Modulation of norepinephrine release by ATP-dependent K⁺-channel activators and inhibitors in guinea-pig and human isolated right atrium

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Abstract

Objective: The aim of this study was to show, whether ATP sensitive K⁺ channels (K ATP channels), are involved in the modulation of norepinephrine (NE) release from the sympathetic nerves innervating the guinea-pig and human right atrium. Methods: The resting and stimulation-evoked release of [3H]norepinephrine ([3H]NE) was measured from the isolated guinea-pig and human right atrium and the effect of activators and inhibitors of ATP sensitive K⁺ channels was studied. Results: Cromakalim (30–300 μM), a K ATP channel-agonist decreased concentration-dependently the stimulation-evoked release of NE from the guinea-pig atrium, an effect, antagonized by glibenclamide, a K ATP channel-antagonist (30 μM). Diazoxide (30–300 μM), another activator of the K ATP channels reduced the resting release of NE, and also attenuated the evoked release at a single concentration (100 μM), and this latter action was also counteracted by glibenclamide (30 μM). Pinacidil, increased dose-dependently the resting and stimulation-evoked release of NE in a glibenclamide-sensitive manner and reversed the inhibitory effect of cromakalim (100 μM), suggesting that it acts as an antagonist. Glibenclamide (30–300 μM), by itself enhanced the stimulation-evoked release of [3H]NE, and also increased the resting release of NE. On the other hand, 5-hydroxydecanoate, an ischemia-selective inhibitor of cardiac K ATP channels did not change NE release. Adenosine, (30–300 μM), an A1-receptor agonist, clonidine (3 μM), an α2-adrenoceptor agonist and oxotremorine, a muscarinic receptor agonist (30 μM) all reduced the evoked release of [3H]NE, but these effects were not modified by glibenclamide (300 μM), indicating that neuronal adenosine (A1), adrenergic (α2) and muscarinic (M1) receptors do not act on K ATP channels. In the human right atrium, cromakalim, and diazoxide did not affect significantly the release of [3H]NE. However, glibenclamide (30–300 μM) and pinacidil (30–300 μM) enhanced dose-dependently the evoked-release of NE, and pinacidil also augmented the resting release. Conclusions: Our results indicate that sympathetic nerve endings of the human and guinea-pig atrium are endowed with ATP-sensitive K⁺ channels. These channels responded to agonists and antagonists under the experimental conditions applied and they could modulate the release of NE thereby affecting the autonomic control of cardiac function under various physiological and pathophysiological conditions. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: ATP sensitive potassium channels; Norepinephrine; Release; Atrium; Human; Guinea-pig

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1. Introduction

ATP sensitive K⁺ channels (K ATP channels), identified

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in cardiac muscle for the first time by Noma [1] in 1983, have received particular attention in cardiovascular research in the recent years. Since their natural activity is regulated by the intracellular ATP/ADP ratio, as a measure of the energy supply of the cell [2,3], they provide a link between the metabolic and electrophysiological states of myocardium, and their role as an endogenous cardioprotectant has been proposed [4–7]. In fact, opening of myocar-
dial $K_{\text{ATP}}$ channels, either by depletion of ATP as an endogenous trigger or by specific $K_{\text{ATP}}$ channel openers, such as cromakalim or pinacidil, results in cardioprotection during ischemia [8,9], while their inactivation by sulphonylurea antidabetics, such as glibenclamide worsens the recovery of post-ischemic contractile function [8] and antagonizes $\alpha_1$ adenosine receptor-mediated cardioprotection [10–12]. In addition to direct effect on myocardial contractility, $K_{\text{ATP}}$ channels are also involved in the regulation of coronary flow both by themselves and as a mediator of the vasodilator action of adenosine [13]. However, due to its complexity, the precise mechanism of cardioprotection by $K_{\text{ATP}}$ channels is not fully understood yet [5,7].

Since ATP-sensitive potassium conductance is also present in neurons [5], the possibility arises that the release of norepinephrine (NE) from sympathetic nerves supplying the heart can be modulated via these channels which also might affect cardiac function during physiological and pathophysiological conditions. It is well known that heart rate and myocardial contractility can be influenced by autonomic innervation. Sympathetic and vagal transmission are in crosstalk: ACh released in response to ongoing activity of the vagus is able to inhibit the release of NE from the nerve terminal, an effect mediated via $M_1$-receptors located presynaptically in guinea-pigs [14,15] and in humans [16]. In addition, the release of NE is also subject to autoinhibitory modulation via $\alpha_2$-autoreceptors [17]. $K_{\text{ATP}}$ channels have been shown to modulate the release of acetylcholine from the isolated atrium of the rat [18], but no data are available on the modulation of NE release. Adenosine, another cardioprotective modulator [19], is able to inhibit NE release from sympathetic nerve in the heart, but not from the vagus, during normoxic [20] and hypoxic conditions [21]. Since adenosine is released in excess during ischemia [22], and part of its actions is mediated via $K_{\text{ATP}}$ channels [12,13,23] the involvement of $K_{\text{ATP}}$ channels in its presynaptic effects has also potential interest.

Therefore, the main question addressed in this study was, whether the release of NE from the sympathetic nerves of the guinea pig and human isolated right atrium taken from patients undergoing heart surgery is regulated by $K_{\text{ATP}}$ channels. In addition their possible interaction with the inhibitory neuromodulation by $\alpha_1$-adenosine-, $M_1$-muscarnic heteroreceptors, and $\alpha_2$-autoreceptors was also examined.

2. Methods

2.1. [$^3$H]Norepinephrine release experiments on the isolated right atrium of the guinea-pig

Male albino guinea-pigs (Richter Gedeon, Budapest, Hungary) of 300 to 500 g body weight were lightly anesthetized with sevoflurane and then sacrificed by a blow on the head, and the right atria were dissected. After dissection, the tissues were incubated for 40 min at 37°C in modified Krebs’ solution containing 10 $\mu$Ci/ml 1-(7,8)-[$^3$H]norepinephrine (2.7 $\times$ 10$^{-3}$ M, specific activity 37 Ci/mmole, Amersham) ascorbic acid (3 $\times$ 10$^{-5}$M) and Na$_2$EDTA (10$^{-4}$ M). The Krebs’ solution was aerated with 95% O$_2$+5% CO$_2$ throughout the experiment. To facilitate the uptake of $^3$H-norepinephrine ([$^3$H]NE), the preparations were stimulated during incubation at 1 Hz with supramaximal (35 V/cm) electrical field stimulation of 1-ms duration, through two platinum electrodes placed above and below the suspended atria, respectively.

After incubation, to remove excess [$^3$H]NE, the atria were transferred to baths of 4 ml and were superfused at a rate of 1 ml/min by a peristaltic pump for 90 min with Krebs’ solution. Following a 90-min washout period, superfusion was continued at the rate of 1 ml/min and 3-min fractions of the superfusate were collected until the end of the experiment. Starting at the beginning of the 10th ($S_1$), 28th ($S_2$), 46th ($S_3$) min, the preparations were stimulated electrically for 2 min at 2 Hz (240 stimuli). Except for the stimulation rate, the method of stimulation was the same as during incubation. Compounds to be investigated were added in perfusing Krebs’ solution 6 min after $S_2$, except for control experiments.

2.2. Measurement of radioactivity

A 1-ml aliquot of each collected fraction was transferred to scintillation vials containing 7 ml of scintillation fluid (Echoscint, National Diagnostics). The radioactivity of the samples was measured in a scintillation beta spectrophotometer (Tri-carb 4530, Packard Instrument). At the end of each experiment, the residual $^3$H content of the atria was determined. The tissue was kept overnight in 1 ml of tissue solubilizer (Soluvable, NEN Research Product) at room temperature, centrifuged and the $^3$H content of the sample was measured. The radioactivity of samples was expressed in terms of disintegrations per gram wet weight of tissue (Bq/g). To normalize the quantity of radioactivity released, the release of [$^3$H]NE was expressed as a percentage of the total radioactivity present in the tissue when the sample was collected (fractional release).

The spontaneous (resting) release of $^3$H is determined by measuring the $^3$H content of two, non-stimulated, 3-min fractions before and after each stimulation period. The regression line of the resting release is determined with exponential curve fitting. The evoked release of $^3$H ($FS_1$, $FS_2$, $FS_3$) is determined by the area under the curve method, i.e. by subtracting the resting release from the actually measured $^3$H release during and after the stimulation. It was reported in previous studies that 88% of the stimulation-induced increase of $^3$H release under identical experimental conditions was due to the increased release of
The effect of drugs on the evoked release of \[^{1}H\]NE was estimated as the \(FS_{2}/FS_{1}\) ratio compared to the \(FS_{2}/FS_{1}\) ratio in the same experiment, which were very similar in control experiments. Therefore, increase or decrease of \(FS_{2}/FS_{1}\) over \(FS_{2}/FS_{1}\) indicates augmentation or inhibition of stimulated NE release, respectively. The effect of drugs on the resting release of \[^{1}H\]NE was assessed as the \(FR_{2}/FR_{1}\) ratio compared to \(FR_{2}/FR_{1}\) ratio in the same experiments, where \(FR_{2}, FR_{2}\) and \(FR_{3}\) are the release measured during the prestimulation sample of the correspondent stimulation (\(S_{1}, S_{2}\) and \(S_{3}\)).

2.3. \[^{1}H\]Norepinephrine release experiments on the isolated human atrial muscle

Right atrial muscle (26.8±0.61 mg) were obtained from operations of 20 male patients, randomly selected from elective cardiac operations performed in the Department of Vascular and Cardiovascular Surgery, Semmelweis Medical University, Budapest, Hungary. The study was not limited to any type of operation or day of the week, no tissue was removed because of this study, and biopsies were done only when technical laboratory personnel and laboratory facilities were available to process the specimens. Thus the operations might be any cardiac operations, and the patients included in the study reflect the general distribution of patients requiring cardiac surgery in our department. The age of patients varied from 46 to 70 years (57±2) and informed consent had been obtained in every case. The applied operation were aorto-coronary bypass (fourteen patients), aortic valve replacement (two patients), mitral valve replacement (three patients) and graft interposition of the ascending aorta (one patient). The daily medication with digitalis, diuretics, antiarrhythmic drugs, glyceryl trinitrate, antihypertensive drugs and \(\beta\)-blocking agents was continued until the cardiac operation. Standard premedication (diazepam+flunitrazepam), induction anesthesia (oxygen+nitrous oxide, fentanyl, dehydrobenzperydol) and muscle relaxation (pipercuronium) were employed. No drugs that can influence tissue catecholamine stores were used, nor was catecholamine support given. The resection specimen from right atrial muscle was excised and were placed in bubbled (95% \(O_{2}\)-5% \(CO_{2}\)) Krebs' solution immediately and kept there during the transport period (30–40 min) at 10–17°C.

After removal and transportation the tissue was cut into 8–12 mg pieces and incubated in 1 ml of modified Krebs buffer (see above) containing 1-(7,8)-\[^{1}H\]norepinephrine (10 \(\mu\)Ci/ml; 37 Ci/mmol, Amersham), ascorbic acid (3·10\(^{-5}\)M) and \(Na\)\(_{2}\)EDTA (10\(^{-4}\) M) for 45 min. Experiments were carried out at 37°C in modified Krebs solution, continuously saturated with carbogen gas (95% \(O_{2}\)-5% \(CO_{2}\)). After incubation, two to three pieces were transferred to each of four thermoregulated plexiglas microvolume chambers (inside volume 100 \(\mu\)l) and the tissues were perfused at a rate of 0.5 ml/min. After 60-min preperfusion, the outflow was collected in 1.5-ml (3-min) fractions for an additional 60 min. During the collection period, the preparations were stimulated electrically twice at 60 V, 2 Hz, 1 ms impulse duration for 2 min (240 pulses), as described above, during the 3rd and 13th collection periods (\(S_{1}\) and \(S_{2}\) ). Drugs were added at the 8th fraction to the perfusion fluid and they were maintained till the end of the experiment. At the end of the perfusion period, the tissues were removed from the perfusion chamber and suspended in 500 \(\mu\)l of 10% trichloroacetic acid and sonicated. Aliquots of the perfusate (0.5 ml) and an aliquot (100 \(\mu\)l) of the tissue were assayed for radioactivity. The measurement of radioactivity and calculation process of the data was the same as described above. The effects of drugs on the electrical stimulation-induced outflow were expressed by the calculated ratio of fractional release \(S_{2}\) over fractional release \(S_{1}\) \((FS_{2}/FS_{1})\) measured in the presence and absence of the drug, whereas their effects on the release of \[^{1}H\]norepinephrine at rest was calculated as the ratio of the values of the second and the 12th fractions \((FR_{2}/FR_{1})\).

The experimental protocol was approved by the Hungarian Research and Ethics Committee (Protocol 30-11/ ETT).

2.4. Statistics

The mean±S.E.M. of data are presented. For statistical analysis, Student \(t\)-test, one way analysis of variance (ANOVA) followed by Dunnett or Bonferroni test was used. A value of \(P<0.05\) was considered to be significant.

2.5. Drugs

The following drugs were used: 1-(7,8)-\[^{1}H\]norepinephrine (specific activity 37 Ci/mmole; Amersham), cromakalim, oxotremorine (SIGMA), adenosine, diazoxide, glibenclamide, clonidine, 5-hydroxidecanoate and pinacidil (RBI). Cromakalim and glibenclamide was dissolved in DMSO (0.3–0.6%), pinacidil and 5-hydroxidecanoate were dissolved in 2-hydroxypropyl-\(\beta\)-cyclodextrin (RBI). Control experiments were performed in the presence of drug-free solvents, using identical conditions applied for drug application.

The composition of the modified Krebs' solution was (mM): \(NaCl\) 113; \(CaCl_{2}\) 1.4; \(KCl\) 4.7; \(KH_{2}PO_{4}\) 1.2; \(NaHCO_{3}\) 25; \(MgSO_{4}\) 0.9; and glucose 11.5.

3. Results

3.1. \(^{1}H\)Norepinephrine release experiments on the isolated right atrium of the guinea-pig

After loading with \[^{1}H\]NE and washout, the tissue
contained $1.068 \times 10^6 \pm 6.457 \times 10^4$ Bq/g radioactivity ($n=91$). The resting release in the first 3-min collection period was $3872 \pm 250$ Bq/g (0.32$\pm 0.02$% of the total radioactivity, $n=91$), and it was relatively constant from one collection period to the next throughout the experiment. Radioactivity released by the first stimulation ($S_1$) was $10.455 \pm 670$ Bq/g, which represents $1.20 \pm 0.03$% ($n=91$) of the radioactivity present at the start of stimulation. The peak response to consecutive stimulation periods were $0.87 \pm 0.09$, $0.85 \pm 0.09$, and $0.83 \pm 0.08$, yielding $FS_1/FS_1$, and $FS_2/FS_2$ ratios of $1.02 \pm 0.01$ and $0.97 \pm 0.01$ ($n=8$) in control experiments, respectively. This indicates that the amount of fractional release in response to consecutive stimulations remained relatively constant. In the presence of tetrodotoxin (1 $\mu$M) there was no detectable increase in $^3$H release in response to stimulation, indicating that evoked release of tritium is due to the propagation of axon potential along the neuronal membrane. In our previous studies it was reported that 88% of the stimulation-induced increase of $^3$H release under identical experimental conditions was due to the increased release of $[^3H]$NE [14,19] i.e it is comparable to endogenous NE.

3.2. Effect of $K_{ATP}$ channel activators and inhibitors on the resting and stimulation-evoked release of $[^3H]$NE

Cromakalim, a $K_{ATP}$ channel activator, reduced the stimulation-evoked release of NE in a concentration-dependent manner, while it did not affect the resting outflow (Figs. 1A and B). The maximal inhibition was observed at 300 $\mu$M concentration, when the evoked release was reduced by $\approx 20$%; no further inhibition above this concentration could be obtained (data not shown). The inhibitory effect of cromakalim (100 $\mu$M) was effectively antagonized by glibenclamide (30 $\mu$M), a selective inhibitor of $K_{ATP}$ channel (Fig. 1A).

Diazoxide, another $K_{ATP}$ channel activator also decreased the stimulation evoked $[^3H]$NE overflow, but significant effect on stimulation evoked release was detected only in a single concentration (100 $\mu$M) (Fig. 2A). In addition, diazoxide remarkably decreased the resting release of NE at all three concentrations tested (30–300 $\mu$M) (Fig. 2B). Glibenclamide (30 $\mu$M) counteracted the effect of diazoxide on the evoked release of $[^3H]$NE, but not on the resting outflow (Figs. 2A and B).

Pacacidil, which is also known as a $K_{ATP}$ channel opener, interestingly did not reduce, but increased dose-dependently the basal and stimulation-evoked release of NE (Figs. 3A and B). In the presence of glibenclamide (300 $\mu$M) pacacidil (100 $\mu$M) was unable to further increase the evoked release of NE (Fig. 3A). Furthermore, at a concentration of 300 $\mu$M, it antagonized the inhibitory effect of cromakalim (100 $\mu$M), a $K_{ATP}$ channel opener on evoked release of NE: the $FS_1/FS_1$ ratios were $0.82 \pm 0.02$ and $2.18 \pm 0.18$ in the presence of cromakalim and cromakalim+pacacidil, respectively ($n=4$, $P<0.01$). These findings indicate that pacacidil acts on neuronal tissue (sympathetic axon terminal) as an antagonist, preventing the effect of an agonist (cromakalim) and competing with

![Fig. 1. Effect of cromakalim on the stimulation-evoked (A) and resting (B) release of $[^3H]$NE from the isolated right atrium of the guinea-pig.](image)
Fig. 2. Effect of diazoxide on the stimulation-evoked (A) and resting (B) release of \[^3\text{H}\]NE from the isolated right atrium of the guinea-pig. Experimental protocol and calculation of data was as in Fig. 1.

Fig. 3. Effect of pinacidil on the stimulation-evoked (A) and resting (B) release of \[^3\text{H}\]NE from the isolated right atrium of the guinea-pig. Experimental protocol and calculation of data was analogue to Fig. 1.

another antagonist (glibenclamide) for a common binding site.

Glibenclamide, (30–300 μM), a K\textsubscript{ATP} channel inhibitor, when added to the superfusion fluid 12 min prior to third stimulation and kept in the fluid throughout the experiment, enhanced both the resting and evoked-release of NE (Figs. 4A and B). These effects were also concentration-dependent between 30 and 300 μM, and the maximal effect obtained by 300 μM, glibenclamide, which resulted in a more than 150% increase of the evoked release.

5-Hydroxidecanoate (5-HD), which is known as another, inhibitory effect of adenosine, oxotremorine or clonidine but can be antagonized by inhibition of K\textsubscript{ATP} channels. Adenosine (30–300 μM) decreased the evoked release of \[^3\text{H}\]H in a concentration dependent manner and the maximum inhibition that could be achieved with adenosine (300 μM) was 34%, which is in agreement with that obtained by earlier studies [20] (data not shown). However, the effect of

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5-Hydroxidecanoate (5-HD), which is known as another, structurally unrelated inhibitor of K\textsubscript{ATP} channels, was also examined on resting and stimulation-evoked NE release. However, 5-HD (100 μM), did not affect significantly the resting and stimulation-evoked release of NE under our experimental conditions, the FR\textsubscript{1}/FR\textsubscript{2} and FS\textsubscript{1}/FS\textsubscript{2} ratios were 0.97±0.01 and 0.97±0.05 in the presence of 5-HD, respectively (P<0.05, n=4 vs. control FR\textsubscript{1}/FR\textsubscript{2} and FS\textsubscript{1}/FS\textsubscript{2} ratios).

3.3. Interaction of the K\textsubscript{ATP} channel inhibitor, glibenclamide with different presynaptically acting drugs

Since it is well established that the release of NE is subjected to presynaptic modulation by endogenous ligands, via stimulation of receptors expressed on noradrenergic terminals [14–17,20], we also studied whether the inhibitory effect of adenosine, oxotremorine or clonidine can be antagonized by inhibition of K\textsubscript{ATP} channels. Adenosine (30–300 μM) decreased the evoked release of \[^3\text{H}\]H in a concentration dependent manner and the maximum inhibition that could be achieved with adenosine (300 μM) was 34%, which is in agreement with that obtained by earlier studies [20] (data not shown). However, the effect of
Fig. 4. Effect of glibenclamide on the stimulation-evoked (A) and resting (B) release of [\(^{3}H\)]NE from the isolated right atrium of the guinea-pig. The effect of glibenclamide on stimulation-evoked release was expressed as $FS_{2}/FS_{1}$ ratios in the presence ($FS_{2}/FS_{1}$, solid bars) and absence ($FS_{2}/FS_{1}$, stippled bars) of glibenclamide. The effect of glibenclamide on resting release was expressed as $FR_{2}/FR_{1}$ ratios, measured in the presence ($FR_{2}/FR_{1}$, solid bars) and absence ($FR_{2}/FR_{1}$, stippled bars) of glibenclamide (for calculation see Section 2). For experimental protocol and statistical analysis, see Fig. 1.

Fig. 6. Release of [\(^{3}H\)]norepinephrine ([\(^{3}H\)]NE) in the absence (control) and in the presence of 300 \(\mu\)M glibenclamide (Glib) measured from isolated human atrial muscle after preincubation of 1 \(\mu\)Ci/ml [\(^{3}H\)]norepinephrine ([\(^{3}H\)]NE). Following 60 min preperfusion 3 min samples were collected. The preparations were field stimulated (2 Hz, 240 shocks) as indicated ($S_{1}, S_{2}$). Glibenclamide was applied to perfusion medium 15 min before $S_{1}$. The release of tritium is expressed as fractional release. For calculation and other experimental details, see Section 2. Data show the mean±S.E.M. of 6–6 identical experiments in the absence and presence of glibenclamide, respectively.

3.4. [\(^{3}H\)]Norepinephrine release experiments on the isolated human atrial muscle

The uptake of radioactivity in the human right atrium was $1.014±0.53\times10^{6}$ Bq/g ($n=55$). Under resting conditions $0.29±0.01\%$ of the actual tritium content was released ($n=55$). Electrical field stimulation resulted in an increase in the amount of [\(^{3}H\)]NE released into the perfusate (stimulation evoked release: $0.743±0.029\%$, $n=55$) which was reproducible during the consecutive stimulations, yielding an $S_{2}/S_{1}$ ratio of $0.80±0.03$ in control experiments ($n=6$) (Fig. 6).

Out of the $K_{ATP}$ channel activators, neither cromakalim, adenosine was not antagonized by the $K_{ATP}$ channel antagonist, glibenclamide (300 \(\mu\)M) (Fig. 5). Similarly, clonidine (3 \(\mu\)M), an $\alpha_{2}$-agonist, and oxotremorine (30 \(\mu\)M), a muscarinic receptor agonist reduced powerfully the evoked release of [\(^{3}H\)]NE but neither of their effect was antagonized by glibenclamide, the $K_{ATP}$ antagonist (Fig. 5).
Table 1
Effect of ATP sensitive K⁺ channels (K<sub>ATP</sub> channel) activators and inhibitors, on the resting and stimulation-evoked release of [³¹H]norepinephrine ([³¹H]NE), measured from the isolated human right atrium

<table>
<thead>
<tr>
<th>Drug (µM)</th>
<th>n</th>
<th>FR&lt;sub&gt;I&lt;/sub&gt;/FR&lt;sub&gt;S&lt;/sub&gt;</th>
<th>FS&lt;sub&gt;I&lt;/sub&gt;/FS&lt;sub&gt;S&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>3</td>
<td>0.787±0.037</td>
<td>0.746±0.068</td>
</tr>
<tr>
<td>Cromakalim 300</td>
<td>4</td>
<td>0.790±0.039</td>
<td>0.690±0.195</td>
</tr>
<tr>
<td>−</td>
<td>4</td>
<td>0.758±0.071</td>
<td>0.899±0.092</td>
</tr>
<tr>
<td>Diazoxide 300</td>
<td>4</td>
<td>0.634±0.038</td>
<td>0.914±0.020</td>
</tr>
<tr>
<td>−</td>
<td>6</td>
<td>0.803±0.036</td>
<td>0.807±0.102</td>
</tr>
<tr>
<td>Glibenclamide 30</td>
<td>4</td>
<td>0.842±0.038</td>
<td>0.769±0.089</td>
</tr>
<tr>
<td>Glibenclamide 100</td>
<td>6</td>
<td>0.803±0.018</td>
<td>1.089±0.061*</td>
</tr>
<tr>
<td>Glibenclamide 300</td>
<td>6</td>
<td>0.906±0.049</td>
<td>1.399±0.107**</td>
</tr>
<tr>
<td>−</td>
<td>3</td>
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</tr>
<tr>
<td>Pinacidil 30</td>
<td>4</td>
<td>0.931±0.022</td>
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<tr>
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<td>5</td>
<td>0.989±0.045</td>
<td>1.260±0.100*</td>
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<tr>
<td>Pinacidil 300</td>
<td>6</td>
<td>1.339±0.049**</td>
<td>1.500±0.112**</td>
</tr>
</tbody>
</table>

* All results show mean±S.E.M. n=number of experiments. The effect of drugs on resting release was expressed as FR<sub>I</sub>/FR<sub>S</sub>, and FS<sub>I</sub>/FS<sub>S</sub> ratios, measured in the presence and absence of the drugs. The effect of drugs on stimulation-evoked release was expressed as FS<sub>I</sub>/FS<sub>S</sub> ratios, measured in the presence and absence of the drug (for calculation see Section 2). All drugs applied into the perfusion fluid 15 min before S.<sub>S</sub>. Control experiments were not pooled; the effect of each drug was compared to values of drug-free control experiments, obtained from the same patients. Asterisks represent significant differences from the respective controls (*P<0.05, **P<0.01) calculated by ANOVA followed by Dunnett test.

nor diazoxide affected significantly the release of [³¹H]NE (Table 1). On the other hand, glibenclamide (30–300 µM), and pinacidil (30–300 µM) enhanced dose-dependently the evoked release of NE, showing that the release of NE in human right atrium could also be controlled by K<sub>ATP</sub> channel modulators, however, the maximal effect, which was reached at 300 µM concentration was less than obtained in the guinea-pig preparation (Table 1, Fig. 6). In addition pinacidil, but not glibenclamide (Table 1) increased also the resting release of tritium.

4. Discussion

In this study, the modulation of NE release from sympathetic nerve endings by K<sub>ATP</sub> channel activators and inhibitors was studied in the isolated, superfused right atrium of the guinea-pig and in isolated human right atrium obtained from cardiovascular surgery specimen. Out of the K<sub>ATP</sub> channel openers, cromakalim and diazoxide reduced the stimulation-evoked release of [³¹H]NE, and their effects could be antagonized by glibenclamide, the specific K<sub>ATP</sub> channel antagonist suggesting that sympathetic nerve terminals supplying the cardiac tissue of the guinea-pig are equipped with K<sub>ATP</sub> channels and the release of NE is subject to modulation by these channels. In our experiments, the maximal inhibitory effect obtained by K<sub>ATP</sub> channel openers i.e. by cromakalim and diazoxide was relatively small ~20%, whereas glibenclamide, the K<sub>ATP</sub> channel antagonist was able to increase the evoked release up to 250%. Therefore it seems likely that the majority of the K<sub>ATP</sub> channels on the noradrenergic nerve endings are open under our conditions, these channels were inactivated by glibenclamide, and potassium ions flowing out these channels might regulate the membrane potential and the amount of noradrenaline released by axonal activity. On the other hand, a much smaller proportion of the channels seems to be inactive – which explains the moderate efficacy of cromakalim and diazoxide to reduce the release of NE – these could be activated by K<sub>ATP</sub> channel openers, or by pathological signals, such as energy depletion. In our study, relatively high concentrations of K<sub>ATP</sub> channel modulators were needed to obtain significant effects, which warrant further explanation. One possible reason is that K<sub>ATP</sub> channel activators and glibenclamide operate not only on K<sub>ATP</sub> channels but also on other ion conductances when applied in high concentrations which also have impact on neurotransmitter release. However, there are several arguments supporting the view that their effects are related to the specific activation of K<sub>ATP</sub> channels: (i) The effective concentration of K<sub>ATP</sub> channel openers and inhibitors (i.e. high micromolar) used in our study is different from studies on vascular smooth muscle or pancreatic beta cells, but similar to concentration range used in other neurotransmitter release studies using K<sub>ATP</sub> channel ligands (see e.g. the paper Takata et al. [24]). Tissue geometry, diffusion properties and K<sub>ATP</sub> channel density and distribution are different in these preparations which might be responsible for the discrepancy; (ii) the effect of agonists (cromakalim, diazoxide) could be prevented by relatively low concentration of glibenclamide (30 µM).

In addition to antagonism of the effect of cromakalim, diazoxide and pinacidil, glibenclamide by itself powerfully enhanced the resting and stimulation-evoked release of NE. Although originally was regarded as a specific K<sub>ATP</sub> channel antagonist, recent observations indicate that glibenclamide can cause effects, unrelated to K<sub>ATP</sub> channel blockade, and a part of these effects may also influence neurotransmitter release [25]. In fact glibenclamide has been shown to affect other ionic conductances present on neurons such as voltage-gated, Ca<sup>2+</sup>- and ATP independent K⁺ current [26] and Ca<sup>2+</sup> activated K⁺ current [27], which may also account for the increase in NE release. Furthermore it also has impact on metabolic processes e.g. it blocks mitochondrial K<sub>ATP</sub> channels [28], as well as 5'-nucleotidase enzyme [29], involved in purine metabolism. In the lack of selective pharmacological tools it is difficult to elucidate whether any of these actions do contribute to the enhancement of NE release by glibenclamide. Nevertheless, the effect of another K<sub>ATP</sub> channel inhibitor, 5-HD was also tested. 5-HD has been widely used to block ischemia-selective activation of K<sub>ATP</sub> channels in cardiovascular research [30]; it is regarded to be a structurally unrelated, but more specific blocker of K<sub>ATP</sub>
channels than glibenclamide. However, 5HD did not alter the resting and the stimulation-evoked release of NE indicating that the effect of glibenclamide to increase NE release was independent on the activation of KATP channel. The affinity of 5-hydroxydecanoate to bind the sulphonylurea receptor or to KATP channel subunits has not been tested so far. Therefore, yet another possible explanation for its ineffectiveness is that KATP channels on sympathetic nerve terminals are pharmacologically different from myocardial KATP channels, and 5-HD binds selectively to mitochondrial KATP channels, rather than to those on plamalemmal ones, sensitive to glibenclamide.

Since both KATP channel openers and inhibitors are used in clinical practice, their effects on autonomic neuroregulation are of potential interest regardless of the fact that they are the consequence of KATP channel activation or not. In spite of the possible clinical relevance, surprisingly few data are available in the literature on the prejunctional modulation of noradrenergic transmission by KATP channel activators and inhibitors. In the periphery, Soares de Silva and Fernandes reported inhibitory effect of pinacidil on the release of NE in the vas deferens [31], while Fabiani and Story [32] failed to find any prejunctional effect of different KATP channel activators in the rat isolated mesenteric artery. On the other hand, consistently with our results, Takata et al. [24], observed inhibitory effect on NE release by diazoxide and cromakalim and facilitatory effect by pinacidil, on rat brain cortical slices and augmentation of NE release by pinacidil from the rabbit pulmonary artery [33] and guinea-pig and rabbit mesenteric arteries [34] was also reported. In our experiments pinacidil was unable to further increase the release of NE in the presence of glibenclamide and it also prevented the inhibitory effect of cromakalim on evoked \[^{1}^{1}H\]NE release indicating that it is able to bind to KATP channels, probably as an antagonist. This paradoxical effect of pinacidil on NE release indicates the pharmacological heterogeneity of the KATP channels located on nerve terminals, vascular smooth muscle, and pancreatic \(\beta\)-cells: opposite from diazoxide and cromakalim, pinacidil did not inhibit, but increased NE release in our study, and in others [24,33,34]; in contrast, pinacidil exert inhibitory effect on vascular smooth muscle contraction [35,36] and on insulin secretion [37] in line with other KATP channel openers. The diversity of KATP channels, expressed in cardiac, pancreatic and neuronal tissue has been recently revealed by molecular biological studies, offering a structural basis for pharmacological heterogeneity [38]. Furthermore, the action of pinacidil to increase NE release from sympathetic nerves supplying the heart corroborates with the tachycardia, a frequent side effect, observed in preclinical studies using pinacidil [39] and provide direct support for the recent observations on the mechanism of proarrhythmic effect of pinacidil in the isolated guinea-pig heart [40].

It is well established that the release of NE in the right atrium is modulated by prejunctional auto- and heteroreceptors [41]; noradrenergic nerve terminals are equipped with inhibitory \(\alpha_{1}\)-adenosine and \(M_{2}\)-muscarinic receptors and \(\alpha_{2}\)-autoreceptors [14-17,20]. The possibility arises, that the action of these prejunctional modulators also involves KATP channels present on the noradrenergic nerve terminals. However, glibenclamide was unable to interact with the inhibitory effect of adenosine, the \(\alpha_{1}\)-receptor agonist, clonidine, the \(\alpha_{2}\)-receptor agonist, and oxotremorine, the muscarinic receptor agonist on stimulation-evoked NE release. Consequently, at least in the guinea-pig atrium, prejunctional receptors and KATP channels provide independent targets to intervene on adrenergic neurotransmission.

The effect of KATP channels activators and inhibitors on NE release from noradrenergic nerves supplying the cardiac tissue was also examined in human right atrium. Although the KATP channel openers (cromakalim and diazoxide) were ineffective to modify the release of NE, glibenclamide and pinacidil increased dose-dependently the evoked-release, suggesting that the release of NE might be subject to modulation by KATP channels. Since the natural activity of KATP channels are regulated by the energy charge of the cell [2], it is reasonable to assume that the energy supply of human cardiac tissue has lowered during the transportation even under the best circumstances, and increased the number of open channels which help to explain the ineffectiveness of KATP channel openers in these specimens. However, the effect of pinacidil and glibenclamide was also relatively moderate in the human tissue in comparison to the guinea pig right atrium, which may reflect to the different density of KATP channels on the sympathetic nerves of the human and guinea pig heart. Finally one has to bear in mind that the human atrial tissue was obtained not from healthy population but from individuals bearing serious cardiovascular disease, and impaired cardiac function.

Our observations has some important pathophysiological and clinical implications. It is widely accepted that autonomic innervation of the heart is an important regulatory mechanism, which modulates cardiac function upon a variety of pathological situations. It is also well known that the natural activity of KATP channels are regulated by [ATP], as an endogenous signal, i.e. the majority of channels are closed under physiological energy charge [2,42]. When intracellular energy stores are depleted, the blockade of KATP channels is relieved and inactive channels are activated. Therefore, KATP channels provide an endogenous protective mechanism, which could play a role during metabolic stress, hypoxia, hypoglycemia and ischemia. Decreased release of NE under these conditions might result in a drop in heart rate and protection of myocardium. However, one has to take into account that during ischemia, in addition to exocytotic release, a massive release of NE occurs in a \(Ca^{2+}\)-independent fashion [43,44], which may overshadow the reduction of exocytotic release. In addition, KATP channels are also
important as the target site of K_{ATP} channel activator drugs used in the cardiovascular research and therapy and as the target site of sulphonylurea antidiabetics. Our findings add information to their precise site of action and indicate that K_{ATP} channels present on neurons, vascular smooth muscle and pancreas β-cells are pharmacologically different, which may help to develop new potential drugs on this field with less side effects.

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