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Sulphonylurea-sensitive Channels and NO-cGMP Pathway Modulate the Heart Rate Response to Vagal Nerve Stimulation *in vitro*

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S. C. Almond and D. J. Paterson. Sulphonylurea-sensitive Channels and NO-cGMP Pathway Modulate the Heart Rate Response to Vagal Nerve Stimulation *in vitro*. *Journal of Molecular and Cellular Cardiology* (2000) **32**, 2065–2073. Sulphonylurea-sensitive K^+ channels (K_{ATP}) have been implicated in the release of acetylcholine (ACh) from the vagus nerve in the heart. Our aim was to establish the functional significance of this and to test whether this modulation could interact with stimulation of the NO-cGMP pathway that facilitates the decrease in heart rate (HR) in response to vagal nerve stimulation (VNS). We studied the effect of activation (diazoxide, 100 μM) and inhibition (glibenclamide 30 μM or tolbutamide 5 μM) of K_{ATP} channels, and activation of the NO-cGMP pathway with the NO donor, sodium nitroprusside (SNP, 20 μM) or the cGMP analogue, 8-Br-cGMP (0.5 mM) on the HR response to VNS in the isolated guinea pig (*Cavia porcellus*) double atrial/right vagus preparation ($n=40$). Tolbutamide increased the bradycardia in response to vagal stimulation at 3 and 5 Hz ($P<0.05$); effects that were reversed by diazoxide. Glibenclamide also significantly increased the HR response to VNS at 1 and 3 Hz ($P<0.05$). Diazoxide alone significantly attenuated the HR response to VNS at 5 Hz ($P<0.05$). Neither glibenclamide nor diazoxide affected the HR response to carbamylcholine (CCh, 50–200 nM). In the presence of a maximal dose of tolbutamide, SNP or 8-Br-cGMP further increased the HR response to VNS at 5 Hz ($P<0.05$). These results are consistent with the hypothesis that inhibition of sulphonylurea-sensitive channels can increase the HR response to VNS by a pre-synaptic mechanism, and that this modulation may be independent of activation of the NO-cGMP pathway.
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Key Words: K_{ATP} channel; Nitric oxide; Autonomic nervous system; Acetylcholine; Heart rate; Guinea pig.

Introduction

Depolarization of the pre-synaptic parasympathetic nerve terminal leads to an influx of calcium through voltage-gated calcium channels and the exocytotic release of the neurotransmitter, acetylcholine (ACh). The release of ACh is inhibited by activation of pre-synaptic M_2,[1] M_1 and M_4[2] muscarinic receptors and is facilitated by neuronally generated nitric oxide.[3]

Neuronal sulphonylurea-sensitive potassium channels (K_{ATP}) have been implicated as pre-synaptic modulators of stimulation-induced release of ACh from the vagus nerve in the isolated trachea[4–7] and in the heart.[8] Activation of these channels has been proposed as the mechanism by which hyperpolarization of the membrane and action potential shortening leads to a decrease in calcium influx and a reduction in transmitter release by exocytosis.[9] However, in rabbit arterial rings, inhibitors of K_{ATP} channels can prevent the vasorelaxing actions of diazoxide and ACh, suggesting that these agents may be acting through a common pathway by opening K_{ATP} channels post-synaptically.[10]

NO donors or analogues of its second messenger,

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cGMP, significantly increase the magnitude of the heart rate response to vagal stimulation.[11] Evidence also suggests that NO and K_{ATP} channels may be able to interact in the modulation of transmitter release; however, the relationship between these pathways in the cardiac vagus nerve is not known. Interestingly, the K_{ATP} inhibitor, glibenclamide, abolishes the hyperpolarization in response to NO in rat mesenteric arteries[12] and in rabbit mesenteric arteries.[13] NO donors directly activate mitochondrial K_{ATP} channels in rabbit ventricular myocytes and, in addition, can potentiate the action of diazoxide to open these channels,[14] suggesting that nitric oxide leads to an increased open probability of K_{ATP} channels. However, others fail to demonstrate an interaction between NO and K_{ATP} channels in arterial smooth muscle cell hyperpolarization[15] or vasodilation.[16,17]

The aims of the investigation were therefore three-fold. First, to establish if sulphonylurea-sensitive channels (K_{ATP} channels) modulate the heart rate response to vagal nerve stimulation in isolated guinea pig atria. Second, to determine whether this modulation is pre- or post-synaptic. Third, to establish if the NO-cGMP pathway and K_{ATP} channels interact to modulate the heart rate response to vagal stimulation.

Materials and Methods

Animal care

The investigation conforms 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 was performed in accordance with Home Office License requirements (PPL 10/1133, Queen Anne's Gate, London, UK) and the Animals (Scientific Procedures) Act 1986 (UK). Fifty-one male guinea pigs (Harlan and Charles River, UK) weighing 220–400 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.*

Isolated guinea pig sino-atrial node/right vagus nerve preparation

Animals were killed by cervical dislocation followed by exsanguination. The heart was exposed and the ventricles removed, allowing the atria to be back perfused with 10 ml of heparinized Tyrode's solution (1000 U/ml). The thorax and mediastinum were rapidly removed and placed in oxygenated (95% O_2, 5% CO_2) Tyrode's solution at room temperature (20–22 °C) in a perspex dissecting dish with a Sylgard base. The heart and right vagus were dissected free and sutures (Ethicon, 5/0 mersilk) fixed at the lateral edges of the two atria. The preparation was transferred to a pre-heated (37 ± 0.1 °C) water-jacketed organ bath containing 100 ml of continuously 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), which had been calibrated prior to the beginning of the experiment using a 10 mN weight. The vagus nerve was placed through a pair of platinum stimulating electrodes connected to a stimulator. The force transducer was attached to an amplifier. Data were acquired on a Power Macintosh 8500 computer (Apple Systems, CA, USA) using a Biopac Systems MP100 data acquisition system (Biopac Systems Inc., CA, USA) and Acqknowledge 3.2 software. The heart rate was triggered from contraction, and the signals displayed in real time. Data were stored on CD for offline analysis.

Solutions and drugs

Tyrode's solution containing (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 °C using a Digitron 1408-K temperature gauge (RS Components Ltd, UK).

K_{ATP} channel modulators glibenclamide, tolbutamide and diazoxide were added from stock solutions of 100 mM (in DMSO), 10 mM (in DMSO) and 50 mM (in 0.1 M NaOH), respectively. Carbamylcholine chloride (CCh), propranolol, sodium nitroprusside (SNP), 8-Bromo-cGMP (8-Br-cGMP) and caesium chloride (CsCl) were added from stock solutions of 1 mM, 0.1 M, 10 mM, 0.1 M and 1 M, respectively, in reagent grade water from an Elga water purification system. Experiments using SNP and 8-Br-cGMP were carried out under dark conditions due to the light sensitivity of these drugs. All chemicals and drugs were obtained from Sigma.

Experimental protocols

The atria were equilibrated in Tyrode's solution for 60–90 min at 37 °C until a stable baseline heart

rate was achieved (average = 209 ± 4 bpm, $n = 51$). Following this period, the vagus was stimulated at 5 Hz, 10–15 V, 1–2 ms duration for 25–30 s at 1 min intervals. Voltage and duration of stimulation were set so as to obtain the maximum vagal response at 5 Hz. Experimental protocols were commenced when three consistent vagal responses were achieved. Fresh Tyrode was placed in the organ bath prior to the commencement of the protocol. The vagus nerve was stimulated three times each at 5, 3 and 1 Hz. A pharmacological modulator was then incubated in the organ bath and the protocol of stimulations repeated. Finally, a second modulator was added to the organ bath and after a period of incubation the vagus nerve was stimulated as before. The preparation was washed with fresh Tyrode's solution at the end of the experiment. The change in heart rate with VNS was calculated by the difference in 5 s averages in heart rate taken prior to the onset and cessation of stimulation. All experiments were carried out in the presence of propranolol (0.1 μM) to prevent any possible positive chronotropic response due to stimulation of cardiac sympathetic nerves.

Effect of K_{ATP} channel inhibition on the heart rate response to vagal nerve stimulation

The effects of the K_{ATP} channel inhibitors, glibenclamide (5–50 μM) (SUR1,Kir6.2 Ki = 27 nM)[18] and tolbutamide (2–10 μM) (SUR1,Kir6.2 Ki = 5 μM)[18] on the heart rate response to VNS were evaluated. After the initial stimulation under control conditions, glibenclamide (5 μM) or tolbutamide (2 μM) were added to the organ bath and allowed to equilibrate until a stable response was seen (approximately 13 min[20]) prior to a second period of VNS. Subsequent concentrations of glibenclamide (10, 30 and 50 μM) or tolbutamide (5 and 10 μM) were added cumulatively at 13 min intervals prior to VNS. In a second set of experiments, after the initial control VNS, one dose of glibenclamide (30 μM)[19,20] or tolbutamide (5 μM) was added to the organ bath and allowed to incubate for 13 min before the second stimulations. To reverse the effect of the K_{ATP} channel inhibitor, 100 μM diazoxide[19,20] was incubated in the organ bath for 6–9 min and a final set of stimulations were completed.

Effect of K_{ATP} channel activation on the heart rate response to vagal nerve stimulation

The effect of K_{ATP} channel activation on the increase in heart rate with VNS was assessed using diazoxide. After the initial period of control stimulation, diazoxide (100 μM) was added to the organ bath and allowed to equilibrate for 6 min. Following a second period of stimulation, glibenclamide (30 μM) was added in addition to the diazoxide and a third round of stimulations were completed.

Effect of K_{ATP} channel inhibition and activation on the heart rate response to bath applied CCh

To test the hypothesis that K_{ATP} channels modulate the heart rate response to vagal stimulation presynaptically, the decrease in heart rate with bath applied CCh (50, 100, 150, 200 nM) was measured in the presence of single doses of glibenclamide (5, 10, 30, 50 μM) or diazoxide (10, 100, 200 μM).

Effect of simultaneous K_{ATP} channel inhibition combined with activation of the NO-cGMP pathway on the heart rate response to vagal nerve stimulation

The effect of tolbutamide on the decrease in heart rate with VNS was examined in the presence of the NO donor, SNP, and also in the presence of the cGMP analogue, 8-Br-cGMP. CsCl (2 mM, 7 min incubation)[21] was present throughout the experiment to minimize changes in baseline heart rate due to SNP activation of I_f. After an initial cycle of stimulations, tolbutamide (5 μM) was incubated in the organ bath for 13 min followed by a second set of stimulations. SNP (20 μM) or 8-Br-cGMP (0.5 mM)[11,21] were then added and incubated for 13 min (in the presence of tolbutamide) and the vagus was stimulated for a third time. The effect of tolbutamide in addition to 8-Br-cGMP on the HR response to bath applied CCh (50, 100 and 200 nM) was also assessed.

Time controls

To eliminate the possibility that the effect of diazoxide on VNS was due to a time-dependent run down of the response, time controls were performed. Vagal stimulations (5, 3 and 1 Hz) were undertaken at 0, 30 and 60 min without the addition of a modulator.

Statistical analysis

Data are presented as mean ± S.E.M. Differences in the data were assessed using a one-way analysis of

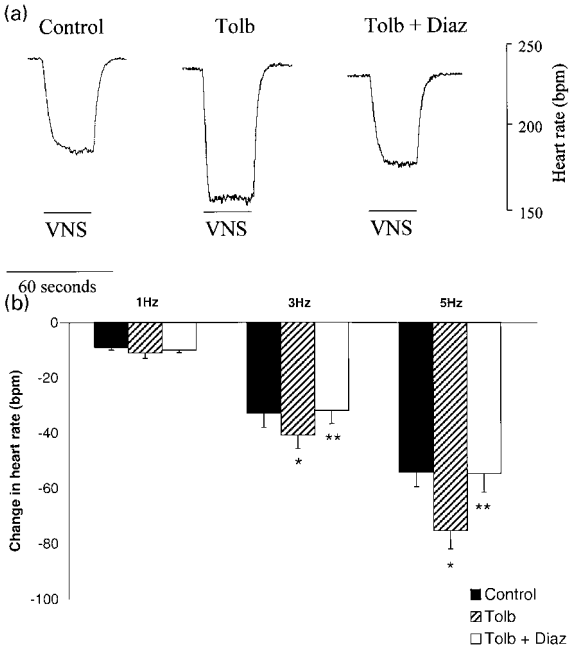

Figure 1 (a) Raw data trace to show the effects of vagus nerve stimulation at 5 Hz on heart rate (bpm) in the isolated guinea pig atria with intact right vagus nerve under control conditions, with the K_{ATP} inhibitor, tolbutamide (5 μM) and with tolbutamide + the K_{ATP} opener, diazoxide (100 μM). (b) Group data showing that tolbutamide significantly increased the HR response to VNS (* $P<0.05$, ANOVA) and this was reversed by the addition of diazoxide (100 μM) at 3 and 5 Hz (** $P<0.05$, ANOVA).

variance with repeated measures followed by a *post hoc* pairwise comparison using the Student–Newman–Keuls test. All data passed a normality test. Statistical significance was accepted at $P<0.05$.

Results

Effect of K_{ATP} channel inhibition on the heart rate response to vagal nerve stimulation

Figure 1(a) shows a representative raw data trace for the effect of vagal nerve stimulation (5 Hz) on heart rate in the presence of tolbutamide (5 μM). Tolbutamide significantly enhanced the decrease in HR in response to VNS ($P<0.05$, $n=5$) at 5 and 3 Hz [Fig. 1(b)]. This response was significantly attenuated by 100 μM diazoxide [Fig. 1(b)]. In the presence of glibenclamide, a trend to increase the response was seen at 1 Hz and this was reversed by diazoxide. No significant changes in baseline heart rate were seen with diazoxide or tolbutamide (Table 1).

The addition of one dose of glibenclamide (30 μM) significantly increased the HR response to VNS at 3 and 1 Hz [$P<0.05$, $n=5$, Fig. 2(b)]. A trend to increase the HR response was seen at 5 Hz and this was significantly reversed by the addition of 100 μM diazoxide [$P<0.05$, Fig. 2(a) and (b)]. Glibenclamide (30 μM) significantly reduced the baseline heart rate from 188.2 ± 15 bpm to 165.6 ± 19 bpm ($P<0.01$, Table 1) and this was unaffected by the addition of diazoxide [Fig. 2(a)].

Table 1 Effect of K_{ATP} inhibition and activation on baseline heart rate

	Heart rate (bpm) Control	with modulator
K_{ATP} inhibition		
Glibenclamide (30 μM), $n=5$	188 ± 15	166 ± 19*
Tolbutamide (5 μM), $n=10$	180 ± 12	176 ± 13
K_{ATP} activation		
Diazoxide (100 μM), $n=7$	216 ± 4	210 ± 5
	CsCl + Tolb	with modulator
NO donor		
SNP (20 μM), $n=5$	135 ± 3	151 ± 3**
cGMP analogue		
8-Br-cGMP (0.5 mM), $n=5$	111 ± 3	126 ± 5**

* $P<0.05$ control *v* modulator (ANOVA).
** $P<0.05$ CsCl + Tolb *v* CsCl + Tolb + modulator (ANOVA).

Effect of K_{ATP} channel activation on the heart rate response to vagal nerve stimulation

Figure 3(a) shows a representative raw data trace for the effect of VNS on heart rate in the presence of diazoxide (100 μM, $n=7$). Diazoxide significantly reduced the HR response to VNS at 5 Hz [$P<0.05$, Fig. 3(b)]; an effect that was reversed by glibenclamide. There was no significant effect at 1 or 3 Hz.

In two time control experiments there was no evidence of any run down of the response at 5 Hz (t = 0, 49 ± 1 bpm; t = 60, 52 ± 1), 3 Hz (t = 0, 24 ± 2; t = 60, 29 ± 5), or 1 Hz (t = 0, 7 ± 1; t = 60, 7 ± 1) stimulation over a 60 min duration.

Effect of K_{ATP} channel inhibition and activation on the heart rate response to bath applied CCh

Glibenclamide (5–50 μM) had no significant effect on the decrease in heart rate in response to the

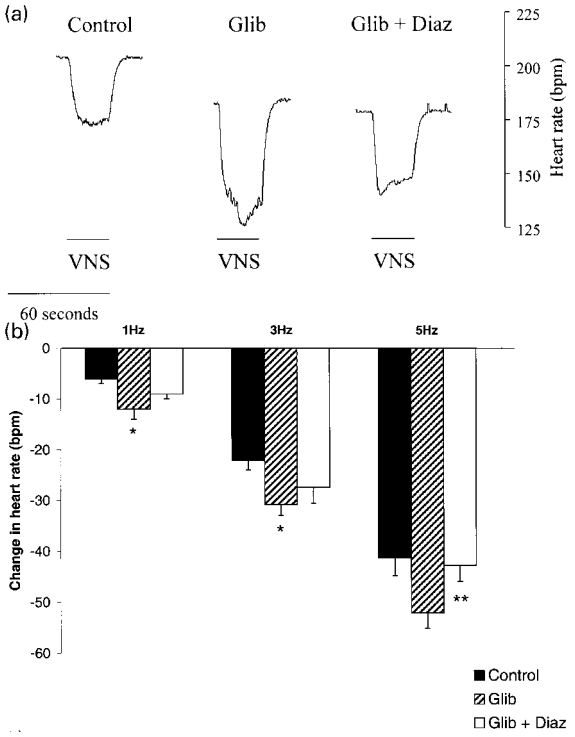

Figure 2 (a) Raw data trace to show the effects of vagus nerve stimulation at 5 Hz on heart rate (bpm) in the isolated guinea pig atria with intact right vagus nerve under control conditions, with the K_{ATP} inhibitor, glibenclamide (30 μM) and with glibenclamide + the K_{ATP} opener, diazoxide (100 μM). (b) Group data showing that glibenclamide (30 μM) significantly increased the HR response to VNS at 1 and 3 Hz (* $P<0.05$, ANOVA). The increase in response with glibenclamide at 5 Hz was significantly reversed by the addition of diazoxide (100 μM) (** $P<0.05$, ANOVA).

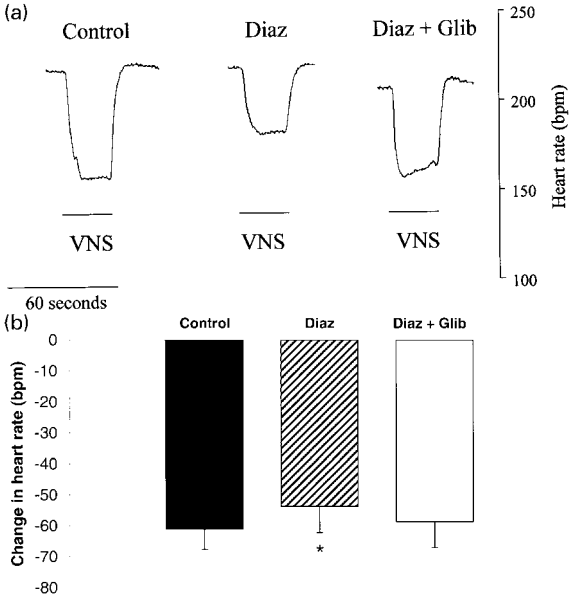

Figure 3 (a) Raw data trace to show the effects of VNS at 5 Hz on heart rate (bpm) in the isolated guinea pig atria with intact right vagus nerve under control conditions, with the K_{ATP} opener, diazoxide (100 μM) and with diazoxide + glibenclamide (30 μM). (b) Group data showing that diazoxide significantly decreased the magnitude of the negative chronotropic response to VNS at 5 Hz (* $P<0.05$, ANOVA).

cumulative addition of CCh (50–200 nM, Table 2). However, glibenclamide (5–50 μM) did significantly reduce baseline heart rate ($P<0.05$). Diazoxide (100 μM) had no significant effect on the negative chronotropic actions of CCh (50–200 nM, $n=5$). However, at 200 μM diazoxide, a significant increase in the HR response to 50 and 100 nM CCh was observed ($P<0.05$, $n=5$, Table 2), suggesting that it may be having a post-synaptic effect at this high concentration in the opposite direction to its effect on VNS.

Table 2 The effect of K_{ATP} activators and inhibitors on the heart rate response to bath applied CCh

	Heart rate response with bath applied CCh (bpm)			
	50 nM	100 nM	150 nM	200 nM
K_{ATP} inhibitor				
Glibenclamide (μM), $n=4$				
Control	-20 ± 3	-41 ± 7	-60 ± 9	-72 ± 10
5	-18 ± 2	-36 ± 6	-54 ± 9	-67 ± 10
10	-16 ± 4	-35 ± 7	-52 ± 10	-66 ± 12
30	-18 ± 4	-36 ± 8	-51 ± 9	-68 ± 11
50	-17 ± 3	-35 ± 6	-53 ± 6	-68 ± 6
Wash off	-13 ± 3	-30 ± 6	-50 ± 6	-69 ± 5
K_{ATP} opener				
Diazoxide (μM), $n=5$				
Control	-14 ± 2	-29 ± 4	-60 ± 10	-83 ± 10
10	-15 ± 32	-36 ± 5	-58 ± 8	-74 ± 6
100	-15 ± 2	-37 ± 6	-58 ± 9	-74 ± 7
200	-20 ± 3*	-41 ± 6*	-58 ± 5	-74 ± 4

Values are expressed as mean $\pm$ S.E.M.
* $P<0.05$ control *v* 200 diazoxide (ANOVA).

Effect of tolbutamide and activation of the NO-cGMP pathway on the heart rate response to vagal nerve stimulation

Tolbutamide (5 μM) increased the heart rate response to nerve vagal stimulation and this was further significantly increased at all frequencies on the addition of the NO donor, SNP (20 μM, $P<0.05$, $n=5$) [Fig. 4(a) and (b)]. Similarly, 8-Br-cGMP (0.5 mM) could still increase the HR response to VNS in the presence of tolbutamide at all frequencies and this additive effect reached significance at 5 Hz

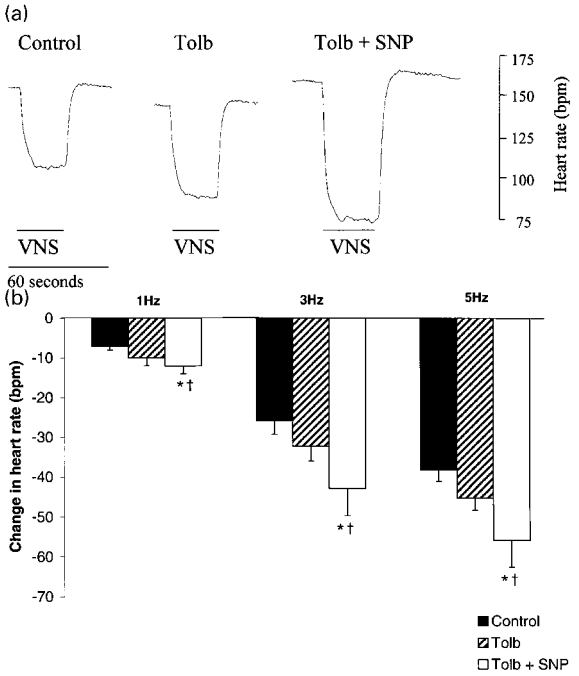

Figure 4 (a) Raw data trace to show the effects of vagus nerve stimulation at 5 Hz on heart rate (bpm) in the isolated guinea pig atria with intact right vagus nerve under control conditions, with the K_{ATP} inhibitor, tolbutamide (5 μM) and with tolbutamide + the NO donor, SNP (20 μM). CsCl (2 mM) was present throughout the experiment to minimize the NO-cGMP dependent stimulation of baseline HR. (b) Group data showing that SNP significantly increased the response relative to control (* $P<0.05$, ANOVA) and relative to tolbutamide († $P<0.05$, ANOVA).

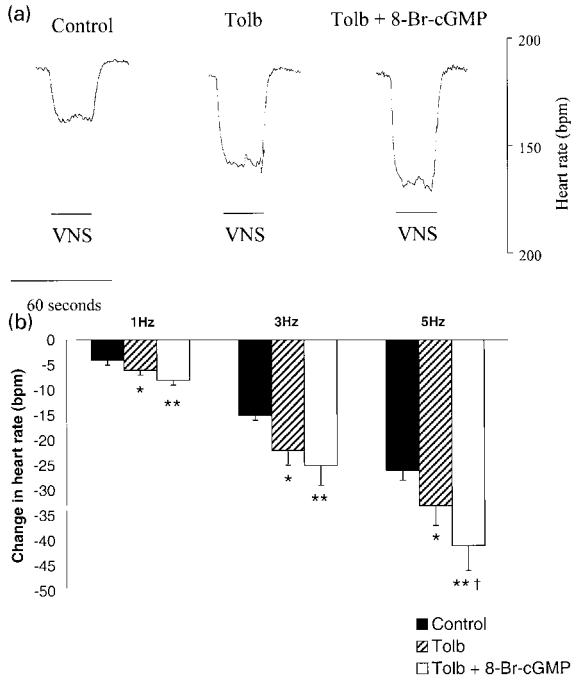

Figure 5 (a) Raw data trace to show the effects of vagus nerve stimulation at 5 Hz on heart rate (bpm) in the isolated guinea pig atria with intact right vagus nerve under control conditions, with the K_{ATP} inhibitor, tolbutamide (5 μM) and with tolbutamide + the cGMP analogue, 8-Br-cGMP (0.5 mM). CsCl (2 mM) was present throughout the experiment to minimize the NO-cGMP dependent stimulation of baseline HR. (b) Group data showing that 8-Br-cGMP significantly increased the response relative to control at all frequencies (** $P<0.05$, ANOVA) and relative to tolbutamide at 5 Hz († $P<0.05$, ANOVA). Tolbutamide significantly enhanced the response relative to control (* $P<0.05$, ANOVA).

[$n=6$, Fig. 5(a) and (b)]. Heart rate responses to cumulative addition of CCh (50, 100, 150 nM) were not significantly different in the presence of tolbutamide (5 μM) or tolbutamide and 8-Br-cGMP (0.5 mM) ($n=5$, data not shown). Despite the presence of 2 mM CsCl, both SNP and 8-Br-cGMP significantly increased the baseline heart rate in the presence of tolbutamide (Table 1).

Discussion

The new finding in this study is that inhibition of sulphonylurea-sensitive channels facilitates the negative chronotropic effect of vagal nerve stimulation in the isolated guinea pig atrial preparation. This is not seen with bath applied CCh, suggesting that the modulation is pre-synaptic. Furthermore, the facilitatory action of sulphonylureas may be independent of the NO-cGMP pathway since an NO donor or 8-Br-cGMP further increase the heart rate response to vagal stimulation in the presence of a maximal blocking dose of tolbutamide.

Effect of K_{ATP} modulators on the heart rate response to cholinergic stimulation

K_{ATP} channels can be activated by decreases in intracellular ATP concentration,[22] hypoxia[23,24] and pharmacological activation by diazoxide,[25] cromakalim and pinacidil.[9,26] In the nervous system, K_{ATP} channels have been identified on pre-synaptic and post-synaptic neurons in the brain.[27] In the substantia nigra, K_{ATP} channels modulate the release of γ-aminobutyric acid[28] suggesting that these channels may be important in neurotransmission.

The present study supports a functional role for K_{ATP} channels in the regulation of the heart rate response to parasympathetic nerve stimulation at physiological frequencies. Glibenclamide and tolbutamide both enhanced the effect of vagal stimulation on the heart, whereas diazoxide attenuated the negative chronotropic response to vagal nerve stimulation. The effects of K_{ATP} channel modulation were less at 1 Hz stimulation (Δ8 bpm) but were

greater at higher frequencies of stimulation (3–5 Hz), indicating that this mechanism may be more important during moderate to severe vagal induced bradycardia. Neither diazoxide or glibenclamide altered the heart rate response to bath applied carbamylcholine at the same dose as their effect on vagal nerve stimulation, suggesting that they act by modulating transmitter release pre-synaptically. These findings are consistent with earlier research, showing altered pre-synaptic release of [^{3}H]ACh, in response to cromakalim and glibenclamide during field stimulation in the isolated rat atrium.[8] However, we cannot conclusively rule out the possibility that other pathways and mediators could be involved in the modulation of the heart rate response to VNS by K_{ATP} channel activators and inhibitors. In the isolated rat trachea, inhibition of [^{3}H]ACh release from the vagus nerve with cromakalim was prevented by glibenclamide, but also by the removal of the endothelium, suggesting that the modulation of transmitter release may be occurring via another, unidentified mediator.[6] Nevertheless, this present study is consistent with the hypothesis that inhibition of K_{ATP} channels leads to depolarization of the membrane potential, reducing calcium influx and decreasing transmitter release by exocytosis.

The role of K_{ATP} channels in the modulation of the heart rate response to vagal stimulation mirrors that seen in the sympathetic nervous system. K_{ATP} channel openers inhibit the release of [^{3}H] NA from rat brain cortical slices in response to transmural stimulation[9] and hypoxia,[29] both effects being antagonized by glibenclamide. In the heart, K_{ATP} openers decrease in a concentration-dependent manner the stimulation-evoked release of [^{3}H] NA from the guinea pig atrium, an effect also antagonized by glibenclamide.[19] Similarly, cromakalim reduces myocardial NA release during global ischaemia in rabbits.[30] Moreover, activation of sulphonylurea-sensitive channels decreases the heart rate response to sympathetic nerve stimulation in the guinea pig double atrial preparation.[20]

It is possible that K_{ATP} openers and inhibitors affect other ionic conductances involved in cardiac autonomic neurotransmission. Glibenclamide has been shown to inhibit the Ca^{2+}-activated K current in a human neuroblastoma cell line,[31] and when the channels are inserted into a lipid bilayer.[32] Glibenclamide may also block the cAMP-activated Cl^- conductance, although at a higher dose (half maximal inhibition at 30 μM) than its effect on K_{ATP} channels.[33] However, other studies using rat skeletal K^+ channels incorporated into lipid bilayers showed that glibenclamide even at high concentrations (100 μM) is a selective and specific blocker of skeletal K_{ATP} channels.[34] Similarly, in human pancreatic β-cells a high concentration of tolbutamide (10 mM) selectively inhibited K_{ATP} channels with no effect on Ca^{2+} activated K^+ channels or voltage gated K^+ channels.[35] The concentrations of sulphonylureas used in the present study were relatively low and similar to that used in other studies where transmitter release was modulated.[9,19] Of interest glibenclamide, but not tolbutamide, significantly decreased baseline heart rate, suggesting that glibenclamide is also having post-synaptic effects on K_{ATP} channels in sino-atrial node cells.[36]

Is there an interaction between the NO-cGMP pathway and K_{ATP} channels?

Individually, NO donors and cGMP analogues significantly increase the magnitude of the heart rate response to vagal stimulation.[11] Conversely, inhibition of NOS attenuates the magnitude of the decrease in heart rate with vagal nerve stimulation in the ferret and dog.[37–39] However, this has not been shown to occur in the rabbit[40] or in young guinea pigs, where there is a slowing of the response but no change in the magnitude.[41] In adult guinea pigs, NOS inhibition attenuates the vagal response. The difference in response within this species appears to be related to the developmental stage of the guinea pig in its expression of nNOS.[42] The effect of activation of the NO-cGMP pathway may be due to NO stimulation of guanylate cyclase, increasing levels of cGMP, which inhibits phosphodiesterase 3. This increases cAMP-PKA dependent phosphorylation of pre-synaptic calcium channels, increasing calcium influx and release of ACh. At 5 Hz vagal stimulation, tolbutamide increased the heart rate response by 18% of that seen with control stimulations and this was further increased in the presence of SNP to 47%. Furthermore, in the presence of CsCl, tolbutamide increased the HR response to VNS by 27% of that seen with control stimulations and 8-Br-cGMP further increased this to 58%. The concentration of tolbutamide used produced maximal enhancement of the bradycardia in response to vagal stimulation, suggesting that the effects of NO and K_{ATP} channel inhibition are additive and that the two pathways may be acting independently. This is consistent with some studies on smooth muscle vasodilation,[15–17] but contrasts with others[12–14] where K_{ATP} channels may mediate hyperpolarization and relaxation in response to NO.

The functional significance of sulphonylurea-sensitive channels and the NO-cGMP pathway in the vagal control of heart rate *in vivo* remains to be established. Evidence presented here suggests that inhibition of K_{ATP} channels and activation of the NO-cGMP pathway facilitate vagal neurotransmission and bradycardia. Both pathways appear to be independent and regulated by different processes. Under physiological conditions, endogenous NO is released during each cardiac cycle[43] and cardiac K_{ATP} channels are predominantly closed, suggesting the possibility that both may be involved in vagal neurotransmission. It is therefore conceivable that pathophysiological states promoting K_{ATP} activation (e.g. ischaemia, hypoxia) and NO production (e.g. sepsis, heart failure), lead to abnormal regulation of cardiac cholinergic activity.

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