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Activation of sulphonylurea-sensitive channels and the NO-cGMP pathway decreases the heart rate response to sympathetic nerve stimulation

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Abstract

Objectives: Activation of ATP sensitive K^+ channels (K_{ATP}) and the NO-cGMP pathway have both been implicated in reducing norepinephrine (NE) release from cardiac sympathetic nerves during stimulation. Our aim was to test whether these pathways could interact and modulate cardiac excitability during sympathetic nerve stimulation (SNS). **Methods:** The effect of inhibitors and activators of K_{ATP} channels and the NO-cGMP pathway on the heart rate (HR) response to cardiac SNS in the isolated guinea pig (*Cavia porcellus*) double atrial/right stellate ganglion preparation was studied ($n=48$). **Results:** The K_{ATP} channel activator, diazoxide (100 μM, $n=6$) or hypoxia (0% O_2/5% CO_2, $n=6$) significantly attenuated the HR response to 3 Hz SNS by -10 ± 4% and -27 ± 6% respectively; an effect that was reversed by the K_{ATP} channel inhibitor, glibenclamide (30 μM). Glibenclamide ($n=6$) on its own enhanced the HR response to SNS by 20 ± 8%. Bath applied NE (0.1–0.7 μM, $n=6$) did not affect the HR response to diazoxide, although an increased response to glibenclamide was observed at 0.3 and 0.5 μM NE. In the presence of 8-Br-cGMP (0.5 mM, $n=7$), diazoxide further decreased the HR response SNS (19 ± 3%). The NO synthase inhibitor, *N*-ω-nitro-L-arginine (100 μM) significantly increased the HR response (13 ± 3%) to SNS in the presence of diazoxide (100 μM, $n=6$). This effect was reversed with excess (1 mM) L-arginine. Conversely, the NO donor, sodium nitroprusside (SNP, 20–100 μM) significantly attenuated the HR response to SNS. The addition of glibenclamide (30 μM, $n=10$) could still enhance the HR response (42 ± 15%) to SNS. Similar results were seen with the cyclic GMP analogue, 8-Br-cGMP (0.5 mM, $n=12$). **Conclusions:** Our results indicate that NO and sulphonlyurea-sensitive channels act in a complementary fashion, but appear to be independent of each other in the regulation of HR during cardiac SNS activation.

Keywords: Nitric oxide; K-ATP channel; Heart rate (variability); Hypoxia/anoxia; Autonomic nervous system

1. Introduction

Depolarization of the presynaptic sympathetic nerve terminal leads to an influx of calcium and exocytotic release of norepinephrine (NE). Once released into the synaptic cleft, NE can influence subsequent transmitter release via activation of presynaptic α_2-adrenoreceptor modulatory pathways (autoinhibition) [7,11]. Similarly, activation of presynaptic muscarinic M_3-receptors [15,18], A_1 adenosine receptors [7,24] and neuronally generated nitric oxide [31] are all able to inhibit NE release from sympathetic nerves in the heart.

More recently, activated neuronal ATP-sensitive potassium channels (K_{ATP}) have been implicated as presynaptic inhibitors of stimulation-evoked NE release in the guinea pig atria [26]. Activation of K_{ATP} channels either by K_{ATP} channel openers or by depletion of ATP has been proposed as the mechanism by which hyperpolarization of the nerve terminal and shortening of action potential duration reduces calcium influx and exocytotic release of NE [34]. Similarly, there is evidence that nitric oxide (NO), like K_{ATP} activation, inhibits peripheral sympathetic activity in the heart. Inhibition of endogenous NO production with non-isoform specific and neuronal NOS (nNOS) inhibitors increases NE release during cardiac sympathetic nerve stimulation (SNS) [31]. However, while the idea of a potential synergistic relationship between K_{ATP} channels and NO as modulators of NE release is attractive, the

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response of these pathways on organ and tissue behaviour is poorly understood.

The interaction between NO and K_{ATP} channels was first proposed by Garland and co-workers [8] who demonstrated that the NO-induced hyperpolarisation in rat mesentery was abolished by K_{ATP} inhibition. In contrast, more recent studies have shown that both NO-induced hyperpolarisation [37] and vasodilation [12,33] are not mediated by activation of K_{ATP} channels. Whether these channels play a functionally significant role in the sympathetic control of cardiac excitability and whether they are modulated by the NO-cGMP pathway is not known.

Therefore, the aims of this study were two-fold. First, to investigate whether modulators of sulphonylurea-sensitive channels (K_{ATP} channels) alter the peripheral sympathetic control of heart rate in the isolated guinea-pig atria. Secondly, to determine whether an interaction between NO and K_{ATP} channels affects the HR response to peripheral sympathetic activation.

2. Methods

2.1. Animal care

All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985) and were performed in accordance with Home Office license requirements (PPL 30/1133, Queen Anne's Gate, London, UK) and the Animals (Scientific Procedures) Act 1986 (UK). Forty eight male guinea pigs (Harlan, UK) initially weighing between 250 and 350 g were housed in a temperature controlled room (20±1°C) with a 12-h light/dark cycle. Chow and water were provided ad libitum.

2.2. Guinea pig sino atrial node/right stellate ganglion preparation

Animals were killed by cervical dislocation followed by exsanguination. The thorax and mediastinum were rapidly removed and placed in Tyrode's solution aerated with 95% O_2/5% CO_2. The ventricles were perfused with heparinised Tyrode's solution (1000 U ml^{-1}) and excised. The atria and right stellate ganglion were dissected free and sutures (Ethicon 6/0 silk) were placed on the lateral edges of both atria. The preparation was then transferred to a preheated (37±0.1°C) water-jacketed bath containing 100 ml of oxygenated Tyrode's solution. The atria were vertically mounted with the suture in the left atrium connected to a stainless steel hook, and the suture in the right atrium attached to an isometric force transducer (Harvard Apparatus, Model # 60-2997, MA, USA). The right stellate ganglion was placed through a pair of platinum ring electrodes connected to a stimulator. Data were collected on a Power Macintosh 7500 computer (Apple Systems, CA, USA) using a Biopac MP100 acquisition system and Acknowledge software (Biopac Systems Inc. CA, USA). Heart Rate (bpm) was triggered from contraction, and signals were displayed in real time. Data were stored on CD Rom for offline analysis.

2.3. Solutions and drugs

Tyrode's solution contained (mM) NaCl 120, KCl 4, $MgCl_2$ 2, NaH_2PO_4 0.1, $NaHCO_3$ 25, $CaCl_2$ 2, and glucose 11. The solution was aerated with 95%O_2/5%CO_2 (pH=7.4) and its temperature maintained at 37±0.1°C (Grant Instruments, Cambridge, UK).

K_{ATP} channel modulators, glibenclamide, tolbutamide and diazoxide were added from stock solutions 100 mM (in DMSO), 10 mM (in DMSO) and 50 mM (in 0.1 M NaOH), respectively. Solutions of cesium chloride (CsCl), sodium nitroprusside (SNP), *N*-ω-nitro-L-arginine (L-NA) and L-arginine (L-arg) were all dissolved in Tyrode's solution and prepared immediately prior to application. Norepinephrine bitartrate (NE), and the membrane permeable cGMP analogue 8-Br-cGMP were added from 1 mM and 0.1 M stock solutions respectively, made up prior to the experiment using reagant grade water from an Elga water purification system. All other chemicals were purchased from Sigma Aldrich, Dorset, UK.

2.4. Experimental protocols

The atria equilibrated in Tyrode's solution for 45–90 min until the heart rate did not alter by 10 bpm over a 20 min period. Following this period, the stellate ganglion was stimulated at 3 Hz, 10 V, 1 ms pulse width for 30 s at 2–3 min intervals. Experimental protocols commenced after three consistent positive chronotropic responses to ganglion stimulation were achieved. Prior to each pharmacological intervention, the ganglion was stimulated at 1, 3 and 5 Hz (10 V, 1 ms). Fresh Tyrode's solution was then placed in the organ bath prior to incubation with either a modulator of the NO-cGMP pathway or K_{ATP} channels. Following this, a second period of 1, 3 and 5 Hz sympathetic stimulation was completed. A second incubation period with a combination of NO-cGMP pathway and K_{ATP} channel modulators preceded the final cycle of SNS. The change in heart rate with SNS was calculated by difference in 5-s averages in heart rate taken prior to the onset and cessation of a stimulation period.

2.4.1. Effect of K_{ATP} channel activation on the HR response to SNS

We evaluated the effects of the K_{ATP} opener, diazoxide (5–100 μM) on the increase in heart rate with SNS. Diazoxide was used as the primary pharmacological K_{ATP} activator since it was shown to be a more potent inhibitor of NE release than both cromokalim and pinacidil, the

latter of which actually enhances adrenergic neurotransmission [26,34]. Concentrations of diazoxide (5, 10, 50 and 100 μM) were added cumulatively and allowed to incubate for 6 min each prior to SNS. To antagonize the effects of the K_{ATP} opener, glibenclamide (30 μM) was added 10 min prior to a single 100 μM addition of diazoxide.

The HR response to SNS was measured following activation K_{ATP} channels by hypoxia. A control 3 Hz stimulation preceded a 6 min in-vitro bout of hypoxia (0% O_2/5% CO_2). During the last 30 s of the in-vitro hypoxic episode, the right stellate ganglion was stimulated at 3 Hz. A second bout of in-vitro hypoxia was introduced after the addition of glibenclamide (30 μM; 10 min incubation) and a third stimulation of the right stellate ganglion during this hypoxic period was completed.

2.4.2. Effect of K_{ATP} inhibition on HR response to SNS

We evaluated the effects of the K_{ATP} inhibitors, glibenclamide (5–30 μM) and tolbutamide (1–5 μM) on the increase in heart rate with SNS. Glibenclamide (5 μM) or tolbutamide (1 μM) was added to the organ bath and allowed 10–12 min incubation prior to SNS. Subsequent concentrations of glibenclamide (10 and 30 μM) or tolbutamide (2 and 5 μM) were added cumulatively at 10–12 min intervals each prior to SNS.

2.4.3. Effect of diazoxide and glibenclamide on the increase in heart rate with bath-applied NE

The increase in heart rate with exogenous NE (cumulative addition: 0.1–0.7 μM) was measured in the presence of single doses of diazoxide (5, 10, 50 and 100 μM) or glibenclamide (5, 10, and 30 μM) to determine whether modulation of K_{ATP} channels altered the HR response to SNS via a pre- or post-synaptic mechanism. The Tyrode's solution was replaced in the organ bath prior to the addition of each concentration of diazoxide or glibenclamide.

2.4.4. Effect of dual activation of NO-cGMP pathway and K_{ATP} channels on the HR response to SNS

We simultaneously activated both the NO-cGMP pathway and K_{ATP} channels to define whether they acted independently to modulate the HR response to SNS. After the first cycle of SNS in the presence of CsCl (2 mM, 12 min equilibration) to minimise the changes in baseline heart rate following activation of the NO-cGMP pathway [23], with the membrane permeable cGMP analogue, 8-Br-cGMP (0.5 mM, 15 min incubation) was added and a second cycle of SNS was repeated. The final cycle of SNS was completed after addition of diazoxide (100 μM).

2.4.5. Role of NOS inhibition on the heart rate response to SNS with K_{ATP} activation

We tested the effects of the NOS inhibitor, *N*-ω-nitro-L-arginine (L-NA, 100 μM) in the presence of the K_{ATP} opener, diazoxide (100 μM) on the increase in heart rate with SNS. Control stimulations were performed in the presence of diazoxide (6 min incubation). For the second cycle of SNS, fresh Tyrode's solution was then placed in the organ bath and L-NA (100 μM) was equilibrated with the tissue for 20 min prior to the addition of diazoxide (100 μM). Following the addition of fresh Tyrode's solution, L-NA (100 μM) and L-arginine (1 mM) (20–25 min incubation), diazoxide (100 μM) was added and the final cycle of SNS was completed. The increases in heart rate with SNS were averaged for diazoxide only, L-NA+diazoxide and L-NA+L-arginine+diazoxide.

2.4.6. Role of K_{ATP} inhibition on the heart rate response to SNS with NO-cGMP activation

We examined the role of glibenclamide (30 μM) in the presence of the NO donor SNP or 8-Br-cGMP, on the increase in heart rate with SNS. The right stellate ganglion was stimulated during a control period prior to addition of SNP (100 μM, 15 min incubation) or 8-Br-cGMP (0.5 mM, 15 min incubation; in the presence of 2 mM CsCl to minimise the change in baseline heart rate caused by 8-Br-cGMP) and again following this equilibration period. Glibenclamide (30 μM; 10 min incubation) was added to the organ bath in the presence of the equilibrated SNP or 8-Br-cGMP and the stellate ganglion was stimulated for a third cycle. The above NO donor protocol was repeated using a lower concentration of SNP (20 μM) in the presence of CsCl (2 mM, 12 min incubation).

2.5. Statistical analysis

Data are presented as the mean±SEM unless otherwise specified. For the double atrial/right stellate ganglion preparations, one-way repeated measures ANOVA followed by a Student Newman-Keuls test for pairwise comparison was used to evaluate intra- and inter-group differences in the positive chronotropic response to SNS at 1, 3 and 5 Hz. Statistical significance was accepted at $P \leq 0.05$.

3. Results

After 90–120 min of equilibration, the spontaneous beating rate of the atrial preparations averaged 184± 3 bpm ($n=48$).

3.1. Effect of K_{ATP} modulators on the increase in heart rate with SNS

Fig. 1 shows a representative raw data trace for the effect of sympathetic stimulation (1, 3 and 5 Hz) on heart rate (bpm) in isolated atrial preparations in the presence of

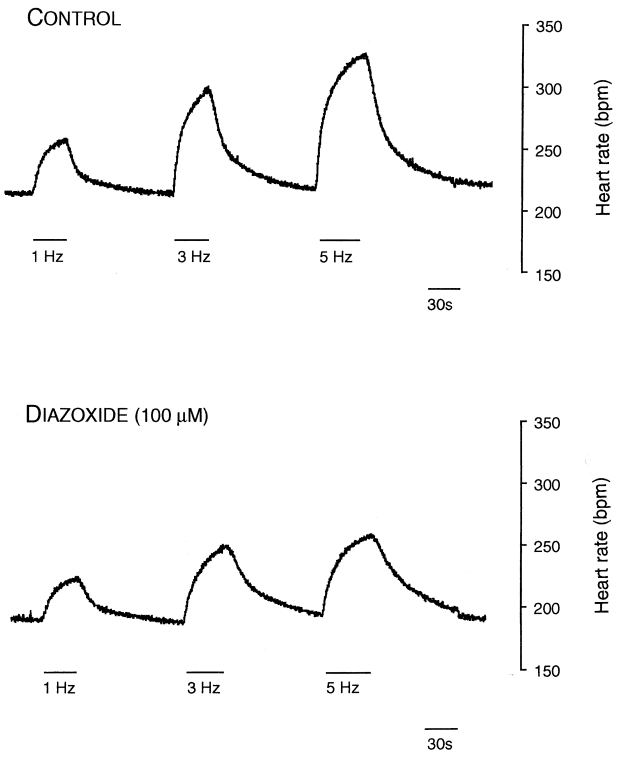

Fig. 1. Representative raw data traces showing the effects of cardiac SNS (1, 3 and 5 Hz, 10 v, 1 ms pulse width) on heart rate (bpm) in a double atrial/right stellate ganglion preparation under control conditions (top trace) and in the presence of the K_{ATP} opener, diazoxide (100 μM; lower trace). The right stellate ganglion was stimulated for 30 s, with 90–120 s between each stimulation period. The time calibration bar refers to both traces.

the K_{ATP} channel activator, diazoxide (100 μM, $n=6$). Diazoxide significantly attenuated the HR response to SNS ($P \leqslant 0.05$). This response was antagonized by the K_{ATP} channel inhibitor, glibenclamide (see Table 1). No effect on baseline heart rate was observed with diazoxide (5–100 μM).

A period of in-vitro hypoxia (0% O_2/5% CO_2) was introduced to examine the effects of hypoxic activation of K_{ATP} channels on the HR response to SNS. After approximately 6 min of hypoxia, the HR response to 3 Hz SNS was significantly reduced ($P<0.05$; $n=6$, Fig. 2). This effect was reversed by the addition of glibenclamide to the atrial preparation prior to a subsequent episode of hypoxia.

Glibenclamide ($n=6$) enhanced the HR response to SNS in a concentration dependent manner, reaching a maximal response at 30 μM (Fig. 3, Table 1). Baseline HR significantly declined from 165±7 bpm during control conditions to 151±7 bpm ($P<0.05$) in the presence of 30 μM glibenclamide. Tolbutamide ($n=5$) enhanced the HR response to SNS at 3 and 5 Hz in a similar manner to glibenclamide (Table 1), however, no significant changes in baseline heart rate were observed.

Table 1
Effect of K_{ATP} activators and inhibitors on the heart rate response to SNS[a]

	Increase in heart rate with SNS (bpm)		
	1 Hz	3 Hz	5 Hz
K-ATP Opener			
Diazoxide (μM), $n=6$			
control	39±1	86±5	105±7
5	36±4	81±3	98±6
10	35±3	79±3	87±15
50	36±2	81±3	97±5
100	31±1*	77±4*	96±8
100+30 Glib	48±5**	96±6**	114±8**
K-ATP inhibitors			
Glibenclamide (μM), $n=6$			
control	37±4	89±7	115±11
5	43±3	97±7	122±12
10	49±4*	104±8*	130±11
30	52±5*	105±5*	133±9*
Tolbutamide (μM), $n=5$			
control	41±10	69±9	76±8
1	40±11	78±16	92±12
2	48±16	86±18*	107±13*
5	51±14	92±17*	107±17*

[a] Values are expressed mean±SEM.
* $P<0.05$, control vs. intervention, ANOVA.
** $P<0.05$, 100 vs. 100 +30 Glib, ANOVA.

3.2. Effect of K_{ATP} modulators on the increase in heart rate with bath-applied norepinephrine

The positive chronotropic effect to the cumulative addition of NE (0.1–0.7 μM) was not significantly different between control and diazoxide (5–100 μM, $n=6$), although, 30 μM glibenclamide significantly enhanced

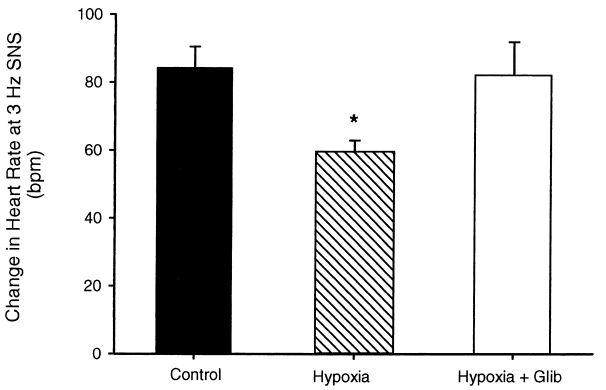

Fig. 2. The effect in-vitro hypoxia (0%O_2/5%CO_2) and its reversal with glibenclamide (30 μM) on the increase in heart rate with right stellate ganglion stimulation (10 V, 1 ms pulse width, 30 s duration) at 3 Hz ($n=6$). Hypoxia significantly decreased the magnitude of the positive chronotropic response to sympathetic activation and this effect was significantly enhanced with glibenclamide (*, $P<0.05$; ANOVA).

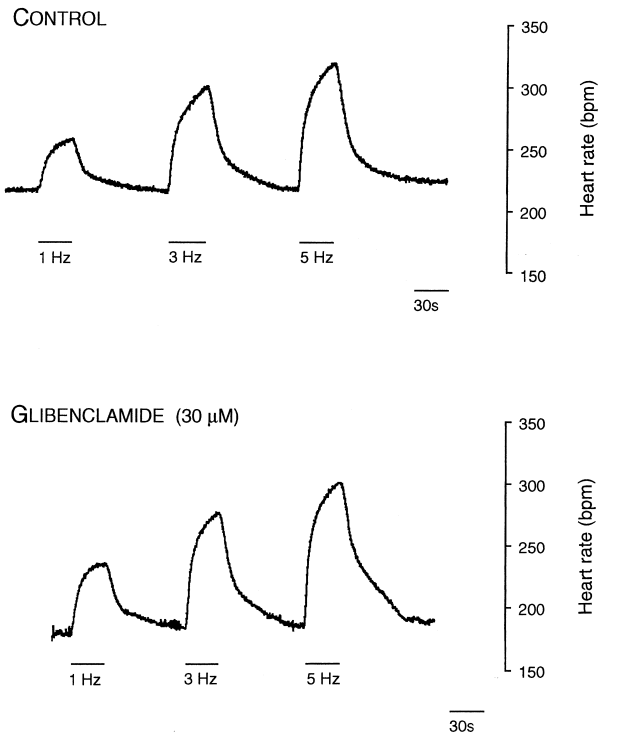

Fig. 3. Representative raw data traces showing the effects of cardiac SNS (1, 3 and 5 Hz, 10 V, 1 ms pulse width) on heart rate (bpm) in a double atrial/right stellate ganglion preparation under control conditions (top trace) and in the presence of the K_{ATP} inhibitor, glibenclamide 30 μM; lower trace). The right stellate ganglion was stimulated for 30 s, with 90–120 s between each stimulation period. The time calibration bar refers to both traces.

($P<0.05$) the chronotropic effect of NE at 0.3 and 0.5 μM ($n=6$, Table 2)

3.3. Effect of activation of both the NO-cGMP pathway and K_{ATP} channels on the HR response to SNS

8-Br-cGMP significantly reduced the HR response 1, 3 and 5 Hz ($P<0.05$) nerve stimulation in the presence of 2 mM CsCl ($n=7$). This effect was then further significantly reduced ($P<0.05$) in the presence of diazoxide at 3 and 5 Hz stimulation (Fig. 4). All effects were fully reversible on wash-off, confirming the preparations were not run down.

3.4. Effect of NO modulators on K_{ATP} mediated heart rate response to SNS

L-NA enhanced the HR response to SNS ($n=5$; change in HR for Control, L-NA, L-NA+L-arginine 35±2, 47±3, 35±2; 86±5, 88±6, 77±4 and 98±5, 111±4, 98±7 at 1 Hz, 3 Hz and 5 Hz respectively). This response was greater than with diazoxide on its own at all frequencies, however only reached statistical significance at 1 Hz. The HR response to SNS with diazoxide was significantly enhanced ($P<0.05$) in the presence of the non-isoform selective NOS inhibitor L-NA ($n=6$) at all frequencies of nerve stimulation (Fig. 5). The effect of L-NA was reversed with excess L-arginine (100 μM L-NA/100 μM Diazoxide+1 mM L-arginine) at 1 and 5 Hz SNS.

Conversely, the NO donor, SNP, significantly attenuated ($P<0.05$) the HR response to SNS, whereas addition of

Table 2
Effect of K_{ATP} activators and inhibitors on the heart rate response to SNS[a]

	Increase in heart rate with Applied Noradrenaline (bpm)			
	0.1 μM	0.3 μM	0.5 μM	0.7 μM
K-ATP Opener				
Diazoxide (μM), $n=6$				
control	27±7	56±8	68±7	80±6
5	25±5	55±8	69±6	77±5
10	30±5	61±6	72±4	81±5
50	28±6	61±6	70±5	79±5
100	31±7	60±8	76±7	83±8
100+30 Glib	37±6	72±5	86±5	89±5
K-ATP inhibitors				
Glibenclamide (μM), $n=6$				
control	24±4	55±5	74±4	77±3
5	33±5	68±6*	84±5	88±5
10	34±8	75±9*	89±7	89±4
30	43±2*	80±10*	95±8*	88±7

[a] Values are expressed mean±SEM.
* $P<0.05$, control vs. intervention, ANOVA.

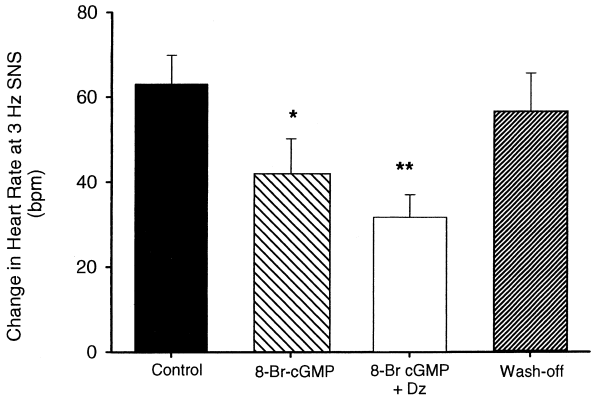

Fig. 4. The effect NO-cGMP pathway activation with 8-Br-cGMP (0.5 mM) and the combined effect of 8-Br-cGMP and Diazoxide (100 μM) on the increase in heart rate with right stellate ganglion stimulation (10 V, 1 ms pulse width, 30 s duration) at 3 Hz ($n=7$). 8-Br-cGMP significantly reduced the HR response to SNS (*, $P<0.05$; ANOVA) and this effect was then further significantly reduced (**, $P<0.05$; ANOVA) in the presence of diazoxide. All effects were fully reversible on wash-off, confirming the preparations were not run down.

glibenclamide had the opposite effect ($n=6$, Table 3). To verify that the effect of K_{ATP} inhibition on the SNP mediated HR response to SNS was not due to alterations in baseline heart rate, experiments were repeated using a lower concentration of SNP (20 μM) in the presence of CsCl. Fig. 6a (top) shows the mean HR response to SNS with SNP (20 μM) and glibenclamide ($n=10$). Glibenclamide enhanced the HR response to SNS in the presence of SNP. Similarly, glibenclamide could still increase the HR response to SNS when the response had been significantly reduced ($P<0.05$) at 1, 3 and 5 Hz nerve

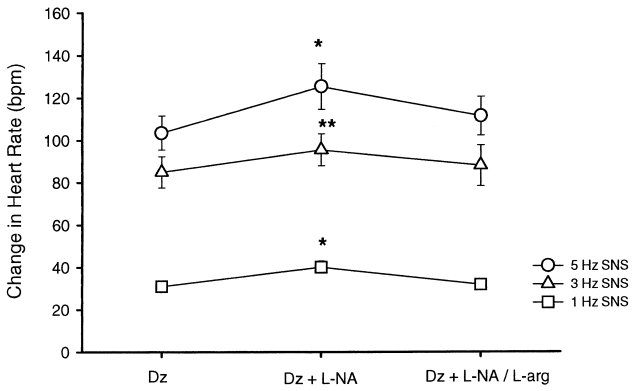

Fig. 5. The effect NOS inhibition with L-NA (100 μM) in presence of diazoxide (100 μM) and its reversal with L-arginine (1 mM) on the increase in heart rate with right stellate ganglion stimulation (10 V, 1 ms pulse width, 30 s duration) at 1, 3 and 5 Hz. L-NA enhanced the HR response to SNS and this response was greater than with diazoxide only ($n=5$; not shown). This trend was observed at all frequencies, however only reached statistical significance at 1 Hz (†, $P<0.05$; ANOVA). The HR response to SNS with diazoxide was signficianc enhanced (*, $P<0.05$; ANOVA) in the presence of L-NA at all frequencies of nerve stimulation. The effect of L-NA was reversed with excess L-arginine (100 μM L-NA/100 μM Diazoxide+1 mM L-arginine) at 1 and 5 Hz SNS (**, $P<0.05$; ANOVA).

stimulation with 8-Br-cGMP (in the presence of 2 mM CsCl; $n=13$, Fig. 6b, Table 3)

4. Discussion

The new finding in this study is that sulphonylurea-sensitive channels (K_{ATP} channels) and the NO-cGMP pathway can significantly regulate the HR response to peripheral cardiac sympathetic nerve stimulation. In addition, activation of both the NO-cGMP pathway and K_{ATP} channels reduced the HR response to SNS further than activation of the NO-cGMP pathway alone, indicating that both pathways can act independently.

4.1. Effect of K_{ATP} modulators on the heart rate response to sympathetic nerve stimulation

Myocardial K_{ATP} channels are closed under normal physiological conditions, but they are activated by either a decrease in intracellular ATP concentration [25], hypoxia [5,39] or by specific K_{ATP} channel activators such as diazoxide [9], cromakalim or pinacidil [3,34]. Their activation during cardiac ischaemia postpones the onset of irreversible damage and reduces the size of myocardial infarct (for review see [17,30,36]). Subsequent to their localization in pre- and postsynaptic neurons [21], recent work has shown that K_{ATP} activation by cromokalim and diazoxide (100 μM) inhibits stimulation-evoked NE release in the guinea pig atria [26]. We provide functional support for this observation and show that similar concentrations of diazoxide decrease the HR response to SNS.

Studies using radiochemical binding to NE (^{3}H-NE) similar to that described by Oe and co-workers [26] found that K_{ATP} activation with either cromokalim or diazoxide inhibited stimulation evoked 2-[^{14C}] NE efflux from the canine/bovine mesenteric artery [3], as well as [^{3}H] NE release from the dorsal metatarsal vein and rat neocortex [34]. Similarly, Remme and co-workers [29] found that cromokalim reduced stimulation evoked NE release in globally ischaemic rabbit hearts and Takata and co-workers [35] reported that 5 min hypoxia in rat cortical slices linearly reduces both tissue ATP content and evoked NE release, the latter effect being reversed by glibenclamide. In our study, the increase heart rate with cardiac SNS was attenuated both in the presence of diazoxide or during hypoxia and this was reversed by glibenclamide suggesting direct modulation of K_{ATP} channels.

It is possible that glibenclamide could be acting on other ionic conductances involved in transmitter release since this sulphonylurea has been shown to inhibit Ca^{2+} activated K^{+} channels in neuroblastoma cell lines [28]. However, inhibition of K_{ATP} channels in human pancreatic β-cells with tolbutamide (10 mM) [2] or in skeletal muscle with glibenclamide (up to 100 μM) has been shown to be

Table 3
Effect of NO modulators on K_{ATP}-mediated heart rate response to SNS[a]

	Increase in heart rate with SNS (bpm)		
	1 Hz	3 Hz	5 Hz
NO DONOR+K-ATP Inhibitor			
SNP (100 μM), n=6			
con	38±8	85±8	108±9
SNP	32±5	65±5*	80±6*
SNP+Glib	45±4**	80±6**	98±9**
SNP (20 μM)+CsCl (2 mM), n=10			
CsCl	20±6	39±7	58±9
SNP	14±4*	30±5*	38±5*
SNP+Glib	20±4**	39±5**	50±6**
cGMP Analogue+K-ATP Inhibitor			
8-Br-cGMP (0.5 mM)+CsCl (2 mM), n=12			
CsCl	19±4	40±4	55±11
8-Br	11±2*	28±3*	37±2*
8-Br+Glib	18±3**	37±4**	48±4**

[a] Values are expressed mean±SEM.
* $P<0.05$, control or CsCl vs. intervention, ANOVA.
** $P<0.05$, SNP or CsCl+intervention vs. Glib, ANOVA.

ineffective in blocking either Ca^{2+} activated K^+ channels or voltage gated K^+ channels [16]. In our study, the concentration of both glibenclamide and tolbutamide were relatively low compared to concentrations needed to inhibit K_{ATP} channels, and they were similar to others who have directly modulated transmitter release with these drugs [26,34]. Nevertheless, we cannot completely exclude the possibility that other ionic conductances were unaffected by glibenclamide since Oe and co-workers [26] reported that the relatively specific K_{ATP} blocker 5-hydroxydecanoate (5-HD) was ineffective in reducing stimulated induced NE release.

In the absence of either pharmacological or physiological K_{ATP} channel activation, application of sulphonylureas to rat substantia nigra slices induces release of transmitter [1] suggesting that activated K_{ATP} channels may play a role in the regulation of transmitter release under normal conditions [19,38]. In the present study, both sulphonylureas, tolbutamide and glibenclamide, significantly enhanced the HR response to 3 Hz SNS by approximately 17% and 25%, respectively. We found that glibenclamide enhanced the HR response to bath applied NE, however, diazoxide did not have any appreciable effect. Taken together, the changes in the HR response to either SNS or exogenous NE elicited by the K_{ATP} inhibition were greater than those seen with K_{ATP} activation. These findings are in agreement with recent studies suggesting that K_{ATP} channels may be partly activated in the isolated atrial preparation [26] and that under normal circumstances they are sensitive to glibenclamide and responsive to β-adrenergic stimulation [38].

The differences in the effect of diazoxide and glibenclamide on the HR response to bath applied NE may be due to their different specificity towards mitochondrial and sarcolemmal K_{ATP} channels. Both types of channel are inhibited by glibenclamide [27], however, diazoxide has a much higher affinity for the mitochondrial K_{ATP} channel [10]. Therefore, it is likely that the enhanced response to bath applied NE in the presence of glibenclamide may occur as a result of sarcolemmal K_{ATP} activation.

4.2. Interaction between K_{ATP} and NO modulators

There is good evidence that NO, like K_{ATP} activation, inhibits peripheral sympathetic activity in the heart. Inhibition of endogenous NO production with non-isoform specific and neuronal NOS (nNOS) inhibitors increases both NE release during cardiac SNS [31] and the heart rate response to SNS [6,32]. Similarly, nNOS inhibition has been shown to enhance, whereas an NO donor or cGMP analogue attenuates, the heart rate and inotropic responses to SNS in the isolated guinea-pig atria [4]. The signalling pathway responsible for NO inhibiting sympathetic neurotransmission is not completely understood, but it could involve cGMP activation of protein kinase G [20] and phosphodiesterases that decrease cAMP-dependent phosphorylation of neuronal Ca^{2+} channels.

Recently, Murphy and co-workers [22] reported that in rabbit vascular smooth muscle, hyperpolarisation elicited by an NO donor was glibenclamide sensitive and suggested that there was activation of K_{ATP} channels via a cGMP mediated pathway. Similarly, it has been shown in guinea pig mesentery that sodium nitroprusside-induced hyperpolarisation was reduced by glibenclamide [38]. In contrast to these studies, hyperpolarisation-induced by either endogenous or exogenous NO in the rat aorta [37] or SNP/cGMP-induced vasodilation in porcine arterioles [12] has been shown to be independent of K_{ATP} activation. We

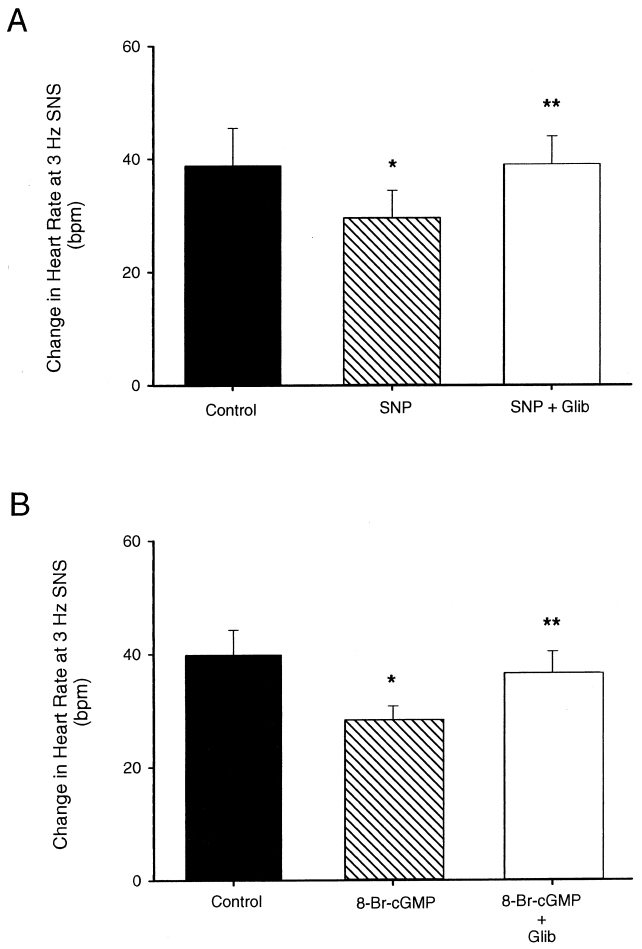

Fig. 6. The effect of glibenclamide (30 μM) in the presence of the NO donor SNP (20 μM, $n=10$:A) or the cGMP analogue, 8-Br-cGMP (0.5 mM; $n=12$:B) on the increase in heart rate with SNS. Glibenclamide could still increase the HP response to SNS (**, $P<0.05$; ANOVA) when the response has been significantly reduced (*, $P<0.05$; ANOVA) at 3 Hz nerve stimulation with SNP and 8-Br-cGMP.

also find no evidence to suggest that K_{ATP} channels and the NO-cGMP pathway are interdependent since we observed a further reduction in the HR response to SNS with K_{ATP} opening in the presence of NO-cGMP activation.

The present study provides functional support for the role of K_{ATP} channels and the NO-cGMP pathway in the presynaptic modulation of cardiac excitability. These pathways appear to be distinct and regulated by different processes and may contribute, with autoinhibition, to the regulation of neurotransmission and post junctional behaviour. The role of these pathways under normal physiological conditions is not established, although in pathophysiological states, they may play a dominant role in modulating the response to sympathetic activation. In particular, conditions that promote K_{ATP} activation (i.e. ischaemia) and NO production (i.e. sepsis, heart failure) may reduce local exocytotic NE release to protect the myocardium by reducing HR to minimise oxygen consumption and cardiac work. This response will have to compete with sympatho-sympathetic reflexes increasing NE release to an ischaemic area. Similarly, the NO-cGMP pathway may directly activate cardiac pacemaking [13,14] as well as reduce NE release [31]. The net effect of these pathways on cardiac excitability is not known. Whether they could provide sites for therapeutic targets to minimise the effects of high sympathetic drive remains to be established. However, any therapeutic use in-vivo would have to counter the effects of sympatho-vagal reflexes activated by the fall in blood pressure caused by the NO-cGMP pathway and K_{ATP} channel activation on vascular smooth muscle.

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