The G. L. Brown Prize Lecture

Nitric oxide and the autonomic regulation of cardiac excitability

David J. Paterson*

University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK

Contents	**Page**

Introduction

Successful communication between the two limbs of the autonomic nervous system has been inextricably linked to our evolution and survival during physiological stress (e.g. exercise, fight-and-flight response). This communication is particularly important in the heart. Abnormalities in sympathetic and parasympathetic signalling have been implicated in the aetiology and progression of cardiac dysfunction. Measurement of autonomic activity is a good independent indicator of clinical outcome (La Rovere & Schwartz, 1997), and a slowed heart rate recovery immediately after exercise is a powerful predictor of mortality (Cole *et al.* 1999). Impaired regulation of cardiac autonomic activity may therefore not only mirror cardiac dysfunction but actively contribute to the aetiology of the dysfunction. This lecture will touch on some of the factors that may be important in peripheral cardiac autonomic regulation during physiological and patho-physiological stress.

High intensity exercise presents one of the major chemical challenges to the myocardium (arterial plasma potassium, 8 mM, arterial pH 6.9, 15-fold increase in circulating catecholamines with local cardiac release of noradrenaline estimated at 0.5–1 μM; for review see Paterson, 1996). Protection of the heart from the chemical consequences of strenuous exercise or the mechanism by which they might trigger exercise-induced arrhythmia (Tofler *et al.* 1990; Mittleman *et al.* 1993) is not firmly established, although there are clearly critical interactions among cardiac autonomic transmitters and metabolites/ions released from contracting muscle (Paterson, 1996). Maintaining cardiac performance during exercise appears to be dependent on the cardiac autonomic nervous system and the by-products of muscle contraction interacting in such a way that each offsets the other's potentially harmful action on the ventricle. Activation of the sympathetic nervous system, and non-adrenergic hormones (e.g. angiotensin II; Ryan & Paterson, 1996), can minimise the harmful effects of simulated exercise-induced hyperkalaemia and acidosis at the level of the single ventricular myocyte (Paterson *et al.* 1993), isolated perfused heart (Paterson *et al.* 1992; Leitch & Paterson, 1994) and whole animal (Paterson *et al.* 1992; O'Neill *et al.* 1993; O'Neill & Paterson, 1995). Stimulation of the inward calcium current in ventricular cells is essential for propagation of the action potential in raised extracellular potassium Tyrode solution (Paterson *et al.* 1993), and this probably accounts for the protective effect of sympathetic activation. However, 'exercise-induced' hyperkalaemia may also be beneficial in preventing the potential pro-arrhythmogenic action of high sympathetic activity on ventricular cells by activating the inwardly rectifying potassium current $I_{K1,}$ thereby minimising a prolonged action potential duration and the

* Email: david.paterson@physiol.ox.ac.uk

likelihood of after-depolarisations caused by adrenergic activation (O'Neill & Paterson, 1995; O'Neill, 1995; Paterson, 1996).

Cardiac sympathetic imbalance and arrhythmia

Although high adrenergic tone and hyperkalaemia are generally well tolerated by the heart during exercise (Paterson, 1996), regional cardiac ischaemia and focal sympathetic imbalance breaks down this tolerance during simulated 'chemical exercise' (O'Neill *et al.* 1995, 1997). Cardiac sympathetic imbalance can cause triggered automaticity that results in hypotension (Podzuweit *et al.* 1989; Nash *et al.* 1998a, 1999, 2001; see Fig. 1). Processes underlying this arrhythmia can be replicated using a 2-dimensional sheet of 256 by 256 resistively coupled ventricular cells, where calcium handling is abnormally high in the central region of the network (Fig. 2). Analysis of the ionic behaviour from a single cell model with upregulated Ca^{2+} conductance reveals that

abnormal activation is a consequence of enhanced Na^+–Ca^{2+} exchange which leads to high intracellular sodium initiating spontaneous depolarisation (Fig. 3). This probably resulted in a new pacemaker developing at the site of high adrenergic tone since the earliest epicardial activation always occurred at the site of the infusion (see Fig. 1; Nash *et al.* 2001). This relatively simple mathematical simulation illustrates the potential power of an integrative experimental-computational approach to elucidate cellular mechanisms underlying arrhythmogenesis.

Interventions that reduce spatial inhomogeneities of intracellular calcium in the ventricle may prevent focal adrenergic-induced arrhythmia. Experimentally, the arrhythmia in Fig. 1 is abolished by propranolol and vagal stimulation (O'Neill *et al.* 1995; Nash *et al.* 2001). It has been known for some time that cholinergic activation exerts an anti-arrhythmic effect on the ventricle, especially against a
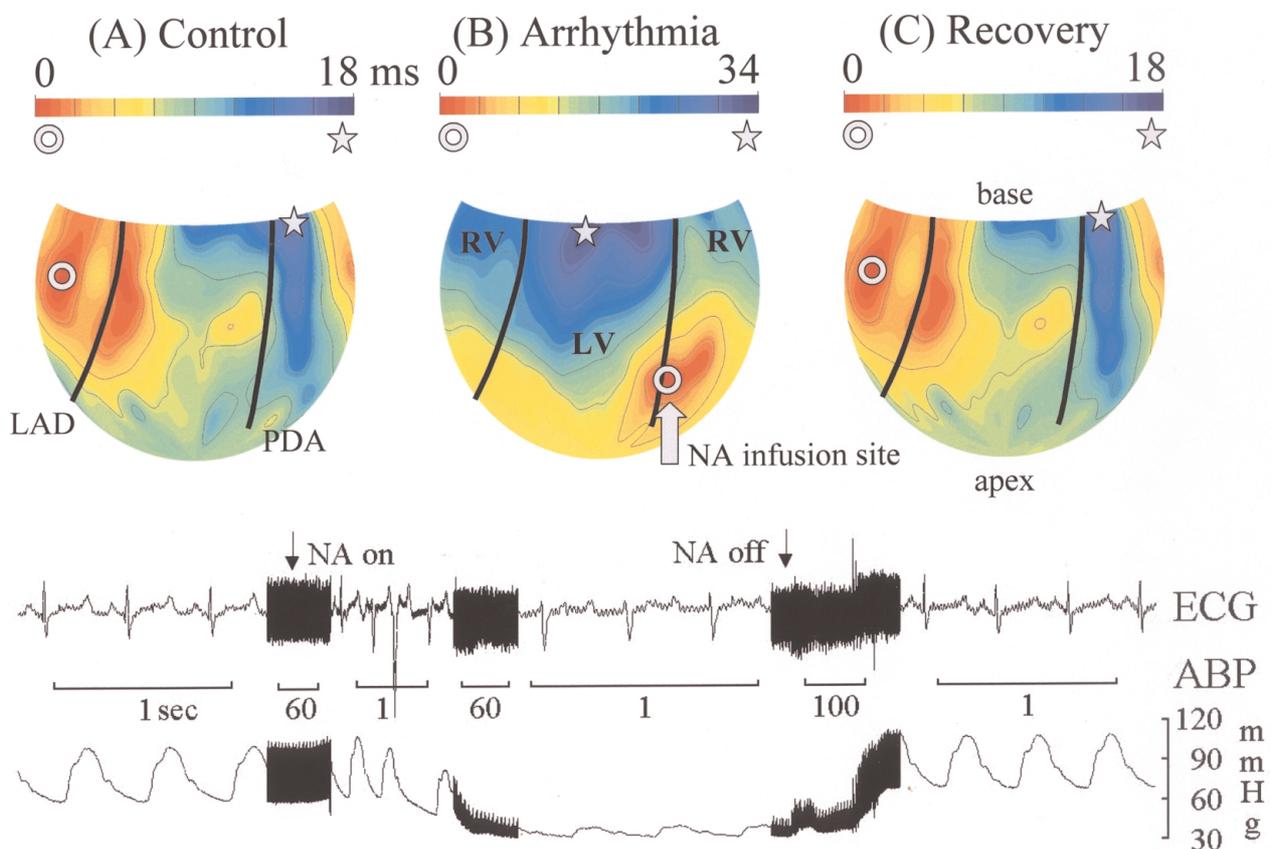
Figure 1

Epicardial activation sequences superimposed on 2-dimensional mathematical model of the ventricles during normal sinus rhythm (*A*), noradrenaline (NA)-induced ventricular arrhythmia (*B*) and recovery (*C*). Earliest epicardial activation (circles) shifted from the apical regions of the right ventricular (RV) free wall during control conditions to the NA infusion site (thick arrow), near the apex of the left ventricular (LV) free wall, during the arrhythmia. Latest epicardial activation (star) occurred adjacent to the posterobasal portions of the interventricular septum under control conditions, but moved to the posterobasal region of the LV free wall during the arrhythmia. At the onset of the arrhythmia, the QRS complex of the ECG inverted and activation time virtually doubled; this was immediately followed by a dramatic decrease in the arterial blood pressure (ABP). All changes were fully reversed after the infusion was stopped. Thick black lines denote the left anterior descending (LAD) and posterior descending (PDA) coronary arteries (modified from Nash *et al.* 1998b, 2001).

background of high sympathetic stimulation (Vanoli *et al.* 1991; Stramba Badiale *et al.* 1991; Hull *et al.* 1994). This is thought to occur through the indirect effect of muscarinic receptor activation decreasing L-type calcium currents ($I_{Ca,L}$) via inhibition of the adenylate cyclase–cyclic AMP system (Hartzell, 1988), and via activation of the nitric oxide–cyclic GMP pathway (Fig. 4) (Han *et al.* 1994, 1995, 1996, 1998). Whether the anti-arrhythmic effects of vagal activation against sympathetic-induced arrhythmias is mediated via an nitric oxide (NO)–cGMP-dependent pathway has not been established.

Endogenous NO is a fundamental intra/intercellular signalling molecule in the cardiovascular system (R. F. Furchgott, L. J. Ignarro & F. Murad; 1998 Nobel Prize in Medicine or Physiology). It is an important mediator of endothelial control of vascular tone, regulation of ATP production and cardiac contractile performance (e.g. Kelly *et al.* 1996; Shah & MacCarthy, 2000). Until recently little was known about its role as a signalling molecule in the autonomic control of cardiac excitability in the peripheral nervous system. However, its overall importance in this control is controversial, with opinion ranging from no effect through to mediation of response (see Balligand, 1999; Vandecasteele *et al.* 1999; Hare & Stamler, 1999).

Nitric oxide–cGMP pathway and the cholinergic modulation of cardiac excitability

In most species including man, endothelial nitric oxide synthase (eNOS) is expressed in cardiac myocytes from the atria, atrio-ventricular node and ventricle (Kojda *et al.* 1997), whereas neuronal NOS (nNOS) has been identified in both

(A) Control activation sequence
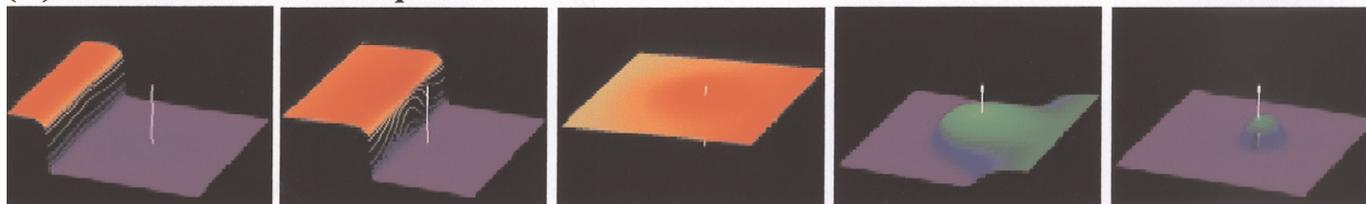
(B) Early indication of automaticity
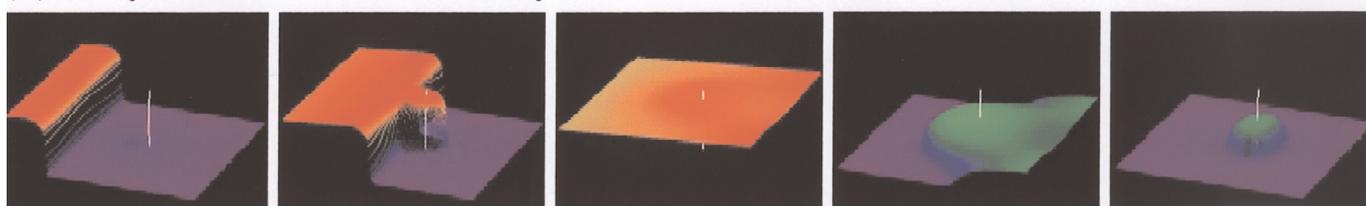
(C) Established automaticity
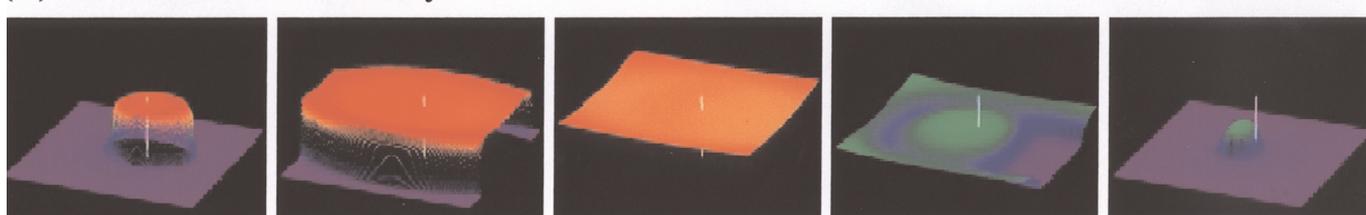
Figure 2

Simulation of the activation sequence using a 2-dimensional mathematical model of ventricular myocardium, for which the central region had high adrenergic tone. Cellular transmembrane ionic currents were incorporated into an arrangement of 256 by 256 resistively coupled myocytes. The L-type calcium channel conductance and the sarcoplasmic reticulum calcium-ATPase uptake current were elevated by a factor of five at the centre of the tissue sheet (white vertical marker). *A*, the control spread of activation proceeded from left to right, where red and indigo represent regions of depolarised and repolarised tissue, respectively. The central cells were last to repolarise, since the plateau phase of their action potentials was extended due to the localised inhomogeneities in the calcium currents. *B*, triggered automaticity became apparent nine cycles later, when the central region spontaneously depolarised just prior to the arrival of the control activation sequence. *C*, a further seven cycles later, the central tissue was the dominant pacemaker and completely captured the activation sequence. The full animation may be viewed at the internet address: http://www.physiol.ox.ac.uk/~djp/NEarrhythmia/2Dnetsim.mpg (from Nash *et al.* 2001).

cholinergic and sympathetic nerve terminals (Klimaschewski *et al.* 1992; Tanaka *et al.* 1993). Balligand *et al.* (1993) first reported that the NOS inhibitor N^{G}-monomethyl-L-arginine (L-NMMA) and methylene blue blocked the negative chronotropic effects of cholinergic agonists in spontaneously beating rat neonatal myocytes. This idea was supported by Han *et al.* (1994, 1996), who showed that inhibition of NOS could prevent cholinergic inhibition of $I_{Ca,L}$ without affecting the atrial muscarinic-activated K^{+} current, $I_{K,ACh}$, in adrenergically pre-stimulated sino-atrial node cells and atrio-ventricular node cells. Moreover, this inhibition was absent in eNOS knockout mice (Han *et al.* 1998) which led them to speculate that NO played an obligatory role in the autonomic control of heart rate. However, others failed to confirm this result (Vandecasteele *et al.* 1998), and more recently a normal

response in eNOS knockout mice to cholinergic and β-adrenergic activation of the inward calcium current has been reported (Vandecasteele *et al.* 1999; Belevych & Harvey, 2000). Some of the reasons for these differences have been discussed in detail elsewhere (Balligand, 1999). In addition, there could be some redundancy of NOS expression where nNOS might substitute for eNOS, as it does in blood vessels (Meng *et al.* 1998), and compensate for the removal of one of the constitutive NOS genes.

Functional data using nNOS inhibitors and non-isoform specific NOS inhibitors are as controversial as the cellular data. Conlon & Kidd (1999) reported that inhibition of nNOS caused a dramatic reduction in vagally mediated bradycardia in the adult ferret and guinea-pig *in vivo*, whereas my group has reported no significant effect in the young guinea-pig *in*
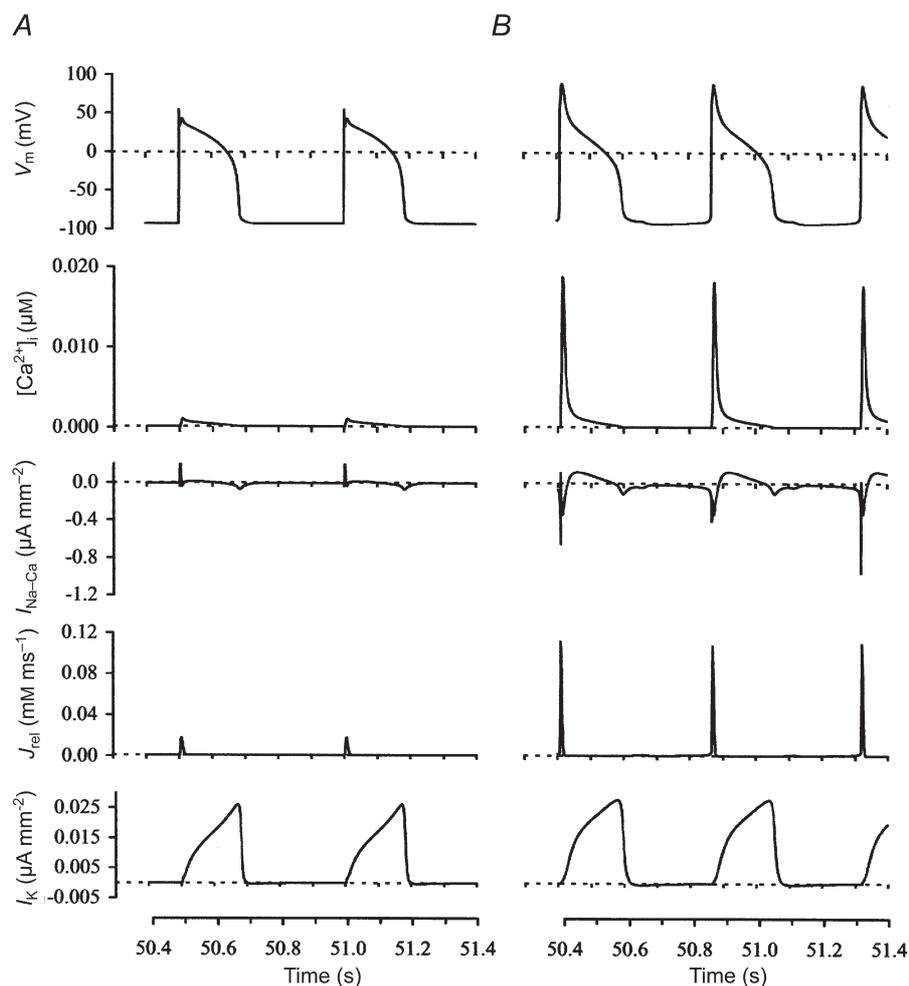
Figure 3

A computational model was used to quantify the differences in ventricular transmembrane potential (V_m) and ionic behaviour between the control state, for which the cell model was continuously paced at 2 Hz with a twice-threshold stimulus for 2 ms (*A*), and under high adrenergic tone (stimulated by a 5-fold elevation of the L-type calcium channel and SR calcium-ATPase uptake conductances), whilst paced as above for 50 s, after which time the stimulus was switched off and the cell exhibited automaticity (*B*). Upregulating these conductances caused the peak cytosolic calcium concentration ([Ca^{2+}]$_i$) and SR calcium release flux (J_{rel}) to markedly increase, compared to the control state, whilst the potassium current ($I_K = I_{Kr1} + I_{K2} + I_{Ks}$) remained similar. This intracellular calcium overload caused the Na^{+}–Ca^{2+} exchanger current (I_{Na-Ca}) to reverse and depolarise the cell, thus acting as an abnormal pacemaker with a spontaneous rate of approximately 2.2 Hz (modified from Nash *et al.* 2001).

Nitric Oxide Pathway

$$Ca^{2+}$$

(+)

calmodulin

NOS Inhibitors (-)

| nNOS | eNOS |

L-citrulline *NO Donors (+)*

L-arginine GTP→NO

GC inhibitor (-) GC

cGMP analogue (+) cGMP ← PDE / Ion channels / PKG
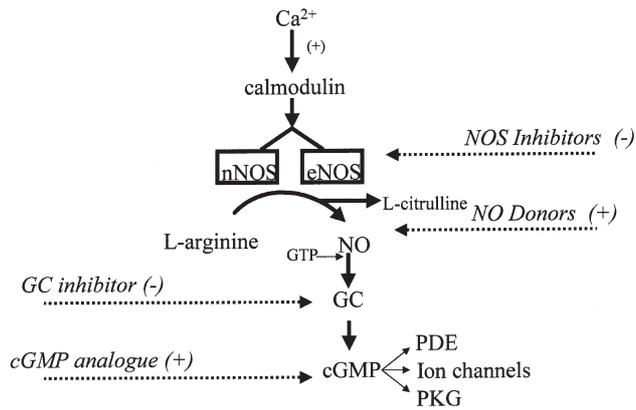
Figure 4

NO–cGMP pathway illustrating sites for pharmacological activation and inhibition.

vitro during vagal nerve stimulation, with or without adrenergic pre-stimulation (Sears *et al.* 1998*a*). Furthermore, no changes in absolute rate are seen in rabbits treated with NOS inhibitors, apart from slowed transients during vagal stimulation in adrenergically pre-stimulated hearts, suggesting a small modulatory role for NO in the indirect cholinergic control of heart rate (HR), i.e. accentuated antagonism (Sears *et al.* 1998*b*). The absence of a significant
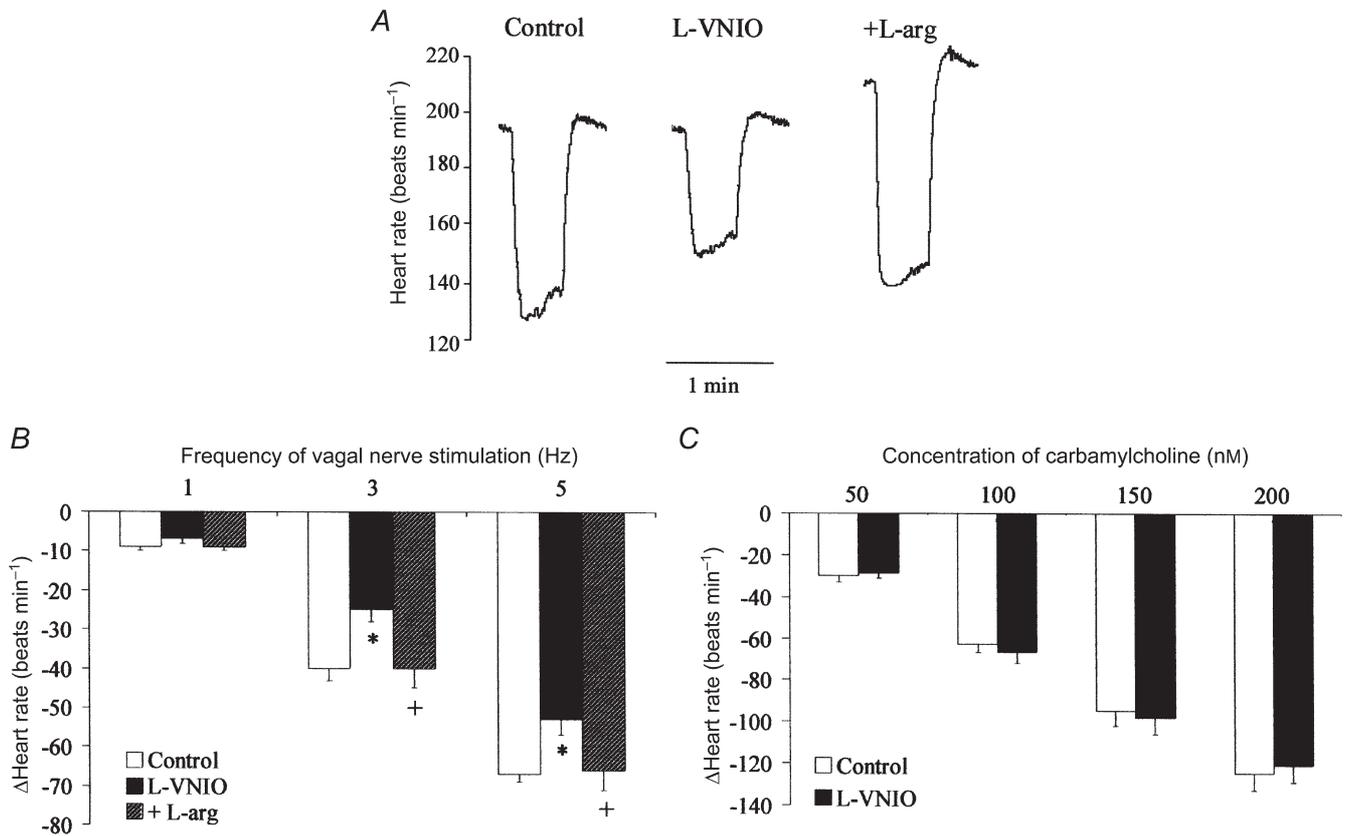
Figure 5

A, representative raw data trace showing the effect of neuronal NOS inhibition (100 μM L-vinyl-N5-(1-imino-3-butenyl)-L-ornithine (L-VNIO)) and its reversal with 1 mM L-arginine on the heart rate response (beats min^{-1}) to vagal nerve stimulation (5 Hz, 10 V, 1 ms pulse width, 30 s duration) in a double atrial–right vagal nerve preparation from an adult guinea-pig. *B*, frequency–response graph for the decrease in heart rate (beats min^{-1}) with right vagal nerve stimulation (1, 3 and 5 Hz) in atria from adult guinea-pigs *(n = 7)*. Neuronal NOS inhibition (100 μM L-VNIO) significantly attenuated (*$P < 0.05$) the negative chronotropic responses at 3 and 5 Hz and this effect was significantly reversed by 1 mM L-arginine ($^+P < 0.05$). *C*, nNOS inhibition (100 μM L-VNIO) had no effect on the heart rate response (beats min^{-1}) to cumulative doses of carbamylcholine in adult guinea-pig atria *(n = 5)* (modified from Herring *et al.* 2000*a*).

effect in the rabbit has also been observed by others (Liu *et al.* 1996; Conlon & Kidd, 1999). However, the discrepancy in response amongst species may be related to the developmental state of the tissue being studied. Herring *et al.* (2000*a*) have reported that whilst nNOS and guanylyl cyclase inhibitiors do not alter the HR response to vagal nerve stimulation in isolated young guinea-pig atria, these agents significantly inhibit the HR response to vagal stimulation in older animals (Figs 5 and 6) that express significantly more nNOS protein (Fig. 7). This suggests that the levels of NOS expression and NO bioavailability are directly coupled to developmental state. Conversely, by-passing endogenous NOS and amplifying NO production with NO donors or its second messenger with 8-bromo-cGMP enhances the drop in HR caused by vagal nerve stimulation *in vitro* and *in vivo*, irrespective of developmental state (Sears *et al.* 1999; Herring *et al.* 2000*a*).

When the stable analogue of ACh, carbamylcholine, is applied in the presence of NOS and guanylyl cyclase inhibitors (Figs 5 and 6) and NO activators, there is no effect on HR (Sears *et al.* 1999; Herring *et al.* 2000*a*), consistent with a presynaptic modulation of vagal neurotransmission. Indeed Neil Herring from my group (unpublished observation and Herring *et al.* 2000*b*) has recently shown that sodium nitroprusside facilitates the release of ^{3}H-acetylcholine during field stimulation in guinea-pig atria via a presynaptic guanylyl cyclase-dependent pathway. This probably results in cGMP stimulation of phosphodiesterase 3 (PDE3) that increases cAMP–protein kinase A (PKA)-dependent phosphorylation of N-type calcium channels. Inhibition of PDE3 with milrinone, of PKA with H89, and of N-type calcium channels with ω-conotoxin all inhibit the HR response to NO donors during vagal activation (Herring *et al.* 2000*b*). When these results are taken together, they support the idea the NO–cGMP-dependent pathway can activate neuronal Ca^{2+}
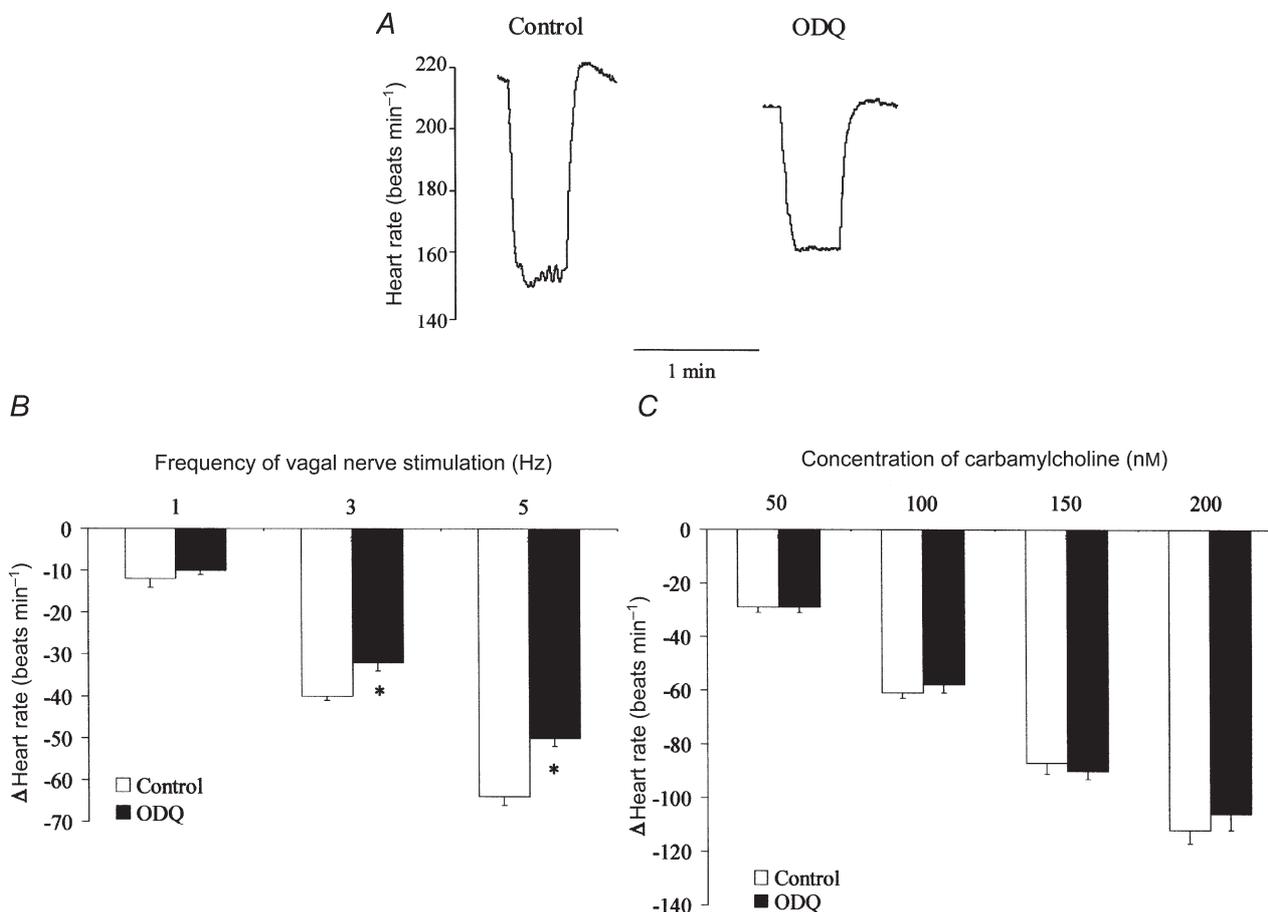
Figure 6

A, representative raw data trace showing the effect of guanylyl cyclase inhibition (10 μM 1H-(1,2,4)-oxadiazolo-(4,3-a)-quinoxalin-1-one (ODQ)) on the heart rate response (beats min^{-1}) to vagal nerve stimulation (5 Hz, 10 V, 1 ms pulse width, 30 s duration) in a double atrial–right vagal nerve preparation from an adult guinea-pig. *B*, frequency–response graph for the decrease in heart rate (beats min^{-1}) with right vagal nerve stimulation (1, 3 and 5 Hz) in atria from adult guinea-pigs *(n = 7)*. Guanylyl cyclase inhibition (with 10 μM ODQ) significantly attenuated the negative chronotropic responses at 3 and 5 Hz (*$P < 0.05$). *C*, guanylyl cyclase inhibition (10 μM ODQ) had no effect on the heart rate response (beats min^{-1}) to cumulative doses of carbamylcholine in adult guinea-pig atria *(n = 6)* (modified from Herring *et al.* 2000*a*).

channels that promote calcium entry and exocytoic release of neurotransmitter. In conscious nNOS knockout mice, baseline HR is high and the HR response to atropine is blunted compared to wild-type controls (Jumrussirikul *et al.* 1998). Moreover, in the isolated atria–vagal preparation from the nNOS$^{-/-}$ mouse, the HR response to vagal nerve stimulation, but not to carbamylcholine, is reduced compared to wild-type litter mates. This further suggests that neuronally generated NO can modulate ACh release during vagal activation (Choate *et al.* 2000). In humans, NO donors have been shown to increase the high frequency component of heart rate variability, an index of cardiac vagal tone (Chowdhary *et al.* 2000), although others do not see this (Hogan *et al.* 1999*b*).

The NO–cGMP pathway is not the only modulator of transmitter release and HR during vagal activation. Inhibition of sulphonylurea-sensitive K$^+$ channels (K$_{ATP}$ channels) also facilitates the release of ACh (Fabiani & Story, 1995) and bradycardia during vagal nerve stimulation (Almond & Paterson, 2000). In mesenteric arteries NO hyperpolarises the cell membrane by opening K$_{ATP}$ channels, whereas dilatation in coronary arterioles by NO donors or 8-bromo-cGMP is unaffected by inhibition of K$_{ATP}$ channels (Hein & Kuo, 1999). Consistent with this latter observation, NO donors or 8-bromo-cGMP further enhance the HR response to vagal nerve stimulation in the presence of a maximal inhibiting concentration of sulphonylurea, suggesting that the two pathways can act independently (Almond & Paterson, 2000).

NO is tonically released in the heart, reaching concentrations of 1–3 μM during diastole (Pinsky *et al.* 1997). In addition to its presynaptic action, NO has a significant postsynaptic action via ACh–M$_2$ receptor coupling to eNOS (Balligand *et al.* 1993; Balligand, 1999). NO-sensitive neurons are also present in stellate and intrinsic cardiac ganglia. These neurons can generate NO and increase beating rate when co-cultured with cardiac myocytes (Horackova *et al.* 1995; Armour *et al.* 1995). NOS inhibition causes a small bradycardia (Kojda *et al.* 1997; Musialek *et al.* 1999) and eNOS knockout mice have a lower basal HR that is independent of high blood pressure (Shesely *et al.* 1996). This suggests that NO directly stimulates basal HR. Musialek *et al.* (1997, 1999) found that low doses of NO donors or 8-bromo-cGMP increases spontaneous beating in isolated guinea-pig atria and rabbit sino-atrial node cells by activation of the hyperpolarisation inward current I_f. Functionally, the increase in HR caused by NO donors is independent of baroreflex activation since the rate response is present in isolated working rabbit hearts (with pre-load and after-load held constant; Hogan *et al.* 1999*a*), cardiac

autonomically denervated and β-blocked rabbits and pigs (Hogan *et al.* 1999*a*; Musialek *et al.* 2000), and in humans where blood pressure has been held constant with an infusion of phenylephrine (Hogan *et al.* 1999*b*). Presynaptically, NO may facilitate ACh release to decrease HR, but post-synaptically it may stimulate HR by activating I_f. This latter idea is supported by functional data that show inhibition of I_f with 2 mM caesium chloride causes a faster drop in the HR response to vagal nerve stimulation compared to control stimulations (Sears *et al.* 1998*b*). When all data are taken together, there may be an important interplay between $I_{Ca,L}$ and I_f that results in an opposing action (Sears *et al.* 1998*b*). NO may act to inhibit $I_{Ca,L}$ (Han *et al.* 1994) to slow the heart rate (Sears *et al.* 1996; Conlon *et al.* 1996; Elvan *et al.* 1997; Han *et al.* 1994), but also stimulate I_f (Musialek *et al.* 1997), thereby attenuating the rapid decrease in HR caused by cholinergic activation (Sears *et al.* 1998*b*). The interplay between $I_{Ca,L}$ and I_f by the NO–cGMP pathway may depend on the amount of background adrenergic activation since inhibition of NOS has little effect on ACh-induced inhibition of $I_{Ca,L}$ in pacemaking cells that have not been pre-stimulated with β-adrenergic agonists (Han *et al.* 1994).

Nitric oxide–cGMP pathway and the sympathetic modulation of cardiac excitability

Increases in cGMP caused by NO can also interfere with β-adrenergic signalling (Kelly *et al.* 1996). Depending on the species, cGMP can inhibit the action of β-receptor stimulated cAMP on pacemaking (Han *et al.* 1994) by catalysing the catabolism of cAMP through activation of phosphodiesterase activity or inhibiting calcium channel activity via the cGMP–PKG pathway (Han *et al.* 1995). In addition, NO can significantly affect cardiac sympathetic neurones. Inhibition of NO synthase with non-isoform and specific neuronal NOS inhibitors enhances noradrenaline release (Schwarz *et al.* 1995) and the HR and contractile responses to cardiac sympathetic nerve stimulation *in vitro* (Choate & Paterson, 1999; see Fig. 8) and *in vivo* (Elvan *et al.* 1997; Sears *et al.* 1998*a*), effects that are all reversed by excess L-arginine. Similarly, intracerebroventricular injection of a NOS inhibitor causes an increase in central sympathetic outflow (Togashi *et al.* 1992; Zanzinger *et al.* 1994, 1995) and an increase in baroreceptor-dependent renal sympathetic nerve activity (Matsumura *et al.* 1998). Inhibition of soluble guanylyl cyclase also enhances the HR response to peripheral sympathetic nerve stimulation, whereas NO donors and 8-bromo-cGMP depresses cardiac excitability during sympathetic nerve stimulation (Choate & Paterson, 1999; see Fig. 8). The HR response to bath-applied noradrenaline or isoprenaline is not affected by NOS inhibition, suggesting that

Figure 7

Western blot showing significantly lower levels of nNOS (120 kDa) protein in right atria isolated from three young compared to three adult guinea-pigs. nNOS was found at both 120 kDa and 160 kDa in guinea-pig fore-brain. Equal amounts of protein (200 μg) were loaded into each lane. (from Herring *et al.* 2000*a*).

-160kDa
-120kDa

1 2 3   1 2 3

Young right atria   Adult right atria   Forebrain Gut
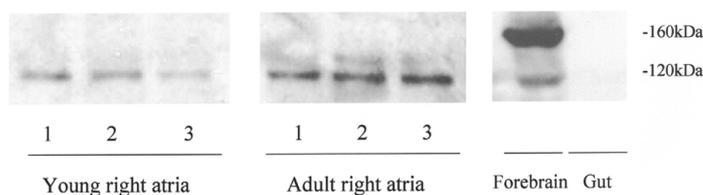
NO can act presynaptically to inhibit transmitter release via a cGMP-dependent pathway (Sears *et al.* 1998*a*; Choate & Paterson, 1999).

The intracellular signalling pathway underlying the pre-synaptic release of noradrenaline by NO has not been firmly established, although it could involve activation of cGMP-dependent phosphodiesterases that have an inhibitory effect on cAMP-dependent protein kinases to decrease calcium

entry and exocytotic release of transmitter (Choate & Paterson, 1999). In addition, the NO–cGMP pathway may modulate other protein kinase-activated ion channels such as K_{ATP} channels. Activation of these channels inhibits NA release (Oe *et al.* 1999) and the HR response to sympathetic nerve stimulation *in vitro* (Mohan & Paterson, 2000). The response to sulphonylureas is not mimicked by bath-applied noradrenaline, suggesting a presynaptic action. In addition,
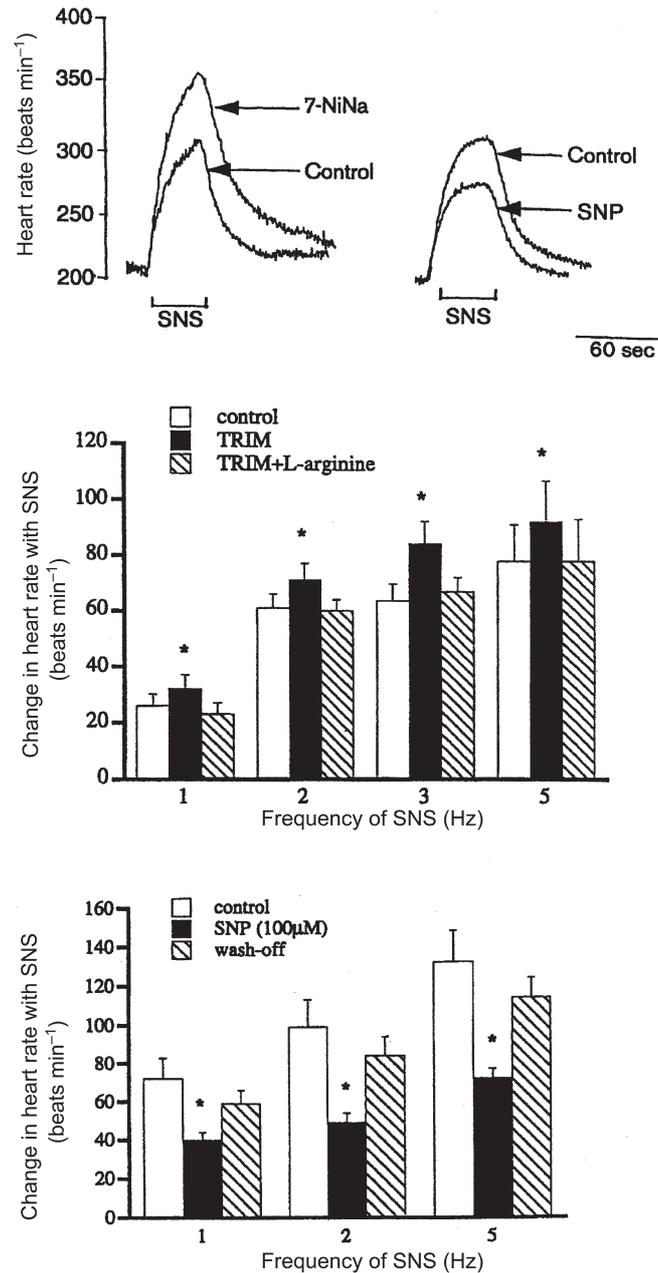
Figure 8

A, effect of NO on the heart rate response to sympathetic nerve stimulation (SNS; 3 Hz, 15 V, 30 s) in isolated guinea-pig atria. Note that inhibition of nNOS with 7-nitro indazole (7-NiNa, 100 μM) enhances the positive chronotropic effect of SNS whereas the nitric donor sodium nitroprusside (SNP, 100 μM) inhibits it. *B,* effect of the nNOS inhibitor 1-2-trifluromethylphenll imidazole (TRIM, 100 μM; *n* = 6) on the heart response to SNS and the addition of excess L-arginine (1 mM); *$*P$ < 0.05. *C,* effect of SNP (*n* = 6) and its wash-off on the heart response to SNS; *$*P$ < 0.05 (modified from Choate & Paterson, 1999).

modulation of the HR response to sympathetic nerve stimulation by the NO–cGMP pathway and by sulphonylurea-sensitive pathways appear to be independent of each other in the isolated guinea-pig atria (Mohan & Paterson, 2000).

Functional significance of nitric oxide in the autonomic regulation of cardiac excitability

Nitrovasoldilators release NO and are commonly used to test the sympathetic component of the baroreflex since they are thought to have no direct action on the heart and the sympathetic nervous system. However, the appropriateness of their use has recently been questioned (Casadei & Paterson, 2000), given that NO can directly stimulate pacemaking via I_f (Musialek *et al.* 1997), inhibit both peripheral (Schwarz *et al.* 1995; Choate & Paterson, 1999) and central sympathetic neurotransmission (Togashi *et al.* 1992; Zanzinger *et al.* 1994, 1995) and interfere with downstream β-adrenergic signalling (Kelly *et al.* 1996). Therefore any quantitative interpretation of the baroreflex using nitrovasodilators must now take into account the extravascular effects of these drugs (Casadei & Paterson, 2000).

Exercise training reduces the HR response to submaximal exercise. Recent work from our group has shown that a significant component of this response occurs via a decreased peripheral presynaptic sympathetic response. This is in part related to increased expression of nNOS in sympathetic ganglia, and presumably increased bioavailability of NO

inhibiting noradrenaline release, although other factors are clearly involved (Mohan *et al.* 2000). In pathophysiological states (e.g. myocardial ischaemia, sepsis, heart failure) that result in high cardiac adrenergic stimulation, activation of cardiac K_{ATP} channels and the NO–cGMP pathway may play an important complementary role in reducing local exocytotic release of noradrenaline to help protect the heart by reducing HR and to minimise oxygen consumption and cardiac work. Activation of these pathways *in vivo* could be of therapeutic value if specific pharmacology could be targeted at the heart. This is important in order to minimise the vascular action of these drugs activating sympathetic reflexes caused by the baroreflex.

Summary

Evidence is now emerging which indicates that the NO–cGMP pathway plays an important role in the sympatho-vagal modulation of cardiac excitability at various levels in the autonomic nervous system. Recent work shows that the NO–cGMP pathway can inhibit the HR response to cardiac sympathetic stimulation by reducing the presynaptic release of NA. Conversely, the NO–cGMP pathway facilitates the release of ACh and the HR response to vagal activation. Functionally, this response predominantly occurs via presynaptic modulation of transmitter release, but the pathway may also be coupled to muscarinic receptor activation and downstream inhibition of calcium currents in pacemaking cells. Factors that amplify NO signalling may
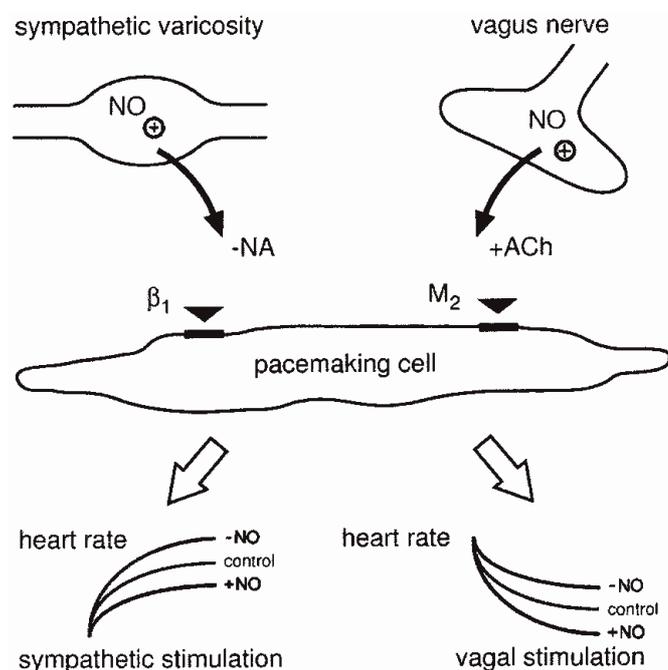
Figure 9

Summary pathway highlighting the functional consequences of the NO pathway on the cardiac autonomic modulation of HR. Amplification of NO–cGMP pathway (+NO) attenuates the HR response to sympathetic activation whereas it facilitates vagal-induced bradycardia. Conversely, inhibition of neuronal NO synthase (–NO) facilitates the HR response to SNS and attenuates the HR response to vagal nerve stimulation. These responses are not mimicked by bath-applied transmitter, suggesting that the dominant functional role of HR control is via presynaptic modulation of transmitter release.

promote sympathetic inhibition and facilitation of cholinergic activity. The reciprocal nature of this signalling pathway in the modulation of peripheral cardiac excitability is illustrated in Fig. 9.

ALMOND, S. C. & PATERSON, D. J. (2000). Sulphonylurea-sensitive channels and NO-cGMP pathway modulate the heart response to vagal nerve stimulation in-vitro. *Journal of Molecular and Cellular Cardiology* **32**, 2065–2073.

ARMOUR, J. A., SMITH, F. M., LOSIER, A. M., ELLENBERGER, H. H. & HOPKINS, D. A. (1995). Modulation of intrinsic cardiac neuronal activity by nitric oxide donors induces cardiodynamic changes. *American Journal of Physiology* **268**, R403–413.

BALLIGAND, J. L. (1999). Regulation of cardiac beta adrenergic response by nitric oxide. *Cardiovascular Research* **43**, 607–620.

BALLIGAND, J. L., KELLY, R. A., MARSDEN, P. A., SMITH, T. W. & MICHEL, T. (1993). Control of cardiac muscle cell function by an endogenous nitric oxide signaling system. *Proceedings of the National Academy of Sciences of theUSA* . **90**, 347–351.

BELEVYCH, A. E. & HARVEY, R. D. (2000). Muscarinic inhibitory and stimulatory regulation of the L-type Ca^{2+} current is not altered in cardiac ventricular myocytes from mice lacking endothelial nitric oxide synthase. *Journal of Physiology* **528**, 279–289.

CASADEI, B. & PATERSON, D. J. (2000). Should we still use nitrovasodilators to test baroreflex sensitivity? [editorial]. *Journal of Hypertension* **18**, 3–6.

CHOATE, J. K., DANSON, E. J. F., MORRIS, J. F. & PATERSON, D. J. (2000). Vagal modulation of heart rate in the nNos knock out mouse in-vitro. *Circulation* **102** (18), 5619 (abstract).

CHOATE, J. K. & PATERSON, D. J. (1999). Nitric oxide inhibits the positive chronotropic and inotropic responses to sympathetic nerve stimulation in the isolated guinea-pig atria. *Journal of the Autonomic Nervous System* **75**, 100–108.

CHOWDHARY, S., VAILE, J. C., FLETCHER, J., ROSS, H. F., COOTE, J. H. & TOWNEND, J. N. (2000). Nitric oxide and cardiac autonomic control in humans. *Hypertension* **36**, 264–269.

COLE, C. R., BLACKSTONE, E. H., PASHKOW, F. J., SNADER, C. E. & LAUER, M. S. (1999). Heart rate recovery immediately after exercise as a predictor of mortality. *New England Journal of Medicine* **341**, 1351–1357.

CONLON, K., COLLINS, T. & KIDD, C. (1996). Modulation of vagal actions on heart rate produced by inhibition of nitric oxide synthase in the anaesthetized ferret. *Experimental Physiology* **81**, 547–550.

CONLON, K. & KIDD, C. (1999). Neuronal nitric oxide facilitates vagal chronotropic and dromotropic actions on the heart. *Journal of the Autonomic Nervous System* **75**, 136–146.

ELVAN, A., RUBART, M. & ZIPES, D. P. (1997). No modulates autonomic effects on sinus discharge rate and AV nodal conduction in open-chest dogs. *American Journal of Physiology* **272**, H263–271.

FABIANI, M. E. & STORY, D. F. (1995). Effects of cromakalim, pinacidil and glibenclamide on cholinergic transmission in rat isolated atria. *Pharmacological Research* **32**, 155–163.

HAN, X., KOBZIK, L., BALLIGAND, J. L., KELLY, R. A. & SMITH, T. W. (1996). Nitric oxide synthase (NOS3)-mediated cholinergic modulation of Ca^{2+} current in adult rabbit atrioventricular nodal cells. *Circulation Research* **78**, 998–1008.

HAN, X., KUBOTA, I., FERON, O., OPEL, D. J., ARSTALL, M. A., ZHAO, Y. Y., HUANG, P., FISHMAN, M. C., MICHEL, T. & KELLY, R. A. (1998). Muscarinic cholinergic regulation of cardiac myocyte ICa-L is absent in mice with targeted disruption of endothelial nitric oxide synthase. *Proceedings of the National Academy of Sciences of the USA* **95**, 6510–6515.

HAN, X., SHIMONI, Y. & GILES, W. R. (1994). An obligatory role for nitric oxide in autonomic control of mammalian heart rate. *Journal of Physiology* **476**, 309–314.

HAN, X., SHIMONI, Y. & GILES, W. R. (1995). A cellular mechanism for nitric oxide-mediated cholinergic control of mammalian heart rate. *Journal of General Physiology* **106**, 45–65.

HARE, J. M. & STAMLER, J. S. (1999). NOS: modulator, not mediator of cardiac performance [news]. *Nature Medicine* **5**, 273–274.

HARTZELL, H. C. (1988). Regulation of cardiac ion channels by catecholamines, acetylcholine and second messenger systems. *Progress in Biophysics and Molecular Biology* **52**, 165–247.

HEIN, T. W. & KUO, L. (1999). cAMP-independent dilation of coronary arterioles to adenosine : role of nitric oxide, G proteins, and K_{ATP} channels. *Circulation Research* **85**, 634–642.

HERRING, N., GOLDING, S. & PATERSON, D. J. (2000a). Pre-synaptic NO-cGMP pathway modulates vagal control of heart rate in isolated adult guinea-pig atria. *Journal of Molecular Cellular Cardiology* **32**, 1795–1804.

HERRING, N., GOLDING, S. & PATERSON, D. J. (2000b). A pre-synaptic role for the NO-cGMP pathway in the vagal control of heart rate. *Autonomic Neuroscience* **82**, 74 (abstract).

HOGAN, N., CASADEI, B. & PATERSON, D. J. (1999a). Nitric oxide donors can increase heart rate independent of autonomic activation. *Journal of Applied Physiology* **87**, 97–103.

HOGAN, N., KARDOS, A., PATERSON, D. J. & CASADEI, B. (1999b). Effect of exogenous nitric oxide on baroreflex function in humans. *American Journal of Physiology* **277**, H221–227.

HORACKOVA, M., ARMOUR, J. A., HOPKINS, D. A. & HUANG, M. H. (1995). Nitric oxide modulates signaling between cultured adult peripheral cardiac neurons and cardiomyocytes. *American Journal of Physiology* **269**, C504–510.

HULL, S. S. JR, VANOLI, E., ADAMSON, P. B., VERRIER, R. L., FOREMAN, R. D. & SCHWARTZ, P. J. (1994). Exercise training confers anticipatory protection from sudden death during acute myocardial ischemia [see comments]. *Circulation* **89**, 548–552.

JUMRUSSIRIKUL, P., DINERMAN, J., DAWSON, T. M., DAWSON, V. L., EKELUND, U., GEORGAKOPOULOS, D., SCHRAMM, L. P., CALKINS, H., SNYDER, S. H., HARE, J. M. & BERGER, R. D. (1998). Interaction between neuronal nitric oxide synthase and inhibitory G protein activity in heart rate regulation in conscious mice. *Journal of Clinical Investigation* **102**, 1279–1285.

KELLY, R. A., BALLIGAND, J. L. & SMITH, T. W. (1996). Nitric oxide and cardiac function. *Circulation Research* **79**, 363–380.

KLIMASCHEWSKI, L., KUMMER, W., MAYER, B., COURAUD, J. Y., PREISSLER, U., PHILIPPIN, B. & HEYM, C. (1992). Nitric oxide synthase in cardiac nerve fibers and neurons of rat and guinea pig heart. *Circulation Research* **71**, 1533–1537.

KOJDA, G., KOTTENBERG, K. & NOACK, E. (1997). Inhibition of nitric oxide synthase and soluble guanylate cyclase induces cardiodepressive effects in normal rat hearts. *European Journal of Pharmacology* **334**, 181–190.

LA ROVERE, M. T. & SCHWARTZ, P. J. (1997). Baroreflex sensitivity as a cardiac and arrhythmia mortality risk stratifier. *Pacing and Clinical Electrophysiology* **20**, 2602–2613.

LEITCH, S. P. & PATERSON, D. J. (1994). Role of Ca^{2+} in protecting the heart from hyperkalemia and acidosis in the rabbit: implications for exercise. *Journal of Applied Physiology*. **77**, 2391–2399.

LIU, J. L., MURAKAMI, H. & ZUCKER, I. H. (1996). Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits. *American Journal of Physiology* **270**, R1361–1370.

MATSUMURA, K., ABE, I., TSUCHIHASHI, T. & FUJISHIMA, M. (1998). Central nitric oxide attenuates the baroreceptor reflex in conscious rabbits. *American Journal of Physiology* **274**, R1142–1149.

MENG, W., AYATA, C., WAEBER, C., HUANG, P. L. & MOSKOWITZ, M. A. (1998). Neuronal NOS-cGMP-dependent ACh-induced relaxation in pial arterioles of endothelial NOS knockout mice. *American Journal of Physiology* **274**, H411–415.

MITTLEMAN, M. A., MACLURE, M., TOFLER, G. H., SHERWOOD, J. B., GOLDBERG, R. J. & MULLER, J. E. (1993). Triggering of acute myocardial infarction by heavy physical exertion. Protection against triggering by regular exertion. Determinants of Myocardial Infarction Onset Study Investigators [see comments]. *New England Journal of Medicine* 329, 1677–1683.

MOHAN, R. M., CHOATE, J. K., GOLDING, S., HERRING, N., CASADEI, B. & PATERSON, D. J. (2000). Peripheral pre-synaptic pathway reduces the heart rate response to sympathetic activation following exercise training: role NO. *Cardiovascular Research* **47**, 90–98.

MOHAN, R. M & PATERSON, D. J. (2000). Activation of sulphonylurea-sensitive channels and the NO-cGMP pathway decreases the heart rate response to sympathetic nerve stimulation. *Cardiovascular Research* **47**, 81–89.

MUSIALEK, P., LEI, M., BROWN, H. F., PATERSON, D. J. & CASADEI, B. (1997). Nitric oxide can increase heart rate by stimulating the hyperpolarization-activated inward current, I(f). *Circulation Research* **81**, 60–68.

MUSIALEK, P., NASH, M. P., THORNTON, J. M., CASADEI, B. & PATERSON, D. J. (2000). The nitric oxide donor sodium nitroprusside increases heart rate in the absence of changes in arterial blood pressure when applied topically to the sino-atrial node in the anaesthetised pig. *Journal of Physiology* **523.P**, 267*P*.

MUSIALEK, P., PATERSON, D. J. & CASADEI, B. (1999). Changes in extracellular ph mediate the chronotropic responses to L arginine. *Cardiovascular Research* **43**, 712–720.

NASH, M., THORNTON, J. M., BANNING, A. P. & PATERSON, D. J. (1998*a*). 3D epicardial activation sequence during a ventricular arrhythmia in the anaesthetised pig. *Journal of Physiology* **513.P**, 162*P*.

NASH, M., THORNTON, J. M. & PATERSON, D. J. (1998*b*). Characterisation of the epicardial electrical activation sequence during ventricular arrhythmia in the anaesthetised pig. *Journal of Physiology* **507.P**, 60–61*P*.

NASH, M. P., THORNTON, J. M., SEARS, C. E., VARGHESE, A., O'NEILL, M. & PATERSON, D. J. (2001). Ventricular activation during sympathetic imbalance and its computational reconstruction. *Journal of Applied Physiology* **90**, 287–298.

NASH, M., THORNTON, J. M., VARGHESE, A. & PATERSON, D. J. (1999). Electromechanical characterisation and computer simulation of a noradrenaline induced ventricular arrhythmia. *FASEB Journal* **13**, A1075 (abstract).

OE, K., SPERLAGH, B., SANTHA, E., MATKO, I., NAGASHIMA, H., FOLDES, F. & VIZI, S. (1999). Modulation of norepinephrine release by ATP-dependent K$^+$ channel activators and inhibitors in guinea-pig and human isolated right atrium. *Cardiovascular Research* **43**, 125–134.

O'NEILL, M. (1995). *Cardiovascular Regulation under Physiological Stress.* DPhil thesis, University of Oxford, UK.

O'NEILL, M., DORRINGTON, K. L. & PATERSON, D. J. (1993). Cardiac sympathetic nerve stimulation enhances cardiovascular performance during hyperkalaemia in the anaesthetized pig [published erratum appears in *Experimental Physiology* **78**, 719 (1993)]. *Experimental Physiology* **78**, 549–552.

O'NEILL, M. & PATERSON, D. J. (1995). Role of the sympathetic nervous system in cardiac performance during hyperkalaemia in the anaesthetized pig. *Acta Physiologica Scandinavica* **153**, 1–11.

O'NEILL, M., SEARS, C. E. & PATERSON, D. J. (1995). Effect of cardiac sympathetic and vagal stimulation on noradrenaline-induced ventricular tachycardia in the anaesthetised pig. *Journal of Physiology* **487**, 173–174 (abstract).

O'NEILL, M., SEARS, C. E. & PATERSON, D. J. (1997). Interactive effects of K$^+$, acid, norepinephrine, and ischemia on the heart: implications for exercise. *Journal of Applied Physiology*. **82**, 1046–1052.

PATERSON, D. J. (1996). Antiarrhythmic mechanisms during exercise. *Journal of Applied Physiology*. **80**, 1853–1862.

PATERSON, D. J., BLAKE, G. J., LEITCH, S. P., PHILLIPS, S. M. & BROWN, H. F. (1992). Effects of catecholamines and potassium on cardiovascular performance in the rabbit. *Journal of Applied Physiology* **73**, 1413–1418.

PATERSON, D. J., ROGERS, J., POWELL, T. & BROWN, H. F. (1993). Effect of catecholamines on the ventricular myocyte action potential in raised extracellular potassium. *Acta Physiologica Scandinavica* **148**, 177–186.

PINSKY, D. J., PATTON, S., MESAROS, S., BROVKOVYCH, V., KUBASZEWSKI, E., GRUNFELD, S. & MALINSKI, T. (1997). Mechanical transduction of nitric oxide synthesis in the beating heart. *Circulation Research* **81**, 372–379.

PODZUWEIT, T., BINZ, K. H., NENNSTIEL, P. & FLAIG, W. (1989). The anti-arrhythmic effects of myocardial ischaemia. Relation to reperfusion arrhythmias? *Cardiovascular Research* **23**, 81–90.

RYAN, D. M. & PATERSON, D. J. (1996). Restoration of cardiac contraction by angiotensin Ii during raised [K$^+$]$_O$ in the rabbit. *Acta Physiologica Scandinavica* **156**, 419–427.

SCHWARZ, P., DIEM, R., DUN, N. J. & FORSTERMANN, U. (1995). Endogenous and exogenous nitric oxide inhibits norepinephrine release from rat heart sympathetic nerves. *Circulation Research* **77**, 841–848.

SEARS, C. E., CHOATE, J. K. & PATERSON, D. J. (1998*a*). Effect of nitric oxide synthase inhibition on the sympatho-vagal contol of heart rate. *Journal of the Autonomic Nervous System* **73**, 63–73.

SEARS, C. E., CHOATE, J. K. & PATERSON, D. J. (1998*b*). Inhibition of nitric oxide synthase slows heart rate recovery from cholinergic activation. *Journal of Applied Physiology* **84**, 1596–1603.

SEARS, C. E., CHOATE, J. K. & PATERSON, D. J. (1999). NO-cGMP pathway accentuates the decrease in heart rate caused by cardiac vagal nerve stimulation. *Journal of Applied Physiology* **86**, 510–516.

SEARS, C. E. & PATERSON, D. J. (1996). Role of nitric oxide in the rate and contraction responses to acetylcholine following adrenergic stimulation in the isolated frog heart. *Journal of Physiology* **495.P**, 167*P*.

SHAH, A. M. & MACCARTHY, P. A. (2000). Paracrine and autocrine effects of nitric oxide on myocardial function. *Pharmacology and Therapeutics* **86**, 49–86.

SHESELY, E. G., MAEDA, N., KIM, H. S., DESAI, K. M., KREGE, J. H., LAUBACH, V. E., SHERMAN, P. A., SESSA, W. C. & SMITHIES, O. (1996). Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proceedings of the National Academy of Sciences of the USA* **93**, 13176–13181.

STRAMBA BADIALE, M., VANOLI, E., DE FERRARI, G. M., CERATI, D., FOREMAN, R. D. & SCHWARTZ, P. J. (1991). Sympathetic-parasympathetic interaction and accentuated antagonism in conscious dogs. *American Journal of Physiology* **260**, H335–340.

TANAKA, K., OHSHIMA, H., ESUMI, H. & CHIBA, T. (1993). Direct synaptic contacts of nitric oxide synthase-immunoreactive nerve terminals on the neurons of the intracardiac ganglia of the guinea pig. *Neuroscience Letters* **158**, 67–70.

TOFLER, G. H., STONE, P. H., MACLURE, M., EDELMAN, E., DAVIS, V. G., ROBERTSON, T., ANTMAN, E. M. & MULLER, J. E. (1990). Analysis of possible triggers of acute myocardial infarction (the MILIS study). *American Journal of Cardiology* **66**, 22–27.

TOGASHI, H., SAKUMA, I., YOSHIOKA, M., KOBAYASHI, T., YASUDA, H., KITABATAKE, A., SAITO, H., GROSS, S. S. & LEVI, R. (1992). A central nervous system action of nitric oxide in blood pressure regulation. *Journal of Pharmacological and Experimental Therapeutics* **262**, 343–347.

VANDECASTEELE, G., ESCHENHAGEN, T. & FISCHMEISTER, R. (1998). Role of the NO-cGMP pathway in the muscarinic regulation of the L-type Ca^{2+} current in human atrial myocytes. *Journal of Physiology* **506**, 653–663.

VANDECASTEELE, G., ESCHENHAGEN, T., SCHOLZ, H., STEIN, B., VERDE, I. & FISCHMEISTER, R. (1999). Muscarinic and beta-adrenergic regulation of heart rate, force of contraction and calcium current is preserved in mice lacking endothelial nitric oxide synthase. *Nature Medicine* **5**, 331–334.

VANOLI, E., DE FERRARI, G. M., STRAMBA BADIALE, M., HULL, S. S. JR, FOREMAN, R. D. & SCHWARTZ, P. J. (1991). Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction. *Circulation Research* **68**, 1471–1481.

ZANZINGER, J., CZACHURSKI, J. & SELLER, H. (1994). Inhibition of sympathetic vasoconstriction is a major principle of vasodilation by nitric oxide in vivo. *Circulation Research* **75**, 1073–1077.

ZANZINGER, J., CZACHURSKI, J. & SELLER, H. (1995). Inhibition of basal and reflex-mediated sympathetic activity in the RVLM by nitric oxide. *American Journal of Physiology* **268**, R958–962.

Acknowledgements