaminergic receptors that has been used to maintain tissue perfusion in critically ill and high-risk surgical patients with the aim of improving clinical outcomes. Postoperative complications occur more frequently in the presence of poor tissue microvascular flow and oxygenation, and dopexamine has been shown to improve these abnormalities. However, the effect of dopexamine on clinical outcomes is less clear, and the findings of randomized trials have proved inconsistent. These conflicting findings might be explained by dose-related differences in the hemodynamic and immunologic effects of dopexamine. The series of investigations that make up this thesis set out to explore the nature of any such dose-related effects and reveal potent anti-inflammatory effects of dopexamine in the absence of haemodynamic effects.

Contents

Figures and Tables

List of Abbreviations

5-HT	5-hydroxytryptamine/serotonin
β-NAG	N-acetyl-β-glucosaminidase
ACh	acetylcholine
ADP	adenosine diphosphate
AKI	acute kidney injury
ALT	alanine aminotransferase/transaminase
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
AST	aspartate aminotransferase/transaminase
ATP	adenosine triphosphate
$[Ca^{2+}]_i$	intracellular calcium concentration
CABG	coronary artery bypass grafting
cAMP	cyclic adenosine monophosphate
CDx	cluster of differentiation x
cGMP	cyclic guanosine monophosphate
CGRP	calcitonin gene related peptide
CI	cardiac index
CO	cardiac output
COMT	catechol-O-methyl transferase
CrC	creatinine clearance
CVP	central venous pressure
D_x	dopamine (main group) receptor
DO_2	rate of delivery of oxygen
DPX	dopexamine
DRx	dopamine (subtype) receptor x
Epac	exchange protein directly activated by cAMP
et al.	*et altera* (Latin)

Fc	fragment, crystallisable region (of an antibody)
fcd	functional capillary density
FITC	fluorescein isothiocyanate
GDP	guanosine diphosphate
GDT	goal-directed therapy
GFR	glomerular filtration rate
G-protein	guanosine nucleotide binding protein
GTP	guanosine triphosphate
HR	heart rate
HV	hepatic venous
ICG	indocyanine green
IFN	interferon
IgX	immunoglobulin type X
IL-x	interleukin-x
ip	intraperitoneal
iv	intravenous
LDF	laser Doppler flowmetry
LFT	liver function test
LPS	lipopolysaccharide
mAb	monoclonal antibody
MEGX	monoethylglycinexylidide
mACh	muscarinic acetylcholine (receptor)
MAO	monoamine oxidase
MAP	mean arterial pressure
MFI	median fluorescent intensity
MPO	myeloperoxidase
NADPH	nicotinamide adenine dinucleotide phosphate
NE	norepinephrine

NO	nitric oxide
NOD	nucleotide oligomerisation domain
e/I NOS	endothelial/inducible nitric oxide synthase
ns	not significant
NYHAx	New York Heart Association class x of heart failure
PBS	phosphate buffered saline
PE	phenylephrine
PEEP	positive end expiratory pressure
PO$_2$/PCO$_2$	partial pressure of oxygen/carbon dioxide
PepG	peptidoglycan
PKA	protein kinase A
pH	negative log (base 10) of hydrogen ion concentration
pH$_i$	the pH of luminal tissue, usually the gastric mucosa
RBC	red blood cell/erythrocyte
RCT	randomised controlled trial
SD/SEM	standard deviation/error of the mean
SMA	superior mesenteric artery
SNP	sodium nitroprusside
SV	stroke volume
SVI	stroke volume index
TMB	tetramethylbenzidine
TLR	toll-like receptor
TNF	tumour necrosis factor
tPO$_2$	tissue oxygen tension
TPR	total peripheral resistance
VIP	vasoactive intestinal peptide
VO$_2$	rate of oxygen consumption
vs.	*versus* (Latin)

Chapter 1 - Introduction

This thesis is about the effects of the catecholamine inotrope dopexamine in the context of endotoxaemia and laparotomy. It is not specifically about goal-directed therapy (where patients are haemodynamically optimised in order to avoid tissue hypoxia) (1). However this study of dopexamine has its origin in questions that have arisen from clinical studies of the use of inotropes to improve oxygen transport and goal-directed therapy. The historical background to this thesis is therefore one whose origins can be traced back to the study of the role of elevating oxygen delivery through goal-directed therapy in order to try and improve patient outcomes. These are themes that have occupied the critical care and anaesthetic communities for several decades. Before exploring how dopexamine has come to be studied, a brief outline of cardiovascular physiology is provided.

1.1   Cardiovascular physiology and determinants of cardiac output

1.1.1   The heart: myocyte excitation and contraction, inotropy and chronotropy

The healthy adult heart is a four-chamber muscular pump that supplies two parallel circulations. Each pumping chamber can be thought of as being filled passively (during diastole) by a flow of blood originating from a reservoir of variable size, and actively ejecting (in systole) in to a network of vessels of variable resistance. The size of the reservoir and resistance of the network are under nervous and hormonal control. Cardiac output is the volume of blood pumped by the heart each minute, and is the mathematical product of stroke volume (itself partly governed by the force of contraction) and frequency of ventricular contraction.

The heart is made up of cardiac muscle fibres (cardiomyocytes) which contract through the sliding filament mechanism. Actin and myosin filaments are propelled past each other through repeated cross-bridge linking and un-linking. At rest, tropomyosin blocks the actin

binding site preventing engagement of myosin heads. Following a cardiac action potential, voltage gated myocyte calcium channels open in the plasma membrane and a rise in $[Ca^{2+}]_i$ occurs, triggering a further release of calcium from the sarcoplasmic reticulum (Figure 1.1). The sarcolemmal derived $[Ca^{2+}]_i$ accounts for around a quarter of the total increase in $[Ca^{2+}]_i$, whereas the sarcoplasmic reticulum accounts for around three quarters of it (2). Calcium ions bind to troponin C within the troponin complex, displacing tropomyosin. This exposes the actin binding site allowing cross-bridge formation with myosin heads. The orientation of the myosin head changes causing filaments to slide past each other in an ATP dependent process. At the end of the action potential, during repolarisation, calcium ions are pumped back into the sarcoplasmic reticulum allowing myocardial relaxation. The force of ventricular contraction is affected by changes in contractility, or the force of contraction for a given resting fibre length. Catecholamine-based inotropes through their actions on adrenoceptors prolong the action potential plateau duration, increasing $[Ca^{2+}]_i$, calcium release from the sarcoplasmic reticulum and hence contractility.

An increase in venous return to the heart causes ventricular (and therefore cardiomyocyte) stretch, resulting in increased filament overlap and hence an increase in the number of available calcium binding sites. Cardiomyocyte stretch also increases myofilament sensitivity to $[Ca^{2+}]_i$ over several beats (the slow force response). These mechanisms, which are the basis of Starling's law of the heart, ensure ventricular output changes in response to changing venous return and that the output of the two ventricles is finely matched.

1.1.2   Vascular tone: preload and afterload

Strictly speaking, preload refers to the stretch induced by a load on a myocyte prior to contraction, a concept made famous by Ernest Starling in his *in vitro* experimental preparations. As cardiomyocyte length cannot realistically be measured at the bedside, other clinical correlates are used. The closest one to this is end-diastolic volume, most commonly derived at the bedside from transthoracic echocardiographic measurements. Importantly this

is not a direct measurement of volume as the derived parameter is based on assumptions regarding ventricular geometry. The easiest surrogate to directly measure is end-diastolic pressure, which applies to all cardiomyocytes in a loaded ventricle. Unfortunately though, end-diastolic ventricular pressure does not predictably relate to end-diastolic ventricular volume (the end-diastolic pressure in a ventricle with amyloidosis or hypertrophy is likely to be greater than that from an identical volume ventricle with no pathology, yet the preload by a volume definition is the same). Furthermore there are other drawbacks to a pressure-based definition of preload; catheters are seldom intentionally inserted in to the ventricle at the bedside, and measurements are therefore usually made in the preceding atrium or great vessels (superior vena cava, or for the left side of the heart the pulmonary capillaries) and assumed to be the same as that in the corresponding ventricle. It is clear that though easily defined *in vitro*, choosing a directly measurable clinical correlate that is valid for all ventricles at all times is not straightforward. Nevertheless it should also be clear that preload must relate to the increase in myocyte length that accompanies the ventricular distension (volume increase) that for any given ventricle is governed by its diastolic filling pressure.

Thus, in health an independent increase in capacitance vessel tone or an infusion of intravenous fluid displaces blood in to the central circulation, thereby increasing end diastolic volume and pressure, myocyte stretch and therefore cardiac output (this is akin to an increased flow rate of blood from the reservoir to the ventricles in the aforementioned model). This link relationship, best described by Guyton when explaining the effects of mean circulatory filling pressure on cardiac output (3), explains much of the confusion clinicians display when they incorrectly speak of volume status, preload and venous capacitance interchangeably.

The resistance the ventricle ejects against is termed afterload and is composed of systolic wall stress, the inertia of ventricular blood (determined by haematocrit), reflected pressure waves from arteries, and arterial bed resistance (total peripheral resistance, or TPR). As TPR is clearly an important component of resistance, haematocrit tends to be stable and the

other parameters are difficult to measure, afterload and TPR are commonly (but imprecisely) used interchangeably in clinical medicine. Cardiac output is directly affected by changes in afterload, though to a lesser degree than preload (3). For a given preload and contractility the direct effect of a decrease in afterload is to increase cardiac output. Systemic blood pressure (mathematically speaking) is the product of cardiac output and TPR $(MAP = CO \times TPR)$, though it is important to understand that CO and blood pressure can be measured, TPR cannot. Therefore cardiac output can also be indirectly affected through complex reflexes such as the baroreceptor reflex (baroreceptors detect changes in blood pressure which provoke stereotypical responses from the autonomic nervous system), critical reductions in coronary flow due to low aortic root pressure, and an adverse myocardial oxygen supply-demand ratio.

.

1.1.3 Microvascular flow (also see 1.6)

The microcirculation consists of regions of the circulation containing blood vessels of diameters less than 100µm (4). This region includes capillaries that link resistance vessels with capacitance vessels, and represents the primary focus of blood-tissue gas and nutrient exchange. The homeostasis of these specialised areas is under myogenic, metabolic, immune and neural controls (2). Due to the large cross-sectional surface area of the microcirculation, changes in arteriolar tone have significant effects on afterload, whereas changes in venular tone (where 60-70% of blood volume resides) has significant effects on preload by altering venous capacitance and therefore mean circulatory filling pressure.

Abnormalities of microvascular flow appear to play an important role in the pathophysiology of critical illness (5-7). Catecholamines may influence this balance by altering both cardiac output, and microvessel tone (8, 9). Indirect effects of these drugs may also exert complex effects on endothelial permeability and hence blood volume which is commonly reduced in critical illness (10, 11). Importantly, during periods of haemodynamic shock the loss of a

number of homeostatic mechanisms may also impair myocardial contractility through both tissue and systemic acidosis.

1.2 The autonomic nervous system modulates cardiovascular physiology through catecholamine effects

The three endogenous catecholamines adrenaline, noradrenaline and dopamine are released by the autonomic nervous system and adrenal medulla and produce characteristic responses in different tissues. These molecules are agonists at widely distributed adrenoceptors and dopaminergic receptors, the receptors varying in density between different tissue beds. In general, the cardiovascular effects of these catecholamines are governed by the number, types and locations of target receptors.

1.2.1 Function and distribution of adrenergic receptors

Adrenergic receptors are classified into α-adrenoceptors and β-adrenoceptors and further into subtypes (12). Agonist binding to adrenoceptors results in G-protein coupling. These G proteins consist of three subunits (α, β and γ), the type of α subunit denoting the type of G-protein (G_s is $G\alpha_{(s)}\beta\gamma$). When coupling, α subunits exchange GDP for GTP, dissociate from the complex and remain active until the GTP is hydrolysed back to GDP. α-GDP then re-associates with the βγ subunit complex and is available to couple with another adrenoceptor. The duration of signalling is inversely related to the speed with which α-GTP is hydrolysed to α-GDP, a process promoted by regulator of G protein signalling (RGS) molecules (13). G-protein-coupled receptors are susceptible to down-regulation and desensitization (12, 14-16), a particular problem in shock states such as sepsis (17). The activation of different G-protein subunits results in different intracellular signals which ultimately result in changes in intracellular calcium handling. These affect the state of inotropy, lusitropy, dromotropy and vascular tone (Figures 1.1 & 1.2).

Although widespread throughout the body, only the cardiovascular distribution of these receptors is discussed here. α-adrenoceptors have a predominantly vascular distribution with a smaller presence in the heart whereas β-adrenoceptors have a heavy presence in the heart but less in the vasculature. In the vasculature adrenergic receptor expression is minimal in capillaries but increases with distance from the capillary in both arterioles and venules. Adrenoceptor density and location within the cardiovascular system also determines the pattern of response to circulating and neuronally released adrenergic agents so that ability to vasodilate is markedly dependent on the pre-existing tone of each vascular bed (18-21). The responses to catecholamines therefore vary across vascular beds, for example between skin, mesenteric beds and skeletal muscle beds (22). To date no α_2 adrenoceptors have been found in the human myocardium though they are found pre-synaptically on innervating nerves. Other adrenoceptors are present in the myocardium. Inotropy is provided predominantly by β-adrenergic mechanisms though α_1 adrenoceptors can bring about small increases in contractility (20).
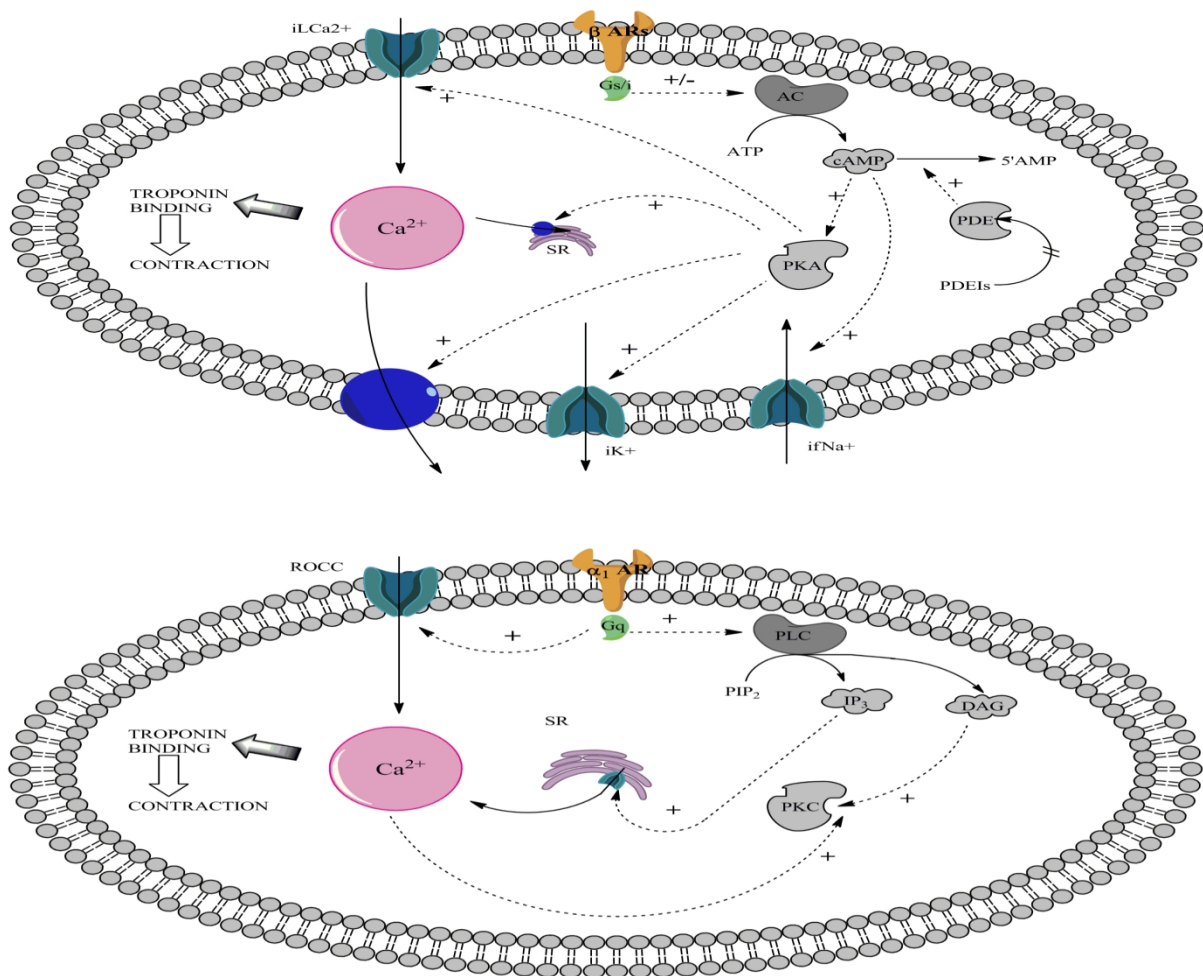
Figure 1.1 Adrenoceptor signalling in the heart. Both β_1 and β_2-adrenoceptors (upper myocyte) activate adenylate cyclase via G_s, increasing intracellular levels of cAMP, PKA and ultimately calcium. This increases the contractility of the heart. The activation of sodium and potassium channels and calcium transporters (filled blue circle) on the sarcoplasmic reticulum and myocyte membrane produces a chronotropic and lusitropic response. Switching to G_i signalling and receptor downregulation may occur, a particular feature of β-adrenoceptors. α_1-adrenoceptors (lower myocyte) activate phospholipase C via G_q, resulting in an increased release of calcium from the sarcoplasmic reticulum and an inotropic response approximately 15% of β-adrenoceptor stimulation. There are no α_2-adrenoceptors in the heart though they are found pre-synaptically on noradrenergic neurons innervating the heart. Agonism of these pre-synaptic receptors results in a decrease in contractility as the neuronal release of noradrenaline is inhibited. This is of no relevance in states of high sympathetic tone (e.g. heart failure) as pre-synaptic inhibition is already maximal and cannot be further increased by higher concentrations of noradrenaline (20).

Figures 1.1 and 1.2 show that although the effect of activating identical adrenoceptors may vary between organs (e.g. opposite effects of myocardial and vascular β-adrenoceptor agonism on intracellular calcium concentrations) the second messengers activated are the same.
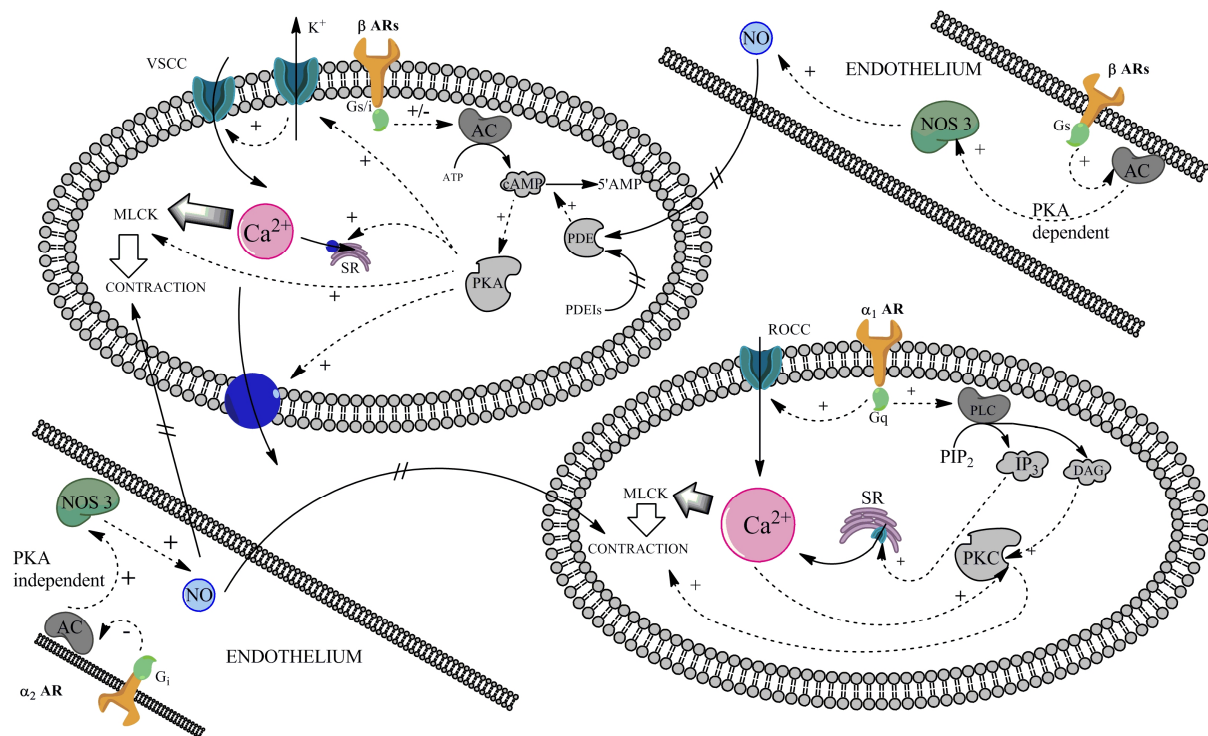
Figure 1.2   Adrenoceptor signalling in the vasculature. β-adrenoceptor signalling (upper vascular smooth cell) activates adenylate cyclase via G_s, cAMP and PKA. This produces hyperpolarisation and a transfer of calcium out of the cytosol and in to the sarcoplasm and interstitium, resulting in decreased vascular tone due to effects on calcium sensitive myosin light chain kinase (MLCK). α_1-adrenoceptor ligation (lower vascular smooth cell) results in activation of the phospholipase C pathway, activation of protein kinase C, an increase in cytosolic calcium and increased vascular tone. Endothelial adrenoceptors also modulate vascular tone through nitric oxide dependent pathways. Endothelial β-adrenoceptor ligation results in adenylate cyclase activation and the PKA dependent generation of NO. Although endothelial α_2-adrenoceptors are negatively coupled to adenylate cyclase they also result in the generation of NO but via non-PKA dependent pathways. Endothelial NO diffuses to vascular smooth muscle where it directly inhibits vascular smooth muscle contraction and also inhibits phosphodiesterase, producing a higher cytosolic level of cAMP, indirectly inhibiting contraction (20).

Although the downstream effects of activating second messenger systems may vary between organs (e.g. opposite effects of β-adrenoceptor ligation on cytosolic calcium concentrations in the heart and vasculature), the second messenger systems shown here are those that are activated by the relevant adrenoceptors throughout the body. Therefore α_2 and β-adrenoceptors are coupled to adenylate cyclase signalling systems whereas α_1-adrenoceptors couple with the phospholipase C pathway. However, the situation is complicated by switching of adrenoceptors between predominantly stimulatory and inhibitory G-protein linkage to adenylate cyclase with prolonged stimulation, and the ability of adrenoceptors to signal via additional intracellular pathways.

1.2.2 Function and distribution of dopaminergic receptors

There are five sub-types of dopaminergic receptor classed in two groups: D_1-like (subtypes DR_1 and DR_5) and D_2-like (subtypes DR_2, DR_3 and DR_4) (12). Whilst dopamine may activate both dopaminergic and adrenoceptors, the former are not activated by other endogenous catecholamines (23, 24). All dopaminergic receptor subtypes have been identified in the kidney where they mediate natriuresis and diuresis (25, 26). Cardiac dopaminergic receptors (DR_1 and DR_4) possess some inotropic actions though less pronounced than β-adrenoceptor mediated responses (27-29). Dopamine receptors can also be identified in the adrenal medulla, autonomic ganglia, endothelium and the renal, mesenteric and splenic vasculature, at both pre- (D_2) and post-synaptic (D_1 and D_2) locations (23). D_1 receptors are found in the media of blood vessels and cause vasodilatation. Vascular D_2 receptor activation can cause vasodilatation or constriction depending on whether medial or adventitial (30). The overall effect of non-selective dopaminergic activation, such as occurs during low dose dopamine or fenoldopam infusion, is to reduce vascular tone.

The structural differences in catecholamines result in the differences in receptor affinity and rates of metabolism. Substitution on the amino group of the catecholamine tail reduces α-receptor affinity but increases β-receptor affinity (31, 32). Furthermore $β_2$ affinity is increased by the size of the substituent. The position of hydroxyl (OH) groups on the aromatic nucleus also alters adrenoceptor affinity as does hydroxyl substitution on the catecholamine tail. These latter groups are key in determining $β_2$ affinity. For example, dopamine lacks a side chain β-OH groups and demonstrate low affinity and intrinsic activity at $β_2$ adrenoceptors despite amino group substitutions (31, 33). Metabolism by COMT is affected by the position of aromatic hydroxyl groups. Resistance to MAO is conferred by substitution of methyl groups on the amino tail with larger groups or introducing small alkyl residues (31). Alkylation of the primary amino group decreases affinity for uptake-1 (34). Although catecholamine structure can determine the degree of adrenoceptor activation, agonists at specific adrenoceptor subtypes may still generate differing concentrations of second

messengers such as cAMP, due to non-selective G-protein coupling (35). Drug-receptor interactions are also influenced by polymorphisms of adrenoceptor genes (36).

1.3 The widespread expression of adrenoceptors and dopaminergic receptors in non-cardiovascular tissue can also affect the cardiovascular system

1.3.1 Metabolic effects

Catecholamines increase metabolic rate and alter the production of metabolically active molecules through perfusion, receptor and second messenger mediated effects. Increased total body oxygen consumption, peripheral insulin resistance, suppression of insulin secretion, increased fatty acid and lactate production and hyperglycaemia are common effects (37, 38). Hyperglycaemia causes denudation of the endothelial glycocalyx and therefore has deleterious effects on the microcirculation (39).

1.3.2 Immune effects (also see 1.6 and 1.7)

Catecholamines have been shown to alter the state of activation of immune cells and may therefore have important effects on immune function which are currently poorly understood (40, 41). These catecholamines may be released neuronally, circulating in the bloodstream, but can also be released by immune cells and act in either an autocrine or paracrine fashion on membrane catecholamine receptors (42). Immune cell-endothelial interactions occur by shear dependent or shear independent mechanisms both of which are influenced by inotropic agents. Shear relates to both the differential rates of flow parallel to, and force imparted perpendicular to, moving layers within non-Newtonian fluids such as blood (shear rate and shear stress, respectively). In the context of shear dependent immune cell-endothelial interactions, shear relates to both the centre-line rate of flow of blood through microvessels and also the resulting stress that is imparted perpendicular to the vessel wall.

26

Shear stress is determined by haematocrit, microvessel capacitance (and the interaction of blood cells with the endothelium). At high shear rates, the probability of immune cells interacting with the endothelium decreases (39). This is because blood cells are more likely to be carried by a high flow rate in the centre-line of the vessel, away from the vessel wall where they could otherwise interact with the endothelium. In microvessels this produces a decrease in viscosity and haematocrit, allows a layer of plasma to internally line the glycocalyx and in this way reduces friction. At any given shear rate a healthy endothelium is also less likely to permit endothelial-immune cell interaction as the negatively charged endothelial glycocalyx repels immune cells from the endothelium and toward the centre-line of the vessel. Endothelial function is therefore an important component of the capacity of the immune system to focus activity in specific tissue areas and microvascular flow is intrinsically related to the immune system.

Shear independent mechanisms describe changes in the activation state of immune cells independent of flow rate. A range of adhesion molecules are expressed by both leucocytes (and the endothelium) following activation by inflammatory mediators. These allow leucocytes initially to loosely attach, or roll, and then bind firmly before trans-migrating between endothelial cells into the tissues. Thus activated cells have a greater chance of endothelial interaction at any given flow rate compared to quiescent cells. Related platelet-endothelial interactions are also important in critical illness illustrating the close relationship between inflammation and coagulation within the microcirculation.

1.4   Dopexamine is a synthetic catecholamine developed for the treatment of heart failure

Dopexamine is a catecholamine molecule and a synthetic structural analogue of dopamine (Figure 1.3) (43, 44). In heart failure cardiac output is inadequate and in order to redress this problem the autonomic system responds with increased adrenergic drive (inotropy and

chronotropy) while the renin-angiotensin-aldosterone system retains salt and water in order to maintain preload. However, this adrenergic stress leads to myocardial β_1-adrenoceptor downregulation, noradrenaline depletion from myocardial nerve terminals, and in combination with preload and afterload increases causes myocardial work eventually leading to myocardial decompensation. In acute heart failure, reductions in preload and afterload are achieved through the use of diuretic and glyceryl trinitrate infusions. In severe heart failure or cardiogenic shock, catecholamine inotropes have traditionally been used to support cardiac function in the short-term. As dopamine possesses natriuretic, diuretic, and inotropic effects at low doses it had traditionally been widely employed for this purpose (45, 46), but unfortunately the unwanted effects of tachyarrhythmias and vasoconstriction proved detrimental. Dopexamine was the product of a search for a synthetic dopamine analogue which would give the same theoretical benefits as dopamine while being devoid of vasoconstrictive and chronotropic effects and providing some degree of afterload reduction. An N-alkylated dopamine analogue was the result.
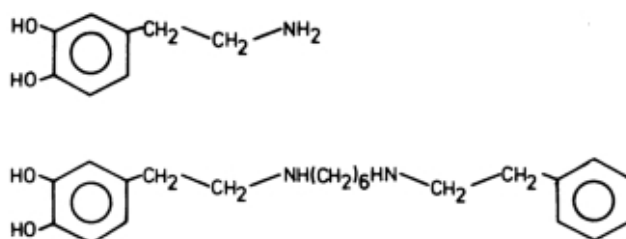
Figure 1.3 Chemical structures of dopamine (upper) and dopexamine (lower)

1.4.1 Dopexamine pharmacodynamics

Although initial studies demonstrated a relatively simple receptor agonist profile, further complexities became obvious (Table 1). In addition to β_2-adrenoceptor and D_1 and D_2

receptor agonism, dopexamine inhibits noradrenaline reuptake at the pre-synaptic uptake 1 transporter. Antagonism at α_1-adrenoceptors and mACh receptors and agonism at 5-HT receptors occur at high doses. There is evidence for VIP and CGRP involvement in the modulation of dopexamine's vascular effects and it has also been demonstrated that dopexamine infusion results in baroreceptor reflex activation. The receptor profile would initially appear confusing, but the clinically relevant receptors where dopexamine operates are β_2-adrenoceptors, D_1 and D_2 receptors and uptake 1 (inhibition). With regards to the relative importance of these, although dopexamine has a ten-fold greater affinity for β_2 adrenoceptors than β_1 in the heart, and these receptors couple with adenylate cyclase and increase cAMP levels (47, 48), the inotropic and chronotropic effects of dopexamine are nevertheless predominantly secondary to increased NE concentrations (from uptake 1 inhibition and baroreceptor reflexes) which exert potent effects on β_1-adrenoceptors (Table 1.1) (49). Although adrenergic and dopaminergic receptor systems in rats and in humans are liable to downregulation, functionally this appears to be less of a problem with β_1 and β_2 systems when dopexamine is infused chronically over a week (50). Nevertheless in the failing heart the effect of dopexamine still rapidly wanes, due to the low neuronal stores of norepinephrine on which dopexamine depends for its inotropic action (49).

1.4.2 *Dopexamine pharmacokinetics*

When dopexamine is incrementally administered to healthy human subjects at infusion rates of up to 4 µg/kg/min, plasma dopexamine levels rise in proportion to the dose of drug infused, peaking at 124 (± 12 [SEM]) ng/ml. On cessation of infusion, a mono-exponential decay is seen with a half-life of 7 (± 1 [SEM]) min, demonstrating a clearance of 36 (± 3 [SEM]) ml/min. A small study from patients undergoing liver transplantation suggests that the liver is responsible for a substantial proportion of dopexamine's clearance (51). Metabolism of dopexamine occurs by O-methylation and sulphation, producing two metabolites that are

both renally and faecally excreted, as is the parent compound. Quantitatively (>90%) the most important excreted metabolite is the 2-methoxy, 1-sulphate molecule. Faecal excretion accounts for approximately 20% of an administered dose whereas renal elimination accounts for >50% (over 12 days).

The values for plasma levels of dopexamine correlate reasonably well with those from clinical studies in anaesthetised patients. Prolonged infusions of 0.5µg/kg/min dopexamine are associated with mean plasma levels of 51 (15 – 122 [range]) ng/ml at 6 h (personal communication, Rupert Pearse), whereas 2µg/kg/min is associated with levels of 85 (69 – 102 [range]) ng/ml (51). Clearance reduces following cardiac surgery to 17ml/min (possibly due to the effects of acidosis and hypothermia on enzyme systems) and following liver transplantation to 24 (20 – 29 [range]) ml/min, while plasma half-life is increased to 11 min in low cardiac output states (51, 52). Dopexamine, like other catecholamines, is therefore short-acting and easily titrated.

Table 1.1 – Summary of receptor, signalling and ion channel studies for dopexamine

Pathway	Study animals	Clinically relevant location	Effect at site	Result of receptor activation *in vivo*	Refs
β_2 adrenoceptor	Humans, dogs, cats, rats, guinea pigs, lambs	Post-synaptic. Heart and vasculature	Activates (high affinity). Increases renal, mesenteric and cardiac cAMP	Systemic, renal and mesenteric vasodilatation Chronotropy, inotropy	(44, 47, 48, 53-60)
β_1 adrenoceptor	Humans, dogs, rats, lambs	Post-synaptic Heart	Poor affinity. Indirect and strong activation by enhancing NE effects	Chronotropy Inotropy	(47, 48, 55, 59-61)
D_1 receptor	Humans, dogs, cats, rats, guinea pigs	Post-synaptic Mesenteric and renal vasculature, nephron	Activates (⅓ affinity of dopamine). Increases renal and mesenteric cAMP	Renal and mesenteric vasodilatation. Natriuresis, diuresis.	(44, 53-58, 60)
D_2 receptor	Humans, dogs, cats, rats, guinea pigs	Pre-synaptic Mesenteric and renal vasculature, nephron	Activates (weaker affinity than D_1)	Decreases NE release (balances effect of uptake 1 inhibition at renal nerve)	(44, 48, 56, 57, 62)
Uptake 1	Humans, dogs, rats, rabbits, guinea pigs	Pre-synaptic. Heart	Inhibition	Potentiates effects of neuronally released NE. Chronotropy, inotropy	(49, 55, 60, 61, 63-65)
Baroreceptor reflex	Dogs	Cardioaccelerator nerves	Activation	Chronotropy Inotropy	(55, 60)
α_1 adrenoceptor	Rats, rabbits	Post-synaptic. Vasculature.	Inhibition	Vasodilatation	(66-68)
mAch receptor	Calves	Post-synaptic (bronchial tissue)	Antagonism	None at clinically relevant concentrations. Can decrease smooth muscle tone.	(69)
5-HT receptor	Rats	Renal vasculature	Agonism	Vasoconstriction – (high doses only)	(70)
VIP/CGRP	Guinea pigs	Pulmonary vasculature	Dopexamine releases VIP and CGRP → agonism at these receptors	Vasodilatation	(47, 53, 58, 71)
Adenylate cyclase	Humans, rats, guinea pigs	Intracellular	Activates	Generate cAMP. Involved in vasorelaxation	
cAMP	Humans, rats, guinea pigs	Intracellular	Increases (direct effect weak)	Activates PKA and membrane ion channels	
PKA	Guinea pigs	Intracellular	Activates	Involved in vasorelaxation	
High and low conductance voltage-sensitive K^+ channels	Guinea pigs	Vascular cell membrane	Activates	Involved in vasorelaxation	
ATP sensitive K^+ channels	Guinea pigs	Vascular cell membrane	Activates	Involved in vasorelaxation	
Verapamil sensitive calcium channels	Guinea pigs	Heart cell membrane	Opens	Involved in myocardial effects of dopexamine	(72)
ANP	Humans, rats			Reduces plasma levels of ANP, though D_1 agonism has a permissive effect on renal ANP effects	(73, 74)

1.4.3 Effects of dopexamine infusion in conscious healthy volunteers

Dopexamine has been infused in man at doses up to 10µg/kg/min (75) and at far higher doses in some animal studies. In order to better focus on the actions of dopexamine at clinically relevant doses (see later) I will explore the effects of dopexamine at doses up to 2µg/kg/min only, though in the case of healthy human volunteers as the data are limited findings at 2.25µg/kg/min have also been included.

Dopexamine's cardiovascular actions include chronotropy, inotropy and vasodilatation particularly in mesenteric, skeletal and renal beds (76, 77). An examination of haemodynamic data from several small studies in healthy volunteers reveals that dopexamine produces modest increases in heart rate (HR, up to 30% over baseline) and MAP up to doses of 2µg/kg/min (78-83), and significant increases in cardiac index (approx. 25% and 45% increases in CO at 1 and 2µg/kg/min, respectively), associated with decreases in total peripheral resistance (TPR) (80, 82). In one study 25% increases in cardiac index (CI) could be achieved with doses as low as 0.125 – 0.5µg/kg/min, although the time required to reach steady state was 15 – 35 min (83). Animal studies across species strongly suggest that increases in cardiac index are predominantly a consequence of afterload reduction rather than inotropy *per se*, though enhanced noradrenergic drive (baroreceptor activation and uptake inhibition) is likely an important factor in any simultaneously seen increased cardiac contractility (60). This is supported by examination of the data from three studies on healthy volunteers measuring norepinephrine levels when dopexamine is infused at 2.25µg/kg/min (81) and TPR when dopexamine is infused at 1µg/kg$^{/}$min (78, 80).

Dopexamine causes peripheral vasodilatation despite the modest increase in MAP. Any increase in MAP is therefore likely a consequence of increased cardiac output. Vasodilatation is notable in renal beds, resulting in decreased vascular resistances and increases in renal blood flows (D and β_2 adrenoceptors). Thus at 1µg/kg/min dopexamine

causes modest increases in GFR, renal plasma flow and diuresis, though not natriuresis (76, 80). During dopexamine infusion an increased filtered and therefore sodium load passes through the glomerulus to reach the proximal convoluted tubule where sodium reabsorption is unaltered. However natriuresis does not occur (unlike in rats), due to sodium reabsorption in the distal convoluted tubule (80). Dopexamine does bind to receptors in areas of the human and rat kidney that are known to cause natriuresis (56). The failure to cause natriuresis in humans may then relate to a greater fractional density of β_2-adrenoceptors in the distal convoluted tubule in humans (which enhance tubular re-absorption) (84, 85). However it is impossible to exclude the importance of other mechanisms that might also account for these differences (e.g. differential dopexamine binding in the loops of Henle, the macula densa and collecting ducts, the renal vasculature, actions on renal nerve discharge, renin release, ANP release and species differences in adrenoceptor behaviour etc.) as these aspects haven't been studied. Regarding splanchnic blood flow in healthy volunteers, at 1µg/kg/min the increase in blood flow relates to increases in cardiac index and not selective splanchnic vasodilatation (78). A significant increase in cardiac index caused by infusion of dopexamine doesn't appear to have any deleterious effects on cerebral haemodynamics and autoregulation (83).

Several other features of dopexamine are notable. In health short-term infusion of dopexamine brings about a modest fall in leucocyte numbers without affecting neutrophil function (86). Dopexamine also appears to inhibit platelet aggregation in response to stimuli and decreases platelet numbers (76, 87). Unlike many β_2 agonists, in man dopexamine does not cause decreased plasma potassium levels below 2µg/kg/min (81, 88). With regards to metabolism, it is associated with only small increases in systemic oxygen consumption (VO_2) and plasma free fatty acids with a small decrease in respiratory quotient (implying increased fatty acid oxidation) (81, 88). Dopexamine results in only minor increases in plasma lactate and relatively stable glucose levels, accounting for the increase in insulin levels only seen above 2µg/kg/min (81, 88). Markers of proteolysis are mildly depressed. This indicates that

up to 2µg/kg/min dopexamine has neutral effects on metabolism. The caveat to all these findings is that they have been made in small studies where the drug was infused on a short-term basis.

1.4.4 Effects of dopexamine in heart failure

Early studies (without comparator controls) in small numbers of patients with NYHA III heart failure demonstrated that in the short-term (10 min), 1µg/kg/min dopexamine caused no effects on blood pressure, significant increases in cardiac index, stroke volume index and indices of contractility, and decreases in systemic vascular resistance without increasing myocardial work significantly (45). Other small studies had similar findings (89, 90). Although some concerns remained about the sustainability of dopexamine effects in this patient group (91), seven small studies had been conducted in 74 NYHA II-III patients at doses up to 6µg/kg/min. Amalgamating this data, the manufacturers were able to demonstrate that at up to 2µg/kg/min, dopexamine dose-dependently increased cardiac index and reduced TPR with only mild increases (13%) in heart rate and neutral effects on blood pressure (82). Studies on small numbers of heart failure patients continued to be published, providing little meaningful data but promising much (92-95). In 1991 a randomised-controlled trial of dopexamine in heart failure over six hours in 45 patients demonstrated that there were concerns with pharmacological tolerance, tachycardia, angina and a lack of convincing renal effects with this drug in NYHA III-IV patients at doses up to 2µg/kg/min. Furthermore the haemodynamic changes seen in earlier studies were not always reproducible (96). By 1995 the next randomised dopexamine trial in heart failure was published but added little useful information as infusions of dopexamine were only run for one hour (97). In conclusion, dopexamine at doses up to 2µg/kg/min produces similar cardiovascular effects in heart failure patients to that in healthy humans. However effects are poorly tolerated in many of these patients due to the underlying intolerance of tachycardia, and effects are short-lived

and less predictable due to changes in adrenoceptor numbers, post-receptor signalling systems and neurotransmitter depletion (49, 98, 99).

Like its catecholamine predecessors dopexamine had failed to open a new chapter in decompensated heart failure treatment, but it had developed a reputation as an inotrope with an interesting cardiovascular profile. As early as 1989 its use had been reported in low cardiac output states due to sepsis and following cardiac surgery (100-103). The 1990s saw a greater interest in the potential renoprotective effects of dopexamine (104, 105), its potential to preferentially direct blood flow to the hepatosplanchnic circulation (106, 107), and as goal-directed therapy came to the fore an interest in its use in that setting also began (108).

1.5   Goal-directed therapy

1.5.1   What is goal-directed therapy?

In several landmark observational studies by Shoemaker and colleagues in the early 1970s, it was noted that following major surgery and in the early stages of severe illness, surviving patients tended to be able to elevate indices of left ventricular performance, oxygen delivery and utilisation above baseline whereas those that couldn't tended not to survive (109, 110). The logical consequence of this was that patients with a poor prognosis could be identified early by their haemodynamic and oxygen transport variables while the assumption was that increasing oxygen delivery to values obtained from a large cohort of survivors could potentially constitute a therapy to improve outcome in these poor outlook patients (111). Normal values (on which treatment goals were based) were determined as the median of the range of various cardiorespiratory variables in survivors - goals could be achieved through the manipulation of preload (volume loading), circulating red cell volume (volume loading and oxygen content) and contractility (inotropic agents, usually catecholamine-based) (1). The biological basis behind this was that a reduced VO_2 was the earliest pathophysiologic

35

event in all types of shock and was actually driven by: *"…low flow, by maldistribution of flow, and by increased metabolic demand…"*, (my emphasis), and furthermore that the length and severity of cumulative VO_2 deficits associated with morbidity and mortality (1). The enforced reduction in VO_2 due to a suboptimal DO_2 would lead to an oxygen debt (the integral difference between resting and current VO_2) that needed to be repaid through an elevated DO_2 (112). This was the explanation provided for the elevations in DO_2 seen post-operatively in high risk surgical patients who survived (1). However, Shoemaker's observations of a supply-dependence of VO_2 and the generation of an "oxygen debt" suffered from the limitations of mathematical coupling (113, 114) and the problem that (particularly in established critical illness and sepsis) it did not always hold true (115-120).

Several prospective trials in high risk surgical and septic patients followed over subsequent years. The means to achieve goals were set in various algorithms, with mixed results (119-124). A literature review of trials of GDT for surgery and sepsis is beyond the scope of this thesis, but it is sufficient to note the following:

> The pathophysiological basis for the use of GDT was that an enhanced oxygen delivery would prevent otherwise critically perfused tissue becoming ischaemic (1, 125, 126). Shoemaker best explains this when he states: *"the essence of this plan is to maintain prophylactically the patient in an optimal haemodynamic state that does not allow him to develop tissue hypoxia…."* (1).

> GDT is now no longer a term specifically describing haemodynamic optimisation with the elevation of oxygen delivery to pre-defined levels, but describes any algorithm/protocol used to achieve haemodynamic end-points (e.g. mean arterial pressure, central venous pressure, changes in stroke volume) with a feedback loop that often includes parameters that are measures or surrogates for tissue perfusion (e.g. mixed venous saturation, lactate etc.), but not involving DO_2 parameters.

Therefore with time the specific goals of GDT have changed though the central tenet of haemodynamic optimisation remains.

Nevertheless GDT for high risk surgical patients, with or without the use of inotropic agents, has been shown to reduce the rate of surgical complications and hospital length of stay, and may decrease short and long-term mortality (127-129).

GDT using central or mixed venous oxygen saturation as the oxygen transport variable, or lactate as a tissue perfusion surrogate is still recommended in the treatment of sepsis, though only in the first 6 h of treatment (130). One could argue that this strategy fits in most precisely with Shoemaker's hypothesis as he gave particular importance to the early prevention of DO_2/VO_2 derangements, arguing that later cardiorespiratory manifestations of critical illness were merely the consequences of an earlier missed opportunity to prevent morbidity/mortality.

1.5.2 Dopexamine and trials of goal directed therapy

In the 1990s the vasoactive agents most commonly used to increase oxygen delivery were the catecholamines dopamine, dobutamine and adrenaline. Each of these agents enhanced oxygen delivery through potent effects on contractility and heart rate, therefore increasing myocardial oxygen demand. They also had metabolic side-effects that included increasing VO_2 (131-133). As GDT was based on improving the supply of oxygen to tissues, and the burden of ischaemic heart disease is also heavy in high risk surgical patients, dopexamine was thought to be better suited for peri-operative GDT as it was able to increase DO_2 with relatively little effect on myocardial or total body VO_2 compared to other agents (108, 133, 134). Although dopexamine does not increase cardiac index by virtue of inotropic action from direct β-adrenoceptor agonism, it does do so indirectly by virtue of its combined vasodilator actions and its indirect β-adrenoceptor stimulating properties (see sections 1.4.1 – 1.4.3). Therefore during dopexamine infusion cardiac index increases because the drug behaves as

an inodilator. Pure vasodilators cannot produce this effect as they can vasodilate but have no direct or indirect β-adrenoceptor mediated inotropic action. The only comparable drugs that can inodilate are methylxanthines and phosphodiesterase inhibitors, drugs with long half-lives which are therefore more difficult to titrate acutely. Dopexamine has therefore been used in many clinical trials due to this titratable inodilator action, coupled with its favourable myocardial VO_2 profile (see above).

Boyd et al.'s study from 1993 was the first of several randomised controlled trials (RCT) comparing GDT with dopexamine against a protocol guided "best treatment", non-dopexamine control group (135). 53 of 107 patients were haemodynamically optimised for a DO_2 of 600ml/min/m^2 in the GDT group (correlating with the median DO_2 of Shoemaker's survivor group). This continued during surgery and for a variable time afterwards (dependent on lactate results) at an average dose that was below 1.5µg/kg/min. The trial found that DO_2 was significantly higher in the GDT group and that significant and large reductions of the complication (>50%) and 28-day mortality rates (75%) were seen in association with this. Furthermore it appeared that the benefits of GDT were translated to a sustained gain in mortality benefit even 15 years later (128). In 1999 Wilson and Woods conducted another RCT in 132 evenly divided patients, comparing GDT using adrenaline against GDT using dopexamine (incremental dose from a start of 0.125µg/kg$^{/}$min) and against a non-GDT control group. GDT was commenced 4 h pre-operatively and ceased 12 h post-operatively. Although haemodynamic data wasn't available for the control group, DO_2 was elevated in the dopexamine GDT group to median levels of 564ml/min/m^2 while the adrenaline GDT group was similarly elevated. Outcome data revealed a significant decrease in mortality when the GDT groups were pooled and compared with controls. On the other hand a significant decrease in morbidity and length of stay was only seen against the control group in the dopexamine GDT group, and even against the adrenaline group.

The following year Takala et al. conducted a multi-centre randomised trial of peri-operative optimisation with or without dopexamine in high-risk patients undergoing abdominal surgery

(136). This involved 412 patients who were admitted pre-operatively to intensive care and optimised to various clinical criteria. Patients were then divided in to three groups, receiving either placebo or dopexamine at 0.5 or 2µg/kg/min, starting 2-12 h before surgery and continued 24 h afterwards. This trial did not find any significant benefits of adding dopexamine to the pre-optimisation protocol, though there was an improvement in the low dose dopexamine group that was statistically not significant (136). In 2003 another RCT of GDT using dopexamine in major elective abdominal surgery was conducted, looking at complications in 100 patients as the primary outcome (137). The dose of dopexamine used was 0.25µg/kg/min and this was continued for 24 h after surgery. Intra-operative haemodynamic monitoring found dopexamine was associated with significant increases in cardiac index CI, SV and HR compared to baseline and controls. There was no significant difference in complication rates between the dopexamine and control group, though the dopexamine group had a higher number of baseline co-morbidities. Two years later a trial conducted by Pearse et al. compared the effect of 8 h of post-operative GDT using stroke volume response and dopexamine at a maximum rate of 1µg/kg/min against a non-dopexamine CVP-led haemodynamic optimisation protocol. This study was stopped at interim analysis after only 122 patients had been recruited due to a significant reduction in complications (44% vs. 68%) and hospital length of stay in the dopexamine GDT group compared to the control group (138).

A 2008 meta-regression analysis of GDT using dopexamine for (non-cardiac) major surgery (including only the five trials above) revealed a reduction in 28-day mortality only when dopexamine was used in low doses (≤ 1µg/kg/min) consistent with the signal detected in Takala's study (Figure 1.4, upper) (139). However, a similar meta-analysis using different methods did not show a significant effect of dopexamine at any dose on (in-hospital) mortality, though the trends were similar (Figure 1.4, lower) (140).

Study	Dopexamine n/N	Control n/N	OR (Peto) 95% CI
Stone et al	2/50	1/50	1.97 (0.2-19.43)
Wilson et al	2/46	6/46	0.34 (0.08-1.43)
Boyd et al	0/27	12/54	0.18 (0.05-0.64)
Takala et al	10/135	18/140	0.55 (0.25-1.21)
Pearse et al	6/62	6/60	0.96 (0.29-3.16)
Total	20/320	43/350	0.50 (0.28-0.88) (derived from logit model) p = 0.016

0.1   0.2   0.5   1   2   5   10
Favours dopexamine   Favours control

Study or sub-category	Dopexamine n/N	Control n/N	RR (random) 95% CI	Weight %	RR (random) 95% CI	Year	Quality
Wilson et al.	2/46	8/46		12.57	0.25 [0.06, 1.11]	1999	A
Takala et al.	10/135	18/140		51.82	0.58 [0.28, 1.20]	2000	A
Stone et al.	3/50	2/50		9.21	1.50 [0.26, 8.60]	2003	A
Pearse et al.	6/62	7/60		26.40	0.83 [0.30, 2.33]	2005	A
Total (95% CI)	293	296		100.00	0.62 [0.37, 1.06]		

Total events: 21 (dopexamine), 35 (control)
Test for heterogeneity: Chi² = 2.76, df = 3 (p = 0.43), I² = 0%
Test for overall effect: Z = 1.75 (p = 0.08)

0.001   0.01   0.1   1   10   100   1000
Favours dopexamine   Favours control

Figure 1.4   Forest plots for:
UPPER. Meta-regression analysis of the use of low dose dopexamine (≤ 1µg/kg/min) and its effects on *28-day* mortality following major surgery. With permission, (139).
LOWER. Meta-analysis of the use of low-dose dopexamine (≤ 1µg/kg/min) and its effects on *in-hospital* mortality following major surgery. With permission, (140) –

A further RCT in 124 high risk general surgical patients was published in 2011, though this time the comparison was between GDT with fixed rates of low-dose dopexamine and an identical GDT protocol without dopexamine (141). In one group dopexamine was administered at a fixed-rate of 0.5µg/kg/min, then for a further 24 h post-operatively. There was no significant difference in stroke volume or cardiac index between the groups, and DO_2

was significantly higher than in controls though only for the last 2 h of the operations. Unusually, the DO_2 measured in this study was well below 600ml/min/m^2 in both groups possibly reflecting the selection of a population that in other trials may have been classed as non-responders to GDT (142, 143). There were no significant differences between groups in terms of peri-operative morbidity, complication rates or hospital mortality, though these were only followed-up for 5 or 15 days.

Recently a multi-centre RCT investigating 30-day post-operative outcomes in 734 high risk surgical patients undergoing major gastrointestinal surgery compared a peri-operative (and 6 hour post-operative) GDT protocol against usual care (129). The GDT protocol consisted of a fixed rate infusion of dopexamine at 0.5µg/kg/min and colloid therapy to achieve and maintain maximal stroke volume. This trial did not find a significant benefit in the composite primary outcome (30-day moderate/major complications and mortality) or secondary outcomes. However there were trends to an improved primary outcome with GDT (relative risk = 0.84 (95%CI 0.71 – 1.01), P=0.07), becoming stronger following adjustment for baseline co-morbidity (odds ratio = 0.71 (95%CI 0.53 – 1.00), P=0.05). Finally, when protocol adherence was adjusted for significant benefits were found in the GDT group (relative risk reduction = 0.8 (95%CI 0.61 – 0.99, P=0.04). This is notable as the power calculation for the trial assumed a relative risk reduction of 25% from an assumed control complication rate of 50%. In fact the control complication rate was only 43.4% and the trial is therefore likely to have lacked the power to detect with significance the differences in complication rates that it did.

Summing up these trials is problematic. The trials were conducted over a period of 21 years during which standard practice changed, and the treatment of control (non-dopexamine) groups has almost certainly improved. Some studies suggest that dopexamine could have a beneficial effect on morbidity and mortality, particularly at lower doses, whereas others do

not support this. If dopexamine does have any effect on outcomes, large trials with lengthy follow-up periods may be necessary to conclusively demonstrate or refute it, as any difference between treatment and placebo is likely to be smaller now than 21 years ago.

1.5.3   Searching for a mechanism of action for dopexamine

The pathophysiological basis of using GDT has centred on increasing oxygen delivery at a time of decreased tissue perfusion. However, Wilson and Wood's trial demonstrated that when DO_2 is increased, reduced rates of complications are seen only with dopexamine and not with adrenaline, leaving open the question of whether systemic DO_2 is really related to dopexamine's mechanism of action at all. The meta-regression analysis by Pearse also noted a lack of effect at higher doses of dopexamine where cardiac index (and by extension DO_2) would be expected to be highest (139).

A *post-hoc* analysis (144) of Takala et al.'s study (136) also revealed some interesting findings, though the analysis in question does have some flaws in its retrospective design and incomplete patient capture. This showed that when surgical patients from the original study were divided in to two groups based on surrogate markers of pre-operative gastric (and by extension splanchnic) perfusion, patients with a poor pre-operative gastric perfusion were at a significantly greater risk of morbidity and mortality compared to those who had a good pre-operative gastric perfusion. Furthermore, those patients who went on to receive dopexamine treatment (at both 0.5 and 2µg/kg/min) in the poor perfusion group experienced an improvement in gastric perfusion post-operatively and a reduced incidence of complications compared to placebo-treated patients from the same group. Curiously these improvements in gastric perfusion and morbidity were not observed in the group with good pre-operative markers of gastric perfusion, though DO_2 was seen to increase with dopexamine. With regards to mortality, no significant differences were seen with dopexamine treatment between any of the poor pre-operative gastric perfusion sub-groups.

This hypothesis-generating analysis also points toward an ambiguous relationship between systemic DO_2, tissue perfusion and complications. It hints that the ability of dopexamine to improve patient outcomes may be related to improving perfusion on a tissue level, and not necessarily at a systemic level. This study (144) was also notable as it correlates with other studies demonstrating that poor pre-operative markers of tissue perfusion are associated with post-operative morbidity (145).

Another side-arm (146) of Takala et al.'s original study (136) prospectively performed gastric tonometry and also compared changes in the endoscopic and histological appearances of gastric mucosa of 38 patients who had been randomised as per the initial protocol. The endoscopically determined health of all gastric tissue deteriorated over the three days, but no statistically significant differences were found between the groups at 72 h. However histologic appearances of gastric tissue demonstrated that dopexamine treated groups showed both statistically significant and non-significant inflammatory change which was less than that in placebo groups (depending on whether assessed by myeloperoxidase staining (a measure of neutrophil infiltration) or by haematoxylin and eosin staining, respectively).

Jhanji et al. in a physiological study (147) explored the effects of a post-operative eight hour fixed rate infusion of dopexamine (0.5μg/kg/min) in a group of high risk surgical patients who were haemodynamically optimised according to a stroke volume guided GDT protocol. This was compared with a similar group optimised identically but without dopexamine, and a third group optimised with a CVP guided protocol similar to that employed in the control group of Pearse et al.'s 2005 trial. The groups were well matched and all patients had epidurals. This study showed that there was a graded response in cardiac index and DO_2 when comparing the three groups, with the dopexamine GDT group at the top. Both GDT groups demonstrated a significant increase in markers of microvascular perfusion (perfused vessel density) with time whereas the control group decreased with time. However, the dopexamine group was the only group to demonstrate a significantly increased tissue oxygen tension (tPO_2). Furthermore while control group endothelium-dependent microvascular behaviour

(measured by post-occlusion hyperaemia) deteriorated over time, GDT with dopexamine enhanced post-occlusion hyperaemia while GDT alone preserved responses at baseline levels (Figure 1.5). Although there were no significant differences between groups in the cytokine profile, a protective effect of dopexamine on endothelium-dependent microvascular responses could not be excluded. Jhanji et al.'s study was not designed to assess the incidence of post-operative complications. These clinical studies take us several steps beyond a simple DO_2 based explanation and open up the possibility of alternative explanations:

- Dopexamine prevents a deterioration of microvascular perfusion in all tissue beds, preventing morbidity (the role of DO_2 in achieving this being potentially irrelevant)
- Dopexamine prevents the deterioration of tissue perfusion in specific tissue beds, namely the renal and/or hepatosplanchnic beds, preventing morbidity
- The anti-inflammatory effect of dopexamine is the basis of preventing morbidity

1.6. *The microcirculation*

1.6.1 *Structure*

The microcirculation comprises blood vessels of less than 100µm diameter and is the part of the circulation that is involved in the delivery and exchange of gases, metabolic substances and hormones and removal of waste products from tissue beds (4). The microcirculation is also closely involved in the regulation of coagulation and immune responses. In general, small arteries divide in to arterioles which decrease in size with further subdivision until capillaries are formed. Capillaries then merge to form venules and eventually veins. The entire circulation is lined by a unicellular layer of cells called the endothelium.

Arterioles primarily regulate microvascular blood flow and are the major resistance vessels in the body. They consist of intimal tubes of endothelial cells surrounded by an outer cylinder of

smooth muscle and connective tissue. Arteriolar tone is regulated by neural, humoral and local factors. The tone of the terminal arteriole allows regulation of capillary perfusion. Arterioles of a low tone will be patent, allowing perfusion of downstream capillaries. Arteriolar constriction will reduce the number of perfused capillaries – thus the terminal arteriole is often referred to as a pre-capillary sphincter. This is an important mechanism as perfused capillary density is a key determinant of tissue oxygenation through effects on the average inter-capillary distance, blood capillary transit time and the surface area available for nutrient and gas exchange. In some vascular beds e.g. skeletal muscle, there is considerable redundancy in the density of capillary beds. This allows a substantial increase in tissue perfusion at times of high metabolic demand. Capillaries are tubes of endothelial cells of approximately 5 µm diameter, with an associated basement membrane, devoid of surrounding smooth muscle and varying in length from 500-1000µm. They are the main site for exchange of gases and metabolic substances between tissue and blood and vary in their permeability according to the organ in which they are found, being classified as continuous, fenestrated or discontinuous (increasing permeability). As with all microvessels, pericytes are found around capillaries. Pericytes are specialised, contractile cells that have long processes wrapped around microvessels (these cells produce constituents of the basement membrane and extracellular matrix and are also involved in regulating the permeability of venular endothelial cell junctions in inflammation). Venules are very distensible but have little smooth muscle and only slightly thicker walls than capillaries. However, venules remain responsive to both circulating and neural vasoconstrictor stimuli. This allows control of venular tone and venous capacitance. Venules play a central role in the evolution of tissue inflammation. In normal conditions, venular endothelium expresses adhesion molecules which may be rapidly upregulated when activated. These molecules facilitate the adhesion and trans-migration of leucocytes to tissue beds.

The microcirculation is not structured according to a single design across diverse tissue beds but is adapted specifically to the function of the organ concerned. The cerebral

microcirculation is adapted to provide the tightly regulated extra-cellular milieu and a guaranteed oxygen supply essential to neuronal integrity. Cerebral endothelial cells lack fenestrations, are bound together by tight junctions while microvessels are linked to neurones by astrocytes which play a role in the coupling of neuronal activation to blood flow. These mechanisms are collectively termed the 'blood brain barrier'. Cerebral blood flow is tightly autoregulated by metabolic factors, and to ensure an adequate blood supply to the brain there is a high capillary density. Similarly the heart is highly metabolically active, mandating a high capillary density which is facilitated by cardiac myocytes being smaller than skeletal muscle fibre counterparts, decreasing diffusion distances. Unlike skeletal muscle with its reserve of recruitable capillaries, even at basal levels of function flow is present in all capillaries in healthy myocardial tissue. Circulating catecholamines released from the adrenal gland act predominantly on coronary endothelial β_2-adrenoceptors promoting vasodilatation and enhancing flow, increasing perfusion. As in the brain the control of coronary microvascular flow is dominated by metabolic autoregulation. Coronary microvessels are poorly innervated so when sympathetic tone and myocardial oxygen demand increase vasoconstriction is minimised. In contrast the cutaneous microcirculation is densely innervated so that when sympathetic tone increases, such as in circulatory compromise, blood flow is re-directed away from skin toward other tissues.

In common with the heart and brain there is a very high capillary density in the pulmonary microcirculation, resulting in tiny diffusion distances and maximising the efficiency of gas exchange. The pulmonary vascular bed receives the entire cardiac output, but is a low pressure and low resistance circulation because arterioles are short and thin walled and autoregulation does not occur to any great degree except for hypoxic pulmonary vasoconstriction which minimises ventilation-perfusion mismatch. This vascular response is opposite to that in systemic tissues (such as skeletal muscle) where a drop in the partial pressure of oxygen leads to vasodilation in order to match oxygen demand and perfusion. The renal microcirculation is extensively adapted to allow selective filtration at the

glomerulus and also to maintain osmotic gradients within the kidney. Unlike in the lung a rich sympathetic innervation of renal arterioles allows coupling of systemic haemodynamics (and tubular flow) to the regulation of microvascular blood flow in the kidney. Both afferent and efferent arteriolar tone allows maintenance of glomerular hydrostatic pressure for filtration, whereas a low capillary hydrostatic pressure is essential to reduce the formation of pulmonary oedema in the lung. The glomerular endothelium contains fenestrations, while podocytes and renal pericytes (mesangial cells) also give structure and function to the glomerular filter. Bowman's space between podocytes and endothelial cells is the entry point for glomerular filtrate in to the nephron. On the other hand endothelial tight junctions in the vasa recta which run parallel to the loops of Henle allow microvessels to act as counter-current exchangers and to maintain the medullary osmolar gradient. Exchangers are also found in the cutaneous microcirculation, though here arterio-venous anastamotic loops serve to regulate the transfer of heat between the body and its environment. On dilation and recruitment (as occurs in exercise) blood flow is directed to the skin surface and cooling occurs.

The microcirculation of the gut is based on a design that varies somewhat along the course of the digestive tract. Generally speaking the gut and its microcirculation is densely innervated with the added sophistication of vasoactive gut peptides acting in the vicinity. In this manner there is similarity with the cutaneous microcirculation where activated sensory nerves can release vasoactive mediators (such as histamine and substance P) which alter local vascular behaviour, and also because circulatory compromise results in the neuronally directed redistribution of blood away from this tissue bed. Millions of villi give the gut mucosa its large surface area for absorbing nutrients, but are also prone to hypoxia. This is because the metabolically active villi are supplied from the base by a single main arteriole travelling to the tip of the villus from where two distributing arterioles descend and supply a villous capillary network that drains in to descending collecting venules. This acts as a counter-current exchanger and invariably results in the diffusion of oxygen from arteriole to venule at

the villous base, reducing villous tip oxygen levels. However despite the dense innervation of the gut, mucosal blood flow is selectively protected from compromise as mucosal microvessels are highly responsive to local metabolic factors and are relatively poorly innervated. Downstream from the gut is the low pressure portal venous system which contains poorly oxygenated blood and the metabolic substrate absorbed from the digestive tract. This supplies 80% of the dual blood supply of the liver. The hepatic microcirculation in common with the pulmonary microcirculation therefore has a low resistance to flow but also performs several metabolic and immune functions to protect the body from potentially absorbed toxins. Hepatic capillaries, or sinusoids, consist of widely spaced endothelial cells with large fenestrae, interspersed with Kupffer cells (specialised macrophages that phagocytose particulate, infective and foreign material originating from the portal blood supply).

1.6.2 Regulating microvascular flow

Microvascular flow is regulated by both local and systemic mechanisms. The central nervous system exerts significant control over the microcirculation, integrating it with baroreceptor reflexes. Through sympathetic innervation of arterioles and venules, afterload and venous return to the heart can be increased or decreased by controlling the neuronal release of perivascular noradrenaline. The response is sophisticated and not uniform across microvascular beds due to differences in innervation, adrenoceptor profile and density. Similarly circulating vasoactive hormones also act on endothelium and vascular smooth muscle to mediate changes in vessel tone.

On a local level, various factors influence blood flow. Myogenic responses are due to the activation of stretch-sensitive ion channels in vascular smooth muscle cells. This causes an increase in vascular contractility. The result is arteriolar constriction in response to pressure

increases and *vice versa*. This ensures a consistency of blood flow to the organ bed over a range of mean arterial pressures - pressure autoregulation. In addition to this, at any given pressure other local mechanisms also regulate microvascular flow (39):

1. Local endothelial shear effect: Shear force is determined by haematocrit, capillary capacitance and interaction of blood cells with the endothelium which activate mechanoreceptors that trigger endothelial nitric oxide (NO) production via eNOS (39, 148). NO is a key mediator of vascular tone resulting in local vasodilatation. Electrical signals transmitted between endothelial cells via gap junctions result in local vasodilatation (whereas transmission across vascular smooth cell gap junctions translates constriction) (149). The consequent increase in local blood flow decreases shear force contributing to the autoregulation of microvascular flow.

2. Vasoactive tissue metabolites (e.g. CO_2, ADP, H^+, adenosine, K+) result in vasodilatation during increased metabolic activity. This allows metabolic autoregulation of microvascular flow may be particularly important in certain vascular beds such as the brain.

3. Paracrine effects on microvessels from red and white blood cells e.g. NO from erythrocytes, catecholamines from leucocytes.

The physics of blood flow through the microcirculation is of great interest and fundamental to the function of the cardiovascular system. Blood is a non-Newtonian fluid. Although arterial blood flow is laminar in nature, in smaller arterioles and capillaries perfusion pressure decreases, in part because of the large numbers of vessels of this size. Unlike larger arteries, microvascular haemodynamics are not determined purely by vessel calibre and driving pressure. Microvascular blood flow is strongly influenced by vascular topology, blood viscosity and the interaction of cellular constituents with each other and the endothelium. The endothelium lines the vessels of the microcirculation and plays a pivotal role in homeostasis of blood flow, inflammation, and coagulation. Just as the endothelium lines the

vessels of the circulation, so the endothelium itself is lined by the glycocalyx, a negatively charged layer of glycoproteins and glycolipids that decreases the permeability of blood vessels and prevents interaction between the endothelium and blood cells. Endothelial cells have an actin cytoskeleton which maintains cellular structure allowing regulation of cellular permeability and hence endothelial barrier function.

Hence despite low perfusion pressure, microvascular blood flow is maintained through the following mechanisms:

1. A thin plasma layer separates the outermost moving blood cells from the vessel wall, decreasing friction
2. The endothelial glycocalyx repels negatively charged molecules including those expressed on cell membranes and plasma proteins
3. Increased axial red cell velocity in capillaries causes an apparent drop in blood viscosity
4. The prevention of blood cell aggregation by 'shear thinning' and the deformability of red and white cells
5. The single file movement of erythrocytes through capillaries termed 'bolus flow' further decreases blood viscosity in the microcirculation

1.6.3 The microcirculation as a target for dopexamine

It had been recognised that there were stereotypical changes in the patterns of systemic oxygen transport variables following major surgery and in sepsis. When the effects of these changes at the tissue level were investigated, it was apparent that microvascular variables also changed stereotypically. It has been shown that the microcirculation becomes impaired following sepsis, that survival is associated with less severe derangements (150), and that improvements in microvascular variables following therapy associate with improved survival (151). Similarly following major surgery reductions in microvascular flow occur and tend to be sustained in those who go on to develop complications (145). One study demonstrated

that the severity of insult correlated with the degree of microvascular alteration (152) – in this regard it is logical to see the microcirculation as a site where dopexamine might be exerting its effects. It is also interesting in this regard that Shoemaker thought that the increases in VO_2 following successful GDT probably represented an opening of microvascular units that allowed oxygen to reach ischaemic cells that could then extract more oxygen (1).

There have been few studies of the effects of dopexamine on the microcirculation in humans. Jhanji et al.'s study demonstrated an ability of dopexamine to increase tissue PO_2, perfused vessel density and the cutaneous hyperaemic response to occlusion when
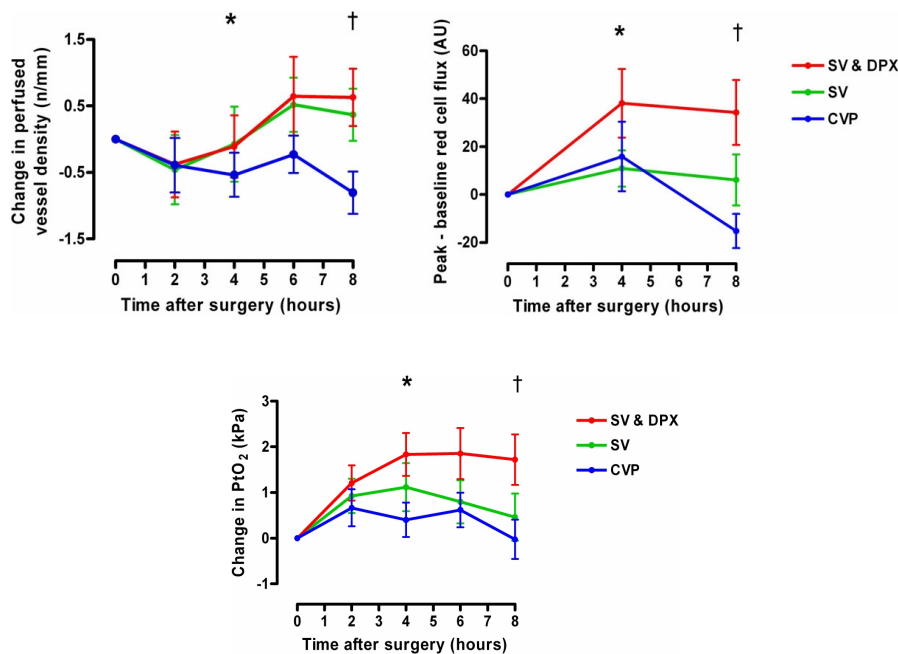
Figure 1.5   The effects of three different 8-hour post-operative haemodynamic optimisation protocols on indices of sublingual perfused vessel density (upper left), forearm cutaneous hyperaemic response (upper right) and forearm tPO2 (lower). The protocols were either: CVP guided (CVP), stroke volume guided (SV) or stroke volume guided with a fixed rate infusion of 0.5 dopexamine (SV & DPX). There was no significant difference in the volume of fluid infused between groups. With permission, (147).

compared to standard or flow-directed haemodynamic optimisation protocols (Figure 1.5) (147).

One other human study (presented across two papers) explored the effect of dopexamine on microvascular flow following free-flap plastic surgery in 24 patients (153, 154). Dopexamine

was infused in 1.25µg/kg/min increments up to 5µg/kg/min after various haemodynamic criteria had been met, and measured change in microvascular perfusion post-operatively (we are only concerned with the doses up to 2.5µg/kg$^/$min). A weakness is that infusions lasted only 5 min at a time, which may not have been enough time to reach steady state conditions (83). Nevertheless what the study found was that tissue microvascular flux, tissue conductance and cardiac index increased in a dose-dependent manner while MAP decreased. However with the same systemic haemodynamic changes, in denervated (free-flap) tissue dopexamine failed to preserve or augment red cell flux though conductance increased. On the other hand it was clearly demonstrated that when noradrenaline was infused in a range of doses which increased cardiac index up to levels seen with 1.25µg/kg/min dopexamine, MAP and microvascular perfusion in the free-flap greatly increased above baseline levels. This indicates that in denervated tissue MAP is an important determinant of microvascular perfusion, not cardiac index/DO$_2$. It is also possible that post-denervation, vessels in the flap are almost maximally dilated so the addition of a vasodilator such as dopexamine has little additional effect in flap vessels, and may produce a steal of flow by the surrounding innervated tissue which can vasodilate (this is supported by the finding that control tissue conductance increased more than free flap conductance when dopexamine was infused). The second paper relating to this study also performed spectral analysis of the Doppler waveforms of perfusion and suggested that myogenic activity was decreased in denervated tissue compared to control tissue at baseline (154). Infusing dopexamine had no effect on myogenic activity (as measured by proportion of power in the myogenic frequencies), but noradrenaline increased it. Given that local myogenic responses are in part governed by flow through blood vessels (2), this may further confirm the importance of MAP in generating flow through denervated tissue. Dopexamine decreased the driving pressure through the flap while noradrenaline increased it (153). Alternatively it may reveal that lower doses of noradrenaline, while increasing MAP and driving pressure in to the flap, also had the effect of increasing tone to normal levels in flap vessels before the highest doses of noradrenaline produced over-constriction. These two

papers are important in revealing that in normal tissue in the post-operative setting dopexamine can improve perfusion. They are also useful in pointing out the importance of maintaining MAP to preserve flow through tissue which is denervated (such as tissue under epidural anaesthesia) or in tissue which is behaving abnormally, such as in sepsis (9, 155). This latter point may also help explain why studies investigating the effects of dopexamine in established sepsis and peri-operative settings have differing results.

Two other small studies (one in five patients, and the other in 10) looked at the effect of dopexamine on microvascular flow in other tissue in pathological states. The first was to investigate microvascular flow in denervated jejunal tissue of stoma fashioned from transplanted small intestine (156). Details of this study are presented below (section 1.6.4). With respect to microvascular flow, dopexamine at doses of 1 and 2µg/kg/min was shown to improve red cell flux in the jejunal tissue that comprised the stoma. The paper does not clearly present data on MAP or cardiac index. The improvement in microvascular flow in the jejunum is also supported by a study post-cardiac surgery where cardiac index was increased by 25% (see 1.6.4) (157). Another small study is also summarised below (1.6.4), and shows using spectrophotometric techniques in patients with hyperdynamic sepsis that a short-term infusion of dopexamine could improve microvascular flow in the gastric mucosa (158).

1.6.4   Effects of dopexamine on regional perfusion

There is evidence that dopexamine may induce splanchnic vasodilatation and enhance renal blood flow (159, 160). Several human studies in various different settings have sought to assess whether or not dopexamine improves regional perfusion, with contrasting findings (161). Table 1.2 excludes those studies that are unable to compare against placebo control though studies where placebo comparator groups aren't included have been included if the studies are of short-duration and comparison against a baseline is both possible and meaningful. On the grounds of relevance studies unambiguously presenting results for dopexamine at doses of 2µg/kg/min or less (even if higher doses were used) are presented.

Table 1.2 Summary of studies examining the effects of dopexamine on regional circulations in humans at doses ≤ 2μg/kg/min

Trial of	Setting	Region Assessed	Method	N	Effect of dopexamine on region	Effect of dopexamine on DO_2	Study limitations	Ref.
5 minute infusion of DPX at 1.25, (2.5, 3.75 and 5μg/kg/min) – comparison with normal tissue here (see region assessed)	Patients with head and neck cancer undergoing free flap surgery	Free flap (denervated) and normal cutaneous tissue (deltoid region)	Laser Doppler velocimetry Mathematically derived tissue conductance (tissue perfusion÷MAP)	24	Compared to baseline: Increase in perfusion in normal tissue, decrease in perfusion in free flap. Increase in conductance in both tissues, though to a greater degree in normal tissue.	Increase	Infusions only lasted 5 minutes, most doses out of normal clinical range. Difficulty of extrapolating behaviour in free flaps to any other tissue type.	(153)
5 minute infusion of DPX at 1.25, (2.5, 3.75 and 5μg/kg/min) – comparison with normal tissue here (see region assessed)	Patients with head and neck cancer undergoing free flap surgery	Free flap (denervated) and normal cutaneous tissue (deltoid region)	Power spectral analysis of Doppler velocimetry waveforms	24	No significant effect on frequencies associated with myogenic activity	Increase	As above. To be able to statistically compare power spectra requires standardising all curves to a defined area under the curve. This may introduce error due to effectively altering the shape of the measured curves. Effects at physiological sites are inferred not directly observed.	(154)
8h of 0.5μg/kg/min DPX vs. two placebo groups haemodynamically optimised either identically or against CVP	Post major upper or lower gastrointestinal surgery	Forearm microcirculation. Sublingual microcirculation.	Laser Doppler flowmetry, Clarke electrode tPO₂, reactive hyperaemia (forearm). Sidestream darkfield imaging (sublingual)	135	Forearm: Higher tPO₂, and enhanced reactive hyperaemic response over time compared to placebo groups. Sublingual: Significantly higher perfused vessel density over time compared to CVP group only.	Significantly increased compared to baseline and other groups.	Insufficiently powered to provide significant data on post-operative outcomes. Therefore this physiological study was unable to directly provide linkage to outcomes. No study of higher doses (1 & 2mcg/kg/min).	(147)
24h of 2μg/kg/min DPX vs. placebo	Infra-renal aortic surgery	Splanchnic (Colon only)	Pre- and (1 week) post-surgical colonoscopic examination of mucosa for signs of ischaemia	30	One week post-operatively DPX group had significantly fewer patients with ischaemic colonic lesions	No data provided	Insufficiently powered study, unable to provide data on DO₂. Colon does not represent entire splanchnic circulation. No direct measurement of tissue blood flow in any specific region.	(162)
0.5-1μg/kg/min DPX vs. placebo intra-op	Abdominal aortic aneurysm resection	Splanchnic (stomach)	Gastric tonometry (pHi)	25	No significant change in pHi in DPX group, though a significant decrease in pHi in placebo group	Increased	pHi is altered by non-perfusion related factors. Therefore no direct measurement of tissue blood flow in any specific region. Gastric region is not representative of entire splanchnic bed. Only intra-operative infusions of dopexamine used.	(106)

0.5 or 2µg/kg/min DPX vs. placebo for 2-12h pre-op, intra-op & until 24h post-op. * *post hoc* analysis of Takala et al.'s trial (136) (Data analysed according to patients' pre-op pHi)	Non-vascular, high risk abdominal surgery	Splanchnic (stomach only)	Gastric tonometry (pHi)	268	pHi did not increase compared to placebo in normal pre-op pHi group at either dose of dopexamine. pHi significantly increased compared to placebo in low pre-op pHi group at both doses of dopexamine	Increase (normal pHi group) No increase (low pHi group)	Study based on *post-hoc* findings. Only gastric tonometry used (see above for shortcomings of this modality) No organ function data provided	(144)
0.5 or 2µg/kg/min DPX vs. placebo for 2-12h pre-op, intra-op & until 24h post-op. * Side-arm of Takala et al.'s trial (136)	Non-vascular, high risk abdominal surgery	Splanchnic (stomach only)	Gastric tonometry (pHi) Endoscopic examination and biopsy of gastric mucosa	38	No significant difference between groups in pHi No significant difference in endoscopic appearance. Biopsy evidence of significantly reduced inflammatory infiltrates in gastric mucosa in dopexamine groups compared to placebo	Not significantly different to placebo at either dose of dopexamine	Insufficiently powered study. Gastric mucosal changes may not have strictly related to gastric perfusion – e.g. no information given on pre- or peri-operative use of non-steroidal anti-inflammatory drugs, proton pump inhibitors etc. No direct measurement of tissue blood flow in any specific region.	(146)
24h of placebo vs. 0.5 or 2µg/kg/min DPX	Pre- and post-major abdominal surgery	Splanchnic (hepatic blood flow)	Indocyanine green (ICG) infusion Splanchnic oxygen kinetics and lactate uptake	21	No significant difference between groups in absolute or fractional splanchnic blood flow, oxygen kinetics or lactate uptake	DPX (not placebo) at any dose increases pre-operatively. Increase not sustained post-op (except high dose group)	Insufficiently powered study No direct measurement of tissue blood flow in any specific region.	(163)
1h sequential infusions of 1 or 2µg/kg/min DPX – comparison against baseline	Post-small intestinal transplant while patients ventilated on ICU	Splanchnic	Jejunal laser Doppler flowmetry Gastric tonometry (pHi, n=2)	5	Significant dose-related increase in jejunal red cell flux pHi wording too ambiguous to be meaningful – also only n=2	Ambiguously worded – suggests cardiac indices (therefore also likely DO_2) increase	Insufficiently powered study Impossible to separate effects of dopexamine on global and regional DO_2	(156)
5 min of: DPX (vs. dopamine vs. dobutamine) DPX vs. baseline considered only	7 h post-CABG involving CPB. 5min infusion only; 20min washout	Splanchnic (hepatic flow and jejunal perfusion)	Hepatic Venous (HV) catheter Intraluminally sited jejunal laser Doppler flowmetry probe	10	Increase in jejunal mucosal perfusion of 20% above baseline Increased HV saturations, lower HV O_2 extraction cf: baseline	Mean increased above baseline of 28%	Five minute infusions only Insufficiently powered study Hepatic vein drains blood from portal circulation – cannot differentiate perfusion effects in different parts of splanchnic circulation	(157)
0.5-2µg/kg/min DPX vs. placebo	Strictly intra-op during aortic surgery	Splanchnic (small intestine)	Urinary recovery of nasogastric administered saccharides	24	No significant difference between groups in recovery of saccharides (both groups increased from baseline)	No data provided.	Insufficiently powered study. No direct measurement of global or tissue blood flow in specific regions.	(164)

1 hour of 1 or 2µg/kg/min DPX vs. placebo (also vs. dopamine)	6 h post CABG (with cardiopulmonary bypass (CPB))	Splanchnic (hepatic blood flow)	ICG infusion	30	% ICG disappearance rate significantly increased only at 2µg/kg/min DPX (no changes in placebo group). Correlates to 38% (disproportionate) increase in liver blood flow.	Cardiac index only significantly increased at 2µg/kg/min (27%). DO_2 likely to follow same pattern.	Insufficiently powered study. Only one hour infusions. ICG disappearance rate is not only related to hepatic blood flow (165).	(166)
~24h of placebo vs. 2µg/kg/min dopexamine (also vs. fenoldopam)	CABG with CPB	Hepatic blood flow	ICG boluses Post-operative liver function tests (LFTs)	42	No significant difference ICG clearance between DPX and placebo at any time. Trends to lower LFTs for 5 days after	ns difference between DPX and placebo at any time	Insufficiently powered study. ICG disappearance rates do not only relate to hepatic blood flow.	(167)
Complex design – 2h dopexamine 1µg/kg/min vs. 2h enteral nutrition (EN) vs. 2h (EN + DPX)	1 day post CABG involving CPB in elderly patients (over 70y.o.)	Splanchnic (hepatic) blood flow Hepatic O_2 kinetics	ICG infusion HV lactate	16	Transient increase hepatic blood flow with DPX (EN failed to augment SBF compared to baseline and can be therefore seen as a control) No significant difference oxygen kinetics, but systemic and HV lactate increased	DO_2 augmented by addition of DPX	Insufficiently powered study. Only 2h infusion. Purely physiological study.	(168)
24h DPX at 0.5, 1 or 2µg/kg/min vs. placebo	CABG with CPB	Splanchnic blood flow Renal function	HV catheter Gastric tonometer (pHi) Creatinine clearance (CrC)	44	pH_i no significant different between groups HV saturation not significantly different HV lactate not significantly different (except 0-6h post-op in 2µg/kg/min group) CrC significantly greater in DPX groups, and inversely related to dose	All groups increased DO_2 with time. Only DPX 2µg/kg/min significantly greater DO_2 than placebo, though not beyond 8h	Insufficiently powered study Gastric tonometry not only affected by gastric blood flow. HV catheter unable to distinguish differential effects in different parts of splanchnic circulation. Unclear what degree of change in regional perfusion will result in a significant change in HV sats and lactate – therefore uncertain sensitivity.	(169)
60-90 min infusion of ~1µg/kg/min DPX vs. placebo	Post CABG (involving CPB)	Splanchnic	Gastric tonometer (PCO_2 gap) ICG	14	Significant increased splanchnic blood flow, DO_2 and fractional splanchnic flow cf: control No significant difference in pHi cf: controls, and significant decrease in pH_i cf:baseline	Significantly increased DO_2 cf: controls (42% above baseline)	See above for shortcomings of ICG & gastric tonometry. Insufficiently powered study. Only 60-90 minutes of infusions.	(170)
18h infusions of: placebo vs. DPX 1µg/kg/min (vs. epidural bupivacaine)	CABG involving CPB	Splanchnic	ICG infusion	40	No significant difference in splanchnic blood flow between control and DPX groups.	ns difference in CI between groups, though significant increase in CI with time	Insufficiently powered study See above for limitations of ICG plasma disappearance rate.	(171)
Approx. 18h either DPX 2µg/kg/min or placebo for patients with pHi<7.3	Post-op heart valve replacement (involves CPB)	Splanchnic	Gastric tonometry (pHi and arterial-mucosa pH gap)	19	No significant difference between groups until 2h. Then pHi significantly lower for significantly longer in DPX group. No significant differences pH gap	No data provided	Insufficiently powered study No cardiac output/DO_2 data. pHi & pH gap data at odds with one another when both are markers of gastric perfusion. Underscores reliability issues.	(172)

56

16hrs of either DPX 1µg/kg/min or placebo (or dopamine)	Following induction and up to 16 h post-CPB for various types of cardiac surgery	Splanchnic	Gastric tonometry (mucosal PCO_2, pHi and arterial-mucosa pH and PCO_2 gap)	35	No significant difference in pH gap between groups No significant effects of dopexamine on pHi, urine output or complications	No data provided	Insufficiently powered study though multi-modal gastric perfusion study	(173)
15 min infusions of DPX at either 1 or 2µg/kg/min (and also at 4µg/kg/min DPX vs. dopamine)	Physiological study in patients anaesthetised for CABG (but not yet undergoing surgery)	Renal blood flow	Right renal vein catheter	20	Mean renal blood flow for each DPX group increases by 41% (1µg/kg/min) and 49% (2µg/kg/min) cf: baseline – less than the corresponding increases in cardiac index	No data on DO_2. Likely to track cardiac indices (increase by 61 and 79% cf: baseline, respectively)	Physiological study with only 15 minute infusion. Can't assess effects on intra-renal haemodynamics. Uncertain whether effects are sustained over longer periods of time, whether they affect post-operative outcome.	(174)
Peri-operative infusion of either 2µg/kg/min DPX or placebo	Aortic surgery	Renal function	Plasma creatinine levels for 3 days post-op CrC	32	Significant increases in plasma creatinine (cf: baseline) in placebo group, but not in DPX group	No data on DO_2 or cardiac index	Insufficiently powered study to assess effects on post-operative complications. No direct measurement of regional blood flow in any tissue bed.	(105)
40 min infusions of either 0.5, 1 or 2µg/min DPX vs. placebo (also 4µg/kg/min DPX)	Post-CABG (using CPB) patients, 1-3 h post-op	Renal resistance of interlobar arteries	Doppler ultrasound	20	No significant difference in renal vascular index between placebo and any DPX group	ns differences in CI between placebo and DPX groups (up to 2µg/kg/min)	Only 40 minute infusions. No ability to assess effects on organ function.	(175)
DPX 1µg/kg/min or placebo, patients pre-divided in to normal or abnormal renal function (i.e. 4 group design)	Strictly intra-operative during CABG (with CPB) and until end of surgery	Renal function	GFR, CrC, various markers of damage specific for different areas of nephron, urine output β-NAG (N-acetyl-β-glucosaminidase)	48	No significant differences between control and DPX groups whether pre-existing normal or abnormal renal function.	No data on DO_2. Cardiac indices in DPX groups increased significantly cf: baseline and controls but only outside of CPB.	No direct measurement of tissue or regional blood flow in the renal or any other bed.	(176)
Up to 72h DPX 2µg/kg/min vs. placebo (and vs. dopamine)	Patients with septic shock resuscitated and on noradrenaline infusion	Renal function	CrC (primary end point) Urine output Free water and Na^+ output	61	No significant differences: CrC, urine output, or any renal outcome	Data not provided	Studied in the setting of septic shock and not post-major surgery. Patients already receiving noradrenaline (not usual for peri-operative settings) No direct measurement of regional/tissue blood flow	(177)

Infusion	Setting/Patients	Region	Measures	n	Results	DO2	Shortcomings	Ref
60 min sequential infusions of 0.5, 1 and 2µg/kg/min DPX (also 4µg/kg/min)	Patients with septic shock already resuscitated to a minimum cardiac index with dobutamine (not halted during DPX)	Splanchnic blood flow	ICG Gastric tonometry (pHi and gastric mucosal - arterial PCO_2 gap)	12	Non-significant increase in splanchnic blood flow cf: baseline in any group. Only significant increase in splanchnic DO_2 in 2µg/kg/min group. Dose-dependent significant increase PCO_2 gap and decrease pH_i cf: baseline	Significant increase from baseline only in 1 and 2µg/kg/min group	Insufficiently powered study Studied in the setting of septic shock. Short-term infusions. Dopexamine added to dobutamine (not usual for peri-operative setting) Shortcomings of ICG & gastric tonometry already stated above	(178)
90 min infusion of DPX 1-4µg/kg/min (NOTE: it is impossible to discern the dose of DPX infused and no mean or median dose of DPX is stated in the paper)	Hyperdynamic septic shock (all patients already on noradrenaline)	Splanchnic blood flow (stomach, hepatic vein)	ICG infusion & hv sampling Gastric tonometry (pHi, gastric mucosal-arterial PCO_2 gap, gastric mucosal-hv PCO_2 gap)	12	Compared to baseline: significantly reduced total splanchnic and pre-hepatic vascular resistance. Significantly increased splanchnic DO_2 (not proportionally greater than increase in systemic DO_2) No significant change in any measured PCO_2 gap	Significantly increased from baseline	Insufficiently powered study Short-term infusions Studied in the setting of septic shock and not post-major surgery. Patients already receiving noradrenaline (not usual for peri-operative settings) Shortcomings of ICG & gastric tonometry already stated above.	(179)
1 hour of 2µg/kg/min DPX. Patients act as own controls. 7 healthy volunteers for "normal physiology" comparison	Hyperdynamic septic shock	Splanchnic perfusion	Reflectance spectrophotometry (for microvascular O2 saturation (Hbi$_{O2}$) and concentration (relHb$_{conc}$)) Gastric tonometry	15 (7)	Hyperdynamic sepsis patients showed broadening and negative skewing of distribution of gastric mucosal Hbi$_{O2}$ and relHb$_{conc}$ cf: healthy controls Significant increase in Hbi$_{O2}$ and relHbconc, with partial normalisation of distributions cf: baseline Significant increase in pH_i cf: baseline.	Significant increase in mean DO_2 over baseline	Insufficiently powered study Short-term infusions Studied in the setting of septic shock and not post-major surgery. Shortcomings of gastric tonometry already stated above.	(158)
2h infusion of either DPX 1µg/kg/min or placebo (or dopamine)	ICU; ventilated patients with SIRS	Splanchnic	Gastric tonometer (pHi) ICG disappearance MEGX formation	25	ns difference between DPX and placebo at baseline (except lower MEGX DPX) and ns change over 2h in placebo group any parameter DPX significant increases in pH_i, ICG and MEGX formation after 2h compared to baseline	No significant change in DO_2	Insufficiently powered study Short-term infusions Studied in the setting of critical illness and not post-major surgery. Shortcomings of ICG & gastric tonometry already stated above.	(180)
Up to 7 days of DPX at 0.5-2µg/kg/min vs. placebo	Critically ill patients predicted a stay >4days	Splanchnic (small intestine) Renal function	Urinary recovery of nasogastric administered saccharides CrC	102	ns difference between groups for any splanchnic or renal parameter ns difference groups for any mortality or length of stay outcomes	Not provided	Not studied in high risk surgical patients. No direct regional blood flow measurements.	(181)

There may be various reasons for the contrasting findings of the studies in Table 1.2. The studies are small and may lack statistical power. Therefore a difference in findings is not unexpected. The studies are heterogeneous in design making a firm conclusion more difficult. Some studies assess patients undergoing non-cardiac surgery whereas others assess patients who have undergone cardiac surgery with cardiopulmonary bypass. Other groups include mixed populations of the critically ill or those with sepsis. The behaviour of the vasculature and the heart and therefore the response to dopexamine, is likely to differ in these populations and the use of other vasoactive medications may differ. The techniques used to assess flow in different regional circulations vary in their specificity. While a hepatic venous catheter may be used to obtain information about blood coming from the splanchnic bed, it is impossible to draw any conclusions from the data about any specific area of the splanchnic bed e.g. small intestine vs. stomach vs. liver etc. Similarly gastric tonometry may be used to obtain different data (e.g. pH_i, gastric PCO_2, PCO_2 gap) but all parameters have been shown to be unreliable indicators of splanchnic perfusion due to non-perfusion related factors (182, 183). The most reliable indicators of perfusion in splanchnic tissue are those that measure microvascular perfusion directly. However it is known from animal studies that different areas in the splanchnic circulation behave differently and therefore unless testing is performed at multiple sites, a complete picture of the effects on the splanchnic bed is impossible to construct (184).

Although the diverse methodology of the papers above must not be overlooked, it is interesting to note that all of the studies above that directly assess microvascular flow demonstrate that dopexamine is associated with improved microvascular perfusion.

1.7   *Immunomodulatory and anti-inflammatory effects*

The evidence for a selective effect on the splanchnic or renal circulations is not compelling. Various investigators have instead looked to the effects of dopexamine on markers of

inflammation. The theoretical basis behind this is that changes in microvascular and cellular behaviour occur only after stereotypical intracellular signalling events have occurred (185-188). Secondly, as already briefly stated in section 1.3.2, catecholamines, including dopexamine, have immunomodulatory effects (40, 189) and therefore theoretically have the ability to influence various parts of the signalling cascades. Before going on to describe the few studies in humans looking at this aspect of dopexamine's actions, immunomodulation by catecholamines is discussed in greater detail.

1.7.1 Catecholamine immunomodulation

Vascular tissue, human peripheral blood mononuclear cells and many other tissue types are covered in adrenoceptors, predominantly β_2-adrenoceptors (40). Many immune cell types contain catecholamine molecules which may be released to act in an autocrine or paracrine fashion, while sympathetic neurones represent another catecholamine pool that can modulate immune response (189, 190). As a result catecholamines have a variety of effects that can affect inflammatory response. Catecholamines induce a short-lived increase in circulating leucocyte numbers, most markedly in the natural killer cell and CD8+ lymphocyte subset followed by a polymorph leucocytosis (40). These are thought to originate from the marginal pool, and the response is thought to be β_2-adrenoceptor mediated (86, 191-193), though the neutrophilia in response to lipopolysaccharide injection has been shown to be α_1-adrenoceptor mediated (194). Chronically elevated levels of plasma catecholamines however have been associated with reduced circulating immune cell numbers and responses (40).

Once released in to the circulation the ability of immune cells to reach tissue from the bloodstream is based on successfully adhering and transmigrating in to the tissue in question. In this regard the interdependence of microvascular flow and immune cell-endothelial interactions has already been touched on. Through complex effects on

microvessel calibre and tone, the vascular effects of catecholamines influence shear rate, shear-dependent autoregulation of microvascular flow and can reduce shear-dependent leucocyte-endothelial adhesion (195-197). Although leucocyte endothelial interaction may be reduced, platelet-neutrophil interactions may be increased, and this likely represents the dose dependent effects of mixed adrenoceptor activation on platelet activation (198-202). Endothelial behaviour e.g. vascular permeability is also under the influence of adrenoceptor signalling and downstream cAMP related signalling seems to be involved in both this and the reduction in immune cell adhesion (10, 195, 203-206). Shear independent factors such as the activation state of neutrophils can also be modified directly by adrenoceptor agonists (207, 208).

The control of neutrophil transmigration from the microcirculation in to the lung following endotoxaemia (but not haemorrhage) has been shown to be α_2-adrenoceptor mediated and may relate partly to the inhibition of diapedisis through endothelial G_i signalling (209-211). However α_1-adrenoceptor signalling can increase both endotoxaemia and haemorrhage induced neutrophil infiltration in the lung (211). This may be explicable in terms of opposite effects on the same intracellular signalling pathway. In the case of α_2-adrenoceptors, a reduction in cAMP mediates an increased activation of downstream Raf-MEK1/2-ERK2-p90rsk pathways whereas the opposite occurs with α_1-adrenoceptor activation, possibly via non-cAMP reactive oxygen species mediated pathways (211-213). The ability of leucocytes to undergo respiratory burst is inhibited by β-adrenoceptor agonists whereas inhibition of phagocytosis by macrophages is mediated by α and β-adrenoceptor pathways (214-216).

Adrenoceptor activation can modulate cytokine responses (217-219). Generally speaking dopaminergic and β_2-adrenoceptor responses promote the secretion of anti-inflammatory cytokines such as IL-10 and reduce the secretion of pro-inflammatory cytokines such as TNF-α. α-adrenoceptor agonism results in the opposite effect. In the case of α_1-adrenoceptor agonism and lung injury it has been shown that pro-inflammatory cytokines increase without an increase in neutrophil infiltration (210, 220). These results may partly explain how organ

function in α-adrenoceptor agonist treated models of sepsis are worse compared to untreated septic controls (221). Although the cytokine response to adrenergic agents is not straightforward, varying according to the cell type in question, (e.g. IL-6 generation occurs in association with β-adrenoceptor agonism in endothelial cells and fibroblasts, but is downregulated in peripheral blood mononuclear cells) (222), the cytokine profile response following adrenergic stimulation in general favours that of a T-helper 2 response over a T-helper 1 (40). The effect of catecholamine stimulation on human tissue may not be favourable and can invoke a cytokine response that mimics that seen with lipopolysaccharide (223, 224).

.Adaptive immunity is affected by adrenergic agents (225, 226). Dopaminergic receptor agonism inhibits the cytotoxicity and proliferation of CD4+ and CD8+ T-cells and in vitro studies demonstrate attenuated B-lymphocyte function and IgG production (40). Adrenergic stimulation via α_1-adrenoceptor and β_2-adrenoceptor mechanisms induces lymphocyte apoptosis (227, 228). It is possible that the increased rates of apoptosis partly relate to intracellular free radical breakdown products of catecholamines (20, 42), and this may also explain the apoptosis seen in the heart, vascular smooth muscle and skeletal muscle myocytes exposed to catecholamines (229-231). On the other hand β_2-adrenoceptor agonism can prevent shiga-toxin induced renal tubular apoptosis and cAMP elevating agents have been shown to reduce LPS-associated apoptosis in endothelial cells via inhibition of caspase pathways (232, 233). The precise intracellular signalling pathways through which adrenergic agents trigger or slow down inflammation, promote cell survival or apoptosis are not known but several have been suggested (211, 232-239).

The effects of catecholamines on immune function and inflammation are clearly complex and myriad. As a broad generalisation though, catecholamine effects are anti-inflammatory when dopaminergic or β-adrenoceptor mediated, whereas the opposite is true for α-mediated effects. Suspecting an anti-inflammatory role for dopexamine would be in keeping with its predominant β_2-adrenoceptor and lesser dopaminergic receptor effects.

1.7.2   Focus on the β2-adrenoceptor

Studies across species support a role for the β2-adrenoceptor (as opposed to β1-adrenoceptor or dopaminergic receptors) in the protection against free-radical mediated injury (240-242). Studies demonstrate that β2-adrenoceptor agonists though not β1-adrenoceptor agonists improve haemodynamic stability and mortality in endotoxaemia and also following caecal ligation and puncture (243-245), and better preserve the pressor response to norepinephrine despite a similar degree of amelioration of TNF-α to β1-adrenoceptor agonists (243). Improvements in organ function compared to controls are also seen and all these effects are partially ascribed to a protection against pro-inflammatory cytokine and free radical generation (244, 246, 247). Similarly β2-adrenoceptor mediated effects have been shown to reduce reactive oxygen species following hypoxic injury and thereby reduce endothelial damage *in vitro* (248). Studies either selectively overexpressing renal β2-adrenoceptors or selectively antagonising renal β2-adrenoceptors and have shown the importance of the β2-adrenoceptor mediated amelioration of renal endotoxaemic injury (249, 250), and note the specific effect of β2-adrenoceptor agonism on cytokine responses in the renal medulla and a cAMP mediated anti-apoptotic effect on renal tubular cells (233, 251). One potential explanation for these observations may be downregulation by β2-adrenoceptors of CD14-TLR4-TNFα signalling cascades (252). These findings are notable as elevated mortality rates in septic shock are associated with both acute kidney injury (253), and also with those β2-adrenoceptor gene polymorphisms that are associated with a greater need for vasoactive drugs and organ dysfunction (36). It is also notable that several animal studies demonstrated either an increase in sepsis mortality following β2-adrenoceptor blockade or a more severe pattern of hepatic injury (221, 254-257).

Other vascular effects of β2-adrenoceptor agonism relate to preserved endothelial barrier function, the amelioration of plasma extravasation and reduction of immune cell adhesion (10, 197, 258). While many of the beneficial effects of β2-adrenoceptor agonism may relate

to the cAMP signalling pathway, there may be cAMP independent pathways that are involved in anti-inflammatory effects (247, 259).

1.7.3 Immunomodulatory effects of dopexamine in human studies

Returning to dopexamine-based studies in humans, some investigators have examined the degree of neutrophil infiltration in various areas of the gut following major abdominal surgery and have found it to be reduced in dopexamine treated groups when compared to placebo treated groups (146, 162). Others have also shown that in comparison to placebo, dopexamine treatment is associated with significantly less circulating plasma tumour necrosis factor (TNF)-α (171), IL-6 (169) and procalcitonin (171) even when splanchnic blood flow is not elevated. However, some investigators have failed to find any convincing anti-inflammatory effects of dopexamine following surgery (147, 163, 260). The evidence base for an anti-inflammatory effect is too small to draw a meaningful conclusion from.

1.8 Broadening the evidence base - effects of dopexamine in animal studies

The effects of dopexamine on regional perfusion and inflammation are not easily studied in humans due to the ethical concerns of placing specialised monitoring in several locations deep inside the body in large numbers of patients. Furthermore a greater degree of standardisation of laboratory conditions, less variation in laboratory animals and the ability to perform more specific and invasive tests allows further information to be drawn from animal studies that is not available from human studies. The difficulty with animal studies is that it can be difficult to compare findings in one species with another, and furthermore although a high degree of standardisation is found within studies, the protocols used vary significantly between groups. In this section the findings of animal studies are presented in order to present further insights regarding mechanisms of action of dopexamine – studies are limited

to those presenting data for doses up to 2.5µg/kg/min (this is just outside our dose range of interest, the maximum dose used in peri-operative haemodynamic optimisation studies being 2µg/kg/min).

1.8.1 Microvascular perfusion and regional circulations

In anaesthetised rats, dopexamine at 1 µg/kg/min increases superior mesenteric artery blood flow and decreases its vascular resistance, probably a result of cAMP increases secondary to activation of both D_1 and β_2 adrenoceptor activation (57). As CO was not measured in this experiment one can only speculate whether the increase in blood flow is proportionate to CO or not. However, when a step-wise application of up to 20cmH$_2$O positive end expiratory pressure (PEEP) is applied in anaesthetised rats, the resultant decreases in MAP and CO are not significantly ameliorated by dopexamine. Nevertheless dopexamine at 1µg/kg/min better preserves ileal arteriolar mean blood flow and diameters. This implies dopexamine can provide some degree of intestinal vasodilatation independently of increases in cardiac output (261). In support of this two porcine studies where superior mesenteric artery (SMA) perfusion pressure was either left free or reduced independently of systemic haemodynamics (262, 263), SMA blood flow and DO$_2$ and jejunal tPO$_2$ decreased with reductions in mesenteric perfusion pressure despite graded reductions in mesenteric resistance (control). Infusing dopexamine at doses of either 0.5 or 1 µg/kg/min significantly increased SMA blood flow by reducing mesenteric resistance, compared to control. At the lowest perfusion pressure (30mmHg) these increases in flow were not reflected in beneficial effects on pH$_i$ (decreased), PCO$_2$ gap (increased), luminal lactate production (increased), jejunal microvascular flux (no significant difference) or tPO$_2$ (decreased) when compared to control. These findings suggest dopexamine may induce selective mesenteric vasodilatation in the mesenteric bed, but in a normally functioning bed which can autoregulate this is of no additional benefit. A further similar study supports this (264). When the vasculature of tissue

beds is unable to autoregulate normally, as occurs in sepsis or after an inflammatory insult, the effect may differ.

In a resuscitated porcine model of sepsis (endotoxaemia), a dose of 2.5µg/kg/min dopexamine induced significant increases in systemic DO_2 which were associated with trends to an increased jejunal tPO_2, a significant increase in jejunal mucosal HbO_2 and a decrease in mesenteric (and skeletal muscle) oxygen extraction (265). Similarly in a resuscitated model of porcine faecal peritonitis 2µg/kg/min dopexamine also increased systemic and mesenteric arterial oxygen delivery above baseline, but failed to have any significant effects on jejunal, colonic, renal or gastric microvascular flux. However gastric and pancreatic microvascular flux were significantly increased compared to baseline with 1µg/kg/min dopexamine and it is notable that following fluid resuscitation but before the infusions of dopexamine, the only microvascular areas where red cell flux hadn't returned to pre-endotoxin levels were the jejunal muscularis and pancreatic beds (266). It is notable that dopexamine infusion at 1µg/kg/min has also been shown to increase pancreatic tissue PO_2 in healthy rats (though not in a model of necrotising pancreatitis) (267).

Bastien et al. showed in a rabbit model of CPB (sterile inflammation) that a 15 minute infusion of dopexamine at 2µg/kg/min was associated with a significant increase in microvascular perfusion in ileal and jejunal tissues, but not the gastric bed (268). Higher doses were not associated with sustained increases in perfusion though the design of the experiment was such that it is impossible to know whether a gradual lack of effect would have occurred at the same dose over time (as comparison was not against a control). In another rabbit model using several different doses of up to 10µg/kg/min dopexamine (in the setting of non-resuscitated endotoxaemia) (269), Lund et al. showed that dopexamine dose-dependently improved liver, gut and skeletal muscle tPO_2 distributions, that this effect began below 2µg/kg/min and that this effect of dopexamine was independent of any increase in DO_2. Furthermore they provided data suggesting that a better distribution of microcirculatory

blood flow was responsible for this, and that the effects on tPO$_2$ in non-septic preparations were not demonstrable.

In support of these findings, microvascular studies in rodent endotoxaemia models using dopexamine at 0.5µg/kg/min show increased intestinal microvascular blood flow and ileal muscularis functional capillary density when compared with untreated endotoxaemic controls (196). In the only study where mucosal blood flow was seen to decrease in response to endotoxin, dopexamine (2.5µg/kg/min) ameliorated these changes almost completely (270). At 2µg/kg/min dopexamine also preserves portal blood flow, liver sinusoidal diameters and blood flow and therefore ameliorates the deterioration in these parameters seen in untreated endotoxaemic controls (271). Dopexamine at 2.5µg/kg/min also shows an ability to preserve erythrocyte velocity at closer to baseline levels for longer (without a change in mesenteric venular diameter) - consequently the mesenteric venular shear rate is higher for longer compared to controls (195). In contrast to untreated endotoxaemic rats, rats treated with dopexamine at this dose demonstrate no depletion in high energy phosphates or any increase in the production of purine compounds in the intestine (272).

1.8.2   Anti-inflammatory effects

Dopexamine (2.5µg/kg/min) ameliorates leucocyte adhesion in rodent mesenteric (195) and (0.5µg/kg/min) intestinal venules (196) when compared to untreated endotoxaemic controls. Possibly as a consequence of these differences there is less plasma extravasation from the intestinal circulation (195). However whereas plasma extravasation appears to be under β$_2$-adrenoceptor control, leucocyte-endothelial interactions were shown not to be so (195). In common with the preservation of vascular barrier function seen in the intestine, in a porcine faecal slurry model dopexamine increased beneficial effects on cerebral microvessel ultrastructure and perivascular oedema formation, and this was blocked by co-administration of a β-adrenoceptor antagonist or an α-adrenoceptor agonist (273). In a rodent model of

endotoxaemia, dopexamine also ameliorated the high levels of plasma TNF-α seen one hour after endotoxaemia in untreated controls (196). The only study performed in a rodent model of necrotising pancreatitis failed to show any histological improvements as a result of the infusion of 1µg/kg/min dopexamine (267). In a porcine model of supracoeliac cross clamping (i.e. ischaemia-reperfusion injury), a dose of 2µg/kg/min dopexamine was associated with a higher systemic DO_2 and a lower production of molecular markers of lipid peroxidation when compared with controls (274). Following on from these studies, various other studies have looked at the effects of dopexamine on free radical mediated injury (240, 275), immunomodulation and organ damage (207, 255, 273, 276). These studies have used dopexamine outside of the dose range of interest, but deserve brief mention as they support anti-inflammatory effects of dopexamine and point to β_2-adrenoceptor agonism being important for this.

Whether these microvascular and immune effects translate in to improved organ function is impossible to assess as none of the animal studies assessed renal or liver function (though plasma markers of pancreatic function were assessed in one study of necrotising pancreatitis, and were not improved by dopexamine) (267). However 1µg/kg/min dopexamine produced an early improvement in GFR and urine volume when compared to untreated endotoxaemic rats (277) and in conscious bacteraemic rats, dopexamine (1µg/kg/min) ameliorated decreases in glomerular filtration rate, urine flow and sodium excretion compared to bacteraemic controls (278). It must be noted that these are unreliable markers of the degree of renal injury though.

1.9 Hypotheses

1. Dopexamine improves microvascular perfusion in the ileum of endotoxaemic rodents

2. The beneficial effects of dopexamine on ileal microvascular perfusion are not related to its effects on cardiac index

3. Dopexamine has anti-inflammatory effects in the setting of endotoxaemia, namely reduced neutrophil activation as assessed by membrane integrin expression, decreased leucocyte-endothelial adhesion in the intestinal microcirculation and decreased neutrophil infiltration in the lung

4. Dopexamine improves organ function in a rodent model of laparotomy and endotoxaemia

5. The anti-inflammatory effects of dopexamine are β_2-adrenoceptor dependent

1.9.1 Novelty of the thesis

In translational medicine the laboratory bench is where drugs are developed for specific purposes, and in general success occurs when those drugs are approved for and used at the bedside. Dopexamine was not specifically designed with the intention of improving outcomes in high risk surgery though it has found a place there. Having said that, the effects of dopexamine in high risk surgery are unclear, and proponents of the drug (as part of a package of peri-operative haemodynamic optimisation) are uncertain as to how exactly dopexamine produces any benefit. In an effort to shed light on what the effects of dopexamine are in this setting, a reverse translational approach has been taken here. Therefore in this thesis a therapy of uncertain benefit for a specific clinical scenario is being studied at the bench through *in vivo* modelling. This unusual and somewhat novel approach has been necessary due to the variability in clinical trial populations and variable trial findings as described previously, the massive expense of investigating this problem through

future large clinical trials, the difficulty of finding a mechanism of action even if a well-designed clinical trial produced a conclusively positive result, and additionally due to the generally poor understanding of catecholamine effects in critical illness. The shortcomings of many human studies are highlighted in Table 1.2.

Although previous *in vitro* and *in vivo* studies have looked at the effects of dopexamine on various aspects of haemodynamics or the microcirculation or inflammation, none have looked at all in an *in vivo* preparation sufficiently similar to major surgery whilst concurrently studying the effects of infusion on organ function (see Table 1.3). Therefore studies demonstrating immunomodulation or improved microvascular perfusion have not been able to demonstrate a translation to improved organ function because this has not been recorded (195, 196, 270, 272). Furthermore many studies have looked at doses outside of the range of clinical interest (240, 255, 273, 275, 276). A more comprehensive review of the limitations of the most relevant animal studies is covered below in Table 1.3. An attempt has been made to overcome many of the limitations highlighted in both Tables 1.2 and 1.3 by the studies that make up this thesis, and this represents in the main the novelty and strength of the studies herein – the multimodal approach to monitoring macrohaemodynamics, microcirculation and innate immune response while marrying the findings to clinically relevant parameters and outcomes – organ function data and biochemical indices of perfusion, in a reproducible and internally consistent model. This has not been done before. Further discussion of the model will be found in Chapter 2.

Table 1.3 *In vivo* animal studies investigating the effects of dopexamine (at 2.5µg/kg/h or less) on regional circulations and inflammation

Ref	Species	Setting	Measurement modalities	Dopex dose (µg/kg/min)	Fluid regime (ml/kg/h)	Principal findings	Drawbacks
(57)	Rats	Physiological and histological study	Combined *in vitro* and *in vivo* elements. Mesenteric blood flow (transit time flow probe).	1 (& 3)	1.2ml/h normal saline	Dopexamine binds to DA1 and β_2 adrenoceptors in mesenteric vasculature and causes a dose dependent increase in mesenteric blood flow	No organ function data. Not clinically relevant model of inflammation therefore unclear whether these effects are maintained following a systemic inflammatory stimulus.
(261)	Rats	Physiological study – incremental PEEP increases to 20cmH$_2$O to reduce CO. Midline laparotomy required.	Thermodilution CO, IVM – red cell velocity and arteriolar diameters recorded	1, (3 & 5)	Unclear	Compared to untreated controls, dopexamine prevents reductions in arteriolar blood flow despite decreases in cardiac output	Not clinically relevant model of inflammation. Unclear what relevance is to clinical situation due to lack of organ function data and short-term infusion of drugs. Furthermore observations were only noted in primary level arterioles and not secondary or tertiary level arterioles.
(262)	Pigs	Physiologic study in animals with significant abdominal surgery – intestinal hypotension induced by clamping of superior mesenteric artery (SMA)	SMA flow (transit time US), laser Doppler flowmetry, tPO$_2$, jejunal luminal dialysate, gastric tonometry	0.5 & 1	600ml bolus, 20ml/kg/h Ringer's acetate	Despite increase in mesenteric DO$_2$ with dopexamine, PCO$_2$ gap increased, jejunal luminal lactate increased, pHi and tPO$_2$ decreased	Short-term infusions of dopexamine. No assessment of effects on organ function/outcome and no control group (crossover design)
(263)	Pigs	Physiologic study in animals with significant abdominal surgery – intestinal hypotension induced by clamping of superior mesenteric artery (SMA)	Mesenteric resistance (derived from transit time US), laser Doppler flowmetry, tPO$_2$	0.5 & 1	600ml bolus, 20ml/kg/h Ringer's acetate	Under conditions of significant intestinal hypotension dopexamine induces mesenteric vasodilation though it has no effect of O$_2$ delivery or extraction	Short-term infusions of dopexamine. No assessment of effects on organ function/outcome and no control group (crossover design)
(264)	Pigs	Physiologic study in animals with significant abdominal surgery – intestinal hypotension induced by clamping of superior mesenteric artery (SMA) while 10cmH$_2$O PEEP applied	SMA flow (transit time US), laser Doppler flowmetry, tPO$_2$, intestinal net lactate production	0.5 & 1	600ml bolus, 20ml/kg/h Ringer's acetate	Dopexamine is unable to rescue the gut from ischaemia below the perfusion pressure threshold that induces intestinal ischaemia	Short-term infusions of dopexamine. No assessment of effects on organ function/outcome and no control group (crossover design)
(265)	Pigs	20 minute LPS infusion: E.Coli (0111:B4) 2 µg/kg. Midline laparotomy and abdominal instrumentation.	Intestinal mucosal tPO$_2$. Mucosal microvascular Haemoglobin oxygen saturation. Global and regional DO$_2$.	2.5 (5, 10 & 20). Also dopamine and dobutamine infused at same doses.	50:50 Ringers and 6% hydroxyethyl starch for pulmonary aortic occlusion pressure 15mmHg	Compared to untreated controls, lowest dose of dopexamine results in significantly higher values of jejunal mucosal Haemoglobin saturations but not tPO$_2$. Mesenteric oxygen extraction significantly reduced. Associated with increase in global DO$_2$.	Short-term infusion only. 3 doses out of normal clinical range. No organ function data. Starches not used clinically any longer. Crossover design.

(266)	Pigs	30 minute infusions of drug four hours after induction of faecal peritonitis by 20g autologous faeces. Midline laparotomy and instrumentation required.	Cardiac index, superior mesenteric artery blood flow. Laser Doppler flowmetry in kidney, pancreas, stomach, colon, and jejunum	1 & 2 (dopamine & dobutamine also used at different times and at varying doses)	10ml/kg pentastarch followed by 15-20ml/kg/h Ringer's acetate for CVP and pulmonary capillary wedge pressure 6-8mmHg	Significant increases in CI and SMA flow. No significant increase in red cell flux in any organs except gastric mucosa and pancreas at 2µg/kg/min dose.	Reasonable model of the emergency laparotomy due to bowel perforation. However crossover design, only short-term infusions of drugs, no organ function data. Laser Doppler flowmetry has poor spatial resolution. Pentastarch now not used clinically.
(267)	Rats	Cerulein and glycodeoxycholic acid induced necrotising pancreatitis	Blood pressure, polarographic pancreatic tPO_2, pancreatic enzymes, pancreatic histology	1	8ml/kg/h normal saline	No effect of dopexamine on tPO_2 (though increases it during non-pancreatitis period). No significant effect of dopexamine on pancreatic injury.	Only one-hour of dopexamine. Necrotising pancreatitis not a model of complications from major surgery.
(268)	Rabbits	Mild hypothermic non-pulsatile cardiopulmonary bypass. Midline laparotomy and sternotomy performed.	Gastric, jejunal, ileal laser Doppler flowmetry. Jejunal biopsy to assess ischaemic damage.	2 (and 4)	3ml/kg/h hetastarch	Lower dose of dopexamine associated with greater LDF values in ileum and jejunum only	Hetastarch no longer used as a peri-operative fluid. Dopexamine only infused for 15 minutes at each dose. Cross-over design and problem of deterioration of sample preparation with time. No organ function data.
(269)	Rabbits	LPS bolus: E.Coli (L-3137) 1.5mg/kg	Tissue PO_2, CO (transpulmonary thermodilution)	0.5, 1, 2 (4 & 10)	20ml/kg/h Normosol	Dopexamine significantly and dose dependently increases tPO_2 distributions in septic shock in gut, liver and skeletal muscle.	2 doses outside of normal clinical range. Short-term infusions only. No organ function data. Hypotensive model – not comparable with haemodynamics seen peri-operatively.
(196)	Rats	15mins LPS infusion: E.Coli (055:B5) 20mg/kg. Midline laparotomy.	Intestinal IVM (functional capillary density, leucocyte adhesion), laser Doppler flowmetry (intestinal circulation), plasma TNF-α	0.5	7.5ml/kg/h normal saline	Compared to untreated controls, significantly: higher intestinal red cell flux, higher fcd in circular and longitudinal layers of intestine, less leucocyte adhesion, reduced levels of TNF-α.	No ability to link changes in microcirculation to global DO_2/CO. No organ function data – unsure whether these improvements in cytokine profile and the microcirculation translate to clinically meaningful parameters.
(271)	Rats	120 mins LPS infusion: E.Coli (026:B6) 2mg/kg with concomitant infusion dopexamine. Midline laparotomy and bowel exteriorisation.	MAP, CO (transpulmonary thermodilution), portal blood flow (transit time flow), intravital microscopy of left lobe of liver	2	25ml/kg/h fluids Ringer's solution	Compared to untreated group: significant attenuation of portal blood flow, liver sinusoidal diameters and sinusoidal blood flow. Significantly elevated CO at 120 mins.	Lack of liver and other organ function data limits clinical significance of findings. Liver IVM can only assess superficial areas of liver and not microcirculation deep in liver parenchyma.

(195)	Rats	60 mins LPS infusion: E.Coli (026:B6) 2mg/kg (with or without dopexamine pre-treatment). Midline laparotomy and bowel exteriorisation.	IVM: leucocyte adhesion, red cell velocity, vessel diameters, vascular permeability	2.5	Unclear though groups received same volumes	Significant attenuation of: decrease in venular shear rate, leucocyte-endothelial adhesion, vascular permeability. Only permeability was β_2-adrenoceptor sensitive	Pre-treatment alone. Use of β_2–adrenoceptor antagonist would have blocked all circulating catecholamines that have an effect on said receptor. Unable to relate microvascular effects to global haemodynamics. No organ function data to assess clinical meaningfulness of observations.
(272)	Rats	60 mins LPS infusion: E.Coli (026:B6)1.5mg/kg	Blood pressure, plasma levels of purines, ileal tissue content of high energy phosphates	2.5	2ml/kg/h normal saline	Normotensive model. Significantly less plasma uric acid and hypoxanthine while significantly greater levels of high energy phosphates in intestinal tissue compared to untreated group.	Study stopped after one hour of dopexamine – uncertain whether longer-term infusion of dopexamine sustains same effects. Uncertain how findings relate to microvascular perfusion and cardiac output. No organ function data therefore no translatability.
(274)	Pigs	Midline laparotomy, 120 mins aortic cross-clamp, 120 mins reperfusion	Cardiac output, blood pressure, plasma levels of malondialdehyde (MDA)	2 & 8	5-10ml/kg/h Ringer's lactate plus colloid boluses for CVP>4mmHg	2µg DPX vs. control: significantly greater CO and significantly less circulating MDA (product of lipid peroxidation during) reperfusion	One dose out of range of interest.Cardiac output not controlled for. No data on translation to clinically relevant parameters e.g. did significantly less lipid peroxidation result in significantly less organ dysfunction? Only one free-radical measured
(277)	Rats	45 mins LPS infusion: 8mg/h E.Coli (0127:B8). Small laparotomy for bladder catheterisation.	Blood pressure, urine output, GFR	1	1.5ml/h 3%creatinine in normal saline	Dopexamine prevents hypotension and preserves urine output and GFR	Renal function not measured (urine output and GFR are not reliable markers of renal function). GFR calculated from creatinine clearance. Control groups and treatment groups differ significantly in MAP. No mechanistic insight possible.
(278)	Rats	60 mins bacteraemia: 4x10^9 E.Coli (06K 13H)/h. Small laparotomy for bladder catheterisation.	Blood pressure, urine output, GFR, sodium excretion, fractional urinary excretion of sodium	1	3 ml/h normal saline	Better preserved MAP, GFR, urine flow compared to controls. Greater absolute and fractional urinary sodium excretion compared to controls	Performed in conscious animals – most other studies in anaesthetised. Short-term model (60 mins bacteria, 80 mins dopexamine). Significant difference in MAP between dopexamine and controls. Plasma urea and creatinine not measured. No mechanistic insight possible.

Chapter 2 - Methods

2.1 General strategies and experimental design

The basic clinical question that has driven this research is: if dopexamine improves outcomes from major surgery, how is this achieved? To design a set of studies to investigate this problem therefore requires some understanding of the issue of post-surgical morbidity and mortality, as clearly any model employed to study this must incorporate key features of the problem for it to hold some clinical relevance.

Predicting surgical morbidity and mortality in the surgical population is not straightforward. Prediction tools such as those produced by Goldman, Lee, Copeland and Gupta only provide estimates of either the risk of major cardiac events or morbidity and mortality (279-282). Nevertheless they suggest that surgical morbidity and mortality is influenced by two main elements:

1. The nature of the patient:
   a. Age, functional status, presence and severity of any co-morbidities
2. The nature of the surgery:
   a. The magnitude of and anatomical location of the surgical insult
   b. The magnitude of peri-operative adversity e.g. haemorrhage, peritoneal soiling, emergency vs elective surgery

UK-based retrospective studies show that in 88% of general surgical patients the risk of death is low (approximately 0.42%). However for the remaining 12% of surgical patients who are significantly older and undergo a significantly greater proportion of emergency procedures, the mortality is 29-30 fold greater, accounting for approximately 84% of all surgical deaths (283). Importantly, hospital length of stay (a surrogate for post-operative complications) is significantly more in this latter group. Furthermore the occurrence of even a single post-operative complication has been shown to deleteriously affect long-term survival

(284). Surgery and either recovery or complications and mortality are therefore best conceptualised as a temporal continuum, with determinants of where a patient falls on this continuum being the magnitude of encountered inflammation (whether traumatic, infective or pre-existing disease severity). The other important feature to note regarding major surgery and complications is that the patient finds his way to recovery, chronicity, multi-organ failure or death over a number of days or weeks.

The strategy for experimental design was therefore to encapsulate these key features of the clinical problem in a reproducible and inexpensive model. Unfortunately limitations of time and expense are a reality in determining model design. Therefore using aged animals, animals that had, for example, renal failure or heart failure experimentally induced, or using a long-term model where recovery, complications and mortality could be assessed over several days was not possible. Unfortunately this led to the use of healthy young animals which does not tally with the nature of the high risk surgical population.

The key elements of the model that were used were:

1. A rodent based model – ease of handling and surgery, inexpensive, ethically more sound than using larger mammals

2. Laparotomy – essential to simulate major surgery, essential to allow placement of various monitoring kits (which in itself further contributed to surgical stress). Intra-abdominal surgery in particular is associated with high risks of morbidity and mortality when compared to, say, knee or hip surgery (282).

3. The use of high dose LPS to
   a. Provide an inflammatory stimulus in addition to the surgery. This would compensate partially for the reduced inflammatory burden of young healthy animals (as opposed to aged, chronically diseased animals), but more importantly provide a potent and accelerated inflammatory stimulus to mimic the inflammatory milieu seen in post-operative patients, particularly those who

experience complications (285-287). The accelerated nature of the stimulus is key to producing a model which can rapidly simulate the complications which usually occur over days/weeks post-operatively, and which evidence shows is associated with endotoxaemia (288, 289)

b. Furthermore act as a model of sepsis and/or SIRS (290), which would additionally serve to mimic the clinical scenario of emergency abdominal surgery for peritonitis or ischaemic bowel

c. Reproducibly produce consistent levels of organ dysfunction (as opposed to caecal ligation and puncture models – see below)

2.1.1 *Consideration of the surgical model*

To model the scenario of major surgery with a significant inflammatory/septic element a rodent model involving significant surgical instrumentation of the abdomen followed by iv delivery of *Escherichia Coli* endotoxin (serotype 0111:B4) is used. Specifically there is laparotomy followed by bowel exteriorisation, blunt dissection down to the infra-renal aorta, then opening of a small section of ileal lumen to the outside (Figure 2.1). This section of the ileum remains outside the abdomen for the duration of the experiment though it is encased in an air-tight barrier most of the time. As the surgery typically lasts one hour and involves significant bowel handling and intra-peritoneal exposure it is clear that the model provides for a reasonable approximation to human surgery involving the gut. Areas of inconsistency when comparing with elective human surgery and modern peri-operative protocols are:

- there is less stringent control of surgical asepsis (lack of skin preparation, sterile gloves, sterile drapes, sterile surgical instruments)
- anaesthesia in this model is quite different to that usually administered in either theatre settings or in intensive care units (intra-peritoneal (ip) sodium thiopentone as

opposed to combination intra-venous (iv) opiate and benzodiazepine/propofol or combination iv opiate and volatile anaesthetic)

- use of Normal Saline as crystalloid fluid as opposed to a balanced salt solution
- the lack of close control of blood sugar

LDF probe (mucosal)
LDF probe (serosal)

Carotid arterial Line

Ileum

Jugular venous line

Mesentery

Doppler US flow monitor

Tracheal tube

Abdominal aorta

Renal arteries
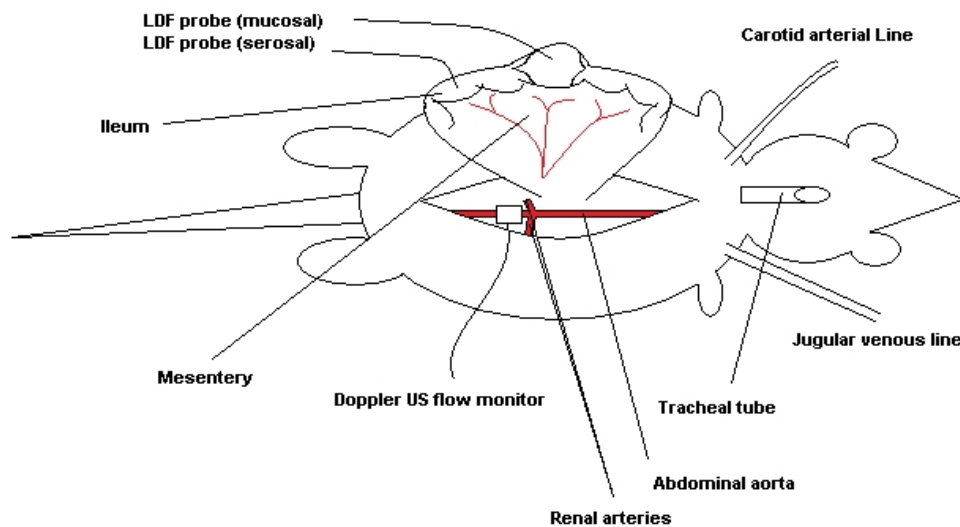
Figure 2.1 – view of surgical interventions in *in vivo* studies.

Laparotomy, bowel exteriorisation and opening of luminal surface of ileum, and also dissection to infra-renal aorta was conducted. Although monitoring modalities varied slightly between studies, abdominal surgery always involved bowel exteriorisation.

2.1.2 Considerations and critique of the use of endotoxins

A recent international study highlights the importance of Gram negative organisms in causing serious infection in the critically ill. 58.5% of patients with any infection had undergone some form of surgery. 62% of positive isolates in infected patients were due to Gram negative organisms, most commonly *Pseudomonas* species and *Escherichia Coli* (291). Furthermore Gram negative pathogens are particularly associated with surgery anywhere along the gastrointestinal tract from the duodenum through the biliary tree to the

rectum (292). Lipopolysaccharide (LPS), is a breakdown product from the cell wall of Gram negative bacteria (Figure 2.2).
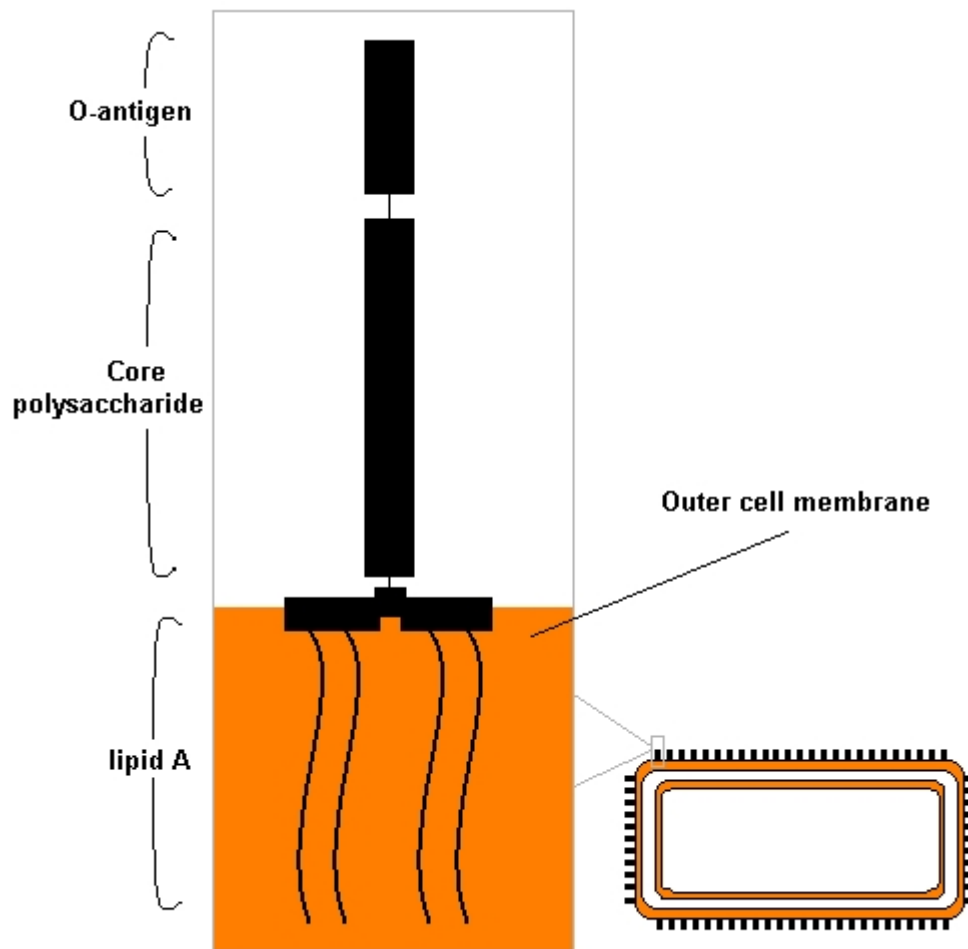
Figure 2.2   The structure of lipopolysaccharide (LPS)

LPS is embedded in the outer cell membrane of Gram negative bacteria (right)

It is not known exactly what causes an infection in one patient to remain relatively quiescent while in another progressing to sepsis, severe sepsis or indeed septic shock. However in the case of Gram negative infections, one likely trigger is LPS, released from bacteria and recognised by pattern recognising receptors on the surface of host cells (293). This leads to a vigorous and widespread cellular response that is clinically indistinguishable from sepsis (293, 294). While unlikely to be the sole determinant of the progress of a Gram negative

infection, the importance of this mechanism is undoubted. Indeed this mechanism, through the translocation of bacteria from the lumen of the gut, may be important in the pathogenesis of peri-operative sepsis and is targeted in critically ill patients with selective digestive decontamination (295-297).

The biological basis for modelling human sepsis with this endotoxin should be apparent, and indeed it is a commonly used laboratory technique. By varying the dose of LPS different stages and severities (warm vs. cold shock) of sepsis can be modelled. Endotoxaemia reproducibly produces both the desired septic phenotype and induces organ dysfunction. I have chosen a dose of LPS designed to mimic normotensive sepsis as would be expected to be the phenotype early on in surgical patients.

Although the haemodynamic, microvascular, metabolic, cellular and immune responses to a challenge of LPS are similar to human sepsis, there are several important points regarding endotoxin based models of sepsis that must be noted:

- Without the presence of microbes, endotoxaemia by definition is not sepsis

- Infection in humans are far more complex entities than endotoxaemia as the presence of whole bacteria, often of varied classes, produces a more complex host response to infection which also changes with time

- Human sepsis usually follows a course of days to weeks rather than hours, allowing for additional factors such as LPS desensitisation to occur

- The fact that organ dysfunction in humans manifests more slowly than in endotoxaemia models suggests a dissimilarity of pathophysiology

- The kinetics of LPS entry in to the circulation from any infective focus in a human is likely to be strikingly different to that modelled by a single iv injection or even infusion of LPS

- There are many different LPS moieties (see Figure 2.2) for each Gram negative bacteria and each can produce a characteristic septic phenotype (298). Modelling

Gram negative sepsis with a single LPS serotype therefore is unlikely to be truly representative of the human picture

- There is a discrepancy between the levels of endotoxin measured in LPS based models and those found in studies of human sepsis. Similarly the levels and patterns of cytokine release when comparing models with human sepsis are different

- The weaknesses of the endotoxaemia model are most strikingly apparent in that those therapies that appear to be of benefit in these models either produce no effects or worsen mortality in studies with humans and in other models of sepsis where live bacteria are used

- Not all features of rodent endotoxaemia mimic human sepsis. For example hypoglycaemia is common in rodent endotoxaemia whereas hyperglycaemia is a more common finding in septic patients

- Species exhibit diverse sensitivities to LPS often of the difference of orders of magnitude. Such is the case when comparing rodents (relatively insensitive to LPS) with humans (highly sensitive to LPS)

- LPS results in intracellular signalling following binding with the pattern recognition receptor TLR 4 (188). Although this seems to be an important aspect of sepsis and post-surgical complications, it is only one of several pathways in the inflammatory signalling network (299). Therefore one criticism of the model, which partly explains the weaknesses described in the foregoing points, is that it relies on an over-stimulation of a single pathway to produce the effect that is seen only after a complex activation of several pathways in a large network.

- There are alternative models which could have been selected to produce a septic phenotype with, arguably, greater clinical validity. These include the models of faecal peritonitis (either caecal ligation and puncture, colon ascends stent peritonitis, inoculation of intraperitoneal autologous faeces), or the administration of live

bacteria. These models have been reviewed extensively elsewhere (290, 300, 301), but are briefly discussed below.

To improve the generalizability of findings, in the third study (intravital microscopy), a combination of lipopolysaccharide and peptidoglycan (PepG) was used. PepG is a breakdown product found in great abundance in the cell wall of Gram positive bacteria, and to a lesser extent in the cell wall of Gram negative bacteria. LPS and PepG are sensed in a host by different pattern recognition receptors (TLR 4 and TLR 2, respectively). The combination of these two stimuli results in a synergistic response from the host though. Although the use of PepG doesn't strictly make the model a sepsis model (microbes are missing), it does make it a better model for polymicrobial sepsis than LPS alone and its effects are based on the activation of two pathways as opposed to one. It is important to note that despite the stimulation of two distinct pathways, the argument of overstimulation of only a very narrow set of signalling pathways out of a network phenomenon still holds.

This may leave one to question why the use of LPS/LPS-PepG as opposed to another, possibly more clinically relevant model. This returns us to some minor theoretical issues and several practical issues. The most clinically relevant animal models in the context of the problem being studied are that of laparotomy for abdominal sepsis – this is not because the interest is in post-operative sepsis *per se*, but because in clinical medicine abdominal sepsis requiring surgery is highly likely to result in post-operative complications. These animal models of sepsis are also much closer to that of the clinical reality as they are recovery-based models, they usually require the use of antibiotics, and they require significant bowel handling and are strongly associated with a bloodstream polymicrobial sepsis. However they possess issues with regards to the reproducibility of organ injury, the reproducibility of degree of peritonitis (being strongly dependent on the length of colon ligated, number of colonic punctures and so on) (290, 301). Furthermore there are practical issues that precluded use in these studies – the completion of one experiment would have taken up to several days due to the nature of the model, incurring significant financial and time-related

costs. Significant faecal contamination of the peritoneal cavity may have made intravital microscopy much more difficult, resulting in an increased loss of animals due to unsuitability for observation (and therefore also an additional cost). Finally and just as importantly, the license permitting practice on such models of sepsis was not held by our institution and is in fact only held by one institution in the UK.

Other than the above, it should also be noted that the model used is 4 or 5 h in duration. This is not a long enough period to assess the effects of endotoxin and treatment on later stage organ and cellular functions. Furthermore the animals used are healthy, young and of one single breed whereas the patients I am modelling are often aged, are carrying co-morbidities and are genetically diverse. Therefore it is important to realise that neither the LPS nor any other model is a perfect match of post-operative sepsis and/or complications. LPS with laparotomy is just one model, with its own limitations, but also its own strengths.

2.2   Flow Cytometry for measurement of leucocyte integrins

Flow cytometry is a powerful and well established technique designed for performing measurements on cells (or other particles) as they pass single file in a flow system past a point of measurement. Light is focused on the cells at the point of measurement and identification of specific cell populations can be made by measuring fluorescence and the scattered light. The versatility of flow cytometry lies in its ability to measure several parameters on thousands of individual cells in a short period of time.

The sample must first be prepared for use in the cytometer in order to produce a suspension of single cells that will flow through the system without clogging up the tubes. In the case of white cells they are vastly outnumbered in the blood stream by erythrocytes. For this reason sample preparation involves lysing erythrocytes, washing out the ghosts then fixing the white cell subset. Just prior to lysing, the cells can be labelled with a fluorescent antibody of interest – in this case fluorescent antibodies against CD11a and CD11b.

Following isolation from other components of blood such as erythrocytes, platelets, plasma proteins and so on, identification of the cellular subsets must be performed. The cells are aspirated from the tube in which they have been prepared and are passed in a hydrodynamically controlled stream passed a beam of laser light. The cells scatter the light in various directions and this is detected. The scattered light gives information on the size/volume of the cells and on its contents (e.g. nature of nuclei). By plotting the side scattered light against forward scattered light (i.e. scattered light in perpendicular orientations), it is possible to identify the subset of interest. Once identified this area of interest is gated (by circumscribing the cells on a plot) and the gate is stored on a template for use later by a computer programme. In this way tests on different samples at different times can reliably look for the exact same cell population by using the stored gate from the initial setup. For statistical significance at least 10,000 counts must be made in the gated area (Figure 2.3).
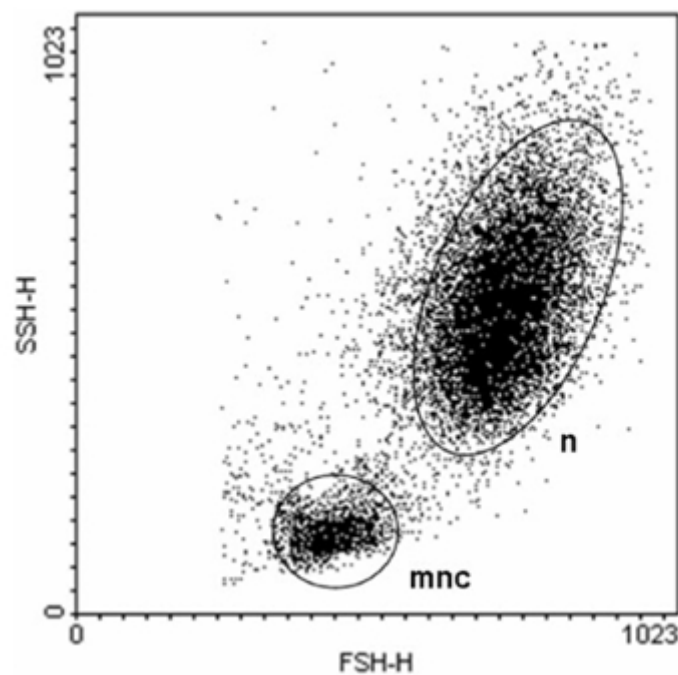
Figure 2.3   Example of a cytogram

This is a cytogram of forward vs. side scatter for a population of leucocytes. As different cells characteristically fall in different areas of the cytogram, a gate can be fixed around the cells of interest. Neutrophils and monocytes have been gated in this paper from our laboratory (302), (with permission). The same parameters were utilised for gating neutrophils in this thesis.

83

A histogram plotting measured fluorescent intensity (logarithmic scale) against counts (of fluorescent intensity - linear scale) is used to determine the median fluorescent intensity. Graphing is automated by computer software, but determination of median fluorescence is somewhat subjective. To determine the median fluorescent intensity of integrins on neutrophils in the bloodstream at a given time point, several measurements must be made. For any integrin on any one sample of blood 3 measurements are made, one on an unadulterated sample, one with an isotype control, and one with the antibody to the integrin of interest (see Figure 2.4). An unadulterated sample is used as all cells auto-fluoresce to some extent and pattern needs to be looked at to ensure there is no abnormal fluorescence in the region of interest.

Similarly an isotype control antibody is used to assess fluorescence due to non-specific binding to other cellular structures (from the constant Fc region of the antibody). This is because the antibodies to CD11a and CD11b are generated as IgG_2 type molecules. While the variable (antigen detecting regions) attach to the integrins of interest, the Fc region of the IgG may also interact non-specifically with other proteins in the sample. For this reason an identically conjugated antibody with an identical Fc region is used to quantify non-specific binding by the antibody to CD11a (or CD11b). This also serves a second useful purpose in demonstrating the specificity of the antibody against CD11a and CD11b. At any time (e.g. baseline) the fluorescence due to binding from, for example, CD11a is expressed as median fluorescent intensity (MFI) and is therefore given by:

$$\text{MFI (CD11a)} = \text{MFI (CD11a}_{Ab}) - \text{MFI (CD11a}_{\text{isotype control}})$$
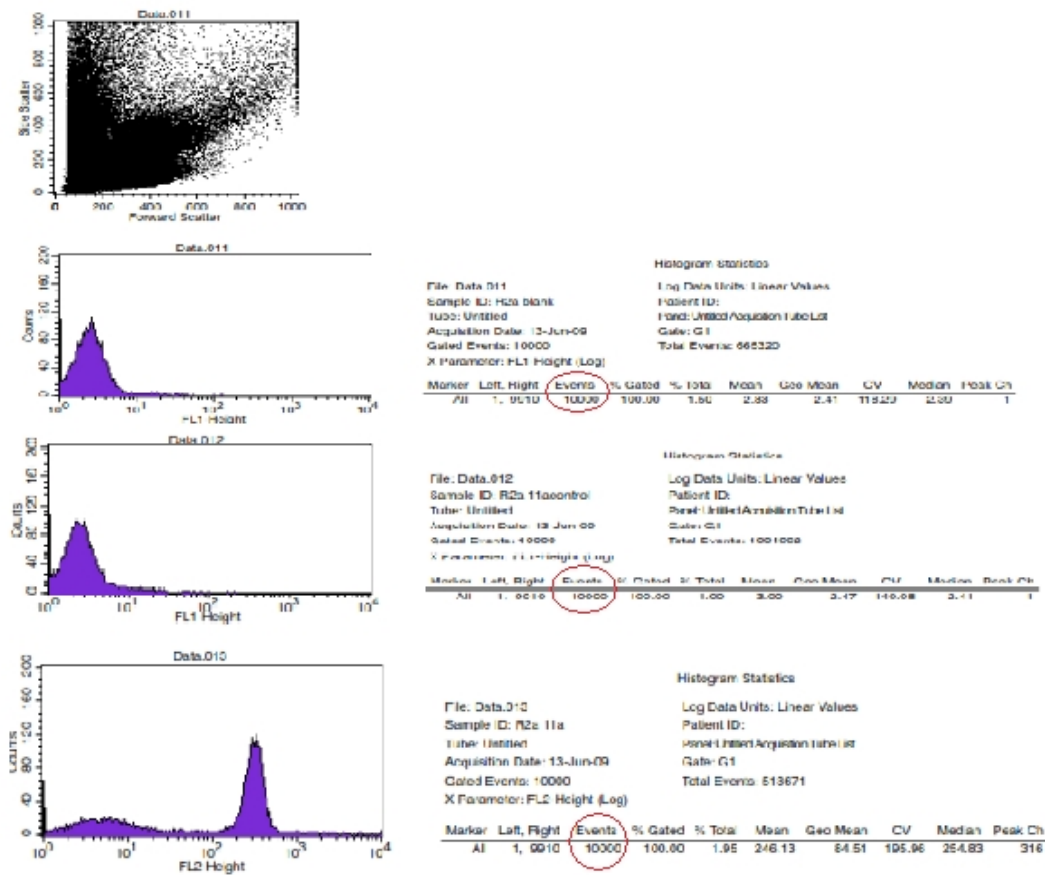
Figure 2.4   Typical cytograms and histograms for neutrophil CD11a pre-LPS

The diagram above shows a cytogram (from the template) and three histograms (with textual data) corresponding to a blood sample taken at baseline from a rat, and treated in three different ways. The numbers circled red show 10,000 counts have been made in the gate. The upper histogram is the fluorescent pattern for unstained blood, whereas the intermediate histogram is for blood stained with an isotype control antibody to CD11a. These should be similar and have low fluorescence. The lower histogram also demonstrates fluorescence for blood from the same sample but treated with an antibody to CD11a. The rightward peak represents (high) fluorescence due to the antibody. The software generates a median for the entire distribution (including the leftward peak). Subjectivity enters when a marker is drawn by the operator across the lower histogram to isolate the peaks from one another (and a new corrected median is calculated).

85
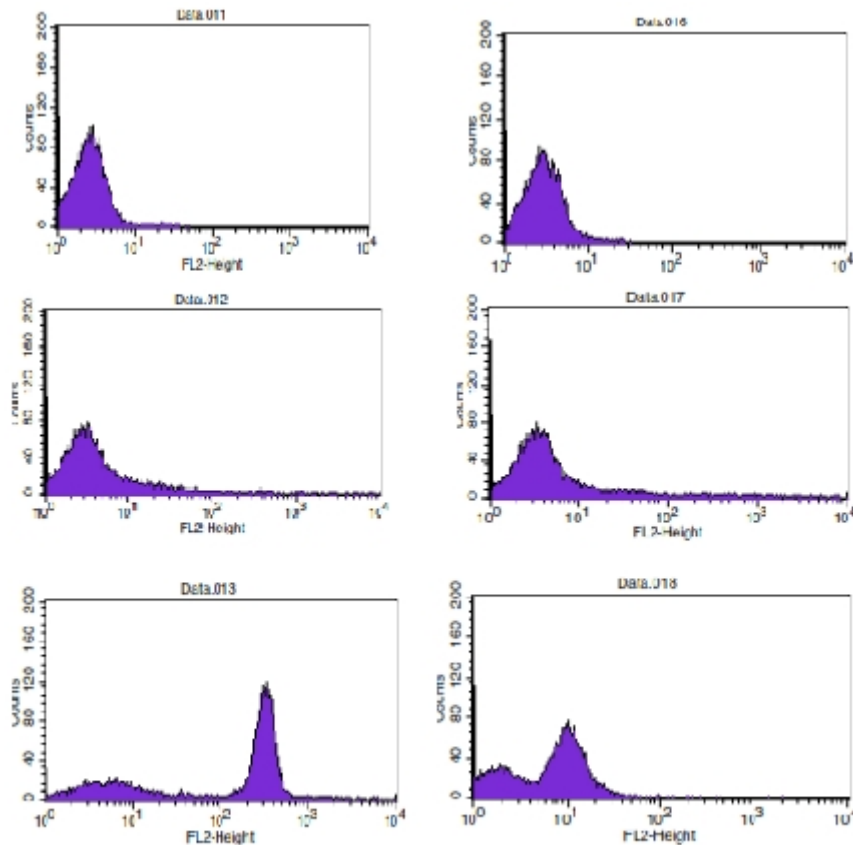
Figure 2.5   Typical histograms for neutrophil CD11a pre- and post-LPS

The distributions in the left column (upper to lower) represent those for blank, isotype control and CD11a at baseline for one rat rendered septic with LPS. Those on the right similarly represent the distributions for identically treated samples following 4 h of sepsis. Looking at the two lower distributions, it is obvious that the there are fewer CD11a positive fluorescent events at the end of the experiment. The distribution on the bottom right also demonstrates how subjectivity can enter the measurement of MFI, as a marker would need to be drawn to separate the peaks (this is clearly less straightforward in the bottom right distribution than the bottom left)

CD11a MFI at the end of the experiment is calculated the same way. Comparing the MFI at

baseline and end of the experiment allows us to understand what is happening to the

number of surface markers on the surface of the cell with time. The distributions in Figure

2.5 (above) demonstrate this visually for CD11a, and were generated for a rat rendered

septic by iv injection of LPS.

2.3 Measuring neutrophil infiltration by measuring myeloperoxidase (MPO) activity

On phagocytosing pathogens such as bacteria, neutrophils undergo respiratory burst (so called due to the great increase their oxygen consumption) where they generate via the action of NADPH oxidase the superoxide ion. This ion is rapidly converted by superoxide dismutase to hydrogen peroxide through the Haber-Weiss mechanism. In the presence of halide ions myeloperoxidase (MPO) catalyses the conversion of hydrogen peroxide to the corresponding hypohalous acid (*in vivo* hypochlorous acid). This acid is an important anti-bacterial mechanism (303). MPO is also released by neutrophils in to the extracellular environment on degranulation where it performs the same task but may also cause unintended damage to host tissue.

MPO is not unique to neutrophils as it is also found in the granules of other polymorphonuclear leucocytes, the lysosomes of monocytes and even in the endothelial cells around sites of inflammation. Although MPO is lost from monocytes on conversion to macrophages, macrophages can come to contain MPO if they pinocytose it from the environment or on ingesting neutrophils (304). However, the MPO content of monocytes is less than that of neutrophils and neutrophils are numerically the largest population of these cells. Indeed myeloperoxidase constitutes 2-5% of the dry mass of neutrophils and constitutes the most abundant inflammatory enzyme within neutrophil azurophilic granules (303, 305). For this reason tissue MPO measurement is commonly used to determine neutrophil infiltration in to tissue (211, 304, 306, 307).

In this study I wished to measure neutrophil infiltration in to the lung. Although this is highly vascular tissue, this was chosen as the pulmonary bed is subject to an intense early neutrophil infiltration, making it a good tissue bed to look for infiltrative changes related to therapy in relatively short-term models.

Organs were harvested en-bloc and doused in ice cold saline before being snap frozen in liquid nitrogen. Samples were sent to colleagues in a laboratory in Italy who performed the measurements of MPO activity. The process is as follows:

Following thawing of tissue samples, MPO must be retrieved from its intracellular location by homogenising the sample and using hexadecyltrimethyl-ammonium bromide to solubilise the enzyme. Centrifugation follows and the soluble enzyme is extracted in the supernatant. Then in order to measure MPO activity it is added to a fixed quantity of hydrogen peroxide in the presence of a tetramethylbenzidine (TMB). This re-creates the conditions in which MPO catalyses the production of hypochlorous acid (though in this case the bromide replaces the chloride). The change in absorbance of light at 650nm is measured spectrophotometrically at 37C and from this MPO activity is measured.

What information we can derive from this technique should be briefly commented on as there are some limitations to it. It is worth clarifying the following:

1. When MPO is measured in homogenates of tissue samples, strictly speaking this represents the total of intracellular immune cell, endothelial cell and extracellular MPO from the sample. It is an extrapolation to say this represents neutrophil infiltration.

2. Measuring MPO enzyme activity is not necessarily the same as measuring MPO enzyme quantity - two tissue samples may have different activities despite the same overall content of MPO due to inter-individual variation in activity and also the variable presence of inhibitors of MPO

3. Measurement of MPO (amount or activity) alone cannot distinguish between MPO in activated and quiescent immune cells – therefore measuring MPO activity in isolation cannot strictly be used to comment on immune cell activation state

4. The reagents used (such as TMB) are not specific for MPO as they can also detect other peroxidases and are affected by naturally occurring inhibitors – therefore false positive results can arise, even when MPO null mice have been used (305)

Nevertheless the technique is both useful and powerful if it is not over-interpreted. When changes in MPO activity follow changes in factors that should determine the inflammatory response (e.g. therapeutic intervention or severity of insult) it becomes more certain that this is tracking inflammation and immune cell responses, and is not a response to confounding variables (308). Under these circumstances, and particularly when confidence intervals are small (reflecting similar intra-group behaviour) and there is additional supportive evidence, we can be quite confident that any changes seen reflect a true difference in neutrophil infiltration in to the tissue sample .

2.4   *Measurement of plasma cytokines*

The study of cytokine responses has been revolutionised by the recent introduction of fluorescent bead-based technologies which allow the measurement of multiple (up to 100) proteins from samples of as small a volume as 25µL (309). The Luminex 200 multi-well analyser uses flow cytometry technology to read samples that have been appropriately prepared for analysis. The principles of the process are as follows. Different "capture antibodies" specific for individual cytokines are linked to different polystyrene beads with pre-defined spectral properties. This allows each bead to be identified (the individual spectral characteristics are due to the blending of different amounts of red and infra-red fluorescent dyes which have been seeded in to the polystyrene beads). When a sample and the beads are mixed, cytokine specific binding occurs (see Figure 2.6) during incubation. Protein-specific biotinylated detection antibodies are added and bind to the specific pre-captured cytokines. Streptavidin (which binds with high affinity to biotin) conjugated to the fluorescent protein R-Phycoerythrin is added and binds to the biotinylated detection antibody, forming a 4-unit complex structure. When the sample is placed in a Luminex reader, correlating the spectral properties of different beads and the amount of R-Phycoerythrin fluorescence allows the software to determine specific cytokine concentrations in the sample. These tests on samples are run in parallel with control samples, and standards containing proteins of known concentration.
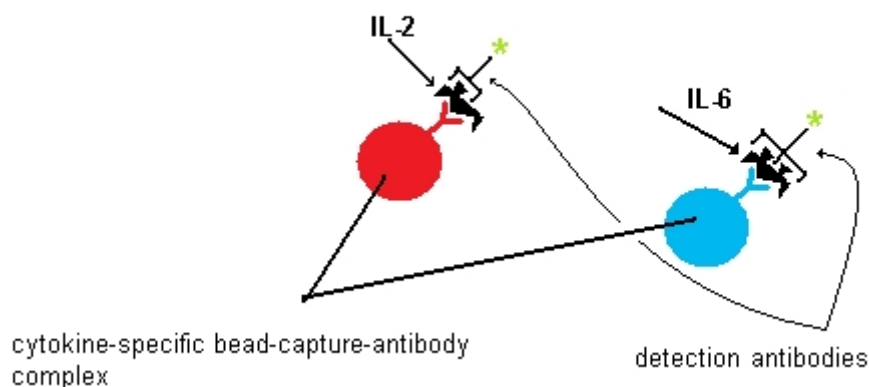
Figure 2.6   Examples of bead-capture-antibody complexes

Schematic of how the bead-capture-antibody complex binds to cytokines (Il-2 and IL-6 as examples), and how biotinylated detection antibodies bind to Streptavidin-conjugated R-Phycoerythrin (green asterisks)

Although it is possible to custom design a panel to measure any cytokines of interest, pre-manufactured kits are available which usually include the cytokines of interest and have been pre-assessed to ensure signals are specific. In this case a rat 10-plex cytokine kit manufactured by Invitrogen (specifically for use in Luminex 200 readers) was used which was designed to measure IL-1α and -β, IL-2, IL-4, IL-6, IL-10, IL-12 (p40/p70), TNF-α, IFN-γ and GM-CSF. The benefit of using pre-designed kits is that the manufacturer has selected an array of bead combinations which minimises spectral overlap and therefore maximises the specificity of fluorescence.

Specific details of sample preparation are included in the relevant experimental chapter.

2.5   Arterial blood gas and lactate measurement

Arterial blood gas measurement was performed using the ABL77 Radiometer (Copenhagen). This machine utilises a cassette containing a miniaturised measuring chamber and several microsensors to measure the partial pressure of oxygen, carbon dioxide, pH, and haematocrit in the sample. Derived data included plasma bicarbonate and

base excess. The measurement of pH and PCO_2 are through potentiometric means whereas the measurement of PO_2 is by amperometric techniques and haematocrit is by conductance technique.

The principle of the potentiometric method for measuring pH in a test solution relies on the following equation relating the pH and potential difference of a solution of known pH (pH_s) and potential (E_s) to that of the test solution.

$$pH = pHs - \frac{E - Es}{k}$$

The constant k is calculated from the Nernst equation and is related to the change in potential per unit change in pH (310). Two electrodes are immersed in the test solution, one sensitive to H^+ and the other being a reference electrode, and the potential difference between them is measured. The apparatus is temperature sensitive and a buffer solution specific to the manufacture is used for calibration.

Similarly the potentiometric method for measuring PCO_2 in the test sample is essentially a modified pH electrode. However, instead of H^+ ions from the test solution permeating the electrode, CO_2 selectively permeates in to a solution of sodium bicarbonate which encases the electrode. The CO_2 reacts with water (according to the reaction below) to form H^+ ions:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$
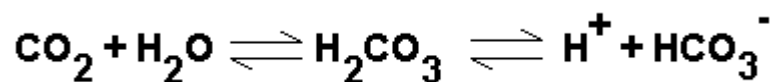
These permeate in to the electrode which is otherwise in the same configuration as a pH electrode – therefore the P_aCO_2 of the test solution can be measured.

The amperometric technique for determining PO_2 in a test sample of blood relies on the principle that dissolved oxygen is proportional to the limiting current when oxygen is reduced by an applied potential across two electrodes immersed in an electrolytic solution (311). The classical Clark electrode utilises a membrane which is highly selective for oxygen and allows

the PO$_2$ in the sample and electrolytic solution to equilibrate. Through this membrane oxygen diffuses from the test sample to an electrolytic solution where a working platinum electrode and a silver/silver chloride reference electrode are sited. Oxygen is reduced at the platinum cathode, resulting in the generation of a current which is measured. These measurement techniques are well established and robust.

Conductivity techniques for measuring haematocrit are based on the principle the conductivity of the test sample is inversely related to the haematocrit. Correction must be made for the electrolyte content of the test sample (and the major electrolytes are measured by the machine using a potentiometric technique).

An important derived parameter is the base excess. The body defends pH within narrow limits and in this regard there are two systems which are useful to offset changes in pH – the respiratory system and the renal system. Both these systems interplay with the bicarbonate buffering system of the blood. By altering alveolar ventilation a change in pH can be offset by altering PCO$_2$ (which in turn alters pH as can be seen from the equation above). Le Chatelier's principle dictates that pH will fall as PCO$_2$ rises, and therefore in circumstances where PCO$_2$ is not normal it becomes difficult to differentiate between respiratory and non-respiratory (metabolic) components of the overall pH. Base excess indicates the contribution of metabolic processes to pH as it refers to the amount of strong acid that must be added to the test sample at 37C in order to normalise the pH to 7.4 if the sample's PCO$_2$ were fixed at 5.3kPa and were fully oxygenated. The base excess is therefore derived by the machine from measured parameters of pH (bicarbonate concentration is derived from pH and PCO$_2$) as:

$$Base\ excess = 0.93 \times \left(\left[HCO_3^- \right] - 24.4 + 14.8 \times (pH - 7.4) \right)$$

When base excess is less than 0 it is termed a base deficit. When base deficit is greater than 2mmol/L (i.e. base excess is less than -2mmol/L) there is said to be an underlying

metabolic acidosis. When pH is normal, the metabolic acidosis has been compensated for by respiratory mechanisms whereas when an acidosis exists the compensation is inadequate. Causes of metabolic acidosis can be divided in to a dysfunction of excretion (e.g. renal failure), an increase in the bodily production of acids (e.g. ketoacidosis, lactic acidosis) or an increase in exogenously administered acids or strong ions (e.g. hyperchloraemia). Other causes also include the excessive loss of alkaline buffers from the body, such as may occur during disorders of the GI tract. In the context of sepsis in previously healthy patients, metabolic acidosis is most commonly attributable to renal failure, shock and hypoperfusion, and very rarely due to severe liver dysfunction.

In the ABL77 an aspiration port is provided through which approximately 70µL of blood is drawn up from the sample. The blood is drawn along in to the measurement chamber where the measurements are performed. Automated calibration of the instrument occurred several times a day and sensor cassettes had expiration dates (following which they were replaced) and were also limited in number of samples they would perform tests on. Failure to calibrate correctly would result in no further tests being permitted by the machine. With respect to pre-analytic errors, the machine wouldn't necessarily detect small air bubbles or clots and could go on to produce erroneous results in these cases. Therefore each test required inspection of the sample in the syringe and the aspirate in the sensor cassette tubing and chamber to ensure the result was reliable.

Although the origin of bloodstream lactate in sepsis is not entirely clear, measurement of lactate has been shown to be useful in human studies as it has been shown to correlate with mortality while a decrease in lactate in response to therapeutic intervention has been shown to correlate with improved outcomes. I elected to measure lactate using the point of care Accutrend Lactate meter (Roche diagnostics). This utilises reflectance photometry to determine the lactate concentration in plasma from a calorimetric lactate-oxidase mediator reaction on a testing strip. The strip has a specific region where the sample of blood is applied (see Figure 2.7). Blood filters through the yellow mesh and then erythrocytes are

separated from plasma at the second glass-fibre layer. Plasma then descends onwards to the detector film where the chemical reaction occurs and over 60s, while the strip is in the analyser, the measurement is performed (312). The measurement range is from 0.8 – 22mmol/L in whole blood and the Accutrend has been validated as both an accurate and reliable machine even approaching the higher end of these concentrations (312-314). Important points to note are that lysis of erythrocytes will tend to overestimate lactate concentration, and that testing a larger volume of blood than recommended can alter the accuracy of the result.
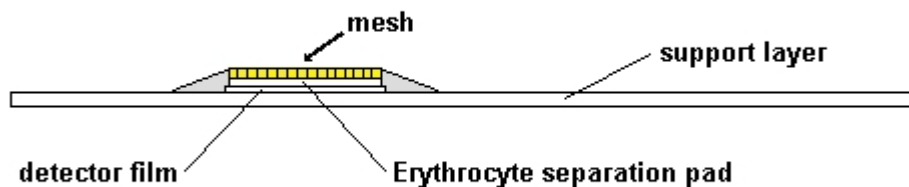
Figure 2.7   Structure of a lactate strip

The mesh region where blood is applied, the erythrocyte separation pad, detector film and support layer are demonstrated

2.6   Measurement of aortic blood flow by transit time ultrasound

Blood flow can be measured in small animals by use of miniature probes that encase the blood vessels of interest. These probes utilise the principle that ultrasonic waves propagated in opposite directions along the same path will take different amounts of time to traverse the path, and the difference in "transit times" depends on the flow rate of blood travelling through the vessel of interest (see Figure 2.8). The mathematical equations that govern this relationship also involve the diameter of the vessel and therefore it is important that the probe selected for use is suitable for the type of vessel it is used on – therefore a flow probe

for the aorta of a small rat may be suitable for use on the hepatic artery of a much larger rat, but the flow probe will be unsuitable if used on the aorta of that larger rat.

To attach the probe a laparotomy had to be performed, the bowel had to be exteriorised (and kept moist to prevent drying) and then dissection of a small portion of the infra-renal aorta from the vena cava and surrounding nervous tissue had to be performed. Once dissection occurred, the probe would be loaded with sonicating gel and carefully hooked around the infra-renal aorta before the latch was fastened. At this point further sonicating gel could easily be applied if the signal was poor. When the animal was turned to its side the signal would occasionally diminish in quality – repeat laparotomy would be unnecessary as gentle manipulation of the probe would usually restore the signal. Variation in probe alignment and position can interfere with the accuracy of the measurements. However, systematic error is minimised by optimising the signal quality indicator (315). Furthermore any random error should distribute evenly between groups (there is no reason to expect the introduction of systematic error in one group and not another).

Use of the blood flow monitor would provide data on aortic blood flow. This obviously excludes blood flow to the brain, coeliac axis and kidneys and most of the upper body of the rat – clearly this is not equivalent to the cardiac output of the rat. Nevertheless this technique has been used as a surrogate marker for cardiac output (relative cardiac output) by others and is widely used for this purpose (316-318), and given that the alternative was a thoracotomy to place the probe elsewhere (pulmonary artery or ascending aorta) which would have necessitated mechanical ventilation, this was deemed more suitable. The probes have been validated as accurate over a range of flows (319).
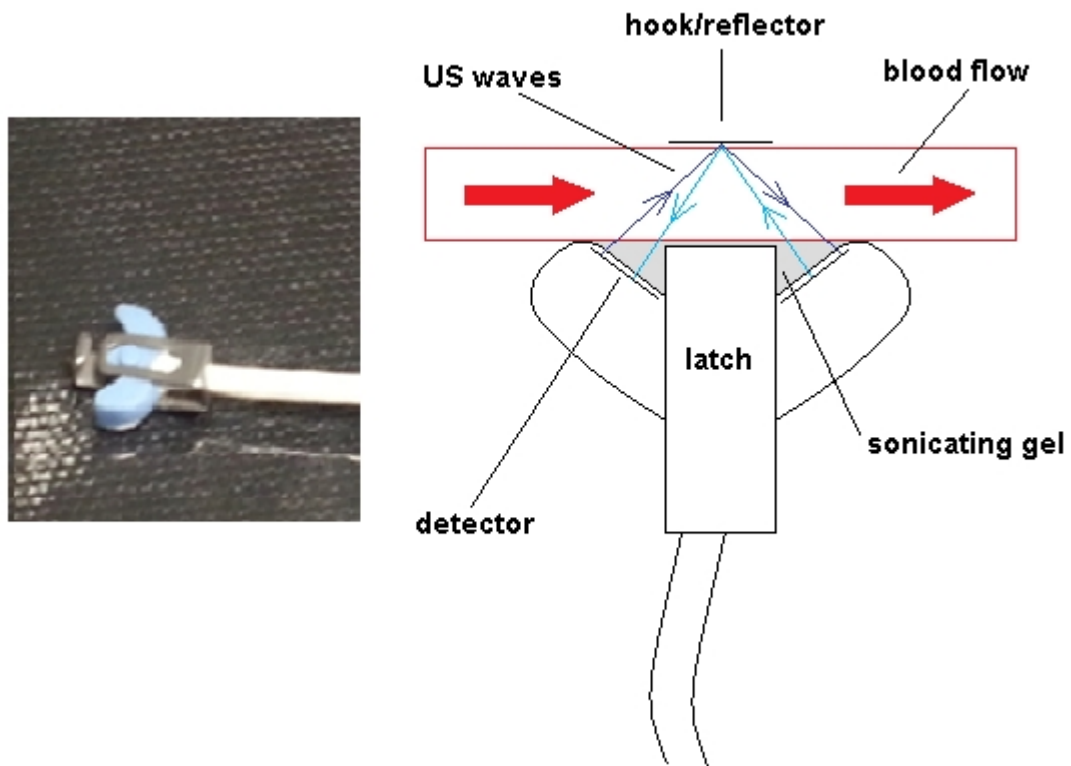
Figure 2.8   Transit time ultrasonic flow probes and their mechanism of action

On the left is one of the ultrasonic aortic flow probes used in the experiments, consisting of a body (light blue) containing two piezoelectric detector/receiver units, a superiorly sited hook/reflector and an inferiorly sited sliding latch. A schematic (right) shows two receiver/detector units (black) angulated to send ultrasonic waves thrugh a blood vessel in opposite directions along the same path length. These waves bounce off the metallic hook at the top which acts as a reflector. Unidirectional flow through the blood vessel (red) will make the transit times differ, and from this measured time difference the processor determines the flow rate. The diagram also demonstrates a grey gap between the blue angled body and the blood vessel – this is filled with a sonicating medium such as sterile gel, ensuring good quality signals can be obtained (see Figure 2.9). The probe is securely fastened around the vessel by gently sliding the latch superiorly.

Figure 2.9   The processing unit for the transit time flow probe

The unit is attached via the blue cable on the bottom left of the unit. The black cable allows output to a desktop computer where the data can be turned in to a graph and stored for retrieval later on (see Figure 2.13). The quality of the signal is displayed on the LED readout (good = 5/5 horizontal bars) – if the probe slipped or sonicating medium dissolved the quality of the signal would fade as a warning.

2.7   Laser Doppler flowmetry

Laser Doppler flowmetry (LDF) is a reasonably well established method of assessing microvascular perfusion in volumes of tissue where measurement is occurring (320). A description of its operation will aid understanding of the scope and limitations of this instrument.

The laser Doppler perfusion monitor comprises fibre optic cables, a monochromatic laser, a photodetector and signal processor. The laser is used to generate light of a single wavelength which is transmitted down an optical fibre to the tissue of interest. The laser light penetrates tissue and interacts with both static and mobile elements of tissue so that some light is scattered in to the tissue and lost while other light is backscattered to a second optical fibre. Backscattered light travels via the optical fibre to a photodetector which generates a current that is processed by the signal processor (320). Some understanding of the physics is useful.

2.7.1 Basic Principles

In Figure 2.10, a corpuscle moving with velocity vector *v* is struck by incident monochromatic laser light represented by wave vector k_i. A scattering vector *q* transforms k_i to the scattered wave vector k_s. In this representation *θ* and *ϕ* are the angles between vectors k_i and k_s, and the Bragg scattering vector *q* and *v*, respectively.

The frequency shift (*Δf*) in k_i brought about through the Doppler effect is expressed as:

$$ f \; = \; \frac{2}{\lambda i} v \sin(\frac{\theta}{2}) \cos(\phi) $$

where *λi* is the wavelength of the incident light.
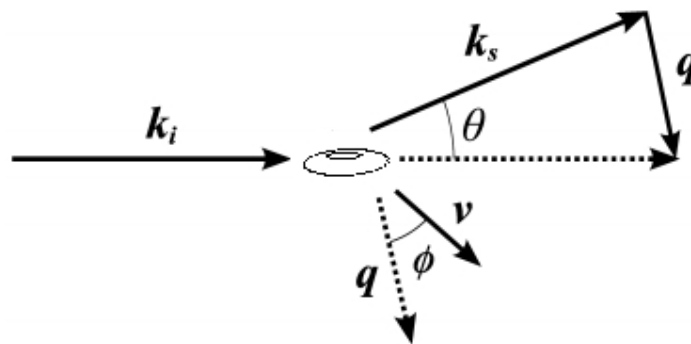
Figure 2.10   Scattering of light by erythrocytes

An erythrocyte moving with velocity vector *v* scatters incident light in a direction that is dependent on the scattering vector *q*

Backscattered light will be a mixture of shifted and unshifted frequencies, the latter arising predominantly from static tissue where *v* is zero. In the simplest case where there is only the

unshifted frequency *f* and one shifted frequency *f* + *Δf*, the summated light wave at the detector will vary in intensity with a frequency of *Δf*: (Figure 2.11):
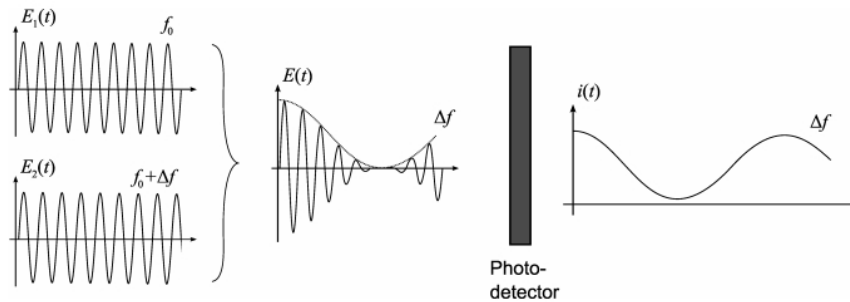
Figure 2.11   Scattering produces a photocurrent at the detector

Two scattered waves, one shifted and one unshifted summate at the photodetector to produce light which varies in intensity at a frequency *Δf*. This generates a photocurrent that also varies with the same frequency.

As the photocurrent (i_t) is proportional to the light intensity it will also vary with frequency *Δf*. When the number of backscattered light waves increases the photocurrent will comprise a spectrum of frequencies corresponding to the Doppler frequencies. In this case the photocurrent is expressed:

$$i(t) = i_{ac}(t) + i_{dc}(t)$$

Where $i_{ac}(t)$ represents the time varying part of the current and $i_{dc}(t)$ the stationary part. Based on the power density spectrum of the photocurrent, *P(ω)*, an estimate of perfusion (defined as being proportional to the mean velocity of red blood cells (RBCs) and the concentration of RBCs) can be made:

$$Perf = \frac{\int_0^\infty \omega P(\omega) d\omega}{i_{dc}(t)^2}$$

where ω is the angular frequency of the photodetector current. The relationship between measured perfusion and actual tissue perfusion is non-linear (due to a widening of the frequency spectrum with greater numbers of RBCs), but for any given concentration of RBCs measured perfusion varies linearly with mean RBC speed. The relationship is also almost linear for low RBC concentrations.

Determinants of perfusion are therefore obviously related to red cell flux, but also to the wavelength of incident light and the optical properties of the tissue being sampled. The volume of tissue being sampled is generally assumed to be <1mm^3 but as this is dependent on tissue properties this is not strictly defined either. Therefore measurements of flux are not absolute, but are relative.

The aim of LDF is to assess perfusion by separating static and dynamic (RBC) components of tissue scattering of incident laser light. Movement of non-RBC tissue (e.g. smooth muscle) introduces non-RBC scattering which is measured as part of the dynamic signal. This motion artefact has been shown to influence LDF signals (320). Another consideration is the temperature dependence of measured perfusion. This arises due to Brownian motion (a temperature dependent phenomenon) of static tissue components and is also responsible for the lack of a biological zero in perfusion signals.

Although the sampling volume of LDF probes is taken as generally <1mm^3, the depth of penetration of the laser is dependent on the wavelength of the light and the separation of emitter and detector fibres. Larger separation distances tend to detect deeper flows and wavelengths in the red and near infra-red region penetrate more deeply than towards green parts of the spectrum. This latter point is important as flux measurements in response to

physiological phenomena such vasodilatation are detected with less sensitivity in red laser flowmetry devices (320).

One of the biggest criticisms of laser Doppler flowmetry is that it provides a crude measure of total flux through the sampling volume. While this is not useless information, it does have some drawbacks. Thus it provides no information on the heterogeneity of flow in a sampled tissue volume, the direction of red cell flow (e.g. oscillatory versus uni-directional flow) and it theoretically cannot distinguish between 100 RBC/s traversing through an arteriolar-venular shunt, 25 RBC/s traversing 4 small venules in a system where 10 should be open, and laminar flow of 10 RBC/s in 10 open venules. Thus for 3 completely different states of tissue perfusion the equipment would generate an equivalent output. For this more detailed type of information more sensitive methods of microvascular monitoring are required.

2.7.2   MoorLAB Laser Doppler Blood Flow Monitor

The LDF device chosen for the purposes of this study was the MoorLAB laser Doppler blood flow monitor (Figure 2.12), in conjunction with slave probes (P10k, Moor Instruments, Axminster, UK) attached to master probes (MP10M200ST, Moor Instruments). Laser light of 780 nm wavelength with a 40Hz sampling rate and a 30˚ angular spread allowed a sampling volume of approximately 1 mm^3. Probes were calibrated daily using PFS flux standard (Moor Instruments, Axminster UK) at 23°C prior to experiments. Amalgamating the haemodynamic and laser Doppler measurements would produce data such as in Figure 2.13.

Figure 2.12   MoorLAB Doppler processing units.

The upper unit is a supplementary unit for an additional probe. The lower unit is the master unit and has a menu that allows the user to switch between probes (as the results can only be displayed for one probe at a time). The probes consist of fibre optic cables which either transmit laser light from the units, or return reflected light back to the units (hence two ports on the left of each unit). The output of the units can be linked to a desktop computer for graphical display, storage and retrieval at a later date (see Figure 2.13).

2.8   Intravital microscopy

Intravital microscopy is a microscopy technique that allows dynamic events to be studied in vivo in real time. The equipment for basic intravital microscopy is similar to that for standard microscopy, but the introduction of the use of fluorescent markers and dyes has increased the sophistication of necessary equipment.

In addition to the equipment, the nature of the specimen places slightly different demands on the way the specimen is catered for compared to standard microscopic techniques. A sufficiently large platform to hold the in vivo specimen is required – this is usually in the form of a modified metallic slab which unfortunately acts as a potent conductor of the specimen's body heat away from the animal. Therefore provision has to be made for maintaining the specimen's core temperature throughout the period of observation. The exposed sample of tissue from the specimen must not be allowed to dry out (and any fluid used to maintain moisture must be removable should it overspill). The tissue must also be held at core temperature, and must be handled in a manner which does not traumatise it. Even following
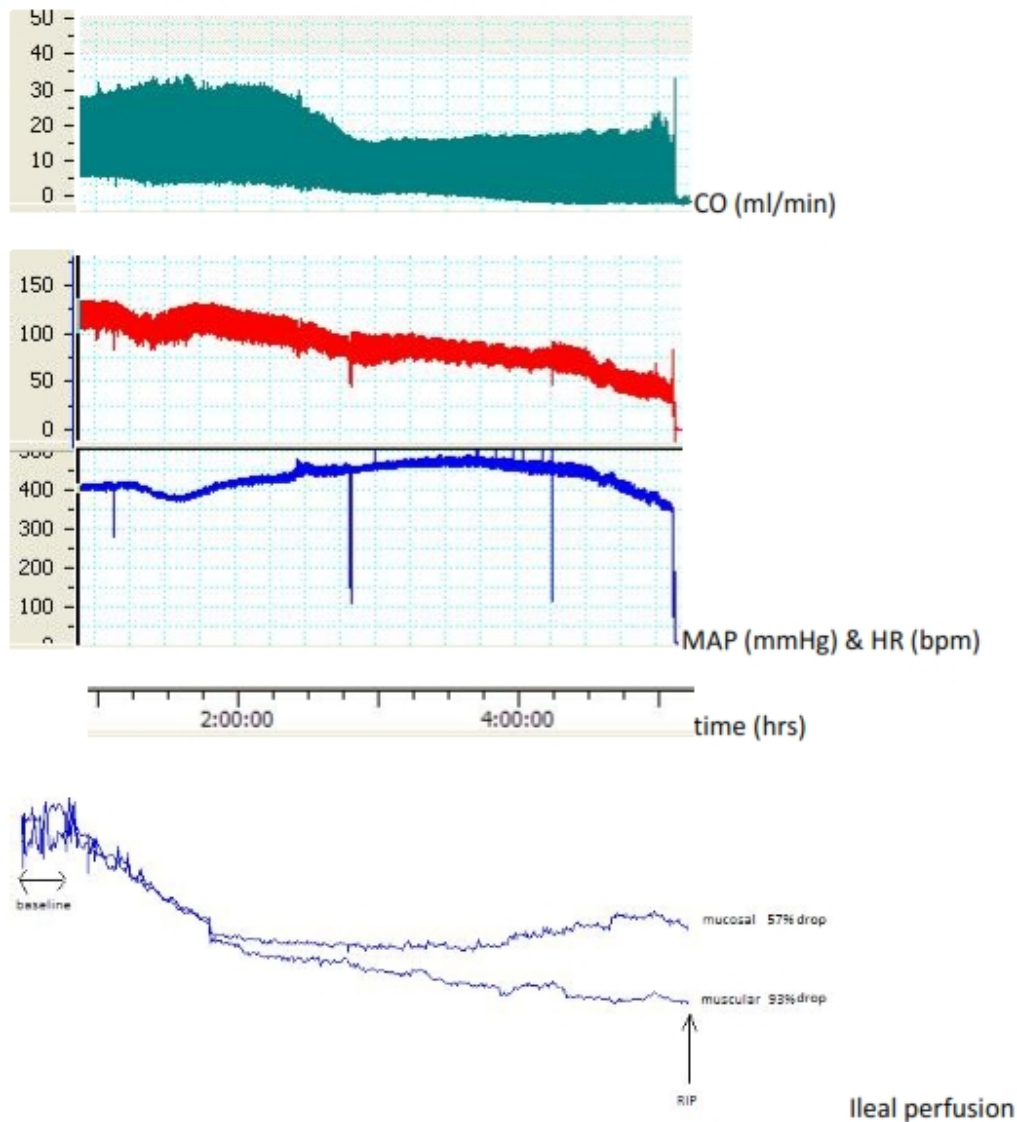
Figure 2.13   Typical haemodynamic and microvascular traces

These were generated simultaneously by a desktop computer from transit time flow probes (uppermost), invasive haemodynamic monitoring (middle 2 graphs) and laser Doppler flux probes (lower) sited on an endotoxaemic rat (preliminary studies only)

atraumatic handling, a period of stabilisation will still be required for the tissue microvessels to return to baseline behaviour. The natural tendency of certain tissues to move e.g. respiratory variation or intestinal peristalsis may need to be countered in order to successfully record observations at high magnification. Finally, when examining the effect of

103

short-acting drugs on the circulation, the drugs must be continually infused during the period of observation.

For all but the briefest of periods of observation these demands are rarely problematic. However the observations required in my studies often led to times of one hour on the microscope stage. In preliminary studies there were significant issues with tissue trauma, drying and animal core temperature (due to the microscope stage acting as a potent conductor of heat away from the rat). In order to combat these issues an in vivo intestinal microscope platform was custom built (by myself) according to a design previously outlined (Figures 2.14 – 2.16) (321).
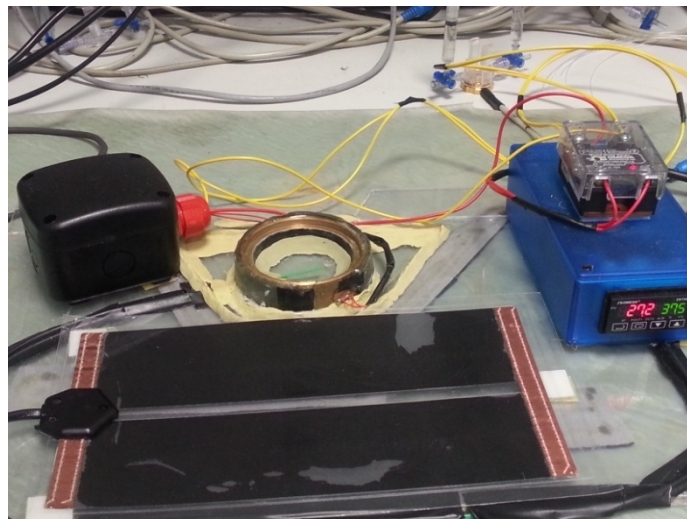
Figure 2.14   The hand-made intravital microscopy platform.

In the foreground is an infra-red heating mat on which the rat would lie. The blue boxed unit on the right contains a thermostatic controller for the foil heater surrounding a bronze cylinder. The cylinder is filled with tap water and covered with a removable circular glass slide. A green thermometer lies in the cylinder and measures the temperature of the tap water as it is heated by the foil heater (through the bronze cylinder). The bowel is placed on the glass slide when the set temperature (green) and measured temperature (red) of the water match.
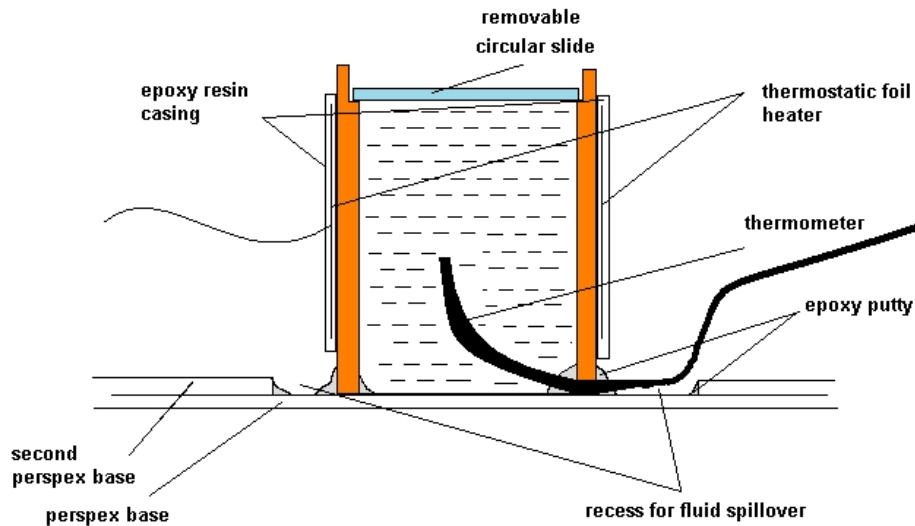
Figure 2.15 Section through the cylindrical portion of the hand-made microscopy platform.

When microscopy occurred irrigating fluid would initially fill the recess between the upper surface of the slide and the top of the cylinder. Excess fluid would spill over in to the epoxy lined recess in the base of the platform and could be suctioned away before it overflowed.
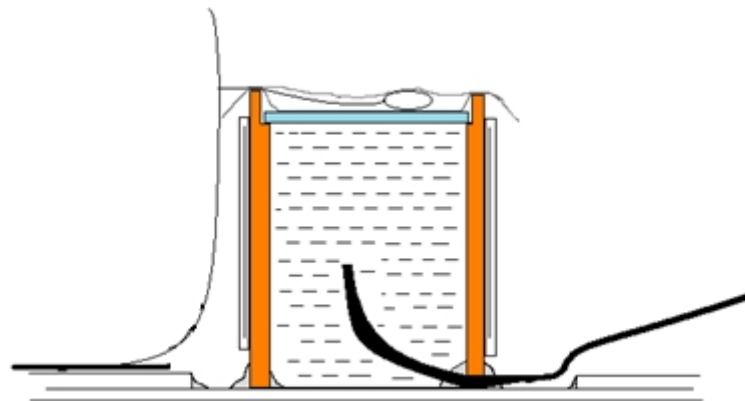
Figure 2.16 Schematic of how rat bowel would lie on the cylindrical stage.

The ventral surface of the rat would face the cylinder and the bowel would be draped over the glass slide which would be covered with Saran wrap. The bowel would be sealed from above with a second layer of Saran wrap to ensure the bowel did not dry out and to maintain microvascular integrity (322). When intravital microscopy was performed, the entire platform could be lifted on to the stage of the microscope, and the superiorly sited Saran wrap would be removed from the bowel so that irrigating fluid could slowly moisten the bowel.

In order to minimise peristalsis, irrigating fluid (warmed normal saline) had a small dose of isoprenaline added to produce a concentration of 0.01µg/ml. This has been used by other investigators in leucocyte rolling/adhesion experiments and has been shown to be below any dose that alters vascular tone (323).

In order to delineate capillaries (for the measurement of functional capillary density) and to better make leucocytes apparent, fluorescent dyes are used which localise to the intravascular compartment (FITC-labelled albumin) or to leucocytes (rhodamine 6G). While these dyes can cause effects that are artefactual (324), studies show that the use of moderate light intensities for short periods of time do not induce additional leucocyte endothelium interaction or alterations in vascular diameters (325). Use of these dyes resulted in images such as shown in Figures 2.17 and 2.18.
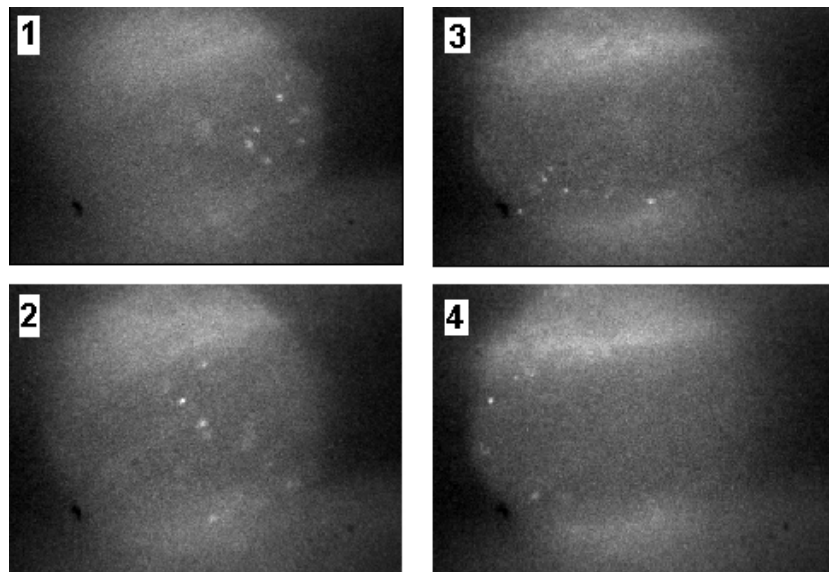
Figure 2.17   Intravital study of leucocyte rolling and adhesion in an intestinal venule from one of the rats.

As the focus is altered slightly, leucocytes appear in different parts of the illuminated circle (1 through 4). By rapidly cycling the focus back and forth over a period of 45s, it is obvious which leucocytes are adherent and which are rolling – this is not obvious from the still images above though.
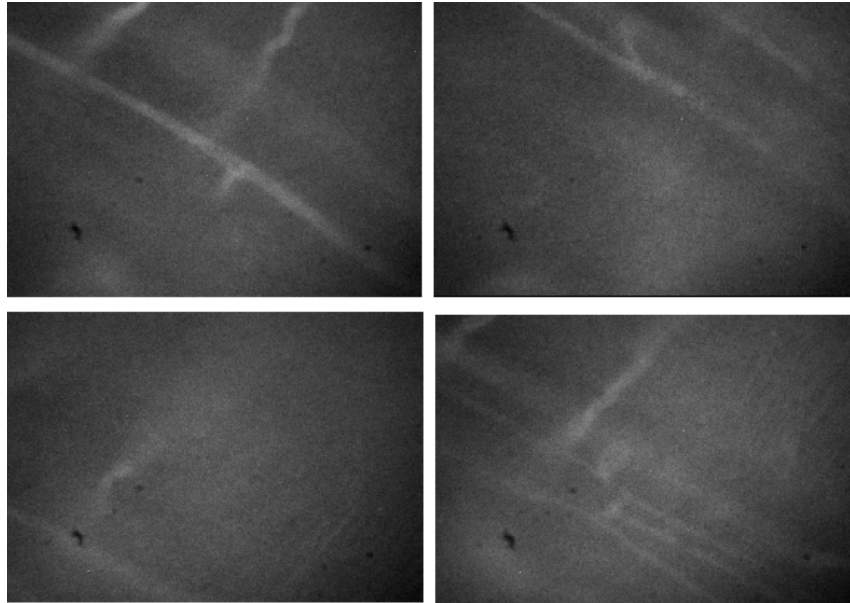
Figure 2.18   Intravital study of capillaries in the intestinal muscularis.

These 4 still images are of the same area, but altering the focus brings different parts of the image and therefore different capillaries in to focus. When played back it is possible to determine the number of vessels in the entire area and to calculate a functional capillary density for the area

Images were recorded for a minimum of 40s, and often for 1 min. These images were stored for offline analysis at a later date by an investigator blinded to the treatment group of each animal. Using the classification of Gore and Bohlen (326) intestinal microvessels were identified and the following measurements were made: diameters of post-capillary (V1 and V3) venules and pre-capillary (A1 and A3) arterioles. For each animal attempts were made to take measurements from a minimum of three different vessels of the same branch order. The number of adherent (n_a) and rolling leucocytes in V1 and V3 venules over a 30s period was observed offline. From these measurements it was also possible to calculate adherent leucocyte density, assuming venules were cylindrical and had an internal area, A, governed by the equation A = $(\pi.d).l$ (where d is the measured diameter of the venule, $(\pi.d)$ gives its circumference and l is the length of the vessel under observation).

The adherent density was then calculated as:

$$\text{Adherent density} = n_a \div A$$

With respect to functional capillary density, recordings from a minimum of three separate areas of intestine were made. In each area capillaries in the circular and longitudinal layer were identified offline. Lengths of individual capillaries of each group (e.g. circular muscle) in the shot were measured using SlideBook 5.0 software. The total length of each type of capillary (L_T) was calculated by adding the measured lengths, and the functional capillary density (fcd) was then calculated as:

$$\text{fcd} = L_T \div A_s$$

where A_s is the area of the screen.

2.9   Wire Myography

Wire myography is a technique used to assess the contractile properties of resistance arteries and has only come to the fore since the 1970s (327, 328). In general, a small chamber forms a volume within which fluid (usually Krebs' solution) bathes a blood vessel under study. The blood vessel is mounted on to two wires attached in a parallel fashion to two jaws in the myography chamber, and changes in vessel tension can be recorded if one jaw is attached to a micrometre screw and the second to an isometric force transducer which measures wall tension. The temperature, oxygenation and carbon dioxide levels in the chamber fluid are maintained to mimic as closely as possible physiological conditions. Vasoactive drugs can be added to the fluid in the chamber to ascertain properties of the vessel by measuring changes in tension at the transducer. Benefits of this technique are that the contractile machinery of the vessel is allowed to perform in its normal orientation (i.e. the vessel remains cylindrical) and the vessel is not affected by any potentially damaging manoeuvres that fix its position such as occur with other techniques.

Once vessels are mounted on to wires, a normalisation procedure is performed to determine the internal diameter of the vessel when fully relaxed under a pressure of 100mmHg or 13.3kPa. Once this internal diameter has been determined, the micrometre is adjusted to this setting as studies demonstrate that rat mesenteric arteries are maximally responsive to vasoactive drugs under these conditions (329). The vessel is then assessed for viability by pre-contraction with a high concentration of extracellular potassium chloride before washing out the chamber and re-filling with fluid. A set of relaxation curves can be obtained following contraction if vasodilators are subsequently added, and from this various calculations (see below and Figure 2.19) can be made.

Handling of myography data: The following measurements were extracted (see Figure 2.19):

- The baseline tension just prior to the addition of the first pre-contracting dose of PE (T_B)

- The tension just prior to the addition of the first dose of ACh (T_M), representing the plateau tension following pre-contraction with the last dose of vasoconstrictor

- The plateau tension for each dose of ACh, measured at the time just prior to the next dose of ACh or SNP (T_{ACh})

- The plateau tension following the addition of SNP (T_{SNP}) – this plateau was easily visible on all myography traces and represented the end of the experiment
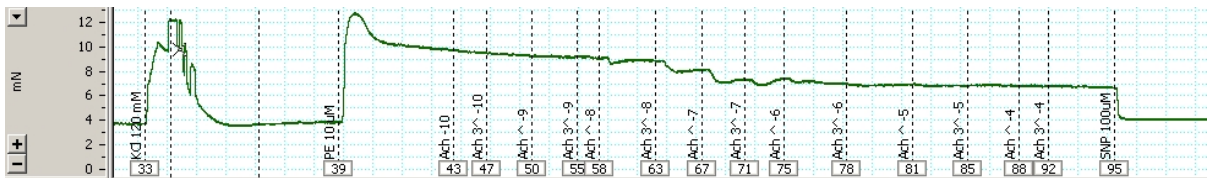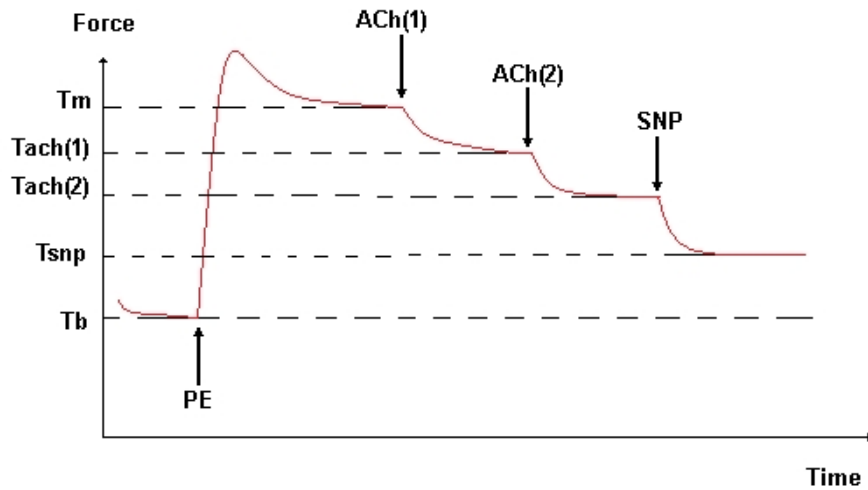
Figure 2.19   Schematic of a myography trace (upper) and a real myography tracing from a control vessel (lower).

In the upper diagram a vessel is pre-contracted with phenylephrine (PE) from a baseline tension (T_b) and plateaus at a tension (T_M). The addition of acetylcholine at increasing doses (ACh(1) and ACh(2)) results in relaxation at two respective plateau tensions ($T_{ACh(1)}$ and $T_{ACh(2)}$). The addition of sodium nitroprusside (SNP) relaxes the vessel maximally at a tension T_{SNP}, which is usually equal to T_b. Similarities should be visible in the lower tracing (39 = addition of PE, 43 – 92 = addition of ACh, 95 = addition of SNP)

The maximum dilator response of the vessel was taken as:

$$T_M - T_{SNP} \qquad (1)$$

whereas the dilator response of a vessel to any given dose of Ach was taken as:

$$T_M - T_{ACh} \qquad (2)$$

110

The percentage decrease in PE-induced tone for each dose of ACh was calculated as:

$$100 \times \left| \frac{T_M - T_{ACh}}{T_M - T_B} \right| \qquad (3)$$

The percentage decrease in PE-induced tone standardised to the maximal vasodilator response of the vessel was calculated as:

$$100 \times \left| \frac{T_M - T_{ACh}}{T_M - T_{SNP}} \right| \qquad (4)$$

The maximal endothelium-dependent vasodilator response was usually taken as the result of equation 4 for the highest dose of ACh. Occasionally a lower dose would produce maximal dilatation as high doses of ACh could be associated with constrictor activity – in these cases the result from the dose of ACh that produced maximal dilatation was used. The percentage SNP-attributable relaxation was calculated at the highest relaxing dose of ACh and was calculated as:

$$100 \times \left(1 - \left| \frac{T_M - T_{ACh}}{T_M - T_{SNP}} \right| \right) \qquad (5)$$

This assumed that SNP-attributable vasorelaxation was 100% of the relaxation possible for the vessel, and generated an output representing the percentage difference between standardised endothelium-dependent mechanisms and the SNP-evoked maximum. On the other hand, when if baseline tone was taken to be the minimum tone attainable by the vessel, the percentage of total relaxation attributable to SNP was given by:

$$\frac{T_M - T_{SNP}}{T_M - T_B} \qquad (6)$$

Therefore:

$$100 - \left| \frac{T_M - T_{SNP}}{T_M - T_B} \right| \qquad (7)$$

represents the percentage tone remaining in the vessel following the addition of SNP to the bath. With respect to percentage decreases on pre-constricted vascular tone in response to ACh and SNP, the data were transformed and a non-linear fit (sigmoidal dose-response, variable slope) approximated by software.

2.10   Statistical Analysis

All data were graphed and statistically analysed using PrismGraph 4.0 (GraphPad Software, Inc., San Diego, CA). D'Agostino and Pearson Omnibus normality testing was performed on all data (Kolmogorov-Smirnov testing if missing data points meant numbers in the group were too small for this). Normally distributed data was tested by using one-way analysis of variance (ANOVA) for comparison across all groups at a given time point, and two-way ANOVA, for changes in multiple groups over time (that is, repeated measurements). Post-testing was performed with Bonferroni corrections. When data were not normally distributed in at least one group for any measurement (for example, urea, experiment 2), the Kruskal-Wallis test was used in place of one-way ANOVA, and appropriate t-tests against controls for post-testing were used, depending on whether the individual groups were normally distributed or not. To assess for significance of changes compared to baseline, paired t-tests were performed. Similarly, when data were not normally distributed in at least one group for any measurement, data were displayed with box and whisker plots. Normally distributed data was displayed using histograms.

Myography data was charted, and significance testing was performed by non-linear regression and comparing best fit curves against one another with respect to a combination of the parameters of top (E_{max}), bottom, $logEC_{50}$ and Hillslope. Analysis was also performed

as a series of one-way ANOVAs for comparison at each ACh dose for further mechanistic

insight. Significance was set for all experiments at P<0.05.

Chapter 3 - Effects of dopexamine in a rodent model of laparotomy and normotensive endotoxaemia: haemodynamics, immune activation and effects on organ dysfunction

3.1 Introduction

A growing body of evidence suggests that the potential exists to reduce the morbidity and unacceptably high mortality rates associated with major surgery in high-risk patients (330). For many years, inotropic and vasoactive agents have been widely used to maintain tissue perfusion in critically ill and high-risk surgical patients with the aim of improving clinical outcomes (331). Dopexamine is a dopamine analogue with agonist activity at β2 and dopaminergic receptors. This spectrum of activity confers vasodilator actions in addition to chronotropic and mild inotropic effects. Postoperative complications occur more frequently in the presence of poor tissue microvascular flow and oxygenation (145, 332, 333), and dopexamine has been shown to improve these abnormalities (147). However, the effect of dopexamine on clinical outcomes is less clear, and the findings of randomized trials have proved inconsistent (135, 136, 138, 141, 147, 334). These conflicting findings might be explained by dose-related differences in the hemodynamic and immunologic effects of dopexamine (20).

Increasing recognition is building that adrenergic agents may have important metabolic and immunologic actions (20, 255), whereas tachycardia and myocardial ischemia may cause significant harm, especially at higher doses. It has been suggested that anti-inflammatory actions may be beneficial (20, 40). Previous work indicated that dopexamine may decrease leucocyte-endothelial adhesion in mesenteric venules (195, 196), a phenomenon dependent on CD11a and CD11b integrins (335). Other adrenergic agents have also been shown to exert anti-inflammatory actions on cytokine responses in immune cells (40, 307, 336). It is possible that the proposed clinical benefits of dopexamine, particularly at low doses, may

relate to actions on inflammatory pathways (337). As outlined in section 1.7.2, there is substantial evidence that β_2-adrenoceptor agonists have potent anti-inflammatory effects. Given these findings were somewhat contradicted by a prior study (195), I also planned to investigate the role of β_2-adrenoceptor agonism on any anti-inflammatory effects of dopexamine. In order to not ablate all β_2-adrenoceptor activation (e.g. by circulating epinephrine), the selective β_2-adrenoceptor agonist salbutamol was used rather than using a selective β_2-adrenoceptor antagonist. The aim of this was to avoid the possibility of unopposed α-adrenoceptor agonism clouding any findings.

The overall objective was to investigate the effects of dopexamine on global hemodynamics, regional microvascular flow, systemic inflammatory response, and organ injury in a rodent model of laparotomy and endotoxemia.

3.2   Methods

48 Male Wistar rats (220-410 g) received a standard diet and water *ad libitum* before experiments. All procedures were performed with Institutional approval and in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. Anaesthesia was induced by ip injection of thiopentone (120 mg/kg) and maintained with supplementary injections administered according to regular testing for limb withdrawal to a standard stimulus or signs of inadequate anaesthesia. Animals were placed on a heated mat and maintained at 37 ± 0.5°C. A tracheostomy was performed with a short section of polyethylene tubing (internal diameter 1.67 mm) to maintain airway patency and facilitate spontaneous respiration. The right carotid artery was cannulated to allow blood sampling and continuous haemodynamic monitoring, and the left jugular vein for drug and fluid administration. A 2cm mid-line incision was then made through the abdominal wall to expose the small intestine. A loop of intestine adjoining the terminal ileum was exteriorised and placed in a Saran receiving pouch. A primary set of arterial blood samples was then taken, the volume taken being replaced with an equal volume of normal saline. Animals were

allowed to stabilize for 15 min before being allocated to one of 6 groups (sham, control, D0.5, D1, D2, S – see Figure 3.1).

Endotoxemia was induced in five of six groups by administration of *Escherichia coli* lipopolysaccharide (LPS) 0111:B4 (6 mg/kg) over a 10-minute period (sham group received 0.9% saline vehicle). Administration of LPS was followed by 4 hours of fluid resuscitation with an infusion of intravenous 0.9% saline at 4.3 ml/kg/h. Sham and control animals received only 0.9% saline infusion. Three different concentrations of dopexamine were added to three of the groups' infusion fluid, producing dopexamine infusion rates of 0.5, 1, and 2 µg/kg/min for groups D0.5, D1, and D2, respectively. The final endotoxaemic group had salbutamol added to its resuscitation fluid to produce a salbutamol infusion rate of 0.2 µg/kg/min (S). The dose of salbutamol was selected on the grounds of previous studies conducted in isolated guinea pig tracheal preparations showing a ten-fold greater potency of salbutamol at the β_2 adrenoceptor when compared with dopexamine (44). The experiments ended after 4 hours of resuscitation when the lungs and heart were harvested *en bloc*. In experiment 1, the lungs were flash frozen in liquid nitrogen before being stored at -80˚C for subsequent analysis.
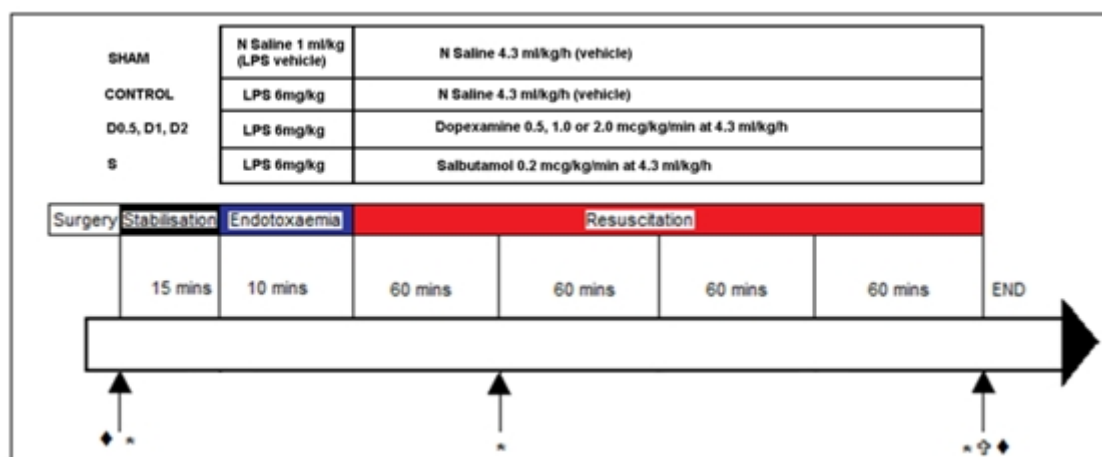
Figure 3.1   Timeline of experimental protocol

★ **Blood sampling for cytokine measurements**

◆ **Blood sampling for base excess, lactate and flow cytometry**

✠ **Blood sampling for organ function; organ harvest**

116

Analysis of plasma lactate, base deficit and renal and hepatic function

200µl of blood was taken at baseline and at the end of the experiment for measurement of plasma lactate concentration (Accutrend Lactate; Roche Diagnostics, Basel, Switzerland) and base deficit (Radiometer ABL77, Copenhagen, Denmark). A 1ml blood sample was also taken at the end of the experiment for measurements of urea, creatinine, alanine aminotransferase and aspartate aminotransferase by a commercial veterinary laboratory (IDEXX Laboratories Ltd, Sussex, UK) who were blinded to treatment.

Analysis of plasma cytokine levels

A 200µl blood sample was taken for measurement of plasma cytokine levels at baseline, 60 minutes after LPS administration, and at the end of the experiment. Samples were centrifuged immediately at 9,900 g for 3 minutes. A minimum of 50 µl of plasma per sample was collected and stored at -80°C for subsequent analysis. Cytokine levels were measured on a Luminex 200 reader (Luminex Co., Austin, TX, USA) by using the Rat Cytokine 10-Plex kit (Invitrogen Corporation, Camarillo, CA, USA) and following manufacturer's instructions. Measurements were expressed as mean fluorescent intensity, which was converted to picograms per milliliter by using a set of nonlinear transforms based on standard curves created in PrismGraph 4.0 (GraphPad Software Inc., San Diego, CA, USA).

Analysis of pulmonary myeloperoxidase (MPO) levels

MPO was measured in samples of renal, hepatic and pulmonary tissue harvested at the end of the experiment. These samples were stored at -80°C and were analyzed in four randomly selected samples per group by colleagues who were blinded to treatment. Samples of tissue

from the (right lung in the case of pulmonary tissue, right kidney in the case of renal tissue) were homogenized in a solution containing 0.5% (w/v) hexadecyltrimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000g at 4°C. An aliquot of the supernatant was then allowed to react with a solution of 1.6 mM tetramethylbenzidine and 0.1 mM H2O2. The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 µmol of peroxide per minute at 37°C and was expressed in µ-units per gram of wet tissue. MPO was also measured in the liver and kidneys in a similar fashion.

Analysis of neutrophil surface CD11a and CD11b marker by Flow Cytometry

The principles of flow cytometry are explained in the methods section. The details of the procedures involved follow. Phycoerythrin and fluorescein isothiocyanate-conjugated mouse monoclonal antibodies (mAbs) against CD11a (IgG2a) and CD11b (IgA) were used to quantify neutrophil cell-surface expression of these markers. Isotype-, fluorochrome-, and protein concentration-matched controls were run in parallel to the mAb (Becton Dickinson, Oxford, UK). Heparinized blood (600 µl) was collected for flow-cytometric analysis of leucocyte adhesion molecules, and 100 µl of whole blood was mixed with mAb against rat CD11a (5 µl) or CD11b (3 µl) in 4 × 75-mm polystyrene test tubes. Blood with no antibody added served as a control for autofluorescence. The test tubes were then incubated on ice for 30 minutes with continuous shaking, protected from light. Erythrocytes were lysed by addition of 2 ml FACSTM lysing solution to the test tubes. The samples were then incubated for a further 10 minutes on ice in the dark, and then centrifuged at 1,000 g for 3 minutes at 4°C. The supernatant was discarded, and the leucocyte pellet was resuspended and washed twice in 2 ml ice-cold optimized PBS cell wash. Finally, leucocytes were fixed in 0.3 ml 1% wt/vol paraformaldehyde in PBS at pH 7.4, and the tubes were stored in the dark at 4°C for up to 24 hours until flow-cytometric analysis could be performed. Samples were analyzed by

using a FACScan flow cytometer equipped with Cell-Quest software. CaliBRITE-3 beads and FACS COMP software were used on a weekly basis to calibrate the fluorescence intensity in accordance with the manufacturer's instructions. Ten thousand neutrophils were collected from each sample with light-scatter gain set in the linear mode and fluorescence gain set in the logarithmic mode. The neutrophil population was identified by light-scatter characteristics (forward versus side-scatter) and enclosed in an electronic gate for fluorescence histogram analysis. Antibody binding was expressed as mean fluorescence intensity (MFI), values for which were corrected for nonspecific binding by subtracting MFI measured for the matched isotype control sample.

Statistical analysis

All data were graphed and statistically analysed using PrismGraph 4.0 (GraphPad Software, Inc., San Diego, CA). D'Agostino and Pearson Omnibus normality testing was performed on all data (Kolmogorov-Smirnov testing if missing data points meant numbers in the group were too small for this). Normally distributed data was tested by using one-way analysis of variance (ANOVA) for comparison across all groups at a given time point, and two-way ANOVA, for changes in multiple groups over time (that is, repeated measurements). Post-testing was performed with Bonferroni tests. When data were not normally distributed in at least one group for any measurement (for example, plasma TNF-α), the Kruskal-Wallis test was used in place of one-way ANOVA, and appropriate t-tests against controls for post-testing were used, depending on whether the individual groups were normally distributed or not. Significance was set at $P<0.05$.

3.3   Results

Absent data points are referred to in the figure legends, and relate to measurements of serum base deficit, lactate, cytokines, and integrin expression. No statistically significant differences were found between control and other animals in terms of weight or anaesthetic dose. Salbutamol treated animals received 1.5 ml/kg more fluid over the course of the entire experiment compared to controls (Table 3.1). This was deemed to be clinically insignificant. No other groups received significantly different volumes of fluid compared to control animals.

	Experiment 1					
	Sham	*Control*	*D 0.5*	*D1*	*D2*	*S*
Weight (g)	300 (285 – 305)	320 (250 – 340)	335 (275 – 365)	255 (220 – 300)	305 (285 - 350)	335 (320 – 355)
Fluid (ml.kg^{-1})	21.6 (21.5 – 21.7)	21.4 (21.0 – 22.6)	22.9 (22.2 – 23.5)	22.8 (22.3 – 22.8)	22.1 (21.7 – 23.5)	23.5 (23.1 – 23.8)*
Thiopental (mg.kg^{-1})	194.2 (182.6 – 198.0)*	158.4 (137.8 – 177.7)	164.7 (140.0 – 179.0)	165.0 (132.7 – 174.9)	157.2 (143.3 – 168.5)	153.6 (145.6 – 168.4)

Table 3.1 Baseline characteristics for experiment 1 (n=8 all groups).

Data presented as mean (SEM) when all groups normally distributed, otherwise median (IQR) if ≥1 group not normally distributed. Kruskal-Wallis test (Fluid – Unpaired t-test with Welch's correction *P<0.05 compared to control group; Anaesthetic – Mann Whitney test *P<0.05 compared to control group)

Haemodynamic data were similar between all groups at outset. Compared with sham animals, control animals had a significantly higher HR ($P < 0.05$) at 4 hours and a lower MAP ($P < 0.01$) compared with baseline at this time (Figures 3.2 and 3.3). At this point, plasma lactate ($P < 0.001$) and base deficit ($P < 0.01$), pulmonary tissue MPO activity ($P < 0.01$), neutrophil cell surface CD11b expression ($P < 0.001$), and plasma TNF-α ($P < 0.05$), IL-1β ($P < 0.05$), IL-6 ($P < 0.001$), IL-10 ($P < 0.05$), IL-2, IL-12 and IFN-γ were also significantly higher in control animals when compared with sham animals (Figures 3.4 –

3.10). Levels of plasma TNF-α at 1 hour (that is, peak levels) were also significantly higher in control animals ($P < 0.001$) (Figure 3.7).

After LPS administration, CD11b expression increased in control animals, whereas CD11a expression decreased (Figure 3.6). Although pulmonary MPO was significantly increased compared to baseline in all endotoxaemic groups, MPO levels were undetectable in the liver and kidney in all groups (not shown). Endotoxaemia resulted in significant organ injury, as evidenced by control-group plasma urea ($P < 0.001$), creatinine ($P < 0.001$), ALT ($P < 0.001$), and AST ($P < 0.01$) being significantly greater than that of the sham group (Figure 3.11 & 3.12).

When compared with control animals, HR was higher in the dopexamine groups at 4 hours after LPS, although this was not statistically significant (Figure 3.3). Although MAP decreased to a similar extent in controls and all dopexamine groups, MAP was slightly better maintained in the D2 group (Figure 3.2). The increase in plasma lactate was less in dopexamine-treated animals than in control animals ($P < 0.001$ controls versus pooled dopexamine) with corresponding changes in base deficit ($P < 0.05$ controls versus pooled dopexamine) (Figure 3.4). Compared with control animals, dopexamine significantly attenuated the increase in TNF-α (D1 $P < 0.05$), IL-1β (D0.5 and D1, $P < 0.05$), and IL-6 (any dose, minimum $P < 0.01$) at 4 hours, whereas the reduction in IL-10 achieved significance only at doses of 0.5 and 1 µg/kg/min ($P < 0.05$ minimum) (Figures 3.7 – 3.9). Peak plasma TNF-α was also attenuated in although significantly in only D2 animals ($P < 0.05$). Although increases in IFN-γ were not significantly ameliorated by dopexamine, the cytokines IL-2 (all doses) and IL-12 (D1) were (Figure 3.10). CD11a expression was unaffected by dopexamine, but in D1 and D2 groups, dopexamine infusion was associated with significantly decreased CD11b expression at 4 hours ($P < 0.01$ minimum) (Figure 3.6), as well as significantly reduced pulmonary MPO activity ($P < 0.05$ minimum) (Figure 3.5). Notably, dopexamine infusion was also associated with significant reductions in renal ($P < 0.005$ pooled dopexamine versus controls) and hepatic injury ($P < 0.05$ pooled dopexamine versus

controls) (Figures 3.11 & 3.12).

Patterns of pulmonary MPO and surface integrin expression in salbutamol treated animals paralleled those of dopexamine treated groups (Figures 3.5 & 3.6), but the plasma cytokine pattern associated with salbutamol treatment differed markedly from dopexamine treated groups, most notably for IL-1β, IL-6, IL-2 and IL-12 (Figures 3.8 -3.10). In association with this, MAP was significantly lower than both control and dopexamine treated groups for significant periods of the experiment in group S (Figure 3.2). Although MAP was significantly reduced compared to controls, indices of organ perfusion were not worse than control animals, though they were not ameliorated either (Figure 3.4). Organ injury was not ameliorated by salbutamol treatment (Figure 3.11 & 3.12).
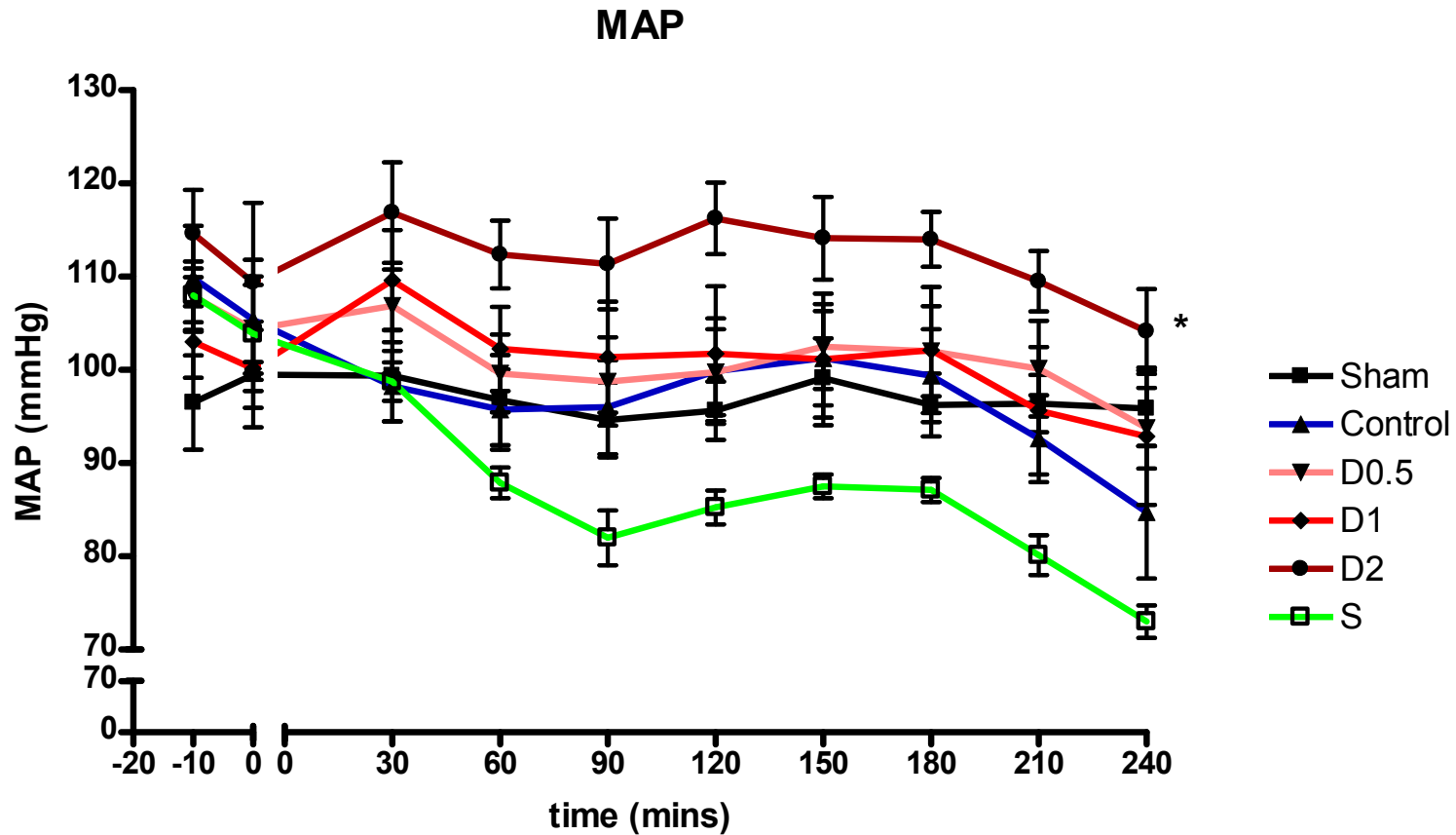
Figure 3.2   MAP for all groups

Two-way ANOVA (Bonferroni's post-tests vs. controls, * P<0.05 [D2 group]). MAP was also significantly reduced compared to baseline at 4 hours in the control (P<0.01) and S (P<0.0001) groups (not shown on graph for clarity). Although not significantly different compared to controls at any time point, S group had significantly decreased MAP compared to all dopexamine groups for the last 30 min, and the D2 group from 60 min onwards (not shown on graph for clarity).
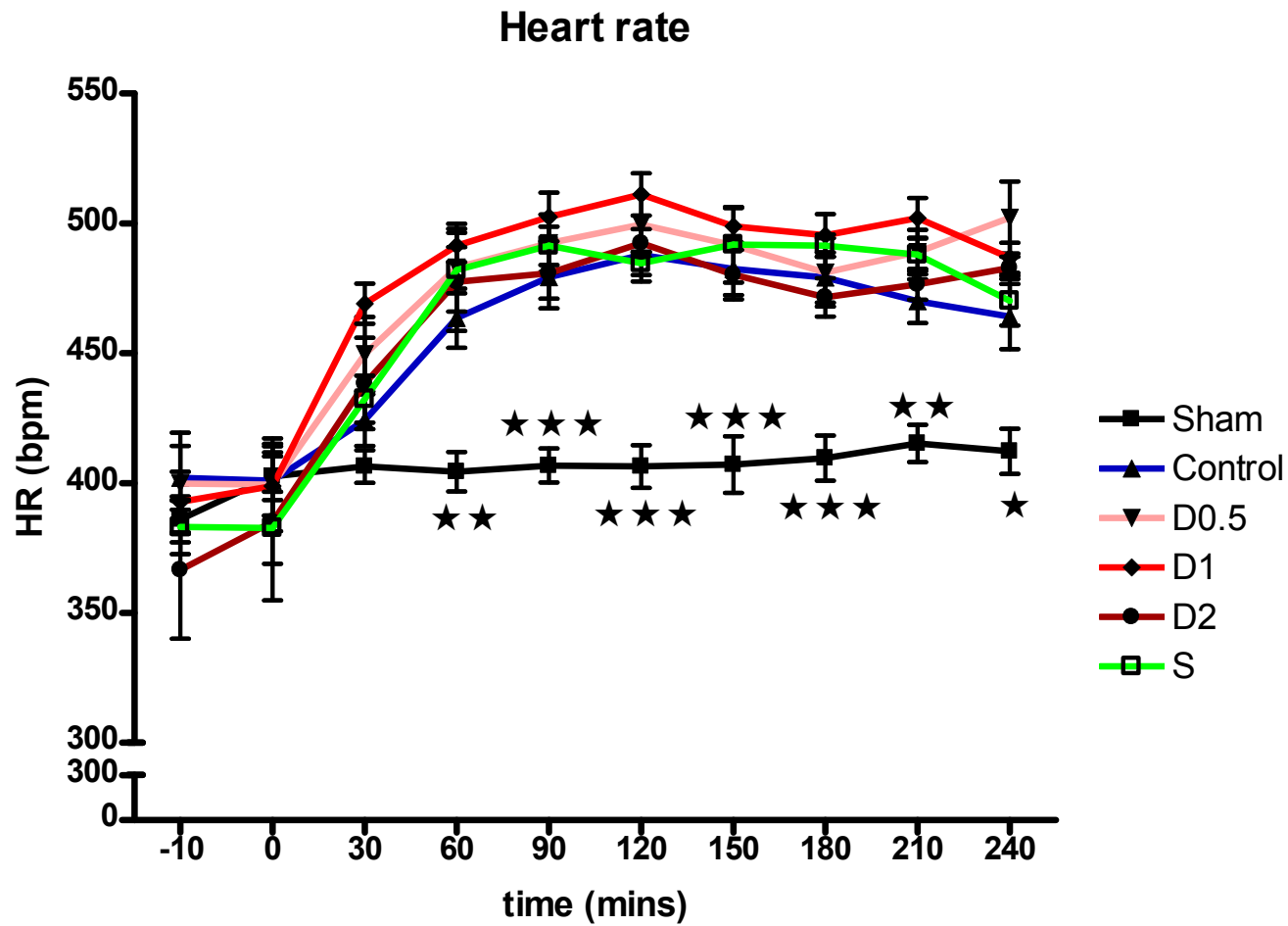
Figure 3.3   Heart rate for all groups

Two-way ANOVA (Bonferroni's post-tests, * P<0.05, ** P<0.01, *** P<0.001 vs. controls)
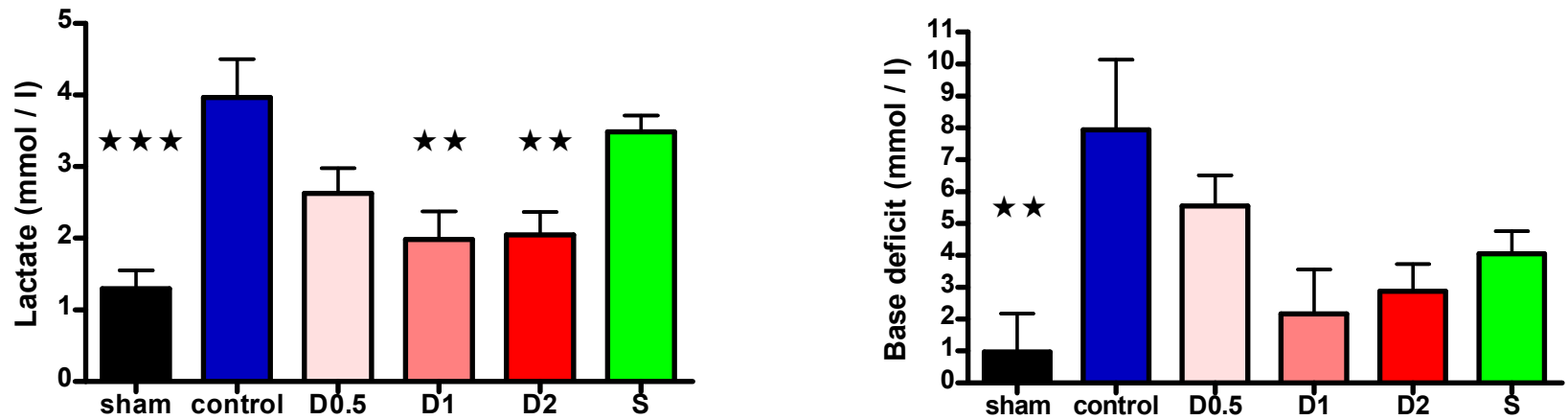
Figure 3.4   **Indices of tissue perfusion**

Plasma lactate (n=8 all groups) and base deficit (D0.5 and D1 n=6 each, all others n=8):
One-way ANOVAs (Bonferroni's post-tests vs. control: ** P<0.01, ***P<0.001)
Indices   of   organ   function   appeared   to   track   markers   of   global   tissue   perfusion.
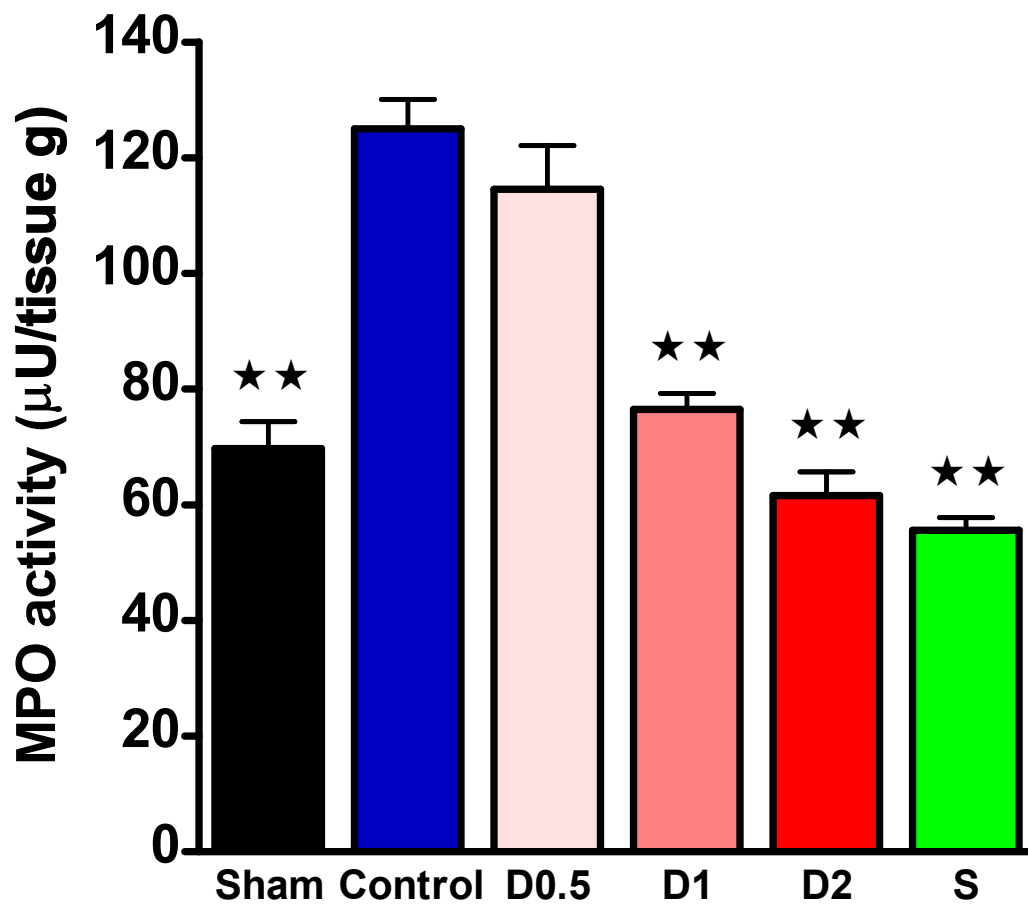
Figure 3.5   Pulmonary MPO

One-way ANOVA P<0.05 (Bonferroni's post-tests vs. controls: ** P<0.01. n=4 all groups)
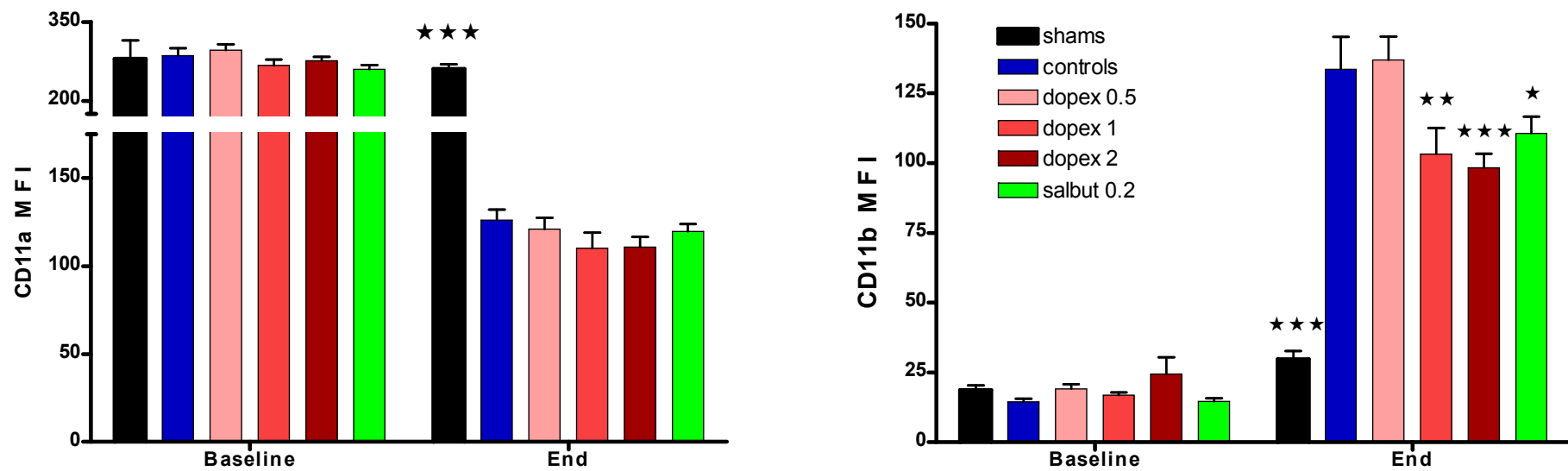
Figure 3.6   CD11a and CD11b mean fluorescent intensity (MFI) expression for all groups

Two-way ANOVA (Bonferroni's post-tests vs. controls *P<0.05 **P<0.01 ***P<0.001)

(CD11a – n=7 D0.5 and S, n=8 all others; CD11b – n=6 controls, n=5 S, n=8 all others)
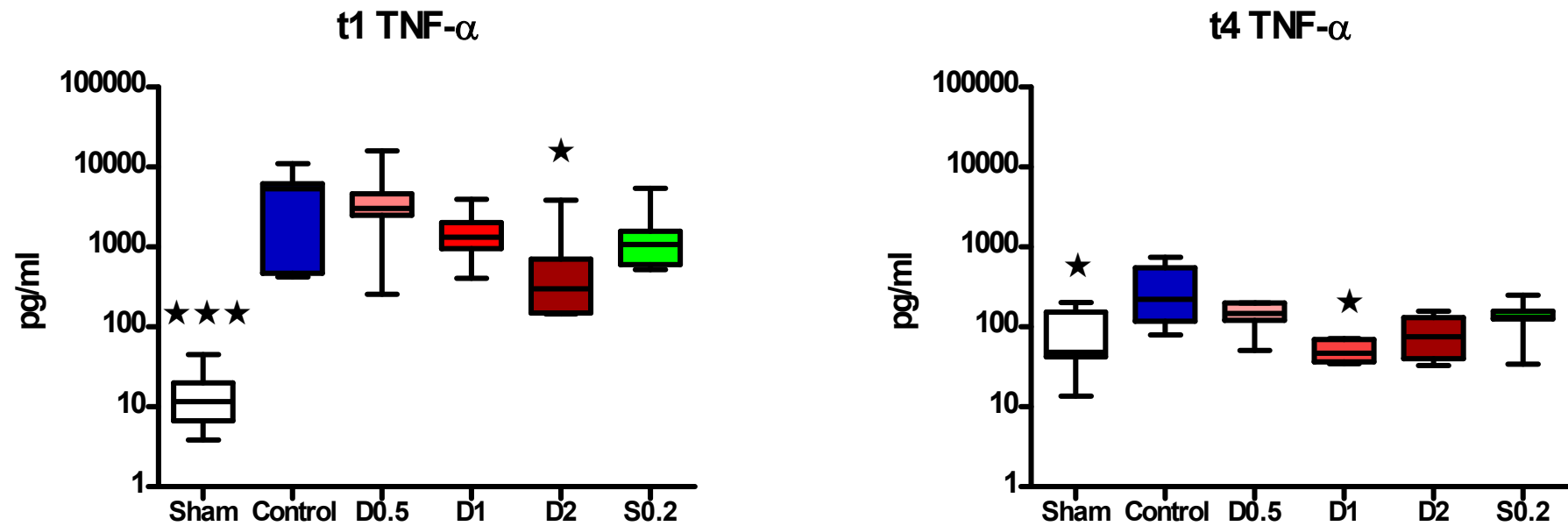
Figure 3.7 **Plasma TNF-α levels for all groups at 1 (t1) and 4 (t4) h post endotoxaemia, respectively**

t1: Kruskal-Wallis test (*post hoc* unpaired t-tests (sham), ***P<0.001 vs. controls, *post hoc* Mann-Whitney test (D2), *P<0.05 vs. controls)

t4: Kruskal-Wallis test (*post hoc* Mann Whitney (sham), *P<0.05 vs. controls, *post hoc* unpaired t-test (D2), *P<0.05 vs. controls)

(t1: n=7 controls and D2, n=8 all others. t4: n=7 D0.5, n=6 D1, n=5 D2, n=8 all others)
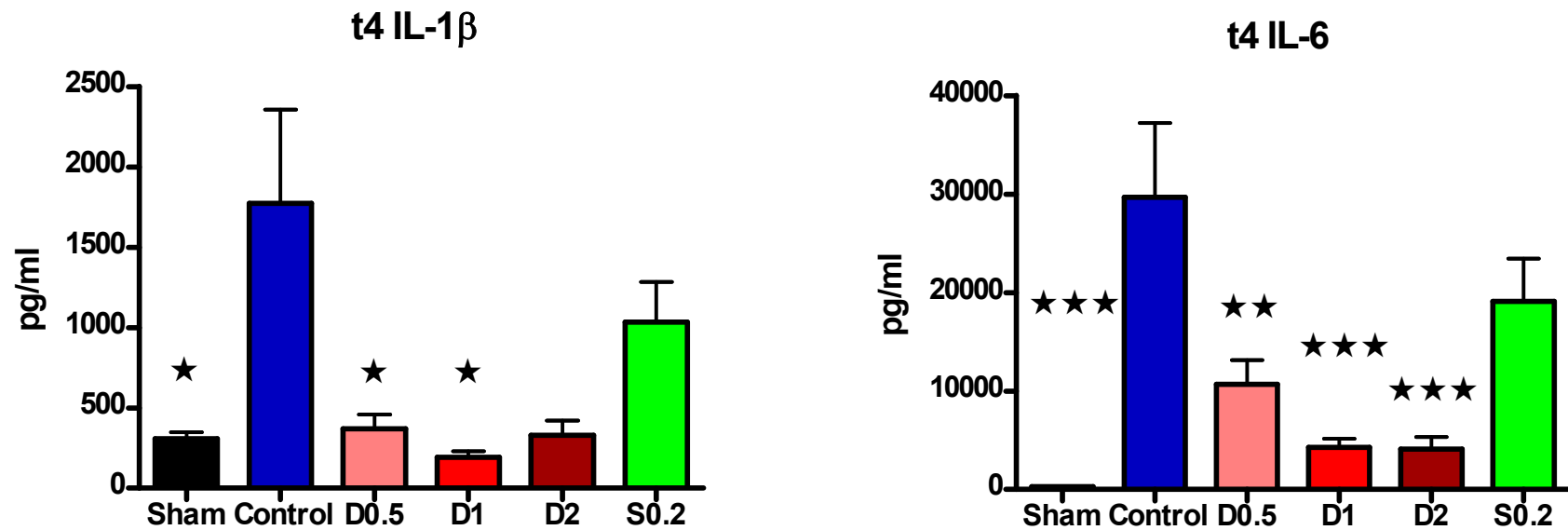
Figure 3.8 Plasma IL-1β and IL-6 for all groups 4 h post endotoxaemia.

One-way ANOVA (Bonferroni's post-tests *P<0.05, **P<0.01, ***P<0.001 compared to control group)

(IL-1β: n=7 D0.5, n=6 D1, n=4 D2, n=8 all others; IL-6: n=7 controls and D2, n=8 all others)
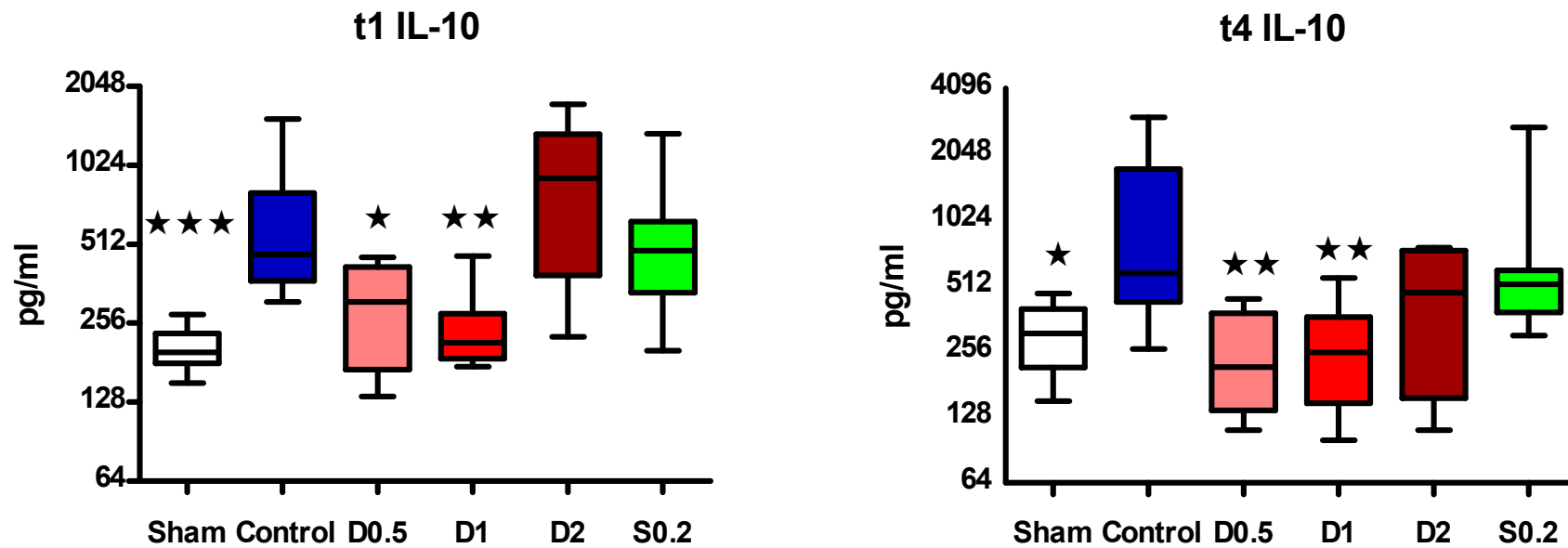
Figure 3.9   Plasma IL-10 levels for all groups at 1 (t1) and 4 (t4) h post endotoxaemia, respectively.

Kruskal-Wallis tests (post hoc tests Mann-Whitney (all groups), *P<0.05, **P<0.01, ***P<0.001)

(t1: D1 n=7, D2 n=5, all others n=8; t4: D0.5 and D1 n=7, D2 n=6, all others n=8)
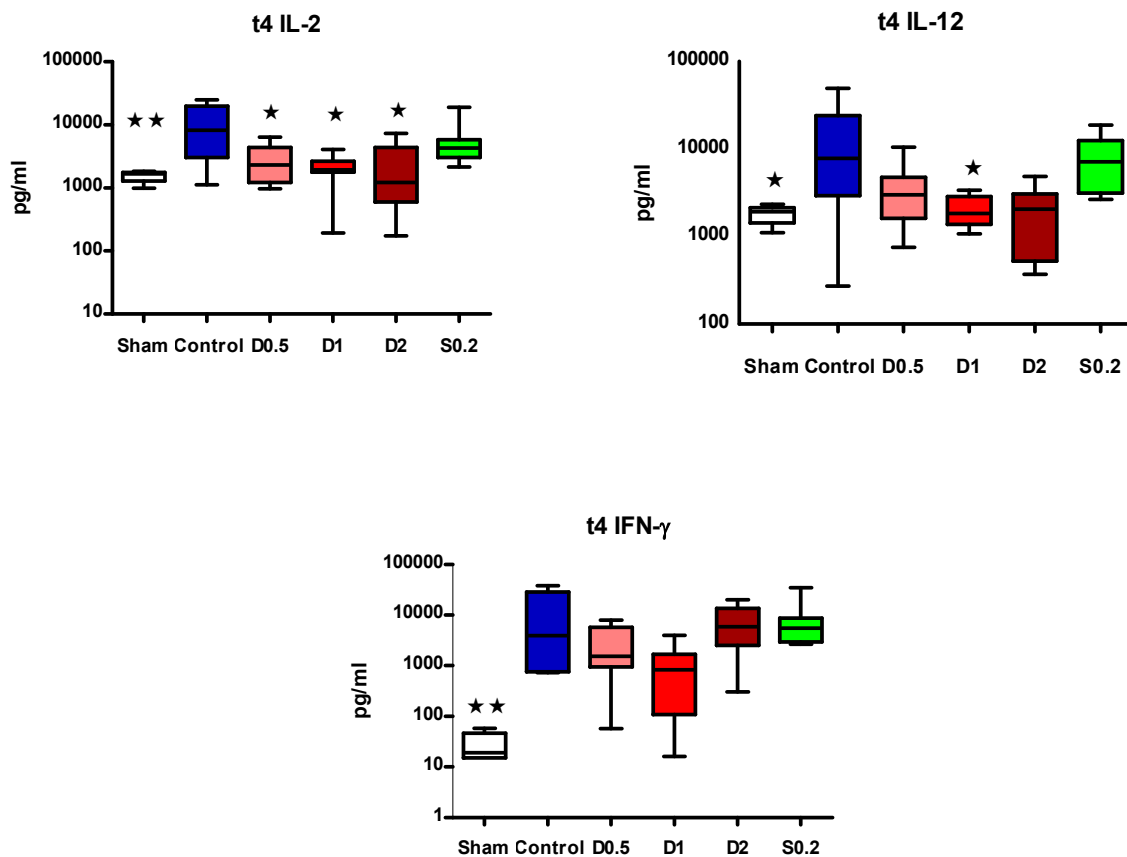
Figure 3.10 **Plasma cytokine levels (IL-2, IL-12 and IFN-γ) for all groups 4 h post-endotoxaemia**

IL-2: Kruskal-Wallis test (*post hoc* Mann Whitney (sham), **P<0.01 vs. controls, *post hoc* unpaired t-tests (D0.5, D1 and D2), *P<0.05 vs. controls)
IL-12: Kruskal-Wallis test (*post hoc* Mann Whitney (sham, D1), *P<0.05 vs. controls)
IFN-γ: Kruskal-Wallis test (*post hoc* Mann Whitney (sham), **P<0.01 vs. controls)

(IL-2 and IL-12: n=7 D2, n=8 all others; IFN-γ: n=8 D0.5 and D1, n=7 sham and S0.2, n=6 controls, n=4 D2)
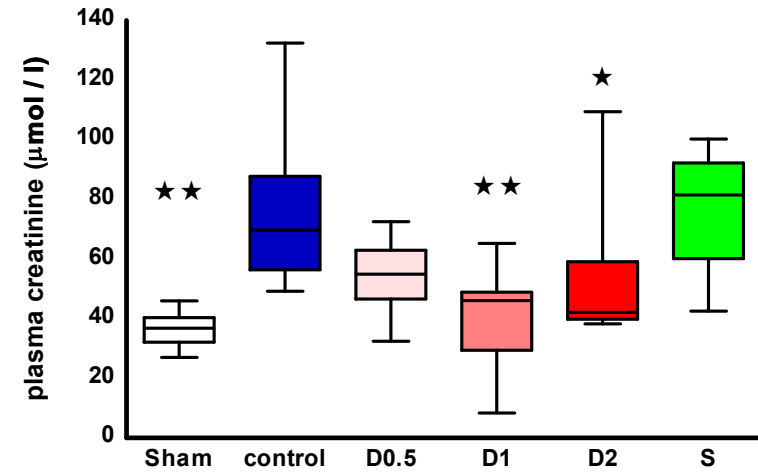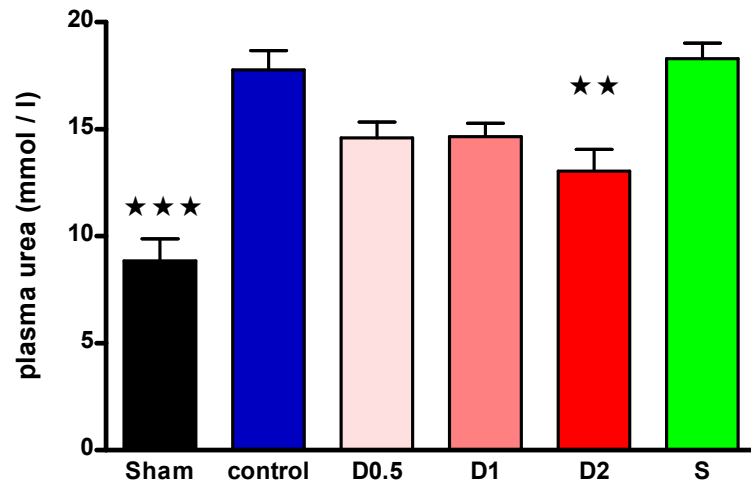
Figure 3.11 Plasma urea and creatinine.

Urea: One-way ANOVA (Bonferroni's post-tests vs. controls: ** P<0.01, ***P<0.001)
Creatinine: Kruskal-Wallis test (*post hoc* unpaired t-tests (sham and D1) **P<0.01 compared with controls; *post hoc* Mann-Whitney test (D2) *P<0.05 compared with controls).
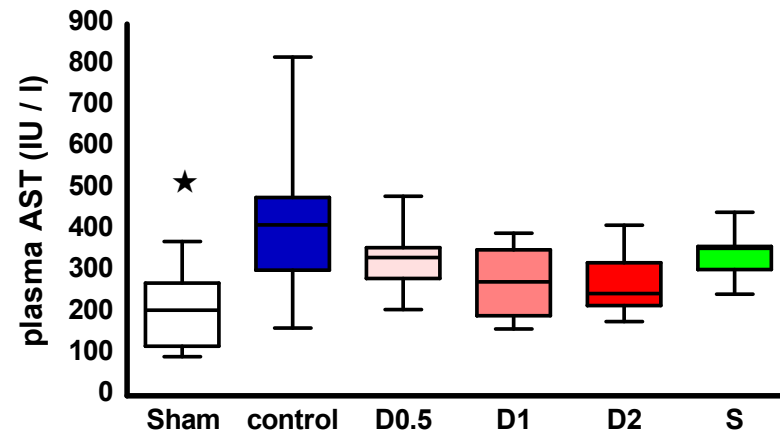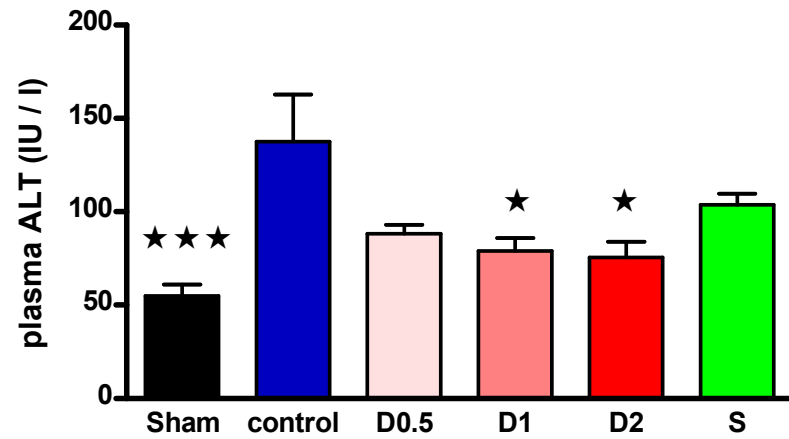
Figure 3.12 Plasma ALT and AST

ALT: One-way ANOVA (Bonferroni's post-tests vs. controls: * P<0.05, ***P<0.001)
AST: Kruskal-Wallis test (*post hoc* unpaired t-tests (sham) *P<0.05 compared with controls)

3.4 Discussion

This study showed that, in a rodent model of laparotomy and endotoxaemia, dopexamine can attenuate the systemic inflammatory response, limit the degree of lactic acidosis, and protect against organ injury. Dopexamine infusion was associated with a reduced systemic inflammatory response, as evidenced by decreased circulating levels of TNF-α, IL-1β, and IL-6, and decreased leucocyte expression of the cell-adhesion molecule CD11b. In turn, this was associated with reduced pulmonary MPO activity, a marker of pulmonary leucocyte infiltration. Overall, dopexamine-treated animals sustained less organ injury than did control animals. Interestingly, these potentially beneficial effects occurred at doses of dopexamine that had little or no effect on blood pressure.

These findings suggest that after a combined surgical and infectious insult, dopexamine can attenuate the increase in circulating levels of inflammatory mediators and reduce leucocyte expression of the cell-adhesion molecule CD11b. As a consequence, leucocyte-endothelial adhesion and transmigration into tissues is decreased, with a reduction in organ injury. This suggestion is consistent with the findings of previous studies indicating that catecholamines can inhibit cytokine release and that CD11b integrins mediate pulmonary neutrophil recruitment (196, 218, 338). Although not intrinsically chemotactic, TNF-α and IL-1β upregulate endothelial and leucocyte expression of adhesion molecules (339, 340), and hence promote leucocyte-endothelial adhesion and migration into tissues. Previous work also showed that adrenergic agents can decrease leucocyte expression of adhesion molecules (207), whereas *in vivo* microscopy suggests that dopexamine may decrease leucocyte-endothelial adhesion in the mesenteric circulation (195, 196). Dopexamine has been shown to decrease free radical-mediated tissue injury in other animal models (242, 274), whereas the β_2-agonist terbutaline reduced nitric oxide and superoxide levels in endotoxaemic rats (246). This latter finding is particularly interesting as the early changes in endotoxaemic renal failure relate to changes in intra-renal haemodynamics (but not renal

blood flow), and changes in tubular transporters – all predominantly mediated by TNF-α, IL-1β and IFN-γ and all ameliorated by β$_2$-adrenoceptor agonists such as dopexamine, fenoterol, clenbuterol and terbutaline (233, 250, 251, 341-346).

Although salbutamol demonstrated similar efficacy in reducing CD11b expression and pulmonary MPO activity, this was neither associated with a similar pattern of cytokine amelioration, nor with an amelioration of organ injury. Cytokines such as IL-1β and stimulators of IFN-γ synthesis (such as IL-12 and IL-2 (347, 348)) were significantly reduced in dopexamine treated animals but not salbutamol treated animals. Indices of perfusion were neither ameliorated nor exacerbated. MAP was significantly reduced compared to dopexamine and control groups. These findings could suggest the beneficial effects of dopexamine on CD11b integrin expression and pulmonary MPO activity are β$_2$-adrenoceptor mediated, though they could possibly point away from cytokines as the intermediary that drives this process. However plasma cytokine levels reflect the subtotal of cytokine responses from several different body compartments that enter the circulation. If neutrophil activation occurs in a compartment of the body where the effects of salbutamol and dopexamine on cytokine release are similar, the hypothesis that neutrophil activation is inhibited by a β$_2$-adrenoceptor mediated reduction in cytokine levels could still be valid. In this case the difference in plasma cytokine levels may reflect a failure of salbutamol to ameliorate cytokine release from other body compartments and may also explain the difference in organ injury. The question arises why the effects of salbutamol and dopexamine on different body compartments is different if they are equipotent at the β$_2$-adrenoceptor? This could relate to more potent vasodilator effects of salbutamol – a reduction in perfusion pressure to some organs (e.g. kidneys) and a re-distribution of blood flow to other tissue beds (such as skeletal muscle) when salbutamol is infused. This might relate to the other differential features of drug action such as noradrenaline reuptake inhibition.

There are additional potential explanations. Should the doses of salbutamol and dopexamine at the β$_2$-adrenoceptor have not been equipotent (Figure 3.3 suggests this is not the case),

there would be no reason to expect an equivalent response. Such a possibility could be supported by other *in vivo* studies where a 6-fold lower salbutamol than dopexamine dose did not result in equivalent amelioration of organ injury, but an equivalent dose did (240). Finally, even if traditional cAMP related signalling pathways were activated to the same degree at these doses, a differential efficacy of these two drugs on non-cAMP related pathways involved in inflammation is not precluded.

Several features of the model are consistent with similar studies and therefore reassuring. Endotoxaemia and bacteraemia are associated with significant increases in HR and usually a significant though only modest reduction in MAP in rodents (though this is dose dependent) (195, 196, 270-272, 277, 278, 349). Furthermore following LPS administration, plasma TNF-α levels peak at approximately 1 hour and continue to remain elevated above baseline for up to 4 h after endotoxin bolus whereas IL-1β and IL-6 do not peak even by 3 to 4 hours post-endotoxin (196, 350-352). Six hours of rodent endotoxaemia has previously been shown to be associated with an upregulation of leucocyte CD11b and also to cause significant renal and hepatic injury (302). Both bacteraemia and endotoxaemia are associated with a 30 -50% decrease in glomerular filtration rate, urine flow and sodium excretion, as soon as 30 minutes following injection (277, 278). Thus profound changes affecting renal filtration occur early following an exposure to bacterial breakdown products (studies suggest this is not due to changes in intravascular volume or renal plasma/blood flow alone) (349, 353-355). The increases in plasma urea and creatinine seen here are consistent with these findings. The findings of the experiment (with respect to dopexamine) are also consistent with the literature in that a reduction in plasma TNF-α, amelioration of CD11b upregulation and amelioration of renal dysfunction was seen (196, 207, 278).

Chapter 4 - Effects of dopexamine in a rodent model of laparotomy and normotensive endotoxaemia: macrohaemodynamics, microhaemodynamics and effects on organ dysfunction

4.1 Introduction

The previous study established that dopexamine could ameliorate organ dysfunction in a normotensive model of laparotomy and endotoxaemia. Although there were demonstrable effects of dopexamine on markers of inflammation and also biochemical markers of perfusion, it is unclear how this relates to indices of global and microvascular blood flow. The following experiment was conducted in order to address those questions.

4.2 Methods

48 Male Wistar rats (220-410 g) received a standard diet and water *ad libitum* before experiments. All procedures were performed with Institutional approval and in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. Anaesthesia was induced by ip injection of thiopentone (120 mg/kg) and maintained with supplementary injections administered according to regular testing for limb withdrawal to a standard stimulus or signs of inadequate anaesthesia. Animals were placed on a heated mat and maintained at 37 ± 0.5°C. A tracheostomy was performed with a short section of polyethylene tubing (internal diameter 1.67 mm) to maintain airway patency and facilitate spontaneous respiration. The right carotid artery was cannulated to allow blood sampling and continuous haemodynamic monitoring, and the left jugular vein for drug and fluid administration. A 2cm mid-line incision was then made through the abdominal wall. Following this the bowel was evacuated into a moist cotton receptacle. Blunt dissection was then performed to access the retroperitoneum and the abdominal vasculature. After isolation

from the vena cava, a 1.5-mm ultrasonic aortic transit time flow probe (MA1.5PRB; Transonic Systems Inc., Ithaca, NY, USA) was placed on the infra-renal aorta to measure aortic blood flow. The bowel was then replaced in the abdominal cavity, except for a loop of ileum just proximal to the cecum. The exposed bowel was kept moist by the application of 0.9% saline drops through a pipette. The laparotomy incision above and below the exit of the terminal ileal loop from the abdomen was then closed with 5.0 Vicryl to prevent excessive insensible losses.

A 1.5-cm incision was subsequently made in the antemesenteric border of the ileum by using unipolar diathermy for later placement of laser Doppler probes. To prevent thermal damage to the ileum, the section was immediately washed with normal saline. The mucosal surface of the bowel was exposed and gently cleansed with 0.9% saline by using cotton-tipped buds in preparation for placement of laser Doppler probes. The animal was then placed on a Perspex stage in the right lateral position so that the ileal loop rested on a raised section of the stage, at the level of the laparotomy incision. Subsequently the bowel was fixed at two points on either side of the incision with a small amount of tissue glue to prevent movement artefact. After application of the laser Doppler probes (see later), an impermeable cover for the ileal loop was created by placing small pieces of pre-cut Saran wrap over the loop and around the probes, until the ileal preparation was airtight. This was followed by a 5-ml/kg bolus of intravenous normal saline to replace insensible fluid losses and by a 15-minute stabilization period. Blood sampling for plasma lactate and arterial blood gases was performed, followed by a second 15-minute stabilization period before measurement of regional microvascular flow. The only other blood samples taken were at the end of the experiment for markers of organ injury.

Endotoxaemia was induced in five of six groups by administration of *Escherichia coli* lipopolysaccharide (LPS) 0111:B4 (6 mg/kg) over a 10-minute period (sham group received 0.9% saline vehicle). Administration of LPS was followed by 4 hours of fluid resuscitation with an infusion of intravenous 0.9% saline at 4.3 ml/kg/h. Sham and control animals received

only 0.9% saline infusion. Three different concentrations of dopexamine were added to three of the groups' infusion fluid, producing dopexamine infusion rates of 0.5, 1, and 2µg/kg/min for groups D0.5, D1, and D2, respectively. Group S had salbutamol added to its resuscitation fluid to produce a salbutamol infusion rate of 0.2µg/kg/min when the fluid was infused at 4.3ml/kg/h. The dose of salbutamol was selected on the grounds of previous studies conducted in isolated guinea pig tracheal preparations showing a ten-fold greater potency of salbutamol at the β_2 adrenoceptor when compared with dopexamine (44). The experiment ended after 4 hours of resuscitation.

Analysis of plasma lactate, base deficit and renal and hepatic function

200µl of blood was taken at baseline and at the end of the experiment for measurement of plasma lactate concentration (Accutrend Lactate; Roche Diagnostics, Basel, Switzerland) and base deficit (Radiometer ABL77, Copenhagen, Denmark). A 1ml blood sample was also taken at the end of the experiment for measurements of urea, creatinine, alanine aminotransferase and aspartate aminotransferase by a commercial veterinary laboratory (IDEXX Laboratories Ltd, Sussex, UK) who were blinded to treatment.

Measurement of ileal red cell flux

Two fibre-optic laser Doppler flux slave probes (P10k; Moor Instruments, Axminster, UK) suspended from clamps were lightly applied to the mucosal and serosal surfaces of the ileum to determine red-cell flux, a measure of regional microvascular blood flow. Two probes were placed on the ileum, one on a mucosal site and one on a serosal site away from visible blood vessels. These were then fixed with tissue glue, a technique that causes minimal interference with tissue microvascular flow. Slave Probes were calibrated daily by using PFS flux standard (Moor Instruments, Axminster, UK) at 23°C before experiments. Slave probes

were reattached to master probes (MP10M200ST; Moor Instruments), which in turn were connected to a satellite monitor (moorLAB; Moor Instruments). Connection of the server to a desktop computer allowed continuous recording of red-cell flux and the direct current or DC signal (index of reflected light intensity and hence quality of probe contact). Laser light of 780-nm wavelength with a 40-Hz sampling rate and a 30-degree angular spread allowed a sampling volume of approximately 1 mm^3. The probe readout was monitored for 2 minutes to ensure adequate contact before fixation. As the thickness of rat ileum is less than the depth of measurement achieved with LDF probes, red-cell flux was averaged between mucosal and serosal sites to minimize bias due to heterogeneity in regional microvascular flow.

Measurement of aortic blood flow

A 1.5-mm perivascular probe was applied with water-soluble sonicating gel and sited as described earlier. The probe was connected to a TS420 monitor (Transonic Systems Inc., Ithaca, NY, USA), which was connected to a Powerlab/8SP monitoring system (AD Instruments). This allowed continuous recording of aortic blood flow and waveform-derived HR and calculation of a measure of stroke volume. Aortic blood flow was indexed to body weight to provide a measure of changes in stroke volume index (SVI) and cardiac index (CI). MAP was indexed to aortic blood flow to give a measure of total peripheral resistance. Probe calibration was performed daily according to the manufacturer's instructions before experiments.

Statistical analysis

All data were graphed and statistically analysed using PrismGraph 4.0 (GraphPad Software, Inc., San Diego, CA). D'Agostino and Pearson Omnibus normality testing was performed on all data (Kolmogorov-Smirnov testing if missing data points meant numbers in the group were too small for this). Normally distributed data was tested by using one-way analysis of

variance (ANOVA) for comparison across all groups at a given time point, and two-way ANOVA, for changes in multiple groups over time (that is, repeated measurements). Post-testing was performed with Bonferroni tests. When data were not normally distributed in at least one group for any measurement (for example, urea), the Kruskal-Wallis test was used in place of one-way ANOVA, and appropriate t-tests against controls for post-testing were used, depending on whether the individual groups were normally distributed or not. Significance was set at $P < 0.05$.

4.3   Results

There was no statistically significant difference between control and other groups regarding animal weight, dose of anaesthetic or volume of fluid received (Table 4.1). There was no significant difference in baseline haemodynamics or haematocrit between controls and any group, and no significant increase in haematocrit over the period of resuscitation for any group (Figure 4.1 to 4.3). There were no significant differences between ileal mucosal or serosal red cell fluxes (Figure 4.4) therefore serosal and mucosal data were pooled to give total ileal red cell flux.

	Experiment 2					
	Sham	**Control**	**D 0.5**	**D1**	**D2**	**S**
Weight (g)	275 (265 – 305)	320 (290 – 320)	320 (285 – 325)	305 (290 – 320)	300 (280 – 300)	330 (310 – 335)
Fluid (ml/kg)	24.3 (23.8 – 24.3)	23.8 (23.8 – 24.0)	23.9 (23.7 – 24.2)	23.8 (23.5 – 23.9)	24.0 (23.8 – 24.1)	23.8 (23.8 – 23.9)
Thiopental (mg/kg)	172.8 (3.2)	156.5 (7.6)	160 (9.3)	152 (6)	157.2 (4.3)	150.9 (5.8)

Table 4.1   Baseline characteristics for experiment 2 (n=8 all groups).

Data presented as mean (SEM) when all groups normally distributed, otherwise median (IQR) if ≥ 1 group not normally distributed.
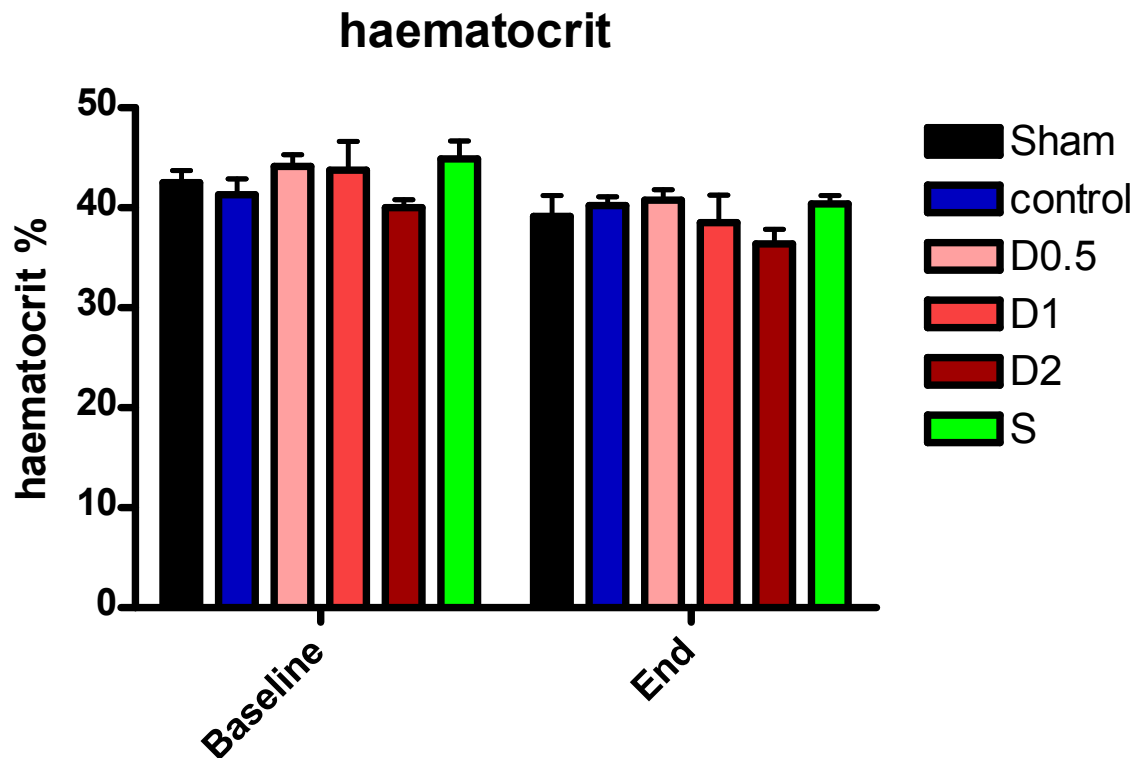
haematocrit

Figure 4.1   Trends in haematocrit from baseline (left) to end (right) for all groups

Baseline MAP, HR, CI, SVI, TPR and lactate were not significantly different between groups. In the sham group, CI and SVI increased progressively (Figure 4.5), whereas ileal flux decreased to a mean of 82% of baseline over a 4-hour period ($P < 0.05$ versus baseline) (Figure 4.6). In control-group animals, ileal flux also decreased over time, but more rapidly and to levels below those observed in sham animals ($P < 0.05$ minimum, from 30 minutes onward) (Figure 4.7). This was associated with a moderate but significant decrease in CI over 4 hours ($P < 0.05$ versus baseline) and a more-marked decrease in SVI over the same period ($P < 0.0005$ versus baseline) and a progressive increase in TPR (Figure 4.5 and 4.6), possibly reflecting the importance of compensatory tachycardia in this model. Even when CI was significantly increased above baseline, control group ileal red cell flux was significantly decreased from baseline (Figure 4.8). By 4 hours, control-group plasma lactate ($P < 0.01$)

and base deficit ($P < 0.05$) were significantly increased compared with sham animals (Figure 4.9). With regard to organ dysfunction, endotoxaemia also resulted in organ injury, although the changes did not reach statistical significance for AST (Figure 4.10 and 4.11).

The addition of dopexamine at any dose did not significantly affect MAP and did not attenuate the LPS-induced decreases in SVI and CI, despite a significant increase in heart rate. Moreover, dopexamine did not influence the decrease in ileal red-cell flux even when CI was significantly increased above baseline (Figures 4.5, 4.7 and 4.8). Dopexamine did not appear to ameliorate the increases in TPR seen in controls. Dopexamine at any dose (and when dopexamine data were pooled) was not associated with any significant differences in end-experiment plasma lactate, base deficit, or organ function when compared with controls, with the exception of ALT in the D2 group ($P < 0.05$ versus controls) (Figures 4.9 – 4.11).

Salbutamol behaved in a similar manner to D2 so that the S group failed to significantly affect MAP, failed to attenuate the LPS-induced decreases in SVI and CI and also failed to influence the decrease in ileal red-cell flux even when CI was significantly above baseline (Figures 4.5 – 4.8). Salbutamol was not associated with any improvement in indices of tissue perfusion or renal function when compared to controls even though salbutamol was not associated with the high TPR seen in control animals (Figure 4.6). Salbutamol ameliorated the increase in plasma ALT when compared to controls.
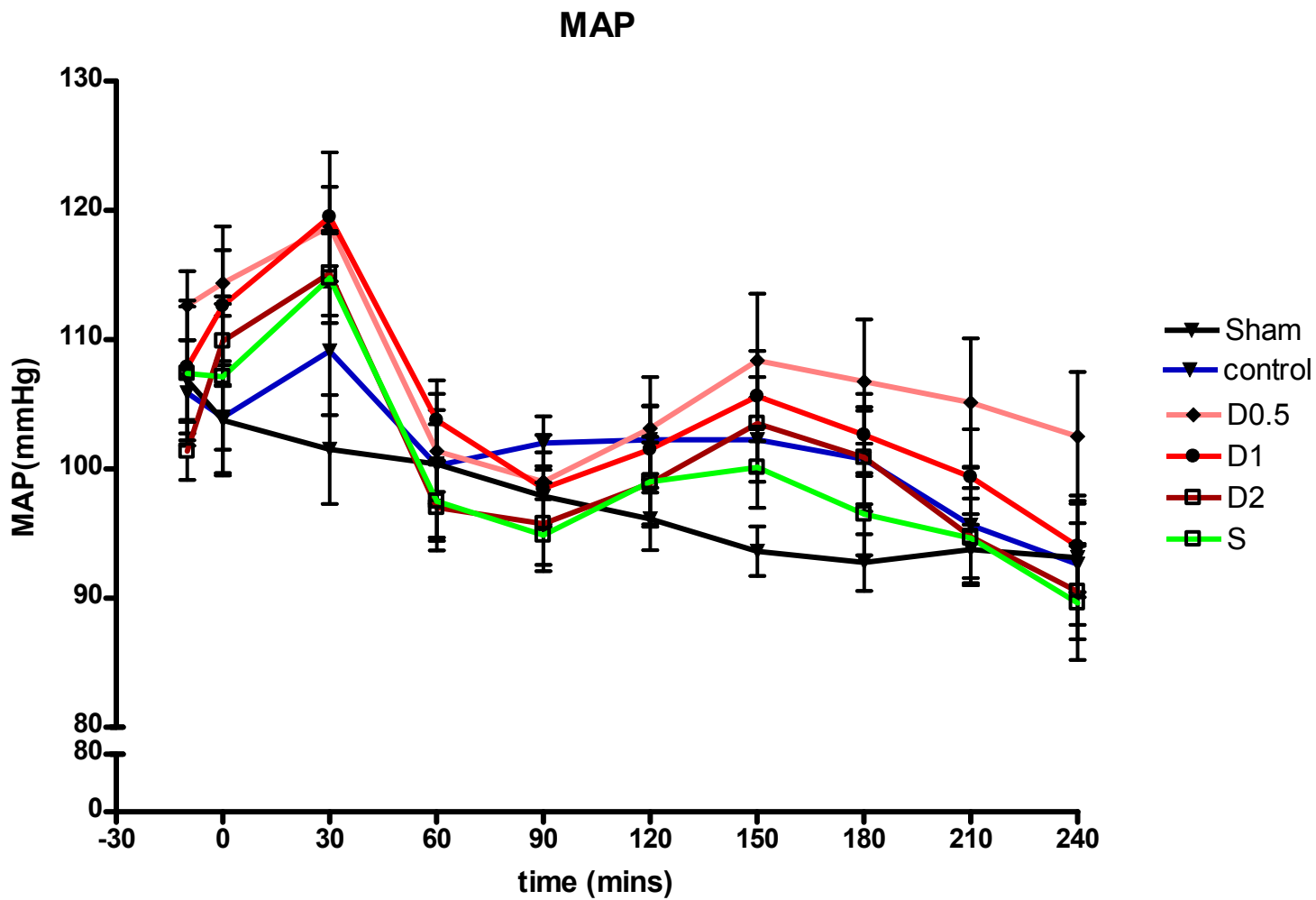
Figure 4.2   MAP for all groups
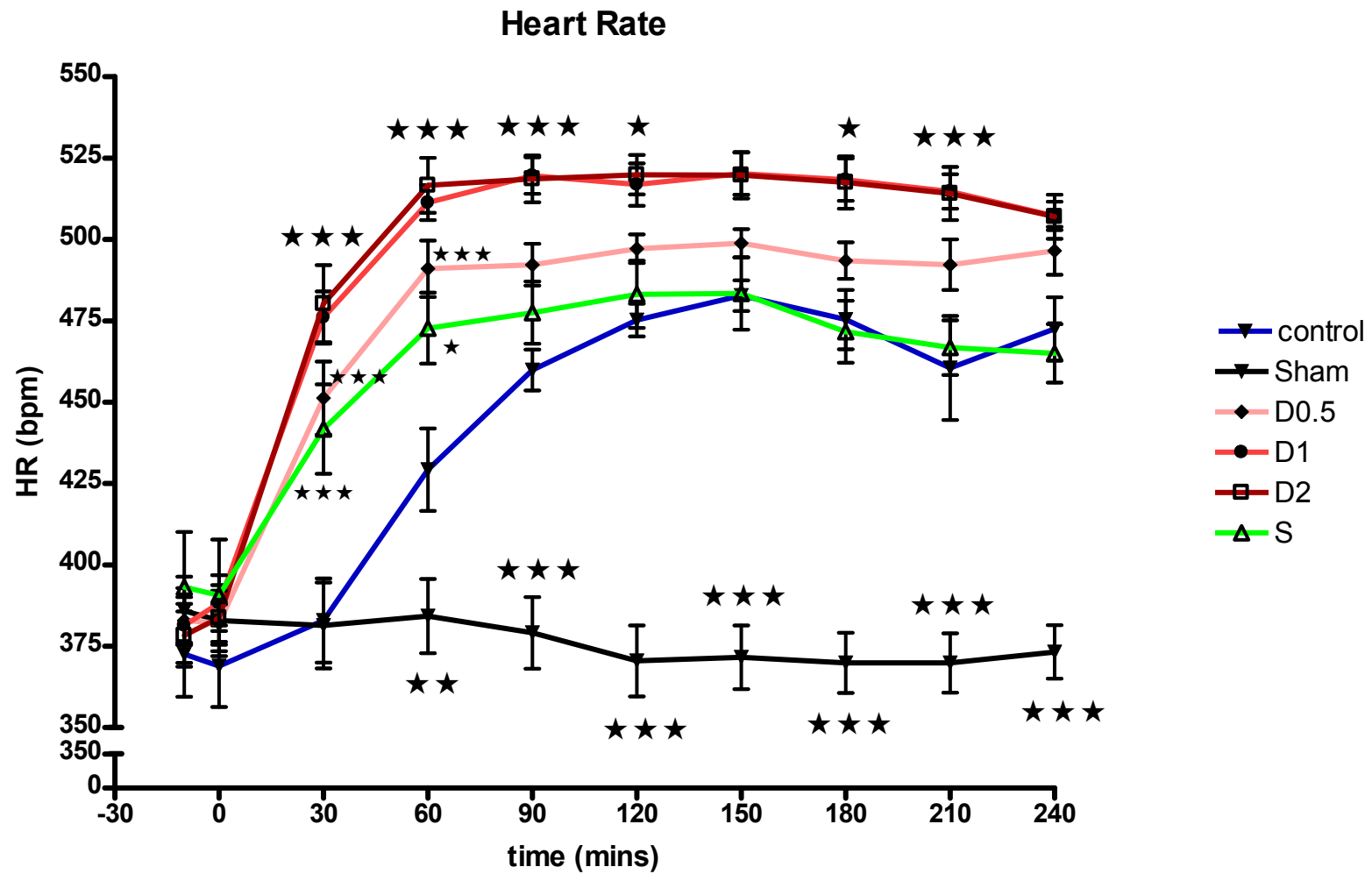
Figure 4.3   Heart rate for all groups

Two-way ANOVA (Bonferroni's post-tests, *P <0.05, **P<0.01, ***P<0.001 vs. controls)
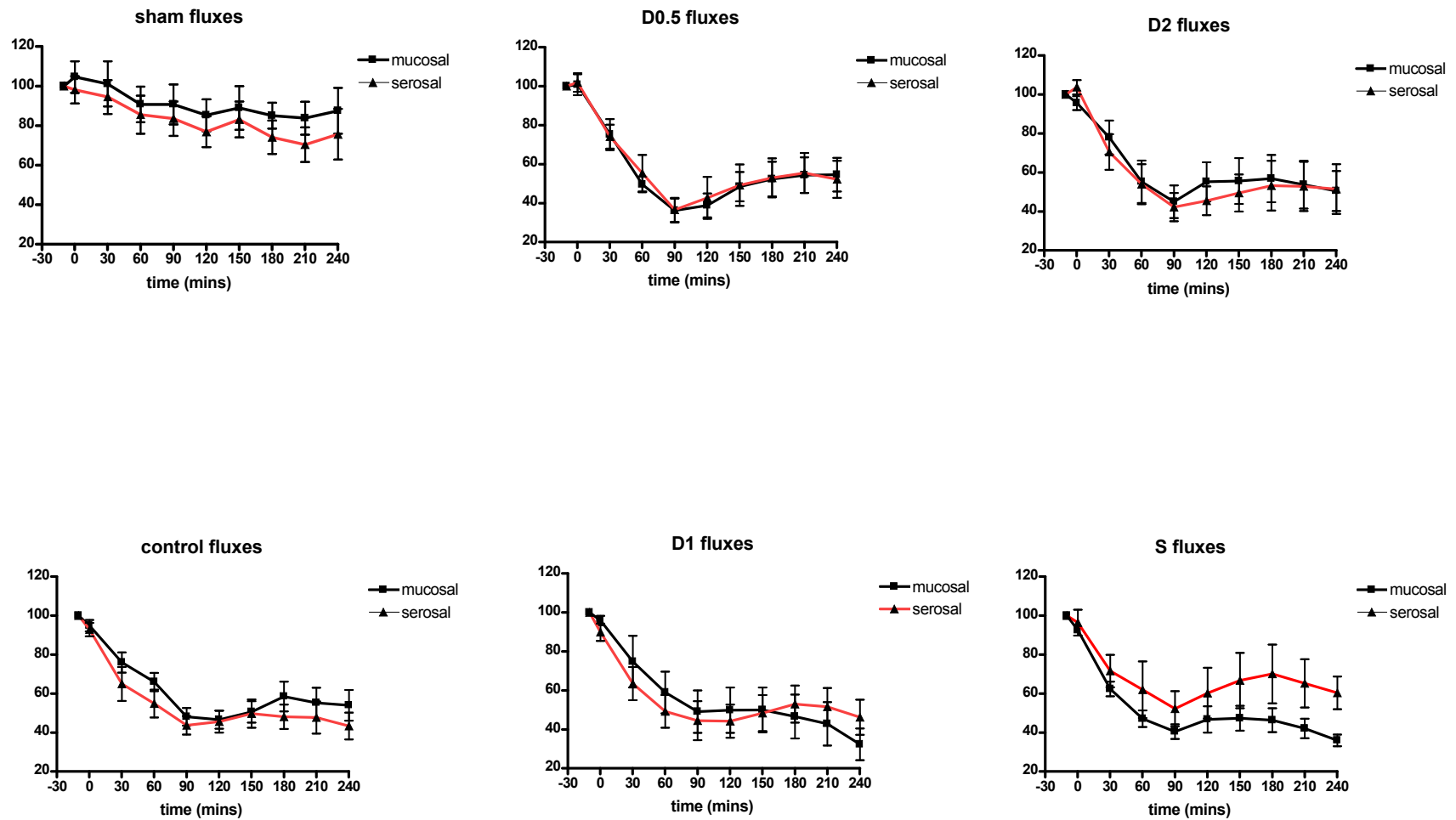
Figure 4.4 **Comparison of microvascular flux from mucosal and serosal probes for each group.**
Two-way ANOVA failed to demonstrate differences between mucosal and serosal red cell flux at any time for any group.

146
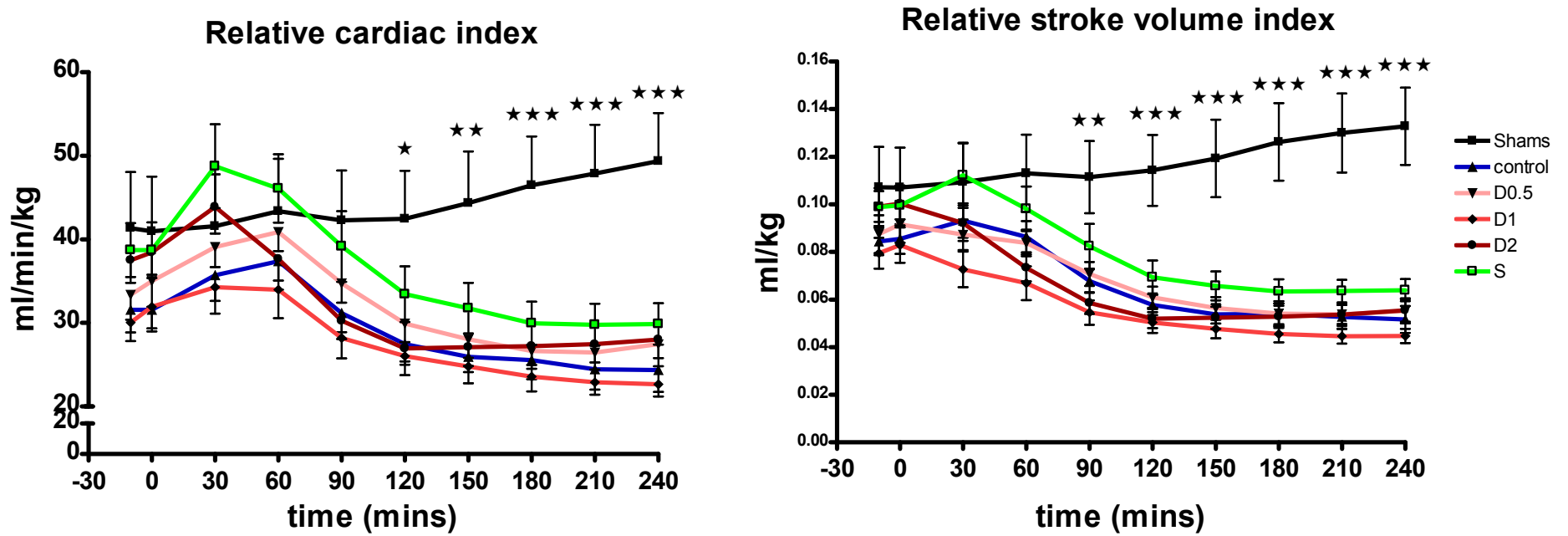
Figure 4.5   Relative cardiac indices and stroke volume indices

Two-way ANOVA (Bonferroni's post-tests *P<0.05, **P<0.01, ***P<0.001, vs. controls).

Stroke volume index significantly increased from baseline in controls only at 30 min. CI increased significantly from baseline in controls, D0.5 and S at 30 and 60 min, and in D2 at 30 min only (see Figure 4.6).
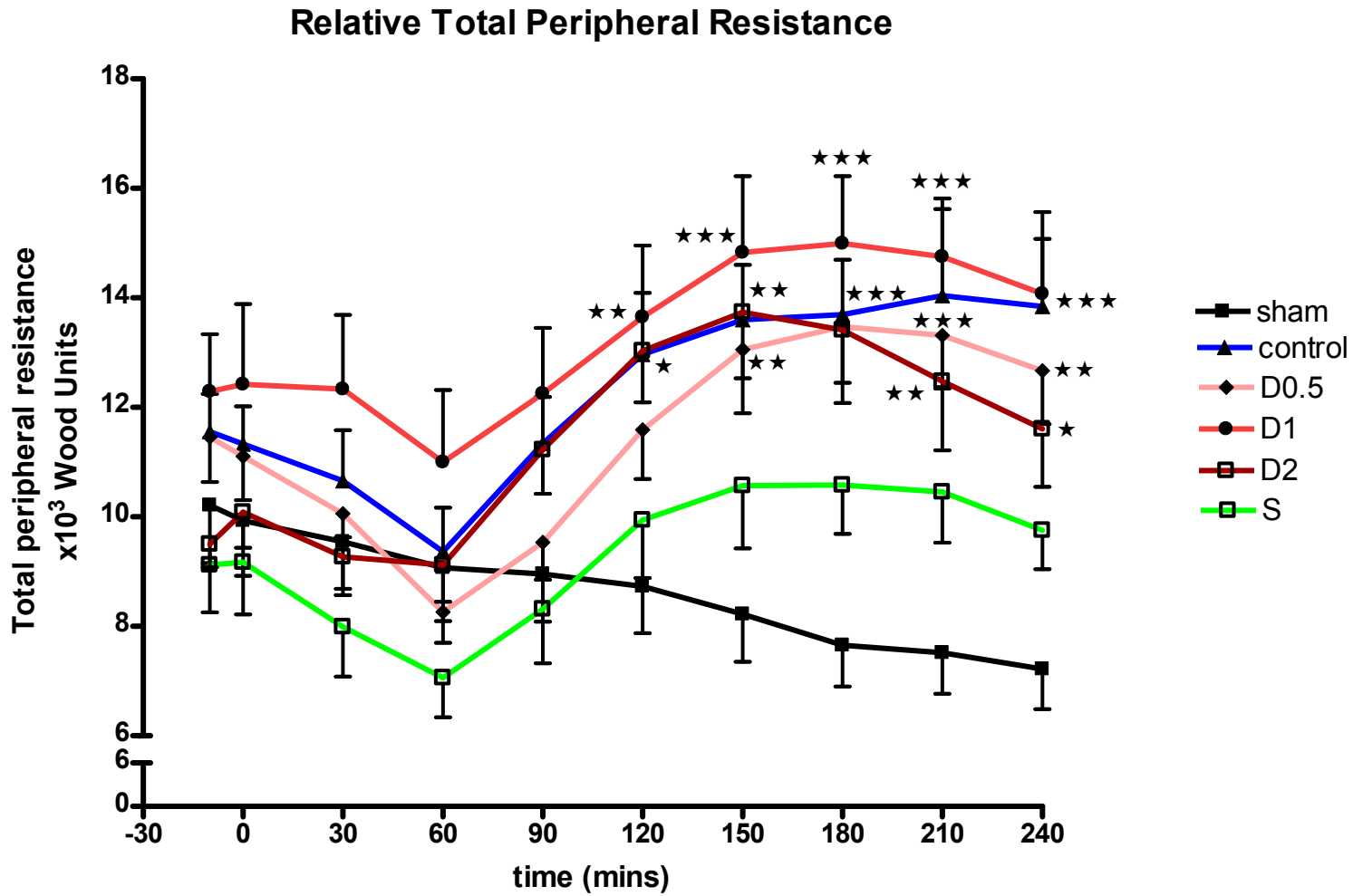
Figure 4.6   Relative TPR for all groups

Two-way   ANOVA   (Bonferroni's   post-tests,   *P<0.05,   **P<0.01,   ***P<0.001   vs.   shams)
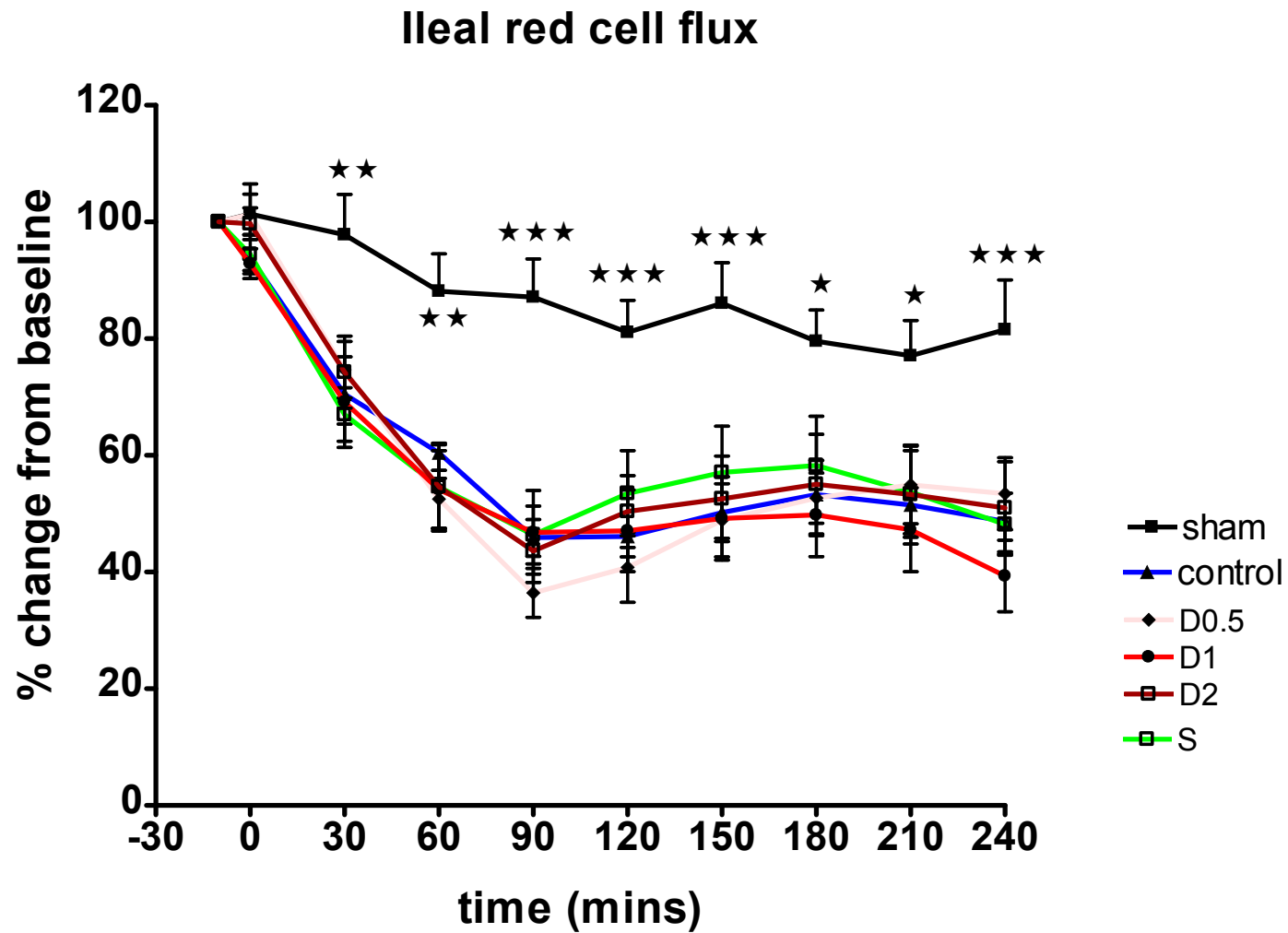
Figure 4.7   **Total ileal red flux for all groups**

Two-way   ANOVA   (Bonferroni's   post-tests   *   P<0.05,   **   P<0.01,   ***   P<0.001   vs.   controls)
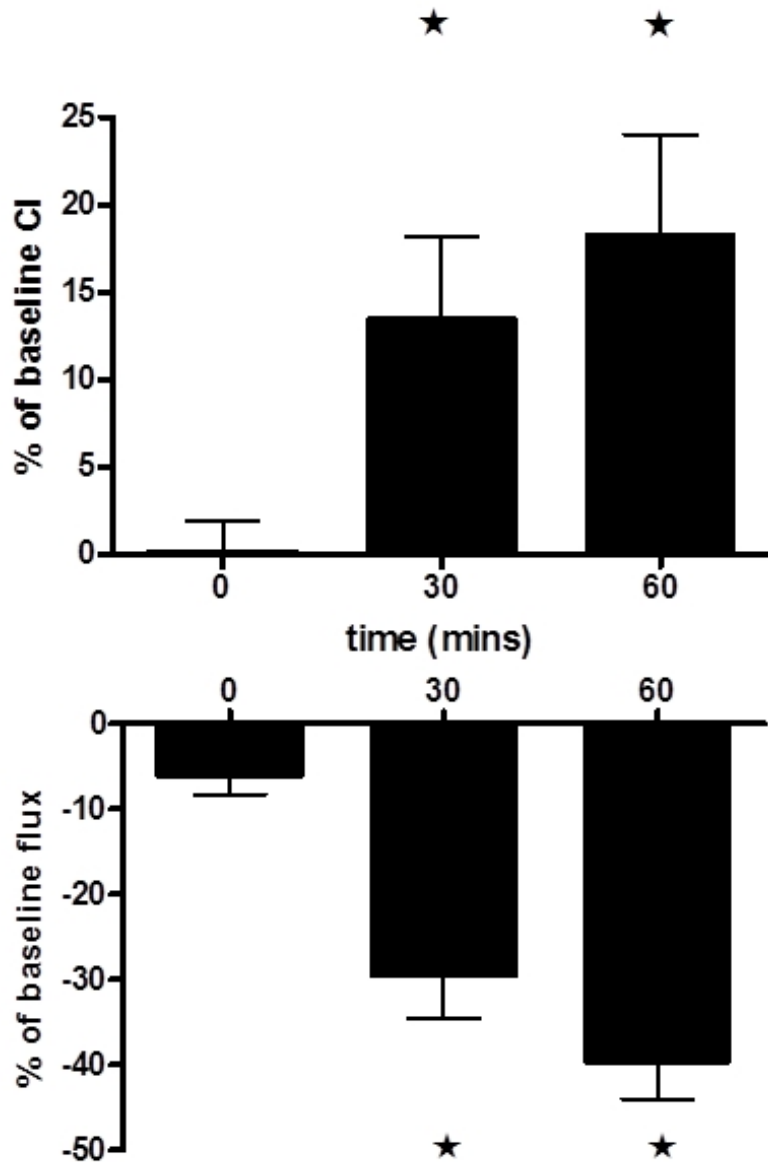
Figure 4.8 – Cardiac index vs. ileal flux in controls (first hour of experiment)

When compared to baseline, despite early and significant increases in relative cardiac index, ileal red cell flux decreased in the control group (Paired t-tests, *P<0.05 vs. baseline). Neither dopexamine at any dose, nor salbutamol significantly altered this profile.
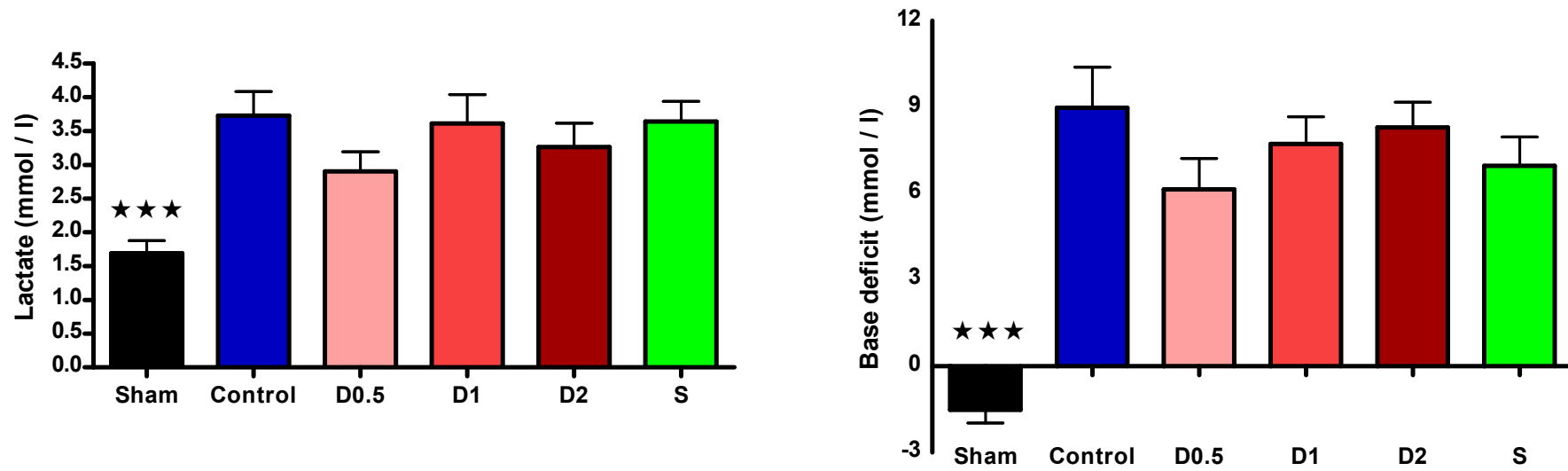
Figure 4.9   Indices of tissue perfusion.

Plasma lactate (n=8 all groups) and base deficit (D1 n=7, all others n=8): One-way ANOVAs (Bonferroni's post-tests vs. control: *** P<0.001)
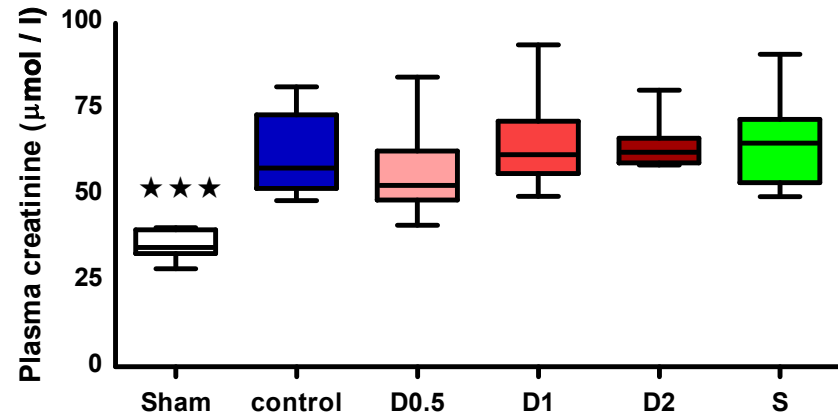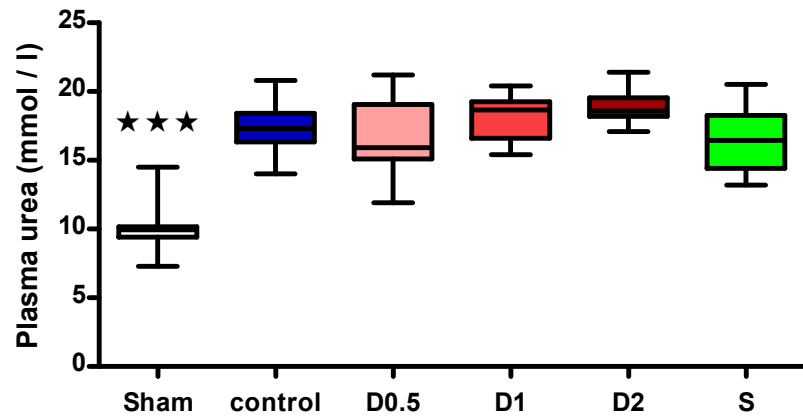
Figure 4.10

Plasma urea and creatinine (n=8 all groups)

Urea: Kruskal-Wallis test (*post hoc* Mann-Whitney (sham), ***P<0.001)
Creatinine: Kruskal Wallis test (*post-hoc* unpaired t-test (sham), ***P<0.001)
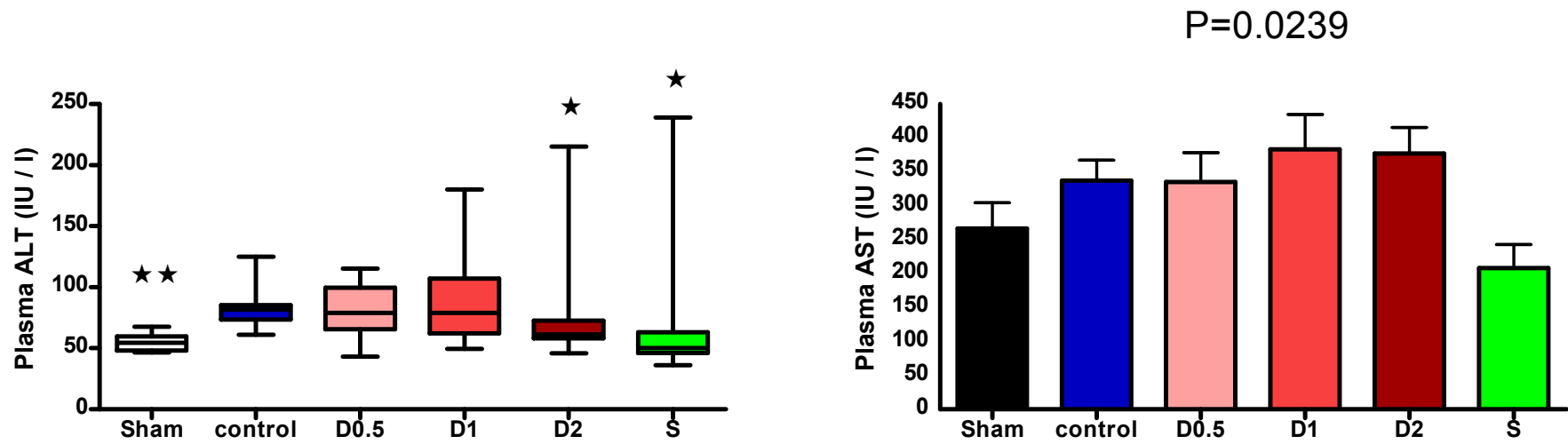
Figure 4.11   Plasma ALT and AST (n=8 all groups)

ALT: Kruskal Wallis test (*post hoc* Mann-Whitney post-testing *P<0.05, **P<0.01 vs. controls).
AST: One-way ANOVA P=0.0239 overall. Post-test not significant for any single group compared to control group

4.4 Discussion

This study showed that, in a rodent model of laparotomy and normotensive endotoxaemia, clinically relevant doses of dopexamine did not significantly alter haemodynamics. Early increases in CI were not sustained and over the four hour period CI and SVI decreased. Furthermore dopexamine failed to improve ileal red cell flux, as assessed by laser Doppler flowmetry. No beneficial effect of dopexamine on indices of tissue perfusion or organ function was demonstrable either, and only at the highest dose of dopexamine was an amelioration of plasma ALT seen. In this preparation salbutamol behaved in the same way as dopexamine, failing to ameliorate any haemodynamic or tissue perfusion indices and only ameliorating the increase in plasma ALT.

Several features of the model are consistent with similar studies, including the previous experiment, and are therefore reassuring. Endotoxaemia and bacteraemia are associated with significant increases in HR and usually a significant though only modest reduction in MAP in rodents (though this is dose dependent) (195, 196, 270-272, 277, 278, 349). Furthermore following LPS administration in rodents, intestinal microvascular blood flow decreases rapidly (by 1 hour) to 50% of baseline and remains depressed up to 4 h following a bolus of endotoxin (196, 356). This is in keeping with findings of intense splanchnic vasoconstriction in shock states (357), and the general increase in TPR seen in endotoxaemic controls here. However, the findings of this experiment (with respect to dopexamine) are not consistent with other studies where dopexamine was shown to preserve intestinal microvascular blood flow at these doses or where it ameliorated renal injury (196, 278). More importantly, the findings of this experiment with respect to the effect of dopexamine on indices of tissue perfusion and organ function are not consistent with those of the previous experiment which is arguably much closer in design to this than any other study.

The effects of dopexamine on biochemical indices of tissue perfusion and organ function in

this experiment go against those of the previous experiment and are therefore somewhat problematic. One possible explanation is that improvements in microvascular perfusion are required for improvements in biochemical indices of tissue perfusion and also in order to ameliorate organ function. Such a suggestion finds support in another study where intestinal microvascular blood flow was ameliorated by dopexamine in association with an amelioration of plasma TNF-α (196), as seen in the previous experiment. This may have been possible if dopexamine had prevented intestinal vasoconstriction, possibly resulting in a reduction in TPR. This did not occur though and partly supports the findings of no significant increase in intestinal microvascular flow with dopexamine in this experiment. On the other hand, salbutamol was not associated with the significantly higher levels of TPR seen in controls yet did not lead to any improvements in intestinal microvascular blood flow, in indices of tissue perfusion or organ function. This could be explained by preferential vasodilatation in other microvascular beds such as skeletal muscle though. If this was the case, minor differences in the experimental protocol may have led to subtle differences in fluid loading conditions, resulting in an improved perfusion in dopexamine treated animals in the first experiment but not this one (there is possibly some support for this when comparing dopexamine heart rates from the current and previous experiment – Figures 3.3 and 4.3). Assuming that microvascular perfusion was only significantly different for dopexamine across the two experiments could also explain why the results for sham, control and salbutamol experimental groups were consistent across the two experiments.

Although ileal red cell flux was not shown to improve in this experiment, subtle changes in microvascular behaviour may have occurred both in this and the previous experiment, but laser Doppler flowmetry may not have detected them (as this monitoring modality cannot provide data on qualitative changes in the microcirculation). However, this possibility would not explain why dopexamine failed to improve organ function in this experiment.

The findings with dopexamine in this experiment are also in contrast to those of a recent clinical study in high risk surgical patients which identified significant increases in tissue

microvascular flow and oxygenation after surgery (147). Although there was no increase in haematocrit in this experiment (suggesting the volume status of the animals did not deteriorate over the course of the experiment), several differences between this experiment and the clinical study (such as the nature and severity of the inflammatory insult, the quantity of fluid delivered and the differences between the subjects under study) could account for these differences.

Chapter 5 – Effects of dopexamine in a rodent model of laparotomy and normotensive endotoxaemia: macrohaemodynamics, intravital microscopy and effects on organ dysfunction

5.1 Introduction

The previous experiments demonstrated that dopexamine has anti-inflammatory actions in models of laparotomy and endotoxaemia - decreasing plasma cytokine levels, modulating neutrophil CD11b expression and decreasing neutrophil infiltration in the lung. Improving microvascular perfusion (and consequently tissue oxygenation) is thought by some to play a key role in how dopexamine exerts its beneficial effects, and indeed in the previous experiment when dopexamine did not augment microvascular blood flow, the striking improvements in organ function seen in the first experiment were not replicated. Why there was a discrepancy between experiments regarding the effects of dopexamine on organ function when the model used was remarkably similar is uncertain.

In this study the previous model of rodent laparotomy and endotoxaemia was modified in two ways. Firstly endotoxaemia involving LPS and PepG (as opposed to LPS alone) was used to assess the validity of the findings in a model closer to polymicrobial sepsis. Secondly the model was altered to allow the use of intravital microscopy to assess the effects of dopexamine on the intestinal microcirculation and on intestinal leucocyte-endothelial adhesion. Finally the comparator salbutamol dose was changed to match 1µg/kg/min dopexamine. Given the tendency to a lower MAP in the first experiment with 0.2µg/kg/min salbutamol it was hoped to avoid this possible confounder by selecting a lower dose of salbutamol. A 2µg/kg/min dopexamine group was no longer strictly necessary and furthermore the beneficial effects of dopexamine were seen at 1µg/kg/min.

By modifying the experiment it was aimed to reconfirm that dopexamine could improve organ function as previously shown, to assess whether there were subtle changes in the

microcirculation that were not detectable by laser Doppler flowmetry and to assess whether or not the improvements in organ function were linked purely to the immune effects of dopexamine.

5.2   Methods

30 male Wistar rats (240-340 g) received a standard diet and water ad libitum before experiments. All procedures were performed with institutional approval and in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. Anaesthesia was induced by intraperitoneal injection of thiopental (120 mg/kg) and maintained with supplementary injections administered according to regular testing for limb withdrawal to a standard stimulus or signs of inadequate anaesthesia. Animals were placed on a heated mat and maintained at 37 ± 0.5°C. A tracheostomy was performed, following which a short section of polyethylene tubing (internal diameter, 1.67 mm) was inserted to maintain airway patency and to facilitate spontaneous respiration. The right carotid artery was cannulated to allow blood sampling and continuous monitoring of heart rate (HR) and mean arterial pressure (MAP). The left jugular vein was cannulated for drug and fluid administration. A 2-cm midline incision was then made through the abdominal wall to expose the peritoneum. Following laparotomy, bowel was evacuated into a moist cotton receptacle. Blunt dissection was then performed to access the abdominal vasculature. After isolation from the vena cava, a 1.5-mm ultrasonic aortic transit time flow probe (MA1.5PRB; Transonic Systems Inc., Ithaca, NY, USA) was placed on the infra-renal aorta to measure aortic blood flow. The bowel was then replaced in the abdominal cavity, except for a loop of ileum just proximal to the cecum. The exposed bowel was kept moist by the application of 0.9% saline drops through a pipette. The laparotomy incision above and below the exit of the terminal ileal loop from the abdomen was then closed with 5.0 vicryl to prevent excessive insensible losses. The animal was then placed on an infra-red animal heating mat on a specially constructed intravital microscopy platform and placed in a right lateral position so

the ileal loop fell on to a raised section of the platform that was at was at the level of the laparotomy incision. The temperature of the raised section was thermostatically controlled at 37.5C in order to ensure the exposed bowel was not at a significantly lower temperature to core temperature. This manoeuvre did not interfere with the ability of the ultrasonic probes to measure aortic blood flow. Subsequently the bowel was covered with Saran wrap to prevent evaporative losses from its surface and maintain bowel microvascular integrity (322). This was followed by a 5ml/kg bolus of normal saline to make up for insensible losses and a 15 min stabilisation period when microvascular flow could settle and the animal could be re-warmed if necessary. A primary set of arterial blood samples was then taken (see below), the volume taken being replaced with an equal volume of normal saline. Animals were allowed to stabilize for 15 min before being allocated to one of five groups (sham, control, D0.5, D1, S).

Endotoxaemia was induced in four of five groups by administration of E*scherichia coli* lipopolysaccharide 0111:B4 (LPS - 1 mg/kg) and peptidoglycan (PepG - 0.3 mg/kg) over a 10 minute period (sham group receiving 0.9% saline vehicle). Sham and control animals received only 0.9% saline infusion at 4.3ml/kg/h. Two different concentrations of dopexamine were added to two of the remaining three groups' infusion fluid, producing dopexamine infusion rates of 0.5 and 1µg/kg/min for groups D0.5 and D1, respectively. Group S had salbutamol added to its resuscitation fluid to produce a salbutamol infusion rate of 0.1µg/kg/min when the fluid was infused at 4.3ml/kg/h. This dose of salbutamol was selected as previous studies conducted in isolated guinea–pig tracheal preparations showing a 10-fold greater potency of salbutamol at the β_2 adrenoceptor when compared with dopexamine (44). Intravital microscopy was performed half way through resuscitation (during which time measurements of global haemodynamics were not possible to perform). The experiment ended after 5 hours of resuscitation.

Analysis of plasma lactate, base deficit and renal and hepatic function

200µl of blood was taken at baseline and at the end of the experiment for measurement of plasma lactate concentration (Accutrend Lactate; Roche Diagnostics, Basel, Switzerland) and base deficit (Radiometer ABL77, Copenhagen, Denmark). A 1ml blood sample was also taken at the end of the experiment for measurements of urea, creatinine, alanine aminotransferase and aspartate aminotransferase by a commercial veterinary laboratory (IDEXX Laboratories Ltd, Sussex, UK) who were blinded to treatment.

Measurement of aortic blood flow

A 1.5-mm perivascular probe was applied with water-soluble sonicating gel and sited as described earlier. The probe was connected to a TS420 monitor (Transonic Systems Inc., Ithaca, NY, USA), which was connected to a Powerlab/8SP monitoring system (AD Instruments). This allowed continuous recording of aortic blood flow and waveform-derived HR and calculation of a measure of stroke volume. Aortic blood flow was indexed to body weight to provide a measure of changes in stroke volume index (SVI) and cardiac index (CI). Probe calibration was performed daily according to the manufacturer's instructions before experiments.

Intravital Microscopy

15 min before the midpoint of fluid resuscitation, 0.2mls of rhodamine 6G (Sigma Aldrich) was administered intravenously in order to better enhance the visibility of leucocytes during IVM. At the midpoint of resuscitation the platform was transferred to the stage of an intravital microscope and microscopy performed to assess leucocyte rolling and adhesion in intestinal venules. A further 0.2mls of FITC labelled albumin (Sigma Aldrich) was then administered intravenously in order to measure functional capillary density, arteriolar and venular

diameters. The platform was then removed from the stage and observations continued as before.

Statistical analysis

Kolmogorov-Smirnov normality testing was performed for all groups. Normally distributed data was tested using one-way analysis of variance (ANOVA) for comparison across all groups at a given time point. Post-testing was performed with Bonferroni's tests. When data was not normally distributed in at least one group for any measurement (e.g. AST), the Kruskal-Wallis test was used in place of one-way ANOVA, and appropriate t-tests against controls for post-testing depending on whether the individual groups were normally distributed or not. Two-tailed paired t-tests were used to compare haemodynamics at baseline with those at other time points for animals within the same group. Data were analysed with PrismGraph 4.0 (GraphPad Software, San Diego, USA). Significance was set at $P<0.05$.

5.3 Results

There was no statistically significant difference between control and other groups regarding weight or volume of fluid received, and only the sham group required significantly more anaesthetic to remain anaesthetised for the duration of the experiments than controls (Table 5.1). There was no significant difference in baseline haemodynamics, base deficit, lactate or haematocrit between controls and any group, and no significant increase in haematocrit over the period of resuscitation for any group (Table 5.2).

In the sham group, MAP and HR did not change significantly but CI and SVI increased progressively (Table 5.3, Figures 5.1 to 5.4). Compared with the sham group and baseline, controls had a significantly higher HR ($P < 0.05$) and a significantly lower SVI and CI at 5 hours (Figures 5.2 to 5.4). MAP was an average of 23mmHg lower compared with baseline

	Sham	*Control*	*D 0.5*	*D1*	*S*
Weight (g)	295 (250 – 318)	305 (290 – 320)	310 (290 – 330)	320 (300 – 335)	300 (290 – 305)
Fluid (ml/kg)	29.9 (29.2 – 30.5)	29.4 (29.3 – 29.5)	29.8 (29.4 – 30.5)	30.1 (29.4 – 30.5)	29.8 (29.4 – 30.4)
Thiopental (mg/kg)	186.6 (8.8)**	141.4 (5.0)	141.6 (6.4)	142.2 (7.0)	157.5 (7.3)

Table 5.1   Baseline characteristics for experiment 3

(n=6 all groups). Data presented as mean (SEM) when all groups normally distributed, otherwise median (IQR) if ≥ 1 group not normally distributed

One-way ANOVA (post hoc Bonferroni's test, **$P<0.01$ vs. controls)

at this time though the result was not significant ($P=0.052$) (Figure 5.1). At this point control group plasma base deficit and lactate were increased compared with sham animals, the latter significantly ($P < 0.05$) (Figure 5.5). With regard to organ dysfunction, endotoxaemia also resulted in organ injury (the changes did not reach statistical significance for ALT on post-testing) (Figures 5.6 and 5.7). When IVM was commenced, control group SVI and CI were significantly less than shams (Figures 5.3 and 5.4) and TPR was significantly greater (Figure 5.8). In association with the increased TPR, intestinal arteriolar A1 and A3 diameters were significantly smaller than in shams (both $P < 0.01$, Figure 5.9). However, neither FCD in any layer of the intestinal muscularis nor intestinal venular diameters were significantly different (Figure 5.10 and 5.11). Compared to shams, the number of leucocytes seen to be rolling or firmly adherent in V3 venules at this time was significantly reduced and increased, respectively, in the control group (Figures 5.12 and 5.13). However, in V1 venules there was no statistically significant difference in median values of rolling or adherent leucocytes.

162

	Sham	*Control*	*D 0.5*	*D1*	*S*
Initial HR (bpm)	378 (357 – 421)	399 (379 – 417)	415 (390 – 420)	379 (356 – 441)	392 (370 – 423)
Final HR (bpm)	371 (8)***	447 (12)	465 (15)	478 (9)	445 (5)
Initial MAP (mmHg)	120 (7)	111 (3)	114 (5)	108 (3)	110 (6)
Final MAP (mmHg)	114 (95 – 133)	93 (69 – 101)	81 (76 – 106)	94 (79 – 106)	78 (70 - 106)
Initial haematocrit (%)	39.0 (35.5 – 42.5)	42.5 (38.0 – 43.5)	45.5 (39.0 – 49.0)	42.0 (41.5 – 43.5)	40.5 (36.0 – 45.5)
Final haematocrit (%)	42.5 (1.4)	41.0 (1.0)	42.8 (0.6)	39.3 (1.0)	37.5 (2.2)
End experiment lactate (mmol/L)	1.7 (0.2)*	3.4 (0.5)	3.1 (0.3)	2.6 (0.4)	3.8 (0.3)
End experiment base deficit (mmol/L)	- 0.6 (1.0)	4.5 (1.6)	2.9 (1.5)	3.3 (1.5)	6.0 (1.0)
End experiment pH	7.38 (0.02)	7.39 (0.02)	7.36 (0.01)	7.39 (0.02)	7.34 (0.02)
End experiment P_aCO_2 (kPa)	5.9 (0.4)*	4.3 (0.4)	4.9 (0.3)	4.7 (0.4)	4.7 (0.1)
End experiment P_aO_2 (kPa)	11.1 (10.0 – 12.0)	12.7 (11.7 – 13.6)	11.6 (10.2 – 12.6)	10.2 (9.9 – 13.8)	11.1 (9.9 – 12.3)

Table 5.2   Baseline and end experiment haemodynamic and blood gas data for experiment 3

(n=6 all groups). Data presented as mean (SEM) when all groups normally distributed, otherwise median (IQR) if ≥ 1 group not normally distributed

One-way ANOVA (post hoc Bonferroni's test, *P<0.05, ***P<0.001 vs. controls)

The infusion of dopexamine at any dose had no significant effect on any haemodynamic parameter when compared to controls, except heart rate (Figure 5.2). Although plasma lactate and base deficit was lower in dopexamine treated animals than in controls, these results were not significant (Figure 5.5). Although plasma creatinine was improved in the D1 group and there were trends to improvements in creatinine kinase, hepatic organ injury was not ameliorated by dopexamine (Figures 5.6 and 5.7). Unlike controls, A1 and A3 arteriolar diameters in dopexamine treated groups were not significantly smaller than shams and TPR was not significantly increased

compared to shams (Figures 5.8 and 5.9). Muscularis FCD and intestinal venular diameters were not affected by dopexamine infusion (Figures 5.10 and 5.11). Although dopexamine infusion had no significant effect on leucocyte rolling and adhesion in V1 intestinal venules, there was a significant amelioration of leucocyte adhesion in V3 venules compared to controls (Figures 5.12 and 5.13).

	Sham	*Control*	*D0.5*	*D1*	*S*
HR	-14bpm (11) NS	49bpm (10) (P<0.005)	57bpm (11) (P<0.005)	87bpm (18) (P<0.005)	54bpm (9) (P<0.005)
MAP	6mmHg (10) NS	-23mmHg (9) (P=0.052)	-27mmHg (6) (P<0.05)	-15mmHg (6) NS	-26mmHg (8) (P<0.05)
SVI	0.044 (0.014) (P<0.05)	-0.036 (0.008) (P<0.01)	-0.050 (0.009) (P<0.005)	-0.054 (0.003) (P<0.0001)	-0.020 (0.011) NS
CI	15.3 (6.2) NS	-10.5 (3.2) (P<0.05)	-16.1 (4.2) (P<0.05)	-14.4 (2.0) (P<0.001)	3.0 (4.9) NS
TPR	-2.23 (1.02) NS	0.49 (0.83) NS	1.33 (0.82) NS	2.17 (0.46) (P<0.01)	-1.48 (0.70) NS

Table 5.3   Mean changes in haemodynamic parameters from baseline to end experiment for experiment 3

(n=6 all groups). Data presented as mean (SEM) when all groups normally distributed, otherwise median (IQR) if ≥ 1 group not normally distributed

P values represent the results of paired t-tests of baseline vs. end experiment values for each group
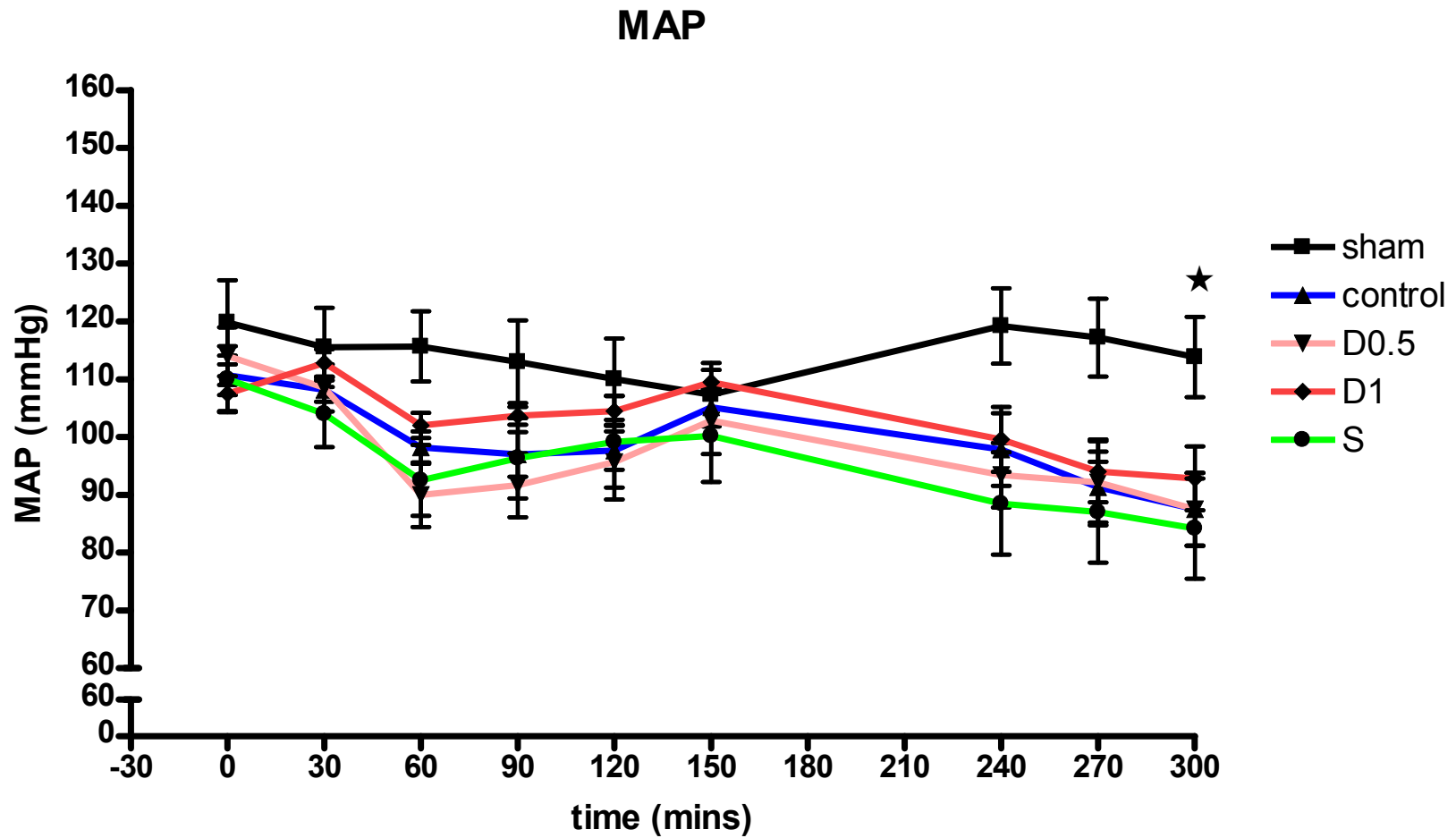
Figure 5.1   MAP for all groups

One-way ANOVA at each time point (Bonferroni's post-tests, *P<0.05 vs. controls)

Values are not plotted for t180 – t210 as animals were undergoing IVM at this time and it was impossible to measure MAP
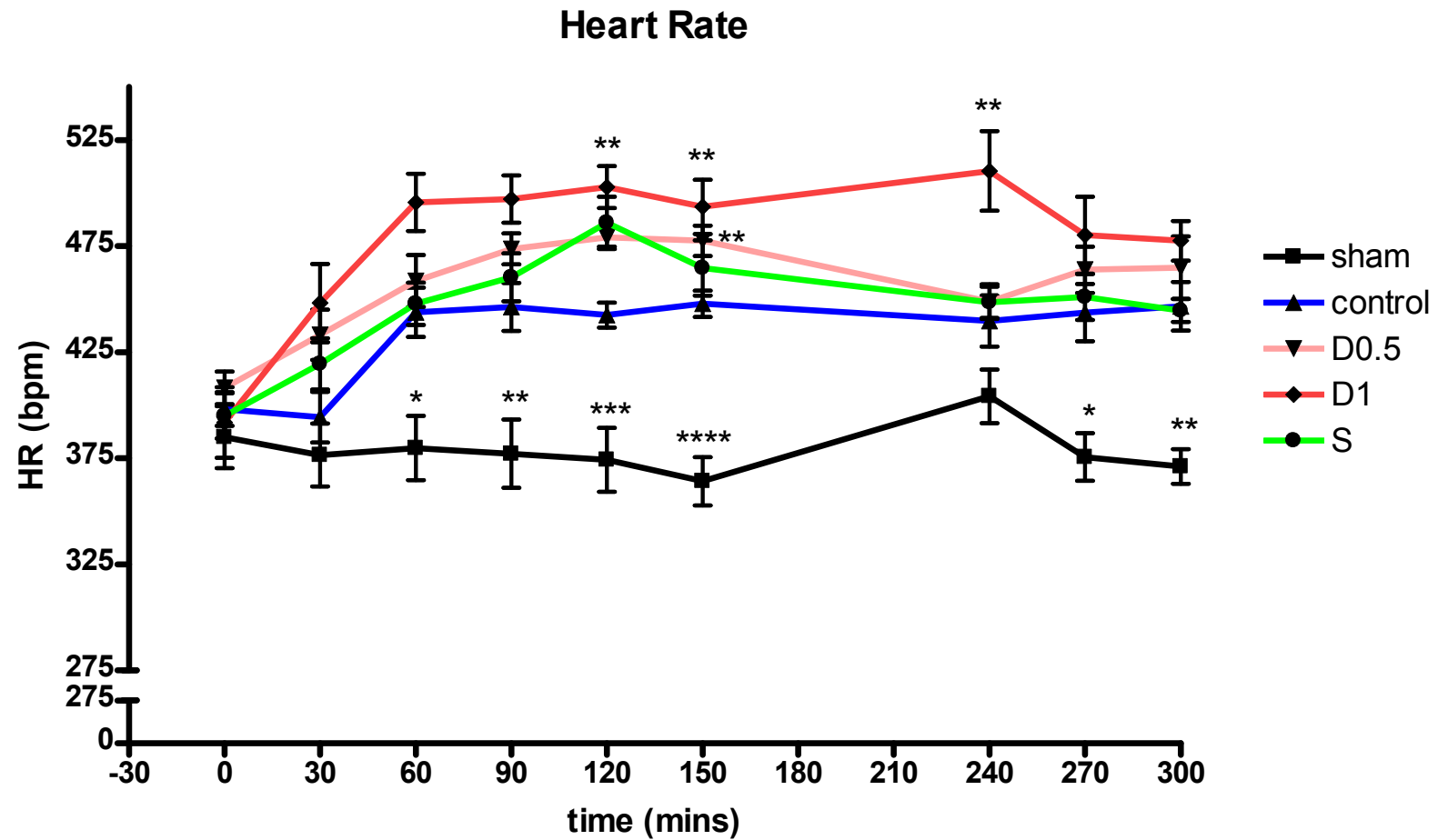
Figure 5.2   Heart rate for all groups

One-way ANOVA at each time point (Bonferroni's post-tests, * P<0.05, ** P<0.01, ***P<0.001 vs. controls)

Values are not plotted for t180 – t210 as animals were undergoing IVM at this time and it was impossible to measure HR

Relative Stroke Volume Index
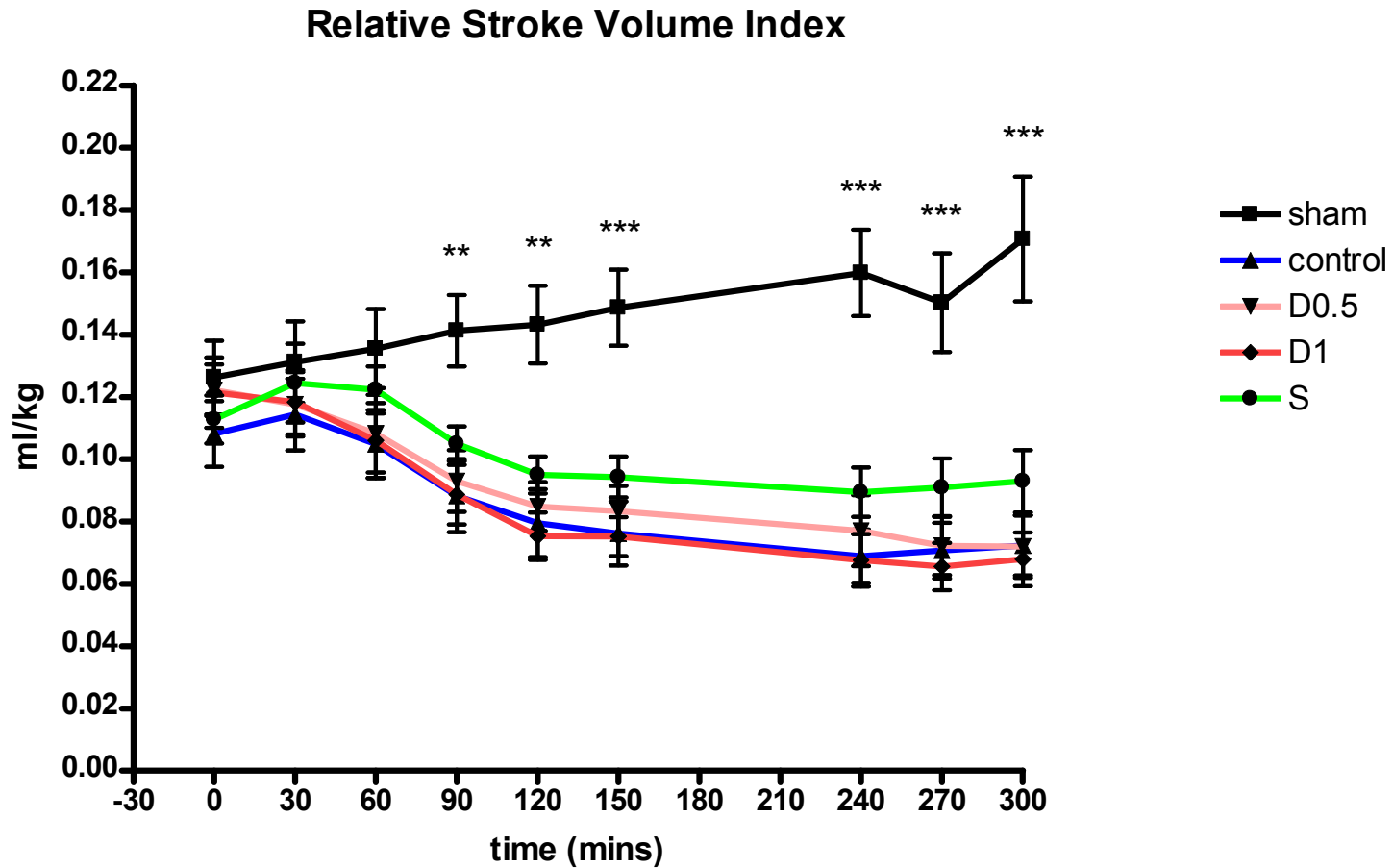
Figure 5.3   Relative SVI for all groups

One-way ANOVA at each time point (Bonferroni's post-tests, *P<0.05, **P<0.01, ***P<0.001 vs. controls)

Values are not plotted for t180 – t210 as animals were undergoing IVM at this time and it was impossible to measure HR and aortic flow (and therefore calculate relative SVI)
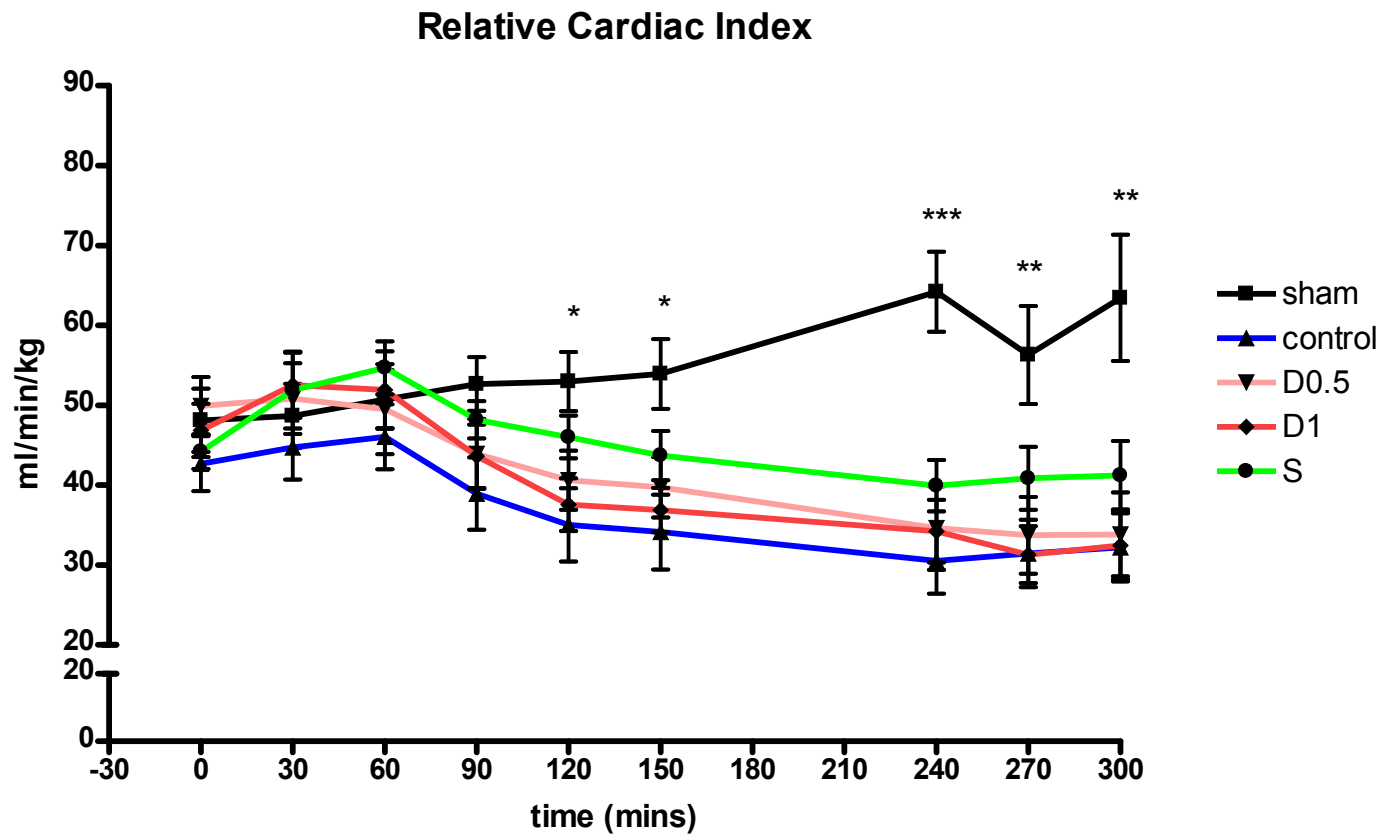
Figure 5.4   Relative CI for all groups

One-way ANOVA at each time point (Bonferroni's post-tests, *P<0.05, **P<0.01, ***P<0.001 vs. controls)

Values are not plotted for t180 – t210 as animals were undergoing IVM at this time and it was impossible to measure aortic flow (and therefore calculate relative CI).
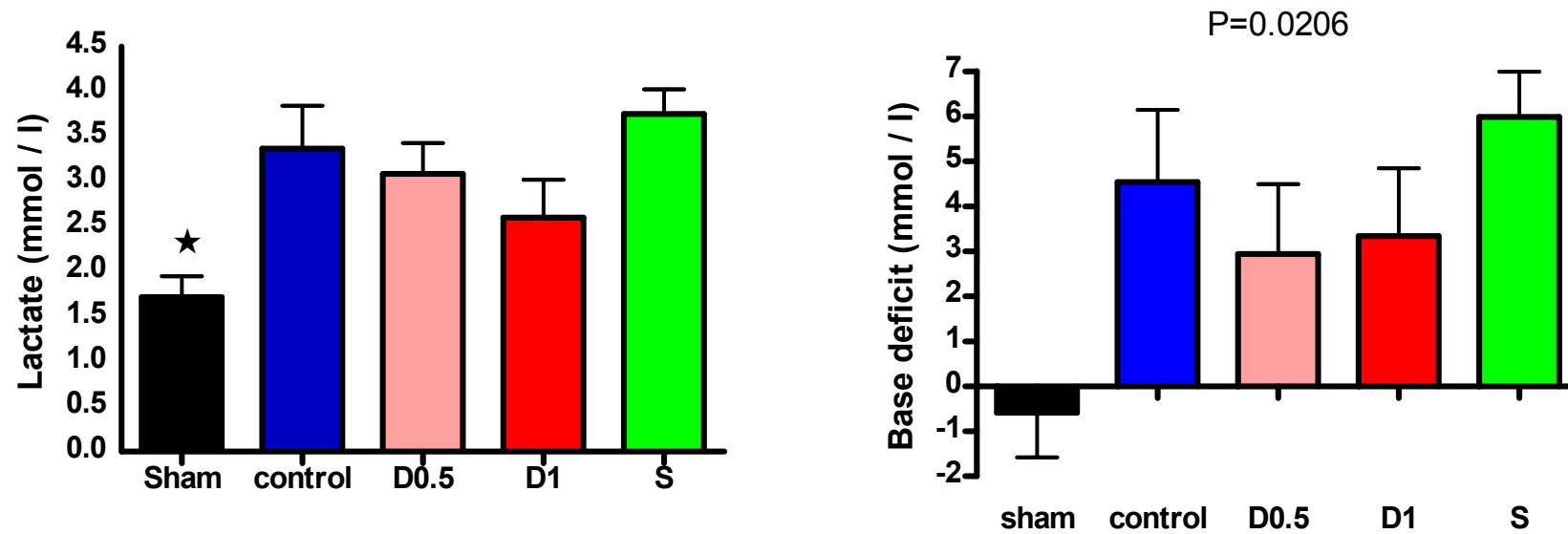
Figure 5.5   Indices of tissue perfusion.

Plasma lactate (n=6 all groups) and base deficit (control and D0.5 n=5 each, all others n=6):
Lactate: One-way ANOVAs (Bonferroni's post-tests, *P<0.05 vs. control)
Base   deficit: One-way   ANOVA   P=0.0206   (no   groups   positive   in   post-test).
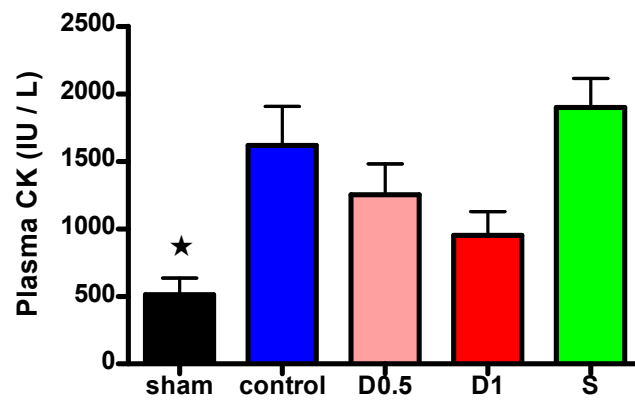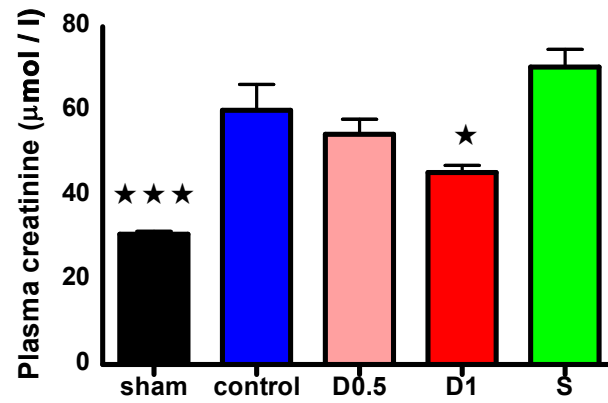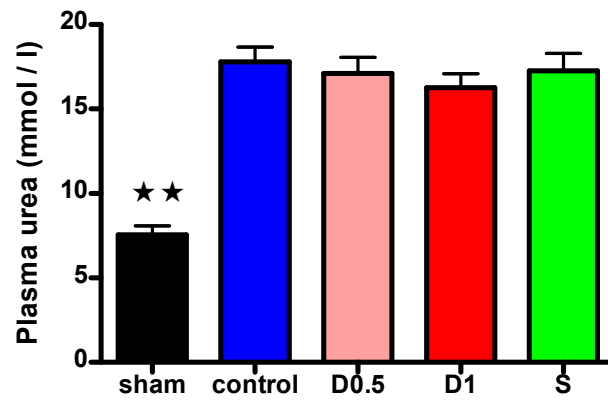
Figure 5.6   Plasma urea, creatinine and creatinine kinase

(n=6 all groups). One-way ANOVA (Bonferroni's post-tests, *P<0.05, **P<0.01, ***P<0.001 vs. controls)
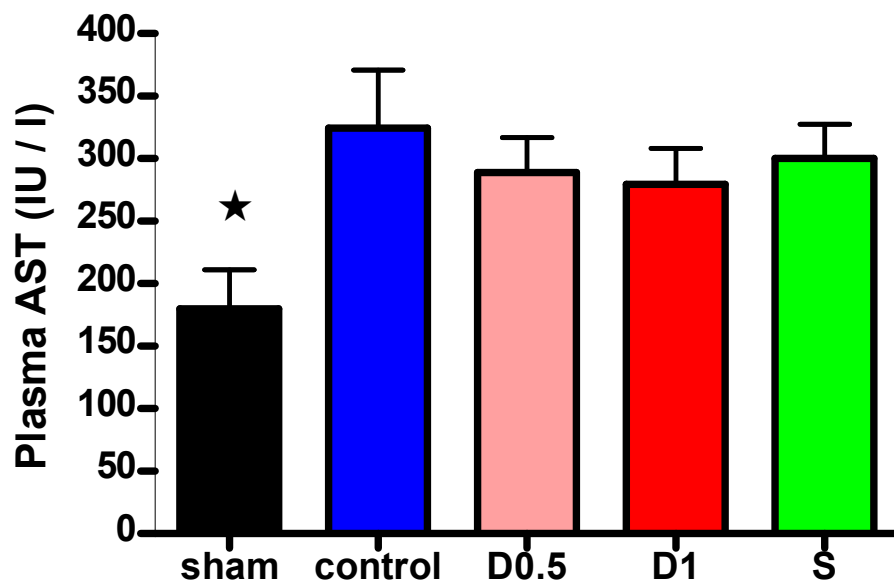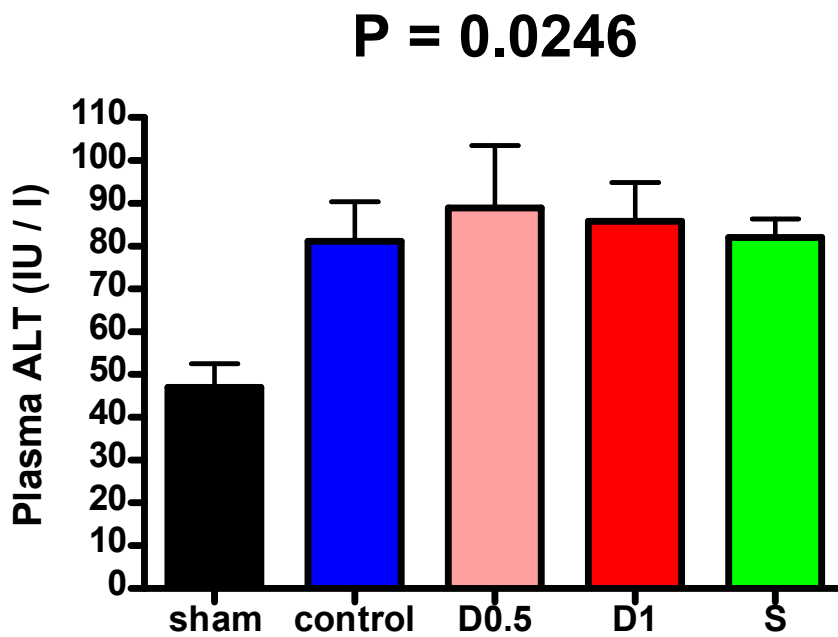
Figure 5.7   Plasma ALT and AST for all groups

(n=6 all groups)

ALT: One-way ANOVA, P=0.0246 (no groups positive in post-tests)

AST: One-way   ANOVA   (Bonferroni's   post-tests,   *P<0.05   vs.   controls)
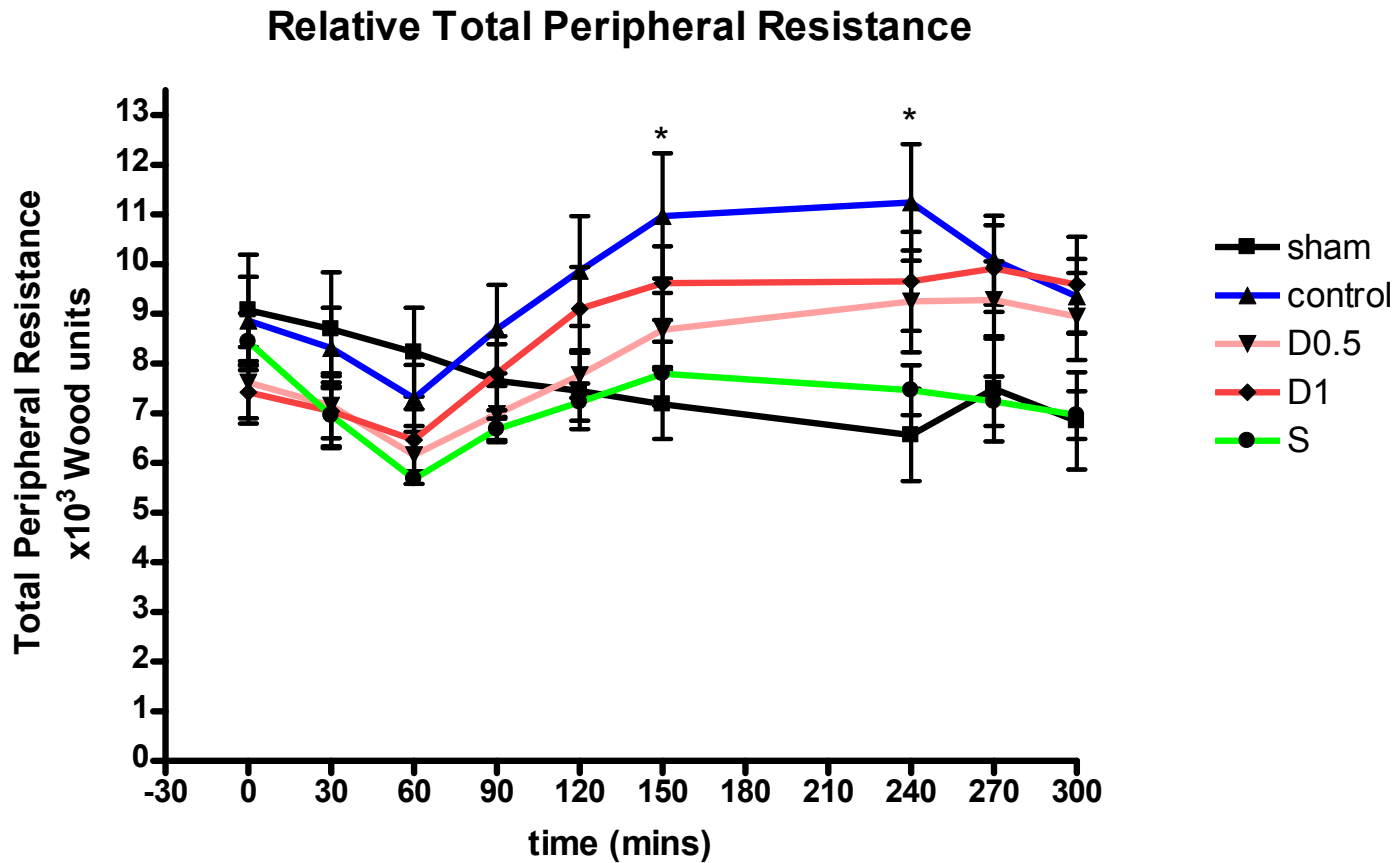
Figure 5.8   Relative TPR for all groups

One-way ANOVA at each time point (Bonferroni's post-tests, *P<0.05 vs. shams)

Values are not plotted for t180 – t210 as animals were undergoing IVM at this time and it was impossible to measure MAP and aortic

flow (and so calculate relative TPR).

The infusion of salbutamol produced a similar pattern of haemodynamics to that seen with dopexamine. However, there was no suggestion of any improvement in indices of tissue perfusion and no evidence of any amelioration of organ injury whatsoever (Figures 5.5 to 5.7). IVM showed that while salbutamol had a similar effect on A3 arteriolar diameters as dopexamine did, it was unable to prevent the significant constriction seen in A1 arterioles (Figure 5.9) though TPR was similar to that seen in shams. There were no discernable effects on muscularis FCD or intestinal venular diameters though. With respect to leucocyte rolling and adhesion, salbutamol significantly ameliorated the decrease in V3 venular leucocyte rolling and significantly decreased V3 venular adherent leucocyte density when compared to controls (Figures 5.12 and 5.13). It showed no significant effect on leucocyte rolling and adhesion in V1 intestinal venules.

A1 diameters
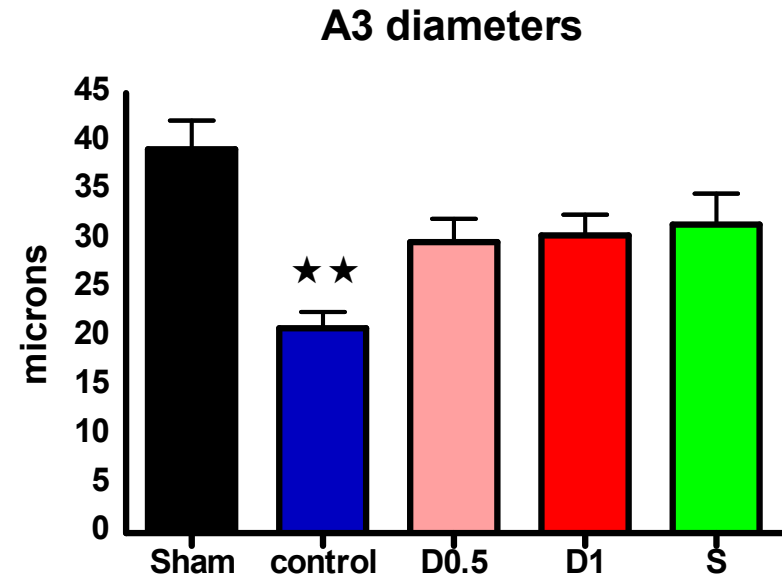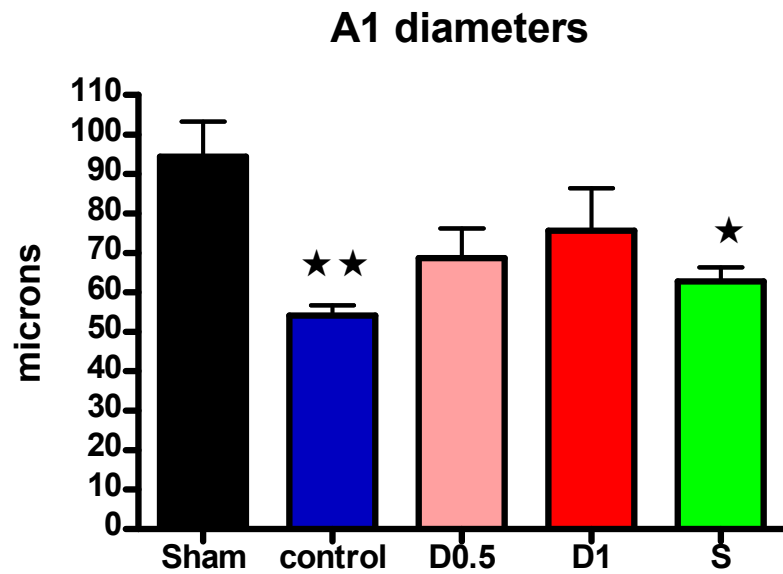
A3 diameters

Figure 5.9   Intestinal arteriolar diameters (n=6 rats per group)

One-way ANOVA (Bonferroni's post-tests, *P<0.05 and **P<0.01 vs. shams)

A1: Numbers of vessels measured per group ranged from 10-17
A3: Numbers of vessels measured per group ranged from 8-19

FCD longitudinal

FCD circular
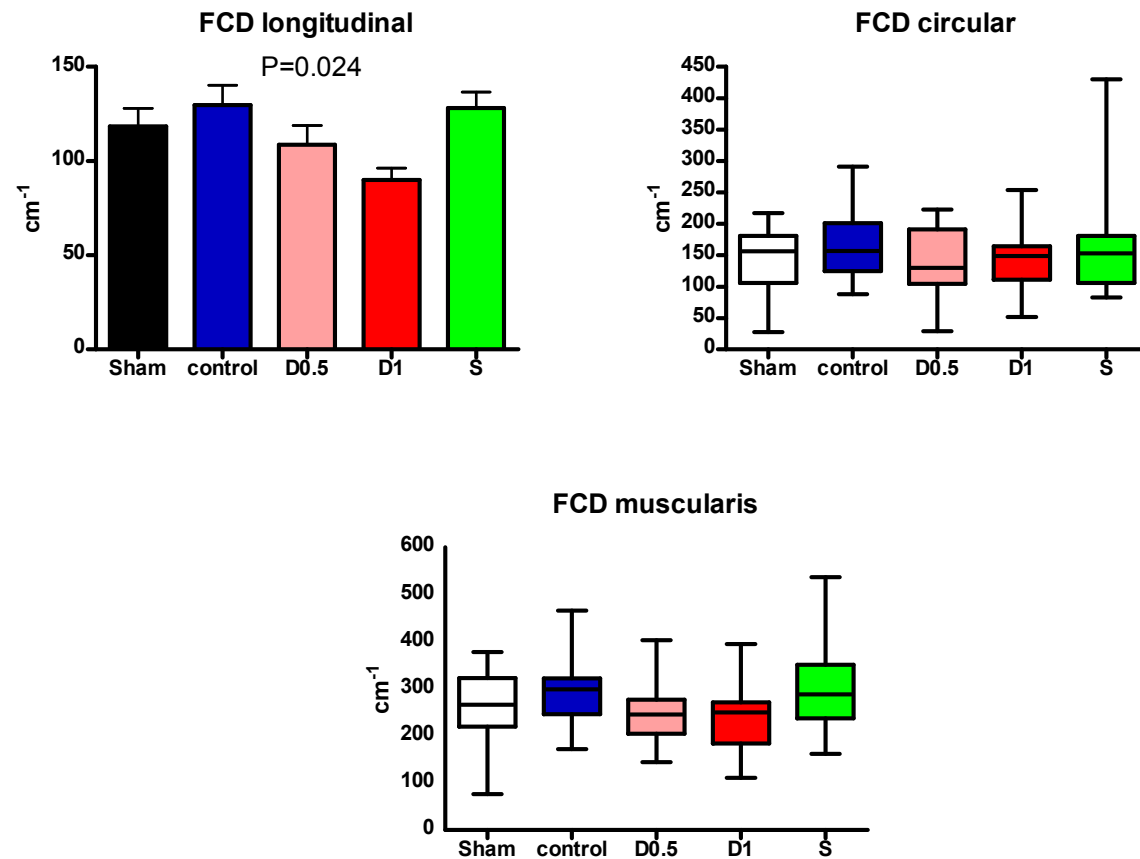
FCD muscularis

Figure 5.10 Intestinal functional capillary density in longitudinal and circular layers of the muscularis (n=6 rats per group)

FCD longitudinal: One-way ANOVA P=0.024 (no groups positive in post-tests)
FCD circular ns, FCD muscularis ns (P=0.058)

Number of images per group ranged from 16-25
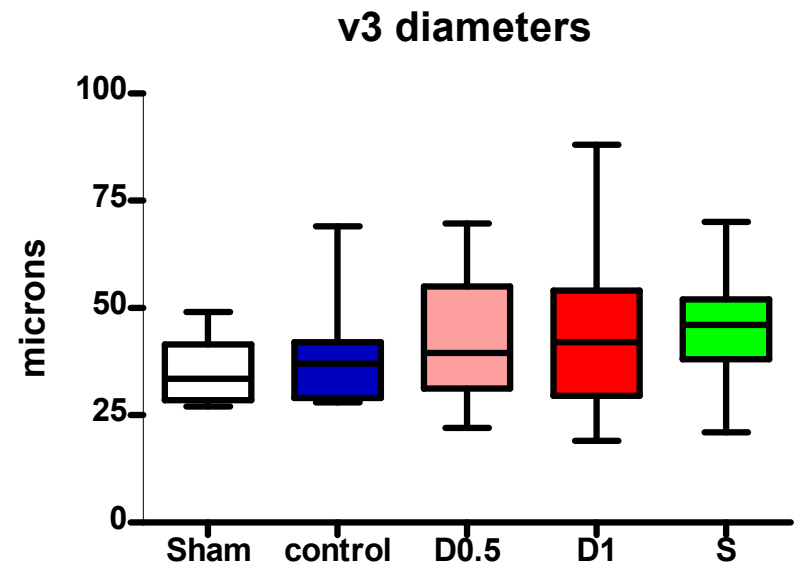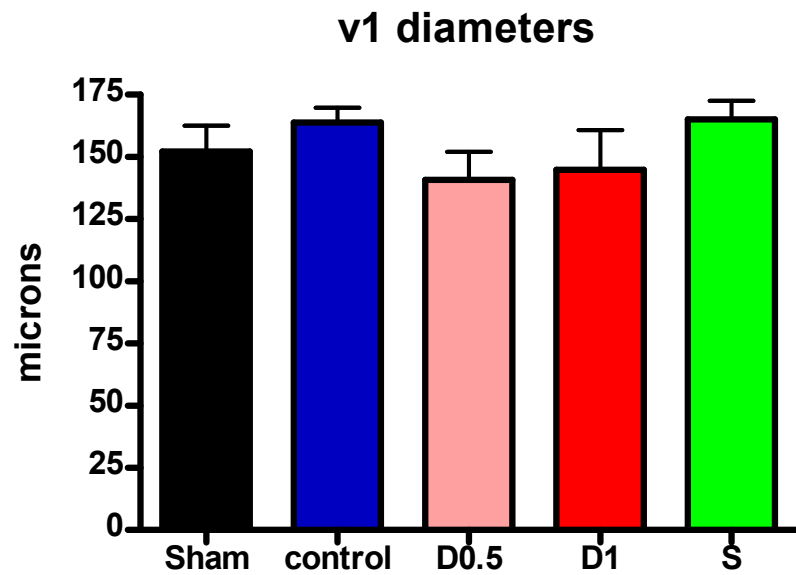
Figure 5.11   Intestinal post capillary venular diameters (n=6 rats per group)

V1: Numbers of vessels measured per group ranged from 13-18
V3: Numbers of vessels measured per group ranged from 8-20
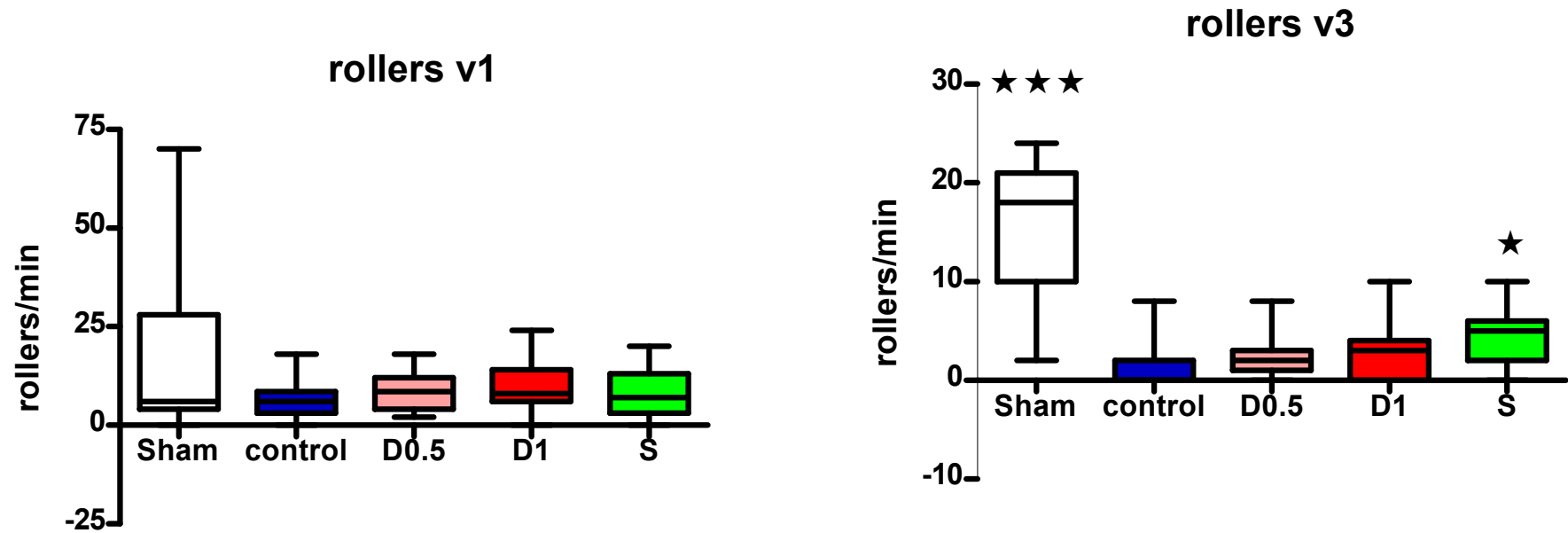
Figure 5.12   Leucocyte rolling in V1 and V3 ileal post-capillary venules (n=6 rats per group)

V3 rollers, Kruskal Wallis test (Mann-Whitney post-tests, *P<0.05, ***P<0.001 vs. controls)

V1: Numbers of vessels observed per group ranged from 15-18
V3: Numbers of vessels observed per group ranged from 8-18
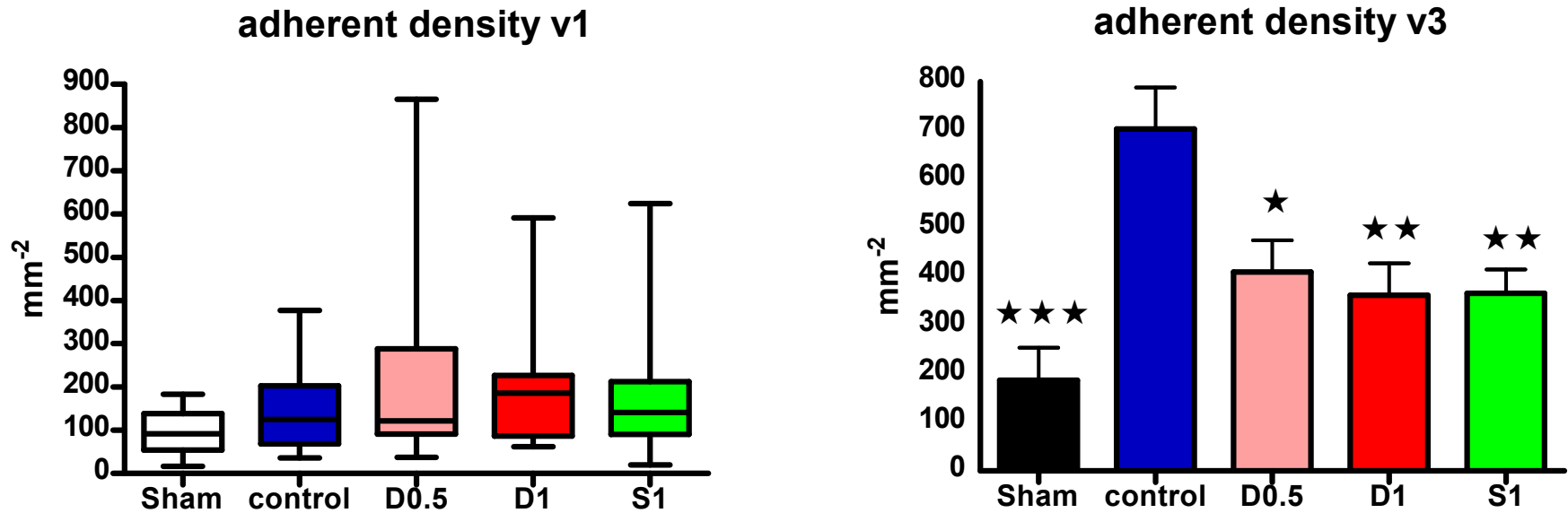
Figure 5.13   Leucocyte adhesion in V1 and V3 ileal post-capillary venules (n=6 rats per group)

V3 adherent, One-way ANOVA (Bonferroni's post-tests, *P<0.05, **P<0.01, ***P<0.001 vs. controls)

V1: Numbers of vessels observed per group ranged from 13-18
V3: Numbers of vessels observed per group ranged from 8-18

5.4 Discussion

This study showed that, in a rodent model of laparotomy and normotensive endotoxaemia, clinically relevant doses of dopexamine could attenuate the systemic inflammatory response and protect against renal injury as evidenced by a reduction in leucocyte adhesion in post-capillary intestinal venules and an amelioration of the rise in plasma creatinine. Although no discernible effect of endotoxaemia could be found on muscularis FCD, dopexamine prevented the significant arteriolar constriction that was seen in control A1 and A3 intestinal arterioles and in this manner altered the microcirculation. No significant effect of dopexamine on indices of tissue perfusion was demonstrable (though there were trends to improvement). Interestingly these effects occurred at doses of dopexamine which had little or no effect on systemic haemodynamics such as MAP, SVI and CI.

Salbutamol produced similar haemodynamics to dopexamine and also ameliorated leucocyte adhesion in post-capillary venules. Nevertheless salbutamol was unable to ameliorate renal (or hepatic) injury, did not improve indices of tissue perfusion and only prevented significant constriction in intestinal A3 arterioles though it was not associated with the high TPR seen in controls. This supports the suggestion made in the previous experiment that salbutamol is also a potent vasodilator in non-splanchnic microvascular beds. The findings with both salbutamol and dopexamine suggest that in this experiment macrohaemodynamics are not related to the improvements in organ function seen with dopexamine, that the inhibition of intestinal leucocyte adhesion is β_2-adrenoceptor mediated, but that these effects are possibly less important in improving organ function than the subtle and specific effects dopexamine produces in the intestinal microcirculation.

In this regard it is worth noting that the distribution of blood flows in the intestinal wall is not uniform. There is a 3-4 fold higher blood flow in the mucosa compared to the muscularis. In response to hypoperfusion splanchnic vasoconstriction occurs and there is a neuronally mediated relative preservation of blood flow to the hypoxia-prone mucosa when compared to

179

the muscularis (358-360). The preservation of blood flow to the mucosa while overall splanchnic vasoconstriction is occurring is the result of autoregulatory mechanisms that allow the mucosa to escape from vasoconstrictive factors that affect the muscularis (361). However, with respect to intestinal mucosal blood flow in endotoxaemia, two studies in rodents show a significant decrease in intestinal arteriolar villous diameters and blood flow (270, 362) while another failed to show any significant changes in that portion of the intestinal microcirculation (196). In this experiment the control group showed intense arteriolar constriction, but no signs of autoregulation (muscularis FCD did not decrease as expected for blood to be re-directed to the mucosa). Dopexamine at 1µg/kg/min on the other hand was associated with a lower longitudinal muscularis FCD. This might support the hypothesis that dopexamine at 1µg/kg/min is capable of preserving mucosal microvascular blood flow in the ileal bed – with better preserved arteriolar diameters and a preferentially constricted muscularis vascular bed serving to direct blood to the mucosal layers of the ileum. This could be due to a unique vasodilator profile of dopexamine (combined β_2-adrenergic and dopaminergic agonist), or alternatively due to an ability of dopexamine to preserve autoregulation due to anti-inflammatory actions in the vasculature. The latter would fit in with the clinical findings of preserved flow-mediated vasodilation following dopexamine treatment (147). It is possible that even if total ileal red cell flux is not dissimilar between groups (such as demonstrated in experiment 2) that dopexamine, through an autoregulatory preservation of mucosal microvascular blood flow and oxygenation, prevents disruption of the hypoxia prone mucosal villi, and in a similar fashion also preserves autoregulation and oxygen supply-demand matching in other vascular beds. In support of this, it has been demonstrated that following endotoxaemia dopexamine ameliorates intestinal ischaemia (assessed by measuring tissue levels of high energy phosphates, levels of ATP breakdown products (hypoxanthine and uric acid) and ATP/ADP ratio) (272). This explanation could also account for the difference in lactate and base deficit between controls and shams given FCD was not significantly different.

Alternative explanations are possible though. Should plasma lactate in this preparation not be derived from hypoperfused tissue, but instead from inflamed tissue with an altered metabolic profile, it would point back to the anti-inflammatory effects of dopexamine rather than any effects of dopexamine on tissue perfusion *per se*.

Several features of the model are consistent with similar studies, including the previous experiments, and are therefore reassuring. Endotoxaemia and bacteraemia are associated with significant increases in HR and usually a significant though only modest reduction in MAP in rodents (though this is dose dependent) (195, 196, 270-272, 277, 278, 349). This, as in the previous two experiments, was replicated here. Following LPS administration in rodents, intestinal microvascular blood flow decreases rapidly (by 1 hour) to 50% of baseline and remains depressed up to 4 h following a bolus of endotoxin (196, 356). This is in keeping with findings of intense splanchnic vasoconstriction in shock states (357), and the general increase in TPR seen in endotoxaemic controls here and in the previous experiment. Various studies exploring rheological and immune events in the microcirculation in endotoxaemia show an increase in adherent leucocyte numbers in intestinal post-capillary venules (196), something that was also found here. Other changes that occur include a leukopenia occurring over two hours (195, 270). This study found a significant increase in adherent leucocytes and a decrease in rolling leucocyte numbers in post-capillary venules, and it is likely this would have resulted in leukopenia - certainly when conducting flow cytometry on endotoxaemic samples in experiment 1, it took significantly longer to capture 10,000 neutrophil counts than in shams. Some findings of this experiment are not consistent with other studies though, such as the failure of endotoxaemia to decrease longitudinal and circular muscularis functional capillary density (196). Not all *in vivo* endotoxaemia studies can produce consistent findings in the intestinal microcirculation though (196, 270, 362), and this is likely the result of differences in the endotoxin serotype, dose, method of administration and fluid conditions of each experiment.

Chapter 6 – Comparison of Experiments 1 to 3

Before presenting the myography experiments, a brief comparison of the preceding *in vivo* experiments is made in order to assess how similar the preparations were compared to one another. This is best done by comparing the behaviour of the control groups:

Table 6.1   Comparison of experimental protocols and outcomes

	Experiment 1 (n=8)	Experiment 2 (n=8)	Experiment 3 (n=6)	P value
Length of experiment	4 h	4 h	5h	n/a
Endotoxaemia	LPS (011:B4) 6mg/kg	LPS 6mg/kg	LPS 1mg/kg / PepG 0.3mg/kg	n/a
Fluid administered (ml/kg)	21.4 (21.0 – 22.6)	23.8 (23.8 – 24.0)	29.4 (29.3 – 29.5) *	<0.05 vs. experiment 1
Fluid administered per hour (ml/kg/h)	5.4 (5.3 – 5.7)	6.0 (5.9 – 6.0) *	5.9 (5.9 – 5.9) *	<0.05 vs. experiment 1
End lactate	4.1 (2.9 – 5.2)	3.9 (3.1 – 4.4)	3.3 (2.2 – 4.6)	ns
End base deficit	7.2 (2.5 – 13.1)	8.6 (6.7 – 11.9)	3.2 (1.8 – 8.0)	ns
End urea	18.6 (15.0 – 19.9)	17.3 (16.4 – 18.4)	18.7 (15.2 – 19.6)	ns
End creatinine	69.7 (56.4 – 87.9)	57.9 (52.0 – 73.4)	57.6 (45.8 – 77.3)	ns
End ALT	128.1 (86.3 – 167.9)	81.8 (73.5 – 85.3)	71.5 (62.4 – 109.4)	ns
End AST	414 (304 – 480)	320 (274 – 403)	302 (243 – 429)	ns

Groups were similar with respect to baseline characteristics (mass, baseline lactate, baseline base deficit). By design animals in experiments 2 received more fluid to compensate for the more sustained and severe surgical insult (5ml/kg bolus at the end of surgery), and in the case of experiment 3 due to the longer experimental time. However when this was corrected to account for differing lengths of the experiment, values for fluid administered differed by statistically but not clinically significant volumes. Baseline lactate

(data not shown), HR and MAP were similar to those of equivalent groups in experiment 1 (see Figure 6.1). Although on first glance there seems to be an overall reduction in the severity of organ injury in control animals in experiment 2 and 3 as compared to experiment 1, this was not significant (ANOVA or individual t-tests). Similarly the development of organ injury in experiment 3 appears to coincide with lower plasma lactates and base deficits than in experiments 1 and 2 – however this was not significant either (ANOVA or individual t-tests).

The placement of an aortic flow probe in experiments 2 and 3 reveals no significant differences between control groups for relative stroke volume, relative cardiac index or markers of total peripheral resistance (though by design there is no data for experiment 2 beyond 4 h) (see Figure 6.2). Given that MAP and HR are similar between the three experiments, it may be reasonably assumed that a similar cardiovascular profile was present in experiment 1 too.

Although the models used for the three experiments were similar it is not certain that the additional surgical preparation required for experiments 2 and 3, and the different nature of the inflammatory insult did not cause haemodynamic differences between the preparations (e.g. in microvascular flow). However, the fact that values for HR, blood pressure and lactate at baseline and throughout the comparable four hour experimental periods were similar argues against this possibility.
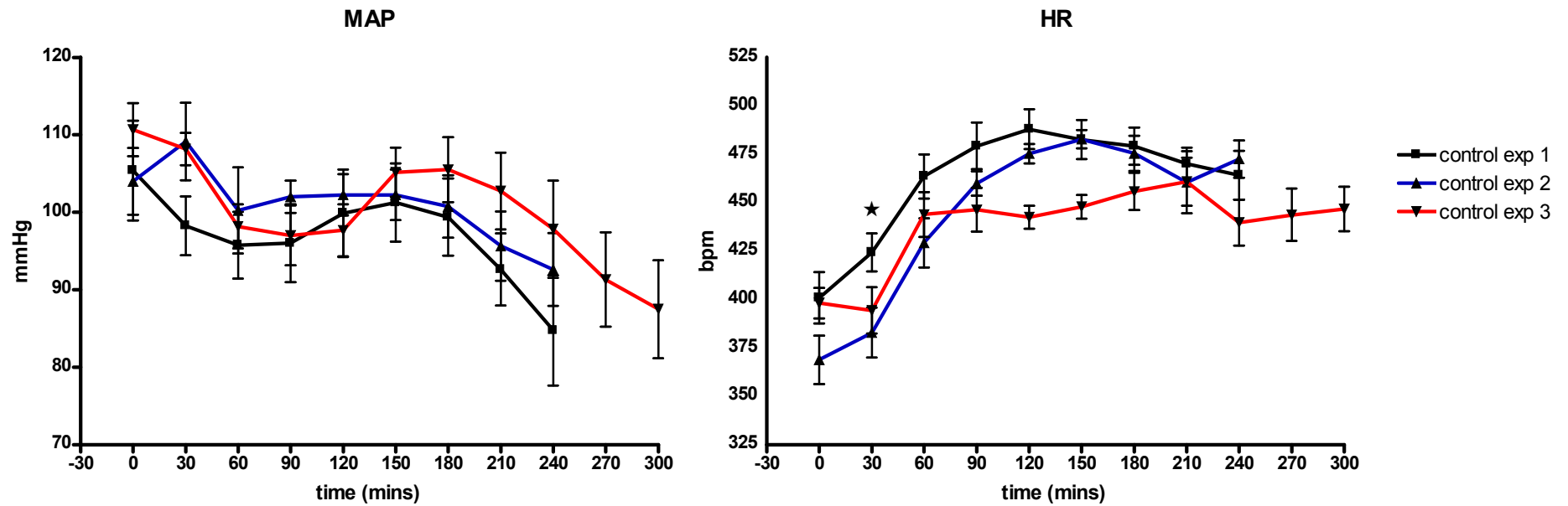
Figure 6.1   HR and MAP for experiments 1, 2 & 3

Two-way ANOVA of HR and MAP for control groups from experiments 1, 2 and 3 (*post hoc* Bonferroni's tests *P<0.05 vs. experiment 2 vs. 1).

In all 3 experiments a biphasic dip in MAP and a tachycardia is seen.
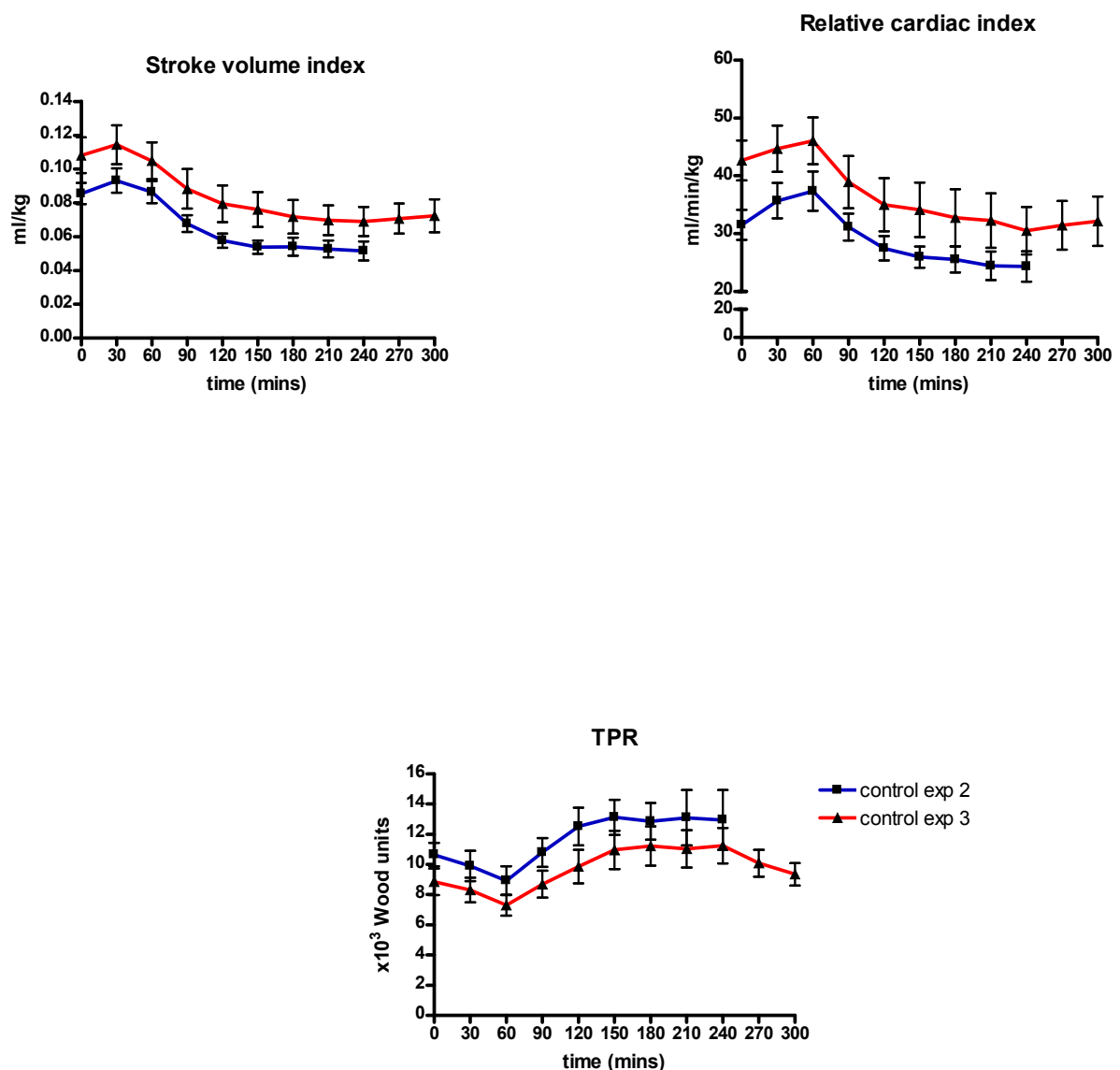
Figure 6.2   Haemodynamic comparison of experiments 2 and 3.

There were no significant differences between groups at any time point. Relative stroke volume was significantly below baseline levels from 90 min onwards whereas relative cardiac index became significantly lower than baseline from 120 min onward (in both experiments; paired t-tests, all comparisons at least P<0.05). TPR was significantly below baseline at 60 min in both experiments, and significantly above baseline in experiment 2 from 120 min onward (paired t-tests, all comparisons at least P<0.05). In experiment 3 TPR only tended to be significantly above baseline from 150 min (P=0.057) but due to the variable time on the intravital microscopy stage the number of measurements made at 180 and 210 min is only 4.

Chapter 7 - Effects of dopexamine on inflamed rodent mesenteric arteries

7.1   Introduction

The previous studies have looked at the effects of dopexamine in endotoxaemia. These studies demonstrated an endotoxaemia associated increase in circulating plasma TNF-α and various other cytokines which were associated with neutrophil activation, markers of inadequate perfusion and organ dysfunction. Dopexamine ameliorated almost all these effects despite its inability to increase cardiac index, ileal microvascular red cell flux and ileal functional capillary density. In cases where organ function improved, anti-inflammatory effects were noted (suppression of plasma cytokine levels, neutrophil activation and infiltration in the first study, and a decrease in neutrophil adhesion in the third study). No evidence of improvement in microvascular function (such as increased functional capillary density or ileal red cell flux) was associated with improved organ function in any studies, though less intense arteriolar vasoconstriction was seen. This leaves open the question of whether the effects of dopexamine relate purely to intrinsic anti-inflammatory effects of dopexamine or some other mechanism.

The previous studies have been *in vivo* studies where it has not been possible to definitively separate any intrinsic anti-inflammatory and rheological/haemodynamic effects of dopexamine on resultant tissue injury. For example, if dopexamine improved microvascular flow under conditions of endotoxaemia through vasodilator actions, or if it improved the barrier function of the endothelium, it could yet appear to have anti-inflammatory effects as leucocyte endothelial adhesion is reduced under such conditions (195). On the other hand if dopexamine did possess anti-inflammatory actions, this could also improve the behaviour of the microcirculation as venular adhesion and arteriolar dysfunction are associated with dysregulated and reduced microvascular blood flow and oxygen delivery (363-366). Although none of my previous studies were able to show simultaneous effects of

dopexamine on microvascular flow and on inflammatory changes, it is not impossible that both occur together as Experiment 1 did not examine effects on microvascular flow, and Experiment 3 did not directly measure microvascular flux, only arteriolar diameters and fcd. Therefore it is important to perform the following studies for an insight in to mechanistic pathways, and for this reason it was also important that vessels were not harvested from animals treated for several hours with LPS +/- dopexamine as it would remain unclear if any differences in the behaviour of blood vessels were due to vascular or anti-inflammatory effects of dopexamine.

In summary, by studying vascular tissue *ex vivo* I planned to investigate whether or not dopexamine has any intrinsic anti-inflammatory activity (in particular on the endothelium), independent of any effects it may have on global haemodynamics, microvascular flow and tissue oxygenation.

7.2   Methods

All procedures were performed in accordance with Institutional and Home Office guidance on the care and use of animals (Animals (Scientific Procedures) Act 1986). Male Sprague-Dawley rats (250-350g) received a standard laboratory chow diet and water *ad libitum* prior to investigations. Single rats were placed in a sealed chamber and killed by exposure to an increasing concentration of carbon dioxide. A midline laparotomy was rapidly performed following transfer of the rat to a workstation. The small intestine was explored for a suitable section of associated mesenteric arcade, approximately 10cm distal to the pylorus. Such an arcade was determined to be suitable if between two and four straight sections of supply arteries were easily visible. This section of small intestine and mesentery was excised *en-bloc* and transferred to a large petri dish containing cold Krebs' solution, consisting of (mM): NaCl (118), KCl (5.4), $MgSO_4.7H_2O$ (1.2), glucose (11), KH_2PO_4 (1.2), $NaHCO_3$ (25) and $CaCl_2.2H_2O$ (2.5) in dH_2O, which had been pre-oxygenated for at least 10 min with 95%O2

and 5%CO2. Small resistance arteries (2nd and 3rd order branches of the superior mesenteric artery, roughly 200 – 350µm in diameter) were carefully cleaned of all fat and connective tissue under a dissecting microscope (Leica) and high intensity illuminator (Cole-Palmer). Lengths of artery of approximately 2mm length were excised and transferred to smaller petri dishes containing Krebs' solution, prior to transfer to culture conditions under a sterile field (HeraSafe Class II microbiological safety cabinet, Heraeus). Vessels were treated in one of four ways. All vessels were incubated for 18 h in 5mL Dulbecco's Modified Eagle's Medium, supplemented with penicillin 100U/mL and streptomycin 0.1mg/mL at 37C / 5% CO2 in a humidified incubator (HeraCell 240, Heraeus). One group of vessels had no intervention (negative control). The remaining three groups of vessels were incubated following the addition of TNF-α (final concentration 10ng/ml). Except for one of these three groups (positive control) the remaining two groups were co-incubated with dopexamine at 1 or 10µg/ml.

A 4-chamber wire myograph (Multiwire Myograph System 620M, DMT Ltd) was prepared the following day by filling each chamber with 5ml of Kreb's solution which was continually oxygenated and kept at 37C. The dissecting microscope was then used to aid in loading a randomly selected pre-incubated vessel on to two wires which were attached in a parallel fashion to the two jaws of each myography chamber, one jaw attached to a micrometre screw for manual adjustment of vessel diameter and the second to an isometric force transducer for measurement of wall tension. Care was taken specifically not to disturb the endothelium when mounting the vessels by ensuring the wires did not irritate the intimal surface of the vessels through rash or aggressive handling. Readouts of tension were via a desktop computer using LabChart Pro V7 software (ADInstruments Ltd.). Once mounted, each chamber was closed by placing a sealed Perspex cover with a small orifice over the top. The orifice allowed for the addition of drugs in to the chamber via pipette. The presence of 4 chambers allowed 4 experiments to be conducted in parallel.

Chambers were allowed to reach operating temperatures (of 36.8 – 37˚C) following which a calibration was performed in order to derive the internal diameter of each vessel and determine the correct amount of basal tension to apply. Vessel tension was increased manually in 0.4mN steps and the change in diameter recorded. These data were analysed using a normalisation software unit (NORM) within LabChart which derives the internal diameter of the vessel and determines the starting distance between the parallel wires (and therefore the resting tension) using the Laplace relationship between internal diameter and wall tension at a given transmural pressure (set to 13.3kPa for systemic arteries, as in this case). The calculated diameter was set accordingly, and the tension was allowed to plateau before commencing experiments.

To assess the suitability of vessels for further study, pre-contraction with 200µl of 3M KCl was initiated (providing a bath concentration of 120mM KCl). Vessels not achieving an increase of at least 0.5mN were deemed unsuitable for study and were discarded. Following this chambers were washed out three times with fresh Krebs' solution before a new 5ml volume of Krebs' solution was added to each myography chamber followed by the addition of 0.3µM nifedipine to reduce spontaneous vasomotor activity. Vessels were given time to plateau at resting tension before the experiments could begin in earnest.

Vessels were then pre-contracted with phenylephrine (PE) to a maximum of 300µM until approximately 70% of the maximal KCl plateau response had been achieved. If this was not sufficient to produce adequate tone the thromboxane receptor agonist U446619 was added at up to 1µM. If this was insufficient the vessel was discarded. Once tension plateaued after pre-contraction, an initial volume of 5µl of 100nM acetylcholine (ACh) was added to the chamber (in-chamber concentration 100pM ACh). Any change in tension was allowed to plateau following which a further 10µl of the same solution of ACh was added to the bath to produce a 3-fold increase in Ach concentration to 300pM. Following any plateau in tension, and in order to maintain approximately half-log increases in ACh concentration, ACh was then added alternately and cumulatively in volumes of 3.5µl and 10µl, each set coming from

a solution of ACh 10 times more concentrated than the previous set. The time of each addition was duly noted. When it became obvious that further ACh was not inducing further vasorelaxation, (or when the ACh concentration reached 300µM), the time was noted and 5µl of 100mM sodium nitroprusside (SNP) was then added to the bath (100µM in-bath concentration) to induce a nominally maximal vasodilatation. Using this technique the total volume of added drugs never exceeded 101µl and that the increase in the volume of the bath was therefore not more than 2%. Following any plateau in tension after the addition of SNP, recording was halted, the cover of the chambers were removed, vessels and wires were discarded following which each chamber was washed out three times with clean Krebs' solution and a further volume of 5ml Krebs' was reloaded in to the bath and experiments repeated with new vessels. In each experiment data were saved for offline statistical analysis.

Statistical analysis

For data analysis, the following measurements were extracted:

- The baseline tension just prior to the addition of the first pre-contracting dose of PE (T_B)
- The tension just prior to the addition of the first dose of ACh (T_M), representing the plateau tension following pre-contraction with the last dose of vasoconstrictor
- The plateau tension for each dose of ACh, measured at the time just prior to the next dose of ACh or SNP (T_{ACh})
- The plateau tension following the addition of SNP (T_{SNP}) – this plateau was easily visible on all myography traces and represented the end of the experiment

Kolmogorov-Smirnov normality testing was performed for all groups. Calculations were performed as described in the methods section. Data were tested using one-way analysis

of variance (ANOVA) for comparison across all groups. When data were not normally distributed in at least one group for any measurement, the Kruskal-Wallis test was used in place of one-way ANOVA, and appropriate t-tests against controls for post-testing depending on whether the individual groups were normally distributed or not. With respect to percentage decreases on pre-constricted vascular tone in response to ACh and SNP, the data were transformed and a non-linear fit (sigmoidal dose-response, variable slope) approximated by software. Data were analysed with GraphPad Prism 4.0 (GraphPad Software, San Diego, USA). Significance was set at $P<0.05$.

7.3   Results

A total of 56 vessels from 11 rats were initially analysed. There were 16 vessels in the untreated group, 3 of which were excluded from analysis. One vessel behaved abnormally during calibration and had to be abandoned. A further two untreated vessels were also excluded as they were clearly abnormal in their behaviour – being unable to sustain contractile and dilator responses to vasoconstrictors and sodium nitroprusside, respectively. None of the 16 vessels were excluded from analysis from the TNF group. There were 12 vessels in the dopexamine groups. In the 1µg/ml dopexamine group one vessel had a faulty calibration and was therefore excluded. No vessels from the 10µg/ml dopexamine group were excluded from analysis.

Vessel diameters were not significantly different and ranged from 235 - 448µm (Figure 7.1). The percentage remaining tone in the vessel after administering SNP was significantly higher in untreated TNF-α vessels compared to control vessels (Figure 7.2).
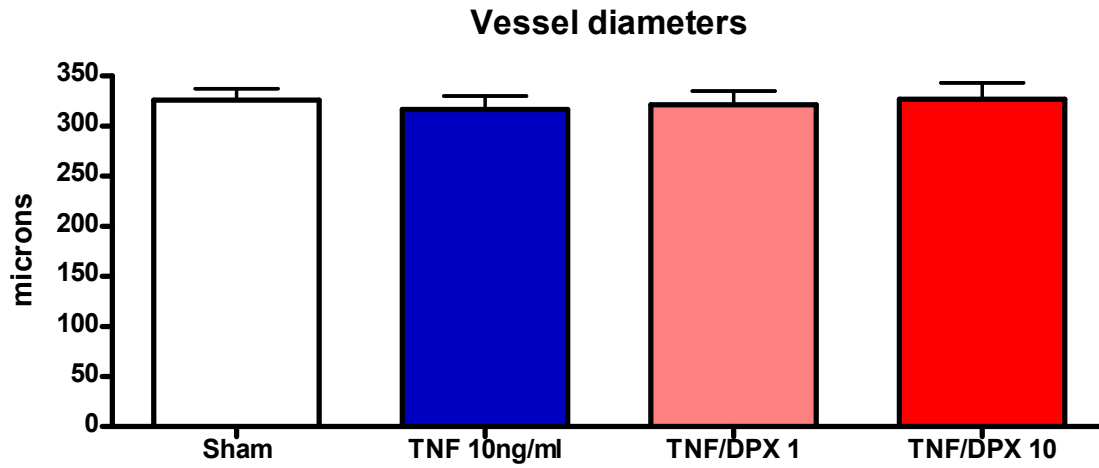
Vessel diameters

Figure 7.1   Vessel diameters

(n=11–16 per group). No significant differences were found between groups in terms of the size of blood vessels

remaining tone after SNP
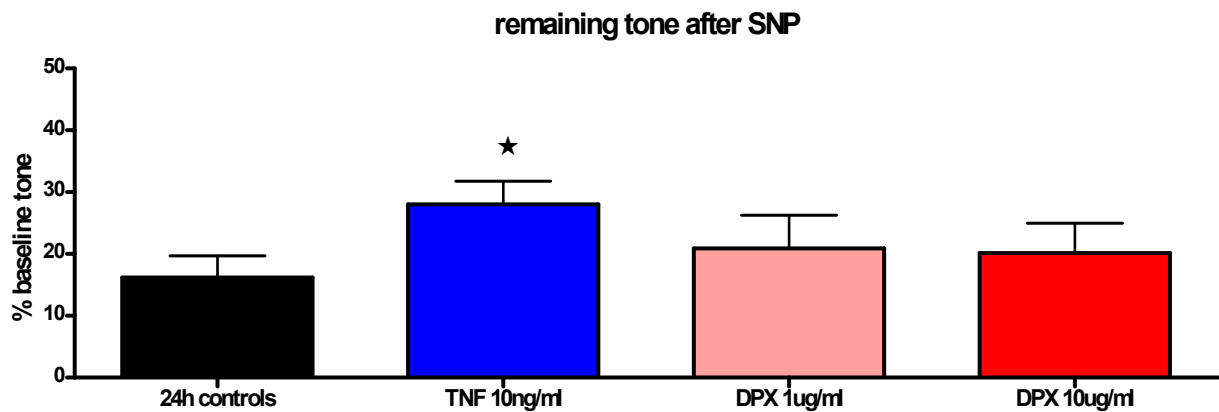
Figure 7.2   Remaining tone in vessels following administration of SNP

(n=11–16 per group). (Unpaired t-tests *P<0.05 TNF-α vs. controls)

Vasorelaxation curves obtained by indexing relaxation to the maximal SNP-inducible relaxation (T_M – T_{SNP}) were significantly different to one another (P<0.0001) (Figure 7.3). One-way ANOVA of the mathematical characteristics of the best fit curves only showed a significant difference in E_{MAX} between incubated controls and the TNF-α group (Table 7.1). An identical set of statistical changes was found, though with less graphical similarity between TNF-α and dopexamine groups (Figure 7.4), when relaxation curves were indexed to the baseline tone (i.e. T_M – T_B) (Figure 7.4 and Table 7.2). Furthermore when compared to incubated controls and as a fraction of the total tone in the vessel (T_M – T_B), there was significantly less relaxation achieved at the four highest dose of ACh in the non-dopexamine co-incubated TNF-α group (but not dopexamine co-incubated groups) (Figure 7.5).

% decrease in PE induced tone

P<0.0001

Legend:
- ◆ TNF 10ng/ml
- ■ incubated controls
- ☐ DPX 1ug/ml & TNF 10ng/ml
- ○ DPX 10ug/ml & TNF 10ng/ml

X-axis: -log [Ach]
Y-axis: % relaxation
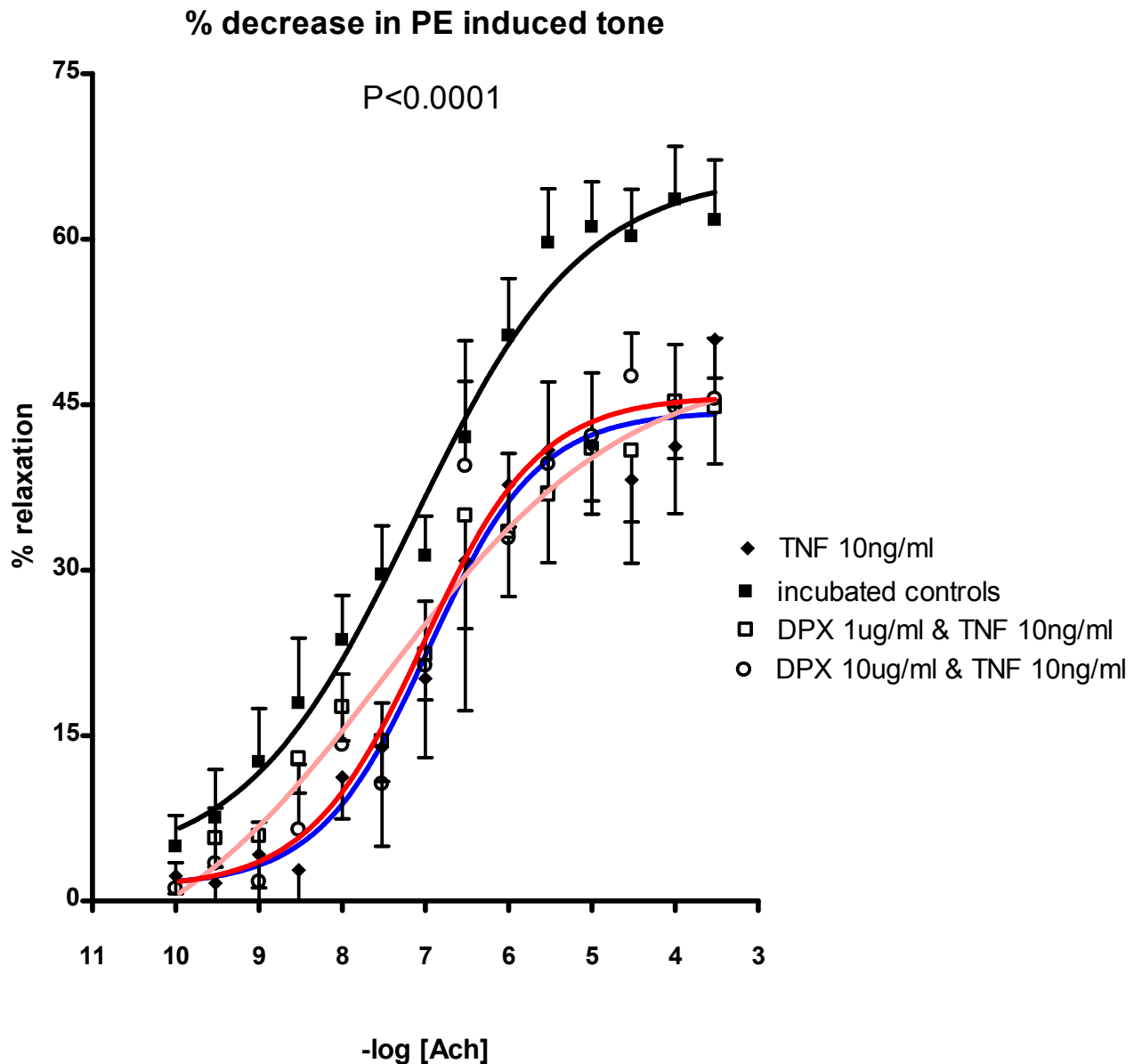
Figure 7.3   Relaxation curves indexed to maximal SNP-induced dilatation

Relaxation curves for incubated control vessels (black), vessels incubated with 10ng/ml TNF-α (blue) and vessels co-incubated with TNF-α and either 1µg/ml (pink) or 10µg/ml (red) dopexamine to increasing doses of ACh following pre-contraction with phenylephrine. These responses have been indexed to the maximum SNP-induced vasodilatation (non-linear regression; comparison of best fit dose-response curves, P<0.0001)

% decrease in PE induced tone
P<0.0001

Legend:
- ◆ incubated controls
- ■ TNF 10ng/ml
- □ DPX 1ug/ml & TNF 10ng/ml
- ○ DPX 10ug/ml & TNF 10ng/ml

(y-axis: % decrease in PE induced tone; x-axis: -log[Ach])
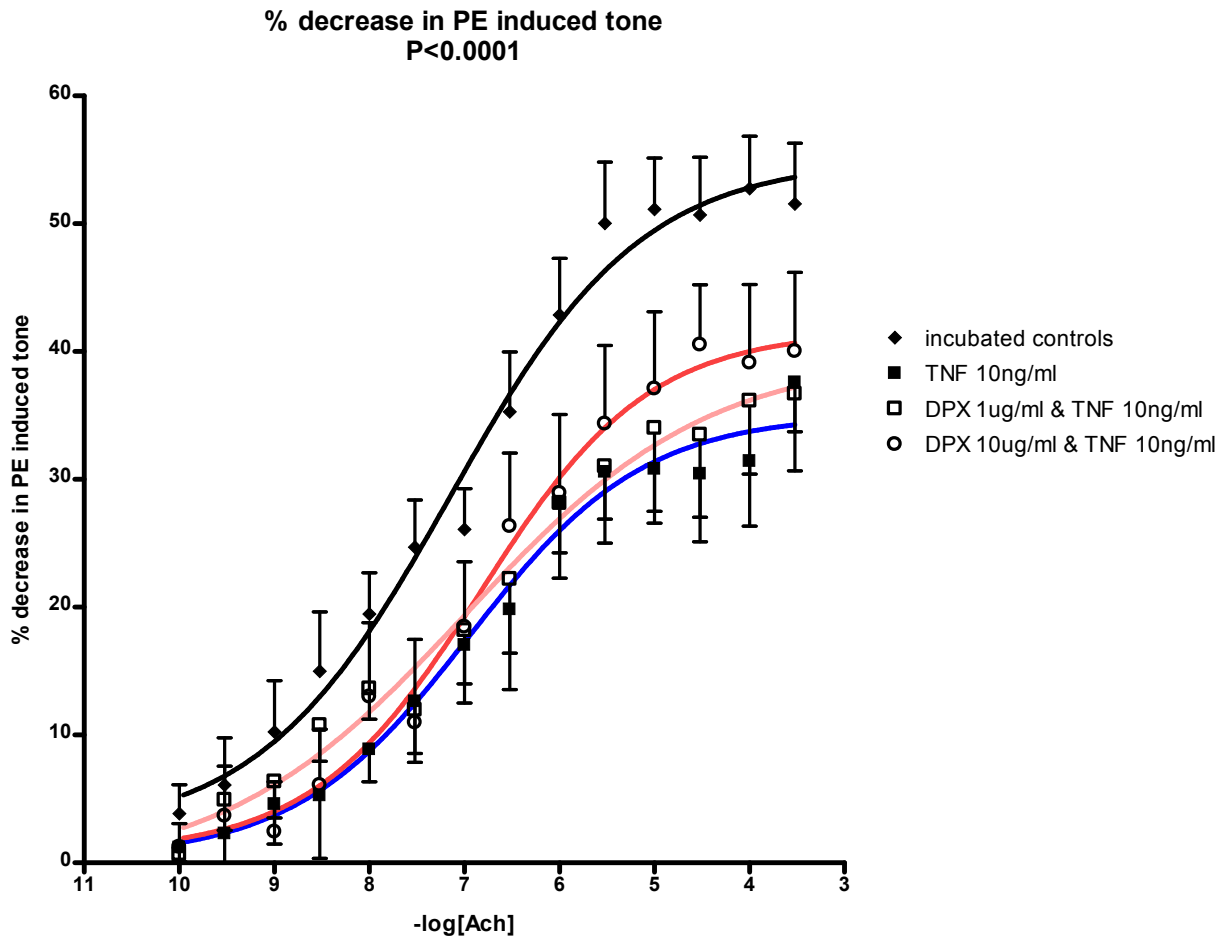
Figure 7.4   Relaxation curves indexed to baseline (pre-PE contraction) tone

Relaxation curves for incubated control vessels (black), vessels incubated with 10ng/ml TNF-α (blue) and vessels co-incubated with TNF-α and either 1µg/ml (pink) or 10µg/ml dopexamine (red) to increasing doses of ACh following pre-contraction with phenylephrine (non-linear regression; comparison of best fit dose-response curves, P<0.0001)

	log EC50	E$_{max}$	N
Incubated controls	7.143 ± 0.2771	66.000 ± 4.5670	13
TNF	6.963 ± 0.3319	44.350 ± 4.2400 *	16
TNF + DPX 1	7.484 ± 0.8459	48.970 ± 9.5410	11
TNF + DPX 10	7.018 ± 0.3188	45.760 ± 4.1150	12

Table 7.1   Mathematical characteristics of relaxation curves indexed to maximal SNP-induced dilatation

One-way ANOVA, Bonferroni's post-tests *P<0.05 vs. incubated controls

	log EC50	E$_{max}$	N
Incubated controls	7.152 ± 0.2858	55.020 ± 3.9100	13
TNF	6.951 ± 0.3809	35.080 ± 3.8210 *	16
TNF + DPX 1	6.988 ± 0.6371	39.780 ± 7.6950	11
TNF + DPX 10	6.832 ± 0.3817	41.610 ± 4.7810	12

Table 7.2   Mathematical characteristics of relaxation curves indexed to baseline (pre PE contraction)

One-way ANOVA, Bonferroni's post-tests *P<0.05 vs. incubated controls
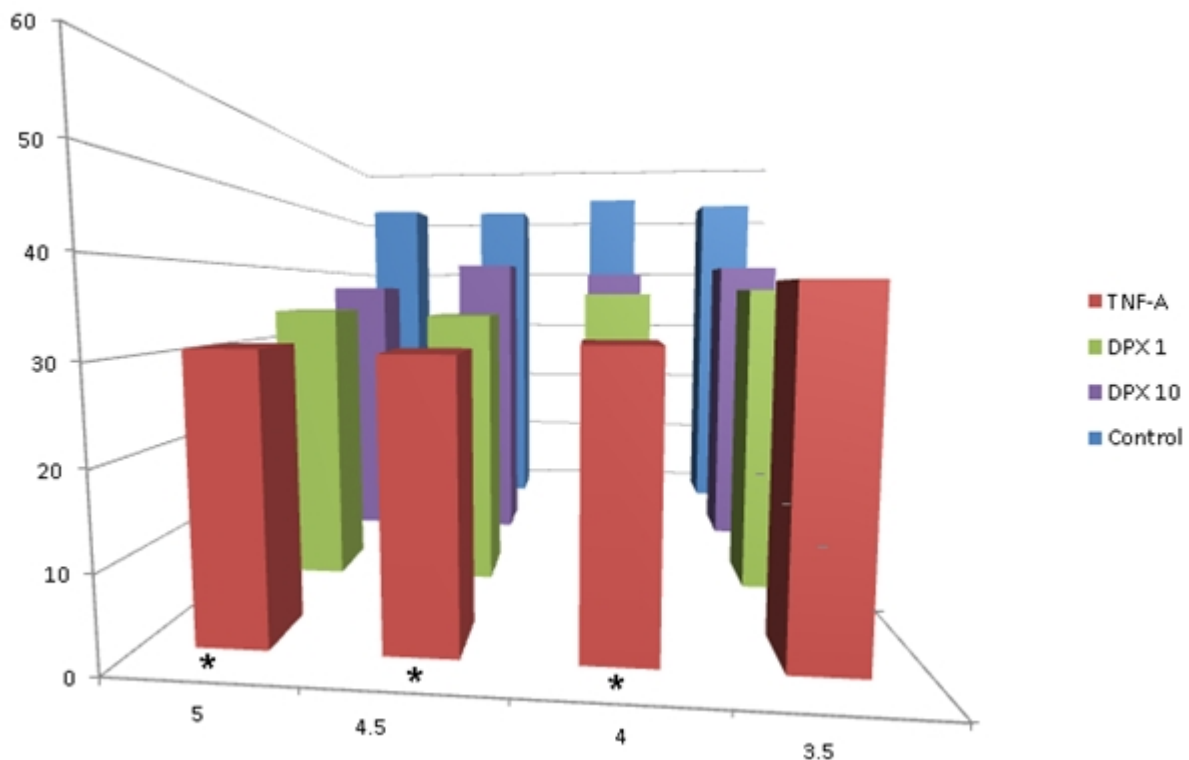
Figure 7.5   % relaxation at highest doses ACh (indexed to baseline tone)

3D bar chart showing mean % relaxation achieved for the 4 highest doses of ACh (-log$_{10}$[Ach]) for control vessels, vessels incubated with 10ng/ml TNF-α and vessels co-incubated with TNF-α and either 1μg/ml or 10μg/ml dopexamine (standard errors omitted for clarity). At each dose TNF-α treated vessels (but not dopexamine co-incubated vessels) demonstrated significantly less relaxation in response to ACh when compared to control vessels (One-way ANOVA, Bonferroni's post-tests *P<0.05). Although one-way ANOVA failed to reveal significant differences between any of the groups at the highest dose of ACh, individual t-testing revealed that only non-dopexamine co-incubated TNF-α vessels relaxed significantly less than controls.

7.4   Discussion

This study shows that vasorelaxation in small mesenteric arteries co-incubated with dopexamine is preserved when compared to the TNF-α group, and also that endothelial dependent vasorelaxation is less impaired.

197

When normalised to SNP responses, there is little apparent difference between TNF-α treated and dopexamine co-incubated vessels (possibly indicating no significant difference in the behaviour of the endothelium). However the non-normalised graph shows some differences in that dopexamine co-incubated vessels better preserve dilator responses. Why this is not visible in the SNP-normalised graphs is due to the proportionally greater tone remaining in TNF-α group after SNP treatment. In other words non-dopexamine co-incubated vessels are closer to their limit of vasodilatation at the highest dose of ACh whereas dopexamine treated vessels are not. This might point to a greater anti-inflammatory effect of dopexamine on smooth muscle cells as opposed to endothelial cells or to a dopexamine mediated anti-inflammatory effect on the endothelium manifested by the amelioration of the limitation of dilator responses to the highest doses of ACh.

The vasodilator effects of ACh are abolished in eNOS deficient mice, abolished by selective inhibitors of eNOS such as L-NAME (N-nitro-L-arginine methyl ester hydrochloride) and also by soluble guanylate cyclase inhibitors (367). Thus ACh (like shear stress) activates eNOS to generate NO which diffuses to smooth muscle where it activates soluble guanylate cyclase, triggering vasodilation. ACh is also able to vasodilate through effects on inward rectifying potassium currents (368). The dilator responses to SNP are also soluble guanylate cyclase dependent and involve activation of vascular smooth muscle inward rectifier potassium channels (369). These similarities suggest that NO species donated by SNP are comparable to and act in a similar fashion to endothelium (ACh) generated nitric oxide species. TNF-α has been shown to result in decreased NO-induced and endothelium dependent vasorelaxation, a decreased synthesis and bioavailability of NO, an increase in vascular superoxide anion, a decreased sensitivity of soluble guanylate cyclase, an upregulation of iNOS and a downregulation of eNOS (370-372). Furthermore TNF-α decreases cAMP levels in endothelial cells (373). β_2-adrenoceptor agonists increase cAMP levels and activate eNOS pathways of NO formation (374), but have also been shown to ameliorate the expression of iNOS following endotoxaemia (245), potentially preventing the

decreased sensitivity of soluble guanylate cyclase. A reduction in the amount of superoxide anion has also been found following β_2-adrenoceptor agonist treatment in endotoxaemia and hypoxia/re-oxygenation injury (243, 246, 248), a similar effect to that seen with free radical scavengers such as superoxide dismutase and inhibitors of xanthine oxidase (371, 375). In this regard it is notable that free radical scavenging by dopexamine has been suggested in several *in vivo* studies (240, 242, 274, 376). The effects of dopexamine in this preparation could therefore represent a β_2-adrenoceptor mediated reduction of free radical production, with the result that NO bioavailability increases and endothelial dysfunction is ameliorated.

TNF-α has also been shown to reduce cell viability in endothelial cells (377), while cyclic nucleotides such as cAMP ameliorate apoptosis via inhibiting caspase activation (378). Cyclic AMP elevating agents also upregulate the enzyme arginase (which competes with NOS for arginine) (379), reducing levels of free radicals and apoptosis when NO is released in pathological quantities by iNOS (380). However the precise role of arginase in improving vascular function is unclear as TNF-α also upregulates arginase, reducing physiological levels of NO released by eNOS, causing endothelial dysfunction (381). Irrespective of the precise mechanism, a decrease in endothelial cell viability would be expected to decrease endothelial function and this provides an alternative or additional mechanism of dopexamine's effects in this preparation.

Important limitations of this experiment are:

- Vessels were incubated with TNF-α and not with LPS. This was because the experience with LPS was an all or nothing response in that either there was no inducible endothelial dysfunction or vessels were so dysfunctional that they were unsuitable for analysis. This led to the use of TNF-α which was able to provide a much more reliable vascular injury.
- Vessels were incubated in TNF-α overnight as opposed to only a brief exposure (e.g. 30 mins). It is arguable whether a 30 minute, 5 hour or 18 hour incubation is more in

keeping with the effects of LPS in the *in vivo* experiments. It is important to note though that shorter durations of incubation with TNF-α have been shown to result in qualitatively different behaviour of small arteries when compared to overnight incubations (375).

- Although the use of this *in vitro* model allows anti-inflammatory effects to be dissected away from any microvascular effects of the drug, there was no comparison of TNF-α treated vessels with those isolated from *in vivo* LPS treated animals to ensure that any extrapolation is justified (this was due to the logistics of these experiments being performed in a different laboratory to the *in vivo* ones, also that a typical *in vivo* experiment occupied an entire working day, rendering transport of isolated vessels from one laboratory to another (across London) difficult). Nonetheless it is clear that endothelial and smooth muscle dysfunction was induced by TNF-α, and therefore any effect dopexamine had in reducing this type of inflammation was a notable observation in itself.

- The experiments could have been made more useful from a mechanistic perspective by utilising various other agents such as dopaminergic, β$_2$-adrenoceptor antagonists, intracellular stimulators or inhibitors of cAMP and PKA. It is hoped to continue such work in the future.

Chapter 8 – Discussion

8.1   Macrohaemodynamics and organ dysfunction

Although the *in vivo* studies conducted for this thesis all differed from one another in certain (arguably minor) respects, all three experiments resulted in significant organ dysfunction in control groups that occurred despite the maintenance of normotension. Paired t-tests show that control group relative cardiac index (in the two experiments where it was measured) was also similar to baseline and occasionally significantly higher for the first 2 h of each experiment (Figure 6.2). Beyond these time points the lowest control relative CI in both experiments was at 240 min and was 21.5% (SEM 8.4%) of baseline in experiment 2, and 28.4% (SEM 8.1%) of baseline in experiment 3 (Figure 6.2). Other haemodynamic changes included a sustained increase in heart rate – a response that can augment cardiac output when stroke volume decreases. This normotensive model of laparotomy and endotoxaemia was also associated with significant increases in end experiment plasma lactate and base deficit, biomarkers that are used to assess end organ perfusion.

When compared to controls, dopexamine had no significant effects on any of these haemodynamic parameters, but most convincingly improved renal function in experiments 1 and 3, at doses of 1µg/kg/min, and improved plasma lactate in the same experiments (the failure to significantly improve lactate in experiment 3 at this dose was very likely due to a small group size as the trend to improved lactate was strong). Dopexamine also improved hepatic function in experiment 1 but this effect could not be reliably reproduced in experiments 2 or 3.

The preceding paragraphs suggest that dopexamine can improve organ dysfunction and the cause of this is not the prevention of hypotension. With regards to relative cardiac index, the suggestion that in this endotoxaemic preparation it is inappropriately low (even when it is above baseline) is supported by several observations in control animals –

1. There is an associated significant increase in plasma lactate. This could be interpreted as representing a type 2 lactic acidosis due to anaerobic metabolism in the context of hypoperfusion

2. There is an associated significant increase in base deficit, which can also be encountered in situations of hypoperfusion

3. In Figure 4.8 there is a mismatch between the early fractional increase in relative cardiac index and the fractional decrease in ileal red cell flux in control endotoxaemic groups – could a higher relative cardiac index have ameliorated this?

Dopexamine appears to improve organ function and lactate without any significant difference in relative cardiac index (or stroke volume) when compared with controls though (the reduction in plasma lactate is also notable considering it might have been expected to increase through dopexamine's β_2-adrenoceptor mediated mechanisms (382)). This argues strongly against an augmented cardiac index (and therefore DO_2) being the mechanism of action for the improvements seen with dopexamine, and by extension it also argues against a suboptimal cardiac index being the mechanism of organ dysfunction and high plasma lactate in untreated endotoxaemic controls.

8.2 The microcirculation and microvascular perfusion

One possibility might be that irrespective of the effects on "global" haemodynamics, there is a fundamental problem at a microvascular level. This could also fit in with the increased lactate and base deficit being due to hypoperfusion and is suggested by the following observations in control animals (experiment 2 and 3):

1. There is a significant decrease in ileal inflow arteriolar diameters (and such reductions have been shown to be associated with decreases in red cell velocity (362))

2. There is a significantly lower ileal red cell flux measured in endotoxaemic animals compared to sham animals – such a reduction is unlikely to be confined to the ileal circulation.

Other studies support this idea of a primary microvascular dysfunction. In endotoxaemia, portal blood flow decreases by an average of 33% by 1 hour, stays depressed for another one hour, and during this time liver sinusoidal diameters steadily increase. Liver sinusoidal blood flow decreases by an average of 27% at 1 hour and 40% at 2 h yet cardiac output remains similar to baseline (271). Furthermore progressive decreases in red cell velocity, venular shear rate and increases in plasma extravasation with time are seen in the mesenteric bed (195).

Although there is some data from animal studies in support of dopexamine significantly improving microvascular perfusion in endotoxaemic rats (195, 196, 270), the studies in this thesis were unable to replicate such findings in any robust manner as neither FCD nor ileal red cell flux were significantly different between dopexamine and control animals in any of our studies. One subtle and possibly important observation relates to the behaviour of intestinal arterioles in experiment 3 though.

As arterioles are more numerous than larger vessels in the arterial tree they make up a large fraction of TPR (383). In keeping with this, at the mid-point of resuscitation when control MAP is comparable to sham animals but control TPR is significantly greater than shams and is at its peak, profound intestinal arteriolar vasoconstriction and a reduction in ileal red cell flux is observed in controls. This corresponds with previous findings of intense splanchnic vasoconstriction also seen in shock states (357), and the fact that endotoxaemia results in an increase in sympathetic outflow such that there is a short-lived surge in plasma

adrenaline and a more sustained increase in plasma noradrenaline (384-386) – features that would be expected to increase TPR. In this regard it is notable that in experiment 3 TPR tends to be lower in dopexamine treated animals, and that intestinal arterioles also tended to be less constricted. This could indicate that red cell velocities and therefore red cell flux (as the product of red cell velocity and arteriolar cross-sectional area) are greater in the intestinal arterioles of dopexamine treated animals than controls. These findings are probably genuine as measurements of arteriolar diameters and the measurements required for calculating TPR are independent of one another. The relatively small numbers in each group in experiment 3 may be the reason why these differences were not shown to be significant. This may also explain why in experiments 1 and 3 organ function was much more convincingly improved with dopexamine than in experiment 2 (where significant differences in microvascular flow were not demonstrated between groups and where TPR was similar to controls). Although salbutamol was associated with a much lower TPR in experiments 2 and 3, it is likely this is due to vasodilatation in non-splanchnic beds, such as skeletal muscle.

One further and related subtle microvascular finding is worth discussion. The distribution of blood flows in the intestinal wall is not uniform. There is a 3-4 fold higher blood flow in the mucosa compared to the muscularis. In response to hypoperfusion splanchnic vasoconstriction occurs and there is a neuronally mediated relative preservation of blood flow to the hypoxia-prone mucosa when compared to the muscularis (358-360). The preservation of blood flow to the mucosa while overall splanchnic vasoconstriction is occurring is the result of autoregulatory mechanisms that allow the mucosa to escape from vasoconstrictive factors that affect the muscularis (361). In this study the control group showed intense arteriolar constriction, but no signs of autoregulation (muscularis FCD did not decrease as expected for blood to be re-directed to the mucosa). Dopexamine at 1μg/kg/min on the other hand demonstrated a lower longitudinal muscularis FCD. This supports the hypothesis that dopexamine at 1μg/kg/min might be capable of preserving microvascular autoregulation in the ileal bed – with better preserved arteriolar diameters and

a preferentially constricted muscularis vascular bed serving to direct blood to the mucosal layers of the ileum. This preserved autoregulation would fit in with the clinical findings of preserved flow-mediated vasodilation (an endothelium dependent phenomenon) following dopexamine treatment (147), but more importantly in the context of these studies it fits in very well with the observation (experiment 4) that dopexamine can improve endothelium-dependent and endothelium-independent vasodilator responses in small inflamed arteries. Such effects have been previously observed with agents that elevate cAMP and possibly relate to cross activation of cGMP related systems (377). It is possible that even if total ileal red cell flux is not dissimilar between groups (such as demonstrated in experiment 2) that dopexamine, through an autoregulatory preservation of mucosal microvascular blood flow and oxygenation, prevents disruption of the hypoxia prone mucosal villi, and in a similar fashion also preserves autoregulation and oxygen supply-demand matching in other vascular beds. This could account for the trends to improved lactate and base deficit in experiment 3 when control and sham FCD was not significantly different.

8.3 Immunomodulation ameliorates organ dysfunction?

Notwithstanding the aforementioned argument, the fact that dopexamine improves organ function, base deficit and plasma lactate without significantly affecting blood pressure, cardiac index or ileal red cell flux and ileal functional capillary density is somewhat problematic when looking for a fundamental cellular mechanism to explain the findings. One approach would be to maintain the argument that oxygen delivery is being improved at a microvascular and tissue level in key microvascular beds – either argued as above, or by arguing that improvements occur in vascular beds not examined in these studies. The alternative is to turn away from hypoperfusion (as suggested by some studies (387)) and look in a completely different place for the explanation. The obvious place to turn to is the potent immunomodulatory effects of dopexamine that have been demonstrated here.

Endotoxaemia produces stereotypical haemodynamic and microvascular changes. At a cellular level there is an early release of vasodilatory NO from eNOS followed by a later (TNF-α and IL-1β mediated (388, 389)) sustained release of nitric oxide from iNOS (390). This pattern is apparent on examining the total peripheral resistance and MAP graphs for all endotoxaemic groups (Figures 6.1 and 6.2). TNF-α has also been shown to mediate superoxide production via NADPH oxidase (391), generating free radicals which impair endothelium-dependent vasorelaxation. Studies have documented that lactate is not necessarily correlated with hypoperfusion (392) and in keeping with this increases in plasma lactate (and other clinical findings) seen in endotoxic shock are replicated by infusion of TNF-α alone (393). Given that TNF-α release from cells occur as a consequence of endotoxin-TLR receptor interactions, and that TNF-α is responsible for setting off a chain of events that is associated with an elevation in plasma lactate, the implication is that the host's inflammatory response to LPS can drive lactate production . It is well documented that endotoxin and sepsis results in an increased activity of the membrane Na^+/K^+ ATPase (394-396), and it has also been demonstrated that activation of this ATPase drives cellular lactate production and aerobic glycolysis (397). Evidence exists that muscle and non-muscle tissue is involved in the generation of lactate in critical illness (398, 399) – the latter including inflammatory cells and areas heavily infiltrated by inflammatory cells (400, 401). The involvement of TNF-α and IL-1β in a host of features observed in all experiments here is notable. TNF-α receptor activation is involved in the acute renal failure induced by LPS (341, 343, 345, 402), is involved in the blunted vasodilator responses to ACh and SNP seen in metabolic syndrome and ischaemia/reperfusion injury (371, 403), drives lactate production and together with IL-1β recapitulates many of the cardiovascular effects seen with LPS (404). Therefore should dopexamine be able to modulate TNF-α receptor signalling directly and/or result in a decreased release of systemic TNF-α by immune cells, it would in theory be able to ameliorate rises in plasma lactate, renal dysfunction and improve vascular reactivity without necessarily increasing global or tissue oxygen delivery. Furthermore a vascular bed which is able to preserve its reactivity should be able to autoregulate and

therefore better balance oxygen supply and demand. This is what these experiments found – not only was dopexamine infusion associated with less TNF-α release, improved organ and (in particular) renal function, lower plasma lactate and less immune activation, but in *in vitro* experiments it was demonstrated that dopexamine could directly interfere with TNF-α induced changes in vascular function and *in vivo* experiments suggested preserved autoregulation. What is more is that dopexamine was associated with reduced levels of IL-1β and IL-12 (a stimulator of IFN-γ synthesis (347)), when salbutamol wasn't.

8.4 Why does salbutamol fail where dopexamine succeeds?

The immunomodulatory effects of catecholamines and in particular β$_2$-adrenoceptor agonists been previously investigated (40, 195, 218, 241). It would be tempting to ascribe the immunomodulatory effects of dopexamine to β$_2$-adrenoceptor agonism given that salbutamol produced almost identical effects on the majority of immune parameters and the results of previous β$_2$-adrenoceptor studies (250, 256). However, all three experiments present problems in adopting this approach. In experiment 1 equipotent doses of salbutamol and dopexamine demonstrated potent effects on neutrophil CD11b, pulmonary MPO and plasma TNF-α. Similarly, in experiment 3 the effects of equipotent salbutamol and dopexamine on intestinal leucocyte-endothelial adhesion were also comparable. Nevertheless, the effects of dopexamine across both experiments resulted in improved organ function, plasma lactate whereas salbutamol didn't. Therefore assuming dopexamine achieves effects via an identical mechanism to salbutamol cannot be strictly correct, notwithstanding the fact that it has already been demonstrated that producing beneficial effects in endotoxaemia can be achieved without reducing TNF-α levels (243). The following explanations could account for some of the difference in outcomes between salbutamol and dopexamine if the β$_2$-adrenoceptor is focused on:

1. The assumption (based on a study using tracheal preparations (44)) that doses of 1 and 2 µg/kg/min dopexamine are equivalent to 0.1 and 0.2µg/kg/min salbutamol at the β_2-adrenoceptor, respectively, may not be correct (405). Should it be the case that the actual dose equivalence is not governed by a fold-difference of 10, this could account for any differences seen in the ability of salbutamol and dopexamine to ameliorate cytokine responses, plasma lactate and organ function in these experiments. However, the fact that neutrophil behaviour measured by 3 separate means (IVM, flow cytometry and MPO activity) was comparable between these groups argues against this possibility.

2. Even if doses of salbutamol were correctly selected, it is possible that the significantly lower MAP in experiment 1 (when comparing salbutamol 0.2µg/kg/min with dopexamine 2µg/kg/min) was responsible for the worse outcomes seen in salbutamol treated groups in that experiment. This is unlikely to be a valid explanation though as in experiment 3, MAP was not significantly different between salbutamol and dopexamine groups yet lactate and renal function showed no trend to improvement whatsoever in the salbutamol group whereas the comparator dose dopexamine group showed strong trends to improvement.

3. It could be argued that the β_2-adrenoceptor is fundamental to the beneficial effects of dopexamine if it is recalled that the classic model of the β_2-adrenoceptor is that agonist binding to it leads to an activation of adenylate cyclase and an increase in intracellular cAMP (20). Any difference in the outcomes between salbutamol and dopexamine could then be ascribed to a non-equivalence at the level of cAMP generation (rather than non-equivalence at the receptor level as point 1 would have argued), as dopexamine additionally signals through dopaminergic receptors which also lead to increases in intracellular cAMP. This argument loses some validity if cAMP generation is not the only cellular basis of the anti-inflammatory action of these β_2-adrenoceptor agonists, and there is some evidence that this could be the case (243, 247).

4. Evidence exists that in addition to the traditional β$_2$-adrenoceptor-adenylate cyclase-cAMP-protein kinase A signalling paradigm, other β$_2$-adrenoceptor-dependent signalling pathways exist which are involved in inflammation (259). β$_2$-adrenoceptor agonists signal via these other pathways in a manner which is not necessarily related to the strength of cAMP signalling (probably due to the promiscuous coupling of β$_2$-adrenoceptors with differing G-protein subunits), but β$_2$-adrenoceptor agonists also have non-β$_2$-adrenoceptor mediated anti-inflammatory effects (241, 344). Differential activation of such pathways by dopexamine and salbutamol may then have been responsible for the differences between salbutamol and dopexamine groups (and may have manifested as higher levels of IL-1β, IL-6, IL-10 and IFN-γ in the salbutamol groups) whereas a similar degree of activation of the traditional pathway could explain neutrophil effects (406).

5. Differences between the efficacy of dopexamine and salbutamol in countering endotoxin induced inflammatory change may have related to a differential ability to reduce cellular levels of free radicals (215, 218, 244, 246, 247). It is also notable that the ability to reduce intracellular levels of free radicals by antioxidants has been linked to the prevention of vascular dysfunction by TNF-α (375). How this ties in with cAMP formation is unknown as this feature has recently been shown to be common to many β$_2$-adrenoceptor agonists (241, 247), though some authors suggest that while blockade of adrenoceptors can abolish this effect (241, 248), various adrenoceptor agonists can reduce superoxide generation independently of adrenoceptor (241), adenylate cyclase, cAMP, protein kinase A or Epac based mechanisms (247). Where dopexamine lies in this signalling spectrum is unknown, though in vivo studies (including the ones in this thesis) point to at least some dependency on the β$_2$-adrenoceptor for effect (240).

6. Salbutamol and dopexamine are thought to have different macrohaemodynamic effects. Salbutamol by virtue of β$_2$-adrenoceptor agonism is understood to preferentially re-distribute blood to skeletal muscle and could shunt cardiac output

away from essential organs, contributing to worse organ injury and higher lactates despite any immunomodulatory effects. Evidence for a different distribution of microvascular blood flow was found in experiments 2 and 3 (TPR graphs, intestinal arteriolar diameters).

8.5   Study Limitations

There are several limitations of the study which deserve mention. The first and most obvious limitation is that the model may not be relevant to humans and the findings therefore may not translate directly. In the methods section of this thesis, it was noted that the model employed is one with an overstimulation of either TLR 4, or TLR 2 & 4 signalling pathways. The model also lacked the presence of bacteria and is a short-term non-recovery model. Furthermore while *in vitro* wire myography studies demonstrate that the mesenteric arteries of humans and rats behave in a very similar fashion (407), the *in vivo* behaviour of rats and humans is by no means necessarily the same (e.g. LPS sensitivities). Studies demonstrate that murine and rodent models (which may very reasonably be expected to be closer to one another than human and rodent models) of sepsis and inflammation behave in quite characteristically different fashions, as do murine and human models (408, 409). However, as commentators point out, the recognition of these differences and the framing of findings in the context of the pathophysiology specific to the species in question should mean that lessons can still be drawn from such studies (410-412).

Further issues relate to the manner in which animal research is conducted (413). Although every effort was taken to minimise bias (e.g. randomly allocating rats to treatment groups, the analysis of IVM and the assessment organ function being conducted in a blinded fashion by persons who were not involved in the experiments), there were several aspects of the study's design that did not involve blinding. As the person conducting the experiments, I was fully aware of which animals were receiving which treatment throughout the experiments,

and was fully aware of which groups were being analysed when performing blood gas analysis, lactate measurements and flow cytometry. Part of this was due to the limitations of performing the studies alone.

It would also have been preferable to have conducted all measurements in one study in order to be more confident about the nature of any mechanisms driving changes seen with dopexamine. Experiment 1 was probably the most revealing yet no measurements of cardiac index or microvascular flow were made and we must infer from experiments 2 & 3 about what was likely to be occurring on those fronts. Similarly in experiment 3 when changes that were in keeping with experiment 1 were seen, no plasma samples were taken to assess the effect on plasma cytokine concentrations and the study was under-powered to detect the changes in organ function due to dopexamine. On the subject of the power of the studies, this was not a factor taken in to the design of the experiments and indeed power calculations have only recently come to the fore as good practice in animal-based research. This was admittedly a weakness.

The appendix lists the data comparing either controls vs. D1 (as the most appropriate dopexamine group) for several key parameters. Comparison is also made with the sham groups in experiment 2 and occasionally with the sham group in experiment 3. This data shows the power of the experiments to demonstrate significant differences between controls and the comparator group in question. It is re-assuring that the majority of significant differences between controls and shams were within the power of the study to be detected. It is notable though that to have the power to detect several changes that were found to be significant would have required occasionally a few and often substantially larger numbers of animals to be tested. Future studies should be designed with this in mind.

211

8.6 Summary

When dopexamine is infused at 1µg/kg/min in combination with a fluid regime that does not augment global oxygen delivery or tissue microvascular flow in the setting of laparotomy and endotoxaemia, dopexamine is seen to have potent immunomodulatory actions, specifically the inhibition of surface neutrophil CD11b expression, an inhibition of post-capillary venular leucocyte-endothelial adhesion, an inhibition of neutrophil infiltration to pulmonary and possibly other tissue beds and an inhibition of the cytokine response to laparotomy and endotoxaemia. There is also an amelioration of the downstream metabolic effects of the cytokine response to laparotomy and endotoxaemia, and a suggestion of a preservation of microvascular autoregulation in ileal beds. The *in vitro* studies show here that dopexamine can limit the TNF-α induced endothelial and smooth muscle dysfunction in small mesenteric arteries, possibly providing a mechanism of action for any such preserved autoregulation. The fact that the comparator β_2-adrenoceptor agonist salbutamol was also able to produce similar immunomodulatory effects but was unable to significantly improve organ function and plasma lactate points to the importance of β_2-adrenoceptor signalling related pathways in immunomodulatory effects of dopexamine, particularly regarding the behaviour of neutrophils (197), but unfortunately leaves us unable to attribute the improved organ function to these effects alone.

The mechanisms behind these consistently observed anti-inflammatory effects cannot reliably be discerned from these experiments alone. Other experiments suggest that ameliorating the rise in free-radicals and circulating cytokines are of key importance. The β_2-adrenoceptor actions of dopexamine (and possibly those at dopaminergic receptors), or functional selectivity of dopexamine at the β_2-adrenoceptor are putative explanations for the differences observed between salbutamol and dopexamine.

8.7 Translation to human studies

Human peri-operative studies differ in several important respects to these studies. Most modern peri-operative studies optimise subjects according to pre-defined stroke volume (and less often cardiac index) based responses to fluid and inotrope therapy. This was clearly not the case in these experiments – cardiac index and stroke volume were allowed to follow their natural course, which was to decrease, while maintenance fluid was infused. Although this did not result in an increase in haematocrit the dynamics of the circulation on a macroscopic and microscopic level are likely to differ. Furthermore human peri-operative studies of dopexamine have not enrolled patients who are all overtly septic. Therefore although these experiments may reveal an aspect of dopexamine that has hitherto been under-appreciated in clinical practice (i.e. immunomodulation), they do not serve to explain how and whether dopexamine synergises with a fluid protocol based on the optimisation of stroke SV/CI. Furthermore, as no significant changes in microvascular flow were seen, which is in contrast to clinical studies (147), some caution must be exercised in ascribing the findings seen with dopexamine in these studies to those seen in clinical studies.

Nevertheless, some findings here are of note for clinicians. Most importantly, these studies have shown that dopexamine can markedly attenuate the inflammatory response to laparotomy and endotoxaemia, and improve organ function without an increase in cardiac index or microvascular flow – this is an important finding in its own right. Secondly, the effects of catecholamines in sepsis cannot be easily predicted even when similar to one another, and so each drug requires individual evaluation given the myriad signalling pathways, specificities of receptor-ligand interactions and differential abilities to signal through different G-protein subunits. Finally, one of the most interesting findings of the thesis was that dopexamine improved the function of different organs to varying degrees across three very similar experiments – this seems to parallel human studies where the effects of dopexamine on morbidity and mortality are not always reproducible. While the results of this thesis provide an additional mechanistic insight in to how the addition of dopexamine to a

peri-operative fluid optimisation strategy in humans can improve outcome, they also reveal how the effects of dopexamine are highly dependent on the clinical conditions under which it is given. If dopexamine can genuinely lead to improved outcomes there is an urgent need to find out what the nature of the variability is that ablates any beneficial effect of dopexamine.

8.8   Hypotheses

With respect to the original hypotheses:

1. Dopexamine improves microvascular perfusion in the ileum of endotoxaemic rodents

2. The beneficial effects of dopexamine on ileal microvascular perfusion are not related to its effects on cardiac index

3. Dopexamine has anti-inflammatory effects in the setting of endotoxaemia, namely reduced neutrophil activation as assessed by membrane integrin expression, decreased leucocyte-endothelial adhesion in the intestinal microcirculation and decreased neutrophil infiltration in the lung

4. Dopexamine improves organ function in a rodent model of laparotomy and endotoxaemia

5. The anti-inflammatory effects of dopexamine are β_2-adrenoceptor dependent

These studies have only weakly supported hypotheses 1 and 2. Hypothesis 2 has not been refuted though improvements in arteriolar diameters were independent of an augmented cardiac index. The experiments strongly support the third and fourth hypotheses. Hypothesis 5 receives strong support from the experiments here. It should be noted that while the experiments here strongly support a role for β_2-adrenoceptor mediated mechanisms as being important in leucocyte endothelial interactions, the experiments could also be used to support the role of other mechanisms being important with regards to the improvements in organ function seen with dopexamine.

8.9   Future directions

The strong immunomodulatory effects of dopexamine seen in these preparations, the unexpected lack of microvascular effects, the lack of a clear mechanism through which dopexamine exerts its effects and the inherent weaknesses of the model itself means that there are a multitude of directions in which this work should be taken in the future.

While the intention was not to look at models of sepsis *per se*, it happens that surgical complications occur with very great frequency in patients who undergo emergency surgery for abdominal sepsis. Therefore to extend these experiments to a similar model (such as faecal slurry or caecal ligation and puncture) would improve the translatability and robustness of any future findings. During any such experiments it would be useful to re-visit many of the original questions. This could be done in several ways:

1. The use of a microsphere technique to assess the distribution of blood flows within the gut, or indeed any other organ - most notably the kidneys and liver. Therefore even in the absence of detectable differences in FCD in specific layers of (for example) the gut, any deterioration in organ function could still be potentially explained by an alteration in the intra-organ distribution of blood flows.

2. A more detailed exploration of how dopexamine modifies oxygen kinetics at the level of the microcirculation and tissues – this could be achieved with tissue oxygen probes (ruthenium based IVM probes or tissue electrodes), the use of IVM to look at NADH autofluorescence, the use of a Doppler camera to measure erythrocyte velocity, and the harvesting and freezing of organs after experiments in order to later determine ATP/ADP ratios.

3. The use of non-invasive laser speckle contrast imaging could also allow surface, whole organ blood flow to be much more easily assessed over a prolonged period of time as compared to laser Doppler flowmetry which is limited in the number of sampling sites and IVM which suffers from the drawback of specimen deterioration with observed time. It may also be useful to use laser speckle imaging to

simultaneously measure the distribution of blood flow in a completely different tissue bed (e.g. liver surface or exposed area of abdominal muscle).

4. Follow up to these experiments must also be made from the perspective of fluid-catecholamine interactions. A flow guided method of volume resuscitation similar to that used in theatre and intensive care units must be employed in a new set of investigations (this will also improve translatability), and an assessment made of whether the addition of dopexamine to such a regime replicates the immunomodulatory findings in these experiments and the microvascular findings in the group's clinical experiments (147).

5. Further mechanistic insights in to the important receptor actions of dopexamine that lead to anti-inflammatory effects can be gained through the use of drugs in *in vitro* preparations where selective blockade of dopaminergic and/or adrenoceptor pathways (e.g. ICI 118551, SCH 23390, domperidone etc.). These experiments should not only be conducted in myography preparations, but also in suspensions of leucocytes, endothelial monolayers and should assess functional aspects of neutrophil function – such as respiratory burst and chemotaxis. Similarly drugs such as Forskolin (an adenylate cyclase activator that increases intracellular cAMP levels) can be administered while surface receptors are blocked to interrogate the importance of downstream parts of the signalling cascade.

Appendix

Data comparing either controls vs. D1 (as the most appropriate dopexamine group) for several key parameters. Comparison is also made with the sham groups in experiment 2 and occasionally with the sham group in experiment 3 – only parameters which were distributed normally for these groups in the experiments have been listed. The software package MedCalc (v12.7.7.0) was used to perform power calculations, setting a significance level of $P<0.05$ and a power of 80% and using the standard deviations for each sample. Where the ratio of numbers in each group was not 1, the same ratio was maintained to show what the appropriate numbers ought to have been for 80% power. It should also be noted that this shows the power of the studies to show a difference between these groups against controls as independent samples t-tests (with unequal variances) as opposed to part of a one-way ANOVA where due to multiple comparisons the power will be even lower.

EXPERIMENT 1				
	Control mean (SD)	D1 mean (SD)	Measurements per group in experiment	Numbers per group for 80% power at P<0.05
lactate	3.96 (1.52)	1.99 (1.11)	8 each	8 each
base deficit	7.94 (6.21)	2.17 (3.39)	8 : 6	13 each
urea	17.78 (2.52)	14.65 (1.76)	8 each	9 each
creatinine	76.2 (27.6)	40.3 (17.8)	8 each	8 each
ALT	137.6 (71.9)	79.1 (19.1)	8 each	14 each
AST	422.6 (195.4)	275.4 (88.0)	8 each	18 each
TNF-α (1h)	4485 (3714)	1611 (1091)	7 : 8	15 : 18
TNF-α (4h)	323.7 (262.3)	50.9 (15.9)	8 : 6	9 : 7
IL-1	1774 (1650)	192.7 (89.7)	8 : 6	10 : 8
IL-6	29668 (19943)	4268 (2555)	7 : 8	6 : 7
MPO	125.02 (10.15)	76.46 (5.55)	4 : 4	2 each
Final CD11b	133.68 (28.46)	103.18 (26.84)	6 : 8	12 : 16

EXPERIMENT 2				
	Control mean (SD)	**Sham** mean (SD)	**Measurements per group in experiment**	**Numbers per group for 80% power at P<0.05**
lactate	3.73 (1.03)	1.69 (0.54)	8 each	4 each
base deficit	8.98 (4.02)	-1.51 (1.30)	8 : 7	3 each
creatinine	62.0 (12.6)	35.5 (4.3)	8 each	3 each
AST	337.0 (86.2)	266.6 (106.1)	8 each	31 each
CI (4h)	24.3 (7.4)	49.4 (16.3)	8 each	4 each
SVI (4h)	0.052 (0.016)	0.133 (0.046)	8 each	5 each
Ileal flux (4h)	48.7 (20.9)	81.6 (34.0)	16 each	13 each

EXPERIMENT 3					
	Control mean (SD)	**D1** mean (SD)	**Sham** Mean (SD)	**Measurements per group in experiment**	**Numbers per group for 80% power at P<0.05**
lactate	3.37 (1.16)	2.60 (1.03)	n/a	5 : 6	31 : 38
base deficit	4.54 (3.58)	3.33 (3.70)	n/a	6 each	144 each
urea	17.80 (2.09)	16.27 (2.02)	n/a	6 each	29 each
creatinine	60.2 (15.1)	45.5 (4.1)	n/a	6 each	10 each
CK	1617 (710)	953 (427)	n/a	6 each	13 each
ALT	81.1 (22.6)	n/a	47.0 (13.4)	6 each	6 each
AST	324.6 (113.2)	279.4 (70.2)	n/a	6 each	69 each
TPR (2.5h)	10.97 (3.10)	9.62 (1.82)	7.18 (1.70)	6 each	56 each (D1), 8 each (sham)
CI (5h)	32.2 (10.4)	n/a	63.4 (19.4)	6 each	5 each
SVI (5h)	0.072 (0.024)	n/a	0.171 (0.049)	6 each	4 each
A1	54.1 (10.0)	75.6 (40.5)	94.4 (28.0)	15 : 14 (D1) 15 : 10 (sham)	33 : 31 (D1), 23 : 16 (sham)
A3	21.0 (5.0)	30.5 (7.4)	39.3 (8.2)	9 : 12 (D1) 9 : 8 (sham)	7 : 10 (D1), 4 each (sham)
fcd longitudinal layer	129.7 (42.2)	89.9 (27.2)	n/a	16 : 19	13 : 16
fcd total muscularis	293.8 (76.0)	235.8 (66.8)	n/a	16 : 22	22 : 31
Adh v3	702.7 (296.9)	360.9 (247.7)	n/a	12 : 14	10 : 12

References

1.   Shoemaker WC. Relation of oxygen transport patterns to the pathophysiology and therapy of shock states. Intensive Care Med 1987;13(4):230-43.

2.   Levick J. An Introduction to Cardiovascular Physiology. 4th Edition ed. London: Hodder Arnold; 2003.

3.   Guyton AC, Lindsey AW, Kaufmann BN. Effect of mean circulatory filling pressure and other peripheral circulatory factors on cardiac output. Am J Physiol 1955;180(3):463-8.

4.   den Uil CA, Klijn E, Lagrand WK, Brugts JJ, Ince C, Spronk PE, et al. The microcirculation in health and critical disease. Prog Cardiovasc Dis 2008;51(2):161-70.

5.   De Backer D, Creteur J, Dubois MJ, Sakr Y, Vincent JL. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. Am Heart J 2004;147(1):91-9.

6.   Ince C. The microcirculation is the motor of sepsis. Crit Care 2005;9 Suppl 4:S13-9.

7.   Spanos A, Jhanji S, Vivian-Smith A, Harris T, Pearse RM. Early microvascular changes in sepsis and severe sepsis. Shock 2010;33(4):387-91.

8.   De Backer D, Creteur J, Dubois MJ, Sakr Y, Koch M, Verdant C, et al. The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. Crit Care Med 2006;34(2):403-8.

9.   Jhanji S, Stirling S, Patel N, Hinds CJ, Pearse RM. The effect of increasing doses of norepinephrine on tissue oxygenation and microvascular flow in patients with septic shock. Crit Care Med 2009;37(6):1961-6.

10.   Adamson RH, Liu B, Fry GN, Rubin LL, Curry FE. Microvascular permeability and number of tight junctions are modulated by cAMP. Am J Physiol 1998;274(6 Pt 2):H1885-94.

11.   Waschke J, Drenckhahn D, Adamson RH, Barth H, Curry FE. cAMP protects endothelial barrier functions by preventing Rac-1 inhibition. Am J Physiol Heart Circ Physiol 2004;287(6):H2427-33.

12.   Alexander SP, Mathie A, Peters JA. Guide to Receptors and Channels (GRAC), 5th edition. Br J Pharmacol 2011;164 Suppl 1:S1-324.

13.   Hendriks-Balk MC, Peters SL, Michel MC, Alewijnse AE. Regulation of G protein-coupled receptor signalling: focus on the cardiovascular system and regulator of G protein signalling proteins. Eur J Pharmacol 2008;585(2-3):278-91.

14.   Summers RJ, McMartin LR. Adrenoceptors and their second messenger systems. J Neurochem 1993;60(1):10-23.

15.   Heck DA, Bylund DB. Mechanism of down-regulation of alpha-2 adrenergic receptor subtypes. J Pharmacol Exp Ther 1997;282(3):1219-27.

16.   Bohm M, Deutsch HJ, Hartmann D, Rosee KL, Stablein A. Improvement of postreceptor events by metoprolol treatment in patients with chronic heart failure. J Am Coll Cardiol 1997;30(4):992-6.

17.   Tang C, Yang J, Wu LL, Dong LW, Liu MS. Phosphorylation of beta-adrenergic receptor leads to its redistribution in rat heart during sepsis. Am J Physiol 1998;274(4 Pt 2):R1078-86.

18.   Brodde OE, Michel MC. Adrenergic and muscarinic receptors in the human heart. Pharmacol Rev 1999;51(4):651-90.

19.   Guimaraes S, Moura D. Vascular adrenoceptors: an update. Pharmacol Rev 2001;53(2):319-56.

20.   Bangash MN, Kong ML, Pearse RM. Use of inotropes and vasopressor agents in critically ill patients. Br J Pharmacol 2012;165(7):2015-33.

21.   Furness JB, Marshall JM. Correlation of the directly observed responses of mesenteric vessles of the rat to nerve stimulation and noradrenaline with the distribution of adrenergic nerves. J Physiol 1974;239(1):75-88.

22.   Marshall JM. The influence of the sympathetic nervous system on individual vessels of the microcirculation of skeletal muscle of the rat. J Physiol 1982;332:169-86.

23.   Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78(1):189-225.

24. Goldberg LI, Rajfer SI. Dopamine receptors: applications in clinical cardiology. Circulation 1985;72(2):245-8.

25. Bertorello A, Aperia A. Inhibition of proximal tubule Na(+)-K(+)-ATPase activity requires simultaneous activation of DA1 and DA2 receptors. Am J Physiol 1990;259(6 Pt 2):F924-8.

26. Lokhandwala MF, Amenta F. Anatomical distribution and function of dopamine receptors in the kidney. Faseb J 1991;5(15):3023-30.

27. Wang N, Zhao DH, Sheng BH. Positive inotropic effect of apomorphine on guinea pig myocardium is mediated by dopamine DA1 receptors. Zhongguo Yao Li Xue Bao 1991;12(3):207-11.

28. Wakita Y. Inotropic, chronotropic, and arrhythmogenic effects of dopamine on the isolated working heart of rabbit. J Physiol Sci 2007;57(3):147-57.

29. Motomura S, Brodde OE, Schumann HJ. No evidence for involvement of dopaminergic receptors in the positive inotropic action of dopamine on the isolated rabbit papillary muscle. Jpn J Pharmacol 1978;28(1):145-53.

30. Zeng C, Zhang M, Asico LD, Eisner GM, Jose PA. The dopaminergic system in hypertension. Clin Sci (Lond) 2007;112(12):583-97.

31. Lullman H MK, Ziegler A, Bieger D. Color Atlas of Pharmacology. 2nd edition ed. Stuttgart: Thieme; 2000.

32. Henkel JG, Sikand N, Makriyannis A, Gianutsos G. Synthesis and adrenoceptor affinity of some highly polar beta-substituted catecholamines. J Med Chem 1981;24(10):1258-60.

33. Mukherjee C, Caron MG, Mullikin D, Lefkowitz RJ. Structure-activity relationships of adenylate cyclase-coupled beta adrenergic receptors: determination by direct binding studies. Mol Pharmacol 1976;12(1):16-31.

34. Graefe KH BH. The Transport of Amines Across the Axonal Membranes of Noradrenergic and Dopaminergic Neurones. In: Trendelenburg U, editor. Catecholamines. Berlin: Springer-Verlag; 1988. p. 193-245.

35.   Xiao RP, Zhang SJ, Chakir K, Avdonin P, Zhu W, Bond RA, et al. Enhanced G(i) signaling selectively negates beta2-adrenergic receptor (AR)--but not beta1-AR-mediated positive inotropic effect in myocytes from failing rat hearts. Circulation 2003;108(13):1633-9.

36.   Nakada TA, Russell JA, Boyd JH, Aguirre-Hernandez R, Thain KR, Thair SA, et al. beta2-Adrenergic receptor gene polymorphism is associated with mortality in septic shock. Am J Respir Crit Care Med 2010;181(2):143-9.

37.   Clutter WE, Bier DM, Shah SD, Cryer PE. Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. J Clin Invest 1980;66(1):94-101.

38.   Galster AD, Clutter WE, Cryer PE, Collins JA, Bier DM. Epinephrine plasma thresholds for lipolytic effects in man: measurements of fatty acid transport with [l-13C]palmitic acid. J Clin Invest 1981;67(6):1729-38.

39.   Lipowsky HH. Microvascular rheology and hemodynamics. Microcirculation 2005;12(1):5-15.

40.   Oberbeck R. Catecholamines: physiological immunomodulators during health and illness. Curr Med Chem 2006;13(17):1979-89.

41.   Elenkov IJ. Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being. Neurochem Int 2008;52(1-2):40-51.

42.   Flierl MA, Rittirsch D, Huber-Lang M, Sarma JV, Ward PA. Catecholamines-crafty weapons in the inflammatory arsenal of immune/inflammatory cells or opening pandora's box? Mol Med 2008;14(3-4):195-204.

43.   Foulds RA. Clinical development of dopexamine hydrochloride (Dopacard) and an overview of its hemodynamic effects. 1988.

44.   Brown RA, Dixon J, Farmer JB, Hall JC, Humphries RG, Ince F, et al. Dopexamine: a novel agonist at peripheral dopamine receptors and beta 2-adrenoceptors. Br J Pharmacol 1985;85(3):599-608.

45.   Dawson JR, Thompson DS, Signy M, Juul SM, Turnbull P, Jenkins BS, et al. Acute haemodynamic and metabolic effects of dopexamine, a new dopaminergic receptor agonist, in patients with chronic heart failure. Br Heart J 1985;54(3):313-20.

46.   Goldberg LI. Cardiovascular and renal actions of dopamine: potential clinical applications. Pharmacol Rev 1972;24(1):1-29.

47.   Bohm M, Pieske B, Schnabel P, Schwinger R, Kemkes B, Klovekorn WP, et al. Reduced effects of dopexamine on force of contraction in the failing human heart despite preserved beta 2-adrenoceptor subpopulation. J Cardiovasc Pharmacol 1989;14(4):549-59.

48.   Napoleone P, Ricci A, Ferrante F, Amenta F. Dopexamine hydrochloride in the human heart: receptor binding and effects on cAMP generation. Eur Heart J 1992;13(12):1709-17.

49.   Port JD, Gilbert EM, Larrabee P, Mealey P, Volkman K, Ginsburg R, et al. Neurotransmitter depletion compromises the ability of indirect-acting amines to provide inotropic support in the failing human heart. Circulation 1990;81(3):929-38.

50.   Martin SW, Broadley KJ. Effects of chronic intravenous infusions of dopexamine and isoprenaline to rats on D1-, beta 1- and beta 2-receptor-mediated responses. Br J Pharmacol 1994;112(2):595-603.

51.   Gray PA, Jones T, Park GR. Blood concentrations of dopexamine in patients during and after orthotopic liver transplantation. Br J Clin Pharmacol 1994;37(1):89-92.

52.   Fitton A, Benfield P. Dopexamine hydrochloride. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in acute cardiac insufficiency. Drugs 1990;39(2):308-30.

53.   Mazzuco TL, Dias MA, Calixto JB. Characterization of the mechanism involved in the relaxant response of dopexamine in the guinea pig pulmonary artery in vitro. J Cardiovasc Pharmacol 1999;33(1):86-92.

54.   Taylor BJ, Rogers BW, Sziszak TL, Sziszak TJ. Effects of dopexamine on hemodynamics and oxygen consumption after beta blockade in lambs. Crit Care Med 1993;21(5):733-9.

55.   Bass AS, Kohli JD, Lubbers N, Goldberg LI. Mechanisms mediating the positive inotropic and chronotropic changes induced by dopexamine in the anesthetized dog. J Pharmacol Exp Ther 1987;242(3):940-4.

56.   Napoleone P, Cavallotti C, Ricci A, Amenta F. Dopexamine hydrochloride in the human kidney: localization, receptor binding and effect on 3'-5'-cyclic adenosine monophosphate generation. Nephron 1993;65(3):385-91.

57.   Amenta F, Ricci A, Napoleone P, Vyas SJ, Lokhandwala MF. Anatomical localization of the binding and functional characterization of responses to dopexamine hydrochloride in the rat mesenteric vasculature. Pharmacology 1991;42(4):211-22.

58.   Vyas SJ, Apparsundaram S, Ricci A, Amenta F, Lokhandwala MF. Biochemical, autoradiographic and pharmacological evidence for the involvement of tubular DA-1 receptors in the natriuretic response to dopexamine hydrochloride. Naunyn Schmiedebergs Arch Pharmacol 1991;343(1):21-30.

59.   Brodde OE. The functional importance of beta 1 and beta 2 adrenoceptors in the human heart. Am J Cardiol 1988;62(5):24C-29C.

60.   Goldberg LI, Bass AS. Relative significance of dopamine receptors, beta adrenoceptors and norepinephrine uptake inhibition in the cardiovascular actions of dopexamine hydrochloride. Am J Cardiol 1988;62(5):37C-40C.

61.   Inarejo M, Himmel H, Ravens U. Effects of catecholamine uptake inhibitors on the positive inotropic responses to isoprenaline, dobutamine and dopexamine in human, rat and guinea-pig atrial heart muscle. Arch Int Pharmacodyn Ther 1993;323:50-61.

62.   Bass AS. Presynaptic effects of dopexamine hydrochloride in the canine kidney. Naunyn Schmiedebergs Arch Pharmacol 1992;345(1):33-6.

63.   Bass AS, Murphy MB, Kohli JD, Goldberg LI. Potentiation by dopexamine of the cardiac responses to circulating and neuronally released norepinephrine: a possible mechanism for the therapeutic effects of the drug. J Cardiovasc Pharmacol 1989;13(4):667-71.

64.   Nedergaard OA. Inhibition of 3H-noradrenaline accumulation by dopexamine hydrochloride in the isolated aorta of the rabbit. Naunyn Schmiedebergs Arch Pharmacol 1989;340(3):270-3.

65.   Mitchell PD, Smith GW, Wells E, West PA. Inhibition of Uptake1 by dopexamine hydrochloride in vitro. Br J Pharmacol 1987;92(2):265-70.

66.   Martin SW, Broadley KJ. Renal vasodilatation by dopexamine and fenoldopam due to alpha 1-adrenoceptor blockade. Br J Pharmacol 1995;115(2):349-55.

67.   Nedergaard OA. Effect of dopexamine hydrochloride on contractions of rabbit isolated aorta evoked by various agonists. Pharmacol Toxicol 1994;74(1):43-9.

68.   Nedergaard OA. Effect of dopexamine hydrochloride on sympathetic neuroeffector transmission in rabbit isolated pulmonary artery. Pharmacol Toxicol 1996;78(3):161-6.

69.   Teschemacher A, Reinhardt D, Lemoine H. Do parasympatholytic effects of long-acting beta 2-sympathomimetics contribute to their relaxant effects in airway smooth muscle cells? Pulm Pharmacol Ther 1998;11(4):253-61.

70.   Martin SW, Broadley KJ. Atypical antagonism of D1-receptor-mediated vasodilator response in the perfused kidney by SCH23390. Pharmacol Res 1995;31(5):289-97.

71.   MacGregor DA, Prielipp RC, Butterworth JFt, James RL, Royster RL. Relative efficacy and potency of beta-adrenoceptor agonists for generating cAMP in human lymphocytes. Chest 1996;109(1):194-200.

72.   Dun W, Zhao RR, Liang Y, Wu BW. Effects of dopexamine hydrochloride on calcium channels in isolated guinea pig ventricular myocytes. Methods Find Exp Clin Pharmacol 1996;18(6):353-7.

73.   Stangl K, Baumann G, Weil J, Gerzer R, Kerscher M, Blomer H. [Acute reduction of increased atrial natriuretic peptide level and cyclic guanosine monophosphate in patients with chronic heart failure caused by beta-adrenergic stimulation with dopexamine hydrochloride. Correlation with hemodynamic parameters]. Z Kardiol 1990;79(6):417-23.

74.   Lokhandwala MF, Vyas SJ, Hegde SS. Renal dopamine and tubular DA-1 receptors in the regulation of sodium excretion. J Auton Pharmacol 1990;10 Suppl 1:s31-9.

75.   van der Starre PJ, Rosseel PM. Dopexamine hydrochloride after coronary artery bypass grafting. Am J Cardiol 1988;62(5):78C-82C.

76.   Mousdale S, Clyburn PA, Mackie AM, Groves ND, Rosen M. Comparison of the effects of dopamine, dobutamine, and dopexamine upon renal blood flow: a study in normal healthy volunteers. Br J Clin Pharmacol 1988;25(5):555-60.

77.   Olsen NV, Lund J, Jensen PF, Espersen K, Kanstrup IL, Plum I, et al. Dopamine, dobutamine, and dopexamine. A comparison of renal effects in unanesthetized human volunteers. 1993.

78.   Bartsch S, Bruning A, Reimann FM, Ludwig D. Haemodynamic effects of dopexamine on postprandial splanchnic hyperaemia. Eur J Clin Invest 2004;34(4):268-74.

79.   Magrini F, Foulds R, Roberts N, Macchi G, Mondadori C, Zanchetti A. Human renovascular effects of dopexamine hydrochloride: a novel agonist of peripheral dopamine and beta 2-adreno-receptors. 1987.

80.   Olsen NV, Lund J, Jensen PF, Espersen K, Kanstrup IL, Plum I, et al. Dopamine, dobutamine, and dopexamine. A comparison of renal effects in unanesthetized human volunteers. Anesthesiology 1993;79(4):685-94.

81.   Geisser W, Trager K, Hahn A, Georgieff M, Ensinger H. Metabolic and calorigenic effects of dopexamine in healthy volunteers. Crit Care Med 1997;25(8):1332-7.

82.   Foulds RA. Clinical development of dopexamine hydrochloride (Dopacard) and an overview of its hemodynamic effects. Am J Cardiol 1988;62(5):41C-45C.

83.   Moppett IK, Wild MJ, Sherman RW, Latter JA, Miller K, Mahajan RP. Effects of ephedrine, dobutamine and dopexamine on cerebral haemodynamics: transcranial Doppler studies in healthy volunteers. Br J Anaesth 2004;92(1):39-44.

84.   Greven J. Evidence for redistribution of filtrate among nephrons after beta-adrenergic stimulation and blockade. Naunyn Schmiedebergs Arch Pharmacol 1974;282(2):171-80.

85.   Bass AS. Contrasting effects of dopexamine hydrochloride on electrolyte excretion in canine kidney. J Pharmacol Exp Ther 1990;253(2):798-802.

86.   Burns AM, Keogan M, Donaldson M, Brown DL, Park GR. Effects of inotropes on human leucocyte numbers, neutrophil function and lymphocyte subtypes. Br J Anaesth 1997;78(5):530-5.

87.   Wagaine-Twabwe D, Hendra TJ, Smith CC, Yudkin JS. The effects of dopexamine, a new dopamine analogue, on platelet function in stress. Br J Clin Pharmacol 1990;30(1):87-95.

88.   Geisser W, Vogt J, Wachter U, Hofbauer H, Georgieff M, Ensinger H. Effects of dopexamine in comparison with fenoterol on carbohydrate, fat and protein metabolism in healthy volunteers. Intensive Care Med 2004;30(4):702-8.

89.   Svensson G, Sjogren A, Erhardt L. Short-term haemodynamic effects of dopexamine in patients with chronic congestive heart failure. Eur Heart J 1986;7(8):697-703.

90.   Svenson G, Strandberg LE, Lindvall B, Erhardt L. Haemodynamic response to dopexamine hydrochloride in postinfarction heart failure: lack of tolerance after continuous infusion. Br Heart J 1988;60(6):489-96.

91.   Murphy JJ, Hampton JR. Failure of dopexamine to maintain haemodynamic improvement in patients with chronic heart failure. 1988.

92.   Jamison M, Widerhorn J, Weber L, Campese V, Vasquez J, Hovanessian L, et al. Central and renal hemodynamic effects of a new agonist at peripheral dopamine- and beta-2 adrenoreceptors (dopexamine) in patients with heart failure. 1989.

93.   Baumann G, Gutting M, Pfafferott C, Ningel K, Klein G. Comparison of acute haemodynamic effects of dopexamine hydrochloride, dobutamine and sodium nitroprusside in chronic heart failure. Eur Heart J 1988;9(5):503-12.

94.   Leier CV, Binkley PF, Carpenter J, Randolph PH, Unverferth DV. Cardiovascular pharmacology of dopexamine in low output congestive heart failure. 1988.

95.   Tan LB, Littler WA, Murray RG. Comparison of the haemodynamic effects of dopexamine and dobutamine in patients with severe congestive heart failure. Int J Cardiol 1991;30(2):203-8.

96.   Gollub SB, Elkayam U, Young JB, Miller LW, Haffey KA. Efficacy and safety of a short-term (6-h) intravenous infusion of dopexamine in patients with severe congestive heart failure: a randomized, double-blind, parallel, placebo-controlled multicenter study. J Am Coll Cardiol 1991;18(2):383-90.

97.   Asanoi H, Sasayama S, Sakurai T, Lee JD, Kinoshita M, Ishimura T, et al. Intravenous dopexamine in the treatment of acute congestive heart failure: results of a multicenter, double-blind, placebo-controlled withdrawal study. Cardiovasc Drugs Ther 1995;9(6):791-7.

98.   Bristow MR, Hershberger RE, Port JD, Minobe W, Rasmussen R. Beta 1- and beta 2-adrenergic receptor-mediated adenylate cyclase stimulation in nonfailing and failing human ventricular myocardium. Mol Pharmacol 1989;35(3):295-303.

99.   Eisenhofer G, Friberg P, Rundqvist B, Quyyumi AA, Lambert G, Kaye DM, et al. Cardiac sympathetic nerve function in congestive heart failure. Circulation 1996;93(9):1667-76.

100.   Colardyn FA, Vandenbogaerde JF. Use of dopexamine hydrochloride in intensive care patients with low-output left ventricular heart failure. Am J Cardiol 1988;62(5):68C-72C.

101.   Vincent JL, Reuse C, Kahn RJ. Administration of dopexamine, a new adrenergic agent, in cardiorespiratory failure. Chest 1989;96(6):1233-6.

102.   Hunter DN, Gray H, Mudaliar Y, Morgan C, Evans TW. The effects of dopexamine hydrochloride on cardiopulmonary haemodynamics following cardiopulmonary bypass surgery. Int J Cardiol 1989;23(3):365-71.

103.   Santman FW, Thomas SM, Hoskins B. Prolonged infusion of varied doses of dopexamine hydrochloride for circulatory failure after cardiac surgery. J Cardiothorac Anesth 1989;3(5 Suppl 1):13.

104.   Gray PA, Bodenham AR, Park GR. A comparison of dopexamine and dopamine to prevent renal impairment in patients undergoing orthotopic liver transplantation. Anaesthesia 1991;46(8):638-41.

105. Welch M, Newstead CG, Smyth JV, Dodd PD, Walker MG. Evaluation of dopexamine hydrochloride as a renoprotective agent during aortic surgery. Ann Vasc Surg 1995;9(5):488-92.

106. Barneschi MG, Rossi R, Pieraccioli E, Girardi G, Novelli GP. [Effect of dopexamine in splanchnic perfusion during surgery of the abdominal aorta]. Minerva Anestesiol 1994;60(5):245-52.

107. Smithies M, Yee TH, Jackson L, Beale R, Bihari D. Protecting the gut and the liver in the critically ill: effects of dopexamine. Crit Care Med 1994;22(5):789-95.

108. Boyd O, Grounds RM, Bennett ED. The use of dopexamine hydrochloride to increase oxygen delivery perioperatively. Anesth Analg 1993;76(2):372-6.

109. Brown RS, Shoemaker WC. Sequential hemodynamic changes in patients with head injury: evidence for an early hemodynamic defect. Ann Surg 1973;177(2):187-92.

110. Shoemaker WC, Montgomery ES, Kaplan E, Elwyn DH. Physiologic patterns in surviving and nonsurviving shock patients. Use of sequential cardiorespiratory variables in defining criteria for therapeutic goals and early warning of death. Arch Surg 1973;106(5):630-6.

111. Shoemaker WC, Appel P, Bland R. Use of physiologic monitoring to predict outcome and to assist in clinical decisions in critically ill postoperative patients. Am J Surg 1983;146(1):43-50.

112. Barbee RW, Reynolds PS, Ward KR. Assessing shock resuscitation strategies by oxygen debt repayment. Shock 2010;33(2):113-22.

113. Walsh TS, Lee A. Mathematical coupling in medical research: lessons from studies of oxygen kinetics. Br J Anaesth 1998;81(2):118-20.

114. Hanique G, Dugernier T, Laterre PF, Dougnac A, Roeseler J, Reynaert MS. Significance of pathologic oxygen supply dependency in critically ill patients: comparison between measured and calculated methods. Intensive Care Med 1994;20(1):12-8.

115.   Hayes MA, Timmins AC, Yau EH, Palazzo M, Watson D, Hinds CJ. Oxygen transport patterns in patients with sepsis syndrome or septic shock: influence of treatment and relationship to outcome. Crit Care Med 1997;25(6):926-36.

116.   Hayes MA, Yau EH, Timmins AC, Hinds CJ, Watson D. Response of critically ill patients to treatment aimed at achieving supranormal oxygen delivery and consumption. Relationship to outcome. Chest 1993;103(3):886-95.

117.   Ronco JJ, Fenwick JC, Tweeddale MG, Wiggs BR, Phang PT, Cooper DJ, et al. Identification of the critical oxygen delivery for anaerobic metabolism in critically ill septic and nonseptic humans. Jama 1993;270(14):1724-30.

118.   Ronco JJ, Fenwick JC, Wiggs BR, Phang PT, Russell JA, Tweeddale MG. Oxygen consumption is independent of increases in oxygen delivery by dobutamine in septic patients who have normal or increased plasma lactate. Am Rev Respir Dis 1993;147(1):25-31.

119.   Gattinoni L, Brazzi L, Pelosi P, Latini R, Tognoni G, Pesenti A, et al. A trial of goal-oriented hemodynamic therapy in critically ill patients. SvO2 Collaborative Group. N Engl J Med 1995;333(16):1025-32.

120.   Hayes MA, Timmins AC, Yau EH, Palazzo M, Hinds CJ, Watson D. Elevation of systemic oxygen delivery in the treatment of critically ill patients. N Engl J Med 1994;330(24):1717-22.

121.   Shoemaker WC, Appel PL, Kram HB, Waxman K, Lee TS. Prospective trial of supranormal values of survivors as therapeutic goals in high-risk surgical patients. Chest 1988;94(6):1176-86.

122.   Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 2001;345(19):1368-77.

123.   Kern JW, Shoemaker WC. Meta-analysis of hemodynamic optimization in high-risk patients. Crit Care Med 2002;30(8):1686-92.

124.   Yu M, Levy MM, Smith P, Takiguchi SA, Miyasaki A, Myers SA. Effect of maximizing oxygen delivery on morbidity and mortality rates in critically ill patients: a prospective, randomized, controlled study. Crit Care Med 1993;21(6):830-8.

125.   Hinds C, Watson D. Manipulating hemodynamics and oxygen transport in critically ill patients. N Engl J Med 1995;333(16):1074-5.

126.   Rixen D, Siegel JH. Bench-to-bedside review: oxygen debt and its metabolic correlates as quantifiers of the severity of hemorrhagic and post-traumatic shock. Crit Care 2005;9(5):441-53.

127.   Grocott MP, Dushianthan A, Hamilton MA, Mythen MG, Harrison D, Rowan K. Perioperative increase in global blood flow to explicit defined goals and outcomes after surgery: a Cochrane Systematic Review. Br J Anaesth;111(4):535-48.

128.   Rhodes A, Cecconi M, Hamilton M, Poloniecki J, Woods J, Boyd O, et al. Goal-directed therapy in high-risk surgical patients: a 15-year follow-up study. Intensive Care Med;36(8):1327-32.

129.   Pearse RM, Harrison DA, MacDonald N, Gillies MA, Blunt M, Ackland G, et al. Effect of a perioperative, cardiac output-guided hemodynamic therapy algorithm on outcomes following major gastrointestinal surgery: a randomized clinical trial and systematic review. Jama 2014;311(21):2181-90.

130.   Surviving Sepsis Campaign. Surviving Sepsis Campaign Responds to ProCESS Trial. In; 2014.

131.   Ensinger H, Weichel T, Lindner KH, Grunert A, Ahnefeld FW. Effects of norepinephrine, epinephrine, and dopamine infusions on oxygen consumption in volunteers. Crit Care Med 1993;21(10):1502-8.

132.   Ensinger H, Weichel T, Lindner KH, Grunert A, Georgieff M. Are the effects of noradrenaline, adrenaline and dopamine infusions on VO2 and metabolism transient? Intensive Care Med 1995;21(1):50-6.

133.   Scheeren TW, Arndt JO. Different response of oxygen consumption and cardiac output to various endogenous and synthetic catecholamines in awake dogs. Crit Care Med 2000;28(12):3861-8.

134.   Boyd O, Lamb G, Mackay CJ, Grounds RM, Bennett ED. A comparison of the efficacy of dopexamine and dobutamine for increasing oxygen delivery in high-risk surgical patients. Anaesth Intensive Care 1995;23(4):478-84.

135.   Boyd O, Grounds RM, Bennett ED. A randomized clinical trial of the effect of deliberate perioperative increase of oxygen delivery on mortality in high-risk surgical patients. Jama 1993;270(22):2699-707.

136.   Takala J, Meier-Hellmann A, Eddleston J, Hulstaert P, Sramek V. Effect of dopexamine on outcome after major abdominal surgery: a prospective, randomized, controlled multicenter study. European Multicenter Study Group on Dopexamine in Major Abdominal Surgery. Crit Care Med 2000;28(10):3417-23.

137.   Stone MD, Wilson RJ, Cross J, Williams BT. Effect of adding dopexamine to intraoperative volume expansion in patients undergoing major elective abdominal surgery. Br J Anaesth 2003;91(5):619-24.

138.   Pearse R, Dawson D, Fawcett J, Rhodes A, Grounds RM, Bennett ED. Early goal-directed therapy after major surgery reduces complications and duration of hospital stay. A randomised, controlled trial [ISRCTN38797445]. Crit Care 2005;9(6):R687-93.

139.   Pearse RM, Belsey JD, Cole JN, Bennett ED. Effect of dopexamine infusion on mortality following major surgery: individual patient data meta-regression analysis of published clinical trials. Crit Care Med 2008;36(4):1323-9.

140.   Gopal S, Jayakumar D, Nelson PN. Meta-analysis on the effect of dopexamine on in-hospital mortality. Anaesthesia 2009;64(6):589-94. (Published by Wiley-Blackwell)

141.   Davies SJ, Yates D, Wilson RJ. Dopexamine has no additional benefit in high-risk patients receiving goal-directed fluid therapy undergoing major abdominal surgery. Anesth Analg 2011;112(1):130-8.

142. Vallet B, Chopin C, Curtis SE, Dupuis BA, Fourrier F, Mehdaoui H, et al. Prognostic value of the dobutamine test in patients with sepsis syndrome and normal lactate values: a prospective, multicenter study. Crit Care Med 1993;21(12):1868-75.

143. Mayeur N, Vallee F, De Soyres O, Mebazaa A, Salem R, Fourcade O, et al. Dopexamine Test in septic shock with hyperlactatemia. Ann Fr Anesth Reanim 2010;29(11):759-64.

144. Poeze M, Takala J, Greve JW, Ramsay G. Pre-operative tonometry is predictive for mortality and morbidity in high-risk surgical patients. Intensive Care Med 2000;26(9):1272-81.

145. Jhanji S, Lee C, Watson D, Hinds C, Pearse RM. Microvascular flow and tissue oxygenation after major abdominal surgery: association with post-operative complications. Intensive Care Med 2009;35(4):671-7.

146. Byers RJ, Eddleston JM, Pearson RC, Bigley G, McMahon RF. Dopexamine reduces the incidence of acute inflammation in the gut mucosa after abdominal surgery in high-risk patients. Crit Care Med 1999;27(9):1787-93.

147. Jhanji S, Vivian-Smith A, Lucena-Amaro S, Watson D, Hinds CJ, Pearse RM. Haemodynamic optimisation improves tissue microvascular flow and oxygenation after major surgery: a randomised controlled trial. Crit Care 2010;14(4):R151.

148. Lush CW, Kvietys PR. Microvascular dysfunction in sepsis. Microcirculation 2000;7(2):83-101.

149. Schmidt VJ, Wolfle SE, Boettcher M, de Wit C. Gap junctions synchronize vascular tone within the microcirculation. Pharmacol Rep 2008;60(1):68-74.

150. Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med 2007;49(1):88-98, 98 e1-2.

151. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, et al. Early increases in microcirculatory perfusion during protocol-directed resuscitation are

associated with reduced multi-organ failure at 24 h in patients with sepsis. Intensive Care Med 2008;34(12):2210-7.

152.   De Backer D, Dubois MJ, Schmartz D, Koch M, Ducart A, Barvais L, et al. Microcirculatory alterations in cardiac surgery: effects of cardiopulmonary bypass and anesthesia. Ann Thorac Surg 2009;88(5):1396-403.

153.   Eley KA, Young JD, Watt-Smith SR. Epinephrine, norepinephrine, dobutamine, and dopexamine effects on free flap skin blood flow. Plast Reconstr Surg 2012;130(3):564-70.

154.   Eley KA, Young JD, Watt-Smith SR. Power spectral analysis of the effects of epinephrine, norepinephrine, dobutamine and dopexamine on microcirculation following free tissue transfer. Microsurgery 2013;33(4):275-81.

155.   Young JD, Cameron EM. Dynamics of skin blood flow in human sepsis. Intensive Care Med 1995;21(8):669-74.

156.   Bellamy MC, Mullane D, O'Beirne HA, Young Y, Pollard SG, Lodge JP. Dopexamine and microcirculatory flow in transplanted small bowel: the Leeds experience. Transplant Proc 1997;29(3):1847-9.

157.   Thoren A, Elam M, Ricksten SE. Differential effects of dopamine, dopexamine, and dobutamine on jejunal mucosal perfusion early after cardiac surgery. Crit Care Med 2000;28(7):2338-43.

158.   Temmesfeld-Wollbruck B, Szalay A, Mayer K, Olschewski H, Seeger W, Grimminger F. Abnormalities of gastric mucosal oxygenation in septic shock: partial responsiveness to dopexamine. Am J Respir Crit Care Med 1998;157(5 Pt 1):1586-92.

159.   Biro GP, Douglas JR, Keon WJ, Taichman GC. Changes in regional blood flow distribution induced by infusions of dopexamine hydrochloride or dobutamine in anesthetized dogs. Am J Cardiol 1988;62(5):30C-36C.

160.   Cain SM, Curtis SE. Systemic and regional oxygen uptake and delivery and lactate flux in endotoxic dogs infused with dopexamine. Crit Care Med 1991;19(12):1552-60.

161. Renton MC, Snowden CP. Dopexamine and its role in the protection of hepatosplanchnic and renal perfusion in high-risk surgical and critically ill patients. Br J Anaesth 2005;94(4):459-67.

162. Baguneid MS, Welch M, Bukhari M, Fulford PE, Howe M, Bigley G, et al. A randomized study to evaluate the effect of a perioperative infusion of dopexamine on colonic mucosal ischemia after aortic surgery. J Vasc Surg 2001;33(4):758-63.

163. Suojaranta-Ylinen RT, Ruokonen ET, Takala JA. The effect of dopexamine on regional tissue oxygenation, systemic inflammation and amino acid exchange in major abdominal surgery. Acta Anaesthesiol Scand 2000;44(5):564-70.

164. McGinley J, Lynch L, Hubbard K, McCoy D, Cunningham AJ. Dopexamine hydrochloride does not modify hemodynamic response or tissue oxygenation or gut permeability during abdominal aortic surgery. Can J Anaesth 2001;48(3):238-44.

165. Merle U, Sieg O, Stremmel W, Encke J, Eisenbach C. Sensitivity and specificity of plasma disappearance rate of indocyanine green as a prognostic indicator in acute liver failure. BMC Gastroenterol 2009;9:91.

166. Sharpe DA, Mitchel IM, Kay EA, McGoldrick JP, Munsch CM, Kay PH. Enhancing liver blood flow after cardiopulmonary bypass: the effects of dopamine and dopexamine. Perfusion 1999;14(1):29-36.

167. Adluri RK, Singh AV, Skoyles J, Robins A, Hitch A, Baker M, et al. The effect of fenoldopam and dopexamine on hepatic blood flow and hepatic function following coronary artery bypass grafting with hypothermic cardiopulmonary bypass. Eur J Cardiothorac Surg 2009;35(6):988-94.

168. Rapp-Kesek D, Joachimsson PO, Karlsson T. Splanchnic blood flow and oxygen consumption: effects of enteral nutrition and dopexamine in the elderly cardiac surgery patient. Acta Anaesthesiol Scand 2007;51(5):570-6.

169. Berendes E, Mollhoff T, Van Aken H, Schmidt C, Erren M, Deng MC, et al. Effects of dopexamine on creatinine clearance, systemic inflammation, and splanchnic oxygenation in patients undergoing coronary artery bypass grafting. Anesth Analg 1997;84(5):950-7.

170. Uusaro A, Ruokonen E, Takala J. Gastric mucosal pH does not reflect changes in splanchnic blood flow after cardiac surgery. Br J Anaesth 1995;74(2):149-54.

171. Bach F, Grundmann U, Bauer M, Buchinger H, Soltesz S, Graeter T, et al. Modulation of the inflammatory response to cardiopulmonary bypass by dopexamine and epidural anesthesia. Acta Anaesthesiol Scand 2002;46(10):1227-35.

172. Gardeback M, Settergren G, Ohquist G, Tiren C. Effect of dopexamine on calculated low gastric intramucosal pH following valve replacement. Acta Anaesthesiol Scand 1995;39(5):599-604.

173. Gardeback M, Settergren G. Dopexamine and dopamine in the prevention of low gastric mucosal pH following cardiopulmonary by-pass. Acta Anaesthesiol Scand 1995;39(8):1066-70.

174. Stephan H, Sonntag H, Henning H, Yoshimine K. Cardiovascular and renal haemodynamic effects of dopexamine: comparison with dopamine. Br J Anaesth 1990;65(3):380-7.

175. Sherry E, Tooley MA, Bolsin SN, Monk CR, Willcox J. Effect of dopexamine hydrochloride on renal vascular resistance index and haemodynamic responses following coronary artery bypass graft surgery. Eur J Anaesthesiol 1997;14(2):184-9.

176. Dehne MG, Klein TF, Muhling J, Sablotzki A, Osmer C, Hempelmann G. Impairment of renal function after cardiopulmonary bypass is not influenced by dopexamine. Ren Fail 2001;23(2):217-30.

177. Schmoelz M, Schelling G, Dunker M, Irlbeck M. Comparison of systemic and renal effects of dopexamine and dopamine in norepinephrine-treated septic shock. J Cardiothorac Vasc Anesth 2006;20(2):173-8.

178. Meier-Hellmann A, Bredle DL, Specht M, Hannemann L, Reinhart K. Dopexamine increases splanchnic blood flow but decreases gastric mucosal pH in severe septic patients treated with dobutamine. Crit Care Med 1999;27(10):2166-71.

179. Kiefer P, Tugtekin I, Wiedeck H, Bracht H, Geldner G, Georgieff M, et al. Effect of a dopexamine-induced increase in cardiac index on splanchnic hemodynamics in septic shock. Am J Respir Crit Care Med 2000;161(3 Pt 1):775-9.

180. Maynard ND, Bihari DJ, Dalton RN, Smithies MN, Mason RC. Increasing splanchnic blood flow in the critically III. Chest 1995;108(6):1648-54.

181. Ralph CJ, Tanser SJ, Macnaughton PD, Sinclair DG. A randomised controlled trial investigating the effects of dopexamine on gastrointestinal function and organ dysfunction in the critically ill. Intensive Care Med 2002;28(7):884-90.

182. Russell JA. Gastric tonometry: does it work? Intensive Care Med 1997;23(1):3-6.

183. Uusaro A, Russell JA, Walley KR, Takala J. Gastric-arterial PCO2 gradient does not reflect systemic and splanchnic hemodynamics or oxygen transport after cardiac surgery. Shock 2000;14(1):13-7.

184. Tenhunen JJ, Uusaro A, Karja V, Oksala N, Jakob SM, Ruokonen E. Apparent heterogeneity of regional blood flow and metabolic changes within splanchnic tissues during experimental endotoxin shock. Anesth Analg 2003;97(2):555-63, table of contents.

185. Into T, Kanno Y, Dohkan J, Nakashima M, Inomata M, Shibata K, et al. Pathogen recognition by Toll-like receptor 2 activates Weibel-Palade body exocytosis in human aortic endothelial cells. J Biol Chem 2007;282(11):8134-41.

186. Riekenberg S, Farhat K, Debarry J, Heine H, Jung G, Wiesmuller KH, et al. Regulators of G-protein signalling are modulated by bacterial lipopeptides and lipopolysaccharide. Febs J 2009;276(3):649-59.

187. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. Cell Mol Life Sci 2008;65(19):2964-78.

188. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998;282(5396):2085-8.

189. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve--an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev 2000;52(4):595-638.

190. Bergquist J, Tarkowski A, Ekman R, Ewing A. Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. Proc Natl Acad Sci U S A 1994;91(26):12912-6.

191. Perkins GD, Nathani N, McAuley DF, Gao F, Thickett DR. In vitro and in vivo effects of salbutamol on neutrophil function in acute lung injury. Thorax 2007;62(1):36-42.

192. Dimitrov S, Lange T, Born J. Selective mobilization of cytotoxic leukocytes by epinephrine. J Immunol 2010;184(1):503-11.

193. Schedlowski M, Hosch W, Oberbeck R, Benschop RJ, Jacobs R, Raab HR, et al. Catecholamines modulate human NK cell circulation and function via spleen-independent beta 2-adrenergic mechanisms. J Immunol 1996;156(1):93-9.

194. Altenburg SP, Martins MA, Silva AR, Cordeiro RS, Castro-Faria-Neto HC. LPS-induced blood neutrophilia is inhibited by alpha 1-adrenoceptor antagonists: a role for catecholamines. J Leukoc Biol 1997;61(6):689-94.

195. Schmidt W, Hacker A, Gebhard MM, Martin E, Schmidt H. Dopexamine attenuates endotoxin-induced microcirculatory changes in rat mesentery: role of beta2 adrenoceptors. Crit Care Med 1998;26(10):1639-45.

196. Birnbaum J, Klotz E, Spies CD, Lorenz B, Stuebs P, Hein OV, et al. Effects of dopexamine on the intestinal microvascular blood flow and leukocyte activation in a sepsis model in rats. Crit Care 2006;10(4):R117.

197. Bowden JJ, Sulakvelidze I, McDonald DM. Inhibition of neutrophil and eosinophil adhesion to venules of rat trachea by beta 2-adrenergic agonist formoterol. J Appl Physiol 1994;77(1):397-405.

198. von Hundelshausen P, Koenen RR, Weber C. Platelet-mediated enhancement of leukocyte adhesion. Microcirculation 2009;16(1):84-96.

199.   Ikarugi H, Shibata M, Shibata S, Ishii H, Taka T, Yamamoto J. High intensity exercise enhances platelet reactivity to shear stress and coagulation during and after exercise. Pathophysiol Haemost Thromb 2003;33(3):127-33.

200.   Horn NA, Anastase DM, Hecker KE, Baumert JH, Robitzsch T, Rossaint R. Epinephrine enhances platelet-neutrophil adhesion in whole blood in vitro. Anesth Analg 2005;100(2):520-6.

201.   Maayani S, Schwarz T, Craddock-Royal B, Tagliente TM. Activation of the alpha(2A)-adrenoceptor mediates deceleration of the deaggregation component of the response to ADP or 5-HT in human platelets in vitro. Platelets 2001;12(6):359-75.

202.   Yu SK, Latour JG. Potentiation by alpha and inhibition by beta-adrenergic stimulations of rat platelet aggregation. A comparative study with human and rabbit platelets. Thromb Haemost 1977;37(3):413-22.

203.   Derian CK, Santulli RJ, Rao PE, Solomon HF, Barrett JA. Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators. J Immunol 1995;154(1):308-17.

204.   Taherzadeh M, Warren JB. Cardiac inotropes inhibit the oedema caused by nifedipine in rabbit skin. Br J Pharmacol 1997;122(6):1160-4.

205.   Schlegel N, Baumer Y, Drenckhahn D, Waschke J. Lipopolysaccharide-induced endothelial barrier breakdown is cyclic adenosine monophosphate dependent in vivo and in vitro. Crit Care Med 2009;37(5):1735-43.

206.   Laudanna C, Campbell JJ, Butcher EC. Elevation of intracellular cAMP inhibits RhoA activation and integrin-dependent leukocyte adhesion induced by chemoattractants. J Biol Chem 1997;272(39):24141-4.

207.   Trabold B, Gruber M, Frohlich D. Synthetic inotropes inhibit the expression of adhesion molecules and augment the expression of L-selectin in polymorphonuclear neutrophils. Resuscitation 2007;74(2):352-6.

208.	Panettieri RA, Jr., Lazaar AL, Pure E, Albelda SM. Activation of cAMP-dependent pathways in human airway smooth muscle cells inhibits TNF-alpha-induced ICAM-1 and VCAM-1 expression and T lymphocyte adhesion. J Immunol 1995;154(5):2358-65.

209.	Pero RS, Borchers MT, Spicher K, Ochkur SI, Sikora L, Rao SP, et al. Galphai2-mediated signaling events in the endothelium are involved in controlling leukocyte extravasation. Proc Natl Acad Sci U S A 2007;104(11):4371-6.

210.	Abraham E, Kaneko DJ, Shenkar R. Effects of endogenous and exogenous catecholamines on LPS-induced neutrophil trafficking and activation. Am J Physiol 1999;276(1 Pt 1):L1-8.

211.	Arcaroli J, Yang KY, Yum HK, Kupfner J, Pitts TM, Park JS, et al. Effects of catecholamines on kinase activation in lung neutrophils after hemorrhage or endotoxemia. J Leukoc Biol 2002;72(3):571-9.

212.	Shenkar R, Abraham E. Mechanisms of lung neutrophil activation after hemorrhage or endotoxemia: roles of reactive oxygen intermediates, NF-kappa B, and cyclic AMP response element binding protein. J Immunol 1999;163(2):954-62.

213.	Abraham E, Arcaroli J, Shenkar R. Activation of extracellular signal-regulated kinases, NF-kappa B, and cyclic adenosine 5'-monophosphate response element-binding protein in lung neutrophils occurs by differing mechanisms after hemorrhage or endotoxemia. J Immunol 2001;166(1):522-30.

214.	Gosain A, Muthu K, Gamelli RL, DiPietro LA. Norepinephrine suppresses wound macrophage phagocytic efficiency through alpha- and beta-adrenoreceptor dependent pathways. Surgery 2007;142(2):170-9.

215.	Weiss M, Schneider EM, Tarnow J, Mettler S, Krone M, Teschemacher A, et al. Is inhibition of oxygen radical production of neutrophils by sympathomimetics mediated via beta-2 adrenoceptors? J Pharmacol Exp Ther 1996;278(3):1105-13.

216.	Lunemann JD, Buttgereit F, Tripmacher R, Baerwald CG, Burmester GR, Krause A. Norepinephrine inhibits energy metabolism of human peripheral blood mononuclear cells via adrenergic receptors. Biosci Rep 2001;21(5):627-35.

217. Lang CH, Nystrom G, Frost RA. Beta-adrenergic blockade exacerbates sepsis-induced changes in tumor necrosis factor alpha and interleukin-6 in skeletal muscle and is associated with impaired translation initiation. J Trauma 2008;64(2):477-86.

218. Szabo C, Hasko G, Zingarelli B, Nemeth ZH, Salzman AL, Kvetan V, et al. Isoproterenol regulates tumour necrosis factor, interleukin-10, interleukin-6 and nitric oxide production and protects against the development of vascular hyporeactivity in endotoxaemia. Immunology 1997;90(1):95-100.

219. Straub RH, Linde HJ, Mannel DN, Scholmerich J, Falk W. A bacteria-induced switch of sympathetic effector mechanisms augments local inhibition of TNF-alpha and IL-6 secretion in the spleen. Faseb J 2000;14(10):1380-8.

220. Bergmann M, Sautner T. Immunomodulatory effects of vasoactive catecholamines. Wien Klin Wochenschr 2002;114(17-18):752-61.

221. Tighe D, Moss R, Bennett D. Porcine hepatic response to sepsis and its amplification by an adrenergic receptor alpha1 agonist and a beta2 antagonist. Clin Sci (Lond) 1998;95(4):467-78.

222. Bergmann M, Gornikiewicz A, Tamandl D, Exner R, Roth E, Fugger R, et al. Continuous therapeutic epinephrine but not norepinephrine prolongs splanchnic IL-6 production in porcine endotoxic shock. Shock 2003;20(6):575-81.

223. Aninat C, Seguin P, Descheemaeker PN, Morel F, Malledant Y, Guillouzo A. Catecholamines induce an inflammatory response in human hepatocytes. Crit Care Med 2008;36(3):848-54.

224. Zhou M, Yang S, Koo DJ, Ornan DA, Chaudry IH, Wang P. The role of Kupffer cell alpha(2)-adrenoceptors in norepinephrine-induced TNF-alpha production. Biochim Biophys Acta 2001;1537(1):49-57.

225. Leposavic G, Pilipovic I, Radojevic K, Pesic V, Perisic M, Kosec D. Catecholamines as immunomodulators: a role for adrenoceptor-mediated mechanisms in fine tuning of T-cell development. Auton Neurosci 2008;144(1-2):1-12.

226. Sanders VM. Interdisciplinary research: noradrenergic regulation of adaptive immunity. Brain Behav Immun 2006;20(1):1-8.

227. Jiang JL, Peng YP, Qiu YH, Wang JJ. Adrenoreceptor-coupled signal-transduction mechanisms mediating lymphocyte apoptosis induced by endogenous catecholamines. J Neuroimmunol 2009;213(1-2):100-11.

228. Bergquist J, Josefsson E, Tarkowski A, Ekman R, Ewing A. Measurements of catecholamine-mediated apoptosis of immunocompetent cells by capillary electrophoresis. Electrophoresis 1997;18(10):1760-6.

229. Burniston JG, Ellison GM, Clark WA, Goldspink DF, Tan LB. Relative toxicity of cardiotonic agents: some induce more cardiac and skeletal myocyte apoptosis and necrosis in vivo than others. Cardiovasc Toxicol 2005;5(4):355-64.

230. Garcia-Cazarin ML, Smith JL, Clair DK, Piascik MT. The alpha1D-adrenergic receptor induces vascular smooth muscle apoptosis via a p53-dependent mechanism. Mol Pharmacol 2008;74(4):1000-7.

231. Garcia-Cazarin ML, Smith JL, Olszewski KA, McCune DF, Simmerman LA, Hadley RW, et al. The alpha1D-adrenergic receptor is expressed intracellularly and coupled to increases in intracellular calcium and reactive oxygen species in human aortic smooth muscle cells. J Mol Signal 2008;3:6.

232. Schildberg FA, Schulz S, Dombrowski F, Minor T. Cyclic AMP alleviates endoplasmic stress and programmed cell death induced by lipopolysaccharides in human endothelial cells. Cell Tissue Res 2005;320(1):91-8.

233. Nakamura A, Imaizumi A, Yanagawa Y, Niimi R, Kohsaka T, Johns EJ. Beta2-adrenoceptor activation inhibits Shiga toxin2-induced apoptosis of renal tubular epithelial cells. Biochem Pharmacol 2003;66(2):343-53.

234. Ballou LM, Cross ME, Huang S, McReynolds EM, Zhang BX, Lin RZ. Differential regulation of the phosphatidylinositol 3-kinase/Akt and p70 S6 kinase pathways by the alpha(1A)-adrenergic receptor in rat-1 fibroblasts. J Biol Chem 2000;275(7):4803-9.

235.   Ballou LM, Tian PY, Lin HY, Jiang YP, Lin RZ. Dual regulation of glycogen synthase kinase-3beta by the alpha1A-adrenergic receptor. J Biol Chem 2001;276(44):40910-6.

236.   Wright CD, Chen Q, Baye NL, Huang Y, Healy CL, Kasinathan S, et al. Nuclear alpha1-adrenergic receptors signal activated ERK localization to caveolae in adult cardiac myocytes. Circ Res 2008;103(9):992-1000.

237.   Costa VM, Silva R, Ferreira R, Amado F, Carvalho F, de Lourdes Bastos M, et al. Adrenaline in pro-oxidant conditions elicits intracellular survival pathways in isolated rat cardiomyocytes. Toxicology 2009;257(1-2):70-9.

238.   Costa VM, Silva R, Tavares LC, Vitorino R, Amado F, Carvalho F, et al. Adrenaline and reactive oxygen species elicit proteome and energetic metabolism modifications in freshly isolated rat cardiomyocytes. Toxicology 2009;260(1-3):84-96.

239.   Del Rio MJ, Velez-Pardo C. Monoamine neurotoxins-induced apoptosis in lymphocytes by a common oxidative stress mechanism: involvement of hydrogen peroxide (H(2)O(2)), caspase-3, and nuclear factor kappa-B (NF-kappaB), p53, c-Jun transcription factors. Biochem Pharmacol 2002;63(4):677-88.

240.   Jacinto SM, Lokhandwala MF, Jandhyala BS. Studies on the pharmacological interventions to prevent oxygen free radical (OFR)-mediated toxicity; effects of dopexamine, a DA1 receptor and beta 2 adrenoceptor agonist. Naunyn Schmiedebergs Arch Pharmacol 1994;350(3):277-83.

241.   Mirza ZN, Kato M, Kimura H, Tachibana A, Fujiu T, Suzuki M, et al. Fenoterol inhibits superoxide anion generation by human polymorphonuclear leukocytes via beta-adrenoceptor-dependent and -independent mechanisms. Ann Allergy Asthma Immunol 2002;88(5):494-500.

242.   Jacinto SM, Chintala MS, Lokhandwala MF, Jandhyala BS. Efficacy and mechanisms of dopexamine in the prevention of ischemia-reperfusion induced organ damage: role of oxygen free radicals. Clin Exp Hypertens 1997;19(1-2):181-90.

243.   Wu JY, Liaw WJ, Tzao C, Chen SJ, Wang JH, Wu CC. Comparison of terbutaline and dobutamine in rats with endotoxemia. Chin J Physiol 2002;45(4):155-62.

244.   Tsao CM, Chen SJ, Shih MC, Lue WM, Tsou MY, Chen A, et al. Effects of terbutaline on circulatory failure and organ dysfunction induced by peritonitis in rats. Intensive Care Med 2010;36(9):1571-8.

245.   Wu CC, Liao MH, Chen SJ, Chou TC, Chen A, Yen MH. Terbutaline prevents circulatory failure and mitigates mortality in rodents with endotoxemia. Shock 2000;14(1):60-7.

246.   Liaw WJ, Tzao C, Wu JY, Chen SJ, Wang JH, Wu CC. Inhibition by terbutaline of nitric oxide and superoxide anion levels of endotoxin-induced organs injury in the anesthetized rat. Shock 2003;19(3):281-8.

247.   Brunskole Hummel I, Reinartz MT, Kalble S, Burhenne H, Schwede F, Buschauer A, et al. Dissociations in the effects of beta2-adrenergic receptor agonists on cAMP formation and superoxide production in human neutrophils: support for the concept of functional selectivity. PLoS One 2012;8(5):e64556.

248.   Pottecher J, Cheisson G, Huet O, Laplace C, Vicaut E, Mazoit JX, et al. Beta2-adrenergic agonist protects human endothelial cells from hypoxia/reoxygenation injury in vitro. Crit Care Med 2006;34(1):165-72.

249.   Nakamura A, Niimi R, Yanagawa Y. Renal beta2-adrenoceptor blockade worsens the outcome of an induced Escherichia coli renal infection. J Nephrol 2010;23(3):341-9.

250.   Nakamura A, Imaizumi A, Yanagawa Y, Kohsaka T, Johns EJ. beta(2)-Adrenoceptor activation attenuates endotoxin-induced acute renal failure. J Am Soc Nephrol 2004;15(2):316-25.

251.   Nakamura A, Johns EJ, Imaizumi A, Yanagawa Y, Kohsaka T. beta(2)-adrenoceptor agonist suppresses renal tumour necrosis factor and enhances interleukin-6 gene expression induced by endotoxin. Nephrol Dial Transplant 2000;15(12):1928-34.

252.   Nakamura A, Niimi R, Yanagawa Y. Renal beta(2)-adrenoceptor modulates the lipopolysaccharide transport system in sepsis-induced acute renal failure. Inflammation 2009;32(1):12-9.

253.   Bagshaw SM, Lapinsky S, Dial S, Arabi Y, Dodek P, Wood G, et al. Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. Intensive Care Med 2009;35(5):871-81.

254.   Markley K, Smallman ET, Briggs LW. Early mortality and temperature regulation in burned mice following administration of catecholamines and adrenergic receptor blocking drugs. J Trauma 1979;19(7):512-8.

255.   Oberbeck R, Schmitz D, Schuler M, Wilsenack K, Schedlowski M, Exton M. Dopexamine and cellular immune functions during systemic inflammation. Immunobiology 2004;208(5):429-38.

256.   Walker-Brown J, Roberts MR. Differential contribution of beta-adrenergic receptors expressed on radiosensitive versus radioresistant cells to protection against inflammation and mortality in murine endotoxemia. Shock 2009;32(5):541-7.

257.   Schmitz D, Wilsenack K, Lendemanns S, Schedlowski M, Oberbeck R. beta-Adrenergic blockade during systemic inflammation: impact on cellular immune functions and survival in a murine model of sepsis. Resuscitation 2007;72(2):286-94.

258.   Bloemen PG, van den Tweel MC, Henricks PA, Engels F, Kester MH, van de Loo PG, et al. Increased cAMP levels in stimulated neutrophils inhibit their adhesion to human bronchial epithelial cells. Am J Physiol 1997;272(4 Pt 1):L580-7.

259.   Giembycz MA, Newton R. Beyond the dogma: novel beta2-adrenoceptor signalling in the airways. Eur Respir J 2006;27(6):1286-306.

260.   Adluri RK, Singh AV, Skoyles J, Robins A, Parton J, Baker M, et al. The effect of fenoldopam and dopexamine on cytokine and endotoxin release following on-pump coronary artery bypass grafting: a prospective randomized double-blind trial. Heart Surg Forum;13(6):E353-61.

261.   Steinberg S, Azar G, Love R, Lee R, Choe E, Flint L. Dopexamine prevents depression of mesenteric blood flow caused by positive end-expiratory pressure in rats. Surgery 1996;120(4):597-601; discussion 601-2.

262. Frojse R, Lehtipalo S, Bergstrand U, Biber B, Winso O, Johansson G, et al. Local metabolic effects of dopexamine on the intestine during mesenteric hypoperfusion. Shock 2004;21(3):241-7.

263. Lehtipalo S, Biber B, Frojse R, Arnerlov C, Johansson G, Winso O. Does dopexamine influence regional vascular tone and oxygenation during intestinal hypotension? Acta Anaesthesiol Scand 2002;46(10):1217-26.

264. Lehtipalo S, Biber B, Frojse R, Arnerlov C, Johansson G, Winso O. Effects of dopexamine and positive end-expiratory pressure on intestinal blood flow and oxygenation: the perfusion pressure perspective. Chest 2003;124(2):688-98.

265. Germann R, Haisjackl M, Schwarz B, Deusch E, Meusburger S, Gruber E, et al. Inotropic treatment and intestinal mucosal tissue oxygenation in a model of porcine endotoxemia. Crit Care Med 1997;25(7):1191-7.

266. Hiltebrand LB, Krejci V, Sigurdsson GH. Effects of dopamine, dobutamine, and dopexamine on microcirculatory blood flow in the gastrointestinal tract during sepsis and anesthesia. Anesthesiology 2004;100(5):1188-97.

267. Alhan E, Kucuktulu U, Ercin C, Deger O, Cicek R. Effects of dopexamine on acute necrotising pancreatitis in rats. Eur J Surg 2001;167(10):761-6.

268. Bastien O, Piriou V, Aouifi A, Evans R, Lehot JJ. Effects of dopexamine on blood flow in multiple splanchnic sites measured by laser Doppler velocimetry in rabbits undergoing cardiopulmonary bypass. Br J Anaesth 1999;82(1):104-9.

269. Lund N, de Asla RJ, Cladis F, Papadakos PJ, Thorborg PA. Dopexamine hydrochloride in septic shock: effects on oxygen delivery and oxygenation of gut, liver, and muscle. J Trauma 1995;38(5):767-75.

270. Schmidt H, Secchi A, Wellmann R, Bach A, Bhrer H, Martin E. Dopexamine maintains intestinal villus blood flow during endotoxemia in rats. Crit Care Med 1996;24(7):1233-7.

271.   Secchi A, Ortanderl JM, Schmidt W, Walther A, Gebhard MM, Martin E, et al. Effects of dobutamine and dopexamine on hepatic micro- and macrocirculation during experimental endotoxemia: an intravital microscopic study in the rat. Crit Care Med 2001;29(3):597-600.

272.   Schmidt H, Weigand MA, Schmidt W, Plaschke K, Martin E, Bardenheuer HJ. Effect of dopexamine on intestinal tissue concentrations of high-energy phosphates and intestinal release of purine compounds in endotoxemic rats. Crit Care Med 2000;28(6):1979-84.

273.   Moss RF, Parmar NK, Tighe D, Davies DC. Adrenergic agents modify cerebral edema and microvessel ultrastructure in porcine sepsis. Crit Care Med 2004;32(9):1916-21.

274.   Kostopanagiotou G, Pandazi A, Andreadou I, Doufas A, Chondroudaki I, Kotsis T, et al. Effects of dopexamine on lipid peroxidation during aortic surgery in pigs: comparison with dopamine. Eur J Vasc Endovasc Surg 2005;30(6):648-53.

275.   Jacinto SM, Lokhandwala MF, Jandhyala BS. Effect of pharmacological interventions in the prevention of lipid peroxidation and respiratory depression induced by oxygen free radicals in anesthetized rats. Free Radic Biol Med 1996;21(6):791-9.

276.   Tighe D, Moss R, Haywood G, Webb A, al-Saady N, Heath F, et al. Dopexamine hydrochloride maintains portal blood flow and attenuates hepatic ultrastructural changes in a porcine peritonitis model of multiple system organ failure. Circ Shock 1993;39(3):199-206.

277.   Yelken BB, Gorenek ES, Uzuner K, Aydin Y, Elmas C. Effects of dopexamine on rat cardiorenal functions during lipopolysaccharide-induced experimental sepsis. J Int Med Res 2004;32(2):109-17.

278.   Palsson J, Ricksten SE, Houltz E, Lundin S. Effects of dopamine, dopexamine and dobutamine on renal excretory function during experimental sepsis in conscious rats. Acta Anaesthesiol Scand 1997;41(3):392-8.

279.   Goldman L, Caldera DL, Nussbaum SR, Southwick FS, Krogstad D, Murray B, et al. Multifactorial index of cardiac risk in noncardiac surgical procedures. N Engl J Med 1977;297(16):845-50.

280.   Lee TH, Marcantonio ER, Mangione CM, Thomas EJ, Polanczyk CA, Cook EF, et al. Derivation and prospective validation of a simple index for prediction of cardiac risk of major noncardiac surgery. Circulation 1999;100(10):1043-9.

281.   Copeland GP, Jones D, Walters M. POSSUM: a scoring system for surgical audit. Br J Surg 1991;78(3):355-60.

282.   Gupta PK, Gupta H, Sundaram A, Kaushik M, Fang X, Miller WJ, et al. Development and validation of a risk calculator for prediction of cardiac risk after surgery. Circulation 2011;124(4):381-7.

283.   Pearse RM, Harrison DA, James P, Watson D, Hinds C, Rhodes A, et al. Identification and characterisation of the high-risk surgical population in the United Kingdom. Crit Care 2006;10(3):R81.

284.   Khuri SF, Henderson WG, DePalma RG, Mosca C, Healey NA, Kumbhani DJ. Determinants of long-term survival after major surgery and the adverse effect of postoperative complications. Ann Surg 2005;242(3):326-41; discussion 341-3.

285.   Roumen RM, Frieling JT, van Tits HW, van der Vliet JA, Goris RJ. Endotoxemia after major vascular operations. J Vasc Surg 1993;18(5):853-7.

286.   Roumen RM, Hendriks T, van der Ven-Jongekrijg J, Nieuwenhuijzen GA, Sauerwein RW, van der Meer JW, et al. Cytokine patterns in patients after major vascular surgery, hemorrhagic shock, and severe blunt trauma. Relation with subsequent adult respiratory distress syndrome and multiple organ failure. Ann Surg 1993;218(6):769-76.

287.   Buttenschoen K, Buttenschoen DC, Berger D, Vasilescu C, Schafheutle S, Goeltenboth B, et al. Endotoxemia and acute-phase proteins in major abdominal surgery. Am J Surg 2001;181(1):36-43.

288.   Xiong J, Wang Y, Zhu Z, Liu J. Expression and significance of toll-like receptor 2,4 of peripheral blood mononuclear cells in acute abdomen patients associated with systemic inflammatory response syndrome. J Huazhong Univ Sci Technolog Med Sci 2006;26(5):570-2.

289.   Valenza F, Fagnani L, Coppola S, Froio S, Sacconi F, Tedesco C, et al. Prevalence of endotoxemia after surgery and its association with ICU length of stay. Crit Care 2009;13(3):R102.

290.   Dyson A, Singer M. Animal models of sepsis: why does preclinical efficacy fail to translate to the clinical setting? Crit Care Med 2009;37(1 Suppl):S30-7.

291.   Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. Jama 2009;302(21):2323-9.

292.   Owens CD, Stoessel K. Surgical site infections: epidemiology, microbiology and prevention. J Hosp Infect 2008;70 Suppl 2:3-10.

293.   Opal SM. The host response to endotoxin, antilipopolysaccharide strategies, and the management of severe sepsis. Int J Med Microbiol 2007;297(5):365-77.

294.   Taveira da Silva AM, Kaulbach HC, Chuidian FS, Lambert DR, Suffredini AF, Danner RL. Brief report: shock and multiple-organ dysfunction after self-administration of Salmonella endotoxin. N Engl J Med 1993;328(20):1457-60.

295.   Kolkman JJ, Mensink PB. Non-occlusive mesenteric ischaemia: a common disorder in gastroenterology and intensive care. Best Pract Res Clin Gastroenterol 2003;17(3):457-73.

296.   Leaphart CL, Tepas JJ, 3rd. The gut is a motor of organ system dysfunction. Surgery 2007;141(5):563-9.

297.   Silvestri L, van Saene HK, Zandstra DF, Marshall JC, Gregori D, Gullo A. Impact of selective decontamination of the digestive tract on multiple organ dysfunction syndrome: systematic review of randomized controlled trials. Crit Care Med;38(5):1370-6.

298.   Ruiz N, Kahne D, Silhavy TJ. Transport of lipopolysaccharide across the cell envelope: the long road of discovery. Nat Rev Microbiol 2009;7(9):677-83.

299.   Rittirsch D, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. Nat Rev Immunol 2008;8(10):776-87.

300.	Doi K, Leelahavanichkul A, Yuen PS, Star RA. Animal models of sepsis and sepsis-induced kidney injury. J Clin Invest 2009;119(10):2868-78.

301.	Rittirsch D, Hoesel LM, Ward PA. The disconnect between animal models of sepsis and human sepsis. J Leukoc Biol 2007;81(1):137-43.

302.	Collin M, Rossi A, Cuzzocrea S, Patel NS, Di Paola R, Hadley J, et al. Reduction of the multiple organ injury and dysfunction caused by endotoxemia in 5-lipoxygenase knockout mice and by the 5-lipoxygenase inhibitor zileuton. J Leukoc Biol 2004;76(5):961-70.

303.	Kalyanaraman B. Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. Redox Biol;1(1):244-257.

304.	Weston RM, Jones NM, Jarrott B, Callaway JK. Inflammatory cell infiltration after endothelin-1-induced cerebral ischemia: histochemical and myeloperoxidase correlation with temporal changes in brain injury. J Cereb Blood Flow Metab 2007;27(1):100-14.

305.	Pulli B, Ali M, Forghani R, Schob S, Hsieh KL, Wojtkiewicz G, et al. Measuring myeloperoxidase activity in biological samples. PLoS One 2013;8(7):e67976.

306.	Toda Y, Takahashi T, Maeshima K, Shimizu H, Inoue K, Morimatsu H, et al. A neutrophil elastase inhibitor, sivelestat, ameliorates lung injury after hemorrhagic shock in rats. Int J Mol Med 2007;19(2):237-43.

307.	Murch O, Abdelrahman M, Collino M, Gallicchio M, Benetti E, Mazzon E, et al. Sphingosylphosphorylcholine reduces the organ injury/dysfunction and inflammation caused by endotoxemia in the rat. Crit Care Med 2008;36(2):550-9.

308.	Breckwoldt MO, Chen JW, Stangenberg L, Aikawa E, Rodriguez E, Qiu S, et al. Tracking the inflammatory response in stroke in vivo by sensing the enzyme myeloperoxidase. Proc Natl Acad Sci U S A 2008;105(47):18584-9.

309.	Djoba Siawaya JF, Roberts T, Babb C, Black G, Golakai HJ, Stanley K, et al. An evaluation of commercial fluorescent bead-based luminex cytokine assays. PLoS One 2008;3(7):e2535.

310.   Europe Co. European Pharmacopoeia (European Directorate Quality). In. 4th Revised edition ed: Council of Europe; 2002.

311.   Laver MB, Seifen A. Measurement of Blood Oxygen Tension in Anesthesia. Anesthesiology 1965;26:73-101.

312.   Baldari C, Bonavolonta V, Emerenziani GP, Gallotta MC, Silva AJ, Guidetti L. Accuracy, reliability, linearity of Accutrend and Lactate Pro versus EBIO plus analyzer. Eur J Appl Physiol 2009;107(1):105-11.

313.   Stevenson CK, Kidney BA, Duke T, Snead EC, Jackson ML. Evaluation of the Accutrend for lactate measurement in dogs. Vet Clin Pathol 2007;36(3):261-6.

314.   Tennent-Brown BS, Wilkins PA, Lindborg S, Russell G, Boston RC. Assessment of a point-of-care lactate monitor in emergency admissions of adult horses to a referral hospital. J Vet Intern Med 2007;21(5):1090-8.

315.   Takata M, Robotham JL. Effects of inspiratory diaphragmatic descent on inferior vena caval venous return. J Appl Physiol (1985) 1992;72(2):597-607.

316.   Rosser DM, Stidwill RP, Jacobson D, Singer M. Oxygen tension in the bladder epithelium rises in both high and low cardiac output endotoxemic sepsis. J Appl Physiol (1985) 1995;79(6):1878-82.

317.   Dyson A, Stidwill R, Taylor V, Singer M. Tissue oxygen monitoring in rodent models of shock. Am J Physiol Heart Circ Physiol 2007;293(1):H526-33.

318.   Lee BY, Al-Waili N, Butler G. The effect of adrenergic beta(2) receptor agonist on paraplegia following clamping of abdominal aorta. Arch Med Sci 2011;7(4):597-603.

319.   D'Almeida MS, Gaudin C, Lebrec D. Validation of 1- and 2-mm transit-time ultrasound flow probes on mesenteric artery and aorta of rats. Am J Physiol 1995;268(3 Pt 2):H1368-72.

320.   Rajan V, Varghese B, van Leeuwen TG, Steenbergen W. Review of methodological developments in laser Doppler flowmetry. Lasers Med Sci 2009;24(2):269-83.

321.   Golub AS, Pittman RN. Thermostatic animal platform for intravital microscopy of thin tissues. Microvasc Res 2003;66(3):213-7.

322.   Horstick G, Kempf T, Lauterbach M, Ossendorf M, Kopacz L, Heimann A, et al. Plastic foil technique attenuates inflammation in mesenteric intravital microscopy. J Surg Res 2000;94(1):28-34.

323.   Campbell JE, Garrison RN, Zakaria el R. Clinical peritoneal dialysis solutions modulate white blood cell-intestinal vascular endothelium interaction. Am J Surg 2006;192(5):610-6.

324.   Saetzler RK, Jallo J, Lehr HA, Philips CM, Vasthare U, Arfors KE, et al. Intravital fluorescence microscopy: impact of light-induced phototoxicity on adhesion of fluorescently labeled leukocytes. J Histochem Cytochem 1997;45(4):505-13.

325.   Harris AG, Sinitsina I, Messmer K. Intravital fluorescence microscopy and phototocicity: effects on leukocytes. Eur J Med Res 2002;7(3):117-24.

326.   Gore RW, Bohlen HG. Microvascular pressures in rat intestinal muscle and mucosal villi. Am J Physiol 1977;233(6):H685-93.

327.   Mulvany MJ, Halpern W. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 1977;41(1):19-26.

328.   Bevan JA, Osher JV. A direct method for recording tension changes in the wall of small blood vessels in vitro. Agents Actions 1972;2(5):257-60.

329.   Mulvany MJ. Procedures for investigation of small vessels using small vessel myograph. . In. August 2004 ed. Aarhus: Danish Myo Technology; 2004.

330.   Pearse RM, Moreno RP, Bauer P, Pelosi P, Metnitz P, Spies C, et al. Mortality after surgery in Europe: a 7 day cohort study. Lancet 2012;380(9847):1059-65.

331.   Pearse RM, Holt PJ, Grocott MP. Managing perioperative risk in patients undergoing elective non-cardiac surgery. Bmj 2011;343:d5759.

332.   Shoemaker WC. Cardiorespiratory patterns of surviving and nonsurviving postoperative patients. Surg Gynecol Obstet 1972;134(5):810-4.

333.   Hopf HW, Hunt TK, West JM, Blomquist P, Goodson WH, 3rd, Jensen JA, et al. Wound tissue oxygen tension predicts the risk of wound infection in surgical patients. Arch Surg 1997;132(9):997-1004; discussion 1005.

334. Wilson J, Woods I, Fawcett J, Whall R, Dibb W, Morris C, et al. Reducing the risk of major elective surgery: randomised controlled trial of preoperative optimisation of oxygen delivery. Bmj 1999;318(7191):1099-103.

335. Liu L, Kubes P. Molecular mechanisms of leukocyte recruitment: organ-specific mechanisms of action. Thromb Haemost 2003;89(2):213-20.

336. Hofstetter C, Boost KA, Flondor M, Basagan-Mogol E, Betz C, Homann M, et al. Anti-inflammatory effects of sevoflurane and mild hypothermia in endotoxemic rats. Acta Anaesthesiol Scand 2007;51(7):893-9.

337. Uusaro A, Russell JA. Could anti-inflammatory actions of catecholamines explain the possible beneficial effects of supranormal oxygen delivery in critically ill surgical patients? Intensive Care Med 2000;26(3):299-304.

338. Asaduzzaman M, Zhang S, Lavasani S, Wang Y, Thorlacius H. LFA-1 and MAC-1 mediate pulmonary recruitment of neutrophils and tissue damage in abdominal sepsis. Shock 2008;30(3):254-9.

339. Lauw FN, Pajkrt D, Hack CE, Kurimoto M, van Deventer SJ, van der Poll T. Proinflammatory effects of IL-10 during human endotoxemia. J Immunol 2000;165(5):2783-9.

340. Doherty GM, Lange JR, Langstein HN, Alexander HR, Buresh CM, Norton JA. Evidence for IFN-gamma as a mediator of the lethality of endotoxin and tumor necrosis factor-alpha. J Immunol 1992;149(5):1666-70.

341. Schmidt C, Hocherl K, Bucher M. Cytokine-mediated regulation of urea transporters during experimental endotoxemia. Am J Physiol Renal Physiol 2007;292(5):F1479-89.

342. Millar CG, Thiemermann C. Intrarenal haemodynamics and renal dysfunction in endotoxaemia: effects of nitric oxide synthase inhibition. Br J Pharmacol 1997;121(8):1824-30.

343. Cunningham PN, Dyanov HM, Park P, Wang J, Newell KA, Quigg RJ. Acute renal failure in endotoxemia is caused by TNF acting directly on TNF receptor-1 in kidney. J Immunol 2002;168(11):5817-23.

344.   Nakamura A, Imaizumi A, Yanagawa Y, Niimi R, Kohsaka T. Suppression of tumor necrosis factor-alpha by beta2-adrenoceptor activation: role of mitogen-activated protein kinases in renal mesangial cells. Inflamm Res 2003;52(1):26-31.

345.   Knotek M, Rogachev B, Wang W, Ecder T, Melnikov V, Gengaro PE, et al. Endotoxemic renal failure in mice: Role of tumor necrosis factor independent of inducible nitric oxide synthase. Kidney Int 2001;59(6):2243-9.

346.   Izeboud CA, Monshouwer M, van Miert AS, Witkamp RF. The beta-adrenoceptor agonist clenbuterol is a potent inhibitor of the LPS-induced production of TNF-alpha and IL-6 in vitro and in vivo. Inflamm Res 1999;48(9):497-502.

347.   Heinzel FP, Rerko RM, Ling P, Hakimi J, Schoenhaut DS. Interleukin 12 is produced in vivo during endotoxemia and stimulates synthesis of gamma interferon. Infect Immun 1994;62(10):4244-9.

348.   Ye J, Ortaldo JR, Conlon K, Winkler-Pickett R, Young HA. Cellular and molecular mechanisms of IFN-gamma production induced by IL-2 and IL-12 in a human NK cell line. J Leukoc Biol 1995;58(2):225-33.

349.   Rosser DM, Stidwill RP, Jacobson D, Singer M. Cardiorespiratory and tissue oxygen dose response to rat endotoxemia. Am J Physiol 1996;271(3 Pt 2):H891-5.

350.   Ma XC, Chen LT, Oliver J, Horvath E, Phelps CP. Cytokine and adrenal axis responses to endotoxin. Brain Res 2000;861(1):135-42.

351.   Taniguchi T, Shibata K, Yamamoto K. Ketamine inhibits endotoxin-induced shock in rats. Anesthesiology 2001;95(4):928-32.

352.   Taniguchi T, Takemoto Y, Kanakura H, Kidani Y, Yamamoto K. The dose-related effects of ketamine on mortality and cytokine responses to endotoxin-induced shock in rats. Anesth Analg 2003;97(6):1769-72.

353.   van Lambalgen AA, Rasker MT, van den Bos GC, Thijs LG. Effects of endotoxemia on systemic plasma loss and hematocrit in rats. Microvasc Res 1988;36(3):291-304.

354. van Lambalgen AA, van den Bos GC, Thijs LG. Whole body plasma extravasation in saline and Haemaccel loaded rats: effects of endotoxemia. Int J Microcirc Clin Exp 1990;9(3):303-18.

355. van Lambalgen AA, van Kraats AA, van den Bos GC, Stel HV, Straub J, Donker AJ, et al. Renal function and metabolism during endotoxemia in rats: role of hypoperfusion. Circ Shock 1991;35(3):164-73.

356. Birnbaum J, Lehmann C, Stauss HM, Weber M, Georgiew A, Lorenz B, et al. Sympathetic modulation of intestinal microvascular blood flow oscillations in experimental endotoxemia. Clin Hemorheol Microcirc 2003;28(4):209-20.

357. Toung T, Reilly PM, Fuh KC, Ferris R, Bulkley GB. Mesenteric vasoconstriction in response to hemorrhagic shock. Shock 2000;13(4):267-73.

358. Adolphs J, Schmidt DK, Korsukewitz I, Kamin B, Habazettl H, Schafer M, et al. Effects of thoracic epidural anaesthesia on intestinal microvascular perfusion in a rodent model of normotensive endotoxaemia. Intensive Care Med 2004;30(11):2094-101.

359. Revelly JP, Ayuse T, Brienza N, Fessler HE, Robotham JL. Endotoxic shock alters distribution of blood flow within the intestinal wall. Crit Care Med 1996;24(8):1345-51.

360. Hiltebrand LB, Krejci V, tenHoevel ME, Banic A, Sigurdsson GH. Redistribution of microcirculatory blood flow within the intestinal wall during sepsis and general anesthesia. Anesthesiology 2003;98(3):658-69.

361. Shepherd AP, Riedel GL. Intramural distribution of intestinal blood flow during sympathetic stimulation. Am J Physiol 1988;255(5 Pt 2):H1091-5.

362. Schmidt H, Secchi A, Wellmann R, Bach A, Bohrer H, Gebhard MM, et al. Effect of endotoxemia on intestinal villus microcirculation in rats. J Surg Res 1996;61(2):521-6.

363. Lidington D, Ouellette Y, Li F, Tyml K. Conducted vasoconstriction is reduced in a mouse model of sepsis. J Vasc Res 2003;40(2):149-58.

364. Lidington D, Tyml K, Ouellette Y. Lipopolysaccharide-induced reductions in cellular coupling correlate with tyrosine phosphorylation of connexin 43. J Cell Physiol 2002;193(3):373-9.

365. Tyml K, Wang X, Lidington D, Ouellette Y. Lipopolysaccharide reduces intercellular coupling in vitro and arteriolar conducted response in vivo. Am J Physiol Heart Circ Physiol 2001;281(3):H1397-406.

366. Drazenovic R, Samsel RW, Wylam ME, Doerschuk CM, Schumacker PT. Regulation of perfused capillary density in canine intestinal mucosa during endotoxemia. J Appl Physiol (1985) 1992;72(1):259-65.

367. Brandes RP, Kim D, Schmitz-Winnenthal FH, Amidi M, Godecke A, Mulsch A, et al. Increased nitrovasodilator sensitivity in endothelial nitric oxide synthase knockout mice: role of soluble guanylyl cyclase. Hypertension 2000;35(1 Pt 2):231-6.

368. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH. K+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 1998;396(6708):269-72.

369. Wanstall JC, Jeffery TK, Gambino A, Lovren F, Triggle CR. Vascular smooth muscle relaxation mediated by nitric oxide donors: a comparison with acetylcholine, nitric oxide and nitroxyl ion. Br J Pharmacol 2001;134(3):463-72.

370. Picchi A, Gao X, Belmadani S, Potter BJ, Focardi M, Chilian WM, et al. Tumor necrosis factor-alpha induces endothelial dysfunction in the prediabetic metabolic syndrome. Circ Res 2006;99(1):69-77.

371. Zhang C, Xu X, Potter BJ, Wang W, Kuo L, Michael L, et al. TNF-alpha contributes to endothelial dysfunction in ischemia/reperfusion injury. Arterioscler Thromb Vasc Biol 2006;26(3):475-80.

372. Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J, et al. Role of TNF-alpha in vascular dysfunction. Clin Sci (Lond) 2009;116(3):219-30.

373. Koga S, Morris S, Ogawa S, Liao H, Bilezikian JP, Chen G, et al. TNF modulates endothelial properties by decreasing cAMP. Am J Physiol 1995;268(5 Pt 1):C1104-13.

374. Xu B, Li J, Gao L, Ferro A. Nitric oxide-dependent vasodilatation of rabbit femoral artery by beta(2)-adrenergic stimulation or cyclic AMP elevation in vivo. Br J Pharmacol 2000;129(5):969-74.

375. Wimalasundera R, Fexby S, Regan L, Thom SA, Hughes AD. Effect of tumour necrosis factor-alpha and interleukin 1beta on endothelium-dependent relaxation in rat mesenteric resistance arteries in vitro. Br J Pharmacol 2003;138(7):1285-94.

376. Jacinto SM, Jandhyala BS. Salutary effects of dopexamine, a DA-1 and beta-2 agonist, on free radical-induced toxicity in anesthetized rats. Ann N Y Acad Sci 1994;723:349-52.

377. Polte T, Schroder H. Cyclic AMP mediates endothelial protection by nitric oxide. Biochem Biophys Res Commun 1998;251(2):460-5.

378. Li J, Yang S, Billiar TR. Cyclic nucleotides suppress tumor necrosis factor alpha-mediated apoptosis by inhibiting caspase activation and cytochrome c release in primary hepatocytes via a mechanism independent of Akt activation. J Biol Chem 2000;275(17):13026-34.

379. Gotoh T, Mori M. Arginase II downregulates nitric oxide (NO) production and prevents NO-mediated apoptosis in murine macrophage-derived RAW 264.7 cells. J Cell Biol 1999;144(3):427-34.

380. Mori M. Regulation of nitric oxide synthesis and apoptosis by arginase and arginine recycling. J Nutr 2007;137(6 Suppl 2):1616S-1620S.

381. Gao X, Xu X, Belmadani S, Park Y, Tang Z, Feldman AM, et al. TNF-alpha contributes to endothelial dysfunction by upregulating arginase in ischemia/reperfusion injury. Arterioscler Thromb Vasc Biol 2007;27(6):1269-75.

382. Levy B, Desebbe O, Montemont C, Gibot S. Increased aerobic glycolysis through beta2 stimulation is a common mechanism involved in lactate formation during shock states. Shock 2008;30(4):417-21.

383. Fenger-Gron J, Mulvany MJ, Christensen KL. Mesenteric blood pressure profile of conscious, freely moving rats. J Physiol 1995;488 (Pt 3):753-60.

384. Zhou ZZ, Jones SB. Involvement of central vs. peripheral mechanisms in mediating sympathoadrenal activation in endotoxic rats. Am J Physiol 1993;265(3 Pt 2):R683-8.

385.   Jones SB, Romano FD. Plasma catecholamines in the conscious rat during endotoxicosis. Circ Shock 1984;14(3):189-201.

386.   Jones SB, Romano FD. Dose- and time-dependent changes in plasma catecholamines in response to endotoxin in conscious rats. Circ Shock 1989;28(1):59-68.

387.   Lobo SM, De Backer D, Sun Q, Tu Z, Dimopoulos G, Preiser JC, et al. Gut mucosal damage during endotoxic shock is due to mechanisms other than gut ischemia. J Appl Physiol 2003;95(5):2047-54.

388.   Thiemermann C, Wu CC, Szabo C, Perretti M, Vane JR. Role of tumour necrosis factor in the induction of nitric oxide synthase in a rat model of endotoxin shock. Br J Pharmacol 1993;110(1):177-82.

389.   Szabo C, Wu CC, Gross SS, Thiemermann C, Vane JR. Interleukin-1 contributes to the induction of nitric oxide synthase by endotoxin in vivo. Eur J Pharmacol 1993;250(1):157-60.

390.   Szabo C, Mitchell JA, Thiemermann C, Vane JR. Nitric oxide-mediated hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxin shock. Br J Pharmacol 1993;108(3):786-92.

391.   Li JM, Mullen AM, Yun S, Wientjes F, Brouns GY, Thrasher AJ, et al. Essential role of the NADPH oxidase subunit p47(phox) in endothelial cell superoxide production in response to phorbol ester and tumor necrosis factor-alpha. Circ Res 2002;90(2):143-50.

392.   James JH, Luchette FA, McCarter FD, Fischer JE. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. Lancet 1999;354(9177):505-8.

393.   Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, et al. Shock and tissue injury induced by recombinant human cachectin. Science 1986;234(4775):470-4.

394.   Karlstad MD, Sayeed MM. Effect of endotoxic shock on basal and insulin-mediated Na+/K(+)-pump activity in rat soleus muscle. Circ Shock 1992;38(4):222-7.

395.   O'Brien WJ, Lingrel JB, Fischer JE, Hasselgren PO. Sepsis increases skeletal muscle sodium, potassium-adenosinetriphosphatase activity without affecting messenger RNA or protein levels. J Am Coll Surg 1996;183(5):471-9.

396.	Jacobs DO, Kobayashi T, Imagire J, Grant C, Kesselly B, Wilmore DW. Sepsis alters skeletal muscle energetics and membrane function. Surgery 1991;110(2):318-25; 325-6.

397.	James JH, Fang CH, Schrantz SJ, Hasselgren PO, Paul RJ, Fischer JE. Linkage of aerobic glycolysis to sodium-potassium transport in rat skeletal muscle. Implications for increased muscle lactate production in sepsis. J Clin Invest 1996;98(10):2388-97.

398.	Levy B, Gibot S, Franck P, Cravoisy A, Bollaert PE. Relation between muscle Na+K+ ATPase activity and raised lactate concentrations in septic shock: a prospective study. Lancet 2005;365(9462):871-5.

399.	Michaeli B, Martinez A, Revelly JP, Cayeux MC, Chiolero RL, Tappy L, et al. Effects of endotoxin on lactate metabolism in humans. Crit Care 2012;16(4):R139.

400.	Traves PG, de Atauri P, Marin S, Pimentel-Santillana M, Rodriguez-Prados JC, Marin de Mas I, et al. Relevance of the MEK/ERK signaling pathway in the metabolism of activated macrophages: a metabolomic approach. J Immunol 2011;188(3):1402-10.

401.	De Backer D, Creteur J, Zhang H, Norrenberg M, Vincent JL. Lactate production by the lungs in acute lung injury. Am J Respir Crit Care Med 1997;156(4 Pt 1):1099-104.

402.	Wu X, Guo R, Chen P, Wang Q, Cunningham PN. TNF induces caspase-dependent inflammation in renal endothelial cells through a Rho- and myosin light chain kinase-dependent mechanism. Am J Physiol Renal Physiol 2009;297(2):F316-26.

403.	Tesauro M, Schinzari F, Rovella V, Melina D, Mores N, Barini A, et al. Tumor necrosis factor-alpha antagonism improves vasodilation during hyperinsulinemia in metabolic syndrome. Diabetes Care 2008;31(7):1439-41.

404.	Gardiner SM, Kemp PA, March JE, Woolley J, Bennett T. The influence of antibodies to TNF-alpha and IL-1beta on haemodynamic responses to the cytokines, and to lipopolysaccharide, in conscious rats. Br J Pharmacol 1998;125(7):1543-50.

405.	Baker JG. The selectivity of beta-adrenoceptor agonists at human beta1-, beta2- and beta3-adrenoceptors. Br J Pharmacol 2010;160(5):1048-61.

406.   Chilcoat CD, Sharief Y, Jones SL. Tonic protein kinase A activity maintains inactive beta2 integrins in unstimulated neutrophils by reducing myosin light-chain phosphorylation: role of myosin light-chain kinase and Rho kinase. J Leukoc Biol 2008;83(4):964-71.

407.   Hutri-Kahonen N, Kahonen M, Jolma P, Wu X, Sand J, Nordback I, et al. Control of mesenteric arterial tone in vitro in humans and rats. Naunyn Schmiedebergs Arch Pharmacol 1999;359(4):322-30.

408.   Zolfaghari PS, Pinto BB, Dyson A, Singer M. The metabolic phenotype of rodent sepsis: cause for concern? Intensive Care Medicine Experimental 2013;1:1-6.

409.   Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 2013;110(9):3507-12.

410.   Radermacher P, Haouzi P. A mouse is not a rat is not a man: species-specific metabolic responses to sepsis - a nail in the coffin of murine models for critical care research? Intensive Care Medicine Experimental 2013;1(1).

411.   Cauwels A, Vandendriessche B, Brouckaert P. Of mice, men, and inflammation. Proc Natl Acad Sci U S A 2013;110(34):E3150.

412.   Osuchowski MF, Remick DG, Lederer JA, Lang CH, Aasen AO, Aibiki M, et al. Abandon the mouse research ship? Not just yet! Shock 2014;41(6):463-75.

413.   Couzin-Frankel J. When mice mislead. Science 2013;342(6161):922-3, 925.