Effects of Glibenclamide on Negative Cardiac Responses to Cholinergic and Purinergic Stimuli and Cromakalim in the Isolated Dog Heart

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ABSTRACT—We investigated the effects of an ATP-sensitive K⁺ channel blocker, glibenclamide, on the negative chronotropic and inotropic responses to intracardiac parasympathetic nerve stimulation, acetylcholine (ACh, a muscarinic receptor agonist), ATP (a P₂-purinergic receptor agonist), adenosine (a P₁-purinergic receptor agonist) and cromakalim (a potassium channel opener) in the isolated, blood-perfused canine right atrium or left ventricle. A high dose of glibenclamide (3 µmol) did not affect the negative chronotropic and inotropic responses to parasympathetic stimulation (frequencies of 1–30 Hz), although it slightly but significantly attenuated the negative cardiac responses to exogenous ACh (0.3–10 nmol). Furthermore, adenosine (0.03–0.3 µmol)-induced negative chronotropic and inotropic responses were significantly inhibited by glibenclamide (3 µmol), but ATP (0.01–1 µmol)-induced negative cardiac responses were not affected. A cumulative administration of cromakalim (0.01–1 µmol) dose-dependently caused much greater decreases in the contractile force of atrial and ventricular muscles than in sinus rate. Glibenclamide (0.3–3 µmol) similarly blocked the negative chronotropic and inotropic responses to cromakalim in a dose-dependent manner. These results suggest that glibenclamide modifies the negative cardiac responses to parasympathetic activation both in pre- and postjunctional sites and the responses to adenosine but not to ATP at K⁺ channels in the dog heart, although the modifications are minor under physiological conditions.

Keywords: Glibenclamide, Parasympathetic nerve, ATP, Negative cardiac effect, Heart (isolated dog)
which directly activates cardiac ATP-sensitive K⁺ channels and shortens the action potential duration (15, 16).

MATERIALS AND METHODS

Isolated, blood-perfused canine heart preparations

An isolated canine right atrium or left ventricle was perfused with heparinized arterial blood from a support dog. The details of the preparations have been described in previous papers (5, 17).

Right atrial or left ventricular preparations were obtained from 25 recipient dogs that weighed 4–17 kg and were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 USP U/kg, i.v.), the right atrium or the left ventricle was excised and immersed in cold Ringer’s solution of the following composition: 154.0 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl₂ and 3.6 mM NaHCO₃. The wet weight of the atrial and ventricular preparations were 7–13 g and 8–16 g, respectively.

The sinus node artery was cannulated via the right coronary artery and was perfused with heparinized blood delivered from the carotid artery of the support dog with a peristaltic pump (model 1210; Harvard Apparatus, Millis, MA, USA). A pneumatic resistance was placed parallel to the perfusion system to maintain the perfusion pressure at 100 mmHg. The venous effluent from the preparation was led to a collecting funnel, from which it was returned continuously to the support dog via an external jugular vein. The ventricular margin of the atrium was attached to a rigid stainless-steel bar, and the preparation was placed in a glass container which was kept at a constant temperature of 37°C. The superior part of the atrium was connected by a silk thread to a force-displacement transducer (AP 620G; Nihon Kohden, Tokyo). The atrial muscle was stretched to a resting tension of 2 g. The isometric tension was recorded on a thermowriting rectigraph (WT 685T, Nihon Kohden). A pair of silver electrodes was brought into contact with the epicardial surface to record the atrial electrogram from which the sinus rate was derived with a cardiotachometer (AT 600G, Nihon Kohden). Another pair of electrodes, placed posteriorly on the caval margin of the atrium was used to stimulate the intracardiac parasympathetic nerve fibers. The blood flow rate to a preparation was recorded by means of a magnetic flowmeter (MFV 2100, Nihon Kohden). The rate of blood flow to the isolated atrium was 3–10 ml/min.

The left ventricular muscle along the anterior descending branch of the left coronary artery was excised, and the anterior descending artery was cannulated. The preparation was perfused with the heparinized blood from the support dog using the same perfusion system as the right atrial preparation. A pair of bipolar silver electrodes was sewn on the ventricular free wall, and the preparation was driven by an electrical stimulator (SEN 7103, Nihon Kohden) at a frequency of 2 Hz with square-wave pulses of 1–3 msec duration and suprathreshold voltage (4–6 V). The left ventricular tension development was measured isometrically by a force-displacement transducer through a fine thread connected to the ventricular surface. The resting tension of the ventricular muscle was 2 g. The rate of blood flow to the isolated ventricle was 6–14 ml/min.

Twenty-five support dogs weighing 6–25 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and supplemental doses of sodium pentobarbital were applied as necessary to maintain stable anesthesia. Dogs were artificially ventilated with room air by use of a Harvard respirator (model 607). Sodium heparin (500 USP U/kg, i.v.) was administered at the beginning of the perfusion of the isolated atrial or ventricular preparation, and 200 USP U/kg was given each hr thereafter. The femoral arterial blood pressure and heart rate derived from lead II of the ECG of the support dog were recorded simultaneously.

Experimental procedures

In the first series of experiments, we investigated the effects of a high dose of glibenclamide (3 μmol) on the cardiac responses to parasympathetic nerve stimulation (frequencies of 1, 3, 5, 10 and 30 Hz, n=6). ACh (0.3, 1, 3 and 10 nmol, n=7), ATP (0.01, 0.1 and 1 μmol, n=5) or adenosine (0.03, 0.1 and 0.3 μmol, n=7) in the isolated right atrium. Intracardiac parasympathetic nerve fibers were stimulated with a pulse duration of 0.01–0.03 msec at a frequency of 1, 3, 5, 10 or 30 Hz for 10 sec by an electrical stimulator. Voltage was used at a subthreshold level for the atrial muscle and sympathetic nerve fibers, but above the threshold for the intracardiac parasympathetic nerve fibers (usually 10 V) (18). The negative cardiac responses to parasympathetic stimulation were inhibited by tetrodotoxin, hexamethonium or atropine (19). The cardiac responses to parasympathetic stimulation, ACh, ATP or adenosine were observed before and 2 min after the administration of glibenclamide.

In the second series of experiments, we investigated the effects of glibenclamide on the negative chronotropic and inotropic responses to cromakalim in the isolated right atrium (n=6) and on the negative inotropic responses to cromakalim in the isolated left ventricle (n=5). The negative cardiac responses to cumulative administration of cromakalim (0.01–1 μmol) were observed before and after the application of glibenclamide (0.3, 1 and 3 μmol). Each dose of glibenclamide was injected at intervals of more than 30 min, and the cardiac responses to cromakalim were observed 2 min after glibenclamide was administered.
Drugs

The drugs used in the present experiments were glibenclamide (Yamanouchi, Tokyo), acetylcholine chloride (ACh; Daiichi, Tokyo), ATP (Kowa, Tokyo), adenosine (Tokyo Kasei, Tokyo) and cromakalim (Beecham, Harlow, UK). Glibenclamide was dissolved in dimethylsulfoxide. Cromakalim was dissolved in 20% ethanol to make a stock solution of 10 mM, which was then diluted with physiologic saline to obtain lower concentrations. Other drugs were dissolved in physiologic saline before the start of the experiments. Drugs were injected into the sinus node artery of the isolated right atrium or into the anterior descending branch of the left coronary artery of the isolated left ventricle through a rubber tube with a microsyringe (Ito, Shizuoka). The amount of drug solution injected was 0.003–0.03 ml. The cardiac responses to repeated administrations of a drug were steady during the experiment.

Statistical analyses

Data are shown as the maximum change in response to each drug and are expressed as means ± S.E.M. Two-way analysis of variance (ANOVA) was used for the statistical analysis of multiple comparisons of each dose-effect curve. When a significant difference was detected by the two-way ANOVA, the data were analyzed further by the least-significant-difference (LSD) method. Student’s t-test for paired or unpaired data was carried out in comparisons between two groups. Fifty-percent inhibition doses (ID_{50}) of glibenclamide for the chronotropic and inotropic responses to cromakalim in the right atrium and the left ventricle were determined for each dose-inhibition curve. One-way ANOVA was used for comparison among the ID_{50} values. A P value less than 0.05 was considered statistically significant.

RESULTS

Effects of glibenclamide on the cardiac responses to parasympathetic nerve stimulation or ACh in the right atrium

Parasympathetic nerve stimulation at frequencies of 1–30 Hz caused decreases in sinus rate and atrial tension of the isolated, blood-perfused right atrium in a frequen-
cy-dependent manner. Glibenclamide at a dose of 3 μmol, the highest dose used in this study, did not significantly affect the negative chronotropic and inotropic responses to parasympathetic stimulation in six isolated atria (Fig. 1A).

ACh (0.3 - 10 μmol) injected into the sinus node artery also induced the negative chronotropic and inotropic effects in a dose-dependent manner. The dose-effect curves for the negative cardiac responses to ACh in the presence of glibenclamide (3 μmol) were significantly different from the control dose-effect curves both in the chronotropism and inotropism (P < 0.05, analyzed by the two-way ANOVA) (Fig. 1B). Neither sinus rate nor atrial contractile force was influenced by glibenclamide (0.3 - 3 μmol) itself significantly. In addition, parasympathetic- and ACh-induced negative cardiac effects were similarly inhibited by atropine (20).

Effects of glibenclamide on the cardiac responses to ATP or adenosine in the right atrium

ATP (0.01 - 1 μmol) induced negative chronotropic and inotropic effects in a dose-dependent manner. A high dose of glibenclamide (3 μmol) did not significantly affect the negative cardiac responses to ATP in five isolated atria (Fig. 2A). In addition, ATP at a dose of 1 μmol induced two-peaked positive chronotropic phases during a negative chronotropic phase, i.e., initially, a brief positive chronotropic effect (t-1) and secondarily, a relatively longer positive chronotropic effect (t-2) (7). Both t-1 and t-2 were not influenced by glibenclamide (3 μmol): in the absence of glibenclamide, t-1 and t-2 were 25.4 ± 11.4% (n = 5) and 21.6 ± 7.8%, respectively, while in its presence, they were 23.3 ± 10.0% and 19.5 ± 6.6%, respectively.

Adenosine (0.03 - 0.3 μmol) dose-dependently decreased the sinus rate and atrial tension of the isolated atrium. Glibenclamide (3 μmol) slightly but significantly inhibited the negative chronotropic and inotropic responses to adenosine (P < 0.05, analyzed by the two-way ANOVA) (Fig. 2B).

**Effects of glibenclamide on the cardiac responses to cromakalim in the right atrium and left ventricle**

When cromakalim at doses of 0.01 - 0.3 μmol was in-

![Fig. 2. Effects of glibenclamide on the negative chronotropic and inotropic responses to ATP (A) or to adenosine (B) in the isolated, blood-perfused canine right atrium. Points show mean values, and vertical bars represent S.E.M. The basal sinus rate and atrial tension were as follows: A, 111 ± 9 (mean ± S.E.M., n = 5) beats/min and 2.3 ± 0.4 g, respectively; B, 111 ± 3 (n = 7) beats/min and 3.2 ± 0.6 g, respectively. ○, Control; ●, after the treatment with glibenclamide (3 μmol). Significant difference from each control: *P < 0.05, **P < 0.01 (analyzed by the LSD method).]
jected cumulatively into the sinus node artery of the isolated right atrium, it caused a dose-dependent negative inotropic effect and a small negative chronotropic effect (Fig. 3A). Cromakalim at a dose of 0.3 μmol markedly suppressed atrial contractility by 90.6 ± 3.1% (n = 6), but decreased sinus rate by only 17.5 ± 4.6%. Glibenclamide (1 μmol) antagonized the negative cardiac responses to cromakalim (Fig. 3B). The dose-effect curves for the negative chronotropic and inotropic responses to cromakalim were significantly inhibited by glibenclamide (0.3–3 μmol) in a dose-dependent manner (P < 0.01) (Fig. 4).

Cromakalim (0.01–1 μmol) injected cumulatively into the anterior descending branch of the isolated left ventricle decreased ventricular developed tension in a dose-dependent manner. Cromakalim at a dose of 1 μmol suppressed ventricular contractility by 87.8 ± 6.1% (n = 5). Glibenclamide (0.3–3 μmol) dose-dependently antagonized the negative inotropic effects of cromakalim (P < 0.05) (Fig. 5).

ID_{50} values of glibenclamide for the negative chronotropic and inotropic effects of cromakalim at a dose of 0.1 μmol, which induced a submaximum negative inotropic response (−72.0 ± 5.4%, n = 6) in the right atrium.
um, were 0.76±0.21 and 0.94±0.25 μmol, respectively. The ID₅₀ value of glibenclamide for the negative inotropic effects of cromakalim at a dose of 0.3 μmol, which induced a submaximum negative inotropic response (−65.6±11.9%, n = 5) in the left ventricle, was 0.54±0.11 μmol. These ID₅₀ values for the sinus rate, atrial tension and ventricular tension decreases evoked by cromakalim were not significantly different among the three groups.

DISCUSSION

In the present study, we demonstrated that the ATP-sensitive K⁺ channel blocker glibenclamide did not change the negative chronotropic and inotropic responses to parasympathetic nerve stimulation, but attenuated the negative cardiac responses to exogenous ACh in the isolated, blood-perfused canine right atrium. Furthermore, ATP-induced negative chronotropic and inotropic effects were not affected by glibenclamide, although adenosine-induced negative cardiac effects were inhibited. Compared with the effects on the negative cardiac responses to ACh or adenosine, glibenclamide showed a greater blocking effect on the cardiac responses to cromakalim. These results suggest that glibenclamide modifies the cardiac effects of parasympathetic activation both in pre- and postjunctional sites, and that ATP-induced cardiac responses are not mediated by an activation of cardiac ATP-sensitive K⁺ channels in the dog heart.

We previously reported that parasympathetic nerve stimulation induced the negative chronotropic and inotropic responses when the pulse duration of stimulation was adequately short (less than 0.3 msec) (19). The negative cardiac responses to parasympathetic stimulation were inhibited by tetrodotoxin, hexamethonium or atropine, suggesting that they are evoked by ACh released from the parasympathetic nerve terminals. Furthermore, glibenclamide at a dose of 3 μmol adequately inhibits cardiac ATP-sensitive K⁺ channels, since the negative chronotropic and inotropic effects of cromakalim or pinacidil, which directly activates ATP-sensitive K⁺ channels, were antagonized by glibenclamide in the isolated atrium and ventricle (Figs. 3–5) (13). The negative chronotropic and inotropic responses to ACh or adenosine are due to an activation of muscarinic or P₁-purinergic receptors, respectively, which interact with receptor-coupled K⁺ channels mediated by GTP-binding proteins (8). A recent study by Belloni and Hintze (14) showed that bradycardia induced by exogenous adenosine was attenuated by glibenclamide, although bradycardia caused by electrical stimulation of the right vagus nerves was not affected in the dog. They suggested that adenosine-induced and vagally-induced bradycardia did not appear to share a common ionic basis, i.e., adenosine activates both receptor-coupled and ATP-sensitive K⁺ channels but ACh does not activate ATP-sensitive K⁺ channels in the sinus node. In the present study, parasympathetic stimulation-induced negative chronotropic and inotropic effects were not changed by glibenclamide (Fig. 1A), which was consistent with the results of Belloni and Hintze (14). However, decreases in the sinus rate and atrial tension evoked by exogenous ACh or adenosine were slightly but significantly inhibited by glibenclamide (Figs. 1B and 2B), suggesting that muscarinic receptors as well as P₁-purinergic receptors are related in part to ATP-sensitive K⁺ channels, as reported previously (11–13). Thus, it is likely that the different effects of glibenclamide between parasympathetic stimulation-induced and ACh-induced negative cardiac responses are related to a simultaneous action of glibenclamide on the prejunctional and postjunctional sites in the heart.

Potassium channels prejunctionally modulate the cholinergic neurotransmission such as the parasympathetic stimulation-induced release of ACh. 4-Aminopyridine, a transient outward K⁺ current blocker, potentiated the negative chronotropic and inotropic responses to parasympathetic nerve stimulation in the isolated, blood-perfused canine right atrium (21). Recently, it was also demonstrated that ATP-sensitive K⁺ channels were involved at least in part of the cholinergic neurotransmission in guinea pig airways (22, 23). Glibenclamide blocks the activation of prejunctional ATP-sensitive K⁺ channels and cardiac ATP-sensitive K⁺ channels at similar concentrations (10–30 μM) in vitro (10, 23). Therefore, a partial explanation for the lack of the effects of glibenclamide on the negative cardiac responses to parasympathetic stimulation may be that glibenclamide similarly affects both the prejunctional and postjunctional ATP-sensitive K⁺ channels. That is, the effects of glibenclamide on the responses to parasympathetic nerve stimulation may be a sum of the augmentation of ACh release due to the inhibition by glibenclamide of the K⁺ current at the prejunctional site and the inhibition of ACh-induced responses by glibenclamide at the postsynaptic site (21). Although the modification by glibenclamide of the negative cardiac responses to parasympathetic stimulation is little under physiological conditions. We need further studies to clarify the role of ATP-sensitive K⁺ channels in the release of neurotransmitters.

Because ATP is rapidly degraded by several enzymes in the tissues, it is suggested that some of the effects of ATP result from its degradation to adenosine (24, 25). However, differences between some cardiac responses to ATP and adenosine have also been demonstrated (7, 25–27). In the present study, glibenclamide attenuated the negative cardiac responses to adenosine but did not
affect the cardiac responses to ATP (Fig. 2). Moreover, ATP-induced negative cardiac effects were not influenced by aminophylline, a $P_2$-purinergic receptor antagonist, although adenosine-induced ones were significantly inhibited in our previous studies (7, 28). Therefore, it is suggested that the negative chronotropic and inotropic responses to ATP may not be due to adenosine after rapid breakdown of ATP; i.e., ATP-induced negative cardiac effects are related neither to $P_2$-purinergic receptors nor to ATP-sensitive K$^+$ channels in isolated perfused dog heart. Further mechanisms of the negative cardiac responses to ATP in the dog heart remain to be investigated.

In the present study, cromakalim induced the negative chronotropic response as well as much greater negative inotropic response, and the cromakalim-induced inhibition of the negative chronotropic and inotropic effects of another potassium channel opener, pinacidil (13). ID$_{50}$ values of glibenclamide for the sinus rate, atrial tension and ventricular tension decreases evoked by cromakalim were not significantly different from the corresponding ID$_{50}$ values of glibenclamide for the negative cardiac responses to pinacidil (13). Thus, it is demonstrated that both cromakalim and pinacidil similarly induce the negative chronotropic effects as well as the negative inotropic effects through ATP-sensitive K$^+$ channels in the sinus node, atrial muscle and ventricular muscle.

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