Pinacidil Attenuates Positive Inotropic but Not Chronotropic Responses to Norepinephrine in Isolated Dog Atrial and Ventricular Preparations

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ABSTRACT—We investigated whether pinacidil, a K⁺ATP channel opener like acetylcholine and adenosine, attenuated the positive chronotropic and inotropic responses to norepinephrine in isolated, blood-perfused dog atrial and ventricular preparations. Pinacidil (0.01 – 0.3 pmol) decreased atrial and ventricular contractile force to a much greater extent than sinus rate in a dose-related manner. Pinacidil dose-dependently attenuated increases in atrial and ventricular forces induced by norepinephrine but not increases in sinus rate. Pinacidil similarly attenuated the positive atrial and ventricular inotropic responses to Bay k 8644 and CaCl₂. The pinacidil doses producing a fifty percent decrease (ED₅₀) of the atrial and ventricular contractile force were not significantly different from the respective pinacidil doses producing a fifty percent inhibition (ID₅₀) of the positive inotropic responses to norepinephrine, Bay k 8644 and CaCl₂. Ouabain (5 and 15 nmol) did not affect the decreases in atrial and ventricular contractile force in response to pinacidil. These results suggest that the K⁺ATP-channel activator pinacidil, unlike acetylcholine or adenosine, functionally attenuates increases in ventricular and atrial contractile force in the responses to norepinephrine and other cardiotonics due to shortening of the action potential duration induced by K⁺ATP-channel activation in the dog heart.

Keywords: ATP-sensitive K⁺ channel, Myocardial contractile force, Norepinephrine, Pinacidil, Sinus rate
tions have been described in previous reports (23, 24). Support dogs, weighing 10 to 22 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated artificially through a cuffed tracheal tube with room air by a Harvard respirator (model 607; Millis, MA, USA). Sodium heparin (500 USP units/kg, i.v.) was administered at the start of perfusion of the isolated atrial or ventricular preparation and 200 USP units/kg was given each hour thereafter.

Isolated right atrial or left ventricular preparations were obtained from other mongrel dogs weighing 7 to 20 kg. Each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After sodium heparin (200 USP units/kg, i.v.) was administered, the right atrium or left ventricle was excised and immersed in cold Ringer’s solution. The sinus node artery of the isolated right atrium or the descending branch of the left coronary artery of the isolated left ventricle was cannulated, and each preparation was perfused with heparinized blood from the carotid artery of the support dog by the aid of a peristaltic pump (model 1210, Harvard Apparatus). A pneumatic resistance was placed with the perfusion system so that the perfusion pressure could be maintained constant at 100 mmHg. The rate of blood flow to the atrial or ventricular preparation was from 3 to 12 ml/min. The venous effluent from the preparation was led to a collecting funnel and returned to the support dog through an external jugular vein.

The preparation was anchored to a stainless steel bar and placed in a cup-shaped glass container kept at 37°C. The upper part of the cardiac preparation was connected to a force-displacement transducer (AP 620G; Nihon Kohden, Tokyo) by a silk thread. The cardiac tissue was usually stretched to a resting tension of 2 g. Isometric tension was recorded on a thermo-writing rectigraph (WT 685, Nihon Kohden). A pair of bipolar silver electrodes was brought into contact with the epicardial surface of the isolated preparation and used to record the atrial electrogram or to drive the left ventricle electrically. The atrial rate was derived from the electrogram with a cardiotachometer (AT 600G, Nihon Kohden). The femoral arterial blood pressure and heart rate derived from lead II of the ECG of the support dog and the rate of blood flow to a preparation were monitored simultaneously.

Experimental protocols

Two series of experiments were carried out. In the first series, we investigated the effects of pinacidil (0.01–0.3 μmol) on the positive inotropic and chronotropic responses to norepinephrine, Bay k 8644 and CaCl2 in isolated, blood-perfused atrial and ventricular preparations. The positive chronotropic and inotropic responses to norepinephrine (0.3 nmol, n = 5), Bay k 8644 (0.9 nmol, n = 5) and CaCl2 (0.9 μmol, n = 5) were determined before and after pinacidil in isolated atria. The dose of each substance, which increased the atrial contractile force by 50% or more, was selected arbitrarily. Each agonist was injected 1 min after treatment with pinacidil because pinacidil usually induces the maximum negative effects within 1 min in the isolated, blood-perfused atrium of the dog. In the isolated ventricles, effects of pinacidil on the positive inotropic responses to norepinephrine (1 nmol, n = 5), Bay k 8644 (0.9 or 2.8 nmol, n = 4) and CaCl2 (0.9–9 μmol, n = 5) were also determined.

In the second series, we investigated the effects of ouabain (5, 15 and 50 nmol) on the negative inotropic responses to pinacidil doses of 0.03–0.3 μmol in 6 isolated right atria and doses of 0.3 and 3 μmol in 5 isolated left ventricles. The cardiac effects of pinacidil was determined 3 min after treatment with ouabain.

Drugs

The drugs used in the present study were pinacidil (generously donated by Shionogi, Osaka), d,l-norepinephrine hydrochloride (Sankyo, Tokyo), Bay k 8644 (generously donated by Dr. F. Seuter, Bayer AG, Wuppertal-Elberfeld, Germany) and ouabain (Takeda, Osaka). Pinacidil was dissolved in 0.1 N HCl to make a stock solution of 0.1 mM and then diluted with physiological saline to obtain low concentrations. Bay k 8644 was dissolved in ethanol, and the other drugs were dissolved in physiological saline before the start of the experiment.

Statistical analyses

Values are shown as the maximum change in response to each drug and expressed as means ± S.E. Statistical analyses were carried out with analysis of variance and Scheffe’s test. P values less than 0.05 were considered statistically significant.

RESULTS

Effects of pinacidil on the positive inotropic and chronotropic responses to norepinephrine, Bay k 8644 and CaCl2 in isolated right atria and left ventricles

Pinacidil (0.03 and 0.3 μmol) decreased atrial contractile force to a greater extent than sinus rate and attenuated the positive inotropic responses to norepinephrine (0.3 nmol, Fig. 1A). However, pinacidil did not attenuate the positive chronotropic responses. When pinacidil (0.01–0.3 μmol) decreased the sinus rate and atrial contractile force in a dose-dependent manner, it significantly (P < 0.01) attenuated the positive inotropic responses to norepinephrine (Fig. 2). When 0.3 nmol of norepinephrine increased the sinus rate and atrial contractile force by 13 ± 3.3% and 86 ± 11.9%, respectively (Table 1), the
Fig. 1. Effects of pinacidil (0.03 and 0.3 μmol) on the positive chronotropic and inotropic responses to norepinephrine (0.3 nmol) (A), Bay k 8644 (0.9 nmol) (B) and CaCl₂ (0.9 μmol) (C) in an isolated, blood-perfused dog atrium.

Fig. 2. Effects of pinacidil on the increases in atrial (closed circles) and ventricular (closed triangles) contractile force in response to norepinephrine, and direct effects of pinacidil on the basal atrial contractile force (open circles), basal ventricular contractile force (open triangles) and basal sinus rate (open squares) in 5 isolated, blood-perfused atria and 5 isolated, blood-perfused left ventricles of the dog. Each response is expressed as the mean change from the control level, and S.E. was omitted for clarity. Asterisks indicate a significant difference from each control (C): *P<0.05, **P<0.01.
A dose of pinacidil producing fifty percent inhibition (ID$_{50}$) of the positive inotropic response to norepinephrine was $0.11 \pm 0.02 \mu\text{mol}$ (Table 2). The pinacidil dose producing a fifty percent decrease (ED$_{50}$) of the atrial contractile force in the norepinephrine experimental group was $0.08 \pm 0.03 \mu\text{mol}$, and it was not significantly different from the ID$_{50}$ for the inotropic response to norepinephrine (Table 2). On the other hand, pinacidil did not attenuate the positive chronotropic responses to norepinephrine: pinacidil at a dose of $0.3 \mu\text{mol}$ did not change the chronotropic responses to norepinephrine (to $96 \pm 36.7\%$ of the control response).

In the isolated, blood-perfused canine left ventricle driven electrically at a frequency of 2 Hz, pinacidil ($0.1-3 \mu\text{mol}$) decreased the ventricular contractile force dose-dependently and attenuated the positive inotropic response to norepinephrine (Fig. 2). The ED$_{50}$ and ID$_{50}$ of pinacidil for the response to norepinephrine were $0.60 \pm 0.15 \mu\text{mol}$ and $0.86 \pm 0.24 \mu\text{mol}$, respectively (Table 2).

Bay k 8644 ($0.9 \text{nmol}$) and CaCl$_2$ ($0.9 \mu\text{mol}$) increased the atrial contractile force, similar to the effect of norepinephrine (Table 1). When pinacidil ($0.01-0.3 \mu\text{mol}$) induced dose-dependent negative inotropic responses in isolated atria, it attenuated the positive inotropic responses to Bay k 8644 (Figs. 1B and 3) and CaCl$_2$ (Figs. 1C and 4) in a dose-dependent manner. ED$_{50}$ of pinacidil for the Bay k 8644 and CaCl$_2$ experimental groups were not significantly different from that for the norepinephrine experimental group.

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**Table 1.** Positive inotropic and chronotropic effects of norepinephrine, Bay k 8644 and CaCl$_2$ before pinacidil treatment in the isolated, blood-perfused dog atrial or ventricular preparation

| Drugs      | Changes in chronotropic effects | Changes in inotropic effects |
|------------|---------------------------------|-----------------------------|
|            | atrium                          | ventricle                   |
| Norepinephrine | $13 \pm 4.2 \text{ beats/min (5)}$ | $2.1 \pm 0.5 \text{ g (5)}$      | $5.0 \pm 1.0 \text{ g (5)}$      |
|            | $13 \pm 3.3\%$                  | $86 \pm 11.9\%$             | $107 \pm 21.5\%$               |
| Bay k 8644  | $10 \pm 1.8 \text{ beats/min (5)}$ | $1.4 \pm 0.4 \text{ g (5)}$      | $1.8 \pm 0.4 \text{ g (4)}$      |
|            | $11 \pm 2.2\%$                  | $70 \pm 20.0\%$             | $47 \pm 13.6\%$               |
| CaCl$_2$   | $3 \pm 1.1 \text{ beats/min (5)}$ | $1.9 \pm 0.3 \text{ g (5)}$      | $2.3 \pm 0.4 \text{ g (5)}$      |
|            | $3 \pm 0.9\%$                   | $81 \pm 11.7\%$             | $62 \pm 10.5\%$               |

Data are shown as means±S.E. Basal sinus rate and atrial contractile force in the norepinephrine, Bay k 8644 and CaCl$_2$ experimental groups were $98 \pm 6.1$, $94 \pm 3.8$ and $96 \pm 6.3 \text{ beads/min}$, respectively and $2.6 \pm 0.6$, $2.0 \pm 0.5$ and $2.6 \pm 0.6 \text{ g}$, respectively, in 5 isolated atria. Basal left ventricular contractile forces in norepinephrine, Bay k 8644 and CaCl$_2$ experimental groups were $4.9 \pm 0.8$, $4.2 \pm 0.7$ and $4.0 \pm 0.8 \text{ g}$, respectively, in 5 isolated left ventricles. Numbers in parentheses show the number of experiments.

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**Table 2.** Fifty percent effective doses (ED$_{50}$) of pinacidil on the atrial and ventricular contractile force and fifty percent inhibition doses (ID$_{50}$) of pinacidil for the atrial and ventricular contractile force in response to norepinephrine, Bay k 8644 and CaCl$_2$ in the isolated, blood-perfused dog heart preparations

| Group       | ED$_{50}$ (\mu\text{mol}) | ID$_{50}$ (\mu\text{mol}) |
|-------------|---------------------------|---------------------------|
|             | atrium               | ventricle              | atrium               | ventricle              |
| Norepinephrine | $0.08 \pm 0.03$ | $0.60 \pm 0.15$ | $0.11 \pm 0.02$ | $0.86 \pm 0.24$ |
| Bay k 8644  | $0.10 \pm 0.07$ | $0.56 \pm 0.16$ | $0.12 \pm 0.02$ | $1.11 \pm 0.28$ |
| CaCl$_2$    | $0.07 \pm 0.01$ | $0.65 \pm 0.19$ | $0.11 \pm 0.02$ | $1.12 \pm 0.27$ |

Data are shown as means±S.E. Each data value was obtained from 5 experiments, except the ID$_{50}$ of pinacidil for the ventricular inotropic response to Bay k 8644 which was from 4 experiments.

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**Fig. 3.** Effects of pinacidil on the increases in atrial (closed circles) and ventricular (closed triangles) contractile force in response to Bay k 8644, and direct effects of pinacidil on the basal atrial contractile force (open circles), basal ventricular contractile force (open triangles) and basal sinus rate (open squares) in 5 isolated, blood-perfused atria and 4 isolated, blood-perfused left ventricles of the dog. Each response is expressed as the mean change from the control level, and S.E. was omitted for clarify. Asterisks indicate a significant difference from each control (C): *P<0.05, **P<0.01.
Experimental group (Table 2). ID$_{50}$s of pinacidil for the isotropic responses to Bay k 8644 and CaCl$_2$ were not significantly different from each respective ED$_{50}$ and were not different from the ID$_{50}$ for the response to norepinephrine (Table 2). When pinacidil dose-dependently decreased the ventricular contractile force, it attenuated the positive inotropic responses to norepinephrine (1 nmol, Fig. 2), Bay k 8644 (0.9 or 2.8 nmol, Fig. 3) and CaCl$_2$ (0.9–9 μmol, Fig. 4). ID$_{50}$s of pinacidil for the inotropic responses to norepinephrine, Bay k 8644 and CaCl$_2$ were not significantly different among them (Table 2). The dose-inhibition curve of pinacidil and the dose-response curve of pinacidil for both atrial and ventricular contractile forces were almost identical in each experimental group (Figs. 2–4).

Effects of ouabain on the cardiac responses to pinacidil in isolated atrial and ventricular preparations

When ouabain (5, 15 and 50 nmol) dose-dependently increased the atrial contractile force by 23 ±4.6%, 120 ±17.9% and 172 ±32.2% (n=3), respectively, in 6 isolated atria, 5 and 15 nmol of ouabain did not change the resting tension, but 50 nmol of ouabain elevated it 0.4±0.3 g from 2.0 g. Ouabain increased the contractile force gradually, and the positive inotropic effect reached the maximum level approximately 3 min after drug injection. Thus, we determined the effects of pinacidil 3 min after ouabain (Fig. 5). The negative inotropic responses to pinacidil (0.1 and 0.3 μmol) were not inhibited by ouabain at doses of 5 and 15 nmol (Fig. 5).

Ouabain (5 and 15 nmol) did not attenuate the negative inotropic responses to pinacidil at low (0.03 or 0.1 μmol) and high (0.3 μmol) doses, but a toxic high dose (50 nmol) of ouabain decreased the negative inotropic responses to pinacidil (P<0.01, Fig. 6). Ouabain did not clearly affect the small negative chronotropic effect of contractile force, and the ED$_{50}$s of pinacidil for the force decrease were almost the same among the norepinephrine, Bay k 8644 and CaCl$_2$ experimental groups (Figs. 2–4, Table 2). These ED$_{50}$s were 5.5 to 8 times larger than those of pinacidil on the baseline contractile force of the isolated atrium. When pinacidil dose-dependently decreased the ventricular contractile force, it attenuated the positive isotropic responses to norepinephrine (1 nmol, Fig. 2), Bay k 8644 (0.9 or 2.8 nmol, Fig. 3) and CaCl$_2$ (0.9–9 μmol, Fig. 4). ID$_{50}$s of pinacidil for the isotropic responses to norepinephrine, Bay k 8644 and CaCl$_2$ were not significantly different among them (Table 2). The dose-inhibition curve of pinacidil and the dose-response curve of pinacidil for both atrial and ventricular contractile forces were almost identical in each experimental group (Figs. 2–4).
pinacidil when it slightly increased.

In 5 isolated, perfused left ventricles, ouabain (5 and 15 nmol) did not affect the negative inotropic responses to pinacidil (0.3 or 3 μmol) when ouabain increased the left ventricular force by 8 ± 4.8% and 34 ± 12.3%, respectively.

**DISCUSSION**

In the isolated, blood-perfused atrial and ventricular preparations of the dog, pinacidil, a K⁺ATP channel opener, decreased the positive inotropic but not chronotropic effects of norepinephrine, Bay k 8644 and CaCl₂ parallel to the dose-dependent decrease in atrial and ventricular contractile force induced by pinacidil itself. IDₕ₅₀ of pinacidil for the inotropic responses to norepinephrine, Bay k 8644 and CaCl₂ were almost the same, and they were not significantly different from the respective EDₕ₅₀ of pinacidil in each experimental group. Ouabain at non-toxic doses did not affect the negative inotropic effect of pinacidil. These results suggest that activation of K⁺ATP channels by pinacidil attenuates increases in ventricular as well as atrial contractile force in responses to norepinephrine and other cardiotonic agents probably due to functional inhibition by shortening of the action potential duration in the heart.

Pinacidil decreases the atrial and ventricular contractile force due to activation of K⁺ATP channels followed by shortening of action potential duration in mammalian heart tissues (19–21), although pinacidil has multiple effects on the membrane ionic currents of cardiac cells (25). In isolated, blood-perfused dog atrial and ventricular preparation, pinacidil induced negative chronotropic and inotropic responses, and the chronotropic response to pinacidil was smaller than the inotropic one (26). Pinacidil (0.01–3 μmol) used in the present study roughly corresponds to the pinacidil concentration (0.01–1 mM) used in the electrophysiological study on cardiac myocytes (21). The negative cardiac responses to pinacidil were blocked by glibenclamide dose-dependently. The negative cardiac responses to cromakalim, another K⁺ATP channel opener, were similarly inhibited by glibenclamide in the isolated dog myocardial preparation (20). Thus, it is most likely that the negative chronotropic and inotropic responses to pinacidil observed in the present study were evoked by activation of K⁺ATP channels in the isolated perfused dog atrial and ventricular preparations.

Pinacidil decreased the positive inotropic response to norepinephrine in the isolated perfused dog atrial and ventricular preparations (Fig. 2). These results indicate that pinacidil attenuates the positive inotropic response to norepinephrine at the postjunctional site, although the sympathoinhibitory effects of cromakalim, another K⁺ATP channel opener, on the systemic and regional vascular responses were most likely prejunctionally located in the spontaneously hypertensive rat (16, 17). Additionally, pinacidil attenuated the positive inotropic responses to Bay k 8644 (a Ca²⁺-channel agonist) and CaCl₂ in a dose-dependent manner in the isolated, perfused dog atrial and ventricular preparations (Figs. 3 and 4). On the other hand, the positive chronotropic responses to norepinephrine and Bay k 8644 were not attenuated by pinacidil. Thus it is likely that pinacidil at the doses used in the present study attenuates the positive inotropic response to norepinephrine at a site distal to the common transduction of the inotropic and chronotropic responses to activation of the Ca²⁺ channels in the isolated dog heart. That is, shortening of the action potential duration by pinacidil attenuates the positive inotropic response to norepinephrine or other cardiotonic agents indirectly and functionally. This is also supported by the following results: The IDₕ₅₀ of pinacidil for the inotropic responses to norepinephrine, Bay k 8644 and CaCl₂ were almost the same, and these values were not significantly different from the respective EDₕ₅₀ of pinacidil in each experimental group (Table 2). Effects of pinacidil on the positive inotropic responses to ouabain also supported the functional inhibition on the positive inotropic responses to cardiotonic substances (Figs. 5 and 6).

When treatment with 50 nmol of ouabain attenuated the negative inotropic response to pinacidil (Fig. 6), it increased the resting tension as well as the atrial contractile force. This suggests that sufficient inhibition of Na⁺-K⁺ ATPase by ouabain inhibits the K⁺ATP channel mediated
action in the dog atrium, although further studies are needed to clarify the precise mechanism of the inhibition. Pinacidil attenuated the action potential duration in the canine ventricular myocyte treated with a toxic dose of ouabain less than that in the ouabain non-treated myocyte (27).

Pinacidil decreased the positive inotropic responses to norepinephrine, Bay k 8644 and CaCl₂ in isolated left ventricles as well as right atria, but did not decrease the positive chronotropic ones (Figs. 1–4). This different inhibition by pinacidil on the chronotropic and inotropic responses to norepinephrine differs from the sensitivity of the chronotropic and inotropic responses to K⁺ATP channel openers as shown in the present study and previous reports (22, 26, 28). This different inhibition by pinacidil on the chronotropic and inotropic responses to norepinephrine probably due to the different sensitivity of the chronotropic and inotropic responses to K⁺ATP channel openers as shown in the present study and previous reports (22, 26, 28). This different inhibition by pinacidil on the chronotropic and inotropic responses to norepinephrine differs from the inhibition by acetylcholine or adenosine on the cardiac responses to norepinephrine. In the isolated, blood-perfused canine atrial preparation, we previously reported that acetylcholine and adenosine attenuated the positive chronotropic and inotropic responses to norepinephrine (14, 15). These sympathoinhibitory effects of acetylcholine or adenosine on the heart are caused at sites of the intracellular signal transduction (12, 13, 29, 30). The sympathetic-parasympathetic interactions are different among the cardiac responses, i.e., heart rate, atrioventricular conduction, atrial contractility, ventricular contractility and refractory period, in the dog heart (14, 31–33). The sympathetic-parasympathetic interaction on the rate is greater than that on the ventricular contractility. The interactions between sympathomimetic substances and adenosine or its analogues on the atrial contractility are also greater than that on the ventricular contractility in the isolated mammalian hearts (12, 15, 30, 34). These differences are partly due to the different receptor density related to the function (35). Thus, we demonstrated that activation of K⁺ATP channels by pinacidil, different from acetylcholine and adenosine, attenuated the ventricular contractile force in response to norepinephrine and other cardiotonic substances without decreasing the chronotropic response in the dog heart.

Under ischemic conditions, K⁺ATP channel activation, adenosine release and parasympathetic nerve activation as well as sympathetic nerve activation occur (1, 4, 8, 9). Therefore, our present results may suggest that during ischemia, activation of K⁺ATP has an important functional antagonistic action on the ventricular as well as atrial myocardial contractile force but not pacemaker activity evoked by catecholamines or exogenously administered cardiotonic agents in the heart.

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