Modulation of Sympathetic Neurotransmitter Release in Acute Myocardial Ischaemia

Xiao-Jun Du

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急性心肌缺血时

交感神经递质释放的调节

谨以此论文献给：

我的祖国 — 中華人民共和國

Declaration

I hereby declare that the work included in this thesis was entirely carried out and written up by myself during my Ph.D. studentship in the Cardiovascular Research Unit, Department of Medicine (Royal Infirmary of Edinburgh), University of Edinburgh. I was the principle contributor to all sections.

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Abbreviations

ACh ····· Acetylcholine
CAD ····· Coronary artery disease
ECG ····· Electrocardiogram
FGX ····· Female gonadectomised
FSH ····· Female sham-operated
GS ····· Left stellate ganglion stimulation
HR ····· Heart rate
LVSP ····· Left ventricular systolic pressure
MAP ····· Mean arterial pressure
MCh ····· Methacholine
MGX ····· Male gonadectomized
MSH ····· Male sham-operated
MI ····· Myocardial infarction
NA ····· Noradrenaline
PUFA ····· Polyunsaturated fatty acids
SCD ····· Sudden cardiac death
VF ····· Ventricular fibrillation
VPB ····· Ventricular premature beats
VS ····· Bilateral vagal nerve stimulation
VT ····· Ventricular tachycardia

Abstract

There is substantial evidence to suggest that the sympathetic nervous system is intimately involved in the development of ventricular arrhythmias during acute myocardial ischaemia. Nevertheless factors controlling myocardial noradrenaline (NA) release during ischaemia are only partly understood. Therefore, the effect of duration and severity of ischaemia on neuronal NA release (left stellate ganglion stimulation, 5Hz) was studied using a perfused, innervated rat heart model. The effect of ischaemia on presynaptic inhibition by α-adrenergic, muscarinic and purinergic receptors was also examined. There is higher density of α-adrenoceptors in female tissues but it is not clear whether there is a gender difference in presynaptic inhibitory mechanisms.

NA overflow progressively declined during 10 min stop-flow ischaemia, but was maintained for up to 60 min during less severe ischaemia (95% flow reduction). α-Adrenergic or purinergic antagonists increased NA overflow during low-flow but not stop-flow ischaemia. In normoxic hearts, neuronal NA overflow was inhibited by vagal nerve stimulation (15 Hz) but not after 10 min low-flow or 1-5 min stop-flow ischaemia. The loss of VS-induced inhibition of NA overflow during ischaemia may be due to enhanced α-adrenergic presynaptic inhibition of acetylcholine release, since an α-adrenoceptor antagonist could restore this effect. The inhibitory effect of the muscarinic agonist methacholine on NA overflow was also attenuated by low-flow or stop-flow ischaemia, indicating a dysfunction of muscarinic presynaptic receptors.

The effect of gender on α_2-adrenergic presynaptic inhibition of neuronal NA release was also studied. In normoxic hearts, NA overflow by control nerve stimulation was similar. The α_2-adrenergic inhibitor rauwolscine potentiated NA overflow more in female than in male hearts, both during normoxia and ischaemia. Ovariectomy attenuated α-adrenergic presynaptic inhibition of NA overflow. During normoxia, presynaptic inhibition of neuronal NA overflow by vagal nerve stimulation was greater in female than in male hearts, but methacholine-reduced NA overflow equally in female and male hearts.

The postsynaptic, heart rate-lowering response to vagal nerve stimulation, but not to methacholine, was more marked in female than male hearts. Castration potentiated, and ovariectomy attenuated the effect of vagal nerve stimulation on heart rate.

Sympathetic nerve stimulation during 10 mins low-flow ischaemia increased the incidence of ventricular fibrillation during early reperfusion (from 8% to 55%). Simultaneous vagal nerve stimulation or pretreatment with methacholine prevented reperfusion ventricular fibrillation *without* a reduction in neuronal NA release. No sex difference was found in the severity of ischaemic arrhythmias after coronary ligation *in vitro* or *in vivo* without nerve stimulation.

Feeding rats n-3 or n-6 polyunsaturated fatty acids changed myocardial fatty acid composition without changing neuronal NA overflow and its adrenergic presynaptic modulation either during normoxia or during ischaemia.

In conclusion, sympathetic nerve stimulation leads to ischaemic-reperfusion arrhythmias. The antiarrhythmic effect of vagal nerve stimulation and of dietary polyunsaturated fatty acids cannot be explained by presynaptic modulation of NA release. Cardiac neuronal function, especially sympathetic neurotransmitter release and presynaptic modulation, is affected by the severity of ischaemia, parasympathetic nervous activity and gender.

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Chapter 1

Introduction and Literature Review

1.1 Sudden Cardiac Death: Challenge to Modern Cardiology

Sudden cardiac death (SCD) continues to be a major problem in current cardiac medicine. In the western world, about 50% of all coronary deaths are in the form of SCD constituting the leading cause of death among men aged 30-65 years [Kannel and Schatzkin 1985, Oliver 1982, Gillum 1989]. It is estimated that each month there will be about 100 SCD events per million population in western society. Most SCD victims (80-90%) have no prodromes within days or hours prior to the fatal event and only a minority recently consult with their doctors presenting vague symptoms [Oliver 1982]. In fact, in a high proportion of victims, SCD is the first and only manifestation of coronary artery disease (CAD) [Kannel and Schatzkin 1985]. Furthermore, most SCDs occur outside hospital [Fraser 1986, Gillum 1989]. These facts imply that subjects with high risk of SCD are not properly identified and therefore do not receive proper medical advice, treatment and monitoring.

1.1.1 The Pathology of SCD

Autopsy and angiographic studies have demonstrated that extensive and severe CAD is the major underlying etiology in over 80% of SCD cases. Significant coronary artery stenosis (>75%) could be identified in 70-90% of cases, and multiple coronary stenoses in about half of all cases [Davies and Thomas 1984, Liberthson *et al* 1974, Baroldi *et al* 1979, Davies and Popples 1979]. Scattered myofibrillar degeneration, coagulative myocytolysis, healed myocardial infarction and myocardial hypertrophy are other common findings [Baroldi *et al* 1979, Liberthson *et al* 1974, Davies and Popples 1979]. The reported frequency of coronary artery thrombosis is not constant, varying from 15% to 74% [Baroldi *et al* 1979, Lovegrove and Thompson 1978, Davies and Thomas 1984, Liberthson *et al* 1974]. However, this figure may not be a true reflection of the nature of the acute ischaemic event due to spontaneous thrombolysis. Coronary spasm may also cause serious arrhythmias and SCD with or without concomitant coronary stenosis [Miller *et al* 1982]. Morphological changes of acute myocardial infarction (MI) are less common (20-35%) [Baroldi *et al* 1979, Lovegrove and Thompson 1978, Liberthson *et al* 1974].

This is in agreement with the finding that about 40% of patients resuscitated from a cardiac arrest have enzymatic evidence of acute myocardial necrosis and only 20% show electrocardiographic (ECG) evidence of a transmural infarction [Greene 1990a]. Some autopsy data suggest a relationship between random damage to the specialized conducting tissue or cardiac nerves and the risk of SCD [Rossi 1985, James 1983]. But because of the difficulties in carrying out detailed investigations and the limited number of cases, it is not possible to come to a definitive conclusion.

A number of other causes or factors also contribute to SCD [Myerburg 1987]. Ventricular hypertrophy is an important and independent risk factor for ventricular arrhythmias and SCD [Kannel and Schatzkin 1985]. Heart failure is another syndrome with a high risk of SCD. According to recent reports, about 40-50% of deaths in patients with heart failure are categorized as SCD [Kannel *et al* 1988, Bigger 1987]. Myocarditis and valvular disease may also be associated with SCD though less frequently. Disorders of the nervous system may predispose the heart to lethal arrhythmias [Oppenheimer *et al* 1990].

Thus, it can be concluded that SCD does not possess a distinctive entity of pathomorphological lesions and a spectrum of minor to extensive myocardial lesions. Therefore, it is difficult to explain the mechanism of SCD by pathological observations alone.

1.1.2 Electrophysiological Substrate of SCD

Ventricular tachycardia (VT) and fibrillation (VF) are the most common electrical disturbances leading to SCD. VF is recorded in 95% of SCD victims attended to within minutes after collapse [Cobb 1988, Hallstrom *et al* 1983]. Similarly, the occurrence of VT and VF is documented in the vast majority of SCD cases (83%) using Holter monitoring [de Lune *et al* 1989]. The average time for VT to degenerate into VF is 96 seconds [Milner *et al* 1985]. Bradyarrhythmia and asystole were seen in 10-20% of patients [de Lune 1989, Milner *et al* 1985, Kempf and Josephson 1984]. Data from Holter monitoring show that VF may be preceded by a sudden increase in HR [Adgey *et al* 1982, McAreavey *et al* 1989].

An inherent ventricular electrical instability in SCD victims is suggested by several observations. About 30% of resuscitated subjects die of another episode of VF within weeks [Greene 1990a]. Men or acute MI patients who have exercise-induced ischaemic ventricular arrhythmias or frequent and complex ventricular premature beats (VPB) during ECG monitoring are at higher risk of SCD [Kannel and Schatzkin 1985, Moss *et al* 1987, Bigger *et al* 1984]. Furthermore, inducibility of VT and VF

by programmed electrical stimulation is high (68-75%) in survivors of cardiac arrest [Horowitz *et al* 1982, Freedman *et al* 1988].

1.1.3 Factors Influencing SCD

A number of factors are associated with increased risk of SCD. Here, only those which have been proved to be important and/or linked to the subject of the thesis are briefly reviewed.

1) CAD risk factors and SCD

The Framingham Study indicates an important relationship between classical risk factors for CAD and the probability of SCD over a 26-year follow-up period (Figure 1.1) [Kannel and Schatzkin 1985]. This emphasizes the importance of underlying CAD in SCD, as has been proved by clinical studies [Surawicz 1987].

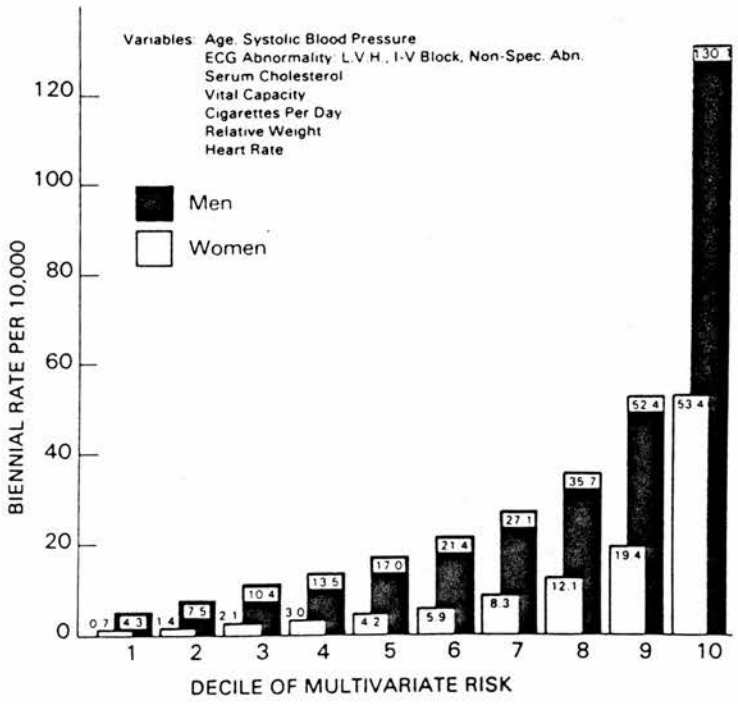


Figure 1.1 *Risk of SCD by decile of multivariate risk: 26-year follow-up, the Framingham Study [Kannel and Schatzkin 1985].*

2) Ventricular dysfunction and extent of myocardial ischaemia

After acute MI, clinical variables reflecting the degree of ventricular dysfunction or ischaemic/infarct size (IS) correlate with frequency and repetitivity of VPB [Coromilas *et al* 1985] and incidence of SCD [Schulze *et al* 1977]. Patients with acute MI requiring early treatment for VT or VF exhibit higher peak plasma creatine

phosphokinase than those without these serious arrhythmias [Abraham *et al* 1989]. Congestive heart failure is also associated with 5-9 fold increase in the risk of SCD [Kannel *et al* 1988]. In animals with experimental MI time of onset and severity of arrhythmias are closely related to IS [Leprán *et al* 1983, Curtis *et al* 1987]. In dogs, frequency of VPBs, incidence of VT, reduction of VF threshold, and the inducibility of VT are all IS-dependent [Coromilas *et al* 1985, Gang *et al* 1982]. Large IS is associated with an increased incidence of VF [Sheehan and Epstein 1983, Bolli *et al* 1986].

3) Ventricular premature beats

Relationship between frequency or type of VPB and risk of SCD has been well defined. The occurrence of VPB during 24 hour ECG monitoring is associated with an increased risk of SCD in men [Kannel and Schatzkin 1985]. In male patients with recent MI, complex (frequent and repetitive) VPB is a strong predictor of SCD during the follow-up period (Figure 1.2) [Moss *et al* 1987, Bigger *et al* 1984, Lown 1979, Ruberman *et al* 1981, Surawicz 1987].

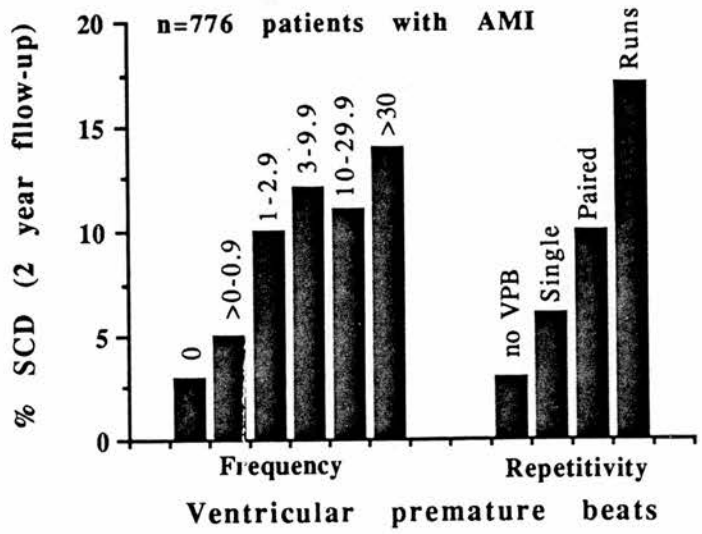


Figure 1.2 *Frequency (per hour) and repetitivity of VPBs during 24 hour Holter monitoring and the incidence of SCD during 2 year follow-up [Bigger et al 1984]*

4) Gender

It is well-known that middle-aged women have a lower risk of CAD, MI, and mortality due to ischaemic heart attack than men. After the menopause, this gender-gap narrows but male dominance persists. As there is a delay of 10-15 years in the development of atherosclerotic lesions in coronary arteries in women in comparison to men, it is impossible to directly compare the incidence of SCD between the sexes on a population basis. However, epidemiological, clinical and experimental data

suggest a gender difference in SCD. In the Framingham cohort, the proportion of SCD out of total coronary deaths during a 30 year follow-up period tends to be higher in men than in women (46% *vs* 34%) [Kannel *et al* 1988]. Recently, a similar gender difference in out-of-hospital deaths as a percentage of total coronary deaths is also indicated by the FINMONICA Study (68% *vs* 56% during 1982-1987) [Romo *et al* 1990] and a national survey in the United States (68.2% vs 54.7% in 45-54 age group during 1980-1985) [Gillum 1989]. In survivors of cardiac arrest or patients with recent VT/VF, male gender is one of the strongest and independent predictor for the inducibility of VT and VF by programmed premature electrical stimulation [Freedman *et al* 1988, Schoenfeld *et al* 1985]. Interestingly, women do not share the increased risk that men demonstrate when VPBs exist [Moss *et al* 1987, Kannel and Schatzkin 1985, Dittrich *et al* 1988]. In anaesthetized or conscious rats, severity of ventricular arrhythmias and early mortality after coronary ligation is higher in males than in females (Figure 1.3) [Siegmund *et al* 1979, Lu *et al* 1984, Du *et al* unpublished]. In a well-designed study the risk for VF during acute coronary occlusion is found to be higher in male than female dogs [Puddu *et al* 1988], although this difference was not observed in another study [Trolese-Mongheal *et al* 1985]. Why gender may influence the propensity to SCD is largely unknown.

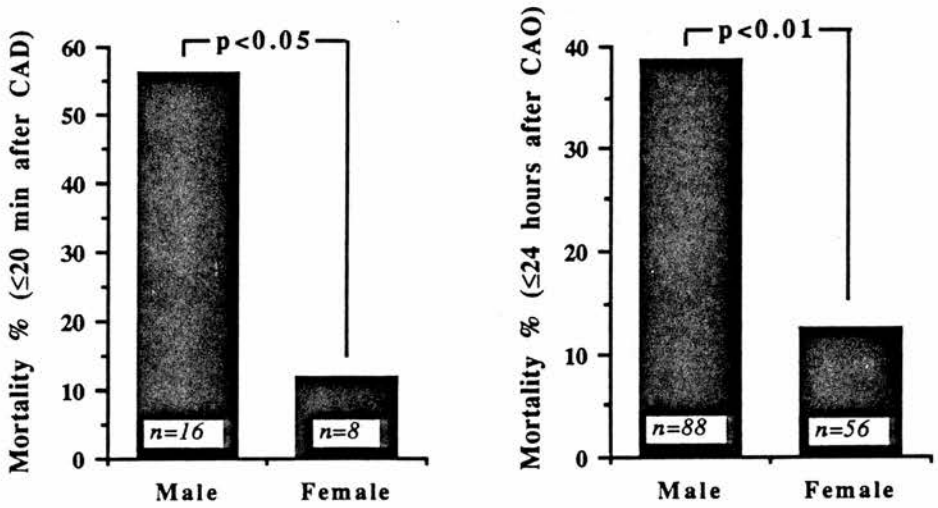


Figure 1.3 *Gender difference in the early mortality induced by left coronary artery occlusion (CAO) in anaesthetized (left panel) [Siegmund et al 1979] and conscious rats (right panel) [Lu et al 1984].*

5) Severity of ischaemia and collateral circulation

Pathological studies show that IS is usually smaller after acute coronary occlusion if functional collaterals are present [James 1978, Baroldi 1970]. In patients with acute MI, well-developed collaterals are accompanied by preserved ventricular function, perhaps due to a reduced IS [Williams *et al* 1976, Nohara *et al* 1983, Rentrop *et al*

1988], and the association of the likelihood of SCD and IS or ventricular dysfunction has been documented. In a group of patients with variant angina, increased severity of ischaemia, estimated by ECG ST-segment elevation, is related to early onset of lethal arrhythmias and subsequent SCD [Miller *et al* 1982]. A postmortem study suggested that a poor pre-existing collateral network increased the risk of SCD [Spain *et al* 1963]. In patients with matched severity of CAD, the presence of a collateral circulation significantly reduces mortality rate during follow-up [Webster *et al* 1974].

Experimental studies also show that severity of ischaemia and collateral circulation influence the vulnerability of the heart to lethal arrhythmias [Meesmann 1982, Bolli *et al* 1986, von Mutins *et al* 1988]. After acute coronary occlusion, dogs with an adequate collaterals had a significant higher VF threshold and were less likely to develop fatal arrhythmias [Garza *et al* 1974]. Bolli and colleagues found in dogs that residual coronary flow of >8% of normal level (*vs* <8%) substantially reduced the incidence of VT (17% *vs* 87%) and VF (0% *vs* 57%) [Bolli *et al* 1986]. Therefore, severity of ischaemia and the status of collateral circulation is a critical factor in the development of SCD.

6) Dietary fat

The strongest evidence suggesting an effect of dietary fat on SCD comes from experimental studies. Incidence of VF and mortality following acute coronary occlusion are reduced by dietary supplementation with polyunsaturated fatty acids for 2-6 months [McLennan *et al* 1985 and 1989, Hock *et al* 1987 and 1990, Riemersma *et al* 1988, Culp *et al* 1980], although the mechanism of this protection remains unclear.

Data from the Framingham Study suggest a plausible and weak relation of serum total cholesterol on the long-term risk of SCD [Kannel and Schatzkin 1985, Kannel *et al* 1988]. Dietary saturated fats have been related to the incidence of CAD [Keys 1980]. Recently, an inverse relation has been demonstrated between dietary polyunsaturated/saturated fatty acid ratio or linoleic acid level in adipose tissue and the relative risk of CAD [Wood *et al* 1987]. Whether a similar relation exists between dietary fat and SCD in man remains to be seen. In patients with acute MI saturated fatty acids in adipose tissue are significantly higher in those with serious ventricular arrhythmias than those without [Abraham *et al* 1989].

1.1.4 Prevention and Prediction of SCD

In the recent two decades, extensive resources and research have been directed towards prevention of SCD. As victims of SCD share most of the major risk factors

for CAD [Kannel and Schatzkin 1985], it might be expected that lowering of these risks could reduce the incidence of SCD. Unfortunately, none of the large scale primary coronary disease prevention trials has led to a statistically significant reduction in SCD [reviews see Oliver 1985, Roelandt and Hugenholtz 1986]. Current emphasis has shifted to various types of secondary prevention. Patients with recent MI or unstable angina are often prone to arrhythmias. Trials with these subjects aimed to lower raised serum cholesterol, blood pressure, platelet aggregation or arrhythmias (lidocaine) have yielded uncertain results on SCD as an endpoint [Oliver 1985, Noneman and Rogers 1978, Furberg and Craver 1988]. Exceptionally, β-adrenergic blockade is effective in reducing the risk of SCD [Oliver 1985, Roelandt and Hugenholtz 1986, Frishman *et al* 1984].

The failure to curb SCD in most primary and secondary prevention trials may be due to the the difficulty to identify those at risk of SCD. Therefore, a large number of studies have been conducted to find clinical variables, which are capable of predicting the probability of subsequent SCD. These parameters, e.g., complex ectopics on ECG Holter monitoring [Moss *et al* 1987, Ruberman *et al* 1981], ejection fraction [Bigger *et al* 1984, Schulze *et al* 1977], inducibility at electrophysiological testing [Freedman *et al* 1988], exercise test [Greene 1990b], R-R interval variability [Kleiger *et al* 1987] and baroreflex sensitivity [Schwartz *et al* 1988a, La Rovere *et al* 1988], are of some value to identify the subgroup at enhanced risk of SCD. But most of them do not have the sensitivity nor the specificity to predict the risk of SCD for an individual.

In summary, since morphological lesions do not provide a simple explanation for the occurrence of the lethal event, SCD is most probably a random event during a short episode of myocardial ischaemia due to underlying CAD [Oliver 1986, Stone 1990]. Thus, questions remaining to be answered should be why some hearts are so vulnerable to ischaemia and what the trigger(s) leading to VT and VF are. Among the transcient and local factors that should be considered are those originating from higher nervous centres or from peripheral neurons.

1.2 Neuronal Mechanisms of Ventricular Arrhythmias in Myocardial Ischaemia

1.2.1 Neuronal Activation during Myocardial Ischaemia

Activation of sympathetic nerve

Within 30 min after the onset of anterior MI, 54% of patients show signs of sympathetic activation, i.e., sinus tachycardia and transcient hypertension [Webb *et al* 1972]. In other patients who show signs of augmented vagal tone atropine treatment may unmask underlying sympathetic activation [Webb *et al* 1972, Neufeld *et al* 1978]. During acute MI, a high level of circulating catecholamines is a common finding in most patients apparently due to enhanced release from sympathetic nerves and adrenal medulla [Goldstein 1981, Nadeau and de Champlain 1979, Bertel *et al* 1982, Karlsberg *et al* 1979, Schömig *et al* 1984b]. Using radiotracer kinetic techniques, McCance and Forfar have reported enhanced spillover of cardiac noradrenaline (NA) in patients with unstable angina or with exertion-induced myocardial ischaemia [1989a and 1989b].

Autopsy studies of hearts from SCD victims show a typical coagulative myocytolysis in 80% of the cases in comparison to 20% in accidental deaths [Baroldi *et al* 1979]. This morphological change is similar to that in experimentally-induced "catecholamine cardiomyopathy" [Rona 1985], thereby providing indirect evidence of enhanced sympathetic activity and NA release.

Several lines of experimental evidence strongly suggest a sympathetic activation during myocardial ischaemia. Direct recording of efferent sympathetic nerve impulses shows a increased firing frequency immediately after coronary artery occlusion [Lombardi *et al* 1984, Malliani *et al* 1980, Gillis 1971], which is accompanied by reduced VF threshold [Lombardi *et al* 1983]. However, NA levels in the ischaemic effluent were not measured in these studies. Increased circulating catecholamine levels indicate an overall activation of sympathetic nervous system, especially in conscious animals [Godin *et al* 1985, Forfar *et al* 1984, Karlsberg *et al* 1979].

An augmented catecholamine release in ischaemic myocardium is indirectly supported by an enhanced NA overflow during ischaemic-reperfusion *in vivo* [Forfar *et al* 1984, Godin *et al* 1985, Yamaguchi *et al* 1990], by increased cAMP levels which is partly prevented by β-blockade [Ohyanagi *et al* 1988, Lubbe *et al* 1981, Thandroyen *et al* 1986], and by the histochemical finding that myocardial catecholamine content is reduced after ischaemia [Abrahamsson *et al* 1982, Krivokapich *et al* 1987, McDonald *et al* 1986, Muntz *et al* 1984]. Usually, reduction in intraneuronal NA (demonstrated by fluorescence method) precedes the drop in myocardial NA content, indicating loss of NA from synaptic vesicles into ischaemic regions [Abrahamsson 1982, Muntz *et al* 1984]. In pig and dog hearts, nearly 20-40% of total myocardial NA may be lost after 5-20 min of ischaemia with or without

reperfusion [McDonald *et al* 1986, Krivokepich *et al* 1987], although the magnitude of this loss may be less according to others [Mukherjee *et al* 1979, Muntz *et al* 1984].

Activation of parasympathetic nerve

Clinical and experimental data also suggest increased cardiac vagal activity (i.e., sinus bradycardia and hypotension) in 55% patients seen within 30 min after the onset of MI [Webb *et al* 1972]. Signs of vagal activation is more common in patients (75%) or dogs with inferio-posterior infarction [Pantridge 1978, Neufeld *et al* 1978, Billman and Marsh 1989]. In experimental studies, increased nerve impulses of afferent and efferent vagal fibres are recorded shortly after ischaemia [Thorén 1976, Uchida and Murao 1974, Corr and Gillis 1978]. Autonomic activation appears to be influenced by the extent of myocardial ischaemia. In the cat, for instance, induction of a small ischaemic area is accompanied by sympathetic activation only, and a large ischaemic lesion results in an enhanced vagal tone overriding a sympathetic activation [Lombardi *et al* 1984]. In man and dog, however, extent of sympathetic activation is proportional to infarct size [Karlsberg *et al* 1979, Goldstein 1981, Bertel *et al* 1982].

Mechanism of neural activation by myocardial ischaemia

In the setting of acute myocardial ischaemia, neuronal activation may occur *via* 1) exciting impulses from high centres to the heart due to pain, anxiety and fear, 2) neuronal reflexes induced by activation of pressor and volume receptors following a decrease in blood pressure and cardiac output [Schömig *et al* 1984b, Karlsberg *et al* 1979], and 3) cardio-cardiac reflexes that are activated by afferents from the ischaemic area. For activation of the cardio-cardiac reflexes, ischaemia-induced chemical (e.g., increase in H^+, K^+, bradykinin, prostaglandins, serotonin) and functional changes (such as left ventricular dilatation, dyskinesis, increased ventricular volume and wall stress) may be important [Shepherd 1985]. Various reflex routes either *via* central nervous system or spinal cord may be activated simultaneously leading to enhanced neuronal tone to the heart [Lombardi *et al* 1983]. Complexity of pathways by which autonomic nerves are activated may constitute the anatomic background of sympatho-vagal interactions and of the simultaneous activation of both branches. Afferent receptors are not evenly distributed in the ventricle, which could explain the observed patterns of autonomic activation in relation to location of ischaemia [Webb 1972, Perez-Gomez *et al* 1979, Thomas *et al* 1978].

1.2.2 Neuronal Activity and Ischaemic Arrhythmias

Effect of enhanced adrenergic activity

Direct clinical evidence documenting a causal relation between sympathetic activation and ischaemic arrhythmias is still rare, but a great amount of indirect data have been accumulated. Increased plasma catecholamines significantly correlate with the severity of ischaemic arrhythmias in some, but not all studies [Nadeau and de Champlain 1979, Karlsberg *et al* 1979, Bertel *et al* 1982, Goldstein 1981]. However, this correlation is only moderate and could be partly explained by a larger ischaemic area and more severe ventricular dysfunction (Section 1.1.3). In patients with unstable angina, ischaemia of the anterior wall is coupled with a marked increase in HR, which precedes the onset of VT (42%) and VF (37%). In contrast, patients with inferior ischaemia show reduction in HR and infrequent onset of VT (8%) and VF (3%) [Perez-Gornez *et al* 1979]. Similarly, increase in HR during ECG monitoring precedes VF in patients with acute MI [Adgey *et al* 1982, McAreavey *et al* 1989].

Psychological stress is associated with increased propensity of sudden cardiac death [Brackett and Powell 1988, Kuller *et al* 1987, DeSilva 1982]. Socioenviromental stress and type A behaviour may be associated with long-term high sympathetic activity which reduces the electrical stability of the heart [Lown 1987, Dimsdale *et al* 1987]. Indeed, mental stress can induce discernable electrophysiological changes and ventricular arrhythmias in patients with uncomplicated MI [Lown 1987]. It is estimated that about 20-40% of SCD events were preceded by acute psychological disturbances [DeSilva 1982].

Detailed analysis of the frequency of SCD events or VT have disclosed a circadian rhythm with significant excessive episodes between 9-12 am [Willich 1990, Lucente *et al* 1988]. This diurnal pattern matches closely with the circadian variation of plasma concentration of catecholamines [Stone 1990, Tofler *et al* 1987], and is abolished by β-blockade [Muller *et al* 1987, Fox and Mulcahy 1990]. A simultaneous reduction in cardiac vagal tone may also be involved [Malpas and Purdie 1990].

The importance of sympathetic activity in the genesis of lethal arrhythmias is not limited to ischaemic conditions. In patients with the long-QT syndrome, cardiac sympathetic activity plays a key role in initiating VF and treatment with β-blockade reduces the incidence of SCD from 71% to 6% [Schwartz 1985]. Moreover, neurogenic disorders are associated with increased risk of SCD [Oppenheimer *et al* 1990]. For example, increased plasma concentration of catecholamines and CK-MB isoenzyme are common in head injury patients, who also show high incidence of ventricular arrhythmias and cardiac mortality [Cruickshank 1989]. Furthermore, during and shortly after vigorous exercise, ventricular arrhythmias and SCD may

happen in apparently healthy subjects [Amsterdam 1990], perhaps due to sympathetic overstimulation as a 10-fold increase in plasma NA level occurs during this vulnerable period [Dimsdale *et al* 1984].

Three lines of experimental evidence strongly support the concept that enhanced cardiac sympathetic activity is a trigger for ischaemic arrhythmias. Firstly, increased sympathetic nerve firing during myocardial ischaemia coincides with the reduction of VF threshold and the onset of VF [Lombardi *et al* 1983, Corr and Gillis 1978]. Secondly, electrical stimulation of central (hypothalamus) or cardiac sympathetic nerves reduces electrical stability of the ischaemic and non-ischaemic heart in several species [Corr and Gillis 1978, Schwartz *et al* 1976, 1985b, Johnasson *et al* 1974]. In dogs, left stellate ganglion stimulation reduces the VF threshold and triggers ventricular arrhythmias, which is in contrast to an antiarrhythmic effect by stimulation of the right stellate ganglion [Schwartz *et al* 1976, Priori *et al* 1988, Corr *et al* 1986]. This difference may be explained by findings that the afferent limbs of most cardio-cardiac sympathetic reflexes may be preferentially distributed through left sided nerves, hence making these reflexes largely dependent on an intact left stellate ganglion [Schwartz *et al* 1976]. Subjecting animals to psychological and physiological stress is also arrhythmogenic [Johnasson *et al* 1974, Verrier and Lown 1984]. Adaptation to these stressors reduces the vulnerability to arrhythmias during ischaemia [Parker *et al* 1987, Billman *et al* 1984, Meerson and Malyshev 1989]. Thirdly, adrenergic agonists increase the incidence of ventricular arrhythmias during ischaemia and reperfusion [Penny 1984, Yamada *et al* 1986, Kimura *et al* 1987, Sheridan *et al* 1980].

Effect of suppressed adrenergic activity

Large amount of studies demonstrate an antiarrhythmic effect by depressing sympathetic tone. Treatment with propranolol reduces the incidence of VF and myocardial damage in the acute phase of MI [Norris *et al* 1984, Peter *et al* 1978]. In patients after acute MI, treatment with β-adrenergic blockers achieves an antiarrhythmic effect with a 33% reduction in mortality due to SCD [Frishman *et al* 1984]. This salutary impact contrasts with the ineffectiveness of class I antiarrhythmic drugs in similar subjects [Furberg 1983].

In experimental studies, α- and β-blockade are effective in reducing the incidence of ventricular arrhythmias either during ischaemia or reperfusion [Penny 1984, Schwartz *et al* 1985, Benfey *et al* 1984, Sheridan *et al* 1980], although there is some controversy [Williams *et al* 1987, Daugherty *et al* 1986]. Adrenergic antagonist may be able to delay the onset of irreversible damage or limit the infarct size [Elson *et al*

1981, Thandroyen *et al* 1990, Opie 1980], thereby providing another explanation for the antiarrhythmic effect. An extreme way to attenuate adrenergic tone is sympathetic denervation either surgically or chemically (pretreatment with 6-hydroxydopamine or α-methylmetatyrosine) with a substantial reduction of cardiac NA content (<10% of normal). Such treatment is associated with a reduction in the severity of ischaemic/reperfusion arrhythmias and ischaemic damage of myocardium [Martin and Meesmann 1985, Abrahamsson 1985, Jones *et al* 1978, Schwartz and Zuanetti 1988]. In dogs, chronic left stellectomy increases VF threshold and is highly protective against ventricular arrhythmias, while right stellectomy may decrease the electrical stability of non-ischaemic and ischaemic hearts [Schwartz *et al* 1976, Corr *et al* 1986]. However, others have reported both right and left stellectomy to be effective in preventing VF during ischaemia in dogs [Puddu *et al* 1988]. In patients after acute MI, left stellectomy achieved significant reduction in the incidence of SCD over 5-year follow-up from 22% to 3.6% [Schwartz *et al* 1985]. In contrast, acute surgical sympathectomy is usually less potent or ineffective [Schwartz et el 1976, Elson *et al* 1981, Euler *et al* 1985]. This may indicate the importance of myocardial catecholamine levels and/or associated changes, such as an increased glycogen content [Daugherty *et al* 1986] in arrhythmogenesis during ischaemia.

Effect of parasympathetic nerve system

Hypotension and bradycardia appearing during acute MI may coexist with ventricular arrhythmias [Webb *et al* 1972, Epstein *et al* 1972]. However, those patients showing signs of vagal activation usually have fewer ventricular arrhythmias and better prognosis [Epstein *et al* 1972, Perez-Gomez *et al* 1979, Neufeld *et al* 1978]. Actually, administration of atropine for the treatment of bradycardia and hypotension may unmask and enhance the effect of underlying sympathetic activation, leading to the development of serious arrhythmias [Pantridge 1978, Epstein *et al* 1972].

Vagal control of the heart may be assessed by measurement of R-R interval variation or baroreflex sensitivity [Schwartz *et al* 1988a]. Those patients with the greatest reduction in vagal activity, defined in the acute phase of MI, are at increased risk of SCD [Kleiger *et al* 1987, La Rovere *et al* 1988]. In postinfarct patients chronic exercise programmes may reduce the risk of ventricular arrhythmias and improve prognosis [Furberg and Craver 1988], an effect possibly due to an improved vagal tone as show in dogs [Billman *et al* 1984].

Experimental results also suggest a protective effect of enhanced vagal tone during MI. In dogs with a healed MI, vagal activity assessed by baroreflex sensitivity

was inversely related to the incidence of SCD triggered by exercise and acute ischaemia (Figure 1.4) [Schwartz *et al* 1988a]. In conscious dogs exposed to an adverse environment, vagal blockade by atropine leads to a substantial increase in vulnerability to VF [Verrier and Lown 1984]. During ischaemia vagal activation, either by electrical nerve stimulation or by enhanced baroreflex, attenuates ischaemia-induced depression in VF threshold [de Ferrari 1987, Verrier 1988, Marshall *et al* 1981] and VF is delayed in onset or prevented [Zuanetti *et al* 1987, Marshall *et al* 1981, Myers *et al* 1974]. Enhanced cardiac vagal tone via baroreflex activation could terminate sustained VT [Wexman and Wald 1977]. Likewise, vagal tonic drugs (cholinesterase inhibitors, cholinergic agonists or enzyme-resistant cGMP analogues) are capable of increasing VF threshold and reducing the incidence of ventricular arrhythmias [Billman 1989, Hohnloser *et al* 1986, Das and Bhattacharya 1972].

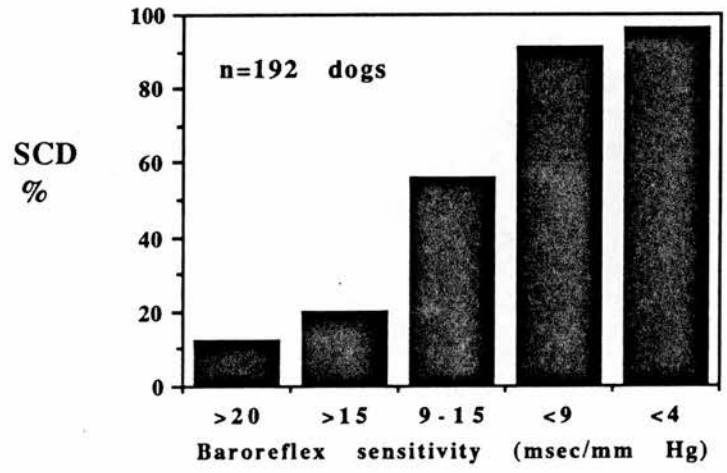


Figure 1.4 *Baroreflex sensitivity and sudden cardiac death in dogs after myocardial infarction (p<0.001 for differences among the groups) [Schwartz et al 1988a]*

Effect of vagotomy on ischaemic/reperfusion arrhythmias is uncertain [Schwartz and Zuanetti 1988, Corr *et al* 1986]. It seems that the resultant influence of vagotomy depends on the preexisting level of vagal tone and sympathetic activity [Verrier and Lown 1984, Corr *et al* 1987, Bergamaschi 1978]. In the cat, coronary occlusion results in a marked vagal activation and vagotomy leads to an increased incidence of VF. In contrast, in conscious dogs with anterior ischaemia vagotomy is ineffective as efferent vagal tone is not increased [Verrier 1988, Corr *et al* 1986].

However, there are some studies which did not find a protective effect of vagal stimulation during myocardial ischaemia [Corr and Gillis 1978, Yoon *et al* 1977, James *et al* 1977]. Some studies even showed a deleterious effect by cholinergic stimulation [Bergamaschi 1978, Scherlag *et al* 1982]. It seems that vagally-mediated

HR reduction *per se* may partly explain these conflicting results [Verrier 1988, James *et al* 1977]. For instance, in dogs with myocardial ischaemia, vagal nerve stimulation or vagomimetic drugs only provides partial protection if HR is kept constant by pacing [Verrier 1988]. Detrimental effect of excessive vagal activation may be owing to resultant hypotension which further reduces blood supply to the ischaemic heart, and to bradycardia which favours arrhythmias due to enhanced automaticity.

1.2.3 Neuronal Influence on Electrophysiology of Ischaemic Myocardium

The electrophysiological substrate for arrhythmogenesis in the setting of ischaemia has been reviewed [Janse and Kléber 1981, Surawicz 1985]. Briefly, they are 1) slow conduction of electrical impulses in the ischaemic region due to reduced action potential and damaged cell-cell coupling; 2) unidirectional block as a result of dispersion in refractoriness and reexitability; and 3) enhanced automaticity partly due to the late-potential-triggered activation. All these aspects are under adrenergic and cholinergic influences.

The electrophysiological effects of adrenergic stimulation in the heart are mediated mainly through β-adrenoceptors. These effects include 1) accelerated diastolic depolarization of sinus cells or potential pacemaker fibres; 2) increased conduction velocity; 3) decreased refractoriness in the A-V junction and His-bundle; and 4) shortened action potential duration of Purkinje fibres and working myocytes. During myocardial ischaemia, β-adrenergic stimulation activates the slow inward current (I_{si}) and decreases exitatory threshold, thereby maintaining the slow conduction [Spinelli and Rosen 1989]. Both changes are in favour of the establishment and maintenance of reentry [Surawicz 1985]. β-Stimulation may also induce and augment late-potentials and automaticity of Purkinje fibres in ischaemic regions *via* an enhanced Ca^{2+} influx [Han and Cameron 1985, Kimura *et al* 1987, Wit and Rosen 1986]. Purkinje fibres within the infarcted zone are more resistant than working myocytes to ischaemia [Cox *et al* 1973, Rossi 1985, Le Marec *et al* 1985], and may show diastolic depolarization and automaticity with an enhanced sensitivity to adrenergic stimulation [Han and Cameron 1985]. Another possibility linking sympathetic activation to the arrhythmogenesis is the hypokaelemic effect of β-adrenergic stimulation [Verrier 1988]. It is well known that hypokalemia increases the ventricular vulnerability to arrhythmias both clinically and experimentally [Nordrehaug and Vander-Lippe 1983, Curtis and Hearse 1989].

In the normal heart, stimulation of α-adrenoceptors opposes the effects of β-stimulation and therefore is antiarrhythmic. During ischaemia, however, α-agonists

enhance inward Na^+ and Ca^{2+} currents, hence accelerate the speed of phase-4 spontaneous depolarization. α-Stimulation may also increase the magnitude of after-depolarization and the resultant triggered-activity [Yamada *et al* 1988]. This may be partly due to the increased α_1-adrenoceptor numbers in ischaemic myocardium [Corr *et al* 1981]. During reperfusion α- but not β-adrenoceptors contribute to the arrhythmogenesis [Penny 1984, Sheridan *et al* 1980, Corr *et al* 1986].

Vagal activation shows profound effect on supraventricular tissues and conduction system. Stimulation of muscarinic receptors decreases slow inward Ca^{2+} and Na^+ currents and increases outward K^+ conductance. As a result, acetylcholine (ACh) exerts a negative chronotropic effect in SA and AV node cells and results in a reduced conduction in the AV junction. In ventricular myocardium, direct electrophysiological effects of vagal stimulation are modest. This is in keeping with a sparse cholinergic innervation of the ventricles [Löffelholz and Pappano 1985]. However, in the presence of an enhanced adrenergic activity, vagal stimulation or ACh antagonizes electrophysiological effects of β-adrenergic stimulation on Purkinje fibres and ventricular myocytes [Corr *et al* 1987]. In intact dogs, vagal nerve stimulation alone or in the presence of β-blockade has no effect on VF threshold. When sympathetic activity is augmented by thoracotomy or by adrenergic stimulation, a definite antiarrhythmic effect is demonstrated [Kent 1982, Verrier and Lown 1978]. It is believed that the protective influence of vagal tone during ischaemia is largely mediated through its anti-adrenergic action, e.g., depressing or abolishing I_{si}, lengthening the effective refractory period, and inhibiting automaticity [Spinelli and Rosen 1989, Corr *et al* 1986]. Speculatively, this may be due to the presynaptic inhibition of NA release and modulation of intracellular cAMP/cGMP production [Löffelholz and Pappano 1985, Levy 1984, Corr *et al* 1986, Thandroyen *et al* 1983].

Part of vagal protection against arrhythmias is mediated by a HR-lowering effect. In normal and ischaemic dog hearts, VF threshold is inversely related to HR [James *et al* 1977]. Although bradycardia may lengthen the vulnerable period and increase the appearance of VPB by the removal of overdrive [Scherlag *et al* 1982], the contribution of these effects to the onset of VF is considered to be small [Epstein *et al* 1972].

Sympathetic activation exacerbates ischaemia-induced metabolic derangement. Increase in heart rate and contractility further deteriorates the relation of energy supply/demand. Enhanced lipolysis of endogenous triglycerides may initiate an energy wasting cycle [Riemersma 1986, Grong *et al* 1986]. Accumulation of lactate, H^+, loss of intracellular K^+, and increased circulating free fatty acids, are also more

marked under adrenergic stimulation [Opie 1985, Knopf *et al* 1988, Gettes *et al* 1986]. All these metabolic factors could interfere with the electrical stability of the ischaemic heart. In contrast, vagal activation is expected to antagonize those changes at different levels.

1.2.4 Autonomic Imbalance and Ischaemic Arrhythmias

It has been well known that inhomogenous changes in electrophysiology, i.e., desynchronized activation and dispersed refractoriness, are critical in the genesis of VF [Surawicz 1985]. If the autonomic influences on these electrophysiological substrates are heterogeneous, the heart would be vulnerable to fibrillate. This possibility has recently gained considerable attention [Inouc and Zipes 1988, Zipes 1990, Gettes *et al* 1986, Verrier 1988, Schwartz *et al* 1988a].

The electrical stability of the heart is influenced by sympatho-parasympathetic interactions which occur at different levels. Impulses from afferent sympathetic and vagal neurons are centrally integrated, a process influenced by high neuronal activities. At the level of the heart, sympathetic and vagal outputs exert opposite effects. The final net response is not simply an algebraic one [Levy 1984]. Presynaptically, ACh released from vagal nerves diminishes NA release from neighbouring sympathetic terminals, and the converse is also true (*vide infra*). Moreover, interactions also take place at the levels of postsynaptic receptors and intracellular biochemical consequences. [Roeske and Yamamura 1983, Levy 1984]. It is believed that the local interactions are important in ischaemic arrhythmogenesis [Corr *et al* 1986, Zipes *et al* 1987], but this aspect has been studied little.

In many cases, sympathetic and parasympathetic nerves are activated simultaneously during myocardial ischaemia and stressful conditions. Disturbing this balance through vagal efferent blockade by atropine or vagotomy may result in a substantial increase in the incidence of arrhythmias [Verrier and Lown 1984, Corr *et al* 1987, Schwartz and Stramba-Badiale 1988]. In contrast, left sided sympathectomy is protective [Schwartz *et al* 1976, Puddu *et al* 1988], as a relatively vagal-dominant seems to be antiarrhythmic [Schwartz *et al* 1988a, Kleiger *et al* 1987]. Indeed, a sudden autonomic imbalance, due either to withdrawal of vagal tone and/or increase in sympathetic output, precedes VF [McAreavey *et al* 1986, Perez-Gornez *et al* 1979, Adgey *et al* 1982, Schwartz *et al* 1988a]. Intracardiac sympathetic and vagal nerves differ in their routes through the ventricular wall [Barber *et al* 1985, Zipes 1990]. Thus, selective myocardial damage to epi- or endo-layers of the ventricular wall may preferentially interrupt one of the autonomic afferent and efferent limbs.

Asymmetry of sympathetic tone during early ischaemia may also occur due to several mechanisms, such as uneven adrenergic innervation [Pierpont *et al* 1984], perineuronal inflammation [Rossi 1985, James 1983], ischaemia-induced regional denervation and resultant changes in adrenergic sensitivity [Zipes 1990, Inoue and Zipes 1987], nonuniform nerve discharge [Lathers *et al* 1978, Neely and Hageman 1990], or selective increase in adrenoceptor numbers [Corr *et al* 1981, Mukherjee *et al* 1982]. It is possible that the antiarrhythmic effects of adrenergic blockade are achieved by a uniform decrease in adrenergic stimulation, thereby preventing the manifestation of uneven neuronal influence.

It is widely assumed that an enhanced catecholamine release in ischaemic myocardium mediates the arrhythmogenic influence of sympathetic activation [Corr *et al* 1986, Schömig 1989]. Prior to a detailed description of local NA release in myocardial ischaemia and the working hypothesis of the project, a brief review of cardiac neurotransmission and its presynaptic modulation will be presented.

1.3 Neurotransmission in the Heart

1.3.1 Innervation of the Heart

The heart receives dual sympathetic and parasympathetic innervation and its function is under constant neuronal control.

The sympathetic efferent limb passes through intermediolateral columns of the upper segment of spinal cord and synapses with preganglionic cardiac sympathetic axons in the lateral grey columns. Preganglionic axons form synapses in the cervical-thoracic ganglia. The inferior cervical and the first thoracic ganglia are fused together forming the stellate ganglion. Cardiac nerves, usually three bundles, arise from these ganglia on each side and form the cardiac plexus at the base of the heart. After leaving the plexus postganglionic sympathetic axons run parallel to the coronary arteries. The SA node and atria are mainly innervated by axons from right sympathetic nerves. In the other parts of the heart, left sided sympathetic innervation is dominant. The density of innervation in atria is about twice of that in the ventricle [Levy and Martin 1979, Pierpont *et al* 1984]. The sympathetic innervation in ventricles is inhomogeneous with a decreasing gradient from base to apex [Pierpont *et al* 1984].

The parasympathetic innervation of the heart originates from the dorsal vagal nuclei in the medulla and forms the cardiac branches on each side. Some axons synapse with postganglionic neurons in the cardiac vagal plexus. Others continue through the plexus and form synapses with postganglionic neurons mainly located within the atrial wall [Löffelholz and Pappano 1985]. The postganglionic neurons give rise to short axons supplying the SA node, atrial myocardium and AV junction. Histological and functional studies demonstrate the presence of ventricular ganglia, although few in numbers, and cholinergic innervation of the His bundle and bundle branches [Levy 1984, Zipes 1990]. Acetylcholine (ACh), choline acetyltransferase and muscarinic receptors all exist in the ventricular myocardium. Vagal nerve stimulation has definite effects on ventricular electrophysiology and contractility, especially when sympathetic tone is high [Levy 1984, Verrier 1988].

1.3.2 Adrenergic Neurotransmission

Details of sympathetic neurotransmission has been reviewed extensively [Philippu and Matthaei 1988, Knight *et al* 1989], and will be summarised here briefly.

Synthesis and storage of NA

In adrenergic neurons, enzymes catalysing the formation of NA are synthesized in the perinuclear region of the cell body and conveyed along the axons to the nerve terminals, where NA synthesis takes place. The starting point, and also the rate-limiting step, is hydroxylation of tyrosine by tyrosine hydroxylase. The formed dopa is rapidly transformed into dopamine by dopa decarboxylase. Dopamine is then transported into storage vesicles by a carrier. In the vesicle dopamine-β-hydroxylase (DBH) catalyses the conversion of dopamine to NA [Philippu and Matthaei 1988].

The storage vesicles contain a very high concentration of NA (>0.6 M) and ATP, together with proteins (e.g. DBH, chromogranin A, calmodulin, synapsin I) and various peptides. It is believed that NA may be bound to ATP resulting in a complex without electrical charge. Chromogranins act as a matrix for the aggregation of NA and ATP to form a gel. Thus, osmolarity of the soluble components and the concentration of "free NA" in the vesicles are low. There is a vesicular NA uptake process, which plays a major role in accumulation and retention of NA from axoplasm. Through the activity of Mg^{2+}-dependent H^+-ATPase located in the vesicular membrane, protons are actively accumulated within the storage vesicle at a concentration of as high as 10 mM, thereby creating a transmembrane H^+ electrochemical potential of about 200 mV. The proton potential is the driving force

of vesicular NA uptake. NA inward transport is coupled with proton extrusion through a special carrier which is sensitive to reserpine-like agents. As a result, most NA reuptaken from the synaptic cleft (*vide infra*) is stored in vesicles for further release (Figure 1.5). This "pH-dependent trapping mechanism" keeps a very low axoplasmic concentration of NA [Philippu and Matthaei 1988].

Exocytotic release

When an action potential is conducted to the nerve terminal by voltage sensitive Na^+ channels, depolarization of the neuronal membrane opens voltage-sensitive Ca^{2+} channels and hence leads to an increase in intracellular Ca^{2+} concentration of approximately 1 μM. Through the activation of Ca^{2+} sensitive proteins, this increased Ca^{2+} facilitates vesicles approaching to and fusing with the presynaptic membrane and spilling their contents into the synaptic cleft. ATP is required perhaps for vesicular movement and to reestablish the ionic distribution afterwards. Together with the release of NA, some co-transmitters like neuropeptide Y, ATP and proteins are also expelled into the synaptic cleft (Figure 1.5) [Knight *et al* 1989].

Exocytotic NA release is 1) calcium and energy dependent, 2) accompanied with co-transmitter release, and 3) modulated by presynaptic mechanisms (*vide infra*) [Knight *et al* 1989].

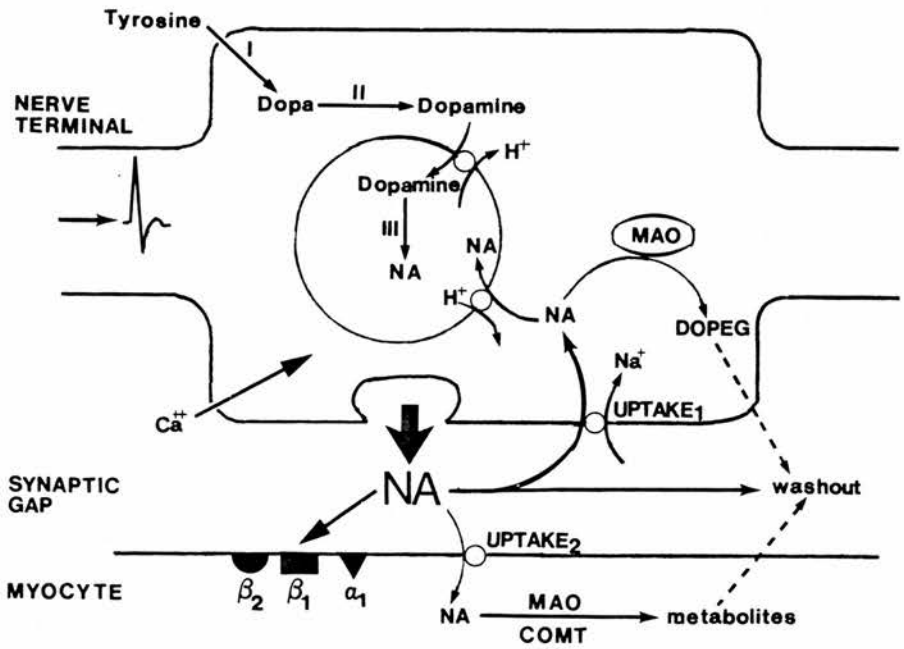


Figure 1.5 *Schematic illustration of NA synthesis, storage, exocytotic release and clearance. I = Tyrosine hydroxylase, II = Dopa decarboxylase, III = Dopamine-β-hydroxylase, MAO=Monoamineoxydase , COMT=Catechol-O- methyltranferase*

Clearance of NA released

Neuronal uptake ($uptake_1$) plays a central role in the clearance of NA from the synaptic cleft [Goldstein *et al* 1988, Iverson 1973]. This active NA transport is energetically dependent on the electrochemical Na^+ gradient (outside >> inside) created by Na^+-ATPase which keeps axoplasmatic concentration of Na^+ low. In the heart, most NA released is uptaken through $uptake_1$ carrier and then returned to the vesicles [Goldstein *et al* 1988, Dart *et al* 1984a]. Only a small fraction of NA, when it is free in the axoplasm, is metabolized by monoamine oxidase (MAO) into inactive 3,4-dihydroxyphenylethylglycol (DOPEG). $Uptake_1$ carrier has a high affinity for NA (Km=0.27 μM) and for some other sympathomimetic amines [Iversen 1973]. As a result, adrenaline (1-2% of total catecholamines) is also stored in the vesicle and released during nerve stimulation. Inhibition of $uptake_1$ process with antidepressants such as desipramine leads to an enhanced NA overflow and increased chronotropic response during sympathetic nerve stimulation [Dart *et al* 1983, Docherty and McGrath 1980]. In the rat heart, desipramine at the concentration of 0.01 μM leads to 50% inhibition of the $uptake_1$ [Iversen 1973].

Released NA may also be uptaken by myocytes through a second uptake mechanism, which is sensitive to corticosteroids but not to desipramine [Iversen 1973]. NA transported into cells is exposed to the action of MAO and catechol-O-methyltransferase (COMT) and transformed into inactive metabolities, such as DOPEG and normetanephrine. However, compared with neuronal $uptake_1$, extraneuronal uptake has a low affinity for NA (K_m=252 μM) and is of minor importance [Iverson 1973, Dart *et al* 1984b, Goldstein *et al* 1988]. In addition, part of the released NA is removed from the synaptic cleft by washout [Dart *et al* 1983].

Postsynaptic effects of NA

The mechanisms of β- and α-adrenergic signal transmission have been studied in great detail and reviewed recently [Berridge 1988, Casey and Gilman 1988, Stiles 1989, Katz 1990, Brown and Birnbaumer 1988].

All 4 subtypes of adrenoceptors coexist in the heart. Three of them, β_1-, β_2- and α_2-receptors, are coupled to the adenylate cyclase complex, which generates the second messenger 3',5'-cyclic adenosine monophosphate (cAMP). Adenylate cyclase complex is composed of at least three distinct protein components: receptors, which recognize and bind ligands and interact with the second component of the complex - a guanine nucleotide binding protein (G protein), that either stimulate (G_s) or inhibit (G_i) the next component - adenylate cyclase. G-proteins are heterotrimers comprising three separate gene products termed α, β, and γ subunits. The β and γ subunits are

identical for both G_s and G_i, while the α subunits differ. α subunits contain guanine nucleotide-binding site and GTPase activity. Binding of NA with β_1- and β_2-receptors leads to activation of G_s with a replacement of GDP by GTP . The α subunit then dissociates from the β and γ subunits. The free $G_{s\alpha}$-GTP activates the catalytic subunit of adenylate cyclase, thereby enhancing the production of cAMP. cAMP in turn, binds to protein kinase (PK) resulting in dissociation of the regulatory subunits from the catalytic subunits, thereby activating the kinase. cAMP-dependent PK catalyses the transfer of phosphate groups from ATP to specific sites on intracellular proteins whose functions are being regulated. A number of proteins are targets of cAMP-dependent PK, including Ca^{2+} and K^+ channels, sarcolemmal proteins like phospholamban, contractile proteins, and metabolic enzymes responsible for glycogenolysis and lipolysis. These changes enhance Ca^{2+} influx, uptake and release leading to increased myocardial contractility and relaxation. Meanwhile, ATP production is stimulated to meet the needs of the increased energy demand. Recent studies have demonstrated that activation of G_s is able to regulate Ca^{2+}, K^+ and Na^+ ionic channels independent of cAMP pathways [Brown and Birnbaumer 1988]. The whole process is terminated by converting of $G_{s\alpha}$-GTP into $G_{s\alpha}$-GDP *via* the intrinsic GTPase activity and inactivation of cAMP by a phosphodiesterase to 5'-AMP. In addition, a tonic inhibition of adenylate cyclase comes from G_i. Stimulation of several receptors, such as α_2-adrenoceptors, muscarinic cholinoceptors, or adenosine purinoceptors, results in the dissociation of $G_{i\alpha}$ from its $\beta\gamma$ subunits, allowing the $\beta\gamma$ subunits to combine with the free $G_{s\alpha}$, thereby inactivating $G_{s\alpha}$.

Stimulation of the α-receptor leads to, *via* an unidentified G protein, enhanced phosphatidylinositol-4,5-bisphosphate (PIP_2) metabolism and turnover with the consequent release of two second messengers, diacylglycerol (DAG) and myoinositol-1,4,5-trisphosphate (IP_3) into the intracellular milieu. IP_3 mobilizes endogenous Ca^{2+} stores, leading to the enhanced contraction of myocardium and vascular smooth muscle. DAG activates protein kinase C, which increases both extracellular Ca^{2+} influx and endogenous Ca^{2+} release from sarcoplasmic reticulum [Berridge 1988].

1.3.3 Cholinergic Neurotransmission

ACh synthesis and storage

ACh is synthesized from choline and acetylcoenzyme A (AcCoA) in the axoplasm of cholinergic nerve terminals. This reaction is catalysed by the choline-acetyltransferase (ChAT). AcCoA is originally synthesized in the mitochondria and

translocated to axoplasm. The other substrate, choline, is taken up from the extracellular space (Figure 1.6). Choline is a charged polar molecule and can not freely cross the cellular membrane. Cholinergic nerve terminals possess a high-affinity choline uptake (HACU) carrier with a K_m of 1-5 μM [Ducis 1988] and choline required for ACh synthesis is almost entirely transported by this carrier. This is the rate limiting step for ACh synthesis. Inhibition of HACU carrier by 3-hemicholinium leads to a parallel decrease in the rate of ACh synthesis and release. Conversely, stimulation of vagal nerves results in an immediate increase in choline transport. HACU carrier does not require energy directly but is highly dependent on Na^+-transport. It also exhibits some sensitivity to K^+ and Cl^- ions. ACh synthesis *per se* is a rather simple process and is capable of supporting a very high rate of synaptic release. This is important as unlike NA, ACh itself is not recycled [Ducis 1988].

Each nerve varicosity contains thousands of vesicles and about 80% of all ACh is stored within them. Concentration of ACh inside the vesicle (500-800 mM) is much higher than that in the axoplasm (0.3 mM). The vesicular membrane contains a bicarbonate stimulated Ca^{2+}- or Mg^{2+}-ATPase that drives the active transport of ACh. Similar to NA storage, ACh transport into vesicles is dependent on a proton gradient (inside pH=5.2-5.5) generated by an ATPase (Figure 1.5) [Zimmermann 1988].

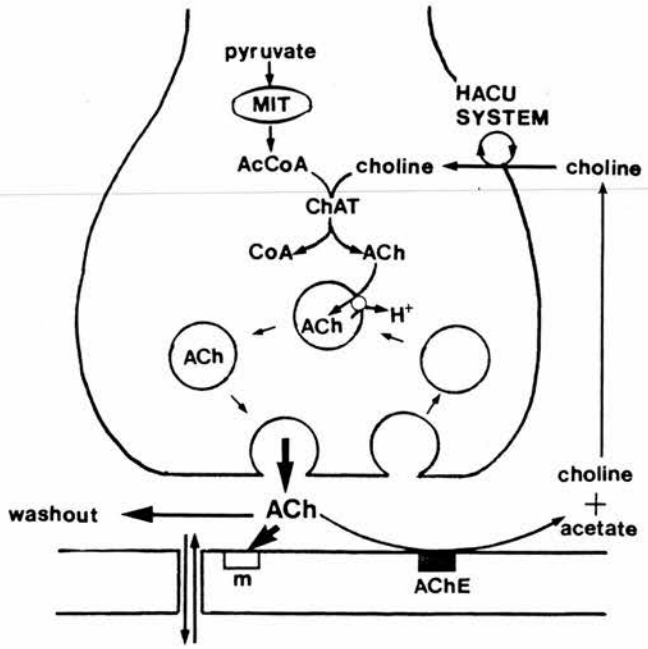


Figure 1.6 *ACh synthesis, storage, exocytotic release and clearance in cholinergic nerve terminals*

ACh release and inactivation

Exocytotic release of ACh occurs when an action potential reaches the terminals and triggeres sufficient influx of Ca^{2+} to "destabilize" the storage vesicle. After vesicles fusing with the presynaptic membrane, ACh and co-transmitters (ATP, peptides and proteoglycan) are extruded into the synaptic cleft [Kilbinger 1988].

ACh diffuses to receptors and also comes in contact with acetylcholinesterase (AChE) located at the cellular surface, where ACh is hydrolysed. Inhibition of AChE by agents like physostigmine raises the overflow of ACh by a factor of 2-3 [Dieterich *et al* 1978, Richardson and Szerb 1974]. The rate of ACh hydrolysis in the heart, however, is much slower than that at neuromotor endplates. Therefore, washout from the synaptic cleft is another important mechanism to terminate the action of ACh. Indeed, in the perfused heart a large fraction of ACh appears in the coronary effluent during nerve stimulation [Löffelholz and Pappano 1985]. About 50% of choline from hydrolyzed ACh is reuptaken for ACh resythesis. Blocking the HACU carrier during nerve stimulation causes enhanced overflow of free choline [Löffelhoz *et al* 1984].

Postsynaptic effects of ACh

Myocardial cholinergic receptors belong to muscarinic$_2$ (M_2) subtype. There are several molecular mechanisms by which cholinergic signal transmission is achieved. Binding of ACh to M_2-receptors may directly activate an inward potassium current (I_{ki}) in sinus and AV node cells. Actually, the M_2-receptor may be an integral part of a K^+-specific membrane channel (ACh-dependent K^+-channel). Therefore, this process is independent of a second messenger. Meanwhile, stimulation of M_2-receptors activates an inhibitory guanine-nucleotide-binding inhibitory protein (G_i), thereby decreasing AC activity and cAMP production. Furthermore, the guanylate cyclase may be activated by binding of ACh to M_2-receptors and thus intracellular cGMP level is increased. The overall effect is that cellular K^+ conductance is increased and Ca^{2+} influx is inhibited [Kilbinger 1988, Watanabe 1984]. Studies have shown a positive correlation between the accumulation of cGMP and the negative inotropic effect of either ACh or vagal nerve stimulation. Furthermore, it is proposed that stimulation of M_2-receptors may activate breakdown of phosphatidylinositol and thus produce IP_3 and diacylglycerol. This is probably responsible for a "rebound" positive inotropic effect after vagal nerve stimulation [Löffelholz and Pappano, 1985]. Vasoactive intestinal polypeptide (VIP) is present in parasympathtic neurones as a co-transmitter with ACh. At high frequency of vagal nerve stimulation, the released VIP mediates atropine-resistant vasodilatation, tachycardia and presynaptic inhibition of ACh release [Hill *et al* 1990, Rand *et al* 1987, Fahrenkrug 1989].

1.3.4 Presynaptic Modulation of Neurotransmitter Release

Concept of presynaptic modulation

Since the first description of enhanced NA overflow by α-adrenergic blockade in 1957 by Brown and Gillespie, the concept of presynaptic receptors which modulate neurotransmitter release is generally accepted. Although efforts have been made to identify and characterize the presynaptic receptors, especially those that regulate NA release in the heart [Rand *et al* 1987, Sharma and Banerjee 1978], much of our knowledge about this aspect comes from pharmacological studies.

Neurotransmitter release may be affected by a series of substances via different pathways. Presynaptic modulation can be classified into four types according to the site of origin of these modulators (Figure 1.7). 1) Automodulation: transmitter released may interact with presynaptic receptors thereby enhance or inhibit further release of the same transmitter; 2) Axo-axonal modulation: a transmitter release may be modulated by a variety of substances released from adjacent neurons of a different type; 3) Trans-synaptic modulation: after being stimulated by a neurotransmitter postsynaptic tissues release a substance which crosses the synaptic cleft and acts on relevant presynaptic receptors; and 4) Substances brought to nerve terminals *via* the circulation and then acting on relevant presynaptic receptors.

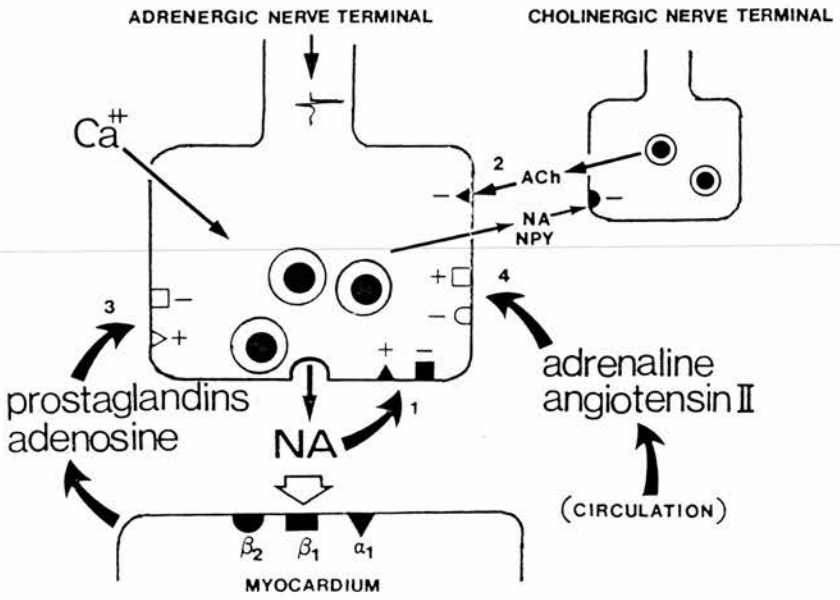


Figure 1.7 *Schematic diagram of presynaptic modulation of NA release. The numbers correspond to the types described in the text.* + facilitatory - inhibitory

Presynaptic receptors modulating neuronal NA and ACh release from peripheral nerve terminals in the heart are listed in Table 1.1 [Fuder 1985, Langer 1981, Kilbinger 1988].

Table 1.1 *Presynaptic receptors activating (+) or inhibiting (-) sympathetic and parasympathetic neurotransmitter release from the heart. ? = no data*

Receptors	Neurotransmitter	
	NA	ACh
α-Adrenoceptors (α_2)	-	-
β-Adrenoceptors (β_2)	+	+
Dopamine receptors (DA_2)	-	?
Muscarinic receptors (M_2)	-	-
Nicotinic receptor	+	?
Purinergic receptors (A_1)	-	-
Prostaglandin receptors	-	?
Histamine receptors (H_2)	-	?
Serotonin receptors (5-HT_1)	+/-	+
Angiotensin II receptors	+	?
Opioid receptors	-	-
Neuropeptide Y receptors	-	-

Automodulation

Feedback inhibition of NA release mediated by presynaptic α_2-adrenoceptors has been well defined. Activation of presynaptic α_2-receptors by NA released suppresses further release of NA in the *in vivo* and *in vitro* heart or atria. α_2-Antagonists could block this inhibition, hence leading to an enhanced release of NA and of large molecular substances like dopamine-β-hydroxylase, chromogranin and neuropeptide Y [Starke 1987, Haass and Schömig 1989]. The operation of this negative feedback mechanism requires a threshold concentration of NA in the synaptic cleft. When the neuronal $uptake_1$ is inhibited, this negative feedback mechanism is enhanced apparently due to an increased NA level in the synaptic cleft [Langer 1981]. The magnitude in reduction of NA by way of α_2-agonist stimulation is more pronounced at low frequency nerve stimulation and disappears at high frequencies [Medgett *et al* 1978, Starke 1987]. On the other hand, the efficacy of α_2-antagonist in increasing NA release is related to stimulation frequency until NA levels are high enough to saturate

all presynaptic α_2-receptors [Fuder *et al* 1984, Langer 1981, St arke 1987]. The mechanism of this autoinhibition is due to G_i-mediated inhibition of Ca^{2+}-dependent intra-axonal mechanisms leading to NA release [Rand 1987]. There have been some studies which suggest the presence of inhibitory α_1-receptors on the adrenergic nerve endings [Strarke 1987, Story *et al* 1985]. Recently, 3 subtypes of α_2-adrenoceptors (α_{2A}, α_{2B} and α_{2C}) have been described based on pharmacological and molecular biological evidence [Bylund 1988]. The implication of these subtype α_2-receptors is to be studied.

The co-transmitter neuropeptide Y (NPY) can stimulate presynaptic NPY-receptors thereby inhibiting the release of NA as well as NPY itself [Potter 1987, Haass and Schömig 1989].

At a low frequency of nerve stimulation, evoked NA release may be facilitated through activation of presynaptic β-adrenoceptors [Yamaguchi *et al* 1977]. Isoprenaline enhances NA release from the heart *in vitro* and *in vivo*. In the presence of propranolol neuronal NA release is slightly reduced [Langer 1981]. It is believed that facilitatory β-receptors are mainly activated by circulating adrenaline [Adler-Graschinsky and Langer 1975, Langer 1981]. The magnitude of this facilitation varies and some studies find that it is rather small or undetectable [Fuder 1985, Cousineau *et al* 1984, Dart *et al* 1984b].

A similar inhibitory feedback mediated by presynaptic muscarinic receptors also exists for cholinergic neurotransmission in the heart. Atropine enhances and ACh or methacholine, an ACh analogue, inhibits vagal stimulation-induced ACh release in the *in vitro* heart [Löffelholz *et al* 1984]. Meanwhile, the co-transmitter VIP may inhibit ACh release presynaptically [Fahrenkrug 1989].

Axo-axonal modulation

Reciprocal modulation between sympathetic and parasympathetic nerves is the most important axo-axonal modulation in the heart. In the *in vivo* and *in vitro* heart, exocytotic NA release can be inhibited by vagal nerve stimulation or ACh infusion [Fuder *et al* 1985, Wetzel and Brown 1985, Levy 1984, Lavallée *et al* 1978]. Preservation of ACh in the biophase by cholinesterase inhibitors leads to enhanced inhibition of NA release [Fuder *et al* 1985]. Similar inhibitory effects can be achieved by using muscarinic agonists like methacholine. Pharmacological and ligand binding studies suggest that pre- and post-synaptic M-receptors are similar.

A number of studies have shown that α_2-adrenergic agonists may also inhibit exocytotic ACh release in the heart [Rand *et al* 1987]. The presynaptic α-receptors mediating this inhibition are α_2-subtype, although presynaptic α_1-receptors may also

be involved [Wetzel *et al* 1985, McGrattan *et al* 1987]. However, there has apparently been no study to demonstrate that endogenous NA released by sympathetic nerve stimulation modulates ACh release from adjacent vagal terminals. In the *in vivo* dog, the influence of the vagus on R-R intervals is attenuated by sympathetic nerve stimulation. This inhibition is mainly mediated by a co-transmitter NPY, not by presynaptic α-adrenoceptors, because the effect remains in the presence of α-antagonists and is mimicked by exogenous NPY [Hall and Potter 1990, Warner and Levy 1989, Potter 1987]. Therefore, these results question the role of α-receptor-mediated inhibition of ACh release during sympathetic nerve stimulation. However, the release of transmitters involved was not directly measured in these studies. Inhibition of ACh exocytosis by NPY in the heart has not been demonstrated.

Trans-synaptic and other modulations

Sympathetic stimulation of the heart is accompanied by increased production of adenosine secondary to an enhanced degradation of adenosine phosphates [Headrick *et al* 1989]. Adenosine can inhibit NA release through activation of A_1-presynaptic receptors, an effect blocked by A_1-receptor antagonist such as 8-phenyltheophylline [Fuder 1985, Richardt *et al* 1989, Rand *et al* 1987]. Under basal conditions, the biophase level of adenosine in the heart is far below the threshold necessary to inhibit NA release (about μM level). However, under conditions of enhanced adrenergic stimulation, myocardial hypoxia or ischaemia, extracellular concentrations of adenosine are high enough to exert this inhibition [Edlund *et al* 1983, Richardt *et al* 1987 and 1989, Headrick *et al* 1989]. This trans-synaptic modulation provides an important feedback control, thereby attenuating the intensity of adrenergic stimulation when myocardial energy supply/demand relation is compromised. ACh release is similarly inhibited by adenosine in other organs [Dunér-Engström and Fredholm 1988, Gustafsson *et al* 1978]. Therefore, it may be an alternative and indirect way by which sympathetic nerve activation affects ACh release in the heart.

Membrane phospholipase-A_2 is also activated by the adrenergic stimulation. This is accompanied by an increased lipolysis of membrane phospholipids and subsequent prostaglandin (PG) production. In the perfused heart, prostacyclin (PGI_2) is the major product from arachidonic acid (20:4n-6) [Wennmalm *et al* 1987, Lamers *et al* 1987]. Infusion of PGI_2 and PGE series into the heart reduces NA release [Langer 1981, Khan and Malik 1982, Fuder 1985]. Locally produced PGs may also have a similar effect [Edlund *et al* 1983]. Blocking the synthesis of PGs by cyclooxygenase inhibitors, like indomethacin, increases NA release in some studies, but not in others [Langer 1981, Fuder 1985].

Serotonin and 5-hydroxytryptamine can also be locally synthesized and released by myocardium, platelets and endothelium, especially during pathological conditions like ischaemia, and facilitate NA release [Thandroyen *et al* 1985, Rand *et al* 1987]. In the heart, the existence of gene expression of renin and angiotensinogen, and local conversion of angiotensin I to angiotensin II have been demonstrated [Lindpaintner *et al* 1988, Dzau 1988]. Therefore, locally generated angiotensin II may facilitate NA release, especially when angiotension II production is increased by adrenergic stimulation [Nakamuru *et al* 1986].

A series of substances carried by the blood stream may reach relevant receptors on sympathetic nerve terminals in the heart, thereby modulating exocytotic NA release. Some of these non-synaptic modulations may be of physiological and pathological importance, such as the facilitatory effect by angiotensin II, adrenaline, serotonin and 5-hydroxytryptamine [Rand *et al* 1987, Langer 1981, Corr *et al* 1986]. Atrial natriuretic factor, a peptide synthesized in the heart, may inhibit exocytotic NA release in non-cardiac tissues [Nakamuru and Inagamin 1986, Kuchel *et al* 1987].

A milieu of high K^+ and low pH interferes with action potential propagation in neurons, thereby presynaptically inhibiting transmitter release [Puig and Kirpekar 1971, Forfar and Riemersma 1987, Sanchez-Prieto *et al* 1987]. This mechanism may not be of importance during normoxic conditions but is of potential significance during myocardial ischaemia when tissue pH falls.

Thus, presynaptic modulation of transmitter exocytosis and subsequent postsynaptic effects are extraordinary complex. Most of our knowledge has been obtained under well perfused, well oxygenated conditions. Endogenous neuromodulators, although present in small amounts under normal conditions, can be synthesized and released in greater quantities during a variety of pathological conditions, thereby playing a more important role in modulation of transmitter release and subsequently of cardiac function and electrophysiology.

1.4 Catecholamine Release in Early Myocardial Ischaemia

In acute myocardial ischaemia, enhanced sympathetic activity may have beneficial as well as deleterious consequences. On the one hand, adrenergic stimulation of the cardiovascular system is necessary to compensate for acute loss of pump function. On the other hand, catecholamines accelerate the progression of ischaemic cell damage [Elson *et al* 1981, Rona 1985] and initiate malignant ventricular arrhythmias [Corr *et al* 1986, Verrier 1988]. Concomitant increase in

adrenoceptor number and perhaps the maintained intracellular signal transduction in ischaemic myocardium (*vide infra*) exaggerate these detrimental influences.

Thus deleterious effects of sympathetic activation on myocardium depend on local catecholamine concentrations and receptor mediated signal transduction. Theoretically, enhanced catecholamine stimulation to the heart could be due to both increased plasma concentration of catecholamines and local release from the sympathetic nerve terminals in the myocardium.

1.4.1 Elevation of Circulating Catecholamines

Plasma catecholamine concentrations are raised in patients with acute MI [Karlsberg *et al* 1981, Bertel *et al* 1982, Goldstein 1981, Nadeau and de Champlain 1979] and remain elevated for at least 48 hours [Benedict and Graham-Smith 1979, Nadeau and de Champlain 1979]. In dogs with experimental MI, progressive elevation in plasma catecholamines occurs within the first 60 min [Corr and Gillis 1978, Forfar *et al* 1984, Godin *et al* 1985, Daugherty *et al* 1986], especially in conscious animals [Karlsberg *et al* 1979]. The magnitude of this increment varies from just above normal range to over ten-fold, largely depending on the extent of MI and impairment of ventricular function [Schömig *et al* 1984b, Karlsberg *et al* 1979 and 1981, Benedict and Graham-Smith 1979].

Some clinical studies revealed an association between plasma levels of catecholamines and severity of ventricular arrhythmias [Nadeau and de Champlain 1979, Bertel *et al* 1982, Goldstein 1981]. However, this association may also be explained by a bigger infarct size and more severe ventricular dysfunction (Section 1.1.3). It is assumed that the initial increase in catecholamine levels at the onset of acute MI may be more meaningful [Goldstein 1981]. But there is no temporal relation between onset of arrhythmias and plasma catecholamine levels, arguing against a direct effect of circulating catecholamines [Riemersma and Forfar 1982]. In experimental studies, adrenalectomy or ligation of the adrenal vein attenuates the elevation of plasma catecholamine but does not reduce the onset of VT or VF [Corr and Gillis 1978, Daugherty *et al* 1986]. Very high levels of plasma catecholamines, which can be toxic to myocardium, are observed only in patients with severe pump failure [Goldstein 1981, Benedict and Graham-Smith 1979]. Moreover, reduced blood supply to the ischaemic region limits the effects of circulating catecholamines there.

Thus, enhanced circulating catecholamines provide evidence for an overall sympathetic activation. However, as discussed above, it may be unimportant for the development of ventricular arrhythmias. Furthermore, as the contribution of cardiac

catecholamines to the circulating pool is small (3%) [Goldstein *et al* 1988] and is limited by a reduced washout from the ischaemic myocardium. Thus circulating catecholamines do not provide a sufficiently sensitive index to reflect local catecholamine spillover in ischaemic myocardium.

1.4.2 Neuronal NA Release in Ischaemic Myocardium

In vivo NA release from ischaemic region

As enhanced circulating catecholamine level and cardiac efferent tone occur soon after myocardial ischaemia, it is generally assumed that there is enhanced neuronal NA release from the heart. However, most clinical and experimental studies do not support this view. In man, a short period of coronary occlusion (0.5-2 min) did not evoke NA overflow from the heart [Schwartz *et al* 1979, Schömig 1988, Richardt *et al* 1990], although an increase in arterial NA level was noticed in one study [Richardt *et al* 1990]. Similarly, experimental studies cannot find increased NA overflow into ischaemic effluent [Forfar *et al* 1984, Godin *et al* 1985, Yamaguchi *et al* 1990], or a small amount if it did occur [Hirche *et al* 1980].

This technique, however, disregards the influence of NA clearance. Actually neuronal uptake increases during early ischaemia in man and in animals [Richardt *et al* 1990, Riemersma and Forfar 1982, Dart 1989], presumably because reduced washout increases the retention of NA in the synaptic cleft. Recently, using ^{3}H-NA kinetic analysis, allowing assessment of NA spillover and clearance separately, McCance and Forfar have found that cardiac NA spillover is higher in patients with recent ischaemic symptoms than those with stable angina. During exercise, cardiac NA spillover is 67% higher in those who developed ischaemia than those who did not [McCance and Forfar 1989a and 1989b].

Experimental and clinical studies *in vivo* all show enhanced NA overflow during the first few minutes of reperfusion following a period of ischaemia [Forfar *et al* 1984 and 1985, Godin *et al* 1985, France-Cereceda *et al* 1990, Yamaguchi *et al* 1990]. The pattern of this reperfusion NA overflow fits the one compartmental model, thus suggesting that this is washout of NA accumulated in the extracellular space, rather than released by reperfusion *per se*. Although the mechanism of this NA release is still unclear, several observations indicate the possibility of exocytosis: 1) Increased NA overflow during reperfusion is accompanied by an enhanced overflow of neuropeptide Y [France-Cereceda *et al* 1990], a co-transmitter released only by exocytosis [Haass and Schömig 1989]; 2) Blockade of α_2-adrenoceptors or neuronal uptake$_1$ enhanced this reperfusion NA overflow [Forfar *et al* 1985]; 3) In the

ischaemic area, a typical ultrastructural change of sympathetic nerve terminals observed is a profound reduction in the number of dense-cored synaptic vesicles [Muntz *et al* 1984] without simultaneous increase in empty vesicles, as in the case of inhibited vesicular storage of NA by reserpine treatment [Tranzer and Richards 1976]. This morphological change suggests enhanced exocytosis.

Although most studies failed to find an increase in local NA level in ischaemic venous effluent during acute myocardial ischaemia, a reduction in myocardial NA content is a consistent observation in various species *in vivo* [Muntz *et al* 1984, Kaufman and Jugdutt 1987, Hirche *et al* 1985, Schwaiger *et al* 1987, Abrahamsson 1982, Mukherjee *et al* 1979]. This change becomes evident within the first 30-60 mins. In some studies, ischaemia of 5-20 min with or without reperfusion reduces myocardial NA content by 30-40% [Schwaiger *et al* 1987, McDonald *et al* 1986]. In dogs reduction of intraneuronal NA precedes that of myocardial NA content, indicating a redistribution of NA within ischaemic area [Muntz *et al* 1984]. Meanwhile, the rate and extent of reduction of NA content vary according to the severity of ischaemia [Muntz *et al* 1984, Schmid *et al* 1982]. Temporary reduction in NA content in non-ischaemic myocardium also occurs in the first few days after acute ischaemia [Mathes *et al* 1971, Seth *et al* 1974].

Nerve stimulation-induced NA release from ischaemic myocardium

In the anaesthaetized dog, when nerve stimulation is given early during a period of coronary artery occlusion, there is significant rise in NA concentrations in the venous blood from both ischaemic and non-ischaemic myocardium. After 30 min ischaemia, however, such stimulation is ineffective in eliciting a rise in NA in ischaemic venous effluent [Forfar *et al* 1984]. These observations are supported by other studies using functional responses (effective refractory period or segmental contraction) as endpoints to nerve stimulation [Martins *et al* 1980, Inoue and Zipes 1988].

Dart *et al* described an *in situ* perfused and innervated rat heart model in which left sympathetic ganglion stimulation evokes a frequency-dependent NA overflow into coronary effluent [Dart *et al* 1983 and 1984b]. This model has advantages of precise control of coronary flow and other relevant experimental conditions and therefore more suitable for quantitative studies than the *in vivo* dog model. By stimulating sympathetic nerves during a period of stop-flow ischaemia followed by reperfusion, the amount of NA overflow can be quantified and compared with that induced by nerve stimulation during normoxic perfusion [Dart *et al* 1984b]. After stopping coronary flow or reducing the flow rate by 95% for 1 to 10 min, a reduction

in NA overflow to about 20-30% of control values was observed (Figure 1.8) [Dart *et al* 1984b, Dart and Riemersma 1985, Schömig 1988]. In the presence of the neuronal reuptake inhibitor desipramine, the suppression of NA overflow during the first few minutes of stop-flow or low-flow ischaemia is restored to some extent, thereby indicating the importance of neuronal reuptake (Figure 1.8) [Dart *et al* 1984b, Dart and Riemersma 1985]. The efficacy of the neuronal reuptake of NA shows progressive decline during energy depletion mimicked by glucose-free perfusion and anoxia or cyanide intoxication [Schömig 1989]. Despite this, neuronal NA overflow during the later period of a 15 min stop-flow ischaemia is again depressed in the presence of desipramine [Dart *et al* 1984b, Dart 1989]. This suggests a failure of neurotransmitter release at this time. When stop-flow ischaemia lasts for more than 15 min there is a progressively enhanced spontaneous NA overflow during reperfusion [Schömig *et al* 1984a]. The magnitude of this NA overflow is too big to allow any detection of additional nerve stimulation evoked NA release at this period of ischaemia [Dart *et al* 1984b]. In contrast to the role of neuronal reuptake, extracellular uptake is of minor importance in the heart both in normoxia and ischaemia [Goldstein *et al* 1988, Dart *et al* 1984b].

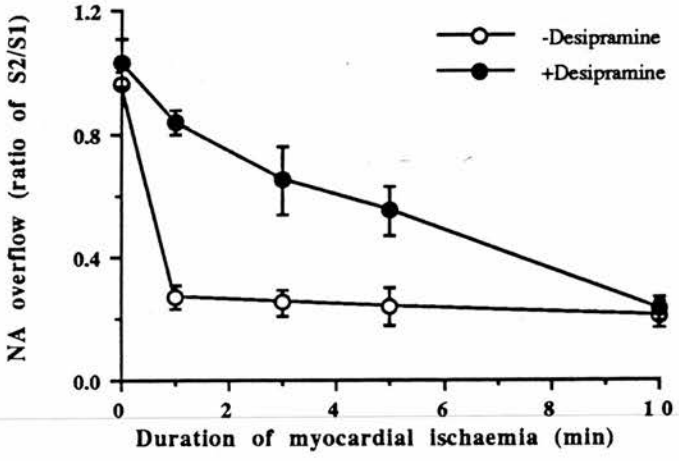


Figure 1.8 *NA overflow induced by electrical stimulation of left stellate ganglion in the perfused rat heart subjected to 1-10 min period of stop-flow ischaemia followed by reperfusion. Data are given as the ratio of the amount of NA overflow evoked by the first (S1 in normoxia) and the second (S2 in ischaemia) stimulations [Schömig 1988, Dart 1989].*

Mechanism of reduced exocytotic NA release

Several possible mechanisms may account for reduced neuronal NA release during early ischaemia.

1) Presynaptic inhibition Of the substances that may inhibit the exocytotic NA release *via* presynaptic receptors (Section 1.3.4), two possibilities have been explored

in the setting of myocardial ischaemia so far. In the *in vivo* dog heart subjected to a period of coronary ligation (<12 min), the rise in the ischaemic venous effluent NA level during sympathetic ganglion stimulation was increased by the α_2-adrenergic antagonist yohimbine [Forfar *et al* 1987]. In the perfused rat heart addition of yohimbine did not lead to a further increase in neuronal NA release within 10 min stop-flow ischaemia [Dart *et al* 1984b]. This discrepancy may be due to a mild ischaemia in the dog study with a residual blood flow of 0.3-0.4 ml/g/min in comparison to normal flow of 0.8-0.9 ml/g/min [Forfar *et al* 1987].

Inhibition of NA release by adenosine in early ischaemia has been examined. Within a few min of stop-flow ischaemia, extracellular adenosine reaches micromolar concentrations capable of inhibiting neuronal NA release [Headqvist and Fredholm 1989, Richardt *et al* 1987]. Adenosine receptor antagonists restored the suppressed NA overflow to control levels within 3 min of ischaemia, but not when ischaemia was extended to 10 min [Richardt *et al* 1987]. Inhibition of sympathetic neurotransmission by exogenous adenosine has also been demonstrated in the *in vivo* dog heart with and without ischaemia [Miyazaki and Zipes 1990, Forfar and Riemersma 1987].

2) High extracellular potassium and acidosis In the first minutes of ischaemia, there is a progressive increase in extracellular concentrations of K^+ and H^+ [Hill and Gettes 1980, Kleber 1984, Lavanchy *et al* 1986]. These may modify the resting and act on potentials of neuronal membranes and Ca^{2+} influx, thereby interfering with NA release.

In the *in vivo* dog heart, NA overflow is inhibited by intracoronary infusion of 10 mM K^+, and facilitated by infusion of high K^+ (75 mM) which leads to a 3-fold increase in K^+ concentration (9 mM) in venous blood [Forfar and Riemersma 1987]. In another study in dogs, sympathetic neurotransmission in the heart is prevented by increasing the extracellular K^+ to 12 mM [Miyazaki and Zipes 1990]. In isolated canine veins, ^{3}H-NA release elicited by electrical stimulation is suppressed by K^+ with a dose-dependent manner in the range of 6-20 mM [Lorenz and Vanhoutte 1975]. In another careful study, however, ^{3}H-NA release from canine coronary arteries is enhanced by K^+ of 10-15 mM and inhibited when exposed to 40 mM K^+ [Borda *et al* 1977].

In the dog heart and cat spleen, a low pH of 6.3-6.4 significantly suppressed sympathetic neurotransmission and NA release [Miyazaki and Zipes 1990, Puig and Kirpekar 1971]. Using the perfused guinea-pig heart, Haass *et al* [1990] show that exocytotic NA release was also suppressed by acidosis (pH=6.0). In the innervated rat heart, however, perfusion with low pH of 6.5, evoked NA overflow was not affected, although its postsynaptic effect is markedly suppressed [Dart and Riemersma 1989].

3) Energy depletion in the nerve terminal Myocardial ischaemia is associated with reduced high energy phosphates. The effect of energy depletion was examined in the innervated rat heart perfused at constant flow rate, thereby avoiding the interference of accumulated H^+, K^+ and metabolites. During anoxia and glucose-free perfusion (replaced by lactate), there is a rapid fall in the amount of neuronal NA overflow [Dart *et al* 1987]. Omission of glucose, but maintaining the oxygen supply, also leads to a progressive decline in NA overflow but less rapid. In contrast, anoxia *per se* has no discernable effect on evoked NA release provided exogenous glucose supply is maintained [Dart *et al* 1987, Dart and Riemersma 1989]. Thus, it seems that exogenous glucose supply plays a vital role in the maintenance of an effective exocytotic release of NA. Similarly, glycolytic energy production is of pivotal importance in preventing the non-exocytotic NA release (*vide infra*) [Dart *et al* 1987, Carlsson 1988, Schömig *et al* 1987]. Indeed, the major route of ATP production in cultured sympathetic neurons is *via* anaerobic glycolysis and only a small amount ATP supply is necessary to maintain normal nerve function [Wakade and Wakade 1985].

In summary, during early ischaemia and enhanced sympathetic activity, an increase in local NA concentration is counteracted by an effective presynaptic inhibition and neuronal reuptake. The ability of neurons to release NA is maintained. Later, there is a failure of the exocytotic release of NA, presumably due to reduced ATP supply, in which impaired glycolytic energy production is of particular importance.

1.4.3 Non-Exocytotic NA Release in Myocardial Ischaemia

In the isolated perfused rat heart subjected to stop-flow ischaemia, there is a progressive "spontaneous" NA overflow during subsequent reperfusion, when ischaemia exceeds 15 min [Schömig *et al* 1984a]. The data indicate that 90% of total NA overflow is washout from the extracellular space [Schömig *et al* 1984a, 1985]. Dart and Riemersma showed a fair correlation between the overflow of NA and washout of an extracellular space marker [1988]. The quantity of NA overflow during late ischaemia greatly surpasses that achieved by tonic nerve stimulation [Dart *et al* 1984b, Schömig *et al* 1984a]. Thus, assuming an uniform NA distribution in the extracellular space, it is estimated that after 20-40 min total ischaemia extracellular NA concentration may reach 1-6 μM range [Schömig *et al* 1984a], a level which is toxic even to non-ischaemic myocardium [Rona 1985, Waldenström *et al* 1978].

Blocking substrate metabolism (oxidative phosphorylation and anaerobic glycolysis) can mimic ischaemia-induced NA release. Anoxia itself or blockade of aerobic metabolism could not trigger such release [Dart *et al* 1987, Schömig *et al* 1987, Carlsson 1988]. Furthermore, non-exocytotic release of NA never occurs within the first 10-15 minutes of stop-flow ischaemia. This delay is believed to be due to an ongoing glycolytic ATP production from endogenous glycogen [Dart *et al* 1987, Schömig *et al* 1987, Carlsson 1988]. However, in the isolated perfused rabbit heart, anoxia in the presence of exogenous glucose supply evokes an immediate overflow of NA which peaks within 10 min of anoxia [Wollenberger and Shahab 1965, Karwatowska-Kryñska and Beresewcz 1983]. The reason for this discrepancy is unclear.

The ischaemia-induced NA release differs from the exocytotic release in several aspects. It is independent of extracellular Ca^{2+} and not modulated by presynaptic mechanisms [Schömig 1988]. The relation of non-exocytotic NA release and co-transmitter release has not been studied during ischaemia, although a co-release of NPY has been found in guinea pig heart during anoxia and glucose-free perfusion [Haass and Schömig 1989]. In contrast to exocytotic release which is increased by blocking neuronal reuptake, inhibition of uptake carrier by desipramine suppresses this release up to 60 min of stop-flow ischaemia [Schömig *et al* 1987]. This indicates the involvement of the uptake carrier in ischaemia-induced NA release. Schömig and his colleagues [1984a, 1987 and 1988] hypothesize that this ischaemic release of NA is mediated by the neuronal uptake carrier reversing its normal transport direction (Figure 1.9).

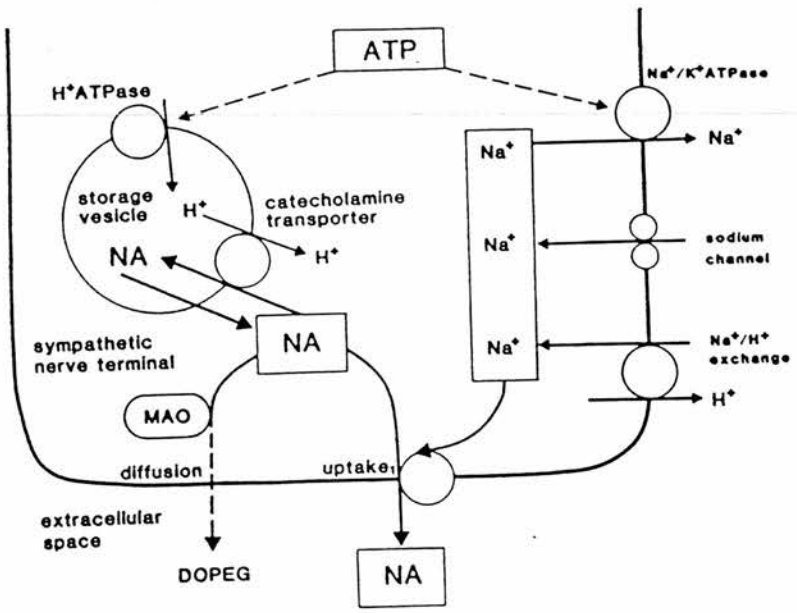


Figure 1.9 *Hypothetical mechanism of non-exocytotic NA release through the neuronal uptake carrier [Schömig et al 1988]. Details see text.*

During myocardial ischaemia energy exhaustion interferes with both NA vesicular storage and reuptake mechanisms. As discussed in Section 1.3.2, a transmembrane proton electrochemical potential, created through the activity of membrane H^+-ATPase, is the driving force of vesicular NA uptake. ATP depletion could lead to reduction of the H^+-induced potential, followed by loss of NA from vesicles into axoplasm. As a result of anoxia, axoplasmic NA is largely protected from the degradation by monoamine oxidase (MAO, K_m oxygen = 50 mm Hg) [Carlsson *et al* 1986, Schömig *et al* 1987]. Meanwhile, energy deficiency inhibits the activity of Na^+/K^+ ATPase on neuronal membranes. Developed intracellular acidosis during ischaemia may result in an enhanced Na^+/H^+ exchange. Both changes could lead to enhanced concentration of Na^+ in the axoplasm. As the sodium gradient is the determinant for the carrier transport direction, accumulation of Na^+ in axoplasm initiates a carrier-mediated efflux of NA from axoplasm into the extracellular space [Schömig *et al* 1984a, 1987, 1989]. When the duration of ischaemia exceeds 40 min, inhibition of neuronal uptake no longer suppresses NA release [Schömig *et al* 1984a], perhaps because NA efflux at this stage is largely due to ischaemia-induced membrane disintegration. The difficulty in proving this hypothesis is that it is not possible to measure axoplasmic NA and Na^+ concentrations. However, experimental simulation of the above mentioned prerequisites, i.e., increase in the axoplasmic NA concentration, inhibition of MAO activity, and intracellular accumulation of sodium, could induce a similar carrier-mediated NA efflux [Schömig *et al* 1988].

Using isolated perfused rat heart model, arrhythmogenic effect of NA release via this mechanism has been demonstrated [Riemersma *et al* 1986b, Dietz *et al* 1989]. However, further work is required to examine whether this carrier-mediated NA efflux occurs in the *in vivo* ischaemic heart.

1.4.4 Effects of Ischaemia on Postsynaptic Adrenergic Signal Transduction

Postsynaptic adrenergic signal transduction in ischaemic myocardium has been intensively studied and reviewed [Thandroyen *et al* 1990, Strasser *et al* 1988, Yamada *et al* 1988, McGrath 1989], and will be briefly summarized here.

Increased β- [Thandroyen *et al* 1990, Mukherjee *et al* 1979 and 1982, Strasser *et al* 1990, Reddy 1989, Vatner *et al* 1988] and α-adrenoceptor density [Corr *et al* 1981, Dillon *et al* 1988, Yamada *et al* 1988] in ischaemic myocardium has been reported in various species and models, although not always [Corr *et al* 1981, Dillon *et al* 1988]. In the dog, rat and guinea pig heart, β-receptor number show progressive increase over the first 1-2 hour period of ischaemia starting within 15 min [Maisel *et al* 1985, Reddy 1989, Strasser *et al* 1990, Mukherjee *et al* 1979]. This increase lasts at least 5

hours in the *in vivo* ischaemic dog heart [Devos *et al* 1985]. The magnitude of increased receptor number varies between 20% to over 100%. In cats, the density of α-receptors increased over 3-fold within 30 mins of ischaemia [Corr *et al* 1981]. One study shows a 49% increase in β_1-receptor number in the perianeurysmic myocardial tissues from patients with recurrent sustained VT [Bevilacqua *et al* 1986]. Mechanisms for this increase in receptor density are still undefined but at least two possible mechanisms have been proposed. One possibility is an increased receptor externalization during ischaemia owing to depletion of energy and an impaired desensitizing process, normally seen during high adrenergic stimulaton [Maisel *et al* 1985, Strasser *et al* 1988, Thandroyen *et al* 1990]. Corr and his co-workers have demonstrated an association of the increase in long-chain acylcarnitine levels and α-receptor numbers in hypoxic myocytes. Both events are completely prevented by the inhibition of carnitine acyltransferase [Yamada *et al* 1988]. Thus, in ischaemic myocardium increased long-chain acylcarnitine may be inserted into membranes, thereby exposing those internal and functionally uncoupled adrenergic receptors. As the receptor affinity and the fraction of receptors coupled with adenylate cyclase is unchanged in ischaemia, the total number of functional β-receptors is increased [Maisel *et al* 1985, Strasser *et al* 1988].

Substantial data show that α_1-receptors mediate arrhythmic effects of catecholamine stimulation during ischaemia and reperfusion, probably *via* an enhanced calcium influx [Yamada *et al* 1989, McGrath 1989, Sheriden *et al* 1980]. This effect is found only during early ischaemia and reperfusion but not during non-ischaemic conditions [Sheridan *et al* 1980], suggesting an enhanced α-adrenergic signal transduction. However, the relevant intracellular events, like increased phosphatidylinositol turnover, release of IP_3 and diacylglycerol, and α_1-stimulation mediated Ca^{2+} influx remain to be defined.

There is some disagreement on the coupling of increased β-receptors and G_s-adenylate cyclase activity during ischaemia. A maintained adenylate cyclase activity is supported by increased cAMP levels in ischaemic myocardium [Thandroyen *et al* 1986, Ohyanagi *et al* 1988]. Isoprenaline-induced stimulation of adenylate cyclase is found to be enhanced in some studies [Maisel *et al* 1985, Mukherjee *et al* 1979], but reduced in others [Strause and England 1982, Devos *et al* 1985, Vatner *et al* 1988, Will-Shahab *et al* 1985, Reddy 1989]. Strasser *et al* [1990] demonstrate an transcient increase in adenylate cyclase activity at 15 mins of ischaemia followed by a progressive decline. This can be is partly explained by an effect of adenosine and acidosis [Reddy 1989]. Thus, the functional significance of increased β-receptors is questionable. However, recent studies suggest that the signal transduction of β-

stimulation may be achieved at least by two pathways (Section 1.3.2). G_s-mediated activation of adenylate cyclase probably remains effective during early ischaemia [Strasser *et al* 1990, Strause and England 1982]. Increased β-receptor density may also couple with Ca^{2+}, K^+ and Na^+ channels *via* cAMP-independent pathways [Brown and Birnbaumer 1988]. The importance of this receptor-G_s-ion channel pathway in arrhythmogenesis remains to be elucidated. The effectiveness of β- and α-adrenoceptor blockers in the protection against ischaemic damage and ventricular arrhythmias during prolonged ischaemia and infarction also indicate an effective postsynaptic signal transduction [Corr *et al* 1986, Norris *et al* 1984, Peter *et al* 1978].

1.5 Questions and Working Hypothesis

In the rat heart subjected to stop-flow ischaemia, three phases of NA release *via* different mechanisms has been proposed: exocytosis (<10 min), carrier-mediated efflux (10-40 min) and leaky diffusion (>40 min) [Schömig *et al* 1984a, 1988]. When extrapolating these findings to the *in vivo* condition, however, one of the major limitations is the severity of ischaemia. Complete cessation of blood flow *in vivo* may be rare both in man and various animal species [Rentrope *et al* 1985, Piek and Becker 1988, Reimer *et al* 1987, Maxwell *et al* 1987]. Considering the importance of energy supply for the maintenance of exocytosis and prevention of non-exocytotic efflux, severity of flow reduction may be critical in determining the time-course of deterioration of sympathetic nerve function.

A variety of substances can modulate the exocytotic release of NA (Section 1.3.4). Although presynaptic α-adrenergic and purinergic inhibition on NA release has been examined in the ischaemic heart to some extent [Forfar *et al* 1983, Dart *et al* 1984b, Richardt *et al* 1987], the importance of presynaptic control of NA release during ischaemia cannot be fully derived from these rather limited data. Of the many other presynaptic modulatory mechanisms not examined, vagal nerve activation mediated inhibition of NA release is of great interest. It is believed that this presynaptic inhibition of exocytotic NA release contributes to vagal protection against ischaemic arrhythmias (Section 1.2.2). But vagal-sympathetic interactions during ischaemia have rarely been studied.

As summarized in Section 1.1.3, several factors may modify the susceptibility of the ischaemic heart to VF. The relevant mechanisms, however, are largely unknown. As neuronal activity have profound impact on the electrical stability of the ischaemic heart, it would be reasonable to speculate that some of these factors may exert their

influence on the arrhythmogenesis through a modulation of autonomic neurotransmission in the heart.

Finally, the role of the exocytotic NA release in the genesis of ischaemic arrhythmias is still unclear. Clinical and experimental studies suggesting such a role have done so largely on the basis of indirect evidence.

WORKING HYPOTHESIS

- Neuronal NA overflow varies depending on the the severity and duration of ischaemia, as will its modulation by presynaptic muscarinic, α-adrenergic and purinergic receptors
- Factors (gender and dietary fats) influencing ischaemic arrhythmias affect neuronal NA release directly or presynaptically.
- Development of ischaemic-reperfusion arrhythmias relate to neuronal NA release and can be modulated by presynaptic mechanisms.

By providing new experimental data, it is hoped that this thesis will make a contribution to the understanding of neuronal mechanism(s) leading to severe ventricular arrhythmias and sudden cardiac death in the setting of ischaemic heart disease.

Chapter 2

Materials and Methodology

2.1 Animals

Male Wistar, and subsequently male and female Sprague-Dawley rats (9-12 weeks old, 200-300 grams) were used for the experiments described in Chapter 3-7. They were purchased from Banting & Kingman Co. (Hull, UK) and housed in an environment with an ambient temperature of 20°C and 12/12 hour light/dark cycle. Four animals were kept per cage. Water and food were taken *ad libitum*. For the gonadectomy study in Chapter 5 and 6, the operation was carried out in rats 4-6 weeks old, an age prior to puberty (7-8 weeks). No attempt was made to ascertain the phase of the estrous cycle of female rats at the time of heart perfusion study.

Male Lew rats (from Banting & Kingman Co.) were used to study the effects of dietary lipids on sympathetic neurotransmission (Chapter 8). The rationale of using Lew rats in this study was that a substantial amount of data from this strain has been collected in this Unit on the effects of dietary lipids on the fatty acid composition of myocardium, ischaemic arrhythmias and postsynaptic α-adrenoceptors [Riemersma *et al*1988, MacLeod and Riemersma 1990, Sargent 1990].

2.2 Innervated and Perfused Heart Model

2.2.1 Preparation

An *in situ* perfused, innervated heart model, described by Dart *et al*[1984b] was used for most of the experiments. Rats were anaesthetized with pentobarbitone sodium (60 mg/kg, ip). Heparin (200 u/rat) was injected intravenously using a femoral vein. The thorax was then opened and a metal cannula (I.D. 0.8 mm) was inserted and tied into the ascending aorta for Langendorff perfusion. The interval from opening the chest to starting heart perfusion was 50-70 seconds. The pulmonary vessels (both sides) were tied off. A polyethylene cannula (I.D. 0.86 mm) was introduced into the right atrium *via* the inferior *vena cava* for collection of coronary effluent. Each heart was perfused at a constant flow rate, adjusted to given flow value of about 5 ml/g/min by the estimated heart weight, with a multichannel Watson-Marlow peristaltic pump (Waston-Marlow Ltd, Cornwall, UK). This flow rate was chosen as it was close to *in vivo* values of coronary blood flow [Malic *et al* 1976]. The perfusate was a modified Krebs-Henseleit solution (composition listed in Table 2.1).

Table 2.1 *Composition of Krebs-Hensenleit solution*

Na^+	148 mM	Glucose	11 mM
K^+	4.0 mM	Pyruvate*	1.8 mM
Ca^{2+}	1.85 mM	EDTA	0.027 mM
Mg^{2+}	1.05 mM	Po_2	550-650 mm Hg
Cl^-	140 mM	Pco_2	36-41 mm Hg
HCO^-_3	25 mM	pH	7.36-7.43
PO^{3-}_4	0.5 mM		

**Present in the experiments examining vagal nerve function. Pyruvate provides acetate for acetylcholine resynthesis [Löffelholz 1981].*

Perfusate was continuously gassed with O_2:CO_2 (95%:5%). The temperature of the perfusate at the point of entry into the aorta was 37°C. pH, Po_2 and Pco_2 in the perfusate were regularly (approximately every hour) checked with an IL 1302 pH/Blood Gas Analyser (Instrumentation Lab, Milan, Italy).

The coronary flow recovery was determined during the stabilizing period. The superior *vena cava* was also tied if the recovery of coronary effluent was low. Recovery of flow was rechecked during the experiment. In most preparations it was found that the recovery rate was nearly complete even without tied superior *vena cava*. In the low-flow ischaemia experiments the superior *vena cava* was tied in all preparations to prevent any possible leakage and to ensure a good recovery.

2.2.2 Measurement of Functional Parameters

The perfusion pressure was monitored by a transducer (Model EM 751, Elcomatic, Glasgow, UK) and was between 40-50 mm Hg at the flow rate of 5 ml/g/min. A 4FG cannula (I.D. 0.8 mm) connected to another pressure transducer (Model EM 751, Elcomatic) was introduced into the left ventricular cavity through the apex. This procedure did not affect cardiac function and the stability of the perfused heart. Left ventricular pressure and the first derivative of the ventricular pressure (dP/dt) were measured with a polygraph (Department of Medical Physics, Royal Infirmary of Edinburgh, University of Edinburgh). Heart rate was derived from the epicardial ECG signal with a built-in chronometer and continuously displayed on the polygraph. Epicardial ECG, ventricular pressure, dP/dt and perfusion pressure were recorded with a U.V. recorder (Model 2106, Visicorder Honeywell, Hemel-Hempstead, UK) or an ink-jet Mingograph 34 (Schönema-Elander, Sweden), and later with a Gould TA 2000 recorder (Gould Electronics Inc, Ohio, USA) with a paper speed of 5-10 mm/min during control periods and 10 mm/sec during nerve stimulations.

2.2.3 Electrical Nerve Stimulation

The left cervico-thoracic stellate ganglion was carefullly exposed with the help of a surgical microscopy (Wild, Chatham. UK), at the level of the first rib [Dart *et al* 1983]. Pre-ganglionic fibres were cut. After a few cardiac sympathetic nerves (usually two branches) were identified, a bipolar platinum electrode was placed underneath the ganglion structure, above the cardiac nerves, with the aid of a micromanipulator (Prior, UK). In the experiments in which vagal nerve stimulation was required, bilateral vagi were dissected free at the cervicothoracic level, and also mounted on a pair of bipolar platinum electrodes held by another micromanipulator. The electrode close to the heart was always the cathode. The nerves were continuously superfused with warmed and oxygenated buffer except when stimulated.

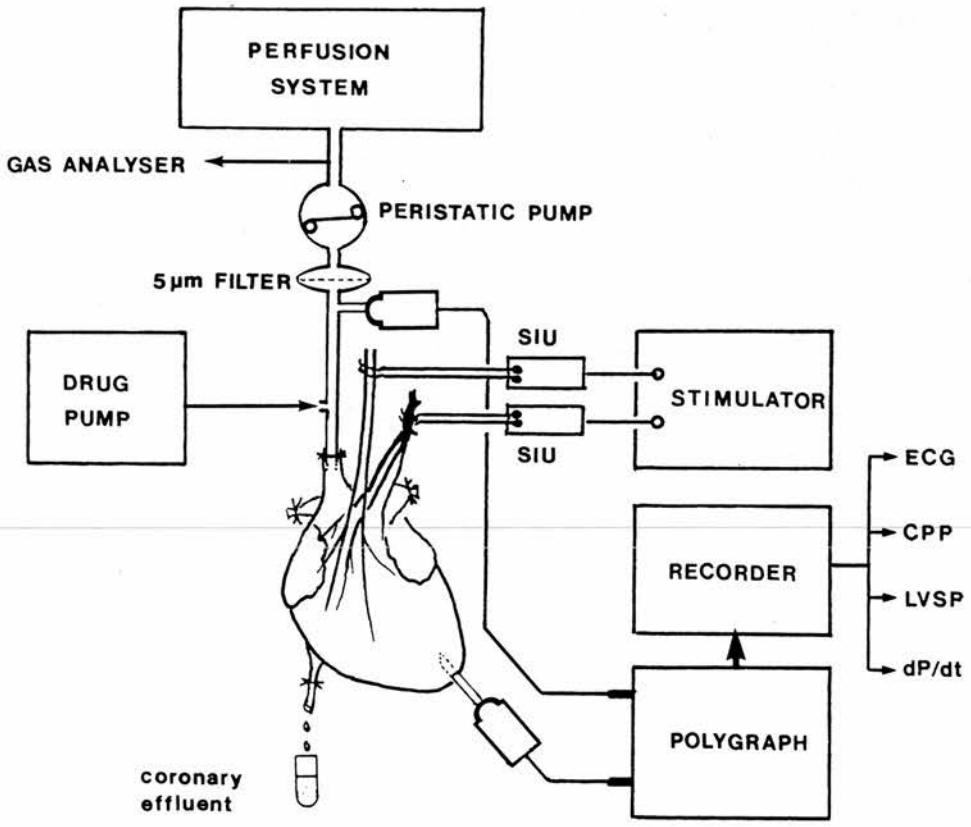


Figure 2.1 *A schematic illustration of the basic model. ECG: electrocardiogram; CPP: coronary perfusion pressure; LVSP: left ventricular systolic pressure.*

Nerve stimulation was performed using a 2-channel Grass S88 stimulator (Grass Instruments, Quincy, Mass, USA) with two stimulus isolation units (Grass SIU 7) to minimize effects of capacitance and ensure a good isolation. The electrical stimulus was a monophasic square wave pulse with a width of 2 msec and a current of 0.8 mA. The frequency was 15 Hz for vagal nerves and 5 Hz for sympathetic nerve stimulation. These had been determined from frequency response studies of cardiac inotropic and chronotropic responses without significant fading of either functional responses and/or NA overflow to several repeated nerve stimulations (*vide infra*).

2.3 Models for Arrhythmia Study

The two models described here were used for arrhythmia studies in Chapter 7.

2.3.1 Isolated Langendorff Perfused Rat Heart Model

After anaesthesia and heparinisation hearts were quickly excised and immersed in ice-cold perfusion buffer to achieve rapid arrest. The aorta was cannulated for retrogradely perfusion with Krebs-Henseleit solution containing 3 mM K^+ and 5.55 mM glucose and continuously gassed with O_2:CO_2 (95%:5%). The perfusion pressure was 60 cm H_2O. Four hearts (two from males and two from females) were mounted within a period of 10 min in each experiment (Figure 2.2). A pair of silver electrodes were attached to the free wall of the left ventricle and the right atrium respectively for the recording of the electrocardiogram (ECG). This electrode arrangement gave a clear P-wave and QRS complex. The ECG was continuously displayed on a oscilloscope (Type 41A, Lan-Electronics Ltd, UK) at 100 mm/s sweep speed and recorded on a four channel Mingograph ink jet recorder at a paper speed of 25 mm/s.

A traction-type coronary occluder consisting of a 4/0 silk suture threaded through a polyethelene guided cannula was used for coronary occlusion [Curtis *et al* 1987]. The suture was positioned loosely around the left main descending coronary artery approx 3 mm from the origin [Spadaro *et al* 1980]. A 10 min period was allowed to stabilize the preparation before the coronary ligation. During this period, the pre-ischaemic coronary flow rate was measured and coronary effluent was collected for the analysis of lactate and a normal ECG was recorded for comparison.

After stabilization the coronary artery was occluded by pulling the thread through the tube and securing them by clamping the tube with a staple. The period of ischaemia was 20 min followed by 5 min reperfusion. The ECG was recorded throughout the ischaemic/reperfusion period. Coronary effluent was collected over 30 secs every 5 mins during ischaemia, and continuously during the first 5 mins of reperfusion.

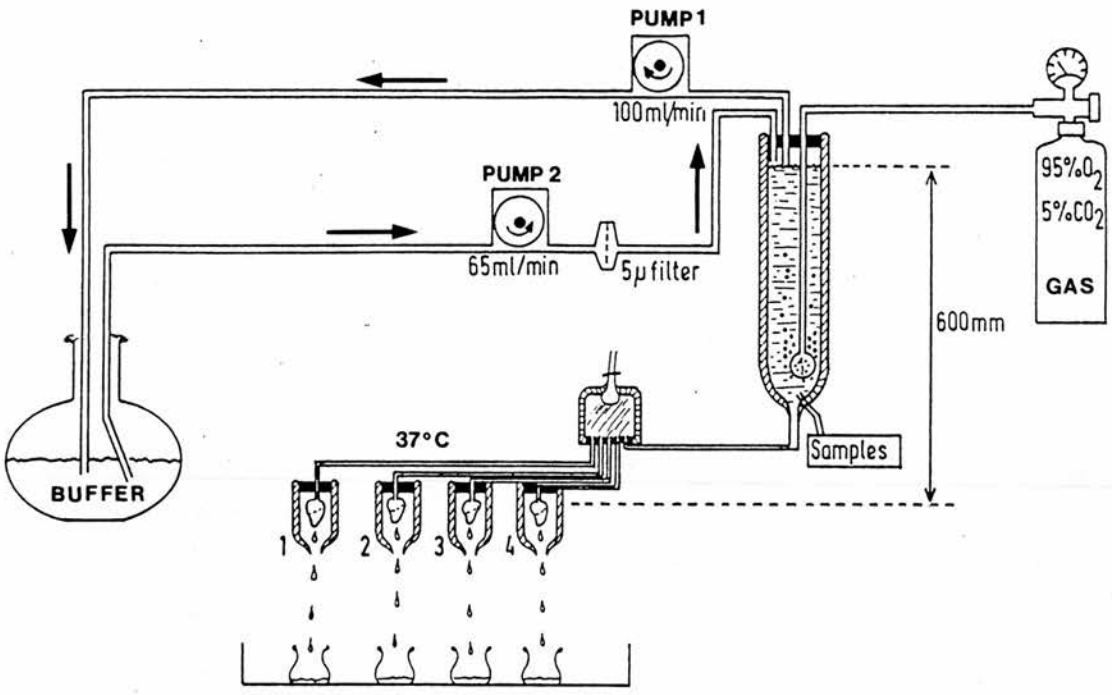


Figure 2.2 *Experimental set-up of the Langendorff perfusion system for the study of arrhythmias in vitro.*

2.3.2 The *In vivo* Anaesthetized, Open-chest Rat Model

The technique used was as described by Johnston *et al* [1983]. After anaesthesia the trachea was cannulated to ventilate with room air (50 strokes/min and 2 ml/100 g body weight) using a Harvard ventilator (Model 683, Harvard Apparatus, South Natick, USA). The chest was opened at the fourth intercostal space and the heart was exposed with a rib expander. Using a non-traumatic needle, a loose 4/0 silk loop was placed around the left coronary artery. The needle was superficially driven into and passed through the tissue between the imaginary midpoint of the left auricular appendage and the left margin of the pulmonary cone (about 3 mm from the origin of the left descending coronary artery). After slight compression of the chest to restore negative intrathoracic pressure, the thorax was then closed by suturing chest muscles and skin . The ends of the loose coronary ligature were left on either side of the chest.

After heparinisation (200 u, iv) the right carotid artery was dissected and cannulated with a 4 FG polyethylene catheter (I.D. 0.8 mm). Arterial blood pressure was measured with an EM751 pressure transducer. A lead II ECG was recorded from subcutaneous steel needles. ECG and blood pressure were displayed on an

oscilloscope (100mm/s sweep speed) and recorded on a Gould TA 2000 recorder with a paper speed of 25 mm/s. Two rats were instrumented and studied in one experiment.

After completion of the surgical procedure, animals were allowed to stabilize for at least 10 mins. Coronary ligation was induced by pulling the ends of the ligature. The period of ischaemia was 20 mins. ECG and blood pressure were continuously monitored during this period. During the experiment, body temperature was kept stable with an electric heating blanket. In rats without sustained ventricular fibrillation, a bolus injection of Evans blue (Sigma, 10% in isotonic saline, 1ml/rat iv), was given at the end of 20 mins ischaemia to measure the amount of ischaemic myocardium. One minute after the injection, hearts were excised. The ischaemic myocardiaum (unstained) was separated from non-ischaemic myocardium (stained) along the epicardially visible border line and both were weighed on a micro-balance.

Different types of ventricular arrhythmias, i.e. ventricular premature beats (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF) were defined according to the Lambeth Conventions [Walker *et al* 1988]. Typical recordings of VPB, VT and VF are shown in Figure 2.3.

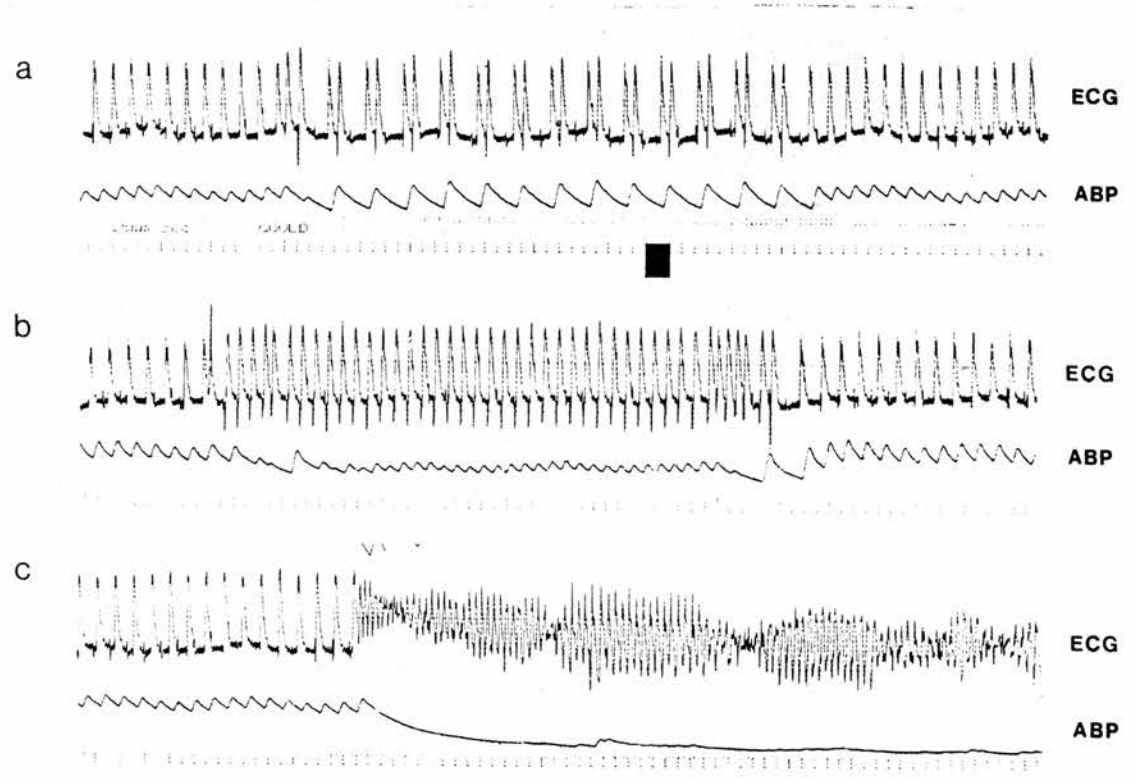


Figure 2.3 *Typical ECG and arterial blood pressure recordings after left coronary artery ligation from a rat in vivo. Paper speed of 25 mm/sec. a) ventricular premature beats in the form of bigeminy; b) unsustained ventricular tachycardia; and c) ventricular flutter and fibrillation.*

2.4 Gonadectomy

Male and female Sprague-Dawley rats were gonadectomised or sham-operated at 4-6 weeks of age according to the techniques described by Waynforth [1980]. Briefly, under light ether anaesthesia and local sterilization, two small skin cuts were made on scrotum (males) or on the back (females). Testis or ovaries were removed and cuts were sutured. Animals were then returned to their cage to recover. Body weights were measured in all animals on a weekly basis. The effects of gonadectomy on the body-weight growth curves of rats have been well-documented [Schaible *et al* 1984]. Heart perfusion experiments were performed 7-8 weeks after the operation. At the time of sacrifice, adequacy of the gonadectomy was determined by inspecting if any residual gonadal tissues were present and whether uterine atrophy in female rats had occurred.

2.5 Experimental Diets

Experimental diets were semi-synthetic and were prepared by mixing dry ingredients and fat. One kg of diet was made up of cornflour 456 g, casein 267 g, cellulose 65 g, salt mix 23 g, vitamin mix 4 g, and fat mix 185 g [Sargent 1990]. The basic control diet had 40% calorie supply from fat, 23% from protein and 37% from carbohydrate, representing the diet consumed by the average Scottish man [Thomson *et al* 1985]. The ingredients for fat mixtures varied in different diets (see Table 2.2). Maxepa was obtained from Seven Seas Health Care Ltd (Hull, UK) and contained 18% of 20:5n-3 and 12% of 22:6n-3 out of total fatty acids. Other oils were pure products obtained from the local market. To minimise peroxidation of the dietary fat, diets were freshly prepared every week and stored at 4°C. Fresh diet was fed and animal cages changed every second day. In order to prevent oxidation of n-3 polyunsaturated fatty acids (PUFA), Maxepa mixed with olive oil was given daily by oral dosing for 10 weeks. The dose was calculated from the amount of diet consumed daily by each animal. Olive oil in the basic diet for the control and n-3 dietary groups was reduced accordingly.

Table 2.2 *Ingredients for the fat mix of different experimental diets (% of total fat by weight).*

Ingredients	Control	n-6	Control	n-3
Beef dripping	74.3%	13.1%	74.1%	74.1%
Olive Oil	13.5%	23.6%	13.1% *	11.4% *
Safflower Oil	12.2%	-	12.8%	12.8%
Corn Oil	-	63,4%	-	-
Maxepa	-	-	-	1.7% *

* *given daily by oral dosing*

2.6 Drugs Used

The drugs used in all the experiments are listed in Table 2.3

Table 2.3 *Drugs used in the experiments*

Drugs	Type	Conc(M)	Source
Atropine	muscarinic receptor agonist	10^{-5}	Sigma
Choline chloride	precursor for acetylcholine synthesis	10^{-5}	Sigma
Clonidine	α_2-adrenoceptor agonist	3×10^{-6}	Sigma
Desipramine	neuronal NA reuptake inhibitor	10^{-7}	Sigma
Isoprenaline	β-adrenoceptor agonist	10^{-9}-10^{-6}	Sigma
Flurbiprofen	prostanoid inhibitor	10^{-5}	Boots
Methacholine	muscarinic receptor agonist	10^{-9}-10^{-4}	Sigma
Phentolamine	α-adrenoceptor antagonist	10^{-6}	CIBA
8-Phenyltheophylline	adenosine$_1$-receptor antagonist	10^{-5}	Sigma
Physostigmine	acetylcholinesterase inhibitor	10^{-6}	Sigma
Rauwolscine	α-adrenoceptor antagonist	10^{-7}-10^{-5}	Carl Roth
Timolol	β-adrenoceptor antagonist	3×10^{-6}	Sigma

Sigma: Sigma Chemicals, St. Louis, USA; CIBA: CIBA Laboratories, Horsham, UK; Boots: Boots Co Ltd., Nottingham, UK; Carl Roth: Carl Roth, Karlsruhe, West Germany

Water insoluble drugs were firstly dissolved in ethanol and the final concentration of ethanol in the perfusate was <0.02%. Desipramine and choline chloride were added directly to the perfusate. Other drugs were infused using a Harvard pump (Model 600/900 and Model 22, Harvard Apparatus Inc., Mass, USA) *via* a side port close to the heart. The ratio of the perfusion flow/infusate was always >40 (40-100) to ensure that the composition and temperature of the perfusate would not be significantly affected. Drug infusion started at least 12 min before nerve stimulation or before the induction of global ischaemia. In those experiments in which hearts were subjected to a period of low-flow ischaemia, drugs were added to perfusate in a separate gassing resevoir. Through a three-way stopcock, it was easy to switch to the perfusate containing drugs and to ensure an accurate concentration.

The drugs used did not affect the spontaneous NA overflow, nor did they interfere with our biochemical measurements.

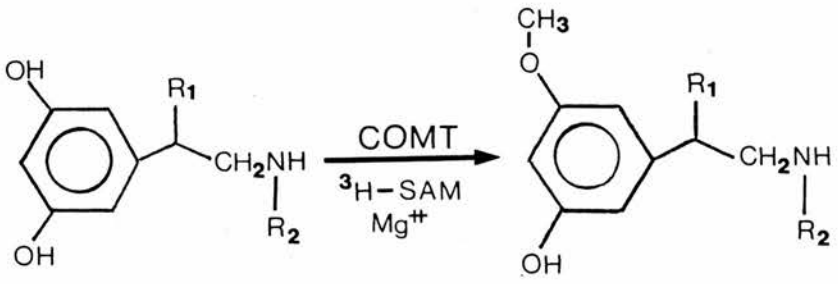
There was a less than 10-second lag between starting the drug pump and the appearance of drug effect on the heart.

2.7 Radioenzymatic Assay of Catecholamines

2.7.1 Principle

NA, adrenaline (A) and dopamine (DA) concentrations in the heart and NA and A levels in the coronary effluent were analysed by a radioenzymatic assay described by Da Prada and Zürcher [1976]. This method has the advantage of simultaneous measurement of NA, A, and DA, high sensitivity and specificity, and small sample volume required.

Catecholamines are converted to 3-O-methylated derivatives catalized by catechol-O-methyltransferase (COMT, EC 2.1.1.6) using tritium labelled methyl-group donor, ^{3}H-methyl-S-adenosyl methionine (^{3}H-SAM). After purification and separation by thin layer chromatography, the products are visualised under ultraviolet light and scraped off.



	R_1	R_2
DA	H	H
NA	OH	H
A	OH	CH_3

The radioactivity of normetanephrine (from NA), and metanephrine (from A) and methoxytyramine (from DA) was determined by liquid scintillation counting

2.7.2 Main Reagents, Materials and Working Solutions

- *COMT*, purified from rat liver according to the method of Axelrod and Tomchick [1958]. The enzyme is stable for at least three months at -40° C.
- *^{3}H-SAM*, purchased from Amersham (Amersham, UK) with the specific activity 10 Ci/mmol and stored at -20° C.
- *TLC plate* LK5 (Whatman, International Ltd., Maidstone, USA)
- *Catecholamine standards*
  Stock: 0.6 mM stirred at 4°C, fresh every 2 months
  Working standard: 60 and 600 fmol/50 µl, fresh daily
- *Carrier solution* (50 l)
  3-Methoxy-4-hydroxphenylethylamine HCl 61 mg (MW=203.7)
  D,L-Methanephrine HC l 59.2 mg (MW=233.7)
  D,L-Normetanephrine HCl 60.0 mg (MW=219.7)
  HCl 0.01N up to 50 ml

- *Solvent for thin-layer chromatography* (TLC)
  Chloroform/Ethanol/Ethylamine (70%) = 80/150/10 v/v/v
- *Scintillation solution*
  Butyl-(PBD) 12.5 g in 2.5L toluene (Merck)
- *Perchloric acid solution* ($HClO_4$) BDH Analar 0.3 or 0.6N

2.7.3 Procedure

Sample preparation

Coronary effluent: Sample (500 µl) was immediately deproteinized by addition of 500 µl of 0.6 N $HClO_4$ and stored at -40°C until assay. Samples were usually analysed within a month.

Heart tissue: hearts were weighed, homogenized in 10 volumes 0.3 N $HClO_4$ (containing 68 mM EGTA). The homogenate was centrifuged at 15,000 rpm for 10 mins and the supernatant decanted. The pellet was resuspended in 0.3 N $HClO_4$ and centrifuged at 15,000 rpm for 10 min. The combined extracts were used for analysis.

Catecholamine methylation and extraction of methylated products

The methylation reaction was carried out at (35°C), in a shaking waterbath, according to the following scheme:

Nature of sample	Sample	Blank	Standard
Deprotenised effluent or tissue extract	100 µl	-	-
0.3 N $HCHO_4$	-	100 µl	100 µl
Reference standards (60, 600 fmol/50 µl)	-	-	50 µl
0.01 N HC1	50 µl	50 µl	-
Enzyme mix	100 µl	100 µl	100 µl

Reaction was stopped after one hour by placing tubes in an ice-cold bath and adding 200 µl of a mixture of three parts borate buffer (1 M, Analar BDH, pH=8.0) and 1 part carrier solution (see 2.7.2). Freshly prepared 100 µl TPB solution (tetraphenylborate 1.5%) was added and the mixture extracted into 10 ml dietylether by shaking for 5 mins and recentrifuged before freezing the water on dry ice. The methylated catecholamines in the ether phase were back-extracted into hydrochloric acid and the acid phase washed with 5 ml butylacetate. After shaking and recentrifugation, the water phase was frozen and the buytylacetate phase was discarded. The acid phase was evaporated to dryness under vacuum and the residue was dissolved in 100 µl of 0.1 N HCl.

Amine separation and radioactive measurement

50 µl of purified extract was carefully spotted onto precut 3×20 cm TLC plates with a preabsorption zone. The plates were developed in a chromatography tank for at least one hour. The plates were then dried under a stream of cold air and the individual catecholamines identified under ultraviolet light. Metanephrine (Rf=0.5), normetanephrine (Rf=0.3), and methoxytyramine (Rf=0.7) were scraped off individually and transferred to liquid scintillation vials with the aid of greaseproof paper. The methylated products were diluted with 1 ml 2 N ammonia (Merck), oxidized by the addition of 50 µl periodate. The oxidation was stopped after 5 mins by the addition of 50 µl 10% glycerol solution. The solution was acidified by the addition of 0.5 ml 10 N acetic acid (BHD Analar) and 10 ml scintillation solution was added, shaken vigorously for 10 mins to extract the oxidized products. Samples were counted using Packard Liquid Scintillation Spectrometer or LKB 1217 Rackbeta Liquid Scintillation Counter (LKB Wallac OY, Finland).

Typically the blank and the 60 and 600 fmol standards (routinely assayed in triplicate) yield the following counts per minute:

	Blank	**60 fmol**	**600 fmol**
NA	20-60	150-300	1200-2000
A	20-50	200-350	1700-2500

In general, each experiment was analysed in the same assay to avoid inter-assay variability and allow a better comparison of NA concentrations before and after an intervention. The intra-assay coefficient of variation by repeated analysis of a sample with 2 pmol/ml was 7% for NA and 9% for A. The inter-assay coefficient of variation by the measurement of the standards at concentrations of 60 fmol and 600 fmol/tube were 29% and 28%, respectively. All samples were analysed in duplicate or triplicate and the mean of the measurements was used. The concentration of adrenaline in the effluent collected during sympathetic stimulation was found to be very low (less than 1 pmol/ml) and therefore not included in the data analysis.

2.8 Other Biochemical Measurements

2.8.1 Lactate

Lactate in coronary effluent was measured using a fully enzymatic kit purchased from Boeringer Mannheim (Kit Cat No. 256773).

The following reactions show the principle of the analysis. Lactate was measured spectrophotometrically (340 nm) at 37° C. The equilibrium of the coupled reaction lies

far to the right and therefore ensuring the complete conversion of the first reaction. The standard used was 1 or 10 mM L-lactate.

$$\text{L-Lactate} + NAD^+ \xrightleftharpoons{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

$$\text{Pyruvate} + \text{L-glutamate} \xrightarrow{\text{GPT}} \text{L-alanine} + \alpha\text{-oxoglutarate}$$

LDH = Lactate dehydrogenase

GPT = Glutamate-pyruvate transaminase

A Cobas Bio centrifugal analyser (Roche Diagnostics, Welwyn Garden City, UK) was used for all the measurements. Precinorm S (880 μM lactate, Boeringer) was used as a quality control in every assay. The inter-assay coefficient of variation determined by repeated analysis of Precinorm S over the entire period of the studies was 3.1%.

2.8.2 Lactate Dehydrogenase (LDH)

LDH activity in the coronary effluent was measured using the optimised standard method produced by Boehringer Mannheim (Kit Cat No 543039). The principle of the measurement is the following forward reaction:

$$\text{Pyruvate} + NADH + H^+ \xrightleftharpoons{\text{LDH}} \text{L-lactate} + NAD^+$$

Thus, LDH was measured spectrophotometrically at 340 nm and 37°C by the disappearance of NADH. All solutions were as described in the commercial kit. The activity was measured on a Cobas Bio centrifugal analyser and the results were calculated from the rate of change in optical density. Samples were stored at 4° C and measured within three hours after collection.

Quality control was monitored using Precipath UBS (445 u/L, Boerhinger, West Germany) throughout all studies. The inter-assay coefficient of variation was 7.5%.

2.8.3 Fatty Acid Composition of Myocardial Phospholipids

Hearts were freeze dried for 48 hrs on a Edwards Modulyo freeze drier. A mortar and pestle was used to crush hearts into a fine powder. The powder was weighed (145 mg) and transferred into Quickfit tubes. The phospholipids were extracted in 21 ml choloroform/methanol (2:1 v/v). The lipid extract was then dried down under vacuum using a Buchi-R rotary evaporator and a water bath at 30°C, redissolved in 6 ml

choloroform/methanol (2:1 v/v), dried down again under vacuum, and finally redissolved in 1.0 ml of chloroform/methanol dispensed with a Hamilton syringe.

Phospholipids were separated by TLC using LK5 plates (Whatman). The plates were saturated with 1.2% boric acid and reactivated at 100° C (60 min). 100 µl of the lipid extract was spotted. The plates were developed in $CHCl_3$:MeOH:H_2O:NH_3 (120:75:6:2 by volume) in a paper-lined tank at room temperature. The plates were left to run for one hour, air dried, sprayed with 0.1% dichlorofluorescein, and visualised under ultraviolet light (365 nm). All bands of the five fractions of phospholipids (phosphatidylinositol, phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine and cardiolipin) were scraped off and placed into a Quickfit tube. The methylesters were obtained by base-catalysed transmethylation using toluene and sodium methoxide (0.5 M) and then redissolved in 25 µl chloroform and stored at -20°C for later gas chromatography. Before transmethylation, 70 µg dimargerinyl (C17:0) phosphatidylcholine was added to each tube as internal standard. The methylesters were separated and quantified using a GLC system (Pye Unicam Series 204, Cambridge, UK) fitted with PU 4700 autoinjector and linked to a Trilab Model II Integrator. A glass column (1.5 m, I.D. 2 mm packed with a stationary phase of GP 10% SP2330 on 100/120 mesh Chromosorb WAW, Supelco, Essex, USA) was used for all separations. The relative retention times with respect to C17:0 methylesters were used to identify individual fatty acid methylesters [Wood *et al*1987].

2.9 Statistical Analysis

Statistical analysis was carried out using MINITAB (CLE.COM Ltd, Birmingham, England) Biochemical measurements are normally distributed and the statistical protocol was one- or two-way analysis of variance (ANOVA), followed by paired or unpaired Student's t-test. For the analysis of NA overflow data each animal served as its own control, whenever possible, to eliminate between animal variation and to improve the statistical power. Therefore, NA data are expressed in absolute terms and also as a ratio of the value measured before and after an intervention.

The incidence of arrhythmias is presented as a frequency (%) and were analysed using a Chi-square test. Other parameters which are not normally distributed were analysed using a non-parametric test (Mann Whitney).

All results are presented as Mean±SEM. Statistical significance was assumed if $p<0.05$ was achieved.

2.10 Nerve Stimulation Experiment: General Protocols and Model Characteristics

2.10.1 Stability of the Model

After preparation, all hearts underwent a stabilization period of at least 20 mins. During this period hearts were perfused at a constant flow rate (about 5 ml/g/min). Heart rate and ventricular contractility were continuously monitored (Figure 2.4). It was found that cardiac function became stable after 10 mins. Ischaemia was made either by reducing the flow rate (low-flow ischaemia) or stopping of the perfusion pump (stop-flow ischaemia). During ischaemia, hearts were covered with a thermostatic chamber thereby keeping the intracardiac temperature between 35.3-37°C monitored with a probe thermometer (Technoterm 9300, Testotem Ltd, West Germany).

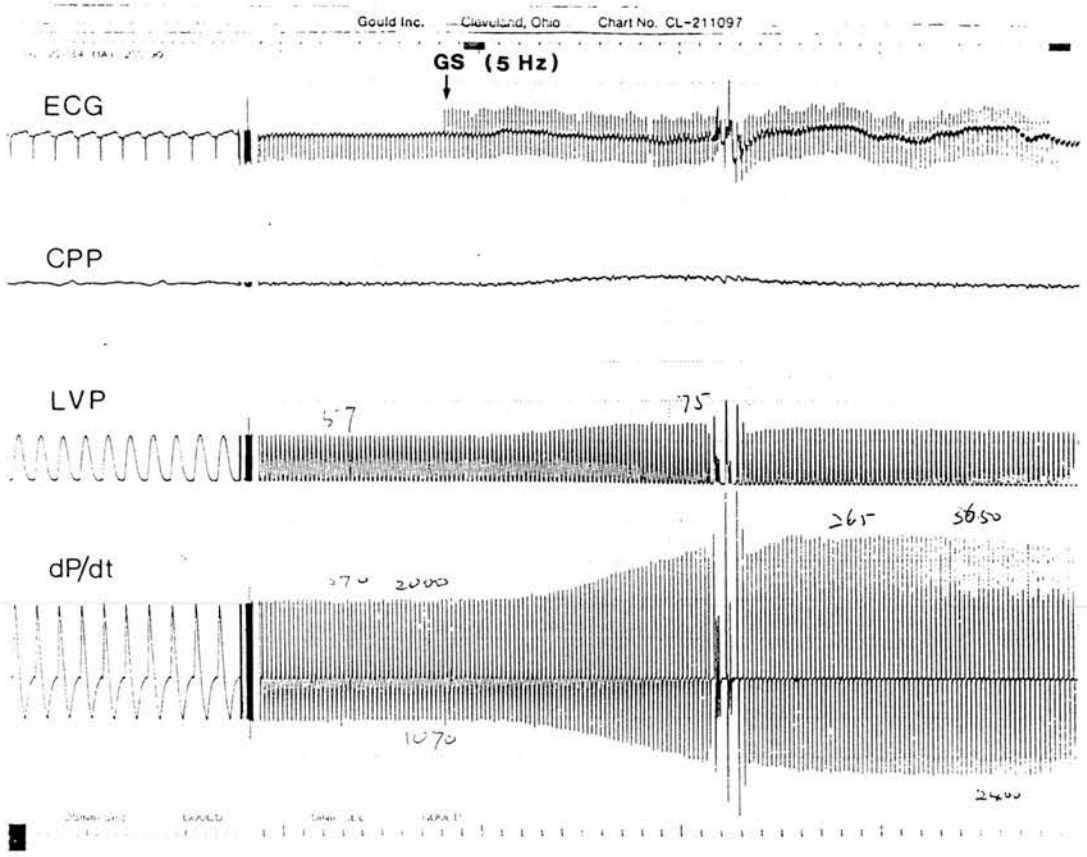


Figure 2.4 *Typical recordings of the epicardial ECG, coronary perfusion pressure, left ventricular pressure, and dP/dt in a normoxically perfused rat heart.*

In some experiments, two hours were necessary to complete the entire protocol. In order to test the stability of the model during this period, seven hearts were perfused

at a flow rate of 5 ml/g/min for 2 hours. Functional and biochemical parameters in coronary venous effluent were monitored throughout (Figure 2.5). All indices remained stable for at least two hours. There was no significant increase in the perfusion pressure. This suggests that critical interstitial oedema did not occur under the experimental conditions. Spontaneous NA overflow in the presence of neuronal reuptake inhibitor desipramine (0.1 μM) was also low and stable. Both vagal and sympathetic nerves remained sensitive to electrical stimulation during the period examined (not shown).

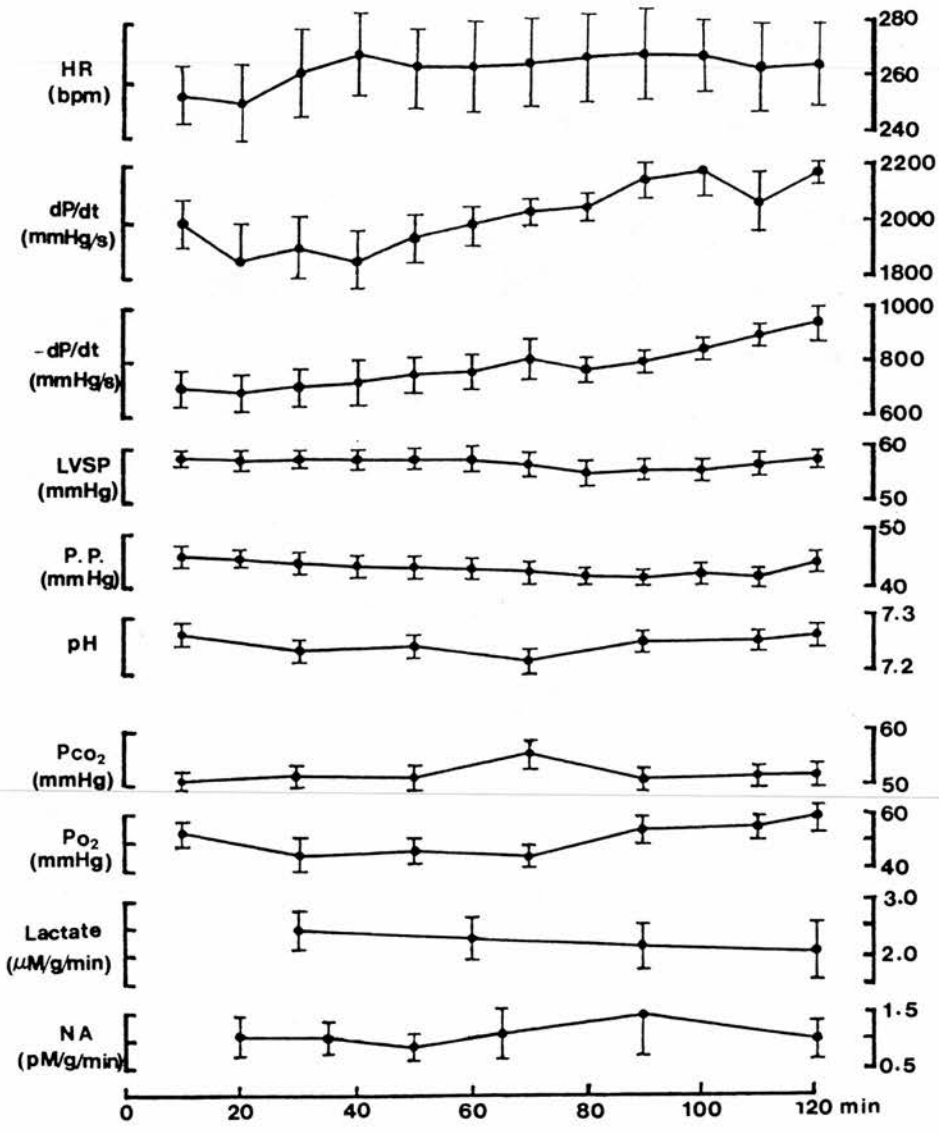


Figure 2.5 *Haemodynamic and metabolic stability of the rat heart perfused at 5 ml/g/min (n=7). No significant changes were observed over a two-hour period in functional indices and biochemical parameters measured in coronary venous effluent (by ANOVA).*

2.10.2 Functional Responses to Nerve or Drug Stimulation

Both sympathetic and vagal nerve stimulation resulted in marked changes in cardiac function depending on the intensity of the stimulation. Peak response of dP/dt, left ventricular systolic pressure (LVSP) and heart rate by sympathetic ganglion stimulation was usually achieved after 20 to 30 secs of nerve stimulation. In contrast, bilateral vagal stimulation resulted in an immediate maximum response (mainly reduction of heart rate, Figure 2.6)

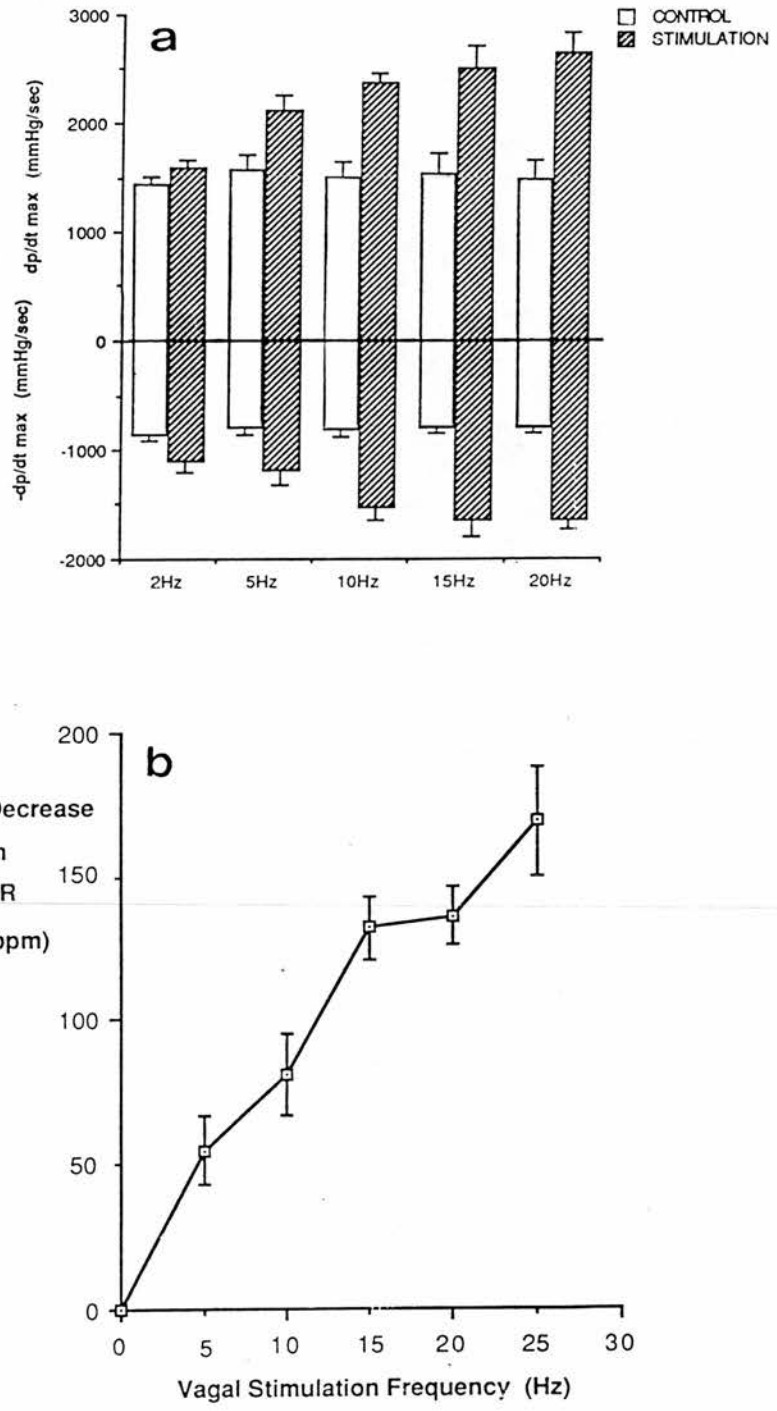


Figure 2.6 *The effects of sympathetic nerve stimulation on dP/dt (A, n=8) and of vagal nerve stimulation on heart rate (B, n=9) in perfused, innervated rat hearts.*

The reliability of the functional measurement in this model was also examined. Normoxic hearts were exposed to concentrations of methacholine (3 groups, n=7-12/group) or isoprenaline (1 group, n=8). Dose dependent changes in ±dP/dt or heart rate were observed (Figure 2.7).

At the end of each experiment, the functional response to a test nerve stimulation was routinely monitored to ensure that an effective nerve stimulation had been maintained.

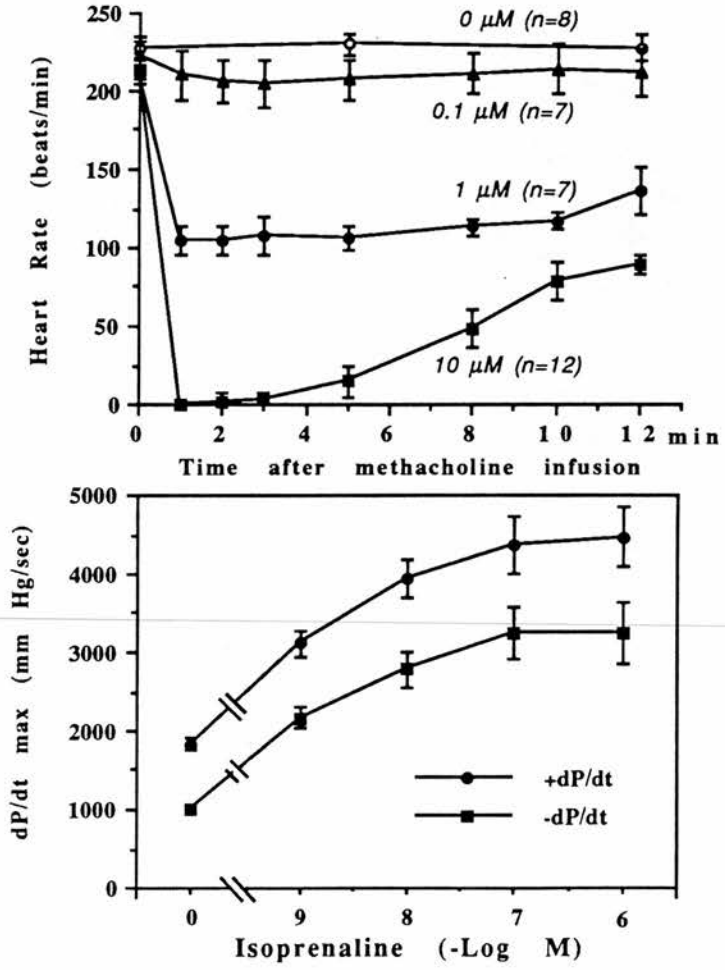


Figure 2.7 *Dose-response relations of the Langendorff perfused rat heart to exogenous cholinergic (methacholine, upper panel n=7-12/group) and adrenergic (isoprenaline, lower panel. n=8) stimulation.*

Preparations were discarded if the following conditions were fulfilled:

- Low recovery of coronary effluent <85% of perfusion flow;
- Poor response to nerve stimulation: <25% increase in peak ±dP/dt (sympathetic ganglion stimulation at 5 Hz), or <30% decrease in heart rate (vagal nerve stimulation at 15 Hz);
- Sustained arrhythmias, including ventricular tachycardia and fibrillation, and atrio-ventricular block during the initial perfusion period prior to nerve stimulations.

In all experiments about 10% of preparations were rejected because of above mentioned reasons, mostly due to a poor response to nerve stimulation.

2.10.3 Nerve Stimulation-Induced NA Overflow

In all experiments, several sympathetic ganglion stimulations (GS), always followed by a 15 min normoxic recovery interval, were delivered during the experimental period, except when specified in Chapter 3, 7 and 8. In four groups of hearts, four consecutive GS (60 sec duration) or 5-6 consecutive GS (30 sec duration) produced a reproducible amount of NA overflow accompanied by a constant increase in heart rate, dP/dt and LVSP (Figure 2.8). In 4 multiple 60 secs nerve stimulation groups, the coefficient of variation (CV%) of NA overflow was smaller if the results were expressed relative to that of the first stimulation (Sn/S1 ratio, Table 2.4). Sn/S1 ratio is generally used for the inter-group comparisons [Dart and Riemersma 1989, Richardt *et al* 1987, Schömig 1988]. Therefore, the inter-group comparisons were made for both NA values and Sn/S1 ratio of NA overflow.

Table 2.4 *Coefficient of variation (CV%) of NA overflow data expressed in absolute terms (pmol/g) or as a ratio of control (Sn/S1)*

	S1	S2	S3	S4
Wistar *(n=8)*				
NA (pmol/g)	55.5%	45.4%	32.5%	59.3%
Sn/S1 Ratio	-	31.7%	28.1%	18.8%
Sprague-Dawley *(n=7)*				
NA (pmol/g)	44.6%	71.3%	56.8%	53.0%
Sn/S1 Ratio	-	52.9%	41.5%	36.7%

In general, collection of coronary effluent started at the beginning of a nerve stimulation for one or two minutes. During ischaemia nerve stimulations were delivered during the final 1 or 0.5 min of ischaemia. Coronary venous effluent was collected during the first min or during the first two mins of reperfusion.

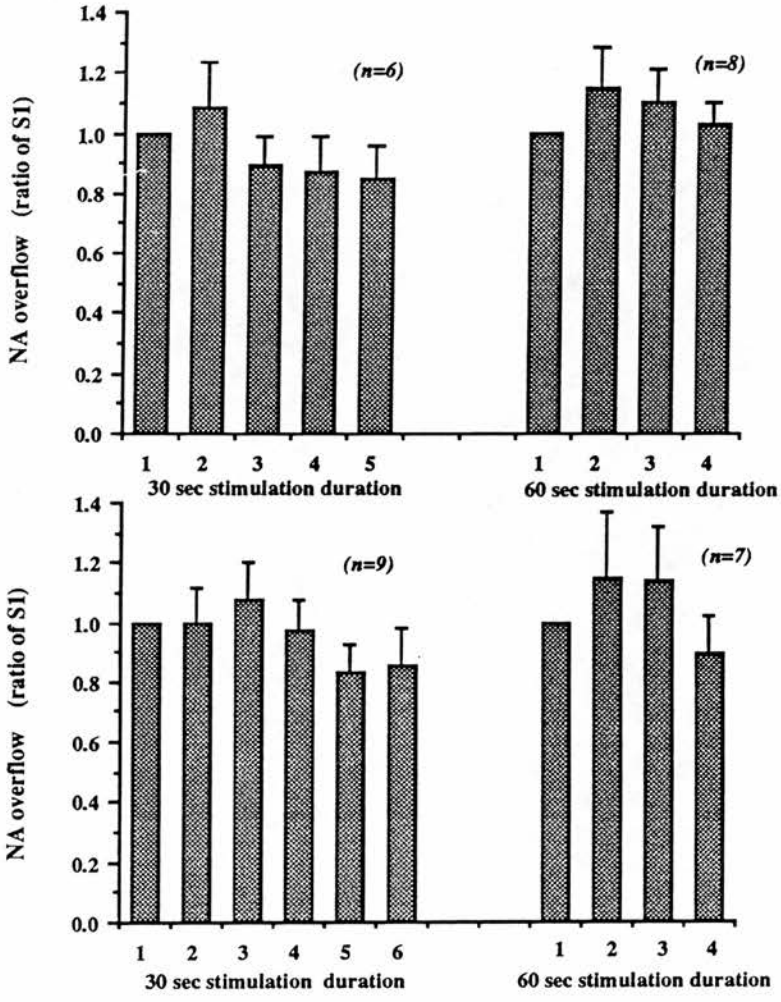


Figure 2.8 *The reproducibility of GS-induced NA overflow in the innervated perfused heart of Wistar and Sprague-Dawley male rats.*

a) NA overflow induced by five times GS (30 sec duration) or four times GS (60 sec duration) in hearts from Wistar rats. S1-induced NA overflow was 16.6±3.9 (30 sec duration) and 32.6±6.4 pmol/g (60 sec duration). Sample collection for 1 min;

b) NA overflow induced by six times GS (30 sec duration) or four times GS (60 sec duration) in hearts from Sprague-Dawley rats. S1-induced NA overflow was 31.0±2.5 (30 sec duration) and 79.7±13.5 pmol/g (60 sec duration). Sample collection for 2 min.

In mid-1989, we switched animals from Wistar to Sprague-Dawley (SD) strain. In order to check whether there was any difference between the strains in sympathetic innervation of the heart, we firstly analysed cardiac NA content. Myocardial NA level in Wistar rats (6.22±0.99 nmol/g, n=5) was about 78% of that in SD rats (7.98±1.54 nmol/g, n=7), but this difference was insignificant. Then we measured NA overflow induced by left stellate ganglion stimulation (GS, 5 Hz for 1 min). Coronary effluent was collected in 1-min aliquots for 2 mins starting at the onset of GS. Under similar perfusion flow rate (SD, 4.9±0.2 ml/g/min, n=7; Wistar, 5.0±0.1 ml/g/min, n=6), NA overflow was significantly higher in SD than in Wistar rat hearts ($p<0.05$, Figure 2.9). Another finding from this experiment was that after 1 min GS, NA overflow during the second min was about 35% of the total collected during the 2 min period and this was not affected by animal strain (Figure 2.9). In another four SD male rat hearts, it was observed that about 98% of total NA overflow induced by a 1 min nerve stimulation was collected within the first 2 mins (Figure 2.10). Therefore, in the studies carried out afterwards, the sampling period of coronary effluent was extended from 1 min to 2 mins in order to collect most of NA released.

Neuronal NA release is greatly affected by ischaemia in this model [Dart *et al* 1984]. In the following chapters, nerve stimulations were applied after one or more episodes of global ischaemia (<10 min) followed by 15 min reperfusion. In order to examine if the effect of ischaemia on neuronal NA release was reversible, eight hearts were subjected to a 10 min period of stop-flow ischaemia plus 10 min reperfusion. GS (5 Hz for 1 min) was given before ischaemia (S1) and after 10 min of reperfusion (S2). NA overflow was 48.0±6.2 pmol/g by S1 and 46.1±7.8 pmol/g by S2 (p=NS). The ratio of NA overflow was 0.98±0.12, indicating a good recovery of neuronal function.

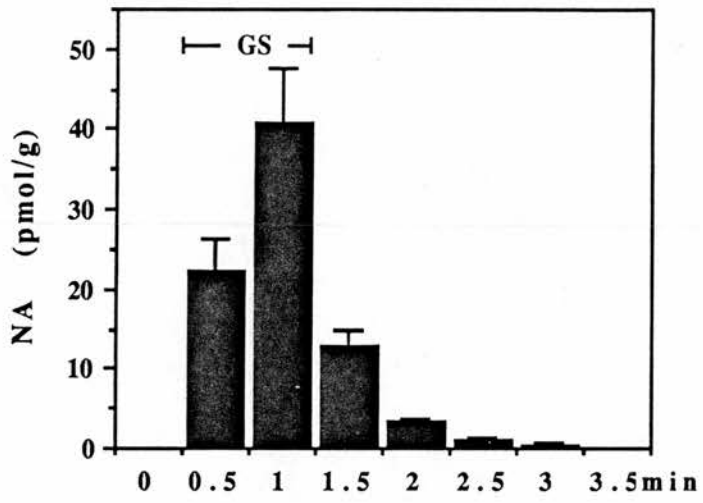


Figure 2.10 *NA overflow in hearts from Sprague-Dawley male rats during normoxia (5.1±0.13 ml/min/g) before, during and after an 1 min sympathetic ganglion stimulation (GS, 5 Hz). Coronary effluent was collected every 30 secs during and after nerve stimulation for a period of 3.5 mins. n=4.*

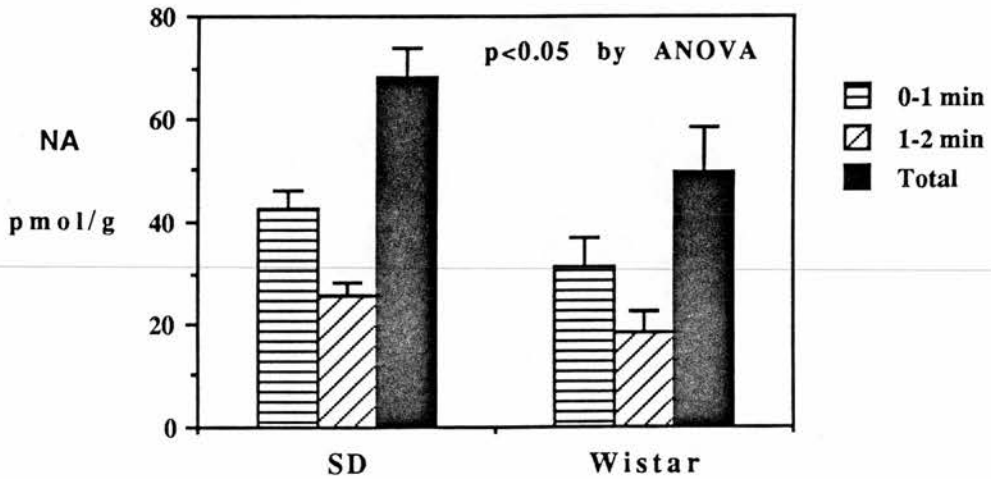


Figure 2.9 *NA overflow induced by a 1 min GS in male Sprague-Dawley (n=7) and Wistar rat hearts (n=6).*

Chapter 3

Neuronal NA Release and the Severity of Myocardial Ischaemia

3.1 Introduction

During early ischaemia, increased efferent sympathetic input to the heart plays an important role in the genesis of ventricular arrhythmias [Corr *et al* 1986, Verrier 1988]. Stimulation of cardiac sympathetic nerves induces ventricular arrhythmias during acute myocardial ischaemia, most probably mediated by neuronal NA release [Corr and Gillis 1978, Schwartz and Vanoli 1981]. However, most of the *in vivo* and *in vitro* studies have shown a reduced neuronal NA release during acute ischaemia when compared normoxic conditions [Dart *et al* 1984b, Schömig 1988, Forfar *et al* 1984]. In the anaesthetized dog, nerve stimulation-induced NA overflow from the ischaemic myocardium fails after 30 mins of coronary ligation [Forfar *et al* 1984]. But an admixture of ischaemic and non-ischaemic effluent collected for the assessment of NA overflow makes the quantitative studies difficult in this model. The effect of stop-flow ischaemia on neuronal NA release has therefore been studied quantitatively in the *in situ* perfused, innervated rat heart. In this model, neuronal NA overflow is largely suppressed within 1-3 mins of stop-flow ischaemia due to combined effects of the presynaptic inhibition, enhanced neuronal reuptake and failure of ATP generation in the nerve terminals [Dart *et al* 1984b, Dart and Riemersma 1985, Richardt *et al* 1987, Schömig 1988]. Other factors like acidosis and extracellular uptake may be of minor importance [Dart and Riemersma 1989, Dart 1989]. This rapid impairment of neuronal NA release is immediately followed by an increasing efflux of NA, mediated by the neuronal reuptake-carrier reversing its normal transport direction [Schömig 1988]. However, extrapolation of these findings to ischaemia *in vivo* may be limited since the experimental condition used was zero flow ischaemia. Reduction of myocardial blood flow within the ischaemic area *in vivo* is neither absolute nor homogeneous [Bolli *et al* 1986, Piek and Becker 1988, Reimer *et al* 1987, Rentrop *et al* 1985, Meesmann 1982]. Until now, there has been no quantitative data on the effect of the severity of ischaemia on neuronal NA release and its presynaptic controlling mechanisms. The effect of low-flow ischaemia on the neuronal reuptake process has apparently not been examined in detail. This is surprising since the role of collateral circulation in preventing ischaemic arrhythmias

has been clearly defined [Bolli *et al* 1986, von Mutius *et al* 1988, Meesmann 1982, Section 1.1.3]. We considered that studying NA release at varying severity of ischaemia *in vitro* may be the best approach to a better understanding of the effect of a heterogeneous ischaemia on sympathetic nerve function *in vivo*. Therefore, using the *in situ* perfused innervated rat heart model (Section 2.2), the effects of the severity of ischaemia on the following aspects were studied:

- nerve stimulation-evoked NA overflow
- neuronal reuptake mechanism
- presynaptic inhibition of neuronal NA release

The flow was selected to reflect the likely flow on the basis of the normal coronary blood flow rate (4-5 ml/g/min) [Malic *et al* 1976] and the reduction in flow during *in vivo* ischaemia [Maxwell *et al* 1987].

3.2 Methods

3.2.1. Materials, Drugs, and Model

Male Sprague-Dawley rats were used for the present study. Global ischaemia of varying severity was made by either reducing coronary flow (low-flow ischaemia) by 90% or 95% to 0.48±0.006 or 0.24±0.003 ml/min/g, respectively, or stopping the perfusion pump (stop-flow ischaemia, 0 ml/min/g). The neuronal NA reuptake inhibitor desipramine, the α-adrenoceptor antagonist phentolamine (1 μM), and the adenosine receptor antagonist 8-phenyltheophylline (10 μM) were used. Desipramine was added from the beginning of all experiments (final concentration 0.1 μM), except when specified. Phentolamine and 8-phenyltheophylline were infused using a Harvard infusion pump. The infusion of these drugs were commenced at least 12 mins before nerve stimulation or the beginning of ischaemia and maintained throughout the period of low-flow ischaemia (Section 2.6).

3.2.2. Protocols

Neuronal NA overflow during low-flow ischaemia and the effect of neuronal reuptake inhibition

After an initial sympathetic ganglion stimulation (GS, 30 sec duration), a 90% (n=7) or 95% (n=6) reduction in coronary flow was induced for 65 mins. GS (30 sec duration) was repeated at 10, 20, 30 and 60 mins of ischaemia. Coronary effluent was collected for 4 mins immediately before and starting with GS. In another 6 hearts subjected to 95% flow reduction, desipramine was omitted from the perfusate, but the rest of the protocol was identical.

Neuronal NA overflow during ischaemia of various severity

Obviously it is impossible to collect effluent during stop-flow ischaemia. Therefore, to compare the effect of stop- with low-flow ischaemia on neuronal NA release, nerve stimulation applied during ischaemia was immediately followed by reperfusion and coronary effluent was collected during the first minute of reperfusion. In eight groups of hearts (n=5-9 per group), after an initial GS (S1, 1 min) during normoxia as individual control, a second GS (S2, 1 min) was carried out during either stop-flow (2 groups) or low-flow ischaemia of 90% (3 groups) and of 95% flow reduction (3 groups). The duration of stop-flow ischaemia were 3 or 10 mins, and of low-flow ischaemia was 10, 30 or 60 mins. For comparison, eight hearts were continuously perfused in normoxic flow rate and GS (1 min) was performed twice with a 45 minute interval between the two stimulations.

In another four groups of hearts, the possibility was tested that NA overflow in this case was caused by the reperfusion process *per se*. In two groups, 90% (n=7) and 95% (n=7) flow reduction was maintained for 60 mins followed by reperfusion. Spontaneous NA overflow was determined in the effluent samples collected during normoxia and during the first minute of reperfusion. The effect of ischaemia on myocardial metabolism was monitored by analysing effluent levels of lactate, pH and Pco_2 during ischaemia and LDH release during reperfusion. In the remaining two groups, hearts were subjected to stop-flow ischaemia of 10 mins (n=9) or 40 min (n=6) followed by reperfusion. NA overflow immediately before ischaemia and during the first min of reperfusion was analysed.

Presynaptic control of NA release during normoxia and ischaemia

In six groups (6-10 hearts per group), after an initial GS (S1, 1 min) as an individual control, hearts were normoxically perfused for a further 10 mins either with phentolamine (1 μM, three groups) or 8-phenyltheophylline (10 μM, three groups). In two groups receiving either of the drugs, a second GS (S2, 1 min) was applied during normoxia. In the remaining four groups, 10 mins stop-flow ischaemia (two groups with either of the two drugs) or 60 mins of 95% flow reduction (two groups) were made and S2 was given during the final minute of ischaemic period followed by reperfusion. Coronary effluent for the determination of neuronal NA overflow was collected for 1 min during GS in normoxia or during the first minute of reperfusion.

3.3 Results

3.3.1. Low-flow Ischaemia and Neuronal NA Overflow

Before and during 60 mins low-flow ischaemia, both NA concentrations in the coronary effluent and heart rate were increased by each GS in the three groups ($p<0.01$, Figure 3.1 and Table 3.1). In the group with 95% flow reduction and in the presence of desipramine, the duration of ischaemia was extended to 90 mins in three hearts and another nerve stimulation was given at the 90 th minute of ischaemia. NA concentration in coronary effluent was increased by stimulation from 0.84±0.29 to 9.79±3.29 pmol/ml. Spontaneous NA overflow was less than 0.5 pmol/min/g throughout the 60-min period of ischaemia and did not differ between the three groups (Figure 3.1).

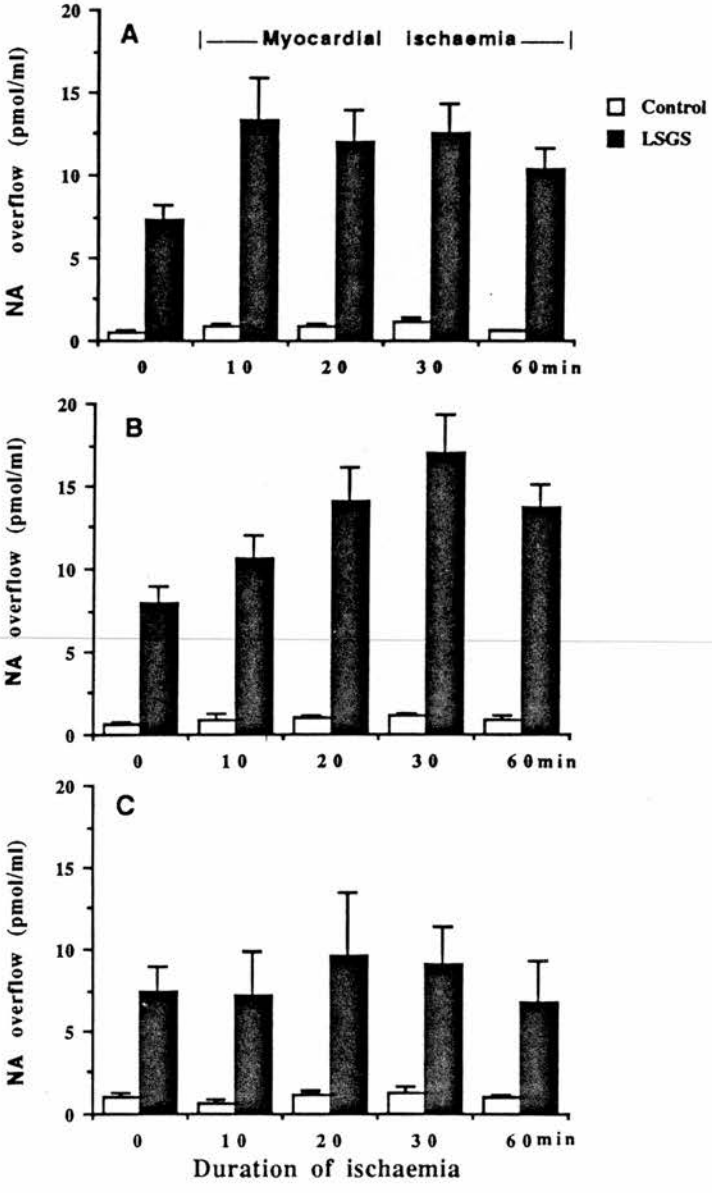


Figure 3.1 *NA concentrations in the coronary venous effluent before and during a 60-min period of low-flow ischaemia and the effect of left sympathetic ganglion stimulation (GS) at 5 Hz for 30 seconds. Basal NA levels remained unchanged throughout the ischaemic period, and each GS significantly elvated NA concentration ($p<0.01$). A: 90% flow reduction with desipramine; B: 95% flow reduction with desipramine; C: 95% flow reduction without desipramine.*

In all the three groups of hearts with 60 mins low-flow ischaemia despite the increase in NA concentrations, GS-induced NA overflow was reduced in amount ($p<0.05$-0.01 *vs* S1) and remained stable throughout the period studied (Figure 3.1 and Figure 3.2). In hearts perfused without the neuronal reuptake inhibitor desipramine, NA overflow was less than in its presence ($p<0.01$). In the presence of desipramine, neuronal NA overflow was lower in the group with 95% flow reduction than in the group with less severe ischaemia ($p<0.01$).

Table 3.1 *Heart rate (beats/min) before and during low-flow ischaemia and the effect of sympathetic ganglion stimulation (GS, 5Hz for 30 secs). DMI denotes desipramine.*

		Duration of Low-flow Ischaemia (min)			
	Normoxia	10	20	30	60
90% Ischaemia (+DMI)					
Control	211±20	55±11	45±13	50±14	47±14
GS	291±31	114±14	89±13	107±13	105±15
95% Ischaemia (+DMI)					
Control	200±8	19±6	11±6	6±3	6±3
GS	263±9	93±22	60±11	53±8	56±11
95% Ischaemia (-DMI)					
Control	207±16	20±13	3±3	9±9	15±15
GS	266±13	76±11	57±15	66±10	73±13

1) The increase in heart rate differed during ischaemia between the 90% and 95% flow reduction groups with desipramine (two-way ANOVA; $p<0.01$); 2) Desipramine did not affect change in heart rate (in comparison to the 95% ischaemia group without DMI , NS).

3.3.2. Severity of Ischaemia and Neuronal NA Overflow

Using a slightly different protocol in which ischaemia was followed by reperfusion and therefore allowed the comparison of stop- with low-flow ischaemia, a flow-dependent reduction in GS-induced NA overflow was observed (Figure 3.3). Stop-flow ischaemia largely inhibited neuronal NA overflow within the first 10 mins ($p<0.001$). NA overflow was reduced within 10 mins in the hearts subjected to 90% flow reduction ($p<0.05$) and no further reduction was found in this group between 10 and 60 mins (NS). In the hearts with 95% flow reduction, a progressive reduction of NA overflow occurred during this period ($p<0.01$) and S2/S1 ratio of NA overflow at 60 mins of ischaemia tended to be lower than in the hearts with 90% ischaemia ($p=0.06$). In normoxic perfused hearts S1- and S2-induced NA overflow was comparable.

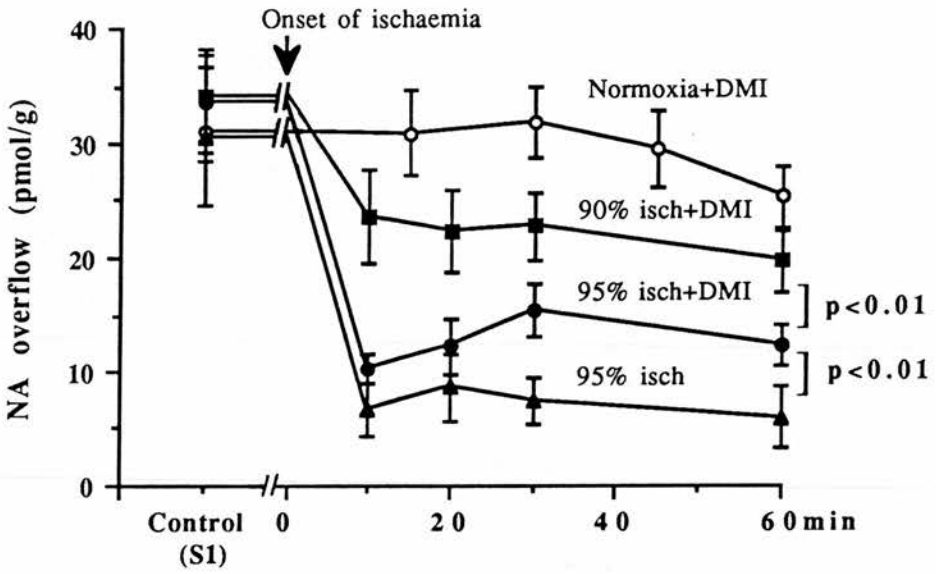


Figure 3.2 *Neuronal NA overflow during 60 mins of 90% and 95% coronary flow reduction to 0.48 and 0.24 ml/min/g, respectively. GS (5Hz for 30 secs) was applied once before and four times during ischaemia. Coronary effluent was collected for over 1 min during normoxia and over 4 mins during ischaemia. P values indicate the difference of the curves by two-way analysis of variance. DMI denotes desipramine (0.1 μM).*

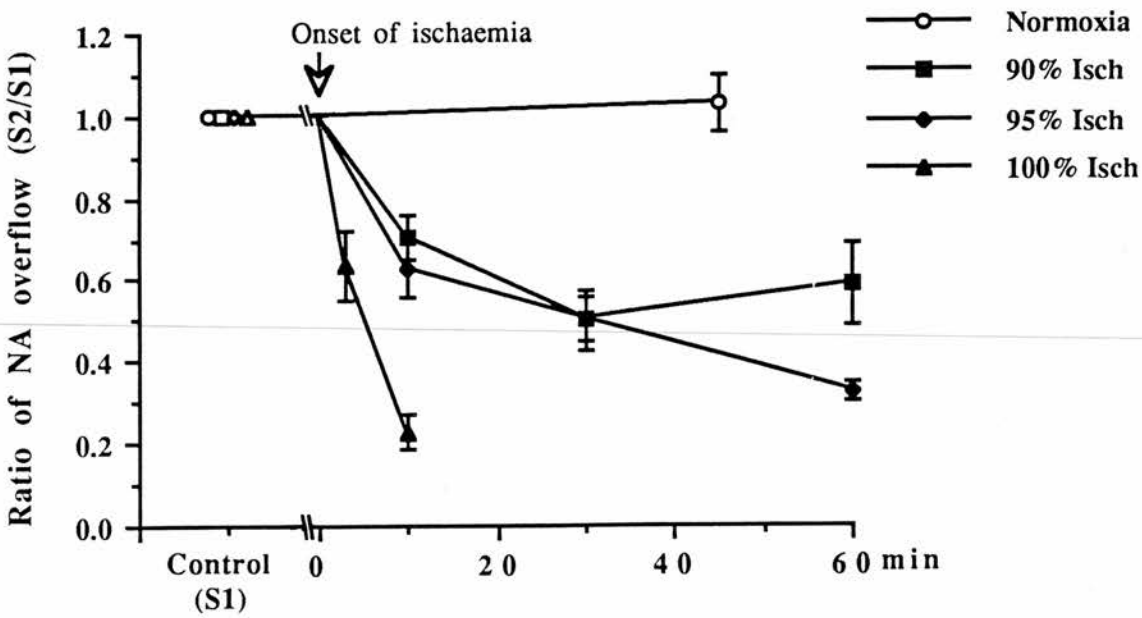


Figure 3.3 *Relation between the severity of ischaemia and neuronally-mediated NA overflow in innervated perfused rat hearts. NA overflow was induced by GS applied before ischaemia (S1) and during the final minute of a period of ischaemia (S2). Results are presented as S2/S1 ratio. There was no significant difference in S1-induced NA overflow between the 9 groups (p>0.4). The progressive decline of S2/S1 ratio during 95% and 100% ischaemia is statistically significant (p<0.01). Each spot represents mean (±SEM) of 5-9 observations. Open symbols denote normoxic values.*

In comparison with pre-ischaemic values, reperfusion did not enhance spontaneous NA overflow after 60 mins low-flow ischaemia of 90% (0.4±0.1 and 0.6±0.2 pmol/min/g, NS) or 95% (1.2±0.5 and 2.9±0.5 pmol/min/g, NS), or after 10 min stop-flow ischaemia (1.1±0.3 and 0.9±0.4 pmol/min/g, NS), except after 40 min stop-flow ischaemia (1.4±0.3 and 104.5±24.0 pmol/min/g, $p<0.001$).

3.3.3. Severity of Ischaemia and Presynaptic Inhibitory Mechanisms

In normoxia, NA overflow was increased over 2-fold by the α-adrenergic antagonist phentolamine from 41.0±8.5 to 96.8±10.8 pmol/min/g ($p<0.01$), but was not significantly affected by the adenosine receptor antagonist, 8-phenyltheophylline (54.7±9.4 to 49.9±3.5 pmol/min/g, NS, Figure 3.4). In the absence of these drugs, 10 mins stop-flow or 60 mins low-flow ischaemia reduced NA overflow from 54.4±6.4 to 12.4±1.8 pmol/min/g ($p<0.001$) or from 47.1±5.3 to 14.9±0.8 pmol/min/g ($p<0.001$), respectively. There was no significant difference in S2/S1 ratio between the two groups (0.24±0.03 *vs* 0.32±0.03, NS). Both phentolamine and 8-phenyltheophylline failed to increase neuronal NA overflow in the hearts with 10 mins stop-flow ischaemia (NS, Figure 3.4). In contrast, with 60 mins 95% flow reduction, NA overflow was partly restored to the pre-ischaemic levels by phentolamine (26.4±4.9 *vs* 45.5±4.0 pmol/min/g, $p<0.05$) and 8-phenyltheophylline (34.1±7.8 *vs* 45.7±9.3 pmol/min/g, NS). Both drugs caused significantly higher S2/S1 ratios than measured in the hearts without drug treatment ($p<0.05$ and 0.01, respectively; Figure 3.4). Meanwhile, S2-induced heart rate increase was also greater in the drug treated hearts than those without drug (+78±13 *vs* +41±11 beats/min, $p<0.05$).

3.3.4. Low-flow Ischaemia, Myocardial Lactate Release and Acidosis

Ischaemia resulted in flow-dependent changes in the coronary effluent levels of lactate, pH and Pco_2 (Figure 3.5). During the first 5 mins of reperfusion following a 60 min period of 95% flow reduction, LDH overflow was 3.84±0.92 U/g. In the hearts subjected to a 65-min period of 90% or 95% flow reduction, the incidence of ventricular fibrillation within the first 2 mins of reperfusion was 15% (3/20) and 51% (29/57, $p<0.01$), respectively.

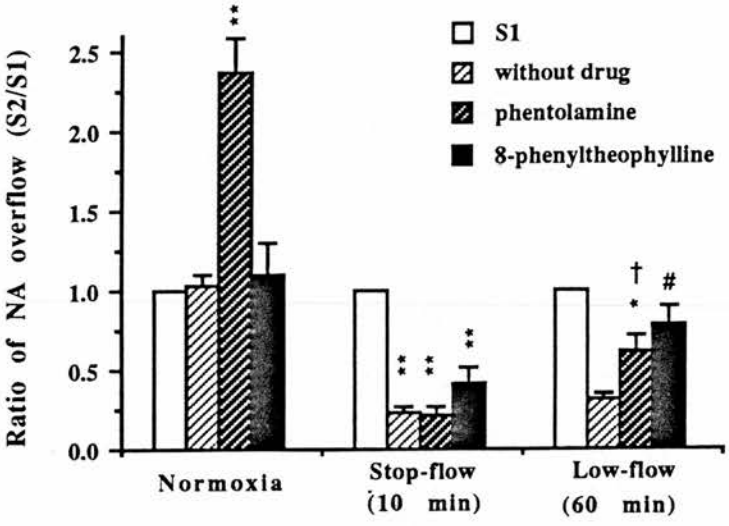


Figure 3.4 *Effect of severity of ischaemia (95% and 100% reduction of coronary flow on presynaptic α–adrenergic and purinergic inhibition of nerve stimulation-mediated NA overflow. GS (5 Hz for 1 min) was performed twice. The first GS (S1) was to determine neuronal NA overflow during normoxic, control conditions. The second GS (S2) was made in the absence (control) or presence of phentolamine or 8-phenyltheophylline, to determine whether the presynaptic receptor antagonists could restore neuronal NA overflow to pre-ischaemic levels (S1, 100%) and the results of NA overflow are presented as S2/S1 ratio. No significant difference in S1-evoked NA overflow was found between the 9 groups (p>0.2). Each column represents the mean (±SEM) of 5-10 observations.*
** p<0.05 and ** p<0.01 vs S1; + p<0.05 and # p<0.01 vs group without drug*

3.4 Discussion

Our study demonstrates the importance of the severity of ischaemia for neuronal NA release and its controlling mechanisms. In the hearts subjected to 60 mins low-flow ischaemia, reduction of NA overflow is more pronounced in the hearts with 95% than in those with 90% flow reduction. In the ischaemia/reperfusion series, the most severe ischaemia (stop-flow) largely suppressed neuronal NA overflow within 10 mins. When coronary flow was reduced by 95%, decline of NA overflow increased with time. In contrast, a higher residual flow (0.48 ml/min/g) prevented this progressive reduction although NA overflow remained significantly depressed. This

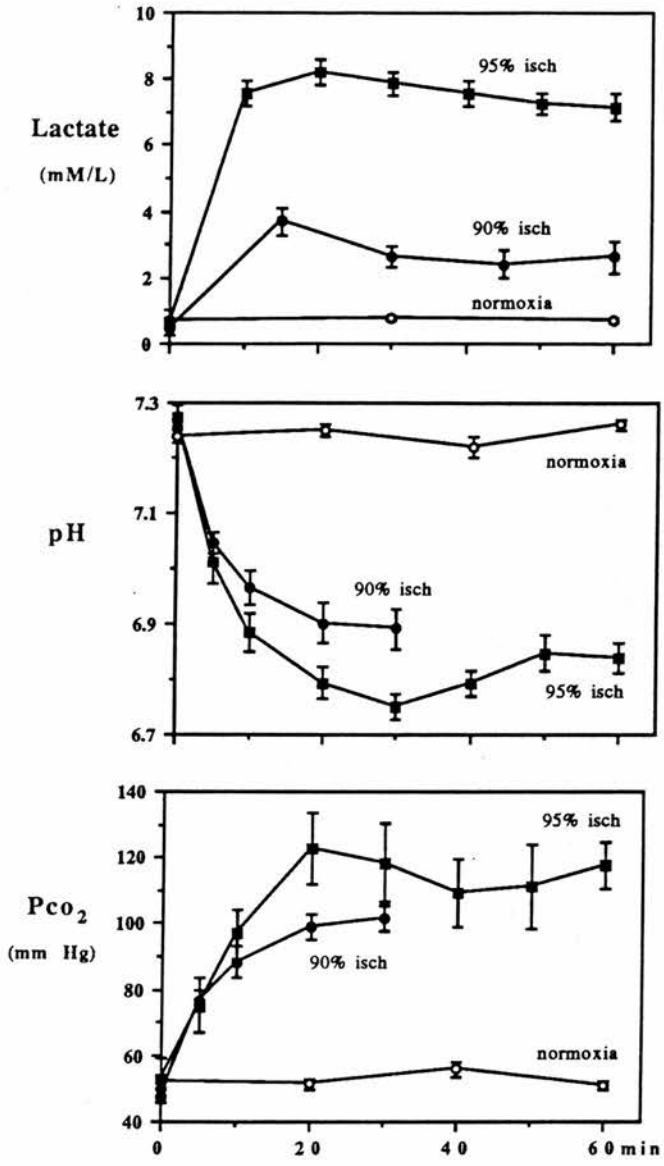


Figure 3.5 Changes in lactate, pH and Pco_2 levels in coronary venous effluent from normoxic and ischaemic rat hearts. The average flow rate was 4.8 ml/min/g for normoxia and 0.48 or 0.24 ml/min/g for 90% and 95% ischaemia, respectively. All changes of these parameters during ischaemia are highly significant and flow-dependent.

flow-dependent phenomenon is important for the understanding of the patho-physiology of the ischaemic heart. An inhibited neuronal NA release may protect the ischaemic myocardium from excessive adrenergic stimulation. However, as the severity of ischaemia is heterogeneous *in vivo* [Bolli *et al* 1986, Reimer *et al* 1987, Pirk and Pecker 1988], it is likely that this suppression is not uniform. Marked metabolic disturbances, such as acidosis, accumulation of lactate and carbon dioxide, intracellular enzyme leakage, and a high incidence of reperfusion VF show that 95% flow reduction is rather severe for the myocardium. However, sympathetic neurotransmission i.e. GS-evoked increases in NA overflow and heart rate, is still

preserved, although depressed, at least within the observation period of 90 mins. It would then seem that adrenergic nerve terminals would be more resistant to ischaemia than the myocardium. Indeed, a more efficient glycolytic ATP production and a low ATP demand for adrenergic nerve fibres to maintain a normal function has been described in the perfused heart and cultured neurons [Dart *et al* 1987, Carlsson 1988, Wakade and Wakade 1985].

In the hearts with 95% flow reduction, inhibition of neuronal reuptake by desipramine doubled NA overflow throughout the 60-min period of ischaemia although the difference in NA overflow during normoxia is significant. This may be due to a prolonged exposure of released NA to the reuptake process during ischaemia. In the present study, we did not compare the effectiveness of desipramine with increasing severity and duration of ischaemia. However data is avaliable from the rat heart with stop-flow ischaemia. There is a progressive reduction in the efficiency of this reuptake mechanism during anoxia and metabolic blockade [Schömig 1988]. Dart *et al* [1984b] have shown that the effectiveness of this drug in restoring neuronal NA overflow is significantly lower during 9-10 mins than that during 0-1 min of stop-flow ischaemia. When stop-flow ischaemia extends to 15 mins, this reuptake mechanism will reverse its normal transport direction to initiate a "carrier-mediated NA efflux", a process independent of neuronal activity [Schömig 1988, Dart *et al* 1987]. Therefore, the neuronal reuptake mechanism is also influenced by the severity of ischaemia and, as shown in our study, remains effective in limiting NA accumulation during prolonged ischaemia, provided a small residual flow is maintained.

Another important observation in this study is that neuronal NA release maintains its sensitivity to the presynaptic inhibition up to 60 mins of 95% flow reduction, but not during the first 10 mins of stop-flow ischaemia. Although neuronal NA overflow was similarly reduced in both experimental conditions, the underlying mechanisms may be different. After 60 mins of 95% flow reduction, phentolamine partially and 8-phenyltheophylline almost completely restored NA overflow to normoxic control levels. Unfortunately, we were not be able to analyse the levels of adenosine in the effluent at this stage. Recently, Headrick *et al* [1989] have shown that the increase of adenosine levels in the coronary effluent correlates well with the extent of flow reduction in the isolated perfused rat heart. Using the *in situ* perfused rat heart model, Richardt *et al* [1987] have also found that within a few minutes of stop-flow ischaemia, effluent concentrations of adenosine increase to levels that inhibit neuronal NA release. Therefore, the effectiveness of 8-phenyltheophylline during prolonged low-flow ischaemia in our study indicates a high interstitial adenosine concentration.

In contrast, blocking adenosine receptors has no detectable effect during normoxia. probably due to a low prevailing level of adenosine [Richardt *et al* 1988, Headrick *et al* 1989]. Failure of adenosine receptors to modulate NA release within the first 10 mins stop-flow ischaemia has been reported [Richardt *et al* 1987]. Two possibilities are postulated by the authors: high interstitial concentration of adenosine which competes with 8- phenyltheophylline for the presynaptic purinergic receptors, and failure of neurotransmission as a result of ATP depletion. Thus the previous and the present studies show that the presynaptic modulatory mechanisms, mediated by α-adrenergic and purinergic receptors, all fail within 10 mins of stop-flow ischaemia, suggesting a common phenomenon during most severe ischaemia [Dart *et al* 1984b, Richardt *et al* 1987]. This is also true for cholinergic presynaptic inhibition [Chapter 4]. Therefore, in the present study carried out in the presence of desipramine, ATP depletion may be the major reason for the failure of NA release after 10 min stop-flow ischaemia and also for the onset of the carrier-mediated NA efflux soon afterwards [Schömig *et al* 1984a, Dart *et al* 1987]. Presynaptic modulation is of minor importance after 10 mins stop-flow ischaemia but is largely responsible for a reduced NA release during prolonged low-flow ischaemia.

Our observations may have some implications for the understanding of cardiac sympathetic activity in the *in vivo* ischaemic heart. Myocardial blood flow reduction is heterogeneous [Reimer *et al* 1987, Piek and Becker 1988, Meesmann 1982]. In moderate ischaemic regions, neuronal NA release is limited by both presynaptic inhibition and enhanced reuptake. Carrier-mediated NA efflux may not occur. In regions with severe ischaemia, energy depletion is mainly responsible for a profound failure of neuronal NA release followed by carrier-mediated NA release soon afterwards. Thus, the resultant spatial and temporal diversity in the adrenergic stimulation of the ischaemic myocardium would contribute to arrhythmia development. The observed dependence of sympathetic nerve function on the severity of ischaemia may be involved, at least in part, in the salutary impact of collateral circulation on early ischaemic arrhythmias [Bolli *et al* 1986, von Mutius *et al* 1988, Meesmann 1982, Webster et al 1974, Section 1.1.3]. Critical amount of collateral flow maintains a normal neuronal uptake function, thereby preventing the onset of carrier-mediated NA efflux [Schömig 1988]. NA release *via* this pathways has been demonstrated to be arrhythmogenic [Riemersma *et al* 1986b, Dietz *et al* 1989].

Chapter 4

Cholinergic Modulation of Noradrenaline Release during Acute Myocardial Ischaemia

4.1 Introduction

The autonomic nervous system plays an important role in the pathogenesis of malignant arrhythmias during acute myocardial ischaemia [Verrier 1988, DeSilva 1982]. The arrhythmogenic effects of enhanced sympathetic stimulation, whether produced by electrical, pharmacological or psychological means are well known. Chronic sympathetic denervation with depletion of noradrenaline (NA) content is protective against subsequent ischaemic arrhythmias [Schwartz and Stone 1980, Puddu *et al* 1988]. The influence of parasympathetic activation is less clearly defined and some studies have shown an inhibitory effect on early ischaemic arrhythmias [Schwartz and Stramula-Badiale 1988, Verrier 1988, Corr and Gillis 1978], which is believed to be due largely to antiadrenergic mechanisms, including the presynaptic inhibition of exocytotic NA release [Corr *et al* 1987, Levy 1984]. Recent clinical and experimental studies have demonstrated that an autonomic imbalance may be critical for the occurrence of lethal ischaemic arrhythmias [La Rovere *et al* 1988, Schwartz *et al* 1988a, Section 1.2.4].

Exocytotic NA release within the heart is largely affected by myocardial ischaemia [Dart *et al* 1984b]. Although presynaptic sympathetic- parasympathetic interactions, mediated by α-adrenergic and muscarinic cholinergic receptors, have been well recognized in normoxic preparations [Levy 1984, Löffelholz and Pappano 1985], and speculated to contribute to the vagal protection against ischaemic arrhythmias [Zuanetti *et al* 1987, Corr *et al* 1986], little is known about how this aspect of sympathetic nerve function may be affected by ischaemia. Coexistence of efferent sympathetic and parasympathetic overactivity may be a frequent pathophysiological event during early myocardial ischaemia [Pantridge 1978, Corr and Gillis 1978]. This study was designed to examine the influence of acute ischaemia on the cholinergic presynaptic modulation of exocytotic NA release in the rat heart.

4.2 Methods

4.2.1 Animals, Drugs and Model Characteristics

Male Wistar rats were used for this study. The experimental model was the *in situ* retrogradely perfused, innervated heart model (Section 2.2). All experiments were performed in the presence of 0.1 μM desipramine to inhibit neuronal reuptake of NA. Choline chloride (10 μM), physostigmine (1 μM), phentolamine (1 μM) and methacholine were also used as specified in Section 2.6. Coronary effluent was collected for 1 min in normoxically perfused hearts starting with the GS, and for 1.5 min in ischaemic-reperfusion experiments as little effluent was collected during the first 30 sec of reperfusion.

To define the concentration of methacholine to be used, we examined the relation between methacholine concentration and exocytotic NA overflow in seven normoxic hearts. After a control sympathetic nerve stimulation (S1, 30 sec duration), methacholine was infused into the perfusate giving final concentrations of 0.1, 1, 5 and 10 μM respectively. Four more consecutive sympathetic nerve stimulations (30 sec duration with 12 min intervals) were then performed at each concentration of methacholine. NA overflow evoked by S2-S5 was significantly inhibited in a dose dependent manner ($p<0.01$-0.001 *vs* 16.5±1.3 pmol/g/min by S1, Figure 4.1). On the basis of these results methacholine was used for all studies, except specified, at a dose of 10 μM, which inhibited over 80% of NA release.

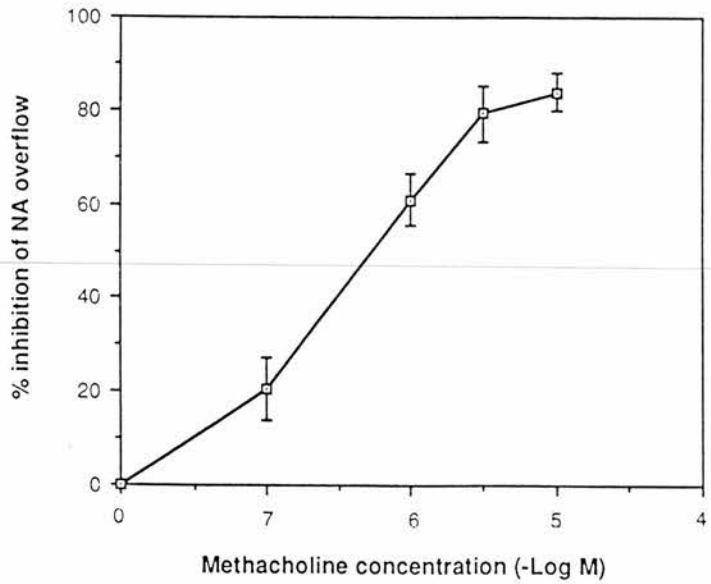


Figure 4.1 *Effects of various concentrations of methacholine on stimulation-mediated NA overflow in normoxic rat hearts (n=7). Left sympathetic ganglion was stimulated five times (30 sec duration) separated by 15 min intervals. Methacholine was added before each of the last four stimulations. Neuronal NA overflow was significantly inhibited by each concentration of methacholine. Points and vertical bars indicate Mean±SEM.*

In 35 normoxic hearts, sympathetic stimulation resulted in a significant increase in heart rate (209±7 to 257±8 beats/min), +dP/dt (1587±28 to 2196±35 mm Hg/sec) and -dP/dt (769±22 to 1175±27 mm Hg/sec). A profound reduction in heart rate and ±dP/dt were recorded during vagal nerve stimulation: heart rate (235±5 to 90±7 beats/min), +dP/dt (1640±35 to 857±32 mm Hg/sec) and -dP/dt (863±39 to 456±18 mm Hg/sec). When combined sympathetic and vagal nerve stimulation was performed, all the functional parameters showed marked reduction from pre-stimulation levels: heart rate (229±16 to 77±14 beats/min), +dP/dt (1589±28 to 904±42 mm Hg/sec), -dP/dt (812±26 to 437±15 mm Hg/sec).

In 12 normoxically perfused hearts with heart rate controlled by pacing at 300-350 beats/min with a pacemaker (APC type E4162, Welwyn Garden City, UK), a significant and stable increase in ±dP/dt and ventricular pressure was achieved by sympathetic stimulation at 2 Hz. Vagal nerve stimulation (15 Hz) superimposed on sympathetic stimulation decreased +dP/dt by 6 %, -dP/dt by 11%, and LVSP by 7% (Figure 4.2).

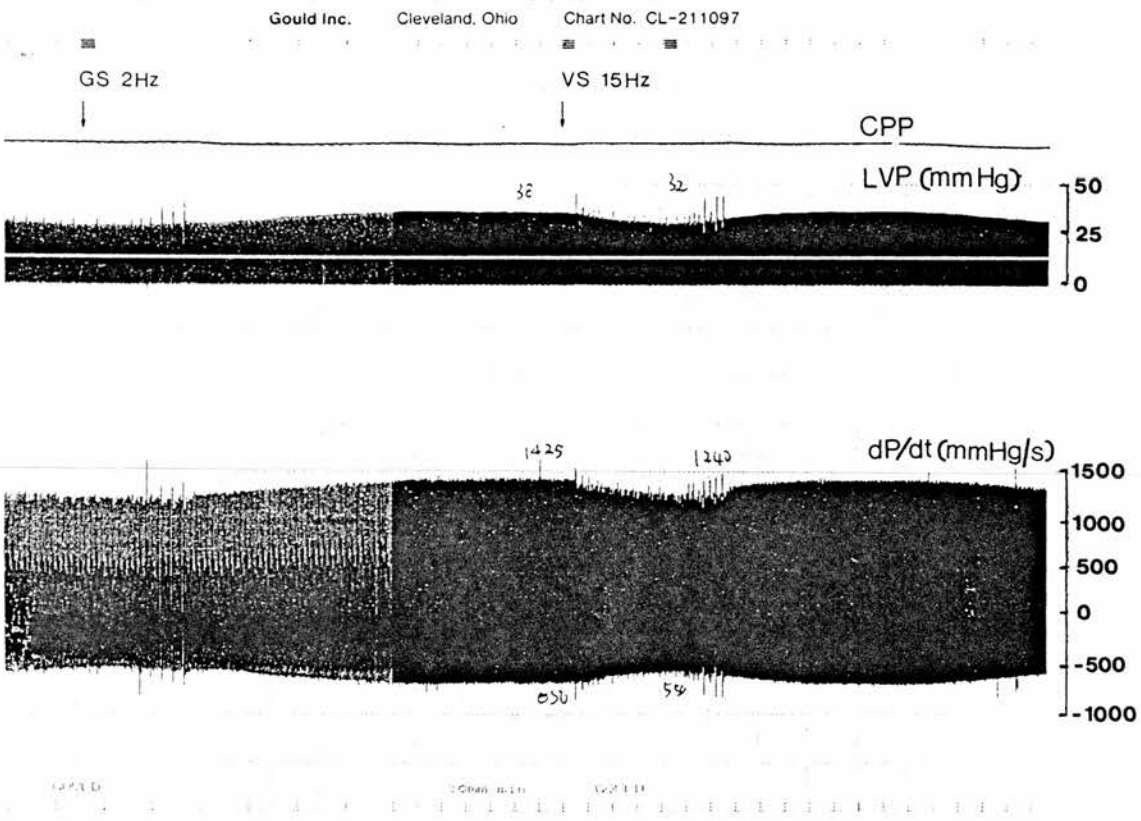


Figure 4.2 *A typical recording of the effect of vagal nerve stimulation on ventricular contractility of a perfused rat heart. Heart rate was kept constant during nerve stimulation by pacing at 330 beats/min. GS (2 Hz) increased LVSP and ±dP/dt and concomitant bilateral vagal nerve stimulation (15 Hz) reduced those parameters.*

Spontaneous NA overflow (in the absence of nerve stimulation) was always found to be very low (about 1 pmol/g/min). To check if vagal nerve stimulation alone could contribute to NA overflow, we collected coronary effluent from six normoxic hearts immediately before and during vagal nerve stimulation (15 Hz for 1 min). Spontaneous NA overflow was 1.41±0.13 pmol/g/min and vagal nerve stimulation did not affect this value (1.43±0.19 pmol/g/min).

4.2.2 Protocols

Vagal nerve stimulation and NA overflow

The effect of vagal nerve stimulation on neuronal NA overflow was examined during normoxia and during either low-flow (0.25 ml/g/min) or stop-flow ischaemia of varying duration.

In 14 hearts, sympathetic or combined sympathetic and vagal nerve stimulation (1 min duration) were each performed, in random order, during normoxic perfusion.

In ischaemic experiments each heart was subjected to two equal periods of low-flow ischaemia separated by a 15 min recovery period of normoxic perfusion. GS or combined sympathetic and vagal stimulation was applied during the final 1 min of each ischaemic period. The order of stimulation was random. The duration of low-flow ischaemia was 3 (n=8) or 10 mins (n=8), and of stop-flow ischaemia was 1 (n=12), 3 (n=8), or 5 mins (n=9). In all experiments physostigmine (1 μM) was added to the perfusate to minimize ACh hydrolysis by cholinesterase, and choline chloride (10 μM) was added as a substrate for ACh resynthesis. Physostigmine at this dose potentiated the heart rate-lowering effect of vagal nerve stimulation (15 Hz for 1 min) from -97±7 to -170±8 beats/min (n=13-16, $p<0.01$).

In order to examine if the effect of ischaemia on vagally-mediated modulation of NA release is reversible, 1 min GS (5 Hz) was given during normoxic perfusion, then a 10-min period of stop-flow ischaemia was made followed by 10 mins reperfusion (n=8/group). A combined sympathetic and vagal stimulation was then applied. In another group (serving as a control), two GS were applied separated by 10 mins stop-flow ischaemia followed by 10 mins reperfusion.

Effect of α-adrenoceptor antagonist on cholinergic inhibition of NA overflow

In eight normoxic hearts the effect of phentolamine on vagally-induced inhibition of NA release was assessed. After an initial 1 min sympathetic nerve stimulation, phentolamine was continuously infused (final concentration 1 μM). Sympathetic or combined sympathetic and vagal nerve stimulation (1 min) were then performed, in random order, separated by a 15-min recovery period. The same protocol was followed during stop-flow ischaemia of 1 (n=8), 3 (n=8), or 5 mins (n=9).

Physostigmine (1 μM) and choline chloride (10 μM) were present throughout.

It is well known that α-blockade increases NA release [Dart *et al* 1984b, Section 1.3.4]. However, it is unclear if cholinergic inhibition of NA release could be potentiated when NA release is enhanced. In order to examine this possibility, GS was performed twice in normoxic hearts in the absence and presence of 0.01 μM (n=5) or 0.1 μM methacholine (n=5). The same protocol was followed in another two groups of hearts (n=6/group) in the presence of 1 μM phentolamine.

Muscarinic agonist and NA overflow

The effect of exogenous muscarinic agonist methacholine (10 μM) on GS-induced NA overflow was examined during normoxia (n=6), low-flow ischaemia of 3 (n=6) and 10 mins (n=5), and stop-flow ischaemia of 1 (n=6), 3 (n=9), and 5 mins (n=8). GS was given twice (1 min duration) in the presence or absence of methacholine.

Methacholine can also be hydrolysed by cholinesterase. Therefore, to exclude this possibility, nine hearts were subjected to two periods of 3 min stop-flow ischaemia in the presence of physiostigmine (1 μM). GS (1 min duration) was given twice with and without methacholine (10 μM). The sequence was randomized.

4.3 RESULTS

4.3.1 Ischaemia and Vagal Inhibition of NA Release

Vagal stimulation reduced exocytotic NA release to approx 75% of control in normoxic hearts ($p<0.01$, Table 4.1 and Figure 4.3). NA overflow was similarly reduced by vagal stimulation during the last minute of a 3-min period of low-flow ischaemia ($p<0.01$), but not if vagal nerves were stimulated during the last minute of a 10-min period of low-flow ischaemia even though the sympathetic stimulation-induced increase in heart rate (12±12 to 74±8 beats/min, $p<0.01$) was prevented (11±11 to 7±7 beats/min, p=NS). During 1 min stop-flow ischaemia, vagal stimulation tended to reduce NA overflow, although this reduction was no longer statistically significant ($p<0.06$). If the duration of stop-flow ischaemia was extended to 3 or 5 minutes, vagal stimulation had no effect on the neuronally mediated NA overflow (Table 4.1 and Figure 4.3), yet a suppression of the chronotropic effect of GS was achieved in the 3-min ischaemic group (sympathetic stimulation 73±18 to 99±8 beats/min, $p<0.01$; combined nerve stimulation 65±9 to 4±4 beats/min, $p<0.001$). At 5 mins stop-flow ischaemia, most hearts were in asystole and heart rate did not significantly change by GS (0±0 to 19±7 beats/min, NS) or combined nerve stimulation (5±5 to 0±0 beats/min).

Table 4.1 *Effects of vagal nerve stimulation (VS) on left sympathetic ganglion stimulation (GS) evoked NA overflow (pmol/g/min) during normoxia, low flow ischaemia and stop-flow ischaemia*

	n	GS	GS+VS
Normoxia	14	30.9±4.1	22.3±3.0**
Low-flow ischaemia			
3 min	8	29.8±2.3	19.4±1.9**
10 min	8	32.8±5.0	31.0±4.3
Stop-flow ischaemia			
1 min	12	36.6±7.2	27.8±6.0
3 min	8	31.3±5.1	29.2±5.1
5 min	9	24.7±2.5	27.9±4.0

** *p<0.01 vs GS value in the same group*

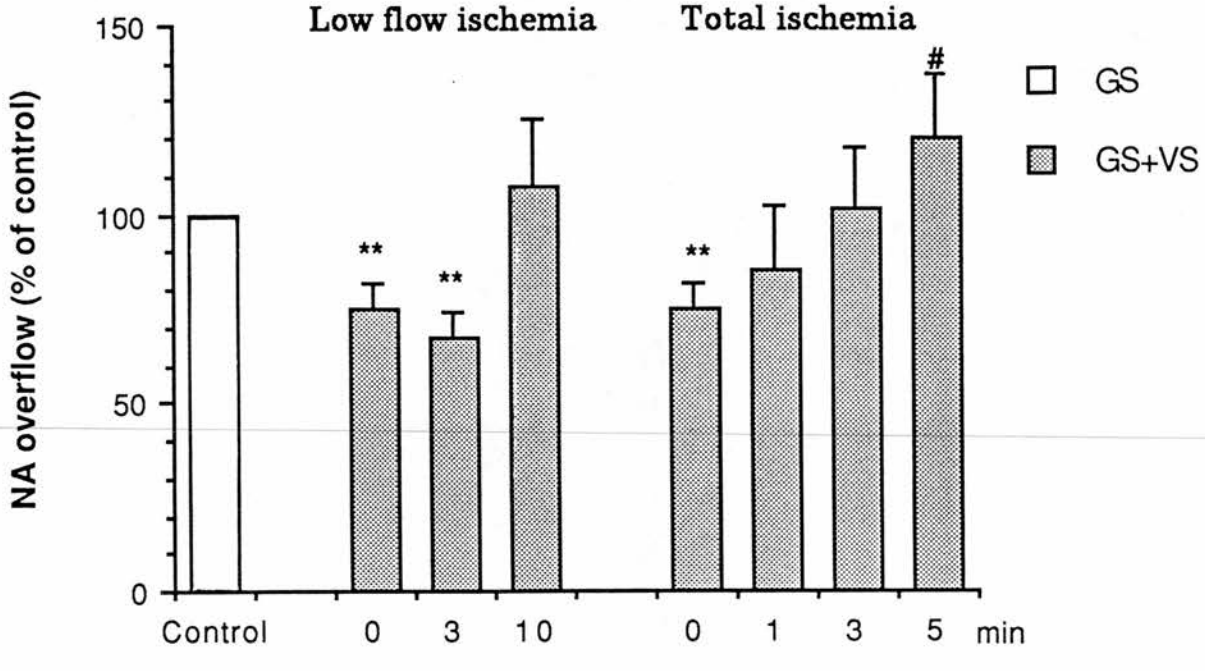


Figure 4.3 *Effects of vagal nerve stimulation (VS) on NA overflow evoked by sympathetic ganglion stimulation (GS) during normoxia, low-flow ischaemia and stop-flow ischaemia. 1 μM physostigmine was used to prevent the hydrolysis of acetylcholine. The NA overflow produced by combined nerve stimulation (GS+VS) has been expressed as a percentage of the overflow produced by GS alone. Group numbers are shown in Table 4.1. Mean±SEM. ** p<0.01 for GS+VS vs GS and # p<0.05 for % of NA overflow vs control (0 min group).*

In the hearts subjected to 10 mins stop-flow ischaemia followed by 10 mins reperfusion, NA overflow was well maintained (48.0±6.2 *vs* 46.1±7.8 pmol/g/min, p=NS) with a S2/S1 NA overflow ratio of 0.98±0.12. After ischaemia/reperfusion vagal nerve stimulation still significantly reduced NA overflow from 41.5±4.0 to 34.2±2.6 pmol/g/min ($p<0.05$) yielding a reduced S2/S1 ratio of 0.85±0.06.

4.3.2 Effect of Phentolamine on Vagal-Sympathetic Interactions

In normoxic hearts phentolamine resulted in a 136% increase in NA overflow ($p<0.001$) corresponding to higher levels of +dP/dt during sympathetic stimulation (1850±253 *vs* 2145±354 mm Hg/sec, $p<0.05$) and increased -dP/dt (800±102 *vs* 1064±115 mm Hg/sec, $p<0.05$). Vagal stimulation in the presence of phentolamine reduced NA release to 74% of control (GA + Phent, $p<0.02$, Table 4.2 and Figure 4.4). This vagally mediated % reduction in NA overflow was similar to that observed under normoxic perfusion conditions in the absence of phentolamine 75%, (see Figure 4.3). In 1-, 3- and 5-min ischaemic groups phentolamine resulted in a 139% ($p<0.001$), 55% ($p<0.01$) and 45% ($p=0.07$) increase in NA overflow. The values were reduced by vagal stimulation to 71% (1 min ischaemia, $p<0.02$), 72% (3 min ischaemia, $p<0.01$) and 88% (5 min ischaemia, $p=0.16$) respectively(Table 4.2 and Figure 4.4). In the presence of phentolamine, sympathetic nerve stimulation increased heart rate from 58±18 to 111±13 beats/min ($p<0.001$) in 3 min ischaemic group , and from 12±9 to 71±12 beats/min ($p<0.001$) in the 5-min ischaemic group; this chronotropic effect was prevented by concomitant vagal stimulation (3 mins ischaemia, 96±12 to 0±0 beats/min, $p<0.001$; 5 mins ischaemia, 30±14 to 9±8 beats/min, p=NS).

Table 4.2 *Effects of the α-adrenergic antagonist phentolamine (1 μM) on vagal stimulation induced inhibition of neuronal NA overflow (pmol/g/min) during normoxia and stop-flow ischaemia*

	n	GS	GS+Phent	GS+VS+Phent
Normoxia	8	26.4±3.1**	62.5±8.8	42.8±5.2*
Stop-flow ischaemia				
1 min	8	24.6±3.0***	58.7±5.6	41.6±4.4**
3 min	8	25.8±4.4**	40.1±3.7	28.7±2.6**
5 min	9	21.0±2.7	30.4±3.6	26.8±4.7

** $p<0.02$, ** $p<0.01$ and *** $p<0.001$ vs GS+Phentolamine in the same group.*

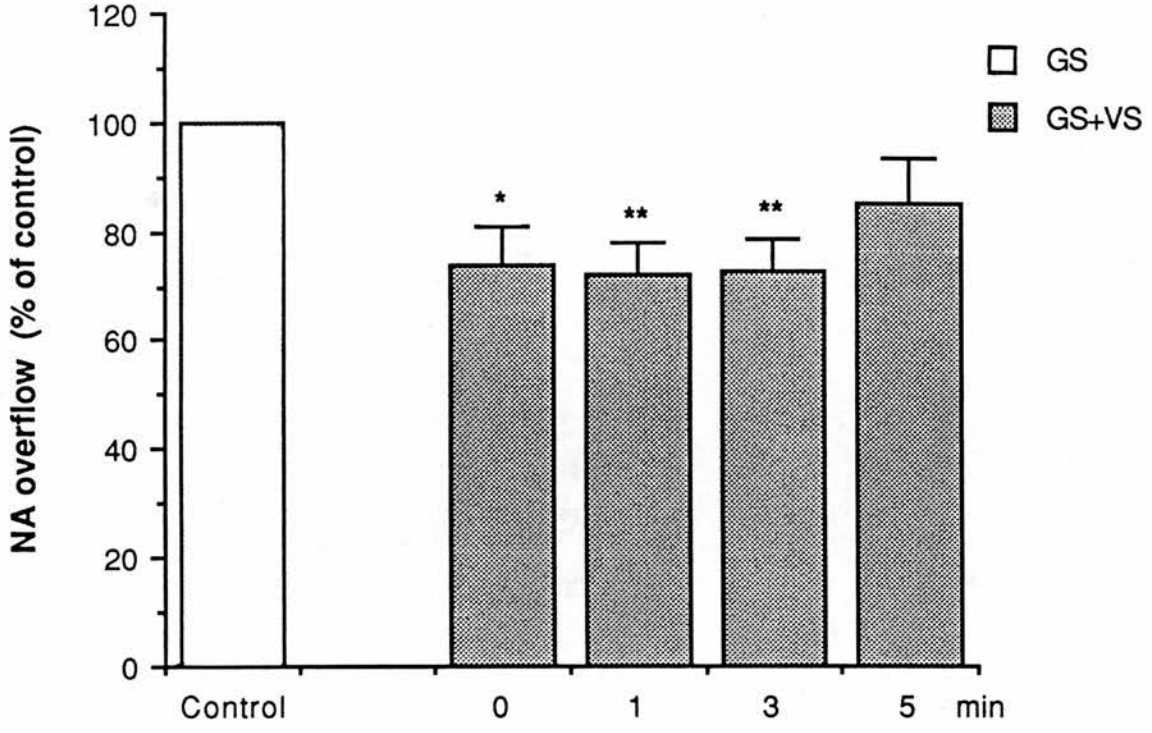


Figure 4.4 *Effect of the α-adrenoceptor antagonist phentolamine (1 μM) on vagally-induced inhibition of NA overflow in rat hearts undergoing normoxic perfusion or various periods of stop-flow ischaemia. Neuronal uptake of NA was inhibited by desipramine (0.1 μM) and 1 μM physostigmine was used to prevent the hydrolysis of acetylcholine. The NA overflow produced by GS+VS has been expressed as a percentage of the overflow produced by GS alone. Group numbers are shown in Table 4.2. Mean±SEM. * p<0.02 and ** p<0.01 for GS+VS vs GS.*

The effect of phentolamine on methacholine-induced inhibition of NA overflow is shown in Figure 4.5. A dose-dependent suppression of NA release was observed in the hearts without phentolamine (methacholine 0.01 μM, 40.1±8.5 to 30.2±5.6 pmol/g/min, p<0.07; 0.1 μM, 44.8±9.3 to 22.8±3.8 pmol/g/min, p<0.025). Although the amount of neuronal NA overflow was significantly increased (p<0.01) by blocking the α–adrenoceptors, the effect of methacholine appeared not to be affected (methacholine 0.01 μM, 92.8±10.0 to 113.1±18.5 pmol/g/min, p=NS; 0.1 μM, 100.9±20.1 to 61.7±15.7 pmol/g/min, p=0.16).

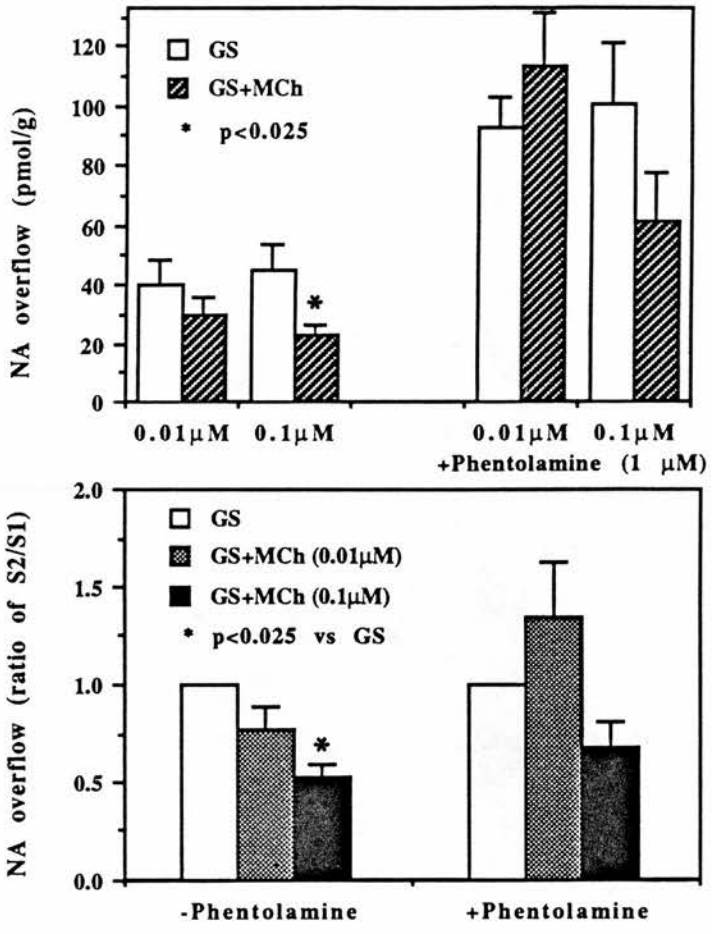


Figure 4.5 *Effect of α-adrenoceptor antagonist phentolamine (1 μM) on the muscarinic receptor agonist methacholine-mediated inhibition of NA overflow in the normoxically perfused rat hearts (n=5-6 per group). GS (5 Hz for 1 min) was performed twice with a 15 min recovery period and methacholine (0.01 or 0.1 μM) was infused at least 12 min before the second GS. * p<0.025*

4.3.3 Ischaemia and Methacholine-induced Inhibition of NA Overflow

In normoxic hearts, NA overflow was reduced to 17% of control by 10 μM methacholine ($p<0.001$) (Table 4.3 and Figure 4.6). In all the ischaemic experiments NA overflow was also significantly reduced by 10 μM methacholine but with less effectiveness ($p<0.05$), depending on the degree of flow reduction and duration of ischaemia (Table 4.3 and Figure 4.6). Addition of the cholinesterase inhibitor physostigmine to the perfusate did not increase the methacholine-induced inhibition of NA overflow after 3 min stop-flow ischaemia. The net increase in the heart rate by GS were similar between the two groups of 3 mins stop-flow ischaemia without (51±12 and 128±18 beats/min, $p<0.01$) and with physostigmine (0±0 *vs* 66±10beats/min, $p<0.01$), although the heart rate immediately before nerve stimulation was significantly lower in the group with physostigmine ($p<0.01$).

Table 4.3 *Effects of the muscarinic agonist methacholine (MCh 10 μM) on sympathetic ganglion stimulation (GS) evoked NA overflow (pmol/g/min) during normoxia, low-flow ischaemia and stop-flow ischaemia.*

	n	GS	GS+MCh
Normoxia	6	42.6±6.4	7.4±1.9**
Low-flow Ischaemia			
3 min	6	29.3±3.0	9.2±2.1**
10 min	5	33.6±7.8	15.5±5.3*
Stop-flow Ischaemia			
1 min	6	45.7±12.5	17.9±7.6*
3 min	9	32.6±5.8	18.8±6.0*
3 min #	9	32.7±5.5	18.1±2.8*
5 min	8	25.2±2.7	13.6±2.3*

p<0.05,p<0.01 vs GS in the same group; # with physostigmine (1 μM).*

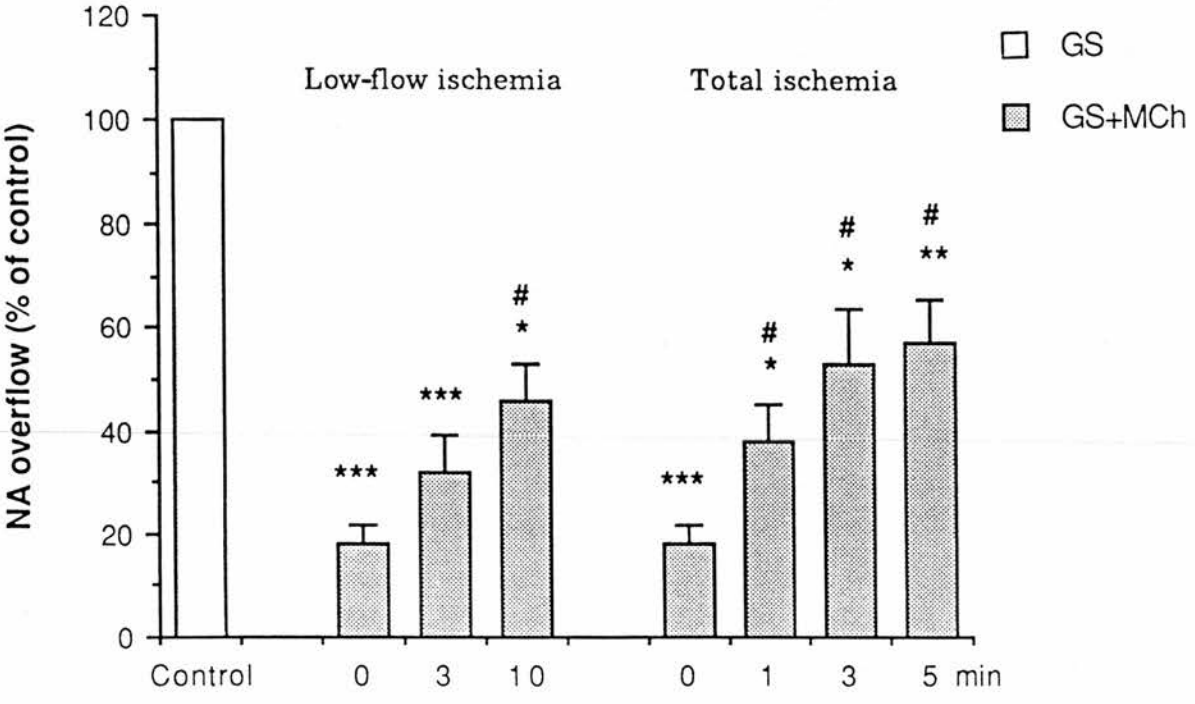


Figure 4.6 *Effects of methacholine (MCh, 10 μM) on stimulation-evoked NA overflow during normoxia, low-flow ischaemia and stop-flow ischaemia. NA overflow produced by GS+MCh has been expressed as a percentage of the overflow produced by GS. Desipramine (0.1 μM) was present throughout the experiments. Group numbers are shown in Table 4.3. Mean±SEM. * p<0.05, ** p<0.01 and *** p<0.001 for GS+MCh vs GS; # p<0.05 for % of NA overflow vs 0 min group.*

4.4 Discussion

These experiments document for the first time that short periods of ischaemia attenuate vagally induced inhibition of exocytotic NA overflow in the rat heart. The results show: (1) that the longer the period of ischaemia the less effective is vagal stimulation in inhibiting NA overflow, and (2) that vagally induced inhibition of NA overflow though still detectable after 3 mins low-flow ischaemia is abolished by 3 mins stop-flow ischaemia. Thus, an essential feature of this ischaemia-induced attenuation is a dependency on the duration and severity of ischaemia. Although vagally induced inhibition on NA release fails within only a few minutes of stop-flow ischaemia. This is a reversible process, as in the hearts subjected to 10 mins stop-flow ischaemia vagal nerve stimulation during reperfusion period still significantly suppressed NA overflow. Two important mechanisms for the clearance of released ACh, namely washout from synaptic clefts and hydrolysis by cholinesterase [Section 1.3.3], are no longer effective in our experimental conditions due to very low or zero flow and the administration of cholinesterase inhibitor. Therefore, a pronounced presynaptic inhibition of exocytotic NA release might have been expected, but the reverse was found.

Several mechanisms could account for this ischaemia-induced failure of vagally-mediated presynaptic inhibition of exocytotic NA release. Hydrolysis of ACh in the ischaemic heart appears not to be responsible because an enzyme inhibitor (physostigmine) was present throughout. In addition, physostigmine did not reverse the diminished inhibitory effect of methacholine on NA overflow observed during 2-3 mins of stop-flow ischaemia. It has been found that cholinesterase activity in normoxic mammalian hearts is low [Löffelholz and Pappano 1985], but nothing is known of the effect of ischaemia on the activity of this enzyme.

A reduction in ACh release from vagal nerve terminals during ischaemia could be a possible mechanism. In principle, at least three factors may cause a reduction in ACh release in the present model:

(1) Ischaemia may directly interfere with exocytotic release of ACh according to the results of studies that used rat brain slices or synaptosomes [Dunér-Engström and Fredholm 1988, Gustafsson *et al* 1978]. Ten minutes of hypoxia or anoxia reversibly inhibited K^+-evoked ACh release and that with concomitant acidosis (pH=6.2), this impairment became irreversible [Gustafsson *et al* 1978]. Lack of ATP may be particularly important in the heart because efferent vagal neurotransmission is dependent on two synapses (i.e. between pre-ganglionic and post-ganglionic neurons and between post-ganglionic neurons and effectors). The main biochemical events in

the vagal nerve terminals, including choline uptake, ACh synthesis, transport and exocytotic release, depend on energy supply and ionic homeostasis [Löffelholz and Pappano 1985, Section 1.3.3]. Indeed, reduced ATP supply has been suggested as a major cause for the failure of sympathetic neurotransmission during ischaemia [Dart *et al* 1984b and 1987] although a concomitant acidosis seems not to contribute to this failure [Dart and Riemersma 1989].

(2) Electrically-evoked ACh release from brain tissue or peripheral vagal terminals can be partly inhibited by micromolar levels of adenosine or the adenosine analogue phenylisopropyladenosine [Jalife *et al* 1980, Halvorsen and Nathanson 1981]. Adenosine may well play a similar role in the present heart model. Within a few minutes of stop-flow ischaemia [Richardt *et al* 1987] micromolar adenosine levels were observed in coronary venous effluent, obtained by reperfusion. Recently using the changes of the effective refratory period by vagal nerve stimulation as an end point, a presynaptic suppression of cholinergic neuro- transmission by 12 mM K^+, low pH of 6.4 or 10 μM adenosine has been documented in the *in vivo* dog heart [Miyazaki and Zipes 1990].

(3) ACh release from cholinergic nerve terminals in the heart can be reduced by exogenous NA *via* activation of the presynaptic α-adrenoceptors [Löffelholz and Pappano 1985, McGrattan *et al* 1987, Section 1.3.4]. However there has been no study to show that exocytotic NA release inhibits exocytotic ACh release. During early ischaemia, a high concentration of NA may accumulate in the synaptic cleft due to the combined effects of sympathetic nerve stimulation, inhibition of neuronal reuptake of NA, and ineffectiveness of washout from the synaptic clefts. Therefore this inhibitory modulation of ACh release is expected to be more pronounced than during normoxia. This possibility is supported by the present results that during 1 and 3 mins stop-flow ischaemia, phentolamine restored the inhibitory efficacy of vagal stimulation on NA overflow to the control level. This is probably by removal of this enhanced transaxonal inhibitory modulation, although NA release is greatly potentiated by the drug at the same time. However, this effect of phentolamine was no longer significant after 5 mins stop-flow ischaemia. As the inhibition of NA by lower doses of methacholine seems not to be changed by phentolamine in our model, the reversibility of vagally-mediated inhibition by α-blockade could not be explained by interactions between α–adrenergic and muscarinic receptors, as suggested by others [Roeske and Yamamura 1983].

Another mechanism contributing to the reduced vagal effect may be ischaemia-induced dysfunction of the presynaptic muscarinic receptors, is suggested by the fact that the modulatory effect of methacholine is similarly attenuated during ischaemia. In an *in vivo* dog study, the number of muscarinic receptors in the ischaemic

myocardium did not change within one hour coronary artery occlusion although the number of β-adrenoceptors doubled [Mukherjee *et al* 1979]. In various cardiac preparations, both the number and affinity state of postsynaptic muscarinic receptors can be modulated by multiple factors, including muscarinic agonists or antagonists, ionic activity, guanine nucleotides and binding state of adjacent non-muscarinic receptors [Watanabe 1984, Jalife *et al* 1980, Birdsall *et al* 1979]. The present study suggests that ischaemia alters the function of presynaptic muscarinic receptors. No effort was made in the present study to examine the mechanism, but an increase in extracellular potassium concentration may be involved. Extracellular K^+ concentration will double or even triple within the first few minutes of myocardial ischaemia [Kléber1984, Hill and Gettes 1980, Miyazaki and Zipes 1990]. Potassium is capable of reducing the affinity state of muscarinic receptors for agonists [Löffelholz and Pappano 1985, Birdsall *et al* 1979]. The results from vagal stimulation and methacholine, although quantitatively similar, were not identical. This could be due to a lower prevailing concentration of ACh than of methacholine in the synaptic cleft. However until ACh levels are measured, the mechanisms whereby ischaemia interferes with vagal inhibition of NA overflow remains speculative.

An interesting finding in the current studies is that when vagal stimulation no longer reduces NA overflow its negative chronotropic effect is still maintained. This may be explained by a denser vagal innervation in atria and conducting system than in ventricles [Lund *et al* 1986] whereas nerves located in the ventricles are probably the source of most NA overflow. In addition, it is not known if there is any difference between presynaptic and postsynaptic muscarinic receptors in their agonist affinity or their sensitivity to ischaemia. However, neuropeptide Y, a co-transmitter released with NA by sympathetic nerve stimulation, inhibits vagal neurotransmission but does not alter the postsynaptic cholinergic action on heart rate [Potter 1987, Warner and Levy 1989, Section 1.3.4]. Similarly, adenosine inhibits ACh release at the presynaptic level, whilst it potentiates the effects mediated by postsynaptic muscarinic receptors [Pelleg *et al* 1988, Belloni *et al* 1989, Potter 1986]. This mechanism may also be involved in our aforementioned dissociation of pre- (inhibition of NA release) and post-synaptic muscarinic effects (reduction in heart rate) by vagal nerve stimulation.

Clinical and experimental observations suggest a simultaneous sympathetic and vagal activation during the very early phase of myocardial ischaemia [Webb *et al* 1972, Lombardi *et al* 1983, Verrier 1988, Corr and Gillis 1978]. If our findings from the perfused rat heart can be extrapolated to the *in vivo* situation, then the presynaptic inhibition of vagal activity on NA release will vary within the ischaemic heart,

the severity and duration of ischaemia. This condition may lead to an autonomic activity within the ischaemic region. Thus, electrical nd arrhythmias may occur as a result of heterogeneous adrenergic These events may happen within the first few minutes of myocardial time when sudden cardiac death frequently occurs [Fraser 1986]. Indeed, experimental studies have also suggested that withdrawal of vagal l lead to ischaemia-induced ventricular fibrillation and sudden cardiac r 1988, Schwartz *et al* 1988a, La Rovere *et al* 1988, Section 1.2.2]. imulation of cardiac sympathetic and vagal nerves during ischaemia her incidence of serious arrhythmias than either alone in an *in vivo* study ii 1978].

usion, these results demonstrate that cholinergic presynaptic inhibition of IA release in the rat heart is impaired during acute ischaemia, and suggest d ACh release and/or dysfunction of the presynaptic muscarinic ors could be responsible. The resulting autonomic imbalance within the myocardium could lead to the development of serious ventricular

Chapter 5

Gender Difference in Presynaptic Adrenergic Inhibition of NA Release during Normoxia and Ischaemia

5.1 Introduction

Clinical studies have suggested a gender-related difference in the adrenergic reactivity to various stressful conditions, including mental stress [Frankenhaeuser *et al* 1976, Lenders *et al* 1987], head-up tilt [Lenders *et al* 1987], lower-body negative pressure [Frey and Hoffler 1988] exercise [Frankenhaeuser *et al* 1976, Lenders *et al* 1987, Claustre *et al* 1980], and insulin-induced hypoglycaemia [Claustre *et al* 1980]. When exposed to these stressful conditions, men show a more marked sympatho-adrenal activation than women with higher plasma catecholamine concentrations and a higher urinary catecholamine output [Lenders *et al* 1987, Frey and Hoffler 1988, Sanchez *et al* 1980]. Under resting conditions, plasma concentrations of adrenaline and NA are higher in males [Lenders *et al* 1987, Frey and Hoffler 1988, Sanchez *et al* 1980].

Results from experimental studies support this sex difference. In rats, cardiac sympathetic activation by drug-induced hypotension is more pronounced in males than in females [Buñag *et al* 1975]. Furthermore, the female cardiovascular system is more tolerant to several pathological conditions, such as circulatory shock [Altura 1976], isoprenaline-induced myocardial necrosis [Wexler *et al* 1974], myocardial ischaemia and infarction [Siegmund *et al* 1979, Lu *et al* 1984, Du *et al* unpublished]. These pathological stimuli could certainly activate the sympatho-adrenal system.

The mechanisms responsible for these sex differences in the adrenergic activation by stressful factors are largely unknown. A lower central sympathetic activity in females has been proposed [Lenders *et al* 1987, von Eiff *et al* 1971]. Catecholamine content and metabolism in the central nervous system and adrenal medulla may also differ [Crowley *et al* 1978, Fernandez-Ruiz *et al* 1989]. Many studies have demonstrated that females have a higher density and activity of α-adrenoceptors in various tissues [Jones *et al* 1983, Morita *et al* 1987, Colucci *et al* 1982]. It is unclear if presynaptic α_2-adrenoceptors are higher in females, as one study may suggest [Wiechman and Borowitz 1979]. Oestrogen treatment increases, and ovariectomy decreases α_2-adrenoceptors [Roberts *et al* 1981, Larsson *et al* 1984, Levin *et al* 1980].

However, testosterone treatment has no such effect [Colucci *et al* 1982]. One clinical study showed that oestrogen attenuated the pressor response to mental stress [von Eiff *et al* 1971].

On account of these findings, it is logical to hypothesize a sex difference in the mechanisms controlling sympatho-adrenal function. One particular possibility is a increased presynaptic inhibition of NA release from sympathetic nerves in females. We therefore examined the sex differences in sympathetic nerve stimulation-induced NA release from the perfused rat heart and the effects of α_2- adrenoceptor antagonist and agonist both in normoxic and in ischaemic conditions. In addition we also examined the effect of ovariectomy on these processes.

5.2 Methods

5.2.1 Animals, Drugs and Model

Male and female Sprague-Dawley rats (9-10 weeks old, 200-290 g) were used. Experiments were performed alternately on male and female rats. No attempt was made in these studies to ascertain the phase of the oestrous cycle of female rats at the time of study. The *in situ* retrogradely perfused, innervated heart model (Section 2.2) was used for this study. Hearts were heavier in male (0.77±0.07 g, n=73) than in female rats (0.72±0.06 g wet weight, n=74, $p<0.01$). Perfusion flow rates were carefully adjusted to the estimated heart weight and were 5.24±0.33 for males and 5.18±0.35 ml/g/min for females, Mean ±SD, NS).

The neuronal NA reuptake inhibitor, desipramine was added to the perfusate (0.1 μM) in all experiments [Dart *et al* 1984b, Iversen 1973]. Rauwolscine and clonidine were used to block or stimulate the α_2-adrenoceptors. Coronary effluent for NA analysis was collected over a 2-min period starting with the nerve stimulation in normoxic perfused hearts. Timolol (3 μM) and isoprenaline were used to block or stimulate β-adrenergic receptors. Coronary effluent was collected during the first 2.5 mins of ischaemic-reperfusion in the ischaemic hearts (as there was little effluent collected during first 30 secs). NA overflow in the absence of nerve stimulation was always low and similar in the two sexes (0.82±0.17 and 0.77±0.14 pmol/g/min, NS).

5.2.2. Protocols

Normoxic series

Experiment 1: Total content of NA, adrenaline and dopamine were measured in eight male and eight female hearts. After 20 mins *in situ* perfusion, hearts were excised, weighed and quickly frozen on solid CO_2 and stored at -40° C until assayed.

Experiment 2: The effect of incremental doses of rauwolscine on neuronal NA overflow was examined in 10 male and 10 female rat hearts. Sympathetic nerve stimulation (30-sec duration) was applied five times (S1-S5) with 15-min intervals. The first (S1) and the last (S5) nerve stimulations served as controls (no drug). Concentrations of rauwolscine were 0.1 μM (S2), 1 μM (S3) and 10 μM (S4).

Experiment 3: Presynaptic α_2-adrenoceptor mediated inhibition of $NA_{release}$ was studied in 12 male and 12 female hearts using rauwolscine and clonidine. After an initial control stimulation (S1, 60 secs), a second stimulation (S2, 60 secs) was performed in the presence of rauwolscine (1 μM) and a third (S3, 60 secs) in the presence of clonidine (3 μM).

Experiment 4: β- and α_1-adrenoceptor mediated inotropic response was examined in male and female hearts. In five male and five female hearts GS (60 secs) was performed three times in the absence (S1 and S2) and presence of timolol (3 μM, S3). In another five male and five female hearts, isoprenaline was infused at concentrations of 2.5×10^{-9}, 10^{-8} and 2.7×10^{-7} M and changes in dP/dt were continuously monitored at each concentration.

Ischaemic series

Experiment 1: The effect of global ischaemia (by stopping coronary perfusion) on nerve stimulation-induced NA release was studied in male and female hearts (n=9 per group). A first nerve stimulation (S1, 30 secs) was used as an individual normoxic control. Then hearts were subjected to three episodes of ischaemia in the sequence of 1-, 3- and 6-min duration and separated by 15-min periods of normoxic reperfusion. Nerve stimulation (S2-S4) was applied during the final 30 secs of each ischaemic period.

Experiment 2: Effect of the α_2-adrenergic antagonist rauwolscine (1 μM) and the agonist clonidine (3 μM) on NA release during ischaemia was studied in male (n=23) and female hearts (n=24). Each heart underwent two episodes of 3 mins of ischaemia separated by a 15-min recovery period. NA overflow was determined during an initial nerve stimulation (60 secs) in the final minute of ischaemia (S1). The hearts were then assigned to treatment with rauwolscine (male n=13, female n=13) or clonidine (male n=10, female n=11). Another nerve stimulation (S2) was performed in the final minute of the second ischaemic period.

Experiment 3: After 20-40 mins of stop-flow ischaemia non-exocytotic NA release occurs in the isolated rat heart and is inhibited by desipramine [Schömig 1988]. This ischaemia-induced NA release was examined in male and female hearts (10 hearts per group). Desipramine was omitted from the perfusate. Hearts were

subjected to 40 mins of ischaemia followed by reperfusion. Coronary effluent was collected during the first 3 mins of reperfusion.

Gonadectomy series

Female Sprague-Dawley rats (6 weeks old) were gonadectomized or sham-operated using the techniques described by Waynforth [1980]. Body weights were measured weekly. The perfusion experiments were conducted during 7-8 weeks after the operation. Adequacy of the gonadectomy was assessed by inspection of possible residual ovarian tissue, atrophy of the uterus, and changes in body weight [Waynforth 1980, Scheuer *et al* 1987]. Nerve stimulation-induced NA overflow and the effects of rauwolscine (1 μM) and clonidine (3 μM) were studied using the same protocol as described for Experiment 3 of the normoxic series.

5.3 Results

5.3.1 GS during Normoxia and Myocardial Catecholamine Content

Sympathetic nerve stimulation (60 secs) caused a marked overflow of NA into the coronary effluent in male and female hearts. This increase in catecholamine release was reflected by an increased inotropic and chronotropic response (Table 5.1). None of these measurements were different between male and female hearts. Myocardial content of NA (7.7±0.8 *vs* 8.1±0.7 nmol/g) and dopamine (0.62±0.12 *vs* 0.63±0.06 nmol/g) were also similar in males and females. However the content of adrenaline was higher in female than male hearts (0.14±0.01 *vs* 0.08±0.02 nmol/g, $p<0.01$).

Table 5.1 *NA overflow and functional response to left sympathetic ganglion stimulation (GS, 5 Hz for 60 secs) in normoxic perfused male and female rat hearts.*

	Male *(n=12)*		**Female** *(n=12)*	
	Basal	GS	Basal	GS
NA overflow *(pmol/g)*	0.7±0.3	86.3±7.9	1.1±0.2	85.3±10.0
$+dP/dt_{max}$ *(mm Hg/sec)*	1728±104	3128±218	1784±144	3626±286
$-dP/dt_{max}$ *(mm Hg/sec)*	946±68	1910±100	908±66	2092±206
Heart rate *(beats/min)*	207±18	233±17	216±17	248±15

All changes induced by nerve stimulation were significant ($p<0.01$) in both groups. No sex difference was observed in any of these parameters.

5.3.2 Gender and Presynaptic Modulation of NA Release during Normoxia

Rauwolscine increased NA overflow at each of the concentrations studied ($p<0.01$) both in male and female hearts. The maximum effect of rauwolscine was observed at 1 μM (Figure 5.1). The increase in NA overflow by rauwolscine was higher in female than in male hearts ($p<0.05$ by ANOVA).

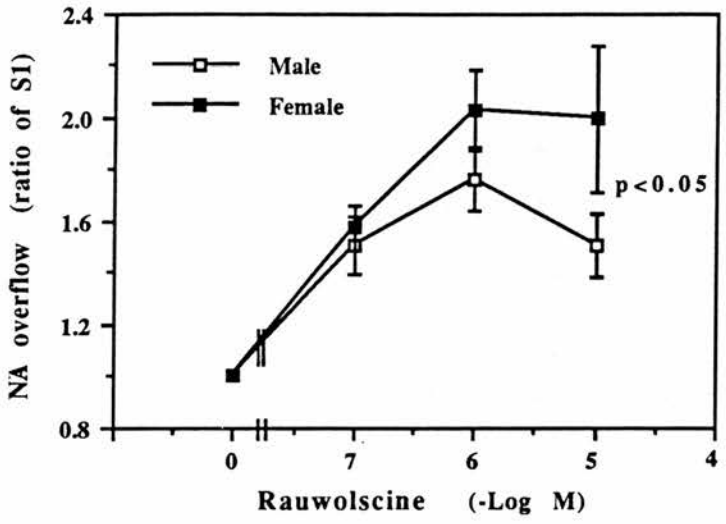


Figure 5.1 *Effect of the α_2-adrenergic antagonist rauwolscine on nerve stimulation-evoked NA overflow in perfused innervated male and female rat hearts (n=10 per group). Sympathetic nerve stimulation (5 Hz for 30 secs) was applied 5 times (S1-S5) with 15 min intervals. The first (S1) was used as a control. Rauwolscine was present before and during S2 (10^{-7}M), S3 (10^{-6}M) and S4 (10^{-5}M). Data are presented as a ratio of that obtained by S1. A second control nerve stimulation was carried out 15 mins after washout of rauwolscine (S5). Similar amount of NA overflow was obtained by S1 and S5 both in males and females (male: 40.9±4.9 and 42.2±5.7 pmol/g; female: 43.5±7.4 and 48.9±8.6 pmol/g).*

At the optimum concentration of 1 μM, rauwolscine significantly enhanced NA overflow during the second nerve stimulation in both sexes ($p<0.01$, Table 5.2). The net increase in NA overflow by rauwolscine was significantly higher in females than in males (+71.0±7.6 *vs* +39.9±7.8 pmol/g, $p<0.01$), and the ratio of NA overflow of the second over the first nerve stimulation (S2/S1) was significantly higher in females than in males ($p<0.05$, Figure 5.2).

Table 5.2 *NA overflow (pmol/g) in male and female rat heart induced by sympathetic ganglion stimulation (GS) during normoxia and ischaemia and effects of the α_2-adrenergic antagonist rauwolscine (1 μM) or agonist clonidine (3 μM)*

	Male	Female	p
Normoxia			
GS	86.3±7.9	85.3±10.0	NS
	(12)	*(12)*	
GS+Rauwolscine	126.3±8.8**	156.3±13.3**	NS
	(12)	*(12)*	
GS+Clonidine	78.3±11.5	65.5±8.2*	NS
	(12)	*(12)*	
Ischaemia			
GS	58.4±6.4	36.2±3.6	<0.005
	(23)	*(24)*	
GS+Rauwolscine	78.3±7.9*	76.9±7.5**	NS
	(13)	*(13)*	
GS+Clonidine	72.9±15.2*	36.1±7.5	<0.05
	(10)	*(11)*	

** $p<0.05$ and **$p<0.01$ vs GS by paired t-test. Numbers in brackets denote group size.*

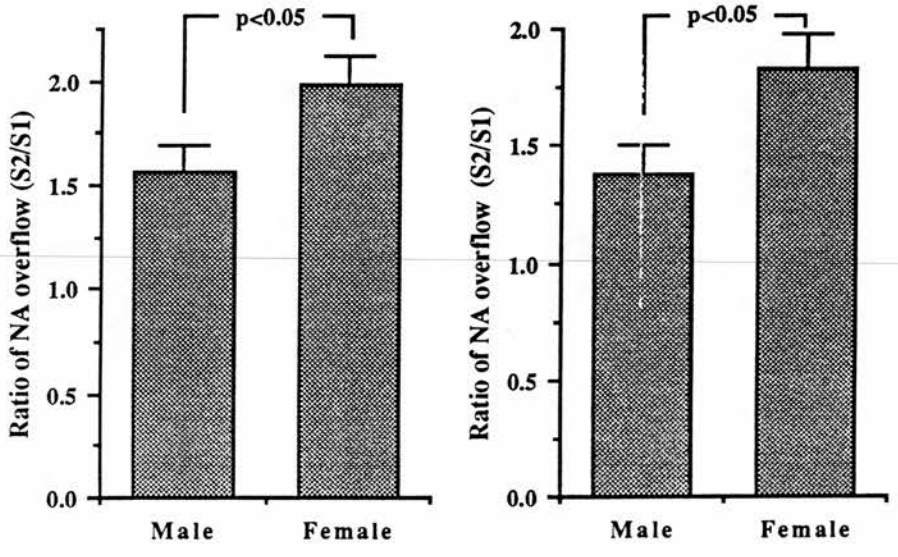


Figure 5.2 *Sex differences in the ratio of NA overflow evoked by GS (5 Hz for 60 secs without (S1) and with (S2) the α_2-adrenoceptor antagonist rauwolscine (1μM) in normoxic (n=12 in each group, left panel) and ischaemic hearts (n=13 per group, right panel). Desipramine (0.1μM) was used to inhibit neuronal reuptake of NA.*

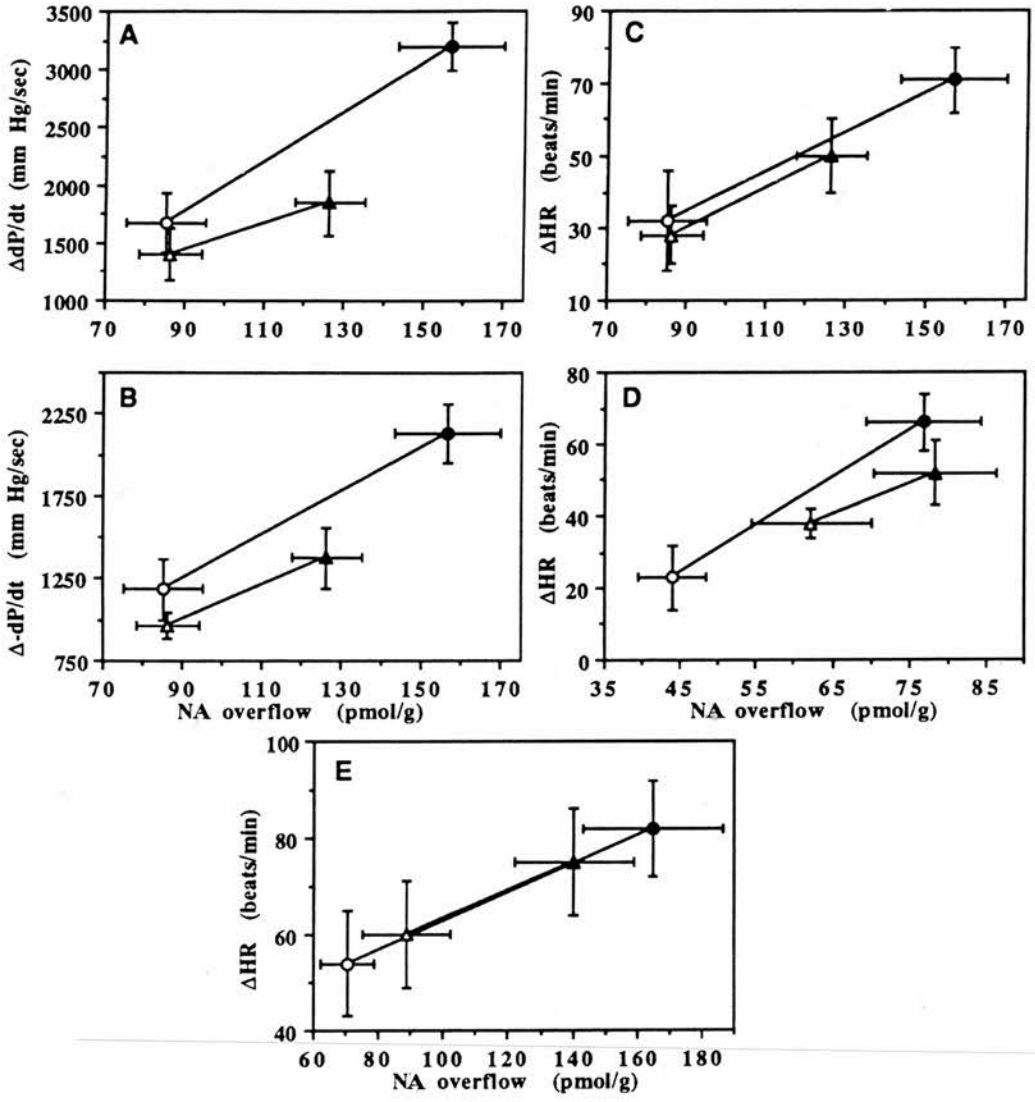


Figure 5.3 *Relationship between NA overflow and increases in left ventricular contractility (Δ±dP/dt) and heart rate (ΔHR) induced by sympathetic nerve stimulation without (open symbols) and with (closed symbols) the α_2-adrenoceptor antagonist rauwolscine. NA overflow data are from the normoxic series (panels A, B and C) in Table 5.2, from the ischaemic series (D) in Table 5.2 and from gonadectomy series (E) in the text. Triangles represent male or gonadectomized female groups, and circles denote female or sham-operated female groups. Ten to 13 hearts in each group. The inotropic and chronotropic effects of rauwolscine are significant ($p<0.05$ or <0.01), except for the rise in heart rate in male rats (panel D) and in gonadectomized female rats (panel E).*

The pre-stimulation levels of ±dP/dt and heart rate were similar between and within groups ($p>0.6$). In the female group, rauwolscine significantly potentiated the inotropic and chronotropic responses to the second (S2) compared with that of the first (S1) nerve stimulation (changes in heart rate and ±dP/dt, all $p<0.01$), but this effect was only of borderline significance in males (Figure 5.3). However, all these sex-related differences in rauwolscine-induced potentiation of functional responses to sympathetic stimulation in normoxic hearts were basically related to differences in NA overflow (Figure 5.3 A, B and C).

In comparison to the effect of the antagonist rauwolscine, the agonist clonidine had little effect on NA overflow in normoxic perfused hearts. A small reduction in NA overflow was observed in females ($p<0.05$) but not in males (Table 5.2). This difference between the two sexes was not significant whether expressed in absolute terms or as a ratio of NA overflow during the control stimulation (Table 5.2 and Figure 5.4).

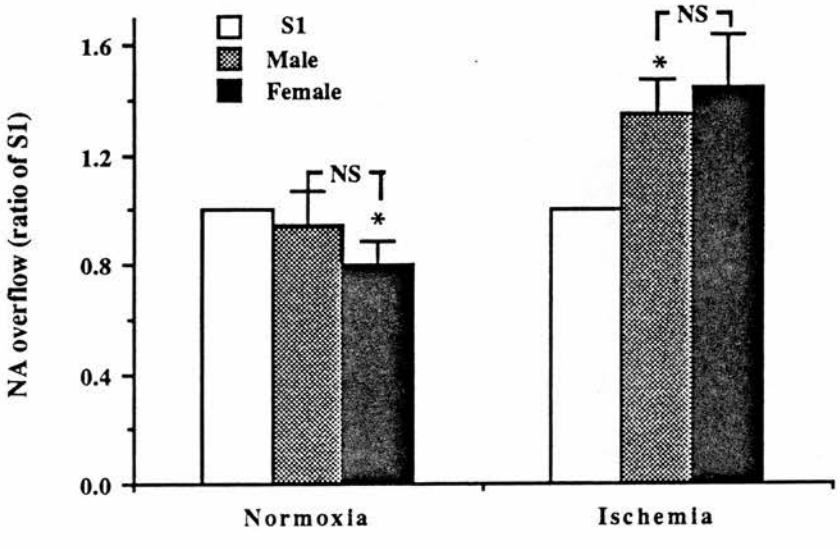


Figure 5.4 *Effect of the α_2-adrenoceptor agonist clonidine (3 μM) on NA overflow induced by sympathetic ganglion stimulation in male and female rat hearts during normoxia and during the final minute of ischaemia (3 mins). Results are presented as the ratio of individual control values measured during normoxia or during ischaemia without clonidine (S1, open bars). Desipramine (0.1 μM) was used to inhibit neuronal NA uptake. Ten to 12 hearts per group.*
**$p<0.05$ vs S1-induced NA overflow.*

In male and female hearts the β-receptor antagonist timolol (3 μM) equally inhibited most of the inotropic and chronotropic responses to GS ($p<0.01$) but a small and significant increase in ±dP/dt was still measured, which usually appeared in the final 20 secs during a 1 min stimulation period in contrast to the earlier appearance (usually in the first 20 secs) of the maximum inotropic response to control stimulation (Figure 5.5). Infusion of concentrations of isoprenaline resulted in a dose-dependent increase in ±dP/dt, which was similar in the male and female groups (Figure 5.6).

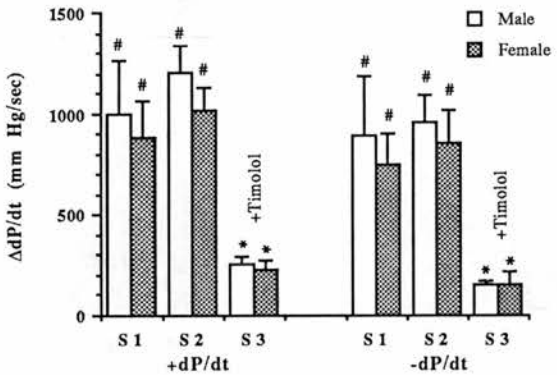


Figure 5.5 *Nerve stimulation-induced increase of ±dP/dt in the absence (S1 and S2) and presence of β-adrenoceptor antagonist timolol (3 μM, S3) in normoxically perfused male and female rat hearts. (NS by ANOVA n=5 per group). * p<0.05 and # p<0.0005 vs pre-stimulation levels, ¶p<0.01 vs S1 and S2.*

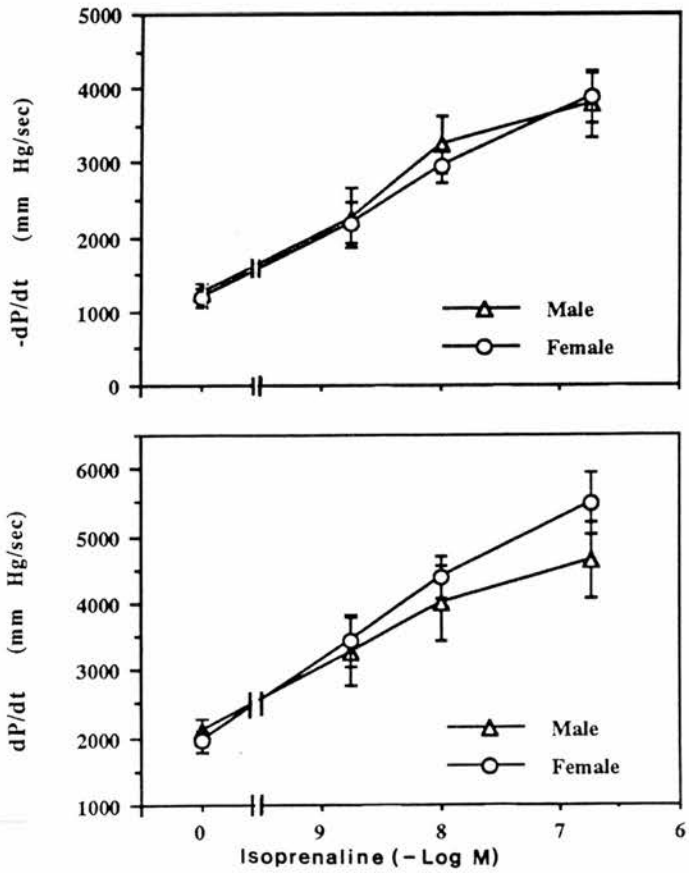


Figure 5.6 *Inotropic responses of perfused male and female rat hearts to different doses of isoprenaline. A similar dose-dependent increase in ±dP/dt is observed in male and female hearts. (NS by ANOVA, n=5 per group).*

5.3.3 Gender and NA Overflow during Ischaemia

A progressive decline in NA overflow was observed within the first 6 mins of ischaemia in male and female hearts ($p<0.001$ by ANOVA). In males this reduction was not significant at 1 min of ischaemia compared with the pre-ischaemic control value (32.5±7.6 *vs* 40.4±6.2 pmol/g, NS), but it was at 3 and 6 mins of ischaemia (27.0±5.5 and 20.1±3.0 pmol/g, respectively, both $p<0.01$). An identical pattern emerged when the results were expressed as the ratio of the pre-ischaemic overflow (Figure 5.7). In females, NA overflow was significantly inhibited by ischaemia at all times examined (1, 3 and 6 mins of ischaemia vs the pre-ischaemic control value: 19.8±3.8, 14.9±2.6 and 10.8±1.5 *vs* 39.3±5.5 pmol/g, $p<0.001$). The ratio of NA overflow during ischaemia relative to the pre-ischaemic control was significantly lower in females than in males ($p<0.01$ by ANOVA, Figure 5.7). Meanwhile, nerve stimulation-induced increase in heart rate was also lower during ischaemia in females than in males (combined data: +35±5 *vs* +52±6 beats/minute, $p<0.05$ by ANOVA).

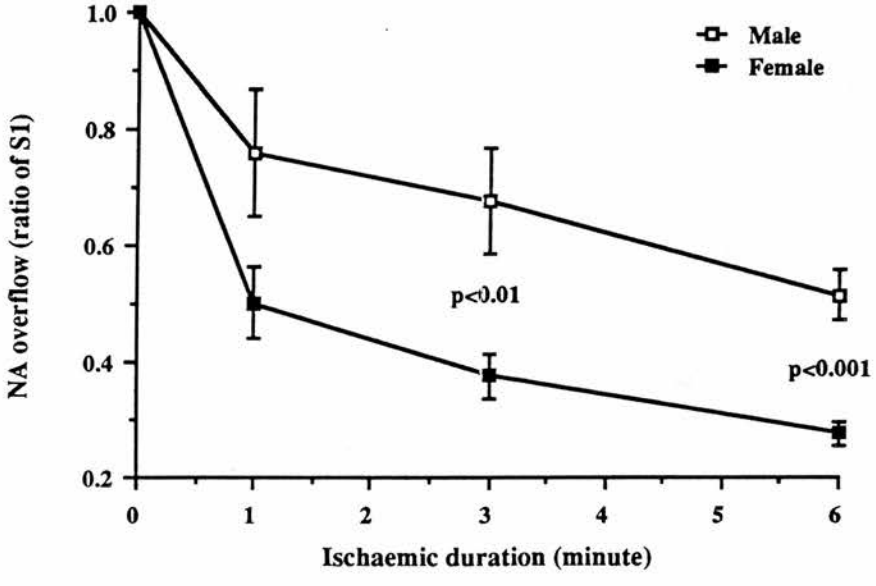


Figure 5.7 *NA overflow from male and female rat hearts induced by sympathetic ganglion stimulation (5 Hz for 30 secs) during normoxia and during 1, 3 and 6 mins of stop-flow ischaemia. NA overflow during ischaemia is expressed as the ratio of individual normoxic values (S1=1). Desipramine (0.1 μM) was used to inhibit neuronal NA uptake. Sex difference was significant (overall $p<0.001$ by ANOVA and at 3 and 6 mins as indicated by t-test). Nine hearts in each group.*

NA overflow was also significantly increased by rauwolscine in male and female groups during ischaemia and the initial difference in both sexes in ischaemic NA overflow was no longer significant (Table 5.2). The net increase of NA overflow by rauwolscine tended to be higher in female than male hearts (+32.8±5.7 *vs* +16.1±6.2 pmol/g, p=0.054). The ratio of NA overflow in the presence and absence of rauwolscine was significantly higher in the female group ($p<0.05$, Figure 5.3), an effect mainly attributable to the difference in NA overflow between males and females in the absence of this drug. Like in the normoxic experiments, changes in heart rate induced by nerve stimulation with and without rauwolscine were also related to the changes in NA overflow during ischaemia (Figure 5.3 D).

During ischaemia, the effect of clonidine differed from that during normoxia. Clonidine increased NA overflow in male hearts with an increased ratio of NA overflow (Table 5.2 and Figure 5.4), whilst no change in NA overflow had been observed during normoxia. In contrast to the clonidine-mediated reduction in NA overflow in normoxic female hearts, an insignificant increase in the ratio of NA overflow (S2/S1) was observed (Figure 5.4). The ischaemia-induced difference in neuronal NA overflow between both sexes was not affected by clonidine (Table 5.2).

Reperfusion after 40 mins of ischaemia evoked spontaneous NA overflow in both male and female hearts (186±21 *vs* 183±31 pmol/g, NS). Overflow of lactate and LDH were also observed in male and female hearts (lactate: 19.6±0.7 *vs* 19.7±1.2 μM/g, LDH: 214±46 *vs* 208±32 μU/g). No sex difference was found in all these changes.

5.3.4 Gonadectomy Series

Gonadectomy was associated with an increase in body weight, which was discernable starting from the second week after the operation. At the time of sacrifice, gonadectomized rats had a higher body weight (320±7 *vs* 258±5 g, $p<0.001$), heart weight (1.02±0.03 *vs* 0.86±0.02 g, $p<0.001$) and a marked uterine atrophy (83±3 *vs* 463±26 mg, $p<0.001$) than sham-operated female rats. The perfusion flow rate was similar in the two groups (4.93±0.13 *vs* 4.73±0.11 ml/g/min, NS).

NA overflow evoked by a control nerve stimulation (S1) did not differ between sham-operated and gonadectomized females. NA overflow was significantly increased by rauwolscine in sham-operated controls (from 70.5±8.4 to 164.7±22.7 pmol/g, $p<0.001$) and in gonadectomized rats (from 88.7±13.2 to 140.5±18.7 pmol/g, $p<0.01$). However, the potentiation of NA overflow by rauwolscine was reduced after gonadectomy as the S2/S1 ratio was significantly lower ($p<0.02$, Figure 5.8).

NA overflow was not significantly affected by clonidine in gonadectomized and sham- operated animals and S3/S1 ratios were similar (0.94±0.17 *vs* 1.04±0.15, NS).

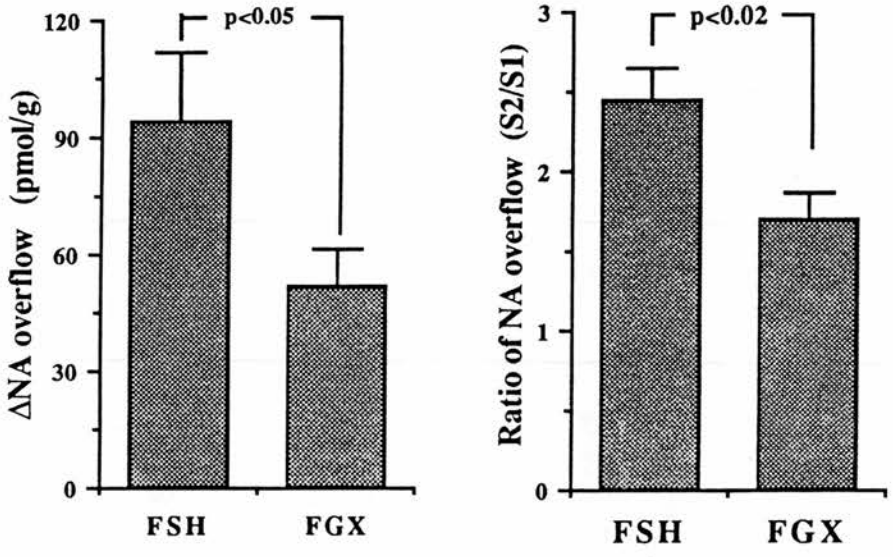


Figure 5.8 *Effect of female gonadectomy (FGX, n=14) or sham-operation (FSH, n=13) on net increase in NA overflow (left panel) and on the ratio of NA overflow (right panel) evoked by sympathetic ganglion stimulation (5 Hz for 60 secs) without (S1) and with (S2) the α_2-adrenoceptor antagonist rauwolscine (1 μM) in normoxic perfused hearts.*

Pre-stimulation heart rate (195±11 vs 183±13 beats/min, NS), +dP/dt (1573±94 *vs* 1544±166 mmHg/sec, NS) and -dP/dt (751±63 *vs* 746±40 mmHg/sec, NS) were similar in sham-operated and gonadectomized rat hearts. Inotropic response to control GS was significantly lower in gonadectomized rat hearts (+dP/dt: 2509±152 *vs* 3323±277 mmHg/sec, $p<0.05$; -dP/dt: 1421±78 *vs* 1934±231 mmHg/sec, $p<0.05$). The chronotropic response to nerve stimulation was not significantly affected by gonadectomy. Changes in heart rate with and without rauwolscine were related to the changes in the amount of NA overflow (Figure 5.3 E).

5.4 DISCUSSION

Gender and Presynaptic α_2-Adrenergic Inhibition of NA Release

The present study has demonstrated, for the first time, a stronger α_2-adrenergic presynaptic inhibition of exocytotic NA release in female than in male rat hearts. In the presence of the α_2-adrenoceptor antagonist rauwolscine, NA overflow was increased proportionally more in female rat hearts. These sex differences are not due to a leftward shift in the dose-response curve of rauwolscine in female rat hearts. The

maximum effect of rauwolscine on NA overflow was achieved at 1 μM concentration in both male and female rats. Clonidine, an α_2-adrenergic agonist, significantly reduced NA overflow in females, but not in males.

One may argue that the gender differences in NA release could simply be caused by a denser sympathetic innervation in female rat hearts. However, if this had been the case then we would have expected NA overflow in females also to be higher in the absence of rauwolscine, and the ratio of NA overflow with and without rauwolscine to remain unchanged. This was not so. Myocardial NA and dopamine levels were also not different in both sexes. Another possibility is a less efficient NA reuptake system in females. Our studies were always carried out in the presence of the neuronal reuptake inhibitor, desipramine, and therefore a gender difference in neuronal reuptake of NA, if it exists, seems an unlikely explanation. There are no published data to support this possibility and one clinical study actually found a higher clearance of catecholamine from the circulation in females [Lenders *et al* 1988]. We found a significantly higher content of adrenaline in female than in male rat hearts. As adrenaline can not be synthesized by adrenergic neurones (Section 1.3.2), this difference is most probably due to sex-related difference in the neuronal uptake of adrenaline. The implication of this difference, however, is unclear as adrenaline constitutes only about 1-2% of total catecholamines in the heart. In addition, oestrogen could inhibit the extraneuronal NA uptake in rat heart *in vitro* [Iversen 1973]. However, since this uptake mechanism plays a minor role in NA clearance from the synaptic cleft in our model [Dart *et al* 1984b], the magnitude of the gender difference in NA overflow seems too large to be explained this way.

Our evidence on the gender difference of presynaptic control of NA release do not rely on the results of NA overflow only. The inotropic and chronotropic potentiation by rauwolscine was also more marked in female than in male hearts. We excluded the possibility that this was due to a difference in postsynaptic adrenergic responsiveness between the sexes. The inotropic response to nerve stimulation is predominantly mediated by β-adrenoceptors and the inotropic effects of increasing concentrations of the β-adrenergic agonist isoprenaline were similar between males and females. When the β–antagonist timolol (3 μM) was used to unmask any α-adrenergic response, the greatly reduced inotropic and chronotropic responses to nerve stimulation did not differ between the two sexes. Thus, neither postsynaptic β- nor α-adrenoceptors in the rat heart are functionally different according to the sex of the animal. Therefore, the sex differences in functional responses to nerve stimulation by rauwolscine can only be explained by the alteration in NA overflow due to this drug. In gonadectomized rats the inotropic state of the heart is reduced, as observed in this nerve stimulation

experiment and other detailed haemodynamic studies [Schaible *et al* 1984, Scheuer *et al* 1987]. Therefore, a difference in the response to nerve stimulation between sham-operated and ovariectomized rat hearts cannot simply reflect NA overflow under these circumstances. Taken together, our results suggest that the presynaptic α_2-adrenergic inhibition of NA release plays a more important role in female than in male rat heart.

Mechanism

Both the increase in NA overflow and the associated greater chronotropic response by rauwolscine was attenuated after gonadectomy, indicating a less effective α_2-adrenergic presynaptic inhibition after removal of the ovaries. The adequacy of gonadectomy was documented by a marked uterine atrophy which has been attributed to the fall in oestrogen levels [Scheuer *et al* 1987]. Therefore, a female hormone-mediated modulation of presynaptic α_2-adrenoceptors is indicated. To the best of our knowledge, this is the first study to suggest such modulation in the heart.

Gender differences in α-adrenoceptors have been extensively studied using pharmacological and radioligand techniques. In various tissues (blood vessels, neurones, platelets, uterus, bladder and urethra), a higher sensitivity and density of α-adrenoceptors has been found in females than in males [Stone *et al* 1989, Jones *et al* 1983, Colucci *et al* 1982, Roberts *et al* 1981, Levin *et al* 1980]. Some studies show that this sex difference in α-adrenoceptors is restricted to the α_2-subtype [Morita *et al* 1987, Hoffman *et al* 1981, Roberts *et al* 1981], but others found that α_1-receptors also differed between the sexes [Stone *et al* 1989, Colucci *et al* 1982]. Where this was studied β-adrenoceptors were found to be similar in the two sexes [Roberts *et al* 1981, Levin *et al* 1980]. Furthermore, this gender difference in α-adrenoceptors does not persist after ovariectomy [Colucci *et al* 1982]. Conversely, elevating oestrogen levels in males and females increases the density of α_2-receptors, but has no such an effect on β-receptors [Roberts *et al* 1981, Colucci *et al* 1982, Hoffman *et al* 1981]. Progesterone prevents the oestrogen-induced rise in myometrial α_2-adrenoceptors [Roberts *et al* 1981]. Testosterone treatment does not affect α-adrenoceptors [Colucci *et al* 1982].

Sex Difference in NA Release during Ischaemia

A reduction in neuronal NA overflow during acute ischaemia has been documented and is explained by presynaptic inhibition, enhanced neuronal reuptake and energy depletion of sympathetic nerves [Dart *et al* 1984b, Dart and Riemersma 1985, Schömig 1988].

Another important observation in our study is that ischaemia reduces neuronal NA release to a greater extent in females than in males. In contrast, no sex difference exists in non-exocytotic NA release induced by a 40-min period of ischaemia. Interestingly, at 3 mins of ischaemia, administration of the α_2-adrenoceptor antagonist rauwolscine led to a higher net increase in NA overflow in females and the initial difference in the total amount of NA overflow between the two sexes was no longer observed. Thus, the lower NA release during ischaemia in female hearts is largely due to the greater α_2-adrenoceptor-mediated presynaptic inhibition in female than in male hearts. This gender difference is only observed during ischaemia but not during normoxia. It is probably the result of a higher prevailing NA level in the synaptic cleft due to the ineffectiveness of washout during stop-flow ischaemia. Our clonidine data also support this proposition. As clonidine is a partial agonist with an efficacy of about 1/5 of that of NA [Medgett *et al* 1978], it will show an antagonistic effect when NA concentrations are high. Indeed, during ischaemia, neuronal NA overflow was increased in males by clonidine. Although the rise in the female group was not significant, it was also not different from the male group. However, the overall effect of clonidine during ischaemia is to enhance neuronal NA overflow ($p<0.01$, combined data from both groups).

The observed sex difference in presynaptic inhibition of neuronal NA release during ischaemia may be an explanation for the early loss in catecholamines from adrenergic nerves after coronary ligation in one study using male rats (0.5 hour) [Abrahamsson *et al* 1982] but not in two other studies of female rats (3-8 hours) [Paessens and Borchard 1980, Ahonen *et al* 1975]. In Chapter 3, we have demonstrated that neuronal NA release is maintained for at least 90 mins of low-flow ischaemia, along with an efficient adrenergic presynaptic inhibition.

Implications

The sympathetic nervous system plays an important role in the development of serious ventricular arrhythmias during acute myocardial ischaemia [Corr *et al* 1986]. Gender may affect the risk of severe ischaemic arrhythmias both in man and experimental animals [Section 1.1.3]. In the anaesthetized rat, the onset of ventricular arrhythmias is delayed and arrhythmia-induced death within the first 20 mins of coronary ligation is less frequent in females than males [Siegmund *et al* 1979]. In the Framingham Study, sudden cardiac death rates are higher for men than for women [Kannel and Schatzkin 1985, Dahlberg 1990]. However, sudden cardiac death, as a percentage of total mortality from coronary heart disease, is not significantly lower in women (46% *vs* 34%, $p<0.1$). Although ventricular tachyarrhythmias are more likely

to be induced in electrophysiological testing in men than in women [Freedman *et al* 1988, Schoenfeld *et al* 1985], these studies were not controlled for differences in the extent of coronary artery disease or extent of impairment of ventricular function. In addition, the majority of women in all these studies were of post-menopausal age. Thus, adequate clinical studies comparing the vulnerability to arrhythmias or sudden cardiac death in men and women appear not to have been conducted. The difference in presynaptic inhibition shown in this study might lead to a reduced adrenergic stimulation and fewer serious arrhythmias and/or a reduction in sudden cardiac death in women. It would be interesting to test this possibility in men and pre-menopausal women.

Chapter 6

Parasympathetic Nervous Control of the Heart: Effect of Gender

6.1 Introduction

We have observed a more potent α-adrenergic presynaptic control of neuronal NA release in female than in male rats (Chapter 5). NA exocytosis is also under modulation of vagal nerve activity [Levy 1984, Lavallée *et al* 1978, Chapter 4]. Although no study has been conducted to test whether gender influences vagal-mediated presynaptic and postsynaptic effects in the heart, several indirect observations indicate that gender may have such effect. The cardiovascular response to stress differs according to gender. An increase in heart rate (HR), blood pressure, peripheral vascular resistance, ventricular contractility and plasma level of catecholamines in response to various stress (e.g. exercise, lower body negative pressure, mental stress, or insulin-induced hypoglycaemia) are common in man. Women usually show an increase in HR only with a less marked increase in plasma catecholamines [Claustre *et al* 1980, Buñag *et al* 1975, Brooks *et al* 1990]. It has been suggested therefore that stress leads to a sympathetic activation in males and a withdrawal of vagal activity in females [Frey and Hoffler 1988, Sanchez *et al* 1980].

Gonadal hormones modulate the activity of choline acetyltransferase (ChAT) and acetylcholine (ACh) content in central nervous system [Muth *et al* 1980, Luine 1985, Luine *et al* 1986]. Oestrogen treatment may increase muscarinic receptors in nervous tissue [Olsen *et al* 1988, Egozi *et al* 1982] and smooth muscle [Levin *et al* 1980]. It is unclear if these effects also occur in the heart, but oestrogen and androgen receptors do exist in myocardium [Stumpf *et al* 1977, McGill *et al* 1980]. Moreover, using ligand binding technique, Williams *et al* demonstrated an increased affinity of muscarinic receptors to ^{3}H-QNB in female than in male rat hearts [1984].

Parasympathetic activation protects against ventricular arrhythmias during acute ischaemia both in man and in experimental animals [Schwartz and Stramba-Badiale 1988, Schwartz *et al* 1988a, Corr *et al* 1986, Section 1.2.2]. A difference may exist between the sexes in the severity of ischaemic arrhythmias or incidence of sudden cardiac death [Dahlberg 1990, Siegmund *et al* 1979, Puddu *et al* 1988, Section 1.1.3]. The mechanisms for this gender difference is largely unclear.

Therefore, the effect of gender on vagal nerve stimulation mediated inhibition of neuronal NA release (presynaptic action) and reduction in HR (postsynaptic action) was examined in the *in situ* perfused and innervated rat heart model (Section 2.2). The influence of gonadectomy on these aspects was also studied.

6.2 Methods

6.2.1 Animals, Drugs and Model

Sprague-Dawley male and female rats (9-10 weeks of age) were used for this study. All experiments were carried out alternatively on male and female rats. Coronary flow rate was adjusted at about 5 ml/g/min. Drugs used were desipramine (0.1 μM, neuronal reuptake inhibitor), choline chloride (10 μM, precursor for ACh synthesis), physostigmine (1 μM, cholinesterase inhibitor), methacholine (0.01-50 μM, muscarinic agonist) and atropine (10 μM, muscarinic antagonist). Desipramine, choline chloride and physostigmine were present throughout the experiment and other drugs were infused as described in Section 2.6. Coronary effluent for the measurement of NA was collected for 2 mins starting from the onset of left stellate ganglion stimulation (GS) in normoxic experiments, or for 2.5 mins in ischaemic-reperfusion experiments.

6.2.2 Protocols

Cholinergic inhibition of NA release during normoxia and ischaemia

Presynaptic inhibition of NA overflow either by bilateral vagal nerve stimulation (VS) or by methacholine was studied in male and female rat hearts.

In 13 male and 14 female normoxically perfused rat hearts, GS (5 Hz for 1 min) was performed three times. The first GS served as a control, followed by GS plus VS (15 Hz for 1 min) or GS in the presence of methacholine (10 μM). The sequence of the last two nerve stimulations were randomized.

Another 10 male and 10 female hearts were subjected to two episodes of 3-min stop-flow ischaemia separated by a 15-min period of normoxic perfusion. GS (5 Hz for 1 min) or combined GS and VS (5 and 15 Hz for 1 min, respectively) was applied, in random order, during the final minute of ischaemia, immediately followed by reperfusion.

Cholinergic postsynaptic effects in normoxic hearts

The effects of endogenous (VS) and exogenous (methacholine) cholinergic stimulation on HR, coronary perfusion pressure were examined in male and female rat hearts.

In 19 male and 22 female hearts, six VS were performed (30 sec duration) at frequencies of 1, 2.5, 5, 10, 15 and 20 Hz, separated by 10-min recovery periods. Epicardial ECG and ventricular pressure were continuously recorded. Ten mins after the last VS, 21 (8 male and 13 female) out of the 41 rat hearts were infused with increasing concentrations of methacholine (0.01, 0.1, 1, 5, 10, 20 and 50 μM). Each dose was maintained for 3 mins. Epicardial ECG and perfusion pressure were continuously recorded. In order to eliminate an effect of myocardial contraction on perfusion pressure recorded during methacholine administration, in another 11 hearts (6 male and 5 female) VF was induced and maintained by electrical stimulation of the left ventricle (1.5 mA, 10-20 Hz) and the effect of concentrations of methacholine (0.01-50 μM) on coronary perfusion pressure was examined as described above. In another 4 preparations, the influence of atropine (10 μM) on the vasoconstrictive effect of 50 μM methacholine was examined.

Gonadectomy and the effects of vagal nerve stimulation

The possibility that gonadal hormones modulate the effects of VS on the heart was examined. Gonadectomy (GX) or sham-operation (SH) were performed in 4 week old male and female rats [Waynforth 1980]. Heart perfusion experiments were carried out 8-9 weeks after the operation (i.e. at 12-13 weeks of age). At the time of sacrifice, effective gonadectomy was verified by visual inspection of residual gonadal tissues and uterine atrophy in ovariectomized female rats (Section 2.4).

GS (5 Hz for 1 min) or combined GS and VS (5 and 15 Hz for 1 min, respectively) were applied separated by a 15-min recovery period. Coronary effluent for the measurement of NA was collected for 2 mins. Afterwards, VS was given for 6 times (30 sec duration with 10-min recovery periods between the nerve stimulations). The frequencies used were 1, 2.5, 5, 10, 15 and 20 Hz, respectively and changes in HR were measured.

6.3 RESULTS

6.3.1 Presynaptic Cholinergic Effects

Hearts of male rats were heavier than those of females (0.80±0.01 *vs* 0.71±0.01 g, $p<0.01$). Perfusion flow was adjusted to the estimated heart weight and did not differ between male and female groups (5.04±0.09 *vs* 5.04±0.05 ml/g/min).

In normoxic hearts, GS-induced NA overflow was similar in the two sexes. Both vagal nerve stimulation and 10 μM methacholine significantly inhibited NA overflow from male and female rat hearts. In female hearts, inhibition of NA overflow by VS was more marked than in male hearts with a bigger net reduction of NA overflow (-38.0±4.8 *vs* -21.9±4.3 pmol/g, $p<0.05$). As a result the ratio of NA overflow in the presence and absence of VS was also lower in females ($p<0.05$, Figure 6.1). Methacholine (10 μM) induced a similar reduction in neuronal NA overflow both in absolute terms or when expressed as a ratio of control NA overflow (Table 6.1 and Figure 6.1). During ischaemia NA overflow by GS was lower in female hearts and VS did not significantly reduce NA overflow in both groups (Table 6.1). However, a tendency of a reduction was noticed in females ($p=0.06$, Figure 6.1).

Table 6.1 *Effects of vagal nerve stimulation (VS, 15 Hz) and methacholine (MCh, 10 μM) on sympathetic ganglion stimulation (GS, 5 Hz) induced NA overflow (pmol/g) in male and female rat hearts in normoxia or in 3 min stop-flow ischaemia*

	Male	**Female**	$p^{\S}$
Normoxia	*(n=13)*	*(n=14)*	
GS	83.4 ± 12.0	88.5 ± 11.9	NS
GS+VS	61.5 ± 11.0*	50.5 ± 9.1**	NS
GS+MCh	18.7 ± 4.8#	22.0 ± 4.9#	NS
Ischaemia	*(n=10)*	*(n=10)*	
GS	53.5 ± 12.9	26.9 ± 4.5	<0.05
GS+VS	58.3 ± 12.9	18.7 ± 2.3	<0.01

§ *unpaired t-test for gender difference.*

**comparison vs GS by paired t-test : *p<0.05 ** p<0.01 # p<0.001.*

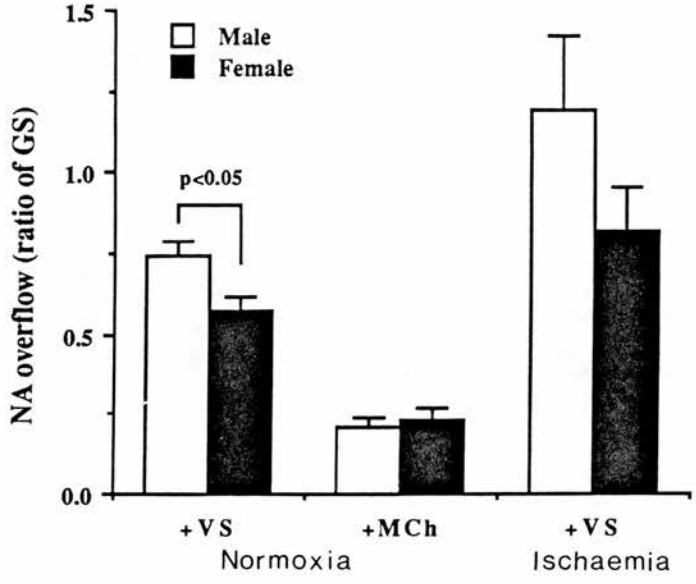


Figure 6.1. *Presynaptic inhibition of neuronal NA overflow by vagal nerve stimulation (VS) or methacholine (MCh) during normoxia or ischaemia and the effect of gender. Results are expressed as ratios relative to NA overflow by a control GS.*

6.3.2 Postsynaptic Cholinergic Effects

Basal HR before each VS was similar in males and females ($p>0.8$). Frequency-dependent reduction in HR by VS was observed in male and female hearts. At each frequency, the HR-lowering effect was greater in females than in males (Figure 6.2). At a frequency of 20 Hz, 50% of female hearts stopped beating whilst asystole was observed only in 3 male hearts (16%). Coronary perfusion pressure was not significantly affected by VS.

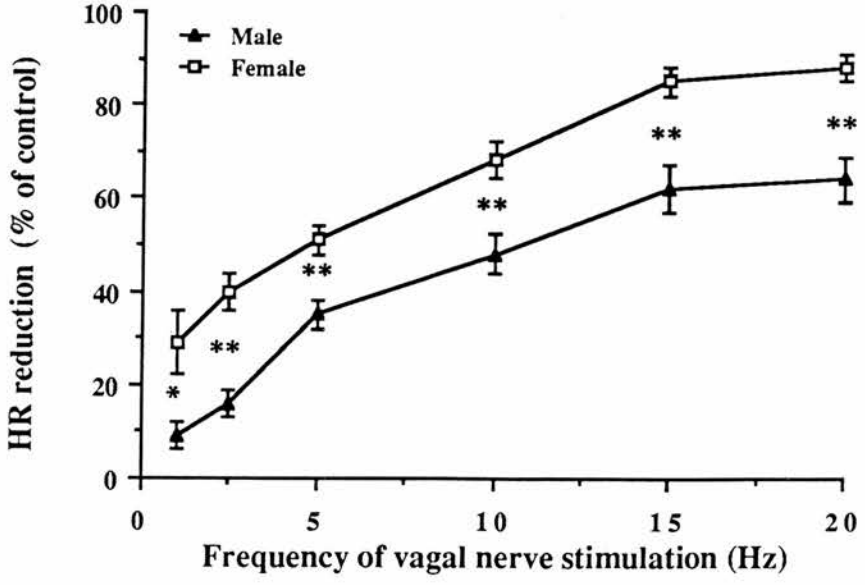


Figure 6.2. *Heart rate-lowering effect of vagal nerve stimulation (1-20 Hz) in normoxically perfused male (n=19) and female rat hearts (n=22). Overall difference $p<0.001$ by ANOVA and significance at individual frequencies *$p<0.05$ **$p<0.01$.*

Administration of methacholine led to a dose-dependent decrease in HR and an increase in perfusion pressure (Figure 6.3). These changes were identical in male and female hearts. In male and female hearts, perfusion pressure before (40±2 *vs* 45±2 mm Hg) and after 3 mins methacholine infusion (40±2 *vs* 46±1 mm Hg) was similar. The changes in coronary perfusion pressure induced by methacholine (>10^{-6} M) were significantly lower ($p<0.01$) in the hearts with induced ventricular fibrillation.

Atropine at 10 μM largely blocked the methacholine-induced (50 μM) increase in perfusion pressure from 118±6 to 46±3 mm Hg ($p<0.01$, n=4, not shown in the figure).

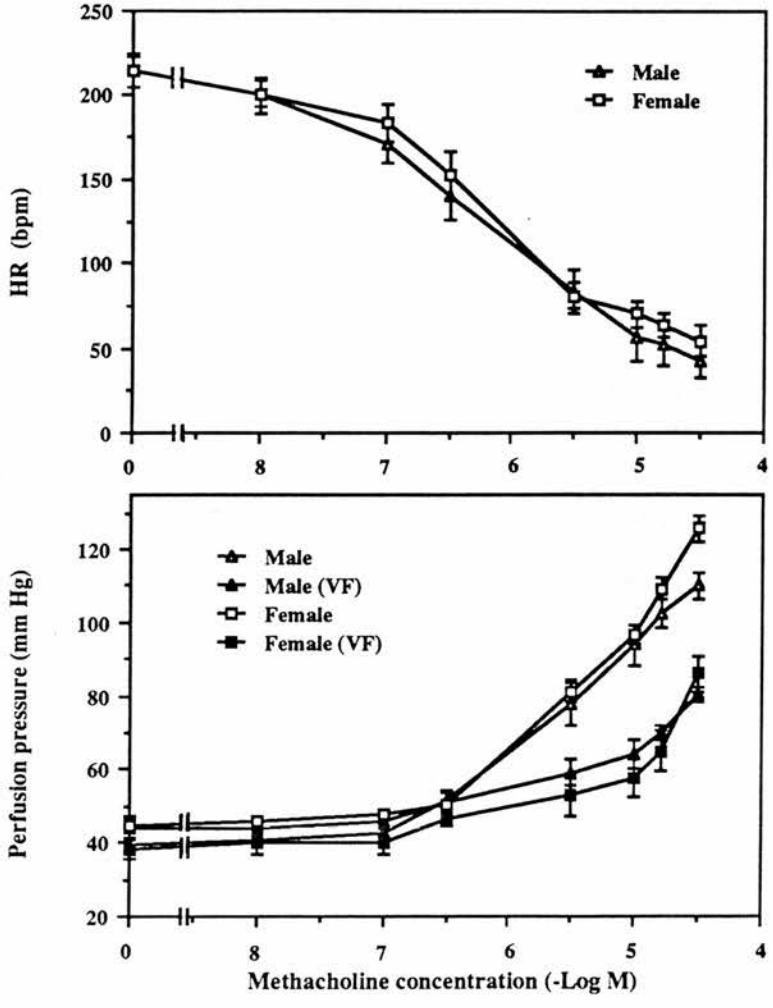


Figure 6.3. *The effect of methacholine on heart rate (HR, upper panel) and coronary perfusion pressure (CPP, lower panel) in male and female rat hearts. No gender difference was observed by ANOVA, either in the absence or presence of ventricular fibrillation (VF). Open symbols: beating hearts. Closed symbols: fibrillated hearts.*

6.3.3 Gonadectomy and Vagal Effects

Body and heart weights increased after ovariectomy and decreased after castration (Figure 6.4). These results and those of uterine weight and perfusion flow rate at the time of sacrifice are presented in Table 6.2.

Table 6.2 *Body, heart, and uterus weights and coronary flow rate (CFR) in sham-operated and gonadectomized rats*

	Body	Heart	Uterus	CFR
	(g)	*(g)*	*(mg)*	*(ml/g/min)*
FSH *(n=12)*	248 ± 5	0.81 ± 0.02	512 ± 18	4.9 ± 0.1
FGX *(n=12)*	312 ± 7*	0.92 ± 0.03*	60 ± 3*	5.0 ± 0.1
MSH *(n=7)*	418 ± 11	1.27 ± 0.04	-	4.8 ± 0.1
MGX *(n=11)*	368 ± 7*	1.09 ± 0.02*	-	5.1 ± 0.1

** $p<0.01$ vs sham-operated rats of the same gender.*

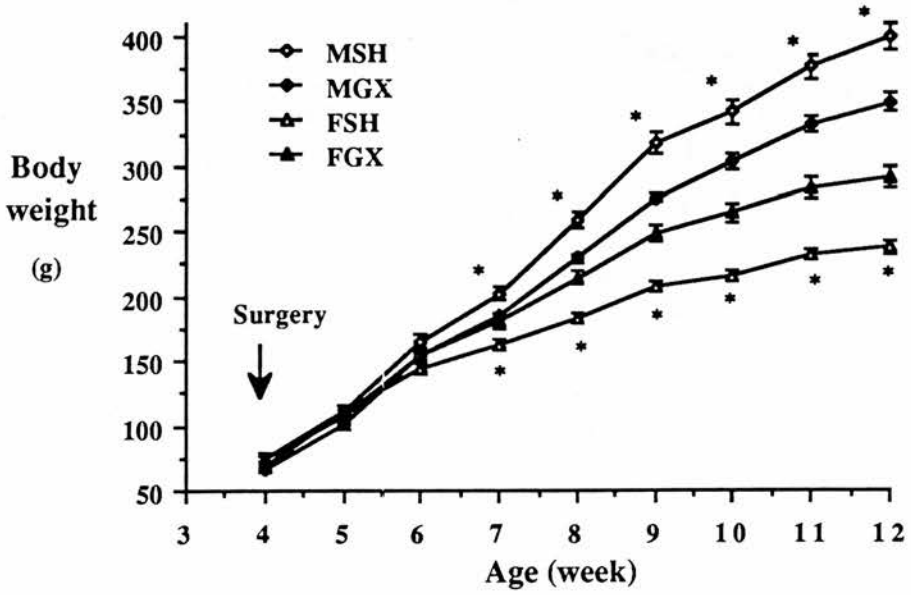


Figure 6.4. *Growth curves in sham-operated and gonadectomized male or female rats. Sham-operated: male (MSH), female (FSH); gonadectomized: male (MGX), female (FGX).*
**$p<0.05$ for MSH vs MGX or FSH vs FGX.*

There was no significant difference in control GS-induced NA overflow between the 4 groups (FSH: 59.9±7.8, FGX: 61.0±10.3, MSH: 71.5±8.6, and MGX: 63.6±5.2 pmol/g). VS significantly reduced this value in FSH group ($p<0.05$), but not in the other three groups (Figure 6.5). Ratios of NA overflow with and without VS did not differ between the four groups.

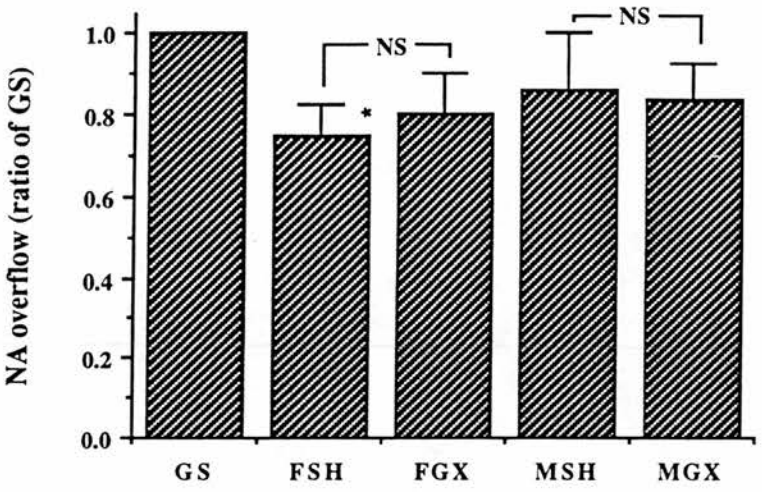


Figure 6.5. *Effect of vagal nerve stimulation (VS, 15 Hz) on NA overflow induced by sympathetic ganglion stimulation (GS, 5 Hz) in hearts of sham-operated and gonadectomized male and female rats. Results are expressed as the ratio of NA overflow induced by a control GS. * p<0.05 vs GS by paired t-test.*

Basal HR was not influenced by gender or gonadectomy ($p<0.6$). The frequency-dependent reduction in HR was demonstrated in male and female hearts of rats whether gonadectomized or not, with similar slopes of the frequency-response curves. However, the extent of HR reduction by VS varied between the four groups and followed the sequence: FSH > FGX > MGX > MSH ($p<0.05$ by ANOVA for the difference between FSH *vs* FGX and MGX *vs* MSH, Figure 6.6).

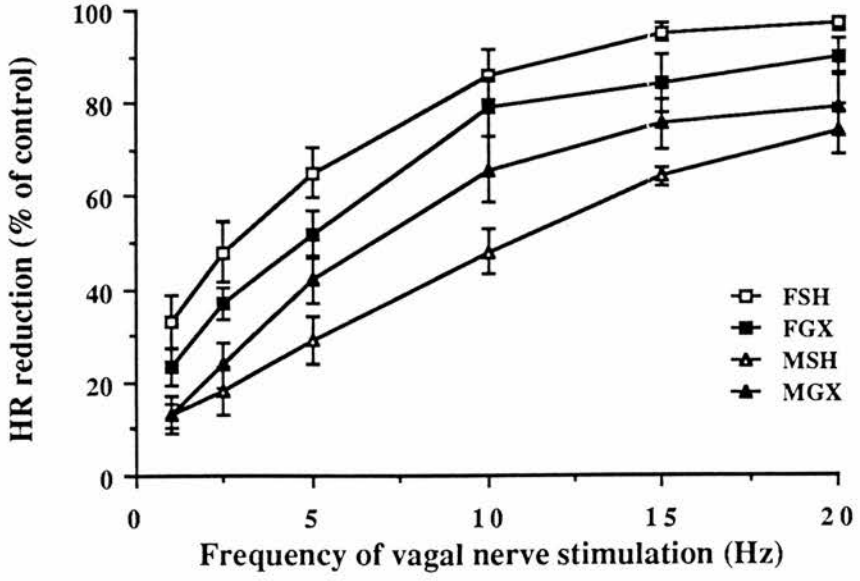


Figure 6.6. *The effect of gonadectomy on HR-lowering response to vagal nerve stimulation (1-20 Hz). p<0.05 for the difference between FSH vs FGX or between MSH vs MGX groups by ANOVA.*

6.4 DISCUSSION

Results from the present study show a more potent vagal effect in the perfused female heart. Vagal nerve stimulation reduced neuronal NA overflow and HR more in female than in male hearts during normoxic perfusion. A failure of vagal-mediated inhibition of NA overflow by a few minutes of stop-flow ischaemia in male hearts has been demonstrated (Chapter 4). In the present study, after 3 mins stop-flow ischaemia, vagal stimulation again did not reduce neuronal NA overflow in male hearts, but a tendency of reduced NA overflow in female hearts was observed. This occurs despite the fact that neuronal NA overflow during ischaemia was significantly lower in females than in males, a finding similar to that presented in Chapter 5.

Mechanisms responsible for this gender difference are not clear. Clearance of released ACh in the heart is achieved *via* two mechanisms: hydrolysis by acetylcholinesterase and washout from the biophase [Löffelholz and Pappano 1985]. The response to VS would change if a gender difference in the capacity of ACh clearance exists. In this study, coronary flow rate was controlled and the studies were performed in the presence of physostigmine. Effectiveness of this drug in preventing hydrolysis of ACh seems reasonable as there was a significant potentiation of HR-lowering response to VS (Chapter 4). Dieterich *et al* [1976] have demonstrated that physostigmine increases evoked output of ACh by 2-3 fold in mammalian hearts. Thus, under our experimental conditions a gender difference in the clearance of ACh seems irrelevant.

Another possibility is a gender difference in the functional status of pre- and post-synaptic muscarinic receptors. It has been demonstrated that oestrogen treatment increases muscarinic receptors in the central nervous tissue of rats [Olsen *et al* 1988, Egozi *et al* 1982]. Oestrogen may also exert a similar effect in smooth muscle and myocardium [Levin *et al* 1980, Williams *et al* 1984]. However, inspection of methacholine-mediated effects, i.e., changes in NA overflow, HR and coronary vasoconstriction, do not suggest a gender difference in muscarinic receptors localized on sympathetic nerve endings, myocytes and smooth muscle cells of coronary arteries.

As ACh overflow by vagal nerve stimulation was not measured in this study, we can not exclude the possibility that the speed of ACh resynthesis is higher in females, thereby leading to a higher degree of ACh pool replenishment during repeated vagal stimulations. In all experiments, 10 μM choline was present to ensure a continuous supply of the precursor for ACh resynthesis. A high affinity choline uptake system

transfers choline into the cholinergic nerve ending [Ducis 1988, Section 1.3.3]. The supply of choline *via* this system meets the demand even under condition of a markedly increased neuronal activity [Ducis 1988, Lindmar *et al* 1980, Wetzel and Brown 1983]. We observed in five separate male hearts that vagal nerve stimulation (15 Hz for 1 min separated by 15-min intervals) reduced HR equally well (% reduction from control: 71±3%, 67±6%, 66±6%, 65±4%, and 63±5%, NS). In the *in vivo* male rats, there is no reduction in ACh content in various parts of the heart after 20 mins intensive vagal stimulation (15 Hz, 0.5 mA and 2 ms) [Lund *et al* 1986]. These observations suggest an efficient ACh resynthesis even in the male rat heart.

Results from the gonadectomy study show that both male and female hormones are involved in the observed gender difference in the HR-lowering effect of vagal nerve stimulation. The frequency-response of HR was attenuated by ovariectomy but strengthened after castration. These opposite effects of gonadectomy in female and male rat hearts seem to indicate that female hormones enhance, and male hormone inhibits the effect of the vagus on the heart. However, an effect of gonadectomy on vagally-mediated inhibition of neuronal NA release was not found. We have no simple explanation for this discrepancy and it is perhaps due to a large individual variation in this specific experiment. Nevertheless, reduced NA overflow by vagal nerve stimulation was only observed in the sham-operated female group.

Modulation of cholinergic nerve function by gonadal hormones has been described for central nervous tissue. The activity of cholinergic enzymes differ between males and females [Luine *et al* 1986. Muth *et al* 1980]. Oestrogen treatment increases ChAT activity and ACh content in certain regions which are sensitive to gonadal hormones [Luine 1985]. Increased ChAT activity may lead to an increased cholinergic function although ChAT may not be the rate-limiting step for ACh synthesis [Ducis 1988, Section 1.3.3]. However, there has been no data to show a gender difference in cardiac content of ACh or ACh overflow evoked by vagal nerve stimulation. We tried to pre-label hearts with ^{3}H-choline (1 μM) and measured radioactive overflow before and during vagal nerve stimulation. The high basal level of radioactivity in coronary effluent and a small increase in overflow of the ^{3}H-radioactivity by nerve stimulation made it unsuitable for quantitative studies. Now, we are setting up a radioenzymatic method for ACh analysis. Data on ACh content in myocardium and release are critical if we wish to explain the mechanism underlying the observed sex difference.

Chapter 7

Sympathetic-Parasympathetic Interactions, Gender and Ischaemic-Reperfusion Arrhythmias

7.1 Introduction

Acute myocardial ischaemia is a potent stimulus for the activation of autonomic nervous system, both in man and in animals [Pantridge 1978, Lombardi *et al* 1983]. Within the first 30 mins after the onset of acute myocardial infarction, 83% of patients show clinical signs of sympathetic and/or parasympathetic activation [Pantridge 1978]. Direct recording of afferent and efferent nerve activity demonstrated enhanced cardiac sympathetic and vagal nerve impulses after coronary ligation [Lombardi *et al* 1983, Corr *et al* 1986].

The role of sympathetic activation in the genesis of ischaemic and reperfusion arrhythmias has been documented by a large body of clinical and experimental studies [Section 1.2.2]. Cardiac sympathetic activation, either by electrical, pharmacological or psychological means, increases the vulnerability of the heart to arrhythmias. On the contrary, chronic sympathectomy or adrenergic antagonists reduce the incidence of ischaemic-reperfusion arrhythmias [Corr *et al* 1986, Verrier 1988, Schwartz and Zuanetti 1988]. Most studies suggest that vagal activation during ischaemia protects the heart from arrhythmias [Verrier 1988, Schwartz and Stramba-Badiale 1988]. A similar salutary effect of vagal activity in the modulation of reperfusion arrhythmias has been suggested by a few recent studies [Schwartz and Zuanetti 1988]. The mechanisms responsible for the vagal protection against ischaemic and reperfusion arrhythmias are considered mainly to be due to its anti-adrenergic effects, including presynaptic inhibition of NA release [Levy 1984, Corr *et al* 1986]. But the contribution by this inhibitory mechanism during ischaemia is largely unknown.

A few studies suggest less severe ischaemic arrhythmias or early mortality in female than in male rats [Siegmund *et al* 1979, Du *et al* unpublished, Lu *et al* 1984] and dogs [Puddu *et al* 1988]. We have found sex differences in the autonomic control of the heart (Chapter 5 and 6). It would then be of interest to examine if these differences affect the severity of arrhythmias during acute ischaemia.

The rat is a commonly used model for the study of ischaemic and reperfusion arrhythmias [Curtis *et al* 1987]. However, the relation between neuronal activity and the genesis of arrhythmias has apparently not been examined in this model.
Therefore this study was designed to examine: 1) modulation of sympathetic and cholinergic stimulation on the pathogenesis of reperfusion arrhythmias linked to NA release, and 2) sex difference in the severity of ischaemic arrhythmias in models without (isolated perfused heart) and with neuronal influence (intact animal).

7.2 Methods and Protocols

The effect of nerve stimulation on reperfusion ventricular fibrillation (VF) was studied using male Wistar rats (9-10 weeks old). Male and female Sprague-Dawley rats (10-12 weeks old) were used for *in vitro* and *in vivo* arrhythmia studies.

7.2.1 Reperfusion Arrhythmia Experiment

The *in situ* perfused innervated rat heart model, described in Section 2.2. was used in this study. Neuronal reuptake inhibitor desipramine (0.1 μM) was present throughout.

After a 30-min stabilization period, low-flow ischaemia (10 mins) was induced by reducing perfusion flow from 5 ml/g/min to 0.25 ml/g/min. Coronary effluent was collected on ice during ischaemia for lactate and NA measurements. At the end of ischaemia perfusion flow was restored to the pre-ischaemic level. Epicardial ECG was recorded during ischaemia and on reperfusion to monitor heart rate (HR) and VF.

Four groups were included:

1) control (n=12), no nerve stimulation;
2) GS (n=11), sympathetic ganglion stimulation (GS, 5 Hz) was performed during 1-10 mins of ischaemia;
3) combined nerve stimulation (n=14), sympathetic (5 Hz) and bilateral vagal nerve stimulation (15 Hz) were applied during 1-10 mins of ischaemia;
4) GS + methacholine (n=12), methacholine (1 μM) was added to perfusate at least 12 mins before ischaemia and maintained throughout the ischaemic period. GS was performed during the last 9 mins of ischaemia.

The end point for these studies was VF occurring within the first 2 mins of reperfusion.

7.2.2 *In vitro* Arrhythmia Experiment

An isolated Langendorff-perfused rat heart model (see Section 2.3.1) was used. After stabilization, coronary artery was occluded and maintained for 20 mins followed by 5 mins of reperfusion. ECG was continuously recorded throughout the ischaemic-reperfusion period. Coronary effluent was collected every 5 mins during ischaemia and during the 5 mins reperfusion. Lactate concentrations were measured in all the samples and NA levels were analysed in the samples collected during the reperfusion.

7.2.3 *In vivo* Arrhythmia Experiment

An anaesthetized open-chest animal model, previously described in Section 2.3.2, was used for this experiment. After stabilizing the preparation and recording of blood pressure and ECG (chest lead), left coronary ligation was induced and maintained for 20 mins. In a pilot experiment, 12 hearts were reperfused 20 mins after coronary ligation according to the technique described by Himori and Matsuura [1989]. A low incidence of reperfusion-induced VF was found (8%). Therefore reperfusion was not routinely performed in this study. ECG and blood pressure were continuously monitored during ischaemia. At the end of 20 mins of ischaemia, those rats without sustained VF received a bolus injection of Evans blue solution intravenously for the determination of non-perfused myocardium (Section 2.3.2).

7.2.4 Verification of Coronary Artery Ligation

In the *in vitro* perfused hearts reduction of coronary effluent by coronary ligation was used to assess the severity of the ischaemic insult. In the *in vivo* study, the unstained ischaemic area was carefully dissected from the blueish non-ischaemic zone and weighed. The percentage of the ischaemic region was expressed as a percentage of left and right ventricular weight (atria were trimmed off before weighing). The extent of reduction in arterial blood pressure immediately after coronary occlusion was also used to estimate the severity of ischaemic insult to the heart.

Two *in vitro* preparations were rejected as the reduction in coronary flow by coronary ligation was less than 20%. Three *in vivo* preparations were discarded due to a failure to occlude the left coronary artery.

7.3 Results

7.3.1 Reperfusion VF and NA Overflow

In the control group, NA levels during the first 10 mins of low-flow ischaemia were less than 1 pmol/ml. Sympathetic nerve stimulation increased NA concentrations to about 30 pmol/ml, and this level was maintained during the 9 mins of nerve stimulation (Table 7.1). In the hearts with combined sympathetic and vagal nerve stimulation, NA level in the effluent collected during the first period (1-4 mins) tended to be lower compared with that measured afterwards, although this difference was not statistically significant ($p=0.06$). During low-flow ischaemia nerve stimulation induced NA overflow was not affected by 1 μM methacholine (Table 7.1 *vs* GS), in contrast to its 50% inhibition of neuronal NA overflow during normoxia (Chapter 4).

Reperfusion VF was 8% in the control group. Sympathetic nerve stimulation significantly increased the incidence of VF during reperfusion ($p<0.05$) accompanied by an enhanced NA overflow during ischaemia (Figure 7.1). Both endogenous and exogenous cholinergic stimulation abolished the sympathetic stimulation-triggered VF during reperfusion ($p<0.05$) but failed to inhibit NA overflow during ischaemia (Table 7.1 and Figure 7.1).

Table 7.1 *NA levels in coronary venous effluent during the first 10 mins of low-flow ischaemia (0.25 ml/g/min) and the effect of nerve stimulation and methacholine.*

	Heart Weight	**NA concentration** *(pmol/ml)*		
	(g)	1-4 mins	4-7 mins	7-10 mins
Control *(n=12)*	0.78±0.02	0.6±0.1	0.9±0.2	0.8±0.2
GS *(n=11)*	0.79±0.02	30.3±7.3	32.1±5.3	23.8±4.6
GS+VS *(n=14)*	0.81±0.03	17.4±2.6	30.7±5.8	30.7±5.6
GS+MCh *(n=12)*	0.81±0.02	28.8±4.6	40.4±6.5	28.1±3.0

During ischaemia there was a marked increase in the concentrations of lactate in coronary effluent and this was similar in the groups with and without nerve stimulation (Figure 7.2). In the control group ischaemia led to a progressive reduction in HR within the first few minutes. GS resulted in a transient and small increase in

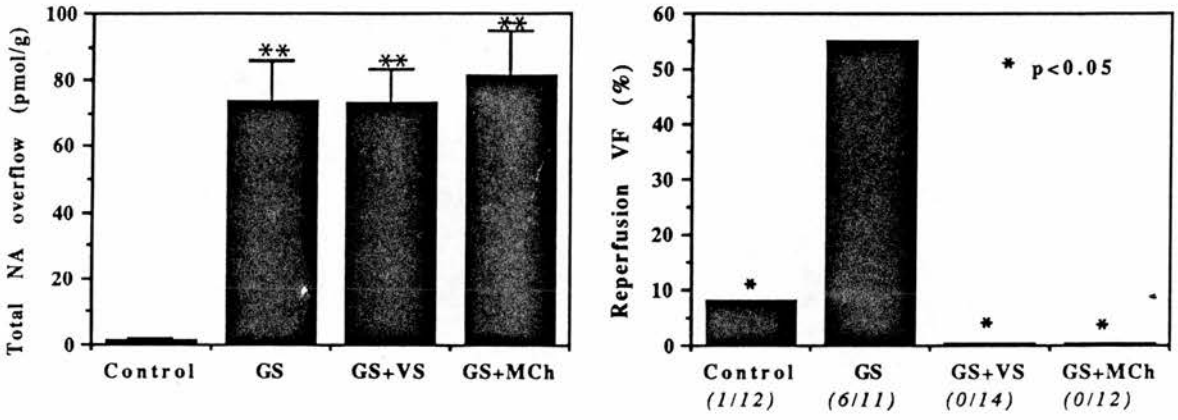


Figure 7.1 *The effect of vagal nerve stimulation (VS, 15 Hz) or methacholine (MCh, 1 μM) on NA overflow induced by sympathetic nerve stimulation (GS) during the first 10 mins of low-flow ischaemia (left panel) and the incidence of VF during reperfusion (right panel). *p<0.05 vs GS group (right panel) and ** p<0.01 vs control group (left panel)*

HR during the first 3 mins of nerve stimulation ($p<0.05$). Combined sympathetic and vagal nerve stimulation significantly reduced HR during the first period of nerve stimulation (1-4 mins, $p<0.05$ vs control and GS groups). Afterwards no difference existed between the three groups (Figure 7.2). Arrhythmias were not found during ischaemia except bradycardia. In the group with methacholine, HR before ischaemia was significantly lower than in the other three groups (136±15 beats/min, $p<0.01$), and did not differ from the GS group during low-flow ischaemia (data not shown).

7.3.2 Gender and Ischaemic Arrhythmias

Coronary ligation *in vitro* resulted in a 40% reduction in coronary flow rate ($p<0.001$) and a significant decrease in HR ($p<0.05$). Lactate concentration in the coronary effluent was higher during ischaemia ($p<0.01$) and further enhanced during the first 5 mins of reperfusion ($p<0.01$). During the first 5 mins of reperfusion, NA overflow was 28.6±6.1 pmol/g in males and 25.1±4.3 pmol/g in females. All these changes were similar in the male and female groups (NS, Table 7.2).

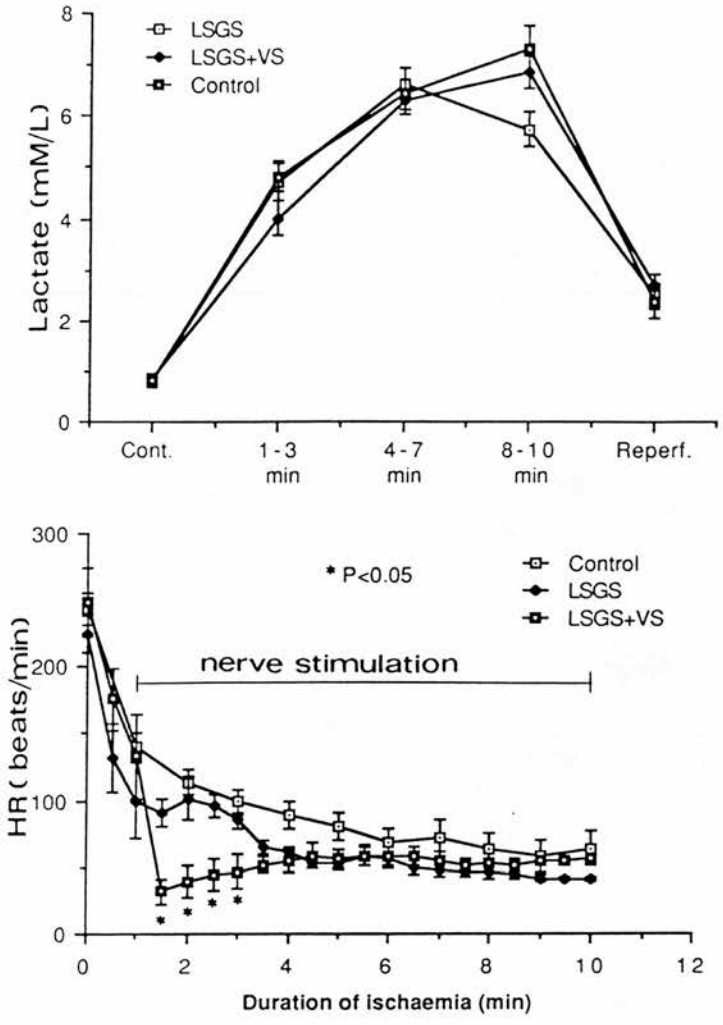


Figure 7.2 *The effect of nerve stimulation on lactate production (upper panel) and heart rate during 10 mins of low-flow ischaemia (lower panel).*p<0.05 vs control and GS groups.*

Table 7.2 *Changes in HR, coronary flow rate (CFR) and lactate levels before and during ischaemia (20 mins) and reperfusion (5 mins) in isolated perfused male and female rat hearts.*

	HR (beats/min)		CFR (ml/min/g)		Lactate (μM)	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*
Control	284±7	272±8	9.1±.23	9.1±5.0	35±4	32±4
Ischaemia						
0.5 min	260±8	249±8	-	-	-	-
5 min	247±6	243±9	5.4±.26	5.5±.27	240±17	217±13
10 min	251±6	247±9	4.8±.27	6.0±.49	355±45	260±30
15 min	243±9	240±10	4.5±.31	5.0±.34	326±69	226±41
Reperf	-	-	-	-	567±67	519±36

All parameters were not significantly different between males (n=24) and females (n=24).

In the *in vivo* experiment, the weight of ventricles (0.83±0.01 and 0.72±0.01 g, p<0.01) and of the ischaemic myocardium (0.27±0.01 *vs* 0.24±0.02 g, p<0.05) was greater in males than in females. The ischaemic myocardium as percentage of total ventricular mass was similar in both sexes (32.7±1.5% *vs* 33.7±2.3%, p=NS).

The development of ventricular arrhythmias during ischaemia and reperfusion in both *in vitro* and *in vivo* studies is summarized in Table 7.3. In these two different experimental models, no sex-related difference was observed in the frequency of ventricular premature beats (VPB), ventricular tachycardia (VT) or VF.

Table 7.3 *Ventricular arrhythmias during 20 mins of coronary occlusion and during reperfusion in in vitro and in vivo hearts from male and female Sprague-Dawley rats.*

	In vitro		*In vivo*	
	Male *(n=24)*	**Female** *(n=24)*	**Male** *(n=26)*	**Female** *(n=24)*
VPB *(beats/20 min)*	80±12	64±19	162±49	118±22
VT %	95.8	87.5	100	83
Onset time (sec)	437±22	439±26	330±26	314±16
Duration (sec)	35±6	29±9	43±8	65±20
VF %	62.5	62.5	28	33
Onset time (sec)	581±23	623±46	473±81	482±51
Duration (sec)	320±83	298±81	84±50	77±39
Reperfusion VF %	93	100	-	-
Onset time (sec)	55±19	43±8	-	-

No significant differences were observed between male and females.

7.3.3 Gender and Haemodynamic Response to Ischaemia

There was no sex difference in both HR and mean arterial pressure (MAP) before coronary ligation in the anaesthetized rats. A small but significant increase in HR between 0.5 and 5 mins after coronary ligation was observed in male rats (p<0.05 or <0.02, Figure 7.3). In female rats, however, HR was significantly reduced 1 min after coronary occlusion (p<0.001 vs pre-ischaemic value, Figure 7.3). As a result there was a highly significant difference in HR between the two groups during 3-20 mins period (Figure 7.3). A profound drop in MAP (p<0.001) was found immediately after coronary ligation. There was no difference in the extent of this acute hypotension in male and female groups (0.5 min: -30±4 *vs* -33±3 mm Hg, NS; Figure 7.3). Males

showed a significant partial recovery in MAP over the first 5 mins of ischaemia and MAP increased by 10 mm Hg compared with the 0.5 min ischaemia level ($p<0.01$, Figure 7.4). In female rats, however, there was a further reduction in MAP ($p<0.05$) within the first minutes of coronary occlusion, although some recovery was observed after 10 mins of ischaemia. The early recovery in MAP during the first 5 mins after coronary occlusion in males was highly significantly different from the decrease in MAP in females ($p<0.001$ Figure 7.4).

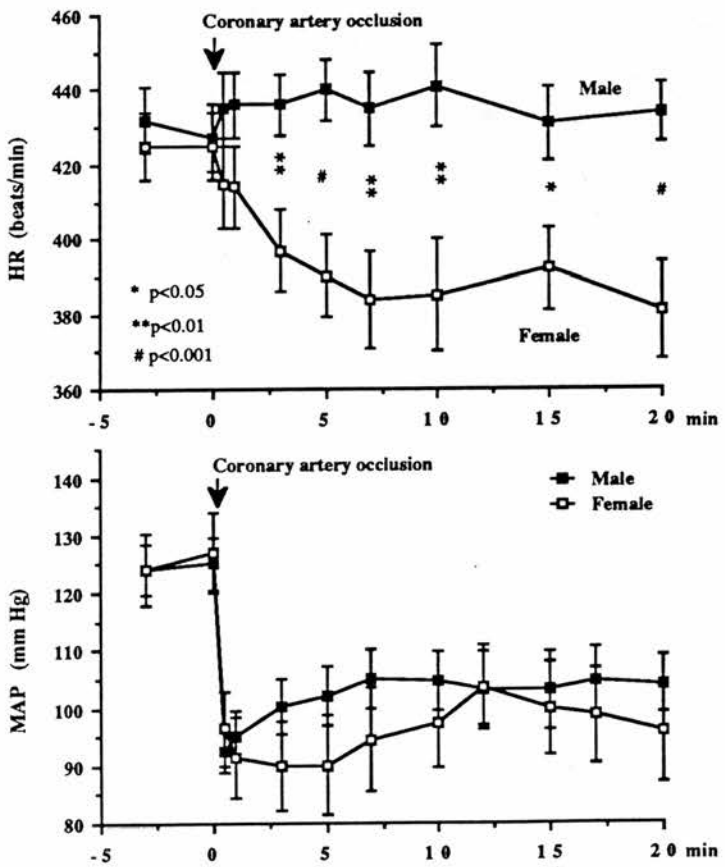


Figure 7.3 *The effect of left coronary ligation on HR (upper panel) and MAP (lower panel) in anaesthetized male and female rats.*

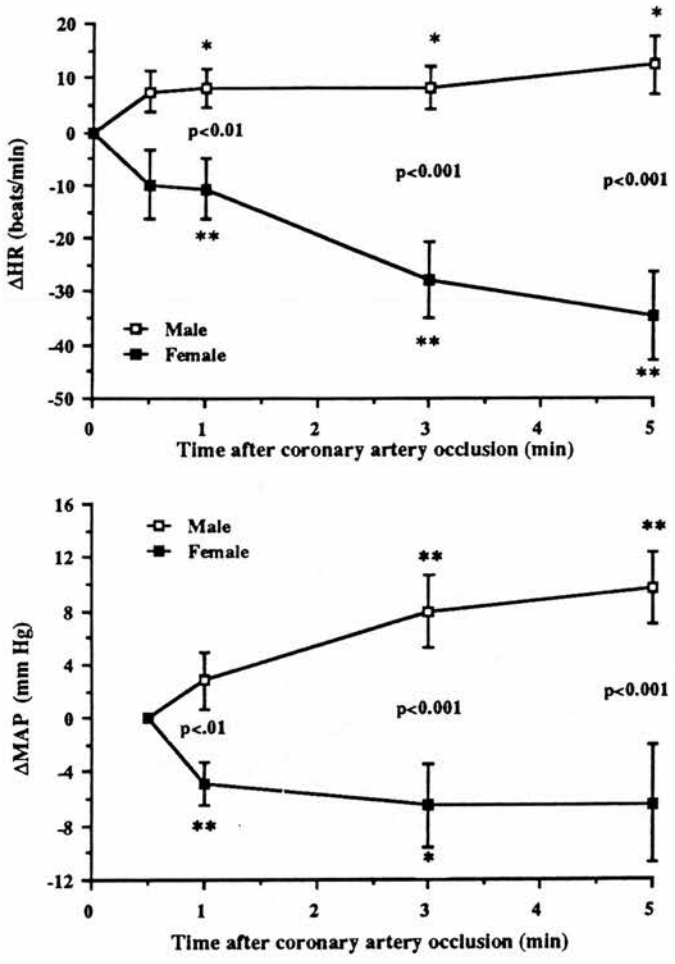


Figure 7.4 *The effect of gender on ischaemia-induced changes in HR (upper panel) and MAP (lower panel) in anaesthetized rats during the first 5 mins of coronary artery ligation. HR results are presented as net changes (ΔHR) from the pre-ischaemic control. MAP data are presented as net changes (ΔMAP) from the level measured at 0.5 min after coronary ligation. *p<0.05 and **p<0.01 vs 0 min values of HR or 0.5 min values of MAP.*

7.4 Discussion

Neuronal Mechanisms and Reperfusion VF

The present study demonstrates that the sympathetic and parasympathetic nerve system exert an opposite influence on the development of reperfusion VF. An enhanced neuronal NA release in the preceding period of ischaemia triggered reperfusion VF. This finding is in agreement with a number of *in vivo* and *in vitro* studies [Section 1.2.2]. In those studies suppression of sympathetic activity by adrenergic antagonists or by sympathectomy reduces the incidence of reperfusion VF

[Penny 1984, Schwartz and Zuanetti 1988, Abrahamsson *et al* 1985]. In an *in vivo* dog study, the amount of spontaneous NA overflow from ischaemic hearts is correlated with the severity of arrhythmias during early reperfusion [Yamaguchi *et al* 1990]. However, a number of other studies also suggest that adrenergic mechanisms are not necessarily involved the arrhythmogenesis during ischaemia and reperfusion in the rat heart [Curtis *et al* 1987, Daugherty *et al* 1986, Botting *et al* 1983]. But none of these studies examined the effect of sympathetic nerve stimulation-induced NA release on the genesis of VF.

An antiarrhythmic effect of cholinergic stimulation (whether by electrical stimulation or using an agonist) on reperfusion VF is observed in the present study. This observation is in keeping with a few existing studies [Schwartz and Zuanetti 1988]. The mechanisms responsible for the salutary impact of cholinergic stimulation on reperfusion VF are considered to be multifactorial, including reduction in HR, presynaptic inhibition of NA release and direct electrophysiological influences [Zuanetti *et al* 1987, Levy 1984, Verrier 1988]. Cholinergic presynaptic inhibition of NA release fails during early ischaemia (Chapter 5). Using a different protocol, a similar failure of cholinergic inhibition of NA release was again observed. Thus in this experiment prevention of reperfusion VF by cholinergic stimulation is associated with a failure of cholinergic presynaptic inhibition of NA release. Therefore, this protection could only be explained by the post-synaptic muscarinic actions. We did find a significant reduction in HR by combined nerve stimulation during 1-4 mins of ischaemia. However, it is uncertain if this early and temporary reduction in HR is important in the suppression of reperfusion VF. This question could be answered by further experiments with HR controlled by pacing. A difference in glycolytic flux, which may be associated with an anti-arrhythmic effect seems not to be involved as lactate levels in coronary effluent during ischaemia were not affected by cholinergic stimulation.

We did not examine the electrophysiological effects of cholinergic stimulation. It has been well documented that vagal stimulation or cholinergic agonists reduce myocardial automaticity, prevent the onset of the delayed after-depolarization, and elongate the effective refractory period [Löffelholz and Pappano 1985, Section 1.2.3]. All these effects may explain the reduced vulnerability to arrhythmias observed. Interestingly, some studies have demonstrated that postsynaptic vagal effects to the heart are potentiated by hypoxia and acidosis, due to increased levels of adenosine [Potter *et al* 1986, Verlato and Borgdorff 1990]. Increased adenosine production during low-flow and stop-flow ischaemia in the perfused rat heart has been well documented [Richardt *et al* 1987, Headrick *et al* 1989]. A negative chronotropic

effect of vagus may also be enhanced by exogenous administration of adenosine [Pelleg *et al* 1988, Belloni *et al* 1989].

Gender and Ischaemic Arrhythmias

In both *in vitro* and *in vivo* experiments, severity of ischaemic arrhythmias during the first 20 mins after coronary artery occlusion was similar in both sexes. As neuronal NA release during ischaemia (Chapter 5) and the function of the vagus (Chapter 6) differ between the sexes, a sex difference in ischaemic arrhythmias in the *in vivo* study was expected. In the *in vitro* study the incidence of VF was sufficiently high to document an anti-arrhythmic effect, but there was none. In the *in vivo* preparation the incidence of VF was generally too low to demonstrate a significant anti-arrhythmic effect with the numbers used [Walker *et al* 1988]. This value is also lower than reported (96-100%) [Curtis *et al* 1987, Johnston *et al* 1983].

The well-defined determinants of VF are infarct size [Curtis *et al* 1987, Bolli *et al* 1986, Coromilas *et al* 1985], heart rate [Bolli *et al* 1986, Lederman *et al* 1987], and potassium level [Curtis and Hearse 1989, Nordrehaug and Vander-Lippe 1983]. In the present study, percentage of ischaemic myocardium, pre-ischaemic HR and MAP are similar in males and females. We did not measure plasma K^+ level. But as all animals were kept on the same laboratory chow, it seems unlikely that the plasma K^+ would differ between the two sexes. Sympathetic nerve stimulation is potent in initiating arrhythmia during ischaemia [Schwartz and Vanoli 1981, Corr and Gillis 1978]. However, direct evidence supporting a causal effect of neuronal NA release on arrhythmogenesis is still limited [Section 1.2.2]. Myocardial NA release during ischaemia may be mediated by efferent sympathetic impulses (exocytosis) or by neuronal reuptake carrier reversing its normal transport direction (non-exocytosis) [Schömig 1988]. We have found a sex difference in neuronal NA release but not in NA non-exocytosis during ischaemia (Chapter 5). In the isolated perfused heart, which is devoid of sympathetic drive, NA release after 20 mins of coronary ligation was also similar in both sexes. If our result from the *in vivo* experiment is a genuine reflection of the arrhythmic vulnerability, it may support the view that the non-exocytotic release of NA is important in initiating VF during ischaemia [Schömig 1988, Dietz *et al* 1989, Riemersma *et al* 1986b]. It is also well-known that chronic sympathectomy with a profound reduction in myocardial catecholamine content is antiarrhythmic but acute sympathectomy is less effective [Corr *et al* 1986, Euler *et al* 1985, Section 1]. However, further studies are required to test whether gender has any significant effect on the development of ischaemic arrhythmias under the condition of enhanced neuronal activity.

Gender and Autonomic Activation After Coronary Ligation

Immediately after coronary occlusion, male rats showed a trans-ient but significant increase in HR and a partial recovery in MAP. A progressive reduction in HR and a further drop in MAP were observed in females during this period. The percentage of ischaemic myocardium and the initial extent of hypotension by coronary ligation are similar in both sexes. In our study, ischaemic area was always restricted to the free anterior wall of the left ventricle. Therefore, differences in these haemodynamic responses to ischaemia can not be explained by differences in the extent or localization of ischaemic area [Curtis *et al* 1987, Billman and Marsh 1989, Corr and Gillis 1978, Section 1.2.1].

Simultaneous increases in HR and MAP are probably due to a sympathetic activation although a withdrawal of parasympathetic tone also lead to an increase in HR. On the other hand, reduction in HR and MAP in females can only be explained by a parasympathetic activation. Sympathetic activation during ischaemia could be triggered by local ischaemia or disturbance in cardiovascular function *via* cardio-cardiac reflex and baroreflex [Shepherd 1985, Section 1.2.1]. Direct stimulation on unmyelinated vagal afferent fibres in the ventricle may ensue an increased parasympathetic activity [Shepherd 1985, Corr *et al* 1986]. Other authors have found an increase in HR and an earlier recovery in blood pressure after coronary ligation in male rats [Johnston *et al* 1983, Siegmund *et al* 1979, Macleod *et al* 1983]. Siegmund *et al* [1979] found a stable but not reduced HR in female rats within the first 20 mins after coronary occlusion, perhaps due to a small group size (n=8). In the present experiment, we included data from all animals (n=24 and 26) in which HR and MAP were reliable within the first 5 mins of coronary ligation, as arrhythmias are rare during this period in this model [Curtis *et al* 1987]. Therefore, the possible distortion by arrhythmias or a chance finding seem unlikely. Additionally, the type of anaesthesia may modify the haemodynamic response to ischaemia [Siegmund *et al* 1979, Macleod *et al* 1983]. But the different response pattern was observed using the same anaesthesia. Thus, result from this study indicate a sex difference in the pattern of autonomic responses to acute myocardial ischaemia, with a relatively sympathetic dominance in males and vagal dominance in females. Interestingly, using the *in vitro* innervated perfused heart model, we have observed a higher neuronal NA release during early ischaemia in males and a more potent cardiac effect of vagal stimulation in females (Chapter 5 and 6).

Men and women may differ in their autonomic response to stress with a more marked sympathetic activation in men (see Section 5.1 and 6.1). Clinical studies also indicate a higher vulnerability to ischaemic arrhythmias in men [Kannel and

Schatzkin 1985, Dahlberg 1990, Freedman *et al* 1988, Section 1.1.3] and a higher incidence of heart failure in women with acute MI [Kimmelstiel and Goldberg 1990]. Sympathetic activation during acute MI provides an acute compensation to maintain haemodynamic stability. But an enhanced sympathetic drive to the heart may increase also the risk of ventricular arrhythmias. On the other hand, vagal activiation may improve the electrical stability of the ischaemic heart at the expense of an incomplete haemodynamic compensation. It would be of interest to examine if the observed sex difference in the autonomic nervous control of the heart is involved in these sex-related differences in humans.

Chapter 8

Dietary Fats and Cardiac Sympathetic Neurotransmission

8.1 Introduction

Epidemiological studies have identified an inverse relation between dietary consumption of n-6 or n-3 polyunsaturated fatty acids (PUFA) and the incidence of ischaemic heart disease [Wood *et al* 1987, Connor and Connor 1990]. A number of factors are believed to be involved in this protection. Systematically, dietary supplement with PUFA can change the profile of plasma lipids, reduce the thrombotic activity, and delay the development of atherosclerosis [Connor and Connor 1990]. As the result of a modified fatty acid composition of cellular membranes, direct influences on the heart may also play an important role [Katz 1986, Lammers *et al* 1987]. Experimentally, diets enriched with PUFAs may reduce prostanoid production [Hartog *et al* 1986], improve coronary circulation [Kenny *et al* 1990, Force *et al* 1989, Hartog *et al* 1986], ameliorate ischaemic damage [McLennan *et al* 1985, Bruckner *et al* 1987, Hock *et al* 1987 and 1990, Black *et al* 1984, Culp *et al* 1980], and reduce the severity of ischaemic arrhythmias both *in vivo* and *in vitro* [McLennan *et al* 1985 and 1989, Hock *et al* 1990, Culp *et al* 1980, Riemersma *et al* 1988, Sargent 1990].

In cardiac tissue, effects mediated by α-, and perhaps β-adrenoceptors are found to be attenuated by diets rich in n-3 and n-6 PUFA [McLennan *et al* 1987, MacLeod and Riemersma 1990, Reibel *et al* 1988, Wince *et al* 1987], although an enhancement of the α-receptor mediated vasoconstriction in dogs has also been reported [Kenny *et al* 1990, Panek *et al* 1985].

Effect of dietary PUFA on catecholamine release has seldomly been investigated. In the atrium of adult rats, both NA content and field stimulation-evoked ^{3}H-NA release are not significantly altered by long-term feeding of sunflower oil (rich in n-6 PUFA) compared with coconut oil (rich in saturated fatty acids). α_2-Adrenoceptor mediated presynaptic inhibition of ^{3}H-NA release was attenuated by n-6 PUFA diet [Semafuko *et al* 1987]. But an enhanced α-adrenergic presynaptic control of ^{3}H-NA release in the perfused rat tail artery is also reported by the same group [Semafuko *et al* 1989]. Another study has found that supplementation with saturated fats reduced neuronal NA release from tail arteries of the rat [Panek *et al* 1985]. Influence of

dietary n-3 PUFA on NA release from the heart during normoxia and ischaemia is unknown.

Sympathetic activation and endogenous NA release play an important role in the pathogenesis of ischaemic arrhythmias [Corr *et al* 1986, Schömig 1988]. As dietary PUFA may modulate both ischaemic arrhythmias and NA release, a modified sympathetic neurotransmission could be involved in this antiarrhythmic effect. Therefore, the purpose of this study was to examine the influence of realistic dietary supplements with n-6 and n-3 PUFA on neuronal NA release and its presynaptic controlling mechanisms in the perfused rat heart. The control diet was rich in fats with a polyunsaturated/saturated ratio (P/S ratio) of 0.3 , as commonly consumed by the average Scottish man [Thomson *et al* 1985]. As several studies suggested that n-3 PUFA supplement could modify vasoactivity [Kenny *et al* 1990, Shimokawa and Vanhoutte 1989, Force *et al* 1989, Hartog *et al* 1986], the effect of n-3 PUFA supplement on coronary vasoconstriction induced by muscarinic agonist was also studied [Kalsner 1989].

8.2. Methods

8.2.1 Dietary Fats, Drugs and Model

Male Lew rats were used for this study. Rats (6 weeks of age) were fed semi-synthetic diets as detailed in Section 2.5. Heart perfusion experiments were carried out 10-11 weeks later. Using the *in situ* perfused, innervated heart model (Section 2.2), effects of dietary fats on neuronal NA release and its presynaptic modulation were examined. Hearts were perfused at a constant flow rate of about 5 ml/g/min except in ischaemic series, in which the flow rate was reduced by 90% to 0.5 ml/g/min. Drugs used were desipramine (0.1 μM), phentolamine (3 μM), isoprenaline (10 nM), froben (10 μM), and methacholine (1-100 μM). Desipramine was added to the perfusate and was present throughout the experiment. Other drugs were infused into the heart *via* a pump (Section 2.6). Coronary effluent was collected for 1 min during nerve stimulation in normoxic experiments, or for 3 mins during low-flow ischaemia starting from onset of nerve stimulation. At the end of experiments, hearts were excised, weighed, frozen and stored in liquid N_2 for fatty acid analysis by gaschromatography (Section 2.8.3).

8.2.2 Protocols

Dietary PUFA and NA release during normoxia

The effects of experimental diets on NA release and its presynaptic control were examined during normoxic perfusion. In the first series, the effect of n-6 PUFA was examined. After 20 mins perfusion to stabilize the hearts, sympathetic ganglion stimulation (GS, 5 Hz for 1 min) was given (S1) as an individual control. The second and third GS were performed in the presence of phentolamine (3 μM, S2) or isoprenaline (10 nM, S3), respectively. In the second series, oral dosing technique was necessary to prevent auto-oxidation of n-3 PUFAs. The control and n-3 PUFA groups each received daily oral dosing of olive oil or Maxepa mixed in olive oil (Section 2.5). The same protocols as used in the first series were followed and drugs presented in S2 and S3 were phentolamine (3 μM) and flurbiprofen (Froben, 10 μM), respectively.

Dietary n-6 PUFA and NA overflow during prolonged ischaemia

In another 2 groups of rats fed control and n-6 diets, the first GS (5 Hz for 30 sec) was performed during normoxic perfusion as a control. Afterwards, coronary flow rate was reduced by 90% to 0.5 ml/g/min and maintained at this level for 60 mins. Another four consecutive GS (5 Hz for 30 sec) were delivered at 8, 18, 28 and 58 mins of ischaemia, respectively. Coronary effluent was collected for 3 mins starting with each nerve stimulation for the analysis of NA overflow. NA and lactate levels in ischaemic venous effluent were also measured before each nerve stimulation.

Dietary n-3 PUFA on methacholine-induced vasoconstriction

In the hearts of rats fed n-3 diet (n=9) and their controls (n=10), a 10-min period was allowed to re-stabilize the preparation after the completion of nerve stimulation experiment. Then, methacholine-induced coronary vasoconstriction was studied. In order to avoid the effect of myocardial contracture on the resistance of coronary vasculature, ventricular fibrillation was induced and maintained by electrical stimulation of the left ventricle (10-20 Hz and 1.5 mA). Accumulating doses of methacholine (1, 5, 10, 20, 50 and 100 μM) were infused into the heart. Each dose was maintained for 3 mins. Froben (10 μM) was present throughout the experiment.

8.3. Results

Body weights of rats fed control, n-3 or n-6 PUFA diets were monitored on a weekly and found to be similar. At the time of sacrifice, body and heart weights were not significantly different between n-6 or n-3 and the respective control groups (Table 8.1).

Table 8.1. *Body and heart weights of rats fed experimental diets*

	n	$Body_A$ (g)	$Body_B$ (g)	Heart (g)
Control	16	160±4	324±12	1.07±0.03
n-6	16	164±6	346±10	1.09±0.03
Control	12	148±8	366±6	1.04±0.03
n-3	10	148±8	370±8	1.07±0.03

A: begining of dietary feeding; B: end of the dietary feeding

8.3.1 Fatty Acid Composition of Myocardial Phospholipids

N-6 or n-3 PUFA enriched diets resulted in a different fatty acid profile of total phospholipids of the myocardium in comparison to that of controls. Supplementation with n-6 PUFA significantly increased 18:2n-6, 20:2n-6, 22:4n-6 and total n-6 fatty acids. N-3 PUFA supplement led to a significant increase in n-3 fatty acids (especially 20:5 and 22:6) accompanied by a decrease in n-6 fatty acids (mainly 20:4, 22:4, and 22:5, Table 8.2). PUFA rich diets, whether n-3 or n-6, did not significantly change the total amount of saturated fatty acids.

Table 8.2. *Fatty acid composition of total phospholipids in hearts of rats fed experimental diets rich in n-3 or n-6 polyunsaturated fatty acids (μg/g wet weight) and their controls*

	Control (*n=9*)	N-6 (*n=12*)	Control (*n=8*)	N-3 (*n=8*)
16:0	795±80	870±57*	947±64	989±47
18:0	2317±236	2419±149	2670±171	2439±149*
18:1 *n-9*	785±72	851±42*	908±39	804±39#
18:2 *n-6*	1381±205	1949±157#	1890±156	1774±93
20:2 *n-6*	7±1	18±3#	7±1	9±1*
20:4 *n-6*	2585±229	2544±137	2964±198	2257±124#
22:4 *n-6*	130±16	152±20*	117±9	52±5#
22:5 *n-6*	372±74	164±25#	198±15	36±5#
18:3 *n-3*	3±1	11±1#	5±1	5±1
20:5 *n-3*	2±2	1±1	6±5	30±5#
22:5 *n-3*	158±27	173±25	218±27	256±19#
22:6 *n-3*	779±115	1044±198**	1087±94	1721±103#
Total sats	3128±314	3305±199	3633±232	3444±194
Total *n-6*	4516±444	4861±259*	5222±337	4183±206#
Total *n-3*	943±114	1229±184**	1316±120	2011±116#

** $p<0.05$ **$p<0.01$ # $p<0.001$ vs the respective control.*

8.3.2 Diets, NA Overflow and Its Presynaptic Modulation

Spontaneous NA overflow was low (<1.5 pmol/g/min) and did not differ between the four dietary groups. Control sympathetic nerve stimulation-evoked NA overflow was similar in dietary n-6 or n-3 PUFA group versus the respective control group (Figure 8.1). In the presence of phentolamine, neuronal NA overflow was significantly enhanced in all groups and this increase was not affected whether animals were supplemented with n-6 or n-3 PUFA. The ratio of NA overflow (S2/S1) was 2.6±0.6 *vs* 1.9±0.3 for n-6 *vs* control (NS) and 2.2±0.2 *vs* 2.1±0.1 for n-3 *vs* control (NS, Figure 8.1). In the presence of isoprenaline (10 nM) or froben (10 μM), neuronal NA overflow was not significantly changed and no diet-related differences in NA overflow were observed (Figure 8.1).

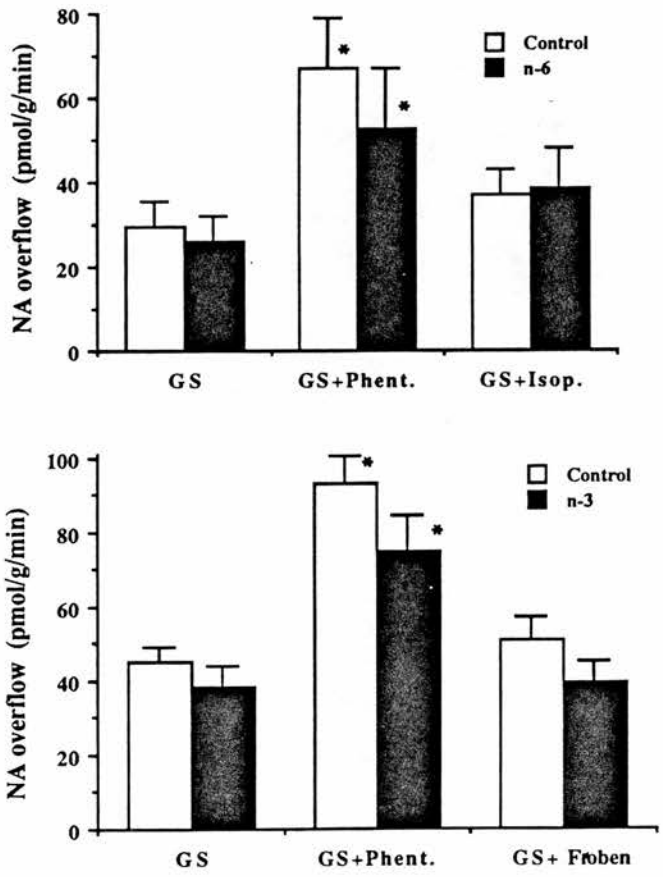


Figure 8.1. *Effect of dietary fat on neuronal NA overflow and its presynaptic controlling mechanisms. The first sympathetic ganglion stimulation (GS) served as control. * $p<0.05$ vs GS in the same group by paired t-test. Note NA overflow was not changed by feeding rats n-6 or n-3 PUFAs (all $p>0.05$ vs controls). Froben= flurbiprofen.*

Heart rate and ±dP/dt did not differ between n-6 or n-3 PUFA and their control dietary groups (NS) during basal conditions and during nerve stimulation either with or without α-adrenergic blockade. In the hearts from rats on control diet, there was a higher increase in ±dP/dt in response to GS in the presence of phentolamine compared with that in its absence ($p<0.05$). This effect of phentolamine was not observed in the two PUFA dietary groups (NS, Table 8.3).

Table 8.3 *Effect of dietary fatty acids on basal and sympathetic ganglion stimulation-mediated haemodynamic responses in perfused hearts and the influence of the α-adrenergic antagonist phentolamine (phent., 3 μM). n=8-12 per group.*

	HR (b/min)		+ dP/dt (mm Hg/s)		-dP/dt (mm Hg/s)	
	-Phent.	+Phent.	-Phent.	+Phent.	-Phent.	+Phent.
Basal						
Control	256±17	239±18	1583±104	1608±104	887±76	875±81
n-6	241±17	244±11	1464±78	1464±89	621±24	614±39
Control	203±14	196±20	1665±54	1722±51	921±50	990±47
n-3	190±8	176±9	1584±67	1521±45	895±37	890±44
Stimulation						
Control	287±16	281±12	2075±126	2492±180*	1217±103	1425±80*
n-6	271±9	271±10	1864±120	2079±122	907±61	979±54
Control	263±14	242±8	2605±114	2918±150*	1659±113	1910±128*
n-3	249±16	244±5	2664±236	2641±241	1685±167	1721±149

1) All changes induced by GS were significant at $p<0.01$.
2) No significant differences were observed between n-6 or n-3 diets and their controls.
*3) *$p<0.05$ vs the stimulation values without phentolamine in the same group by paired t-test.*

8.3.3 N-6 Diet and Neuronal NA Overflow during Ischaemia

The control nerve stimulation during normoxia resulted in similar amount of NA overflow in n-6 PUFA dietary animals and their controls (34.4±4.0 *vs* 35.4±6.1 pmol/g, NS). During 60 mins of 90% flow reduction, spontaneous NA overflow was maintained and did not differ between the two groups (Figure 8.2). Lactate concentration in coronary effluent was significantly increased by ischaemia and did not differ according to the diet of the rats. Each nerve stimulation applied during ischaemia evoked an over 10-fold increase in NA overflow accompanied by a significant increase in HR (Figure 8.2 and Table 8.4). During ischaemia, concentrations of lactate in the ischaemic venous effluent increased from 0.4 ±0.1 to

2-3.5 mM ($p<0.01$) in control animals and from 0.5±0.1 to 2.6-3.3 mM ($p<0.01$) in n-6 PUFA animals. No diet-related differences were found in these observations.

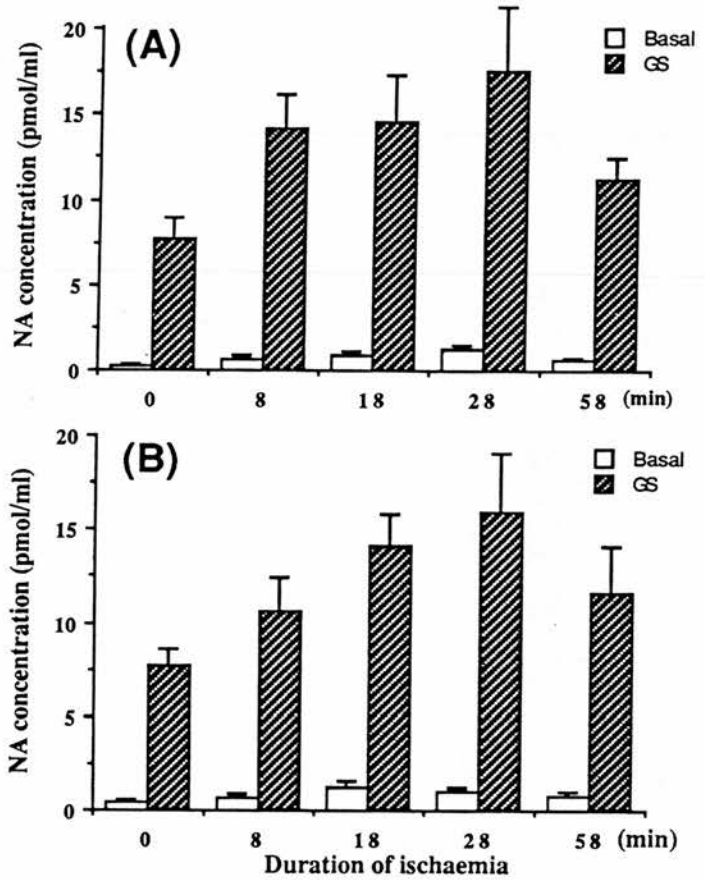


Figure 8.2. *Effect of n-6 PUFA diet on spontaneous and nerve stimulation-induced NA overflow during normoxia (0 min) and during 60 mins of 90% flow reduction. The neuronal reuptake inhibitor desipramine (0.1 μM) was present throughout the experiment. (n=8/group). The increase in NA concentration during GS is highly significant ($p<0.001$) and did not vary according to the diet: control (panel A) and n-6 PUFA diet (panel B).*

Table 8.4 *The effect of sympathetic ganglion stimulation (GS, 30 sec duration) on heart rate before and during 60 mins low-flow ischaemia.*

			Duration of Ischaemia (min)			
Diet	**Condition**	**Normoxia**	8	18	28	58
Control	*Basal*	215±28	55±11	54±17	37±11	43±11
	GS	289±19	133±17	109±18	93±8	106±16
n-6	*Basal*	219±15	66±17	45±8	47±9	65±12
	GS	268±34	132±12	97±12	104±11	116±16

$p<0.01$ for the increase in heart rate by each GS ; n=8 per group.

8.3.4 N-3 PUFA and Methacholine-Induced Coronary Vasoconstriction

Under our experimental conditions (5 ml/g/min perfusion flow, inhibition of prostanoid production by flurbiprofen , and minimizing the effect of contraction by induced VF), there was no significant difference in basal coronary perfusion pressure of the hearts from animals fed n-3 PUFA or control diets. A dose-dependent increase in perfusion pressure was observed during methacholine infusion. The extent of this coronary vasoconstriction was independent of the diet (NS by ANOVA, Figure 8.3).

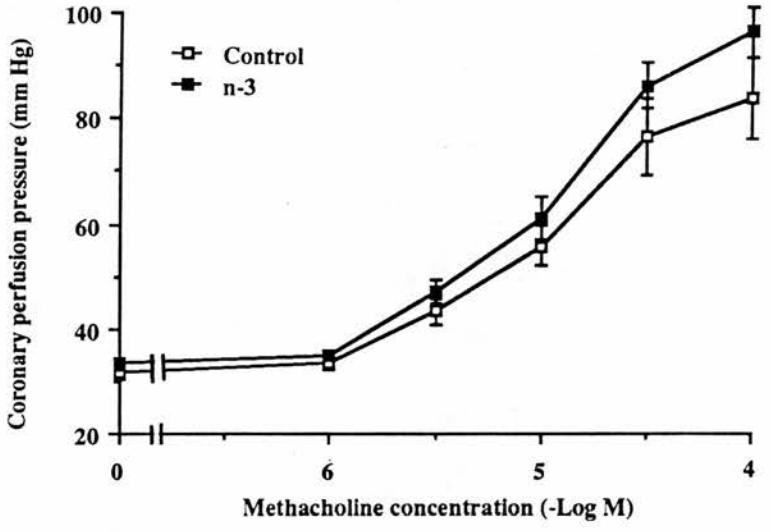


Figure 8.3. *The effect of the muscarinic agonist methacholine on coronary perfusion pressure of hearts from rats fed n-3 PUFA (n=9) or their control (n=10) diet.*

8.4. Discussion

The present study shows that 10 weeks feeding rats on diets enriched with PUFA resulted in significant changes in fatty acid composition of myocardial total phospholipids. The n-6 PUFA enriched diet increases the total n-6 PUFA, which is mainly due to a 40% increase in 18:2. Dietary supplementation with Maxepa leads to about 50% increase in total n-3 fatty acids, especially 22:5 and 22:6, accompanied by a significant reduction in n-6 fatty acids. All these changes are in agreement with previous reports [Hock *et al* 1987, Reibel *et al* 1988, Sargent 1990].

As the cell membrane plays a central role in the exitation-secretion coupling and agonist-receptor interactions [Katz 1986], concomitant change in sympathetic neurotransmission was expected, but was not observed. Spontaneous and control nerve stimulation-evoked NA overflow were similar in the control and dietary PUFA groups. Nor was the presynaptic modulation of NA release mediated by α- and β-adrenoceptors or by prostaglandin receptors affected by diets. This can not be explained by an insensitivity of the model, since the increase in NA overflow by blocking presynaptic α-receptors with phentolamine is highly significant. Postsynaptic effects of sympathetic nerve stimulation were also not significantly affected by the diets. Stimulation of sympathetic ganglion at 5 Hz equally increase heart rate and ±dP/dt in all groups. In control rat hearts, ±dP/dt response to sympathetic stimulation is potentiated by blocking α-adrenoceptors with phentolamine which is in accordance with a significantly increased NA overflow by this drug. However, this was not observed in hearts of the rats fed PUFA enriched diets although neuronal NA overflow is similarly enhanced as in controls. It may indicate an attenuation of the β-adrenoceptor-mediated inotropic response to enhanced endogenous adrenergic stimulation by dietary PUFA, as demonstrated by others [Wince *et al* 1987, McLennan *et al* 1987]. However, a between-group difference is not observed. Several studies have demonstrated that arterial vasodilation can be enhanced by dietary PUFA [Kenny *et al* 1990, Panek *et al* 1985, Shimokawa and Vanhoutte 1989]. In this study, the dose-response curve of perfusion pressure to methacholine was not shifted by dietary n-3 PUFA. Taking all these findings together, this study provides no evidence to suggest that dietary fatty acid composition affects sympathetic neurotransmission or the function of pre- and post-synaptic receptors in the normoxic heart of the rat.

Myocardial NA release is believed to be important for the genesis of ischaemic arrhythmias [Lombardi *et al* 1984, Schömig 1988, Section 1.2.2]. Neuronal NA overflow during prolonged low-flow ischaemia is under the effective presynaptic modulation (Chapter 3). Differences in this modulation would be reflected by a different NA overflow level. However, during a 60-min period of low-flow ischaemia, increases in NA overflow and HR by nerve stimulation do not differ in hearts of rats fed n-6 or their control diet. In another study from this laboratory, neuronal activity-independent NA release during ischaemic-reperfusion is also not altered by n-3 or n-6 dietary PUFA compared with the control diet [Sargent 1990].

Semafuko and co-workers [1987] have demonstrated that dietary supplementation with sunflower oil (rich in n-6 PUFA) attenuates the α–adrenergic presynaptic modulation of NA release in isolated rat atrium of adult rats *vs* a supplement of saturated fat. Meanwhile, control ^{3}H-NA release and NA content are not affected.

Interestingly, this dietary modulation seems to be organ-dependent as they have also reported an enhanced α-adrenergic inhibition of NA release in the tail artery of rats [Semafuko *et al* 1989]. There are several methodological differences in their studies compared with the present one. ^{3}H-NA labelling technique and field stimulation were used by Semafuko *et al* [1987, 1989] to study NA release and its modulation, whilst we measured endogenous NA overflow evoked by electrical nerve stimulation in the presence of neuronal reuptake inhibitor. Another important issue may be the feeding period. In their study, dietary feeding started from pregnancy period until adulthood [Semafuko *et al* 1987, 1989]. Central and peripheral sympathetic nerves develop very rapidly within the first 2-3 weeks [De Champlain *et al* 1970, Semafuko *et al* 1987]. Therefore, it is possible that an earlier supplementation with dietary PUFA may lead to a higher incorporation of PUFA into the nervous membranes. Obviously, it is difficult to know to what degree our measured alteration in fatty acid composition in myocardial phospholipids reflect the fatty acid profile in the nerve membranes.

In summary, the results from this study indicate that dietary feeding of n-6 and n-3 PUFA results in major changes in fatty acid composition of membrane phospholipids in the heart. However, these changes are not accompanied by a significantly modified sympathetic neurotransmission or function of the receptors examined. Therefore, any protective effects of dietary PUFA in myocardial ischaemia may be mediated by factors which are independent of efferent sympathetic neurotransmission. However, a possible modulation of the central nerve activity by long-term dietary fats [Mills and Ward 1986] can not be excluded.

Chapter 9

General Discussion

9.1 Major Findings

The principle aim of this project was to examine the effect of acute myocardial ischaemia on neuronal NA release and its modulation.

The severity and duration of ischaemia is an important determinant for changes of the function of the sympathetic nerve terminal (Chapter 3). During stop-flow ischaemia, NA exocytosis is reduced and fails within 10 mins, accompanied by the loss of presynaptic modulation and neuronal reuptake mechanism. However, exocytosis, presynaptic modulation and neuronal reuptake are all maintained for at least 60-min period of low-flow ischaemia (residual flow similar to collateral flow *in vivo*). Carrier-mediated NA efflux does not occur during low-flow ischaemia. The presynaptic parasympathetic inhibitory mechanism appears even more sensitive to severe ischaemia. A rapid failure of vagally-mediated inhibition of neuronal NA release is observed after 1 min of stop-flow ischaemia, in contrast to a maintained inhibitory effect at 3 mins of low-flow ischaemia (Chapter 4 and 7). Thus, this study demonstrates a dependency of NA release on the severity of ischaemia, a conclusion which is critical for the understanding of local sympathetic nerve function during acute ischaemia.

In the perfused rat heart, inhibition of NA release by vagal nerve stimulation is abolished within a few minutes of low-flow ischaemia (Chapter 4 and 7) due to a reduced ACh release, which in turn is at least partly due to an adrenergic presynaptic inhibition, and dysfunction of presynaptic muscarinic receptors. Therefore, ischaemia affects the sympathetic-parasympathetic interactions in such a way which is in favour of NA release. Despite this failure, sympathetic stimulation-triggered ischaemic-reperfusion VF in this model is prevented by superimposed cholinergic stimulation (Chapter 7). Thus, under conditions of simultaneous vagal and sympathetic activation during acute myocardial ischaemia, cholinergic presynaptic inhibition of NA release may no longer exist within the ischaemic area shortly after the interruption of coronary flow. Cholinergic postsynaptic actions, however, are maintained during longer periods of ischaemia and are responsible for the antiarrhythmic effect by vagal nerve activation.

During normoxic perfusion, presynaptic inhibition of cardiac NA release by α-adrenoceptors or vagal nerve stimulation is more potent in female than in male rat . This mechanism leads to a lower exocytotic NA release during early stop-flow ischaemia in females than in males (Chapter 5). Meanwhile, neuronal NA release and heart rate in the female heart are under stronger parasympathetic influence than that in the male (Chapter 6). These gender related differences may be responsible for the different heart rate and blood pressure responses to acute coronary artery occlusion in male and female rats *in vivo* (Chapter 7). Gonadectomy studies indicate that gonadal hormones, especially oestrogen, may be responsible for these differences (Chapter 5 and 6). Thus, the autonomic control of the heart is also influenced by gender.

In contrast, feeding rats diets enriched with n-6 or n-3 polyunsaturated fatty acids for 10 weeks does not exert a significant effect on sympathetic neurotransmission, despite major changes in fatty acid composition of myocardial phospholipids (Chapter 8).

9.2 Limitations of the Study

A flow rate of 5 ml/min/g, which is close to coronary blood flow measured *in vivo* [Malic *et al* 1976], was used to perfuse the innervated heart "normoxically". Nevertheless, hearts under such perfusion produced more lactate (about 2-3 μM/g/min) than those receiving the higher flow rate of 9 ml/g/min (<0.5 μM/g/min). This is due to the low oxygen capacity of the perfusate, despite the fact that Po_2 is as high as 600 mm Hg. The implication of this higher lactate production by myocytes is not entirely clear, as this level is still much lower than that observed during ischaemia-reperfusion. The main purpose of this project is to study the autonomic nerve function in the heart and cardiac nerves are rather resistant to hypoxia [Carlsson 1988, Dart *et al* 1987, Wakade and Wakade 1985]. The myocardial contractility in such perfused hearts may be low due to the limited oxygen supply. However, there are no signs of declining ventricular contractility and response to sympathetic or vagal nerve stimulation over a 2-hour perfusion period (Section 2.10).

The perfused rat heart, compared to the *in situ* dog heart, is a suitable model for *quantitative* study of NA release [Dart 1989]. However, this study and also previous studies [Schömig 1989, Dart *et al* 1983] show a wide individual variation in NA overflow by control sympathetic nerve stimulation. This limits the sensitivity to detect a difference between groups and a larger group size is required to show such a difference statistically. In contrast, the variation in the amount of NA overflow from the same heart by repeated nerve stimulations is relatively small [Section 2.10,

Schömig 1988]. Therefore, in this study between-group comparisons were made using both absolute values and the Sn/S1 ratio of NA overflow by a test stimulus (Sn) over that obtained by a control nerve stimulation (S1).

In preparations subjected to stop-flow ischaemia, reperfusion is necessary to washout NA released by nerve stimulation during ischaemia. The effect of reperfusion *per se* on neuronal function can not be excluded but is difficult to estimate, although assumed to be small [Dart *et al* 1987]. Non-exocytotic NA overflow occurs when stop-flow ischaemia extends to 15-20 mins [Schömig 1988], or perhaps during prolonged low-flow ischaemia, although not observed in these studies. This restricts the duration of severe ischaemia that can be applied to study the neuronal release of NA.

Due to the limitations of time and laboratory facilities, it was not possible to measure some important parameters, i.e., myocardial content and neuronal release of acetylcholine in Chapter 4 and 6, and adenosine concentration in coronary venous effluent in Chapter 3. Thus, some of our findings are not fully explained and some of the conclusions depend in part on data from others, and therefore remain somewhat speculative.

Finally, the relation between neuronal NA release/presynaptic modulatory mechanisms and arrhythmogenesis during ischaemia has been explored little. Nevertheless, the preliminary results indicate that neuronal NA release is associated with an increased incidence of VF and that the vagus also plays an important role. Therefore, the innervated perfused rat heart is a suitable model for such studies.

9.3 Presynaptic Modulation of NA Release during Ischaemia

Three different types of presynaptic inhibitory mechanisms were examined: automodulation by α-adrenoceptors (Chapter 3-5 and 8), axo-axonal modulation by vagal nerve stimulation (Chapters 4, 6 and 7), and trans-synaptic modulation by purinoceptors (Chapter 3). These inhibitory mechanisms differ in some aspects during normoxia and during ischaemia.

Firstly, the inhibition of NA release mediated by vagal nerve stimulation is the most sensitive to ischaemia and fails within 10 mins of low-flow ischaemia (Chapter 4 and 7). In contrast, both α-adrenergic and purinergic inhibitory mechanisms remain effective for at least 60 mins of low-flow ischaemia (Chapter 3). Obviously, sensitivity of presynaptic inhibitory process to ischaemia reflect to some extent the complexity of the mechanisms involved. Inhibition by vagal nerve stimulation requires more steps than that by α-adrenergic or purinergic receptors, e.g. ACh

exocytotic release, diffusion and binding of ACh to presynaptic muscarinic receptors on sympathetic nerve endings, and an effective intraneuronal signal transmission. Each of these links may be affected by ischaemia, thereby rendering vagally induced inhibitory mechanism more vulnerable to ischaemia than automodulation of NA release.

Secondly, presynaptic mechanisms also differ in their relatively contribution to the suppression of neuronal NA release. In normoxically perfused rat hearts, the most important influence is mediated by α-adrenoceptors (Chapter 3-5, 8). Vagal activity may also play a role (Chapter 4 and 6) but less so. Blocking purinergic receptors or inhibiting prostanoid production was without significant effect in this model (Chapter 3 and 8). During low-flow ischaemia, however, vagal inhibitory mechanism fails rapidly (Chapter 4 and 7). The potency of α-adrenoceptor mediated inhibition is not changed for at least 60 mins (Chapter 3). In contrast, purinergic inhibition becomes the most important mechanism during ischaemia (Chapter 3), presumably due to increased prevailing concentrations of adenosine [Richardt *et al* 1987]. The presynaptic facilitatory modulation of NA release in the ischaemic heart, like those mediated by β-adrenoceptors, angiotensin II receptors, serotonin receptors, have not been studied. It would be of interest to do comparative studies on the effects of ischaemia on both inhibitory and facilitatory modulations.

Intervention at the level of presynaptic receptors is accompanied not only by modified NA release, but also by parallel changes in the functional response, i.e.±dP/dt and heart rate, to sympathetic nerve stimulation both in normoxia and ischaemia (Chapter 3-5, 8). Therefore, the functional significance of the presynaptic modulation of NA release, especially inhibitory, is stressed.

9.4 NA Release in Ischaemia in Relation to Arrhythmogenesis

An important feature of myocardial ischaemia *in vivo* is heterogeneity in flow reduction across the ischaemic area [Bolli *et al* 1986, Piek and Becker 1988, Reimer *et al* 1987]. It is assumed that the dependency of sympathetic nerve function on the severity of ischaemia, demonstrated in the present study, may also pertain *in vivo*. Therefore, in regions with near zero blood flow, rapid failure of neuronal NA release may occur followed by "carrier-mediated NA efflux" [Schömig 1988]. In non-ischaemic or mildly ischaemic regions NA release is preferentially *via* exocytosis. Meanwhile, in regions with severe but incomplete cessation of blood flow, excytosis is maintained but inhibited presynaptically. Non-exocytotic NA efflux may not happen or be substantially postponed. Consequently, a spatially and chronologically

inhomogeneous NA release results in uneven adrenergic stimulation in closely related myocardial regions with unequal flow. In the very early stage, adrenergic stimulation is probably stronger in non-ischaemic and mildly ischaemic areas. At a later stage of ischaemia, non-exocytotic release of NA in regions with the most severe ischaemia may cause a reversal of this gradient in adrenergic stimulation with intense stimulation in central ischaemic areas but not in non- and mild-ischaemic areas (Figure 9.1). This may favour the induction of electrophysiological dispersion leading to the development of VF.

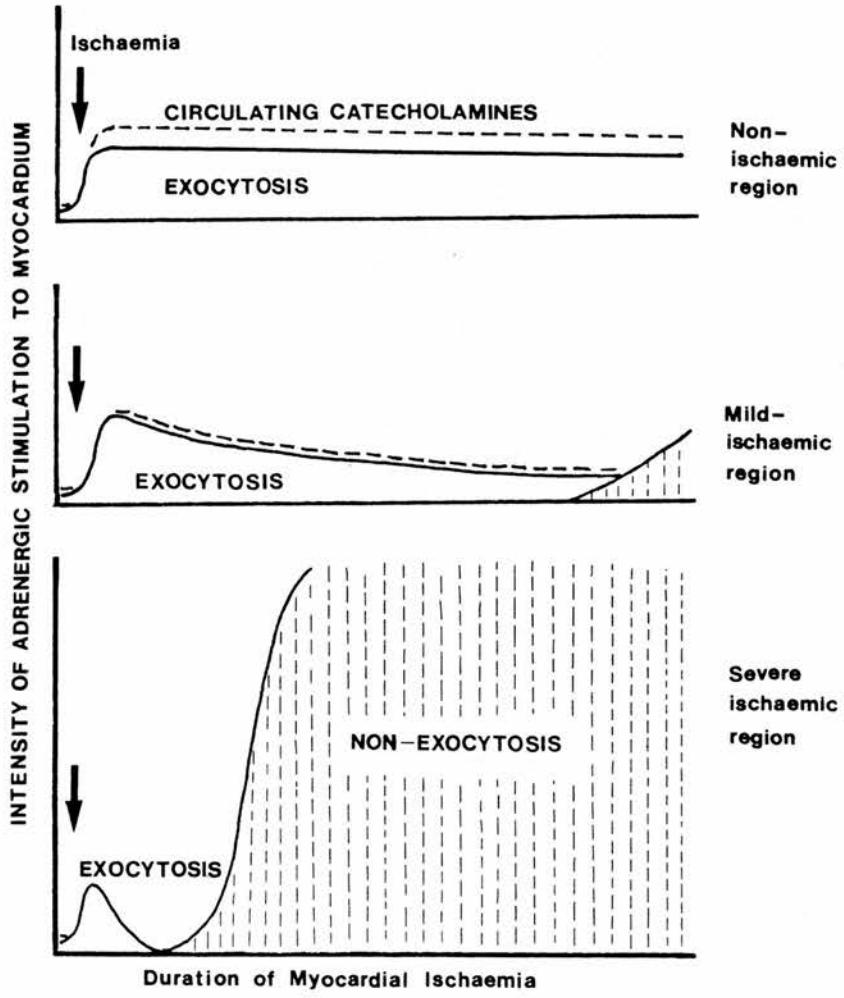


Figure 9.1 *Schematic representation of the different intensity of adrenergic stimulation to ischaemic and non ischaemic myocardium in vivo due to various sources of catecholamines.*

It is generally believed that acute myocardial ischaemia is associated with an enhanced adrenergic stimulation to ischaemic myocardium [Corr and Gillis 1978, Verrier 1988]. The effectiveness of adrenergic blockade during myocardial ischaemia

in vivo supports this view (see Section 1.2.2). However, using nerve stimulation technique, a reduced amount of NA overflow has frequently been observed during early ischaemia in previous *in vitro* and *in vivo* studies [Dart 1989, Forfar *et al* 1984] and also in the present study (Chapter 3-6).

The intensity of adrenergic stimulation is dependent on NA concentration in the synaptic space which is difficult to estimate. Assuming that neuronal reuptake is largely inhibited by desipramine in our study, then the amount of NA overflow equals roughly to that released. During low-flow ischaemia (0.25 ml/g/min), neuronal NA release is reduced to 30-50% of normoxic control value (Chapter 3). In contrast, NA concentration in the coronary effluent during nerve stimulation is much higher during ischaemia (10-25 pmol/ml) than during normoxic perfusion at 5 ml/g/min (5-10 pmol/ml, Chapter 3, 7, 8). A higher synaptic concentration of NA in ischaemic than in normoxically perfused myocardium is also indicated by clonidine's facilitation of NA release, which was only observed during ischaemia (Chapter 5). In normoxia, NA released into the extracellular space (about 0.2 ml/g wet weight) [Frank and Langer 1974] may diffuse into capillary blood flow and thus removed. In other words, capillary blood flow constitutes a pool, which "buffers" increased NA levels in the synaptic cleft. During ischaemia however, this buffering and washout mechanism is markedly impaired. Therefore, a "concentrating effect of ischaemia" may exist by which NA levels in the biophase are increased in myocardial ischaemia, despite a reduced quantity of neuronal NA release. Thus, postsynaptic adrenergic stimulation of the ischaemic myocardium may be enhanced during the early period. The neuronal reuptake mechanism could attenuate this NA accumulation in ischaemic myocardium during early phase of ischaemia. However, this protective mechanism may fail quickly under anoxic and stop-flow ischaemic conditions [Schömig 1989, Dart *et al* 1984b], or be interfered with *in vivo* by a number of drugs [Goldstein *et al* 1988, Docherty and McGrath 1980, Iversen 1973].

Arrhythmogenic effect of sympathetic nerve stimulation during myocardial ischaemia has been well-documented in various animal species [Corr and Gillis 1978] except in the rat. Using the perfused and innervated rat heart model, it was demonstrated that sympathetic nerve stimulation during ischaemia enhances both NA release and incidence of VF during early reperfusion (Chapter 7).

Several factors that influence ischaemic arrhythmogenesis (Section 1.1.3) may do so *via* an effect on sympathetic neurotransmission, as indicated by data from this project.

Firstly, our results on the effect of severity of ischaemia on neuronal NA release and the controlling mechanisms (Chapter 3) may explain the salutary effect of

collateral flow in the development of severe ventricular arrhythmias (Section 1.1.3). It is suggested that a critical amount of residual flow preserves normal nerve function with inhibited neuronal NA release by presynaptic mechanisms. Under these conditions, carrier-mediated NA efflux is prevented for at least 60 mins of ischaemia well beyond the electrically vulnerable period after acute coronary ligation in the rat heart model [Curtis *et al* 1987].

Secondly, clinical and experimental data suggest that females may be less susceptible to severe ventricular arrhythmias and sudden cardiac death (Section 1.1.3). In the present study, it has been demonstrated that neuronal NA release in the female rat heart is under a more tonic presynaptic α-adrenergic control, thereby leading to a lower NA release during early ischaemia (Chapter 5). Vagal influence on the heart at pre- and post-synaptic sites is also stronger in female than in male rats (Chapter 6). The different haemodynamic response to coronary ligation *in vivo* in male and female rats with coronary artery occlusion supports a gender difference in the autonomic response to acute myocardial ischaemia (Chapter 7). All these observations may help to explain the gender difference in the severity of arrhythmias and early mortality after experimental myocardial ischaemia and infarction [Siegmund *et al* 1979, Lu *et al* 1984].

Thirdly, the incidence of VT and VF is found to be higher in Sprague-Dawley than in Wistar rats during the first 30 mins of coronary artery occlusion *in vivo* [Curtis *et al* 1987, Johnston *et al* 1983]. One possible mechanism may be a higher neuronal NA release in hearts of Sprague-Dawley rats than that of Wistar rats, as demonstrated in Section 2.10.

However, negative data were also obtained. For example, there is no significant influence of dietary PUFA on efferent sympathetic neurotransmission in the heart (Chapter 8). Thus, the well-defined antiarrhythmic effect of dietary PUFA (see Section 1.1.3 and 8.1) can not be explained this way. Moreover, VF triggered by sympathetic nerve stimulation is prevented by simultaneous cholinergic stimulation, despite the fact that NA release is unchanged (Chapter 4, 7). Therefore, under certain circumstances, arrhythmogenic effect of adrenergic activation may be less important, or antagonized by other coexisting factors. These results underline the multifactorial nature of arrhythmogenic mechanisms in acute myocardial ischaemia.

Presentations and Publications

Meeting Presentations

Du XJ, Dart AM, Riemersma RA, Oliver MF: Failure of presynaptic cholinergic inhibition of noradrenaline release during myocardial ischaemia. *J Mol Cell Cardiol* 1989;21:21(suppl IV): S42

Du XJ, Riemersma RA: Dietary linoleate and presynaptic alpha adrenergic modulation of noradrenaline release. *J Mol Cell Cardiol* 1989;21(suppl 21):S42

Du XJ, Riemersma RA, Dart AM: Myocardial ischaemia attenuates presynaptic alpha adrenergic modulation of noradrenaline release. *J Mol Cell Cardiol* 1989;21(suppl IV):S43

Du XJ, Dart AM, Riemersma RA: Reduced reperfusion arrhythmias despite impaired presynaptic vagal-sympathetic interaction. *J Mol Cell Cardiol* 1989;21(suppl IV):S50

Du XJ, Riemersma RA: Maintenance of exocytotic noradrenaline overflow during prolonged myocardial ischaemia in the rat. *Acta Physiol Scand* 1989;136(suppl 584):11

Du XJ, Riemersma RA, Dart AM: Alpha adrenoceptor antagonist reverses ischaemia-induced failure of vagal stimulation mediated inhibition of noradrenaline release. *Acta Physiol Scand* 1989;136(suppl 584):11

Du XJ, Riemersma RA: Mechanisms controlling myocardial noradrenaline exocytosis: importance of severity of ischaemia. *J Mol Cell Cardiol* 1990;22(suppl III):S15

Du XJ, Riemersma RA: Severity of myocardial ischemia and neural norepinephrine (NE) release. *Circulation* 1990;82(suppl):III-641

Du XJ, Riemersma RA: Presynaptic control of cardiac noradrenaline release: effect of gender. *Eur Heart J* 1990;11(suppl):36

Du XJ, Riemersma RA: Vagal control of the heart: The importance of gender difference.
(Submitted to 1991 Annual Meeting of the British Cardiac Society)

Publications

Du XJ, Dart AM, Riemersma RA, Oliver MF: Failure of the cholinergic modulation of norepinephrine release during acute myocardial ischemia in the rat. *Circ Res* 1990;66(4):950-956

Du XJ, Riemersma RA: Reduced neuronal noradrenaline overflow in the ischaemic rat heart: importance of the severity of coronary flow reduction. *Basic Res Cardiol* 1991;86 (in press)

Du XJ, Dart AM, Riemersma RA, Oliver MF: Sex difference in presynaptic adrenergic inhibition of norepinephrine release during normoxia and ischemia in the rat heart. *Circ Res* 1991;68(3)

References

Abraham R, Riemersma RA, Wood D, et al: Adipose fatty acid composition and the risk of serious ventricular arrhythmias in acute myocardial infarction. *Am J Cardiol* **1989**;63:269-272

Abrahamsson T, Holmgren S, Almgren O: Noradrenaline release in acute myocardial ischaemia, a fluorescence-histochemical and biochemical study. in Parratt JR (ed): *Early Arrhythmias Resulting from Myocardial Ischaemia.* Macmillan Press Ltd, London, **1982**;pp153-169

Abrahamsson T, Almgren O, Carlsson L: Ischemia-induced noradrenaline release in the isolated rat heart: Influence of perfusion substrate and duration of ischemia. *J Mol Cell Cardiol* **1983**;15:821-830

Abrahamsson T, Almgren O, Carlsson L: Antiarrhythmic effect of reducing myocardial noradrenaline stores with α-methyl-meta-tyrosine. *J Cardiovasc Pharmacol* **1985**;7(suppl 5):S81-S85

Adams KF, Vincent LM, McAllister SM: The influence of age and gender on left ventricular response to supine exercise in asymptomatic normal subjects. *Am Heart J* **1987**;113:732-742

Adgey AAJ, Devlin JE, Webb SW, et al: Initiation of ventricular fibrillation outside hospital in patients with acute ischaemic heart disease. *Br Heart J* **1982**;47:55-61

Adler-Graschinsky E, Langer SZ: Possible role of a β-adrenoceptor in the regulation of noradrenaline release by nerve stimulation through a positive feedback mechanism. *Br J Pharmac* **1975**;53:43-50

Ahonen A, Härkönen M, Juntunen J, et al: Effects of myocardial infarction on adrenergic nerves of the rat heart muscle, a histochemical study. I. Fluorescence microscopical findings. *Acta Physiol Scand* **1975**;93:336-344

Altura BM: Sex and estrogens and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. *J Pharmacol Exp Ther* **1975**;193:403-412

Altura BM: Sex and estrogens in protection against circulatory stress reactions. *Am J Physiol* **1976**;231:842-847

Amsterdam EA: Sudden death during exercise. *Cardiology* **1990**;77:411-417

Axelrod J, Tomchick R: Enzymatic O-methylation of epinephrine and other catechols. *J Biol Chem* **1958**;233:702-705

Baroldi G: Functional morphology of the anastomotic circulation in human cardiac pathology. *Methods Arch Exp Pathol* **1970**;5:438-473

Baroldi G, Falzi G, Mariani F: Sudden coronary death. A postmortem study in 208 selected cases compared to 97 "control" subjects. *Am Heart J* **1979**;98:20-31

Belloni FL, Brown I, Hintze TH: Mechanism of the apparent parasympathetic inhibition of adenosine induced heart rate slowing in the dog. *Cardiovasc Res* **1989**;23:239-248

Benedict CR, Graham-Smith DG: Plasma adrenaline and noradrenaline concentrations and dopamine-β-hydroxylase activity in myocardial infarction with and without cardiogenic shock. *Br Heart J* **1979**;42:214-220

Benfey BG, Elfellah MS, Ogilvie RI, et al: Anti-arrhythmic effects of prazosin and propranolol during coronary artery occlusion and reperfusion in dogs and pigs. *Br J Pharmac* **1984**;82:717-725

Bergamaschi M: Role of the sympathetic and parasympathetic innervation in the genesis of ventricular arrhythmias during experimental myocardial ischemia. in Schwartz PJ, et al (eds): *Neural Mechanisms in Cardiac Arrhythmias*. Raven Press, New York, **1978**;pp139-154

Berridge MJ: Inositol lipids and calcium signalling. *Proc R Lond B* **1988**;234:359-378

Bertel O, Bühler FR, Baitsch G, et al: Plasma adrenaline in patients with acute myocardial infarction relationship to ventricular arrhythmias of varying severity. *Chest* **1982**;82:64-68

Bevilacqua M, Norbiato G, Vago T, et al: Alterations in norepinephrine content and beta adrenoceptors regulation in myocardium bordering aneurysm in human heart: their possible role in the genesis of ventricular tachycardia. *Eur J Clin Invest* 1986;16:163-168

Bigger JT, Fleiss JL, Kleiger R, et al: The relationship among ventricular arrhythmias, left ventricular dysfunction, and mortality in the 2 years after myocardial infarction. *Circulation* **1984**;69:250-258

Bigger JT Jr: Why patients with congestive heart failure die: arrhythmias and sudden cardiac dearth. *Circulation* **1987**;75:IV-28-35

Billman GE, Schwartz PJ, Stone HL: The effects of daily exercise on susceptibility to sudden cardiac death. *Circulation* **1984**;69:1182-1189

Billman GE, Marsh DH: Effect of myocardial ischemia on hemodynamic response to carotid occlusion. *Am J Physiol* **1989**;256:H672-H680

Billman GE: Effect of the cholinergic agonist cabachol and cyclic guanosine monophosphate on sudden cardiac death: Protection from ventricular fibrillation (abstr). *J Am Coll Cardiol* **1989**;13:91A

Birdsall NHM, Burgen ASV, Hulme EC, Wells JW: The effects of ions on the binding of agonists and antagonists to muscarinic receptors. *Br J Pharmac* **1979**;67:371-377

Black KL, Hsu S, Radin NS, Hoff JT: Effect of intravenous eicosapentaenoic acid on cerebral blood flow, edema and brain prostaglandins in ischemic gerbils. *Prostaglandins* **1984**;28:545-556

Bohus B: Acute cardiac responses to emotional stressors in the rat; the involvement of neuroendocrine mechanisms. in Orlebeke JF, et al (eds): *Psychophysiology of Cardiovascular Control*, Plenum Press, New York and London, **1983**;pp131-150

Bolli R, Fisher DJ, Entman ML: Factors that determine the occurrence of arrhythmias during acute myocardial ischemia. *Am Heart J* **1986**;111:261-270

Borda L, Shuchleib R, Henry PD: Effects of potassium on isolated canine coronary arteries modulation of adrenergic responsiveness and release of norepinephrine. *Circ Res* **1977**;41:778-786

Botting JH, Johnston KM, Macleod BA, et al: The effect of modification of sympathetic activity on responses to ligation of a coronary artery in the conscious rat. *Br J Pharmac* **1983**;79:265-271

Brackett CD, Powell LH: Psychosocial and physiological predictors of sudden of cardiac death after healing of acute myocardial infarction. *Am J Cardiol* **1988**;61:979-983

Brooks S, Nevill ME, Meleagros L, et al: The hormonal responses to repetitive brief maximal exercise in humans. *Eur J Appl Physiol* **1990**;60:144-148

Brooks WW, Verrier RL, Lown B: Influence of vagal tone on stellectomy-induced changes in ventricular electrical stability. *Am J Physiol* **1978**;234:H503-H507

Brown AM, Birnbaumer L: Direct G protein gating of ion channels. Am J Physiol **1988**;254:H401-H419

Bruckner G, Webb P, Greenwell L, et al: Fish oil increases peripheral capillary blood cell velocity in humans. *Atherosclerosis* **1987**;66:237-245

Buñag RD, Walaszek EJ, Mueting N: Sex differences in reflex tachycardia induced by hypotensive drugs in unanesthetized rats. *Am J Physiol* **1975**;229:652-656

Bylund DB: Subtypes of α_2-adrenoceptors: pharmacological and molecular biological evidence converge. *TIPS* **1988**;91:356-361

Campbell RWF, Bourke J: Beta blocking agents in post myocardial infarction patients. *Eur Heart J* **1986**;7(suppl A):119-121

Carlsson L, Abrahamsson T, Almgren O: Release of noradrenaline in myocardial ischemia - importance of local inactivation by neuronal and extraneuronal mechanisms. *J Cardiovasc Pharmacol* **1986**;8:545-553

Carlsson L: A crucial role of ongoing anaerobic glycolysis in attenuating acute ischemia-induced release of myocardial noradrenaline. *J Mol Cell Cardiol* **1988**;20:247-253

Casey PJ, Gilman AG: G protein involvement in receptor-effector coupling. *J Biol Chem* **1988**;263:2577-2580

Castillo JD, Webb GD: Rapid desensitization of acetylcholine receptors of eel electroplaques following iontrophoretic application of agonist compounds. *J Physiol* **1977**;270:271-282

Claustre J, Peyrin L, Fitoussi R, et al: Sex differences in the adrenergic response to hypoglycemic stress in human. *Psychopharmacology* **1980**;67:147-153

Cobb LA: Survival after cardiac arrest. in Higgins MW and Luepker RV (eds): *Trends in Coronary Heart Disease Mortality: The Influence of Medical Care*, Oxford University Press, New York, Oxford, **1988**;pp152-159

Collis MG, Vanhoutte PM: Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats. *Circ Res* **1977**;41:759-767

Colucci WS, Gimbrone MAJr, McLaughlin MK, et al: Increased vascular catecholamine sensitivity and α-adrenergic receptor affinity in female and estrogen-treated male rats. *Circ Res***1982**;50:805-811

Connor WE, Connor SL: Diets, atherosclerosis and fish oil. *Adv Intern Med* **1990**;35:139-171

Coromilas J, Bigger JTJr, Gang ES, et al: Relationship between infarct size and ventricular arrhythmias. in Zipes DP, Jalife J (eds): *Cardiac Electrophysiology and Arrhythmias*, Grune & Stratton Inc., Orlando, **1985**;pp523-530

Corr PB, Gillis RA: Autonomic neural influences on the dysrrhythmias resulting from myocardial infarction. *Circ Res* **1978**;43:1-9

Corr PB, Shayman JA, Kramer JB: Increased α-adrenergic receptors in ischemic rat myocardium. *J Clin Invest* **1981**;67:1232-1236

Corr PB, Yamada KA, Witkowski FX: Mechanisms controlling cardiac autonomic function and their relation to arrhythmogenesis. in Fozzard HA, et al (eds): *The Heart and Cardiovascular System. Scientific Foundation.* Raven Press, New York, **1986**;pp1360-1374

Corr PB, Pitt B, Natelson BH,et al: Task force 3: Sudden cardiac death: Neural-chemical interaction. *Circulation* **1987**;76(suppl I):I208-I214

Cousineau D, Goresky CA, Bach GG, Rose CP: Effect of β-adrenergic blockade on *in vivo* norepinephrine release in canine heart. *Am J Physiol* **1984**;246:H283-H293

Cox JL, Daniel TM, Boineau JP: The electrophysiologic time-course of acute myocardial ischemia and the effects of early coronary artery reperfusion. *Circulation* **1973**;48:971-983

Crowley WR, O'Donohue TL, Jacobowitz DM: Sex differences in catecholamine content in discrete brain nuclei of the rat: Effects of neonatal castration or testosterone treatment. *Acta Endocrinol* **1978**;89:20-28

Cruickshank JM: The adrenergic system and prevention of myocardial damage by β-blockade: A clinical overview. *J Cardiovasc Pharmacol* **1989**;14(suppl 9):S20-S24

Culling W, Penny WJ, Cunliffe G, et al: Arrhythmogenic and electrophysiological effects of alpha adrenoceptor stimulation during myocardial ischaemia and reperfusion. *J Mol Cell Cardiol* **1987**;19:251-258

Culp BR, Lands WEM, Lucchesi BR, et al: The effect of dietary supplementation of fish oil on experimental myocardial infarction. *Prostaglandins* **1980**;20:1021-1031

Curtis MJ, Macleod BA, Walker MJA: Models for the study of arrhythmia in myocardial ischaemia and infarction: the use of the rat. *J Mol Cell Cardiol* **1987**;19:399-419

Curtis MJ, Hearse DJ: Ischaemia-induced and reperfusion-induced arrhythmias differ in their sensitivity to potassium: Implication for mechanisms of initiation and maintenance of ventricular fibrillation. *J Mol Cell Cardiol* **1989**;21:21-40

Cusey PJ, Gilman AG: G protein involvement in receptor-effector coupling. *J Biol Chem* **1988**;263:2577-2580

Da Prada M, Zürcher G: Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline and dopamine within the femtomol range. *Life Sci* **1976**;19:1161-1174

Dahlberg ST: Gender difference in the risk factors for sudden cardiac death. *Cardiology* **1990**;77(suppl 2):31-40

Dardinal R, Savard P, Armour JA, et al: Mapping of ventricular tachycardia induced by thoracic neural stimulation in dogs. *Can J Physiol Pharmacol* **1986**;64:411-418

Dart AM, Dietz R, Kübler W, et al: Effects of cocaine and desipramine on the neurally evoked overflow of endogenous noradrenaline from the rat heart. *Br J Pharmac* **1983**;79:71-74

Dart AM, Dietz R, Hieronymus K, et al: Effects of α- and β-adrenoceptor blockade on the neurally evoked overflow of endogenous noradrenaline from the rat isolated heart. *Br J Pharmac* **1984a**;81:475-478

Dart AM, Schömig A, Dietz R, et al: Release of endogenous catecholamines in the ischemic myocardium of the rat. Part B: Effect of sympathetic nerve stimulation. *Circ Res* **1984b**;55:702-706

Dart AM, Riemersma RA: Neurally mediated and spontaneous release of noradrenaline in the ischaemic and reperfused rat heart. *J Cardiovasc Pharmacol* **1985**;7(suppl 5):S45-S49

Dart AM, Riemersma RA, Schömig A, Ungar A: Metabolic requirements for release of endogenous noradrenaline during myocardial ischaemia and anoxia. *Br J Pharmac* **1987**;90:43-50

Dart AM, Riemersma RA: Origins of endogenous noradrenaline overflow during reperfusion of the ischaemic rat heart. *Clin Sci* **1988**;74:269-274

Dart AM, Riemersma RA: Effects of acidosis on anoxic and exocytotic noradrenaline release from the heart. *J Mol Cell Cardiol* **1989**;21:75-83

Dart AM: Influence of myocardial ischaemia on exocytotic noradrenaline release. in Brachmann J, Schömig A (eds): *Adrenergic System and Ventricular Arrhythmias in Myocardial Infarction.* Springer-Verlag, Berlin, **1989**;pp34-43

Das PK, Bhattacharya SK: Studies on the effect of physostigmine on experimental cardiac arrhythmias in dogs. *Br J Pharmac* **1972**;44:397-403

Daugherty A, Frayn KN, et al: The role of catecholamines in the production of ischemia-induced ventricular arrhythmias in the rat in vivo and in vitro. *Br J Pharmac* **1986**;87:265-277

Davies MJ, Popple AW: Sudden unexpected cardiac death- a practical approach to the forensic problem. *Histopathology* **1979**;3:255-277

Davies MJ, Thomas A: Thrombosis and acute coronary-artery lesions in sudden cardiac ischaemic death. *N Engl J Med* **1984**;310:1137-1140

De Champlain J, Malmfors T, Olson L, Sachs C: Ontogenesis of peripheral adrenergic neurons in the rat: pre- and post-natal observations. *Acta Physiol Scand* **1970**;80:276-288

De Ferrari GM: Vagal stimulation and sudden death in conscious dogs with a healed myocardial infarction (abstr). Circulation **1987**;76(suppl IV):IV-107

De Luna AB, Coumel P, Leclercq JF: Ambulatory sudden cardiac death: Mechanisms of production of fatal arrhythmia on the basis of data from 157 cases. *Am Heart J* **1989**;117:151-159

Deckers EAM, Hoor FT: Influences of dietary fats on coronary flow rate and left ventricular work of the isolated rat heart: sunflower seed oil versus lard. *Nutr and Metab* **1980**;24:396-400

DeSilva RA: Central nervous system risk factors for sudden cardiac death. *Ann NY Acad Sci* **1982**;382:143-161

Devos C, Robberecht P, Nokin P, et al: Uncoupling between beta-adrenoceptors and adenylate cyclase in dog ischaemic myocardium. *Naunyn-Schmiedeberg's Arch Pharmacol* **1985**;331:71-75

Dieterich HA, Kaffei H, Kilbinger H, et al: The effects of physostigmine on cholinesterase activity, storage and release of acetylcholine in the isolated chicken heart. *J Pharmacol Exp Ther* **1976**;199:236-246

Dieterich HA, Lindmar R, Löffelholz K: The role of choline in the release of acetylcholine in isolated hearts. *Naunyn-Schmiedeberg's Arch Pharmacol* **1978**;301:207-215

Dietz R, Offner B, Dart AM, Schömig A: Ischaemia-induced noradrenaline release mediates ventricular arrhythmias. in Brachmann J, Schömig A (eds): *Adrenergic System and Ventricular Arrhythmias in Myocardial Infarction.* Springer-Verlag, Berlin, **1989**;pp313-321

Dimsdale JE, Hartley LH, Guiney T, et al: Postexercise peril plasma catecholamines and exercise. *JAMA* **1984**;251:630-632

Dimsdale JE, Ruberman W, Carleton RA, et al: Task Force 1: Sudden cardiac death: Stress and cardiac arrhythmias. *Circulation* **1987**;76(suppl I): I-198-I-214

Dillon JS, Gu XH, Nayler WG: Alpha1 adrenoceptors in the ischaemic and reperfused myocardium. *J Mol Cell Cardiol* **1988**;20:725-735

Dittrich H, Gilpin E, Nicod P, et al: Acute myocardial infarction in women: Influence of gender on mortality and prognostic variables. *Am J Cardiol* **1988**;62:1-7

Docherty JR, McGrath JC: An examination of factors influencing adrenergic transmission in the pithed rat, with special reference to noradrenaline uptake mechanisms and post-junctional α-adrenoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **1980**;313:101-111

Du XJ, Sargent CA, Riemersma RA, Winslow E: Dietary and gender effects on the ischaemic ventricular arrhythmias in the rat. *(submitted for publication)*

Ducis I: The high-affinity choline uptake system. In Whittaker VP (ed): *The Cholinergic Synapse*. Springer-Verlag, Berlin, **1988**;pp409-437

Dunér-Engström M, Fredholm BB: Evidence that prejunctional adenosine receptors regulating acetylcholine release from rat hippocampal slices are linked to an N-ethylmaleimide-sensitive G-protein, but not to adenylate cyclase or dihydropyridine-sensitive Ca^{2+} channels. *Acta Physiol Scand* **1988**;134:119-126

Dzau VJ: Circulating versus local renin-angiotensin system in cardiovascular homeostasis. *Circulation* **1988**;77(suppl I):I-4-I13

Edlund A, Fredholm BB, Patrignani P, et al: Release of two vasodilators, adenosine and prostacyclin, from isolated rabbit hearts during controlled hypoxia. *J Physiol* **1983**;340:487-501

Egozi Y, Avissar S, Sokolovsky M: Muscarinic mechanisms and sex hormone secretion in rat adenohypophysis and preoptic area. *Neuroendocrinology* **1982**;35:93-97

El-Sherif N, Gough WB, Zeiler RH, Mehra R: Triggered ventricular rhythms in 1-day-old myocardial infarction in the dog. *Circ Res* **1983**;52:566-579

Elson JJ, Eick RET, Singer DH: Autonomic nervous system and cellular injury from circumflex ligation in dogs. *Am J Physiol* **1981**;240:H738-H745

Euler DE, Nattel S, Spear JF, et al: Effect of sympathetic tone on ventricular arrhythmias during circumflex coronary occlusion. *Am J Physiol* **1985**;249:H1045-H1050

Euler DE, Murdock D, Scanlon PJ: Sympathetic influence on canine reperfusion arrhythmias (abstr). *Circulation* **1988**;76:IV-108

Fahrenkrug J: VIP and autonomic neurotransmission. *Pharmac Ther* **1989**;41:515-534

Fernandez-Ruiz JJ, Bukhari AR, Hernandez ML, et al: Sex- and age-related changes in catecholamine metabolism and release of rat adrenal gland. *Neurobiol Aging* **1989**;10:331-335

Fink GD, Paddock RJ, Rodgers GM, et al: Elevated cyclic GMP levels in rabbit atria following vagal stimulation and acetylcholine treatment. *Proc Soc Exp Biol Med* **1976**;153:78-82

Force T, Malis CD, Guerrero JL, et al: N-3 fatty acids increase postischemic blood flow but do not reduce myocardial necrosis. *Am J Physiol* **1989**;257:H1204-H1210

Forfar JC, Riemersma RA, Oliver MF: α-Adrenoceptor control of norepinephrine release from acutely ischaemic myocardium: Effects of blood flow, arrhythmias, and regional conduction delay. *J Cardiovasc Pharmacol* **1983**;5:752-759

Forfar JC, Riemersma RA, Russell DC, Oliver MF: Relationship of neurosympathetic responsiveness to early ventricular arrhythmias in ischaemic myocardium. *Cardiovasc Res* **1984**;18:427-437

Forfar JC, Riemersma RA: Metabolic modulation of cardiac neurosympathetic activity *in vivo*: effects of potassium and adenosine. *Cardiovasc Res* **1987**;21:821-829

Fox KM, Mulcahy DA: Therapeutic rational for the management of silent ischemia. *Circulation* **1990**;82(suppl II):II-155--II-160

Franco-Cereceda A, Öwall A, et al: Release of neuropeptide Y and noradrenaline from the human heart after aortic occlusion during coronary artery surgery. *Cardiovasc Res* **1990**;24:242-246

Frank JS, Langer GA: The myocardial interstitium: its structure and its role in ionic exchange. *J Cell Biol* **1974**;60:586-601

Frankenhaeuser M, Dunne E, Lundberg U: Sex differences in sympathetic-adrenal medullary reactions induced by different stressors. *Psychopharmacology* **1976**;47:1-5

Fraser GE: The epidemiology of sudden death. in Fraser GE (ed): *Preventive Cardiology*, Oxford University Press, New York, Oxford, **1986**;pp195-205

Freedman RR, Sabharwal SC, Desai N: Sex differences in peripheral vascular adrenergic receptors. *Circ Res* **1987**;61:581-585

Freedman RA, Swerdlow CD, Soderholm-Difatte V, et al: Clinical predictors of arrhythmia inducibility in survivors of cardiac arrest: Importance of gender and prior myocardial infarction. *J Am Coll Cardiol* **1988**;12:973-978

Frey MAB, Hoffler GW: Association of sex and age with responses to lower-body negative pressure. *J Appl Physiol* **1988**;65:1752-1756

Frishman WH, Furberg CD, Friedewald WT: β-adrenergic blockade for survivors of acute myocardial infarction. *N Engl J Med* **1984**;310:830-837

Fuder H, Bath F, Wiebelt H, et al : Autoinhibition of noradrenaline release from the rat heart as a function of the biophase concentration. Effects of exogenous α-adrenoceptor agonists, cocaine and perfusion rate. *Naunyn-Schmiedeberg's Arch Pharmacol* **1984**;325:25-33

Fuder H, Muscholl E, Wolf K: Cholinesterase activity and exposure time to acetylcholine as factors influencing the muscarinic inhibition of [^{3}H]-noradrenaline overflow from guinea-pig isolated atria. *Br J Pharmac* **1985**;86:905-914

Fuder H: Selected aspects of presynaptic modulation of noradrenaline release from the heart. *J Cardiovasc Pharmacol* **1985**;7(suppl 5):S2-S7

Furberg CD: Effect of antiarrhythmic drugs on mortality after myocardial infarction. *Am J Cardiol* **1983**;52:32C-36C

Furberg CD, Craver DM: Secondary prevention: Life-style interventions. in Higgins MW and Luepker RV (eds): *Trends in Coronary Heart Disease Mortality. The influence of Medical care.* Oxford University Press, New York and Oxford, **1988**;pp227-231

Gang ES, Bigger JT, Livelli FDJr: A model of chronic ischemia arrhythmias: The relation between electrically inducible ventricular tachycardia, ventricular fibrillation threshold and myocardial infarct size. *Am J Cardiol* **1982**;50:469-477

Garza D, White F, Hall R, Bloor C: Effect of collateral development on ventricular fibrillation threshold. *Basic Res Cardiol* **1974**;69:371-382

Gettes LS, Symanski JD, Fleet WF, et al: The intracellular and extracellular changes associated with ischaemia-effects of catecholamines in arrhythogenesis. *Eur Heart J* **1986**;7:(suppl A): 77-84

Gibson GE, Peterson C: Decreases in the release of acetylcholine in vitro with low oxygen. *Biochem Pharmacol* **1982**;31:111-115

Gillis RA: Role of the nervous system in the arrhythmias produced by coronary occlusion in the cat. *Am Heart J* 1971;81:677-684

Gillum RF: Sudden coronary death in the United States 1980-1985. *Circulation* **1989**;79:756-765

Godin D, Campeau N, Nadeau R, et al: Catecholamine release and ventricular arrhythmias during coronary occlusion and reperfusion in the dog. *Can J Physiol Pharmacol* **1985**;63:1088-1095

Goldstein DS: Plasma norepinephrine as an indicator of sympathetic neural activity in clinical cardiology. *Am J Cardiol* **1981**;48:1147-1154

Goldstein DS, Brush JEJr, Eisenhofer G, et al: *In vivo* measurement of neuronal uptake of norepinephrine in the human heart. *Circulation* **1988**;78:41-48

Greene HL: Sudden arrhythmic cardiac death- mechanisms, resuscitation and classification: The Seattle perspective. *Am J Cardiol* **1990a**;65:4B-12B

Greene JH: *The Autonomic Nervous System and Exercise.* Chapman and Hall, London, **1990b**;pp166-177

Grong K, Jodalen H, Stangeland L, et al: Cellular lipid accumulation in different regions of myocardial infarcts in cats during beta adrenergic blockade with timolol. *Cardiovasc Res* **1986**;20:248-255

Gustafson AB, Kalkhoff RK: Influence of sex and obesity on plasma catecholamine response to isometric exercise. *J Clin Endocrinol Metab* **1982**;55:703-708

Gustafsson L, Hedqvist P, Fredholm BB, et al: Inhibition of acetylcholine release in guinea pig ileum by adenosine. *Acta Physiol Scand* **1978**;104:469-478

Gutstein WH, Anversa P, Guideri G: Spasm of small coronary arteries and ischemic myocardial injury induced by hypothalamic stimulation in the rat. *Am J Pathol* **1987**;129:287-294

Haass M, Schömig A: Neuropeptide Y and sympathetic transmission. in Brachmann J, Schömig A (eds): *Adrenergic System and Ventricular Arrhythmias in Myocardial Infarction*, Springer-Verlag, Berlin, **1989**;pp21-33

Haass M, Hock M, Richardt G, et al: Acidosis suppresses exocytotic noradrenaline and neuropeptide Y release in the energy-depleted heart (abstr). *Circulation* **1990**;82(suppl III):III-454

Hall GT, Potter EK: Attenuation of vagal action following sympathetic stimulation is modulated by prejunctional α_2-adrenoceptors in the dog. *J Auto Nerv Sys* **1990**;30:129-138

Hallstrom AP, Eisenberg MS, Bergner L: The persistence of ventricular fibrillation and its implication for evaluating emergency medical services. *Emerg Health Serv Q* **1983**;1:41-49

Halvorsen SW, Nathanson NM: *In vivo* regulation of muscarinic acetylcholine receptor number and function in embryonic chick heart. *J Biol Chem* **1981**;256:7941-7948

Hamer A, Vohra J, Hunt D, et al: Prediction of sudden death by electrophysiologic studies in high risk patients surviving acute myocardial infarction. *Am J Cardiol* **1982**;50:223-229

Han J, Cameron JS: Effects of epinephrine on automaticity of Purkinje fibers from infarcted ventricles. in Zipes DP, Jalife J (eds): *Cardiac Electrophysiology and Arrhythmias*, Grune & Stratton Inc, Orlando, **1985**;pp331-335

Hartog JM, Lamers JMJ, Verdouw PD: The effects of dietary mackerel oil on plasma and cell membrane lipids, on hemodynamics and cardiac arrhythmias during recurrent acute ischemia in the pig. *Basic Res Cardiol* **1986**;81:567-580

Harvey SAK, Booth RFG, Clark JB: The effect of [Ca^{++}] and [H^+] on the functional recovery of rat brain synaptosomes from anoxic insult in vitro. *Biochem J* **1983**;212:289-295

Headrick J, Clarke K, Willis RJ: Adenosine production and energy metabolism in ischaemic and metabolically stimulated rat heart. *J Mol Cell Cardiol* **1989**;21:1089-1100

Heal DJ, Bristow LM, Hurst EM, et al: Sex-related differences in central adrenergic function and responsiveness to repeated administration of desipramine or electroconvulsive shock. *Br J Pharmac* **1989**;97:111-118

Henning RJ, Cheng J, Levy MN: Vagal stimulation decrease rate of left ventricular relaxation. *Am J Physiol* **1989**;256:H428-H433

Henning RJ, Khalil IR, Levy MN: Vagal stimulation attenuates sympathetic enhancement of left ventricular function. *Am J Physiol* **1990**;258:H1470-H1475

Herbert J, Fuller L: Sex differences in neuroendocrine responses to stress with particular reference to the cardiovascular system. in: Ginsburg J (ed): *The Circulation in the Female*. The Parthenon Publishing Group, Lancs, New Jersey, **1989**;pp143-159

Hill JL, Gettes LS: Effect of acute coronary artery occlusion on local myocardial extracellular K^+ activity in swine. *Circulation* **1980**;61:768-778

Hill MRS, Wallick DW, Martin PJ, Levy MN: Attenuation of noradrenergic, nonmuscarinic, vagally induced tachycardia (abstr). *Circulation* **1990**;82(4 suppl III):III-454

Himori N, Matsuura A: A simple technique for occlusion and reperfusion of coronary artery in conscious rats. *Am J Physiol* **1989**;256:H1719-H1725

Hirche HJ, Franz CHR, Bös L, et al: Myocardial extracellular K^+ and H^+ increase and noradrenaline release as possible cause of early arrhythmias following acute coronary artery occlusion in pigs. *J Mol Cell Cardiol* **1980**;12:579-593

Hirche HJ, McDonald FM, Polwin W, et al: Vicious cycle of catecholamines and K^+ in cardiac ischemia. *J Cardiovasc Pharmacol* **1985**;7(suppl 5):S571-575

Hock CE, Holahan MA, Reibel DK: Effect of dietary fish oil on myocardial phospholipids and myocardial ischemic damage. *Am J Physiol* **1987**;252:H554-H560

Hock CE, Deck LD, Bodine RC, Reibel DK: Influence of dietary n-3 fatty acids on myocardial ischemia and reperfusion. *Am J Physiol* **1990**;259:H1518-H1526

Hoffman BB, Lavin TN, Lefkowitz RJ, et al: Alpha adrenergic receptor subtypes in rabbit uterus: Mediation of myometrial contraction and regulation by estrogens. J *Pharmacol Exp Ther* **1981**;219:290-295

Horowitz LN, Spielman SR, Greenspan AM, et al: Mechanisms in the genesis of recurrent ventricular tachyarrhythmias as prevailed by clinical electrophysiologic studies. *Ann NY Acad Sci* **1982**;382:116-132

Inoue H, Zipes DP: Results of sympathetic denervation in the canine heart: supersensitivity that may be arrhythmogenic. *Circulation* **1987**;75:877-887

Inoue H, Zipes DP: Time course of denervation of efferent sympathetic and vagal nerves after occlusion of the coronary artery in the canine heart. *Circ Res* **1988**;62:1111-1120

Inoue H, Skale BT, Zipes DP: Effects of ischemia on cardiac afferent sympathetic and vagal reflexes in dog. *Am J Physiol* **1988**;255:H26-H35

Iversen LL: Catecholamine uptake processes. *Br Med Bull* **1973**;29:130-135

Jalife J, Hamilton AJ, Moe GK: Desensitization of the cholinergic receptor at the sinoatrial cell of the kitten. *Am J Physiol* **1980**;238:H439-H448

James RGG, Arnold JMO, Allen JD, et al: The effects of heart rate, myocardial ischemia and vagal stimulation on the threshold for ventricular fibrillation. *Circulation* **1977**;55:311-317

James TN: Primary and secondary cardioneuropathies and their functional significance. *J Am Coll Cardiol* **1983**;2:983-1002

Janse MJ, Kléber AG: Electrophysiological changes and ventricular arrhythmias in the early phase of regional myocardial ischemia. *Circ Res* **1981**;49:1069-1081

Johansson G, Jonsson L, et al: Severe stress-cardiopathy in pigs. *Am Heart J* **1974**;87:451-457

Johnston KM, MacLeod BA, Walker MJA: Responses to ligation of a coronary artery in conscious rats and the actions of antiarrhythmics. *Can J Physiol Pharmacol* **1983**;61:1340-1353

Jones CE, Devous MD, Thomas JXJr, et al: The effect of chronic cardiac denervation on infarct size following acute coronary occlusion. *Am Heart J* **1978**;95:738-746

Jones SB, Bylund DB, Rieser CA, et al: α_2-Adrenergic receptor binding in human platelets: Alteration during the menstrual cycle. *Clin Pharmacol Ther* **1983**;34:90-96

Kalsner S: Cholinergic constriction in the general circulation and its role in coronary artery spasm. *Circ Res* **1989**;65:237-257

Kannel WB, Thomas HEJr: Sudden coronary death: the Framingham Study. *Ann NY Acad Sci* **1982**;382:3-21

Kannel WB, Schatzkin A: Sudden death: Lessons from subsets in population studies. *J Am Coll Cardiol* **1985**;5:141B-149B

Kannel WB, Plehn JF, Cupples LA: Cardiac failure and sudden death in the Framingham Study. *Am Heart J* **1988**;115:869-875

Karlsberg RP, Penkoske PA, Cryer PE, et al: Rapid activation of the sympathetic nervous system following coronary artery occlusion: relationship to infarct size, site, and haemodynamic impact. Cardiovasc Res **1979**;13:523-531

Karlsberg RP, Cryer PE, Roberts R: Serial plasma catecholamine response early in the course of clinical acute myocardial infarction: Relationship to infarct extent and mortality. *Am Heart J* **1981**;102:24-29

Karwatowska-Kryñska E, Beresewicz A: Effect of locally released catecholamines on lipolysis and injury of the hypoxic isolated rabbit heart. *J Mol Cell Cardiol* **1983**;15:523-536

Katz AM: Membrane structure. in Fozzard HA, et al (eds): *The Heart and Cardiovascular System. Scientific Foundations*, Raven Press, New York, **1986**;pp101-110

Katz AM: Interplay between inotropic and lusitropic effects of cyclic adenosine monophosphate on the myocardial cell. *Circulation* **1990**;82:I-7-I-11

Kaufman WR, Jugdutt BI: Left ventricular catecholamines during acute myocardial infarction in the dog. *Can J Physiol Pharmacol* **1987**;65:172-178

Kempf FCJr, Josephson ME: Cardiac arrest recorded on ambulatory electrocardiograms. *Am J Cardiol* **1984**;53:1577-1582

Kenny D, Brooks HL, Warltier DC: Enhanced α-adrenergic vasoconstriction by n-3 fatty acids in conscious dogs. *Am J Physiol* **1990**;258:H1660-H1667

Kent KM: Neurogenic control of the heart or sympathetic-vagal interaction. in Riemersma RA, Oliver MF (eds): *Catecholamines in the Non-ischaemic and Ischaemic Myocardium*. Elsevier Biomedical Press, Amsterdam, **1982**;pp13-19

Keys A: *Seven countries. A Multivariate Analysis of Death and Coronary Heart Disease*. Cambridge, MA Harvard University Press, Massachusetts and London, **1980**;pp248-262

Khan MT, Malik KU: Modulation by prostaglandins of the release of [^{3}H] noradrenaline evoked by potassium and nerve stimulation in the isolated rat heart. *Eur J Pharmacol* **1982**;78:213-218

Kilbinger H: The autonomic cholinergic neuroeffector junction. in Whittaker VP (ed): *The Cholinergic Synapse*, Springer-Verlag, Berlin, **1988**;pp581-595

Kimmelstiel C, Goldberg RJ: Congestive heart failure in women: Focus on heart failure due to coronary artery disease and diabetes. *Cardiology* **1990**;77(suppl 2):71-79

Kimura S, Bassett AL, Kohya T, et al: Automaticity, triggered activity, and responses to adrenergic stimulation in cat subendocardial Purkinje fibers after healing of myocardial infarction. *Circulation* **1987**;75:651-660

Kléber AG: Extracellular potassium accumulation in acute myocardial ischemia. *J Mol Cell Cardiol* **1984**;16:389-394

Kleiger RE, Miller JP, Bigger JTJr, et al: Decreased heart rate variability and its association with increased mortality after acute infarction. *Am J Cardiol* **1987**;59:256-262

Kliks BR, Burgess MJ, Abildskov JA: Influence of sympathetic tone on ventricular fibrillation threshold during experimental coronary occlusion. *Am J Cardiol* **1975**;36:45-49

Knight DE, von Grafenstein H, Maconochie DJ: Intracellular requirements for exocytotic noradrenaline release. in Brachmann J, Schömig A (eds): *Adrenergic System and Ventricular Arrhythmias in Myocardial Infarction.* Springer-Verlag, Berlin, **1989**;pp3-20

Knopf H, Theising R, Hirche H: The effect of desipramine on ischemia-induced changes in extracellular K^+, Na^+, and H^+ concentrations and noradrenaline release in the ischaemia. *J Cardiovasc Pharmacol* **1988**;12(suppl 1):S8-S14

Kolman BS, Verrier RL, Lown B: The effect of vagus nerve stimulation upon vulnerability of the canine ventricle: role of sympathetic-parasympathetic interactions. *Circulation* **1975**;52:578-585

Krause EG, England PJ: Loss of the cyclic AMP accumulation induced by isoproterenol during acute ischaemia in the isolated rat heart. *J Mol Cell Cardiol* **1982**;14:611-613

Kuchel O, Debinski W, Racz K, et al: An emerging relationship between peripheral sympathetic nervous activity and atrial natriuretic factor. *Life Sciences* **1987**;40:1545-1551

Kuller LH, Talbott EO, Robinson C: Environmental and psychosocial determinants of sudden death. *Circulation* **1987**;76(suppl I):I-177-I-184

La Rovere MT, Specchia G, Mortara A, et al: Baroreflex sensitivity, clinical correlates, and cardiovascular mortality among patients with a first myocardial infarction: A prospective study. *Circulation* **1988**;78:816-824

Laduron PM: Presynaptic heteroreceptors in regulation of neuronal transmission. *Biochem Pharmacol* **1985**;34:467-470

Lamers JMJ, Hartog JM, Verdouw PD, et al: Dietary fatty acids and myocardial function. in Stam H and van der Vusse GJ (eds): *Lipid Metabolism in the Normoxic and Ischemic Heart.* Steinkopff Verlag Darmstadt, Springer-Verlag, New York, **1987**;pp209-211

Langer SZ: Presynaptic regulation of the release of catecholamines. *Pharmacol Rev* **1981**;32:337-362

Lanier SM, Malik KU: Attenuation by prostaglandins of the facilitatory effect of angiotensin II at adrenergic prejunctional sites in the isolated Krebs-perfused rat heart. *Circ Res* **1982**;51:594-601

Larsson B, Andersson KE, Batra S, et al: Effects of estradiol on norepinephrine-induced contraction, alpha adrenoreceptor number and norepinephrine content in the female rabbit urethra. *J Pharmacol Exp Ther* **1984**;229:557-562

Lathers CM, Kelliher GJ, Roberts J, Beasley AB: Nonuniform cardiac sympathetic nerve discharge: mechanism for coronary occlusion and digitalis-induced arrhythmia. *Circulation* **1978**;57:1058-1065

Lavallée M, De Champlain J, Nadeau RA, et al: Muscarinic inhibition of endogenous myocardial catecholamine liberation in the dog. *Can J Physiol Pharmacol* **1978**;56:642-649

Le Marec H, Dangman KH, Danilo PJr, et al: An evaluation of automaticity and triggered activity in the canine heart one to four days after myocardial infarction. *Circulation* **1985**;71:1224-1236

Lederman SN, Wenger TL, Harrell FEJr, et al: Effects of different paced heart rates on canine coronary occlusion and reperfusion arrhythmias. *Am Heart J* **1987**;113:1365-1369

Lenders JWM, Willemsen JJ, de Boo T, et al: Lower increase in plasma catecholamines in both normo- and hypertensive women than in men after adrenergic stimulation. *J Hypertension* **1987**;5:S337-S339

Lenders JWM, de Boo T, Lemmens WAJ, et al: Comparison of blood pressure response to exogenous epinephrine in hypertensive men and women. *Am J Cardiol* **1988**;61:1288-1291

Leprán I, Koltai M, Siegmund W, et al: Coronary artery ligation, early arrhythmias, and determination of the ischemic area in conscious rats. *J Pharmacol Meth* **1983**;9:219-230

Levin RM, Shofer FS, Wein AL: Estrogen-induced alteration in the autonomic responses of the rabbit urinary bladder. *J Pharmacol Exp Ther* **1980**;215:614-618

Levy MN, Martin PJ: Neural control of the heart. in American Physiological Society (ed): *Handbook of Physiology*. Section 2, The Cardiovascular System, Vol.1: The heart, **1979**;pp581-620

Levy MN: Cardiac sympathetic-parasympathetic interactions. *Fed Proc* **1984**;43:2598-2602

Liberthson RR, Nagel EL, Hirschman JC, et al: Pathophysiological observations in prehospital ventricular fibrillation and sudden cardiac death. *Circulation* **1974**;49:790-798

Lindmar R, Löffelholz K, Weide W, et al: Neuronal uptake of choline following release of acetylcholine in the perfused heart. *J Pharmacol Exp Ther* **1980**;215:710-715

Lindpaintner K, Markus MJ, Suzuki FO, et al: Intracardiac generation of angiotensin and its physiologic role. *Circulation* **1988**;77(suppl I):I-18-I-23

Löffelholz K, Brehm R, Lindmar R: Hydrolysis, synthesis, and release of acetylcholine in the isolated heart. *Fed Proc* **1984**;43:2603-2606

Löffelholz K, Pappano AJ: The parasympathetic neuroeffector junction of the heart. *Pharmacol Rev* **1985**;37:1-24

Loiacono RE, Rand MJ, Story DF: Interaction between the inhibitory action of acetylcholine and the α-adrenoceptor autoinhibitory feedback system on release of [^{3}H]-noradrenaline from rat atria and rabbit ear artery. *Br J Pharmac* **1985**;84:697-705

Lombardi F, Verrier RL, Lown B: Relationship between sympathetic neural activity, coronary dynamics, and vulnerability to ventricular fibrillation. *Am Heart J* **1983**;105:958-965

Lombardi F, Casalone C, Bella PD, et al: Global versus regional myocardial ischaemia differences in cardiovascular and sympathetic responses in cats. *Cardiovasc Res* **1984**;18:14-23

Lombardi F: Acute myocardial ischaemia, neural reflexes and ventricular arrhythmias. *Eur Heart J* **1986**;7(suppl A):91-97

Lombardi F, Sandrone G, Pernpruner S, et al: Heart rate variability as an index of sympathovagal interaction after acute myocardial infarction. *Am J Cardiol* **1987**;60:1239-1245

Lorenz RR, Vanhoutte PM: Inhibition of adrenergic neurotransmission in isolated veins of the dog by potassium ions. *J Physiol* **1975**;246:479-500

Lovegrove T, Thompson P: The role of acute myocardial infarction in sudden cardiac death. *Am Heart J* **1978**;96:711-713

Lown B, Verrier RL: Neural factors and sudden death. in Schwartz PJ, et al (eds): *Neural Mechanisms in Cardiac Arrhythmias.* Raven Press, New York, **1978**;pp87-98

Lown B: Sudden cardiac death: The major challenge confronting contemporary cardiology. *Am J Cardiol* **1979**;43:313-328

Lown B: Sudden cardiac death: biobehavioral perspective. *Circulation* **1987**;76:(suppl I):I186-I196

Lu X, Han CD, Lei LC, Du XJ: Sex differences in the pathophysiological process after experimental myocardial infarction in the rat. *Chinese Med J* **1984**;64:87-90

Lubbe WF, Nguyen T, West EJ: Modulation of myocardial cyclic AMP and vulnerability to fibrillation in the rat heart. *Fed Proc* **1983**;42:2460-2464

Lucente M, Rebuzzi AG, Lanza GA, et al: Circadian variation of ventricular tachycardia in acute myocardial infarction. *Am J Cardiol* **1988**;62:670-674

Luine VN: Estradiol increases choline acetyltransferase activity in specific forebrain nuclei and projection areas of female rats. *Exp Neurol* **1985**;89:484-490

Luine VN, Renner KJ, McEwen BS: Sex-dependent differences in estrogen regulation of choline acetyltransferase are altered by neonatal treatments. *Endocrinology* **1986**;119:874-878

Lund DD, Oda RP, Pardini BJ, et al: Vagus nerve stimulation alters regional acetylcholine turnover in rat heart. *Circ Res* **1986**;58:372-377

MacLeod BA, Augereau P, Walker MJA: Effects of halothane anesthesia compared with fentanyl anesthesia and no anesthesia during coronary ligation in rats. *Anesthesiology* **1983**;58:44-52

MacLeod DC, Riemersma RA: Dietary fish oil attenuates the myocardial α_1– adrenoceptor response independently of endogenous catecholamine (abstr). *J Mol Cell Cardiol* **1990**;22(suppl III):S25

Maisel AS, Motulsky HJ, Insel PA: Externalization of β-adrenergic receptors promoted by myocardial ischemia. *Science* **1985**;230:183-186

Malic AB, Kaplan JE, Saba TM: Reference sample method for cardiac output and regional blood flow determination in the rat. *J Appl Physiol* **1976**;40:472-475

Malliani A, Schwartz PJ, Zanchetti A: Neural mechanisms in life-threatening arrhythmias. *Am Heart J* **1980**;100:705-715

Malpas SC, Purdic GL: Circadian variation of heart rate variability. *Cardiovasc Res* **1990**;24:210-213

Marshall RJ, Muir AW, Winslow E: Development of a severe model of early coronary artery ligation-induced dysrhythmias in the anaesthetized rat. *Br J Pharmac* **1981**;73:951-959

Martin C, Meesmann W: Antiarrhythmic effect of regional myocardial chemical sympathectomy in the early phase of coronary occlusion in dogs. *J Cardiovasc Pharmacol* **1985**;7(suppl 5):S76-S80

Martin GJ, Magid NM, Myers G, et al: Heart rate variability and sudden death secondary to coronary artery disease during ambulatory electrocardiographic monitoring. *Am J Cardiol* **1987**;60:86-89

Martins JB, Kerber RE, Marcus ML: Inhibition of adrenergic neurotransmission in ischaemic regions of the canine left ventricle. *Cardiovasc Res* **1980**;14:116-124

Maseri A, Severi S, De Nes M, et al: "Variant" angina: One aspect of a continuous spectrum of vasospastic myocardial ischemia. *Am J Cardiol* **1978**;42:1019-1035

Mathes P, Cowan C, Gudbjarnason S: Storage and metabolism of norepinephrine after experimental myocardial infarction. *Am J Physiol* **1971**;220:27-32

Maxwell MP, Hearse DJ, Yellon DM: Species variation in the coronary collateral circulation during regional myocardial ischaemia: a critical determinant of the rate of evolution and extent of myocardial infarction. *Cardiovasc Res* 1987;21:737-746

McAreavey D, Neilson JMM, Russell DC: Evidence for reduced vagal tone preceding ventricular fibrillation in man (abstr). *Eur J Clin Invest* **1986**;16:A6

McAreavey D, Neilson JMM, Ewing DJ, et al: Cardiac parasympathetic activity during the early hours of acute myocardial infarction. *Br Heart J* **1989**;62:165-170

McCance AJ, Forfar JC: Cardiac and whole body [^{3}H] noradrenaline kinetics in ischaemic heart disease: contrast between unstable angina syndromes and pacing induced ischaemia. *Br Heart J* **1989a**;61:238-247

McCance AJ, Forfar JC: Selective enhancement of the cardiac sympathetic response to exertion by myocardial ischemia (abstr). *Circulation* **1989b**;80:II-551

McDonald FM, Knopf H, Hartono S, et al: Acute myocardial ischaemia in the anaesthetized pig: local catecholamine release and its relation to ventricular fibrillation. *Basic Res Cardiol* **1986**;81:636-645

McGill HC, Anselmo VC, Buchanan JM, Sheridan PJ: The heart is a target organ for androgen. *Science* **1980**;207:775-777

McGrath JC: Alpha-adrenoceptors in arrhythmogenesis. in Williams EMV (ed): *Antiarrhythmic Drugs*. Handbook of Experimental Pharmacology vol 89, Springer-Verlag, Berlin, **1989**;pp475-508

McGrattan PA, Brown JH, Brown OM: Parasympathetic effects on in vivo rat heart can be regulated through an α_1-adrenergic receptor. *Circ Res* **1987**;60:465-471

McLennan PL, Abeywardena MY, Charnock JS: Influence of dietary lipids on arrhythmias and infarction after coronary artery ligation in rats. *Can J Physiol Pharmacol* **1985**;63:1411-1417

McLennan PL, Abeywardena MY, Charnock JS, et al: Dietary lipid modulation of myocardial β-adrenergic mechanisms, Ca^{2+}-dependent automaticity, and arrhythmogenesis in the marmoset. *J Cardiovasc Pharmacol* **1987**;10:293-300

McLennan PL, Abeywardena MY, Charnock JS: The influence of age and dietary fat in an animal model of sudden cardiac death. *Aust NZ J Med* **1989**;19:1-5

Medgett IC, McCulloch MW, Rand MJ: Partial agonist action of clonidine on prejunctional and postjunctional α-adrenoceptors. *Arch Pharmacol* **1978**;304:215-221

Meerson FZ, Malyshev IY: Adaptation to stress increases the heart resistance to ischemic and reperfusion arrhythmias. *J Mol Cell Cardiol* **1989**;21:299-303

Meesmann W: Early arrhythmias and primary ventricular fibrillation after acute myocardial ischaemia in relation to pre-existing coronary collaterals. in Parratt JR (ed): *Early Arrhythmias Resulting from Myocardial Ischaemia.* Macmillan Press Ltd. New York, **1982**;pp93-124

Miller DD, Waters DD, Szlachcic J, et al: Clinical characteristic associated with sudden death in patients with variant angina. *Circulation* **1982**;66:588-592

Mills DE, Ward RP: Effects of essential fatty acid administration on cardiovascular responses to stress in the rat. *Lipids* **1986**;21:139-142

Milner PG, Platia EV, Reid PR, et al: Ambulatory electrocardiographic recordings at the time of fatal cardiac arrest. *Am J Cardiol* **1985**;56:588-592

Miyazaki T, Zipes DP: Presynaptic modulation of efferent sympathetic and vagal neurotransmission in the canine heart by hypoxia, high K^+, low pH, and adenosine. Possible relevance to ischemia-induced denervation. *Circ Res* **1990**;66:289-301

Morady F, DiCarlo L, Winston S, et al: Clinical features and prognosis of patients with out-of hospital cardiac arrest and a normal electrophysiologic study. J Am Coll Cardiol **1984**;4:39-47

Morita T, Latifpour J, O'Hollaren B, et al: Sex differences in function and distribution of α_1- and α_2-adrenoceptors in rabbit urethra. Am J Physiol 1987;252:F1124-F1128

Moss AJ, Carleen E, Multicenter Postinfarction Research Group: Gender differences in the mortality risk associated with ventricular arrhythmias after myocardial infarction. in Eaker ED, et al (eds): *Coronary Heart Disease in Women.* Haymarket Doyma Inc., New York, **1987**;pp204-207

Mukherjee A, Wong TM, Buja LM, Lefkowitz RJ: Beta adrenergic and muscarinic cholinergic receptors in canine myocardium: Effects of ischemia. *J Clin Invest* **1979**;64:1423-1428

Mukherjee A, Bush LR, McCoy KE, et al: Relationship between β-adrenergic receptor numbers and physiological responses during experimental canine myocardial ischemia. *Circ Res* **1982**;50:735-741

Muller JE, Ludmer PL, Willich SN, et al: Circadian variation in the frequency of sudden cardiac death. *Circulation* **1987**;75:131-138

Muntz KH, Hagler HK, Boulas J: Redistribution of catecholamines in the ischemic zone of the dog heart. *Am J Pathol* **1984**;114:64-78

Muth EA, Crowley WR, Jacobowitz DM: Effect of gonadal hormones on luteinizing hormone in plasma and on choline acetyltransferase activity and acetylcholine levels in discrete nuclei of the rat brain. *Neuroendiocrinology* **1980**;30:329-336

Myerburg RJ: Sudden cardiac death: Epidemiology, causes, and mechanisms. *Cardiology* **1987**;74(suppl 2):2-9

Myers RW, Pearlman AS, Hyman RM, et al: Beneficial effects of vagal stimulation and bradycardia during experimental acute myocardial ischemia. *Circulation* **1974**;49:943-947

Nadeau RA, de Champlain J: Plasma catecholamines in acute myocardial infarction. *Am Heart J* **1979**;98:548-554

Nakamaru M, Inagami T: Atrial natriuretic factor inhibits norepinephrine release evoked by sympathetic nerve stimulation in isolated perfused rat mesenteric arteries. *Eur J Pharmacol* **1986**;123:459-461

Neely BH, Hageman GR: Differential cardiac sympathetic activity during acute myocardial ischemia. *Am J Physiol* **1990**;258:H1534-H1541

Neufeld HN, Zivner Z, Eldar M, et al: Sinus bradycardia in acute myocardial infarction. in Schwartz PJ, et al (eds): *Neural Mechanisms in Cardiac Arrhythmias*. Raven Press, New York, **1978**;pp19-30

Newman PE: The coronary collateral circulation: Determinants and functional significance in ischaemic heart disease. *Am Heart J* **1981**;102:431-445

Nohara R, Kambara H, Murakami T, et al: Collateral function in early acute myocardial infarction. *Am J Cardiol* **1983**;52:955-959

Noneman JW, Rogers JF: Lidocaine prophylaxis in acute myocardial infarction. *Medicine* **1978**;57:501-515

Nordrehaug JE, Vander-Lippe G: Hypokalaemia and ventricular fibrillation in acute myocardial infarction. *Br Heart J* **1983**;50:525-529

Norell MS, Lyons JP, Gardener JE, et al: Protective effect of collateral vessels during coronary angioplasty. *Br Heart J* **1989**;62:241-245

Norris RM, Barnaby PF, Brown MA, et al: Prevention of ventricular fibrillation during acute myocardial infarction by intravenous propranolol. *Lancet* **1984**;2:883-886

Ohyanagi M, Matsumori Y, Iwasaki T: Beta-adrenergic receptors in ischemic and non-ischemic canine myocardium: Relation to ventricular fibrillation and effects of pretreatment with propranolol and hexamethonium. *J Cardiovasc Pharmacol* **1988**;11:107-114

Oliver MF: Sudden cardiac death-An overview. in Parratt JR (ed): *Early Arrhythmias Resulting from Myocardial Ischaemia,* Macmillan Press Ltd, New York, **1982**;pp1-14

Oliver MF: Lack of impact of prevention on sudden cardiac death. *J Am Coll Cardiol* **1985**;5:150B-154B

Oliver MF: Prevention of coronary heart disease-propaganda, promises, problems, and prospects. *Circulation* **1986**;73:1-9

Olsen KL, Edwards E, Schechter N, Whalen RE: Muscarinic receptors in preoptic area and hypothalamus: effects of cyclicity, sex and estrogen treatment. *Brain Res* **1988**;448:223-229

Opie LH: Comparison of anti-infarct effects of beta-blockade, glucose-insulin-potassium, and hyaluronidase. *Am Heart J* **1980**;100:520-531

Opie LH: Products of myocardial ischaemia and electrical instability of the heart. *J Am Coll Cardiol* **1985**;162B-165B

Oppenheimer SM, Cechetto D, Hachinski VC: Cerebrogenic cardiac arrhythmias: Cerebral electrocardiographic influences and their role in sudden death. *Arch Neurol* **1990**;47:513-519

Packer M: Sudden unexpected death in patients with congestive heart failure: a second frontier. *Circulation* **1985**;72:681-685

Paessens R, Borchard F: Morphology of cardiac nerves in experimental infarction of rat hearts. I. Fluorescence microscopical findings. *Virchows Arch A Path Anat Histol* **1980**;386:265-278

Panek RL, Dixon WR, Rutledge CO: Modification of sympathetic neuronal function in the rat tail artery by dietary lipid treatment. *J Pharmacol Exp Ther* **1985**;233:578-583

Pantridge JF: Autonomic disturbance at the onset of acute myocardial infarction. in Schwartz PJ, et al (eds): *Neural Mechanisms in Cardiac Arrhythmias,* Raven Press, New York, **1978**:pp7-17

Parker GW, Michael LH, Entman ML: An animal model to examine the response to environmental stress as a factor in sudden cardiac death. *Am J Cardiol* **1987**;60:9J-14J

Pelleg A, Mitsuoka T, Mazgalev T, et al: Interacting negative chronotropic effects of adenosine and the vagus nerve on the canine sinus node. *Cardiovasc Res* **1988**;22:55-61

Penny WJ: The deleterious effects of myocardial catecholamines on cellular electrophysiology and arrhythmias during ischaemia and reperfusion. *Eur Heart J* **1984**;5:960-973

Perez-Gomez F, De Dios RM, Rey J, et al: Prinzmetal's angina: reflex cardiovascular response during episode of pain. *Br Heart J* **1979**;42:81-87

Peter T, Norris RM, Clarke ED, et al: Reduction of enzyme levels by propranolol after acute myocardial infarction. *Circulation* **1978**;57:1091-1095

Philippu, Matthaei H: Transport and storage of catecholamines in vesicles. in Trendelenburg U and Weiner N (eds): *Catecholamines* I.Handbook of Experimental Pharmacology, Springer-Verlag, Berlin, **1988**;pp1-42

Piek JJ, Becker AE: Collateral blood supply to the myocardium at risk in human myocardial infarction: A quantitative post-mortem assessment. *J Am Coll Cardiol* **1988**;11:1290-1296

Pierpont GL, DeMaster EG, Cohn JN: Regional differences in adrenergic function within the left ventricle. *Am J Physiol* **1984**;246:H824-H829

Podrid PJ, Fuchs T, Candinas R: Role of the sympathetic nervous system in the genesis of ventricular arrhythmia. *Circulation* **1990**;80(suppl I):I-103-I-113

Potter E, McCloskey DI, Courtice GP: Effects of hypoxia, hypercapnia and acidemia on vagal action at the heart in the dog. *J Auton Nerv Syst* **1986**;16:79-83

Potter EK: Presynaptic inhibition of cardiac vagal postganglionic nerves by neuropeptide Y. *Neurosci Lett* **1987**;83:101-106

Priori SG, Zuanetti G, Schwartz PJ: Ventricular fibrillation induced by the interaction between acute myocardial ischemia and sympathetic hyperactivity: Effect of nifedipine. *Am Heart J* **1988**;116:37-43

Priori SG, Schwartz PJ: Sympathetic nervous system and malignant arrhythmias: evidence for further links. in Refsum H, et al (eds): *Heart & Brain,* Springer-Verlag, Berlin, **1989**;pp98-107

Puddu PE, Jouve R, Langlet F, et al: Prevention of postischemic ventricular fibrillation late after right or left stellate ganglionectomy in dogs. *Circulation* **1988**;77:935-946

Puig M, Kirpekar SM: Inhibitory effect of low pH on norepinephrine release. *J Pharmacol Exp Ther* **1971**;176:134-138

Rabinowitz SH, Verrier RL, Lown B: Muscarinic effects of vagosympathetic trunk stimulation on the repetitive extrasystole (RE) threshold. *Circulation* **1976**;53:622-627

Rand MJ, Majewski H, Wong-Duting H, et al: Modulation of neuroeffector transmission. *J Cardiovasc Pharmacol* **1987**;10:S33-S44

Rapaport E: Sudden cardiac death. *Am J Cardiol* **1988**;62(suppl 12):3I-6I

Recordati G, Schwartz PJ, Pagani M, et al: Activation of cardiac vagal receptors during myocardial ischaemia. *Experientia* **1971**;27:1423-1424

Reddy MH: *Beta Adrenergic Function in Acute Myocardial ischaemia.* Ph.D. Thesis, University of Edinburgh, **1989**

Reibel DK, Holahan MA, Hock CE: Effects of dietary fish oil on cardiac responsiveness to adrenoceptor stimulation. *Am J Physiol* **1988**;254:H494-H499

Reimer KA, Long JB, Murry CE, et al: Three-dimensional distribution of collateral blood flow within the anatomic area at risk after circumflex coronary artery occlusion in dogs. *Basic Res Cardiol* 1987;82:473-485

Rentrop KP, Cohen M, Blanke H, et al: Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. *J Am Coll Cardiol* **1985**;5:587-592

Rentrop KP, Thornton JC, Feit F, et al: Determinants and protective potential of coronary arterial collaterals as assessed by an angioplasty model. *Am J Cardiol* **1988**;61:677-684

Richards DA, Cody DV, Denniss AR, et al: Ventricular electrical instability: A predictor of death after myocardial infarction. *Am J Cardiol* **1983**;51:75-80

Richardson IW, Szerb JC: The release of labelled acetylcholine and choline from cerebral cortical slices stimulated electrically. Br J Pharmac **1974**;52:499-507

Richardt G, Waas W, Kranzhöfer R, et al: Adenosine inhibits exocytotic release of endogenous noradrenaline in rat heart: A protective mechanism in early myocardial ischemia. *Circ Res* **1987**;61:117-123

Richardt G, Waas W, Kranzhöfer R, et al: Interaction between the release of adenosine and noradrenaline during sympathetic stimulation: a feed-back mechanism in rat heart. *J Mol Cell Cardiol* **1989**;21:269-277

Richardt G, Kranzhöfer R, Blessing R, et al: Systemic and coronary venous noradrenaline concentration during PTCA. Circulation **1990**;82(suppl III):III-456

Riemersma RA, Forfar JC: Effects of experimental ischaemia on myocardial catecholamines. in Riemersma RA, Oliver MF (eds): *Catecholamines in Non-ischaemic and Ischaemic Myocardium.* Elsevier Biomedical Press, Amsterdam, **1982**;139-154

Riemersma RA, Wood DA, Butler S, et al: Linoleic acid content in adipose tissue and coronary heart disease. *Br Med J* **1986a**;292:1423-1427

Riemersma RA, Dart AM, Oliver MF, et al: Noradrenaline release, metabolism and myocardial ischaemia induced arrhythmias (abstr). *Clin Sci* **1986b**;70:(suppl 13):34p

Riemersma RA: Raised plasma non-esterified fatty acids (NEFA) during ischaemia:implications for arrhythmias. in Stam H, van der Vusse GJ (eds): *Lipid Metabolism in the normoxic and ischaemic heart.* Steinkopff Verlag Darmstadt, New York, **1986**:pp177-185

Riemersma RA, Sargent CA, Saman S, et al: Dietary fatty acids and ischaemic arrhythmias. *Lancet* **1988**;ii:285-286

Roberts JM, Insel PA, Goldfien A: Regulation of myometrial adrenoceptors and adrenergic response by sex steroids. *Mol Pharmacol* **1981**;20:52-58

Robinson K, Conroy RM, Mulcahy R, et al: Risk factors and in-hospital course of first episode of myocardial infarction or acute coronary insufficiency in women. *J Am Coll Cardiol* **1988**;11:932-936

Roelandt J, Hugenholtz PG: Sudden death: prediction and prevention. *Eur Heart J* **1986**;7(suppl A):169-180

Roeske WR, Yamamura HI: Adrenergic-cholinergic interactions. in Kunos G (ed): *Adrenoceptors & Catecholamine Action, Part B*. John Wiley & Sons, New York, **1983**;2:pp109-122

Romo M, Salomaa V, FINMONICA Study Group: Coronary heart disease deaths outside hospital (abstr). *Eur Heart J* **1990**;11(suppl):172

Rona G: Catecholamine cardiotoxicity. *J Mol Cell Cardiol* **1985**;17:291-306

Rossi L: Cardioneuropathy and extracardiac neural disease. *J Am Coll Cardiol* 1985;5:66B-70B

Rothschild M, Rothschild A, Pfeifer M: Temporary decrease in cardiac parasympathetic tone after acute myocardial infarction. *Am J Cardiol* **1988**;62:637-639

Ruberman W, Weinblatt E, Goldberg JD, et al: Ventricular premature complexes and sudden death after myocardial infarction. *Circulation* **1981**;64:297-305

Sanchez J, Pequignot JM, Peyrin L, et al: Sex differences in the sympatho-adrenal response to isometric exercise. *Eur J Appl Physiol* **1980**;45:147-154

Sanchez-Prieto J, Harvey SAK, Clark JB: Effects of *in vitro* anoxia and low pH on acetylcholine release by rat brain synaptosomes. *J Neurochem* **1987**;48:1278-1284

Sargent CA: *Dietary Fat and Ischaemic Arrhythmias*. Ph.D. Thesis, University of Edinburgh, **1990**

Schaible TF, Penpargkul S, Scheuer J: Cardiac responses to exercise training in male and female rats. J *Appl Physiol* **1981**;50:112-117

Schaible TF, Malhotra A, Ciambrone G, et al: The effects of gonadectomy on left ventricular function and cardiac contractile proteins in male and female rats. *Circ Res* **1984**;54:38-49

Schatzkin A, Cuppls LA, Heeren T, et al : The epidemiology of sudden unexpected death: risk factors for men and women in the Framingham Heart Study. *Am Heart J* **1984**;107:1300-1306

Scherlag BJ, Kabell G, Harrison, et al: Mechanisms of bradycardia induced ventricular arrhythmias in myocardial ischaemia and infarction. *Circulation* **1982**;65:1429-1434

Scheuer J, Malhotra A, Schaible TF, et al: Effects of gonadectomy and hormonal replacement on rat hearts. *Circ Res* **1987**;61:12-19

Schmid PG, Greif BJ, Lund DD, et al: Tyrosine hydroxylase and choline acetyltransferase activities in ischemic canine heart. *Am J Physiol* **1982**;243:H788-H795

Schoenfeld MH, McGovern B, Garan H, et al: Determinants of the outcome of electrophysiologic study in patients with ventricular tachyarrhythmias. *J Am Coll Cardiol* **1985**;6:298-306

Schömig A, Dart AM, Dietz R, et al: Release of endogenous catcholamines in the ischemic myocardium of the rat. Part A: locally mediated release. *Circ Res* **1984a**;55:689-701

Schömig A, Ness G, Mayer E, et al: Sympathetic activity in patients with acute myocardial infarction before and after intracoronary thrombolytic therapy (abstr). *Eur Heart J* **1984b**;5(suppl 1):39

Schömig A, Fischer S, Kurz T, et al: Nonexocytotic release of endogenous noradrenaline in the ischemic and anoxic rat heart: Mechanism and metabolic requirements. *Circ Res* **1987**;60:194-205

Schömig A: Adrenergic mechanisms in myocardial infarction: Cardiac and systemic catecholamine release. *J Cardiovasc Pharmacol* **1988**;12(suppl 1):S1-S7

Schömig A, Kurz T, Richardt G, et al: Neuronal sodium homoeostasis and axoplasmic amine concentration determine calcium-independent noradrenaline release in normoxic and ischemic rat heart. *Circ Res* **1988**;63:214-226

Schömig A: Increase of cardiac and systemic catecholamine in myocardial ischemia. in: Brachmann J, Schömig A (eds): *Adrenergic System and Ventricular Arrhythmias in Myocardial Infarction.* Springer-Verlag, Berlin, **1989**;pp61-77

Schömig A, Kranzhöfer R, Richardt G, et al: Dual mechanism of noradrenaline release in human heart (abstr). *Circulation* **1990**;82(suppl III):III-455

Schrör K, Funke K: Prostaglandins and myocardial noradrenaline overflow after sympathetic nerve stimulation during ischemia and reperfusion. *J Cardiovasc Pharmacol* **1985**;7(suppl 5):S50-S54

Schulze RAJr, Strauss HW, Pitt B: Sudden death in the year following myocardial infarction: Relationship of ventricular premature contractions in the late hospital phase and left ventricular ejection fraction. *Am J Med* **1977**;62:192-199

Schwaiger M, Araujo L, Luxen A, et al: Sustained catecholamine depletion in stunned canine myocardium (abstr). Circulation **1987**;76:(suppl IV):IV-378

Schwartz L, Sole MJ, Vaughan-Neil EF, et al: Catecholamines in coronary sinus and peripheral plasma during pacing-induced angina in man. *Circulation* **1979**;59:37-43

Schwartz PJ, Snebold NG, Brown AM: Effects of unilateral cardiac sympathetic denervation on the ventricular fibrillation threshold. *Am J Cardiol* **1976**;37:1034-1040

Schwartz PJ, Stone HL: Left stellectomy in the prevention of ventricular fibrillation caused by acute myocardial ischemia in conscious dogs with anterior myocardial infarction. *Circulation* **1980**;62:1256-1265

Schwartz PJ, Vanoli E: Cardiac arrhythmias elicited by interaction between acute myocardial ischemia and sympathetic hyperactivity: A new experimental model for the study of antiarrhythmic drugs. *J Cardiovasc Pharmacol* **1981**;3:1251-1259

Schwartz PJ, Vanoli E, Zaza A, et al: The effect of antiarrhythmic drugs on life-threatening arrhythmias induced by the interaction between acute myocardial ischemia and sympathetic hyperactivity. *Am Heart J* **1985a**;109:937-948

Schwartz PJ, Motolese M, Pollavini G, et al: Surgical and pharmacological antiadrenergic interventions in the prevention of sudden death after a first myocardial infarction (abstr). *Circulation* **1985b**;72(suppl III):III-358

Schwartz PJ: Idiopathic long QT syndrome: Progress and questions. *Am Heart J* **1985**;109:399-410

Schwartz PJ, Stramba-Badiale M: Parasympathetic nervous system and malignant arrhythmias. in Kulbertus HE, Frank G (eds): *Neurocardiology*. Futura Publishing Co., New York, **1988**;pp179-200

Schwartz PJ, Zuanetti G: Role of the autonomic nervous system in reperfusion arrhythmias. *J Moll Cell Cardiol* **1988**;20(suppl II):113-118

Schwartz PJ, Vanoli E, Stramba-Badiale M, et al: Autonomic mechanisms and sudden cardiac death: New insights from analysis of baroreceptor reflexes in conscious dogs with and without a myocardial infarction. *Circulation* **1988a**;78:969-979

Schwartz PJ, Zaza A, Pala M, et al: Baroreflex sensitivity and its evolution during the first year after myocardial infarction. *J Am Coll Cardiol* **1988b**;12:629-636

Semafuko WEB, Rutledge CO, Dixon WR: Effect of dietary lipids on myocardial norepinephrine content and field stimulation-mediated release of norepinephrine from perfused neonatal and adult rat hearts. *J Cardiovasc Pharmacol* **1987**;10:16-23

Semafuko WEB, Rutledge CO, Dixon WR: Modulation of adrenergic neurotransmission in the rat tail artery by dietary lipids. *J Cardiovasc Pharmacol* **1989**;13:138-145

Seth SS, Jagadeesh G, Siddiqui HH, et al: Changes in myocardial norepinephrine in Indian domestic pigs after two-stage coronary ligation. *Eur J Pharmacol* **1974**;27:175-179

Sharma VK, Banerjee PS: Presynaptic muscarinic cholinergic receptors. *Nature* **1978**;272:276-278

Sharma AD, Saffitz JE, Lee BI, et al: Alpha adrenergic-mediated accumulation of calcium in reperfused myocardium. *J Clin Invest* **1983**;72:802-818

Sheehan FH, Epstein SE: Determinations of arrhythmic death during coronary artery reperfusion: Effect of perfusion bed size. *Am Heart J* **1983**;105:911-914

Shepherd JT: The heart as a sensory organ. *J Am Coll Cardiol* **1985**;5:83B-87B

Sheridan DJ, Penkoske PA, Sobel RE, Corr PB: Alpha adrenergic contribution to dysrhythmia during myocardial ischemia and reperfusion in cats. *J Clin Invest* **1980**;65:161-171

Shimokawa H, Vanhoutte PM: Dietary n-3 fatty acids and endothelium-dependent relaxations in porcine coronary arteries. *Am J Physiol* **1989**;256:H968-H973

Siegmund W, Leprán I, Szekeres L: Effect of pentobarbitone anesthesia, age and sex in the acute phase of myocardial infarction in rats. in Tardis L, et al (eds): *Pharmacological Control of Heart and Circulation,* Budapest, **1979**;pp63-66

Simon JR, Kuher MJ: High affinity choline uptake: ionic and energy requirements. *J Neurochem* **1976**;27:93-99

Smith EF, Lefer AM, Smith JB: Influence of thromboxane inhibition on the severity of myocardial ischaemia in cats. *Can J Physiol Pharmacol* **1980**;58:294-300

Spadaro J, Fishbein MC, Hare C, et al: Characterization of myocardial infarcts in the rat. *Arch Pathol Lab Med* **1980**;104:179-183

Spain DM, Bradess VA, Iral P, et al: Intercoronary anastomotic channels and sudden unexpected death from advanced coronary atherosclerosis. *Circulation* **1963**;27:12-17

Spielman SR, Greenspan AM, Kay HR, et al: Electrophysiologic testing in patients at high risk for sudden cardiac death. I. Nonsustained ventricular tachycardia and abnormal ventricular function. *J Am Coll Cardiol* **1985**;6:31-39

Spinelli W, Rosen MR: Autonomic mechanisms in cardiac rhythm and arrhythmias. in Refsum H, et al (eds): *Heart & Brain*, Springer-Verlag, Berlin, **1989**;pp621-639

Spratto GR, Miller JW: An investigation of the mechanism by which estradiol-17β elevates the epinephrine content of the rat uterus. *J Pharmacol Exp Ther* **1968**;161:7-13

Starke K: Presynaptic α-autoreceptors. *Rev Physiol Biochem Pharmacol* **1987**;107:73-146

Steenbergen C,DeLeeuw G, Barlow C, et al: Heterogeneity of the hypoxic state in perfused rat heart. *Circ Res* **1977**;41:606-615

Stiles GL: Mechanisms of receptor activation in adenylate cyclase systems. *J Cardiovasc Pharmacol* **1989**;14(suppl 5):S1-S5

Stone JD, Crofton JT, Share L: Sex differences in central adrenergic control of vasopressin release. *Am J Physiol* **1989**;257:R1040-R1045

Stone PH: Triggers of transient myocardial ischemia: circadian variation and relation to plaque rupture and coronary thrombolysis in stable coronary artery disease. *Am J Cardiol* **1990**;66:32G-36G

Story DF, Standford-Starr CA, Rand MJ: Evidence for the involvement of α_1-adrenoceptors in negative feedback regulation of noradrenergic transmitter release in rat atria. *Clin Sci* **1985**;68(suppl 10):111s-115s

Strasser RH, Krimmer J: Regulation of β-adrenergic receptors: Impaired desensitization in myocardial ischemia. *J Cardiovasc Pharmacol* **1988**;12(suppl 1):S15-S24

Strasser RH, Krimmer J, et al: Dual sensitization of the adrenergic system in early myocardial ischemia: independent regulation of the β-adrenergic receptors and the adenylate cyclase. *J Mol Cell Cardiol* **1990**;22:1405-1423

Stumpf WE, Sar M, Aumüller G: The heart: a target organ for estradiol. *Science* **1977**;196:319-321

Surawicz B: Ventricular fibrillation. *J Am Coll Cardiol* **1985**;5:43B-54B

Surawicz B: Prognosis of ventricular arrhythmias in relation to sudden cardiac death: Therapeutic implications. *J Am Coll Cardiol* **1987**;10:435-447

Thandroyen FT, Worthington MG, Higginson LM, et al: The effect of alpha- and beta-adrenoceptor antagonist agents on reperfusion ventricular fibrillation and metabolic status in the isolated perfused rat heart. *J Am Coll Cardiol* **1983**;1:1056-1066

Thandroyen FT, Swan S, Opie LH: Serotonin and the heart: Pharmacological evaluation with the S_2-serotonergic antagonist ketanserin. in Vanhoutte PM (ed): *Serotonin and the Cardiovascular System.* Raven Press, New York, **1985**;pp87-93

Thandroyen FT, Higginson L, Opie LH, et al: The influence of verapamil and its isomers on vulnerability to ventricular fibrillation during acute myocardial ischemia and adrenergic stimulation in isolated rat heart. *J Mol Cell Cardiol* **1986**;18:645-649

Thandroyen FT, Muntz KH, Buja LM, Willerson JT: Alterations in β-adrenergic receptors, adenylate cyclase, and cyclic AMP concentrations during acute myocardial ischemia and reperfusion. *Circulation* **1990**;82:II-30-II-37

Thomson M, Fulton M, Wood DA, et al: A comparison of the nutrient intake of some Scotsmen with dietary recommendations. Human Nutr. *Appl Nutr* **1985**;39A:443-455

Thorén PN: Activation of left ventricular receptors with nonmedullated vagal afferent fibers during occlusion of a coronary artery in the cat. *Am J Cardiol* **1976**;37:1046-1051

Tofler GH, Brezinski D, Schafer AI, et al: Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N Engl J Med* **1987**;316:1514-1518

Tranzer JP, Richards JG: Ultrastructural cytochemistry of biogenic amines in nervous tissue: Methodology improvements *J Histochem Cytochem* **1976**;24:1178-1193

Trolese-Mongheal Y, Duchene-Marullaz P, Troles JF, et al: Sudden death and experimental acute myocardial infarction. *Am J Cardiol* **1985**;56:677-681

Uchida Y, Murao S: Excitation of afferent cardiac sympathetic nerve fibers during coronary occlusion. *Am J Physiol* 1974;226:1094-1099

van Doornen LJP, Boomsma DI: Sex differences in catecholamine reactions to stress and its relevance to coronary heart disease. in Orlebeke JF, et al (eds): *Psychophysiology of Cardiovascular Control.* Plenum Press, New York and London, **1983**;pp809-827

Vatner DE, Knight DR, Shen Y, et al: One hour of myocardial ischaemia in conscious dogs increase beta-adrenergic receptors, but decreases adenylate cyclase activity. *J Mol Cell Cardiol* **1988**;20:75-82

Verlato G, Borgdorff P: Endogenous adenosine enhances vagal negative chronotropic effect during hypoxia in the anaesthetized rabbit. *Cardiovasc Res* **1990**;24:532-539

Verrier RL, Lown B: Behavioral stress and cardiac arrhythmias. *Ann Rev Physiol* **1984**;46:155-176

Verrier RL: Autonomic substrates for arrhythmias. Prog in Cardiol **1988**;1:65-85

von Eiff AW, Plotz EJ, Beck KJ, et al: The effect of estrogens and progestins on blood pressure regulation of normotensive women. *Am J Obstet Gynecol* **1971**;109:887-892

von Mutius S, Neumann M, Meesmann W: Early changes in collateral blood flow to ischemic myocardium and their influence on bimodal vulnerability during the first 30 min of acute coronary artery occlusion in dogs. *Basic Res Cardiol* **1988**;83:94-106

Wakade AR, Wakade TD: Sympathetic neurons grown in culture generate ATP by glycolysis: correlation between ATP content and [^{3}H]noradrenaline uptake and storage. *Brain Res* **1985**;359:397-401

Waldenström AP, Hjalmarson AC, Thornell L: A possible role of noradrenaline in the development of myocardial infarction. *Am Heart J* **1978**;95:43-51

Walker MJA, Curtis MJ, Hears DJ, et al: The Lambeth Conventions: guide-lines for the study of arrhythmias in ischaemia, infarction and reperfusion. *Cardiovasc Res* **1988**;22:447-455

Wan DCC, Powis DA, Marley PD, et al: Effects of α- and β-adrenoceptor agonists and antagonists on ATP and catecholamine release and desensitization of the nicotinic response in bovine adrenal chromaffin cells. *Biochem Pharmacol* **1988**;37:725-736

Waris T, Lähteenmäki T, Hukki J, et al: Congruence of adrenergic and cholinergic intrinsic innervation of human and rat atria. *Basic Res Cardiol* **1987**;82:445-453

Warner MR, Levy MN: Neuropeptide Y as a putative modulator of the vagal effects on heart rate. *Circ Res* **1989**;64:882-889

Watanabe AM: Cellular mechanisms of muscarinic regulation of cardiac function, In Randall WC (ed): *Nervous Control of Cardiovascular Function.* Oxford University Press, New York, Oxford, **1984**;pp130-164

Waxman MB, Wald RW: Termination of ventricular tachycardia by increase in cardiac vagal drive. *Circulation* **1977**;56:385-389

Waynforth HB: *Experimental and surgical techniques in the rat.* Academic Press, New York, **1980**

Webb SW, Adgey AAJ, Pantrige JF: Autonomic disturbance at onset of acute myocardial infarction. *Br Med J* **1972**;3:89-92

Webster JS, Moberg C, Rincon G: Natural history of severe proximal coronary artery disease as documented by coronary cineangiography. *Am J Cardiol* **1974**;33:195-200

Wennmalm M, FitzGerald GA, Wennmalm A: Prostacyclin as neuromodulator in the sympathetically stimulated rabbit heart. *Prostaglandins* **1987**;33:675-691

Wetzel GT, Brown JH: Relationships between choline uptake, acetylcholine synthesis and acetylcholine release in isolated rat atria. *J Pharmacol Exp Ther* **1983**;226:343-348

Wetzel GT, Brown JH: Presynaptic modulation of acetylcholine release from cardiac parasympathetic neurons. *Am J Physiol* **1985**;248:H33-H39

Wetzel GT, Goldstein D, Brown JH: Acetylcholine release from rat atria can be regulated through an α_1-adrenergic receptor. *Circ Res* **1985**;56:763-766

Wexler BC, Willen D, Greenberg BP: Progressive electrocardiographic changes in male and female arteriosclerotic and non-arteriosclerotic rats during the course of isoproterenol-induced myocardial infarction. *Cardiovasc Res* **1974**;8:460-468

Wiechman BE, Borowitz JL: Effect of steroid hormones and diethylstilbestrol on adrenomedullary catecholamine secretion. *Pharmacology* **1979**;18:195-201`

Williams DO, Amsterdam EA, Miller RR, et al: Functional significance of coronary collateral vessels in patients with acute myocardial infarction: Relation to pump performance, cardiogenic shock and survival. *Am J Cardiol* **1976**;37:345-351

Williams RS, Schaible TF, Bishop T, Morey M: Effects of endurance training on cholinergic and adrenergic receptors of rat heart. *J Moll Cell Cardiol* **1984**;16:395-403

Williams FM, Rochette L, Kane KA, Parratt JR: Effects of alterations in sympathetic nervous activity on the severity of reperfusion-induced arrhythmias in anesthetised rats. *J Cardiovasc Pharmacol* **1987**;10:555-561

Willich SN: Epidemiologic studies demonstrating increased morning incidence of sudden cardiac death. *Am J Cardiol* **1990**;66:15G-17G

Will-Shahab L, Krause EG, Bartel S, et al: Reversible inhibition of adenylate cyclase activity in the ischemic myocardium. *J Cardiovasc Pharmacol* **1985**;7(suppl 5):S23-S27

Wince LC, Hugman LE, Chen WY, et al: Effect of dietary lipids on inotropic responses of isolated rat left atrium: attenuation of maximal responses by an unsaturated fat diet. *J Pharmacol Exp Ther* **1987**;241:838-845

Wise BC, Shoji M, Kuo JF: Decrease or increase in cardiac muscarinic cholinergic receptor number in rats treated with methacholine or atropine. *Biochem Biophys Res Commun* **1980**;92:1136-1142

Wollenberger A, Shahab L: Anoxia-induced release of noradrenaline from the isolated perfused heart. *Nature* **1965**;207:88-89

Wood DA, Butler S, Riemersma RA, et al: Adipose tissue and platelet fatty acids and coronary heart disease in Scottish men. *Lancet* **1984**;i:117-121

Wood DA, Riemersma RA, Butler S, et al: Linoleic and eicosapentaenoic acids in adipose tissue and platelets and risk of coronary heart disease. *Lancet* **1987**;i:178-183

Yamada KA, Saffitz JE, Corr PB: Role of alpha- and beta- adrenergic receptor stimulation in the genesis of arrhythmias during myocardial ischaemia. *Eur Heart J* **1986**;7(suppl A):85-90

Yamada KA, Heathers GP, Pogwizd SM, Corr PB: Sympathetic influences on arrhythmogenesis in the ischemic heart. in Refsum H, et al (eds): *Heart & Brain,* Springer-Verlag, Berlin, **1988**;pp79-97

Yamaguchi N, de Champlain J, Nadeau RA: Regulation of norepinephrine release from cardiac sympathetic fibers in the dog by presynaptic α- and β-receptors. *Circ Res* **1977**;41:108-117

Yamaguchi N, Kimura T, Lamontagne D, et al: Occlusion time dependency of regional noradrenaline release and cardiac arrhythmias during reperfusion of acutely ischaemic heart in the dog *in vivo*. *Cardiovasc Res* **1990**;24:688-696

Yoon MS, Han J, Tse WW, et al: Effects of vagal stimulation, atropine, and propranolol on fibrillation threshold of normal and ischemic ventricles. *Am Heart J* **1977**;93:60-65

Zimmermann H: Cholinergic Synaptic Vesicles. in Wittaker VP (ed): *The Cholinergic Synapse.* Handbook of Experimental Pharmacology Vol. 86 Springer-Verlag, Berlin, **1988**;pp349-375

Zipes DP, Levy MN, Cobb LA, et al: Task Force 2: Sudden cardiac death: Neural-cardiac interactions. *Circulation* **1987**;76(suppl 1):I-202-I-207

Zipes DP: Influence of myocardial ischemia and infarction on autonomic innervation of heart. *Circulation* **1990**;82:1095-1105

Zuanetti G, de Ferrari GM, Priori SG, et al: Protective effect of vagal stimulation on reperfusion arrhythmias in cats. *Circ Res* **1987**;61:429-435