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NO-cGMP pathway increases the hyperpolarisation-activated current, I_f, and heart rate during adrenergic stimulation

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Abstract

Objectives: The role of the nitric oxide (NO)-cGMP pathway in the autonomic modulation of cardiac pacemaking is controversial and may involve an interplay between the L-type calcium current, I_{CaL}, and the hyperpolarisation activated current, I_f. We tested the hypothesis that following adrenergic stimulation, the NO-cGMP pathway stimulates phosphodiesterase 2 (PDE2) to reduce cAMP dependent stimulation of I_f and heart rate (HR). **Methods:** In the presence of norepinephrine (NE, 1 μM), the effects of the NO donor sodium nitroprusside (SNP) were evaluated in sinoatrial node (SAN)/atria preparations and isolated SAN cells from adult guinea pigs. **Results:** Contrary to our hypothesis, SNP (10 and 100 μM, $n=5$) or the membrane permeable cGMP analogue, 8Br-cGMP (0.5 mM, $n=6$) transiently increased HR by 5 ± 1, 12 ± 1 and 12 ± 2 beats/min, respectively. The guanylyl cyclase inhibitor 1*H*-(1,2,4)-oxadiazolo-(4,3-a)-quinoxalin-1-one (ODQ, 10 μM, $n=5$) abolished the increase in HR to SNP (100 μM) as did the I_f blockers caesium chloride (2 mM, $n=7$) and 4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino)-pyrimidinium chloride (ZD7288, 1 μM, $n=7$). Addition of SNP (10 μM) also transiently increased I_f in SAN cells ($n=5$). After inhibition of PDE2 with erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA, 10 μM, $n=5$), the increase in HR to SNP in the presence of NE was significantly augmented and maintained. RT-PCR analysis confirmed the presence of PDE2 in addition to cGMP inhibited PDE3 mRNA in central SAN tissue. **Conclusions:** These results suggest that during adrenergic stimulation, activation of the NO-cGMP pathway does not decrease HR, but has a transient stimulatory effect that is I_f dependent, and is limited in magnitude and duration by stimulation of PDE2.

Keywords: Sinus node; Heart rate (variability); Chronotropic agents; Adrenergic (ant)agonists; Nitric oxide

1. Introduction

The role of nitric oxide (NO) in the modulation of cardiac pacemaking mechanisms is controversial. Presynaptically, NO produced by neuronal nitric oxide synthase (nNOS) inhibits norepinephrine (NE) release [1] and the heart rate (HR) response to sympathetic nerve stimulation [2]. Conversely, in the cholinergic innervation of the heart, NO enhances vagal induced bradycardia [3–7] by facilitating acetylcholine release [8]. However, the postsynaptic actions of NO generated by muscarinic stimulation of sinoatrial node (SAN) endothelial NOS are less clear (compare Ref. [9] with Ref. [10]). Functionally the role of NO is complicated by the opposing action of NO decreasing L-type calcium current (I_{CaL}) [11] whilst increasing the hyperpolarisation-activated current (I_f) [12,13].

Independent of the autonomic nervous system, application of exogenous NO causes a marked tachycardia due to guanylyl cyclase-cGMP dependent stimulation of I_f in guinea pig SAN cells [12]. Similar results have been observed in the cardiac denervated rabbit [14] and in human subjects when arterial blood pressure is held constant [15]. NO increases HR via cGMP dependent inhibition of phosphodiesterase 3 (PDE3) to increase cAMP, mobilisation of intracellular calcium and also by a direct action of cGMP itself [16]. These well documented

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pathways all contribute to an increase in I_f [17,18]. However, inhibitors of protein kinase G or I_{CaL} have no effect on the tachycardia caused by NO [12,16].

Yoo et al. [19] have recently shown that although NO increases I_f in basal conditions, when cAMP is raised in the presence of a high concentration of isoproterenol, the NO donor sodium nitroprusside (SNP) may decrease I_f in rabbit SAN cells. This effect could be abolished using the non-isoform selective PDE inhibitor IBMX suggesting that cGMP stimulation of PDE2 decreases cAMP levels and I_f. We therefore tested two hypotheses. First, does the pathway proposed by Yoo et al. functionally translate to a decrease in HR during physiological levels of adrenergic stimulation? Second, does PDE2 play a role in mediating the HR changes to NO during adrenergic stimulation?

2. Methods

Experiments conformed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the Animals (Scientific Procedures) Act 1986 (UK) and were performed under British Home Office Project License PPL 30/1133.

2.1. Isolated guinea pig sinoatrial node/atrial preparation

Adult (400–500 g) female guinea pigs were killed by cervical dislocation and exsanguinated. The thorax was opened and ventricles removed so that heparinised Tyrode's solution (1000 U/ml) could be rapidly perfused into both atria. The heart was removed and placed in a dissecting dish with Tyrode's solution aerated with 95% O_2/5% CO_2 at room temperature. The atria were dissected and sutures (Ethicon 5/0 silk) were placed at the lateral edges of the two atria. The preparation was then transferred to a preheated (37±0.2°C), continuously oxygenated, water-jacketed organ bath containing 60 ml of Tyrode's solution. The atria were mounted vertically with the suture in the left atrium attached to a stainless steel hook and the right atrium attached to an isometric force transducer (HDE F30) connected to an amplifier. Before starting each protocol, the mounted atria were kept in Tyrode's solution for at least 60 min until their beating rate stabilised. The Tyrode's solution in the organ bath was replaced approximately every 20 min throughout each protocol. Data were acquired on a Power Macintosh 8500 computer using a Biopac Systems MP100 data acquisition system and Acqknowledge 3.5 software. Beating rate was triggered from contraction, and the signals displayed in real time. Data were stored on compact disk for off-line analysis.

2.2. Isolation of guinea pig sinoatrial node cells

SAN cells were isolated according to previously described techniques [20]. Briefly, the heart was rapidly removed and perfused by the Langendorff technique with a solution containing no calcium for 3 min (composition (mmol/l): NaCl 137, KCl 5, $NaHCO_3$ 12, glucose 5, sodium pyruvate 1, NaH_2PO_4 0.4, $MgCl_2$ 1, NaOH 1, EGTA 0.1, aerated with 95% O_2/5% CO_2 (pH 7.4, 36°C). This was subsequently replaced with a solution of the same composition lacking EGTA but containing 25 mg/50 ml collagenase (Type I, activity 172 U/mg, Worthington Biochemicals). Following collagenase perfusion for 12× the weight of the heart in grams in min (maximum exposure, 36 min), the atria were removed and further dissected to reveal the SAN region bordered by the crista terminalis, superior and inferior vena cava and inter-atrial septum. This region was cut into small strips (2×5 mm) perpendicular to the crista terminalis and dispersed in 2 ml of a solution high in potassium (composition (mmol/l): KCl 70, K_2ATP 5, $MgSO_4$ 5, K^+ glutamate 5, taurine 20, trisphosphocreatine 5, EGTA 0.04, succinic acid 5, KH_2PO_4 20, glucose 10, HEPES 5, pH 7.2 with KOH) and stored at 4°C for at least an hour before use.

Cells were allowed to settle to the base of a glass-bottomed flow chamber. This was placed on the stage of an inverted microscope (Leica DMIRB) and superfused with normal Tyrode at a flow rate of 3.5 ml/min. SAN cells were visualised using a ×100 oil immersion objective and those used in this study were either characteristically spindle or spider shaped, with faint striations, well defined membranes and regular spontaneous activity.

2.3. Electrophysiology

I_f was recorded from single SAN cells using the perforated-patch clamp technique [21] and an Axopatch 200B amplifier in voltage clamp mode (Axon instruments). Patch pipettes (3–5 MΩ) were filled with a solution containing (mmol/l): KCl 150, $MgCl_2$ 5, K_2ATP 1, HEPES 3, pH 7.2 with KOH. Amphotericin B (Sigma) was used at 240 mg/ml. Access resistance measured using 10 mV hyperpolarisation steps (20 ms in duration) from −40 mV every 30 s was allowed to stabilize for at least 5 min before both capacitance (cell and pipette, 25–45 pF) and the series resistance (20–40 mΩ) were compensated electronically (by at least 50%). Current signals were filtered at 1 kHz, recorded onto compact disc and analysed using pCLAMP software (Axon instruments). I_f current was elicited by step hyperpolarisations (2 s duration, 10 s apart) from a holding potential of −40 to −80 mV. This current has been shown to be completely blocked by the specific I_f blocker ZD7288 (which does not effect I_K) [22]. However, to rule out the possibility that there is any remaining I_K that will deactivate on hyperpolarisation from −40 mV, we measured I_{Ks} on depolarisation from −50 to

−40 mV and changes in the holding current at −40 mV on application of the I_{Kr} blocker E4031 (5 μM).

2.4. Solutions and drugs

The Tyrode solution contained (mmol/l) NaCl 120, KCl 4, $MgCl_2$ 2, $NaHCO_3$ 25, $CaCl_2$ 2, Na_2HPO_4 0.1 and glucose 11. The solution was aerated with 95% O_2/5% CO_2 (pH 7.4) and its temperature was continuously monitored (Digitron 1408-K gauge) and kept at 37±0.2°C.

SNP (10 and 100 μM, Sigma) was used as a NO donor that has previously been shown to produce maximal tachycardia in guinea pig sinoatrial node/atria preparations [12]. Unlike many NO donors, SNP has been shown not to cause cGMP independent effects on myocyte contractility [23]. 8Br-cGMP (0.5 mM, Sigma) was used as a membrane permeable analogue of cGMP that produces a similar degree of tachycardia in the same preparation [12]. These drugs were applied in the presence of norepinephrine (NE, Sigma) at 1 μM which produces a submaximal to maximal tachycardia based on dose response curves previously performed in this laboratory and is similar to NE concentrations reported in the synaptic cleft during sympathetic activation [24]. Control experiments showed that this concentration produced a tachycardia that was sustained for at least 10 min and we measured changes in HR to SNP or 8Br-cGMP from this stable baseline. To investigate the mechanism for the effects of NO in the presence of NE, 1*H*-(1,2,4)-oxadiazolo-(4,3-a)-quinoxalin-1-one (ODQ, 10 μM, 40-min incubation, Tocris Cookson, UK) was used to inhibit guanylyl cyclase. This concentration abolishes the response to the doses of SNP we use in a similar preparation [12] and is above that reported for inhibition of guanylyl cyclase activity produced by endogenous NO activity [25]. Erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA, 10 μM, Sigma) was used to inhibit PDE2 at a concentration that has previously been shown to be effective [26,27] without altering the HR response to NE [28]. Caesium chloride [29] (2 mM, Sigma) was used as a blocker of I_f although it may also alter Na^+/K^+ pump activity and block potassium channels at this and higher concentrations [29,30]. We therefore also used the structurally different and more specific I_f blocker 4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino)-pyrimidinium chloride (ZD7288, 1 μM, Tocris Cookson, UK). ZD7288 does not block potassium channels at the concentration used and affects the SAN action potential by slowing the rate of diastolic depolarisation consistent with a specific effect on I_f [22,31].

Drugs were dissolved in reagent grade water from an Elga purification system except ODQ and EHNA which were dissolved in dimethylsulfoxide (DMSO). A control experiment showed that DMSO at the concentrations used (<0.02%) did not affect baseline HR. Since SNP, 8Br-cGMP and ODQ are light sensitive, all experiments were carried out in a darkened room.

2.5. Total RNA extraction and RT-PCR

The central region of the guinea pig sinoatrial node was dissected and immediately frozen in liquid nitrogen. Total RNA was isolated from the tissue after homogenisation in an Eppendorf pestle and mortar (Anachem, UK) using Total RNA Extraction reagents (Ambion, USA). A 1-μg sample of RNA was reverse transcribed (RT) using the Expand Reverse Transcriptase System with oligo dT_{15} (Roche Diagnostics, UK). Oligonucleotide PCR primers were designed based on consensus sequences within published nucleotide sequences (GenBank database, NIH, USA) for PDE2 (forward: 5′-CCC ATC AAG AAC GAG AAC CAG GAG-3′ and reverse: 5′-GAA AAG GCG TGC ATC CAG TTG TGG-3′), PDE3 (forward: 5′-GAT ATA GGG ATA TTC CTT ATC ATA AC-3′ and reverse: 5′-TGC AGC AGC TGC GTG ATG ATT CTC-3′) and β-actin (forward: 5′-TTC AAC TCC ATC ATG AAG AAG TGT GAC GTG-3′ and reverse: 5′-CTA AGT CAT AGT CCG CCT AGA AGC ATT-3′). Primers were synthesised commercially by MWG Biotech, Germany. Roche Diagnostics, UK, supplied PCR enzymes and buffers. Reactions contained 2 μl cDNA from each RT reaction, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP mix, 20 pmol each primer, 1.0 U *Taq* DNA polymerase in 50 μl volumes with nuclease-free H_2O. The cycling parameters were 30 cycles of denaturation at 95°C for 30 s, primer annealing at 57°C for 30 s, primer extension at 72°C for 1 min, followed by one final extension at 72°C for 5 min in a DNA Engine thermal cycler with heated lid (MJ Research). Then 20 μl from each reaction were separated on a 2% Aquapor HR agarose (National Diagnostics, UK) gel containing 500 μg/ml ethidium bromide in TAE buffer (40 mM Tris/acetate, 1 mM EDTA) for 2 h at 40 V and DNA visualised under UV illumination.

2.6. Statistical analysis

Data are presented as mean±S.E.M. One-way repeated measures ANOVA followed by Tukey's post hoc analysis was used to evaluate the effect of an intervention. An unpaired Student's *t*-test was used to evaluate differences between groups. All data passed a normality test. Statistical significance was accepted at $P<0.05$.

3. Results

After the equilibration period, mean HR was 185±4 beats/min ($n=29$) in the spontaneously beating SAN/double atrial preparation.

3.1. The effect of NO-cGMP dependent pathway on HR and the hyperpolarisation activated current in the presence of adrenergic stimulation

A control experiment showed that NE produced a

sustained tachycardia of 109±10 beat/min above equilibration HR (n=5) over a 10-min period. Contrary to our hypothesis, in the presence of the new stable baseline with NE, SNP (n=5) at 10 and 100 μM produced a further transient increase in HR over a 10-min period that could be mimicked by 8Br-cGMP (n=6) as shown in Fig. 1. After application of NE and SNP or 8Br-cGMP we were able to return to equilibration HR after two changes of the bath solution over a period of ~20 min. In three experiments we repeated the control response to NE after this wash-off to show that it was still sustained and of the same magnitude.

In a separate set of experiments, the increase in HR to 100 μM SNP in the presence of NE was completely abolished by pre-incubating the preparation with ODQ (n=5) suggesting that the response to SNP was due to stimulation of guanylyl cyclase and production of cGMP.

To see if the transient increase in HR to SNP in the presence of NE was due to an increase in I_f, we repeated the experiment in the presence of the I_f blocker caesium chloride (n=7). Caesium chloride significantly reduced baseline HR (−55±3 beats/min) and the HR response to NE (from 96±4 (n=31) to 45±6 beats/min). In these conditions the HR response to SNP after NE was significantly reduced as shown in Fig. 2. However, caesium chloride may not be completely selective for I_f at this concentration (2 mM). We therefore repeated the experiment using the structurally different and more specific I_f blocker ZD7288 (n=7). ZD7288 was significantly more potent than caesium chloride at reducing baseline HR (−98±8 beats/min) as has been shown previously [32] and also reduced the HR response to NE (from 96±4 (n=31) to 58±9 beats/min). However, in these conditions the increase in HR to SNP in the presence of NE was abolished. To take into account the effects of caesium chloride or ZD7288 on baseline HR, the response to SNP after NE can be expressed as a percentage increase in HR from the respective baselines. This does not affect statistical significance and the percentage increase in HR to SNP in the presence of NE with ZD7288 is not significantly different from the response with ODQ (ΔHR control +4.4±0.4%, caesium chloride +2.1±0.4%, ZD7288 +1.3±0.5%, ODQ +0.4±0.3%). These results suggest

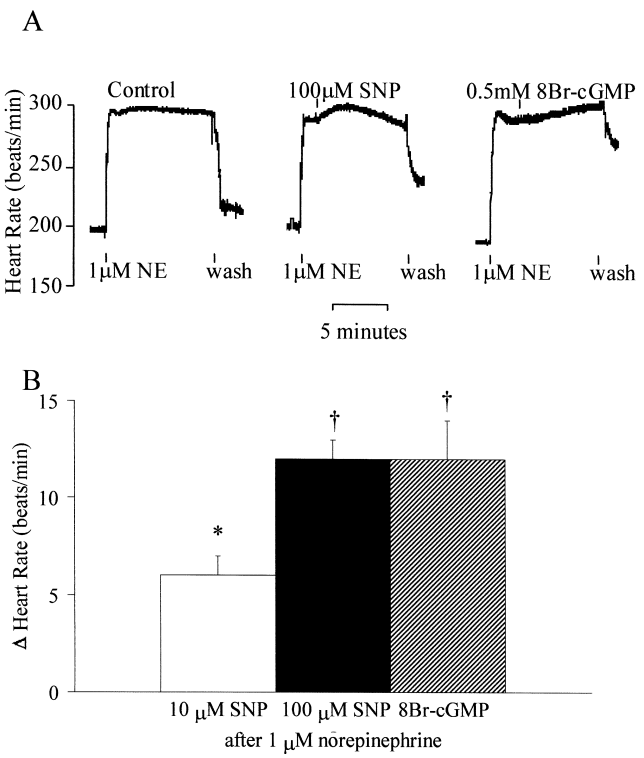

Fig. 1. SNP and 8Br-cGMP increase heart rate during adrenergic stimulation. Norepinephrine (NE, 1 μM) caused a sustained tachycardia over a 10-min period. SNP (10 μM, n=5) significantly increases (*P< 0.05 vs. NE control) heart rate during adrenergic stimulation. Higher concentrations of SNP (100 μM, n=5) and the membrane permeable analogue of cGMP, 8Br-cGMP (0.5 mM, n=6) produce a significantly larger effect ($^{\dagger}P$<0.05 vs. 10 μM SNP and NE control).

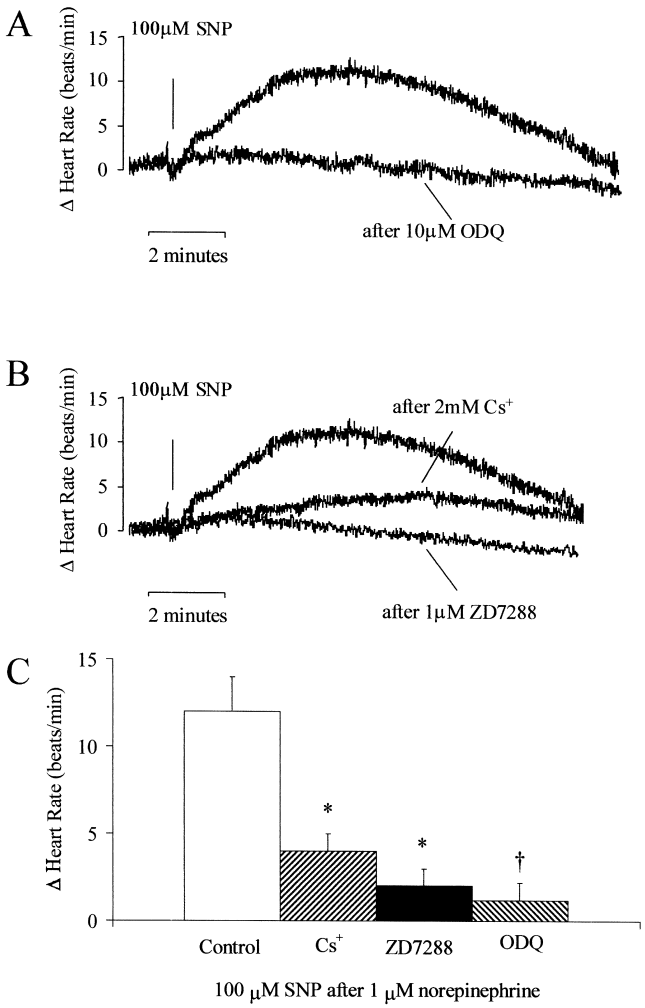

Fig. 2. Inhibitors of guanylyl cyclase and the hyperpolarisation-activated current, I_f prevent the increase in heart rate to SNP during adrenergic stimulation. The increase in heart rate to SNP (100 μM, n=5) during adrenergic stimulation (norepinephrine, 1 μM) is significantly reduced (*P<0.05, unpaired t-test) by the I blockers caesium chloride (2 mM, n=7) and ZD7288 (1 μM, n=7) as well as by the guanylyl cyclase inhibitor ODQ (10 μM, n=5) ($^{\dagger}P$<0.05 vs. Control and Cs^+, unpaired t-test).

that the increase in HR due to SNP in the presence of NE is mediated by a guanylyl cyclase dependent increase in I_f.

To test this possibility more directly we measured changes in I_f under similar conditions in isolated sinoatrial node cells from the guinea pig ($n=5$) using the permeabilised patch clamp technique. In the presence of NE, SNP (10 μM) also produced a transient but significant increase in the magnitude of I_f hyperpolarising from −40 to −80 mV as shown in Fig. 3. Depolarisation from −50 to −40 mV activated no I_{Ks} ($n=4$) and there was no change in holding current at −40 mV on application of the I_{Kr} blocker E4031 (−17±11 to −21±16 pA, $n=9$). This suggests that the I_f current we measure is not contaminated by deactivation of I_K on hyperpolarisation from −40 mV.

3.2. The role of cGMP stimulated phosphodiesterase 2 in the NO-cGMP modulation of HR during adrenergic stimulation

To see if the increase in HR to SNP was limited by cGMP dependent stimulation of PDE2, we repeated the experiment in the presence of EHNA ($n=5$). As can be seen in Fig. 4, the HR response to SNP (100 μM) was significantly augmented and maintained following inhibition of PDE2. These results suggest that NO activation of PDE2 limits the increase in cAMP and tachycardia to SNP in the presence of NE.

RT-PCR analysis of RNA isolated from tissue isolated from the central SAN region of a guinea pig showed the presence of PDE2 in addition to cGMP inhibited PDE3 (Fig. 5). An identical result was observed in tissue isolated from another guinea pig.

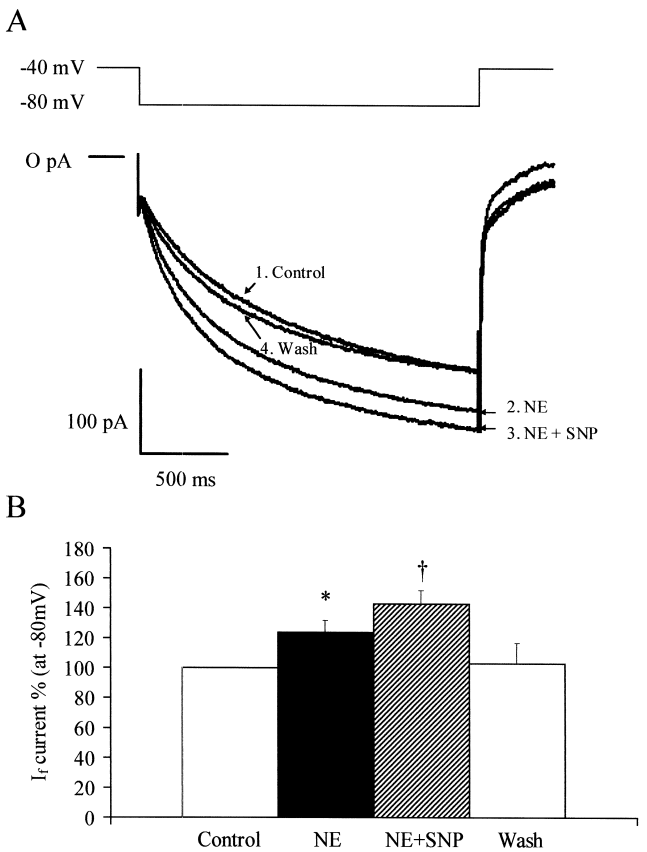

Fig. 3. SNP increases I_f during adrenergic stimulation in guinea pig sinoatrial node cells. Norepinephrine (NE, 1 μM, $n=5$) significantly (*$P<0.05$ vs. Control) increased the time dependent current I measured by hyperpolarisation voltage steps from −40 to −80 mV. However, SNP (10 μM) transiently increased I_f further in the presence of NE ($^{\dagger}P<0.05$ vs. NE and Wash).

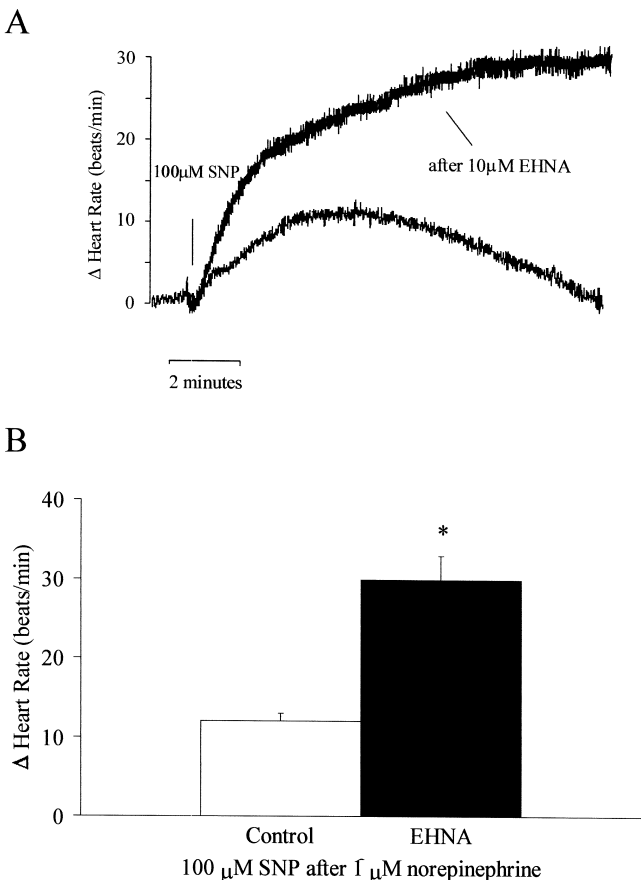

Fig. 4. Inhibition of phosphodiesterase 2 augments the increase in heart rate to SNP during adrenergic stimulation. The increase in heart rate to SNP (100 μM, $n=5$) during adrenergic stimulation (norepinephrine, 1 μM) is significantly (*$P<0.05$, unpaired *t*-test) augmented and maintained by the PDE2 inhibitor EHNA (10 μM, $n=5$).

4. Discussion

The results from this study suggest that: (i) in the presence of physiological levels of adrenergic stimulation, the NO-cGMP pathway causes a transient increase in HR due to cGMP dependent stimulation of I_f; and (ii) the magnitude and duration of the HR response is limited by stimulation of PDE2.

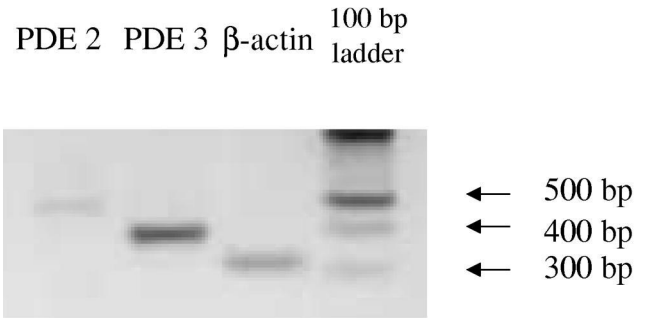

Fig. 5. Expression of phosphodiesterase isoforms in guinea pig central sinoatrial node tissue. Electrophoresis gel showing the PCR products (15 μl from a 50 μl reaction volume) for phosphodiesterase 2 (PDE2, 427 base pairs), PDE3 (374 base pairs) and β-actin (positive control, 310 base pairs) from RNA isolated from the central sinoatrial node tissue of a guinea pig. Equal amounts of cDNA from the same sinoatrial node were used in each reaction that underwent the same submaximal number of PCR cycles. Identical results were observed with central SAN tissue from another animal.

4.1. NO-cGMP dependent pathway increases I_f and HR during adrenergic stimulation

NO donors can increase HR independent of the autonomic nervous system [14,15] by cGMP dependent inhibition of PDE3 to increase cAMP levels, mobilisation of intracellular calcium or by a direct effect of cGMP itself [16]. This leads to stimulation of I_f in SAN cells and inhibitors of this current can abolish the tachycardia to a variety of NO donors [12,16]. Conversely, Yoo et al. [19] have reported that SNP may decrease I_f by cGMP dependent stimulation of PDE. We tested the hypothesis that a reduction in I_f during adrenergic stimulation would translate to a decrease in HR. Surprisingly, SNP at concentrations as high as 100 μM transiently increased HR. The effect of SNP was abolished by guanylyl cyclase inhibition and was mimicked by the membrane permeable analogue of cGMP 8Br-cGMP, suggesting that SNP was acting via release of NO and stimulation of guanylyl cyclase (rather than nitrosylation or generation of superoxide radicals caused by some NO donors [23]). The increase in HR to SNP was rapid whereas the tachycardia to 8Br-cGMP developed more slowly. This may be due to the chemical nature of the two reagents, NO released from SNP being a freely diffusible, highly reactive gas compared to 8Br-cGMP which is less able to diffuse across cell membranes and is not easily hydrolysed. In addition, 8Br-cGMP may act directly on I_f [17] rather than by modulating phosphodiesterase activity [33].

PDE2 may still play a role in shaping the HR response to NO during adrenergic stimulation as inhibition of PDE2 significantly augmented and sustained the increase in HR to SNP in these conditions. These results suggest that the transient nature of the response to SNP was due to activation of PDE2 that may limit and eventually reduce cAMP levels raised by NO dependent inhibition of PDE3. This effect may also eventually overcome the other stimulatory effects of NO on HR including mobilisation of intracellular calcium and direct actions of cGMP. RT-PCR revealed that PDE2 is present in central SAN tissue in addition to cGMP inhibited PDE3. Equal amounts of cDNA from the same SAN were used for each reaction that underwent the same submaximal number of PCR cycles. This methodology although only semi-quantitative, along with repetition of the result in a different SAN suggests that PDE3 is the more dominant isoform in this tissue as the PCR product of this isoform is of higher optical density than that of PDE2. The SAN tissue will contain fibroblasts and neural elements in addition to pacemaker cells so we cannot precisely determine the location of the PDE2 mRNA. However, the effect of PDE2 inhibition on the HR response to SNP suggests that the small amount of PDE2 mRNA we detect is probably from pacemaker cells. Pharmacological data also support a similar pattern of expression of PDE isoforms in SAN cells as PDE2 inhibition causes no change in baseline HR whilst the PDE3 inhibitor milrinone produces a substantial tachycardia [8]. A lower expression of PDE2 compared to PDE3 may explain why the stimulatory effect of SNP initially predominates even in the presence of adrenergic stimulation.

Two structurally different inhibitors of the hyperpolarisation-activated current I_f abolished the increase in HR to SNP. We also show that SNP causes a transient increase in the magnitude of I_f in the presence of similar levels of adrenergic stimulation in SAN cells from the guinea pig. We have previously shown that NO shifts the activation curve of I_f to more positive potentials [36] in guinea pig SAN cells, but in this study, as the effect of SNP was only transient, we were unable to perform a full I/V curve in these circumstances. The contrasting results reported by Yoo et al. [19] may be explained by several methodological differences. We used a submaximal to maximal concentration of the physiological agonist NE similar to that reported in the synaptic cleft during sympathetic activation [24] rather than a high concentration of isoproterenol. We also used the permeabilised rather than whole cell patch clamp technique. The use of the permeabilised patch clamp technique prevents diffusion of calcium buffers from the pipette solution into the cytosol that may occur with whole cell configuration. This is important as the positive chronotropic effect of NO donors is partly due to mobilisation of intracellular calcium [16]. Calcium can directly activate I_f [18] and recent work has shown it to be an important mediator in the control of cardiac pacemaking [40,42] and its adrenergic modulation [20].

4.2. Implications for the role of NO in the autonomic control of HR

Han et al. [11,37] have suggested that NO generated by

muscarinic receptor stimulation of eNOS activity is essential for cholinergic inhibition of L-type calcium current (I_{CaL}) in rabbit SAN cells. Prior adrenergic stimulation is required for this mechanism as when cAMP levels are elevated, NO via cGMP may stimulate PDE2 to decrease cAMP-protein kinase A (PKA) dependent stimulation of I_{CaL} [26]. Some groups show that NOS inhibition [11,37] or eNOS gene knockout [9] abolishes the reduction in I_{CaL} to acetylcholine after prior adrenergic stimulation, whilst others observe no effect [10,35,41]. These negative results suggest that NO may not be essential for cholinergic reduction of I_{CaL} although they do not necessarily rule out the possibility that the NO pathway plays a role in addition to muscarinic inhibition of adenylyl cyclase [38,39]. These conflicting results may be due to different experimental approaches (whole cell vs. perforated patch clamping), the temperature at which experiments are performed, the degree of prior adrenergic stimulation and cholinergic antagonism as well as the developmental stage of tissue and species (see Ref. [34] for a review).

However, vagal slowing of HR appears to involve an interplay between several ion currents including I_{CaL}, I_f and I_{KAch}. Functionally, post-synaptic inhibition of eNOS appears only to slow the HR response to acetylcholine following adrenergic pre-stimulation with NE [13], an effect that can be reversed with excess L-arginine. These conditions simulate cardiac autonomic control immediately following the cessation of exercise when vagal rebound occurs despite sympathetic tone remaining high. Sears et al. [13] also provide indirect evidence that NO may decrease I_{CaL} but also stimulate I_f during cholinergic activation. Our data are consistent with this hypothesis as during physiological levels of adrenergic stimulation, NO increased I_f via a cGMP dependent pathway to transiently increase HR. This action of NO may be a protective mechanism to minimize the severity of vagal bradycardia.

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