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Negative inotropic effect of pinacidil on the rat left atria

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Abstract—Cromakalim at 50 μM and pinacidil at 0·1–10 μM had no effect, but pinacidil at 0·1 mM had a negative inotropic effect on the rat electrically-driven left atria without altering the positive inotropic responses to isoprenaline or phenylephrine alone. Glibenclamide had no effect but 4-aminopyridine, procaine (30 μM) and tetraethylammonium (3 mM) augmented the cardiac stimulation response. The ability of pinacidil to attenuate the cardiac stimulation response was not altered by glibenclamide, 4-aminopyridine or procaine but was prevented by pretreatment with tetraethylammonium. Thus, on the rat left atria, pinacidil has a negative inotropic effect which is unrelated to the opening of ATP-sensitive potassium channels, but may be due to opening the inward rectifying potassium channels.

Some of the membrane-associated potassium channels (e.g. the inward and outward rectifying channels) have a major role in the threshold, frequency and duration of cardiac action potential and of cardiac contractility. However, the role of ATP-sensitive potassium channels in the cardiac action potential and cardiac contractility remains to be clearly defined. ATP-sensitive potassium-channel openers (e.g. pinacidil and cromakalim) shorten cardiac action potentials (pinacidil (Martin & Chinn 1990), cromakalim (Bril & Man 1990)) and may have effects on cardiac contractility. Thus, negative inotropic effects of modest concentrations of pinacidil dissolved in HCl and of cromakalim dissolved in ethanol were described for the canine atrial wall (Yanagisawa et al 1989) and these effects were reversed by glibenclamide, an ATP-sensitive potassium-channel blocker (Satoh et al 1990). Pinacidil, dissolved in HCl but not in dimethylsulphoxide, also had a negative inotropic effect on the rabbit left atria and antagonized the positive inotropic responses of this tissue to phenylephrine but not to isoprenaline (Ray & MacLeod 1990). In a more recent study we have demonstrated that pinacidil dissolved in ethanol did have a negative inotropic effect on the rat right ventricle but that this negative inotropic effect was solely due to the ethanol (Bishop & Doggrell 1992). Recent studies have also failed to demonstrate negative inotropic effects of cromakalim on the rabbit left atria (Ray & MacLeod 1990) or the rat right ventricle (Bishop & Doggrell 1992). Thus it is not clear whether opening the ATP-sensitive potassium channel does have effects on cardiac contractility.

The aim of the present study was to characterize the effects of pinacidil and cromakalim on the responses of the rat left atria to cardiac stimulation and of the rat electrically-driven left atria to isoprenaline and phenylephrine. The positive inotropic responses of the rat left atria to phenylephrine are mediated by α_1-adrenoceptors (Chess-Williams et al 1990). I report a negative inotropic effect with a high concentration of pinacidil but not with cromakalim, and have investigated the effects of glibenclamide, 4-aminopyridine, procaine and tetraethylammonium (potassium-channel blockers) on this negative inotropic effect of pinacidil.

Materials and methods

Conractile responses of the electrically-driven rat left atria (Doggrell 1988). Male Wistar rats, 250–350 g, were stunned and exsanguinated. The heart was rapidly removed and placed in Krebs solution saturated with 5% CO_2 in oxygen. All experiments were performed in the presence of a modified Krebs solution (composition (mM); NaCl 116, KCl 5·4, $CaCl_2$ 2·5, $MgCl_2$ 1·2, NaH_2PO_4 1·2, $NaHCO_3$ 22·0, D-glucose 11·2, Na_2EDTA 0·04) at 37 °C which was bubbled with 5% CO_2 in oxygen. Contractile responses were measured isometrically with force displacement transducers (Grass model FTO3.C) and displayed on a polygraph (Grass model 79B). In each series of experiments, the individual values obtained were subject to Student's *t*-test. Differences were considered significant when $P < 0.05$. Mean values ± s.e.m. were also obtained.

Left atria were removed from the heart and halved. Each half-atrium was mounted longitudinally between two platinum electrodes (approx. 3 cm apart, above and below the tissue) under 1 g tension in 5-mL organ baths containing Krebs solution (with 10 μM guanethidine to prevent the release of noradrenaline from nerve endings, and atropine at 1 μM) and allowed to equilibrate for 60 min. During the equilibration period, the tissues were washed by overflow. Tissues were electrically stimulated at 4 Hz (5 ms, 10 V). After 9 min of stimulation, a cumulative challenge with isoprenaline or phenylephrine was initiated. Further additions were made at 3-min intervals and the cycle was continued until a maximum response was obtained. This was the first challenge to the agonist.

One of the tissues was then treated with a drug (pinacidil, cromakalim, glibenclamide, 4-aminopyridine, procaine or tetraethylammonium) for 75 min while the other tissue of the pair remained untreated throughout. During this 75 min about 1 L of drug-free or drug-containing Krebs solution overflowed the tissue. The tissues were electrically stimulated and cumulatively challenged with isoprenaline or phenylephrine until a maximal response was obtained (second challenge). This procedure was repeated with one tissue remaining untreated throughout and the paired tissue being treated with a higher concentration of the same drug before a third challenge to electrical stimulation and agonist. When experiments were performed to investigate the effects of pinacidil in the presence of potassium-channel blockers, one of the potassium-channel blockers (glibenclamide, 4-aminopyridine, procaine or tetraethylammonium) was present in the Krebs solution of all the tissues throughout the experiment.

The contractile responses to cardiac stimulation just before the second and third challenges with an agonist were calculated as a percentage of the response to stimulation before the first challenge with the agonist. For each challenge with an agonist, the response to cardiac stimulation just before challenge with the agonist was subtracted from the combined response to cardiac stimulation and the agonist. The maximal combined responses to cardiac stimulation and an agonist were calculated as a percentage of the maximum of the first challenge to the agonist. If the maximum responses to cardiac stimulation and an agonist between treated and untreated tissues were not significantly different, response curves were calculated as a percentage of the maximum of the individual curves. If the maximum responses to cardiac stimulation and an agonist between treated and untreated tissues were significantly different (e.g. pinacidil at 0·1 mM attenuated the maximal combined responses to cardiac stimulation and phenylephrine), response curves were calculated as a percentage of the maximum of the first curve.

The drugs used, which were dissolved in distilled water unless otherwise stated, were (±)-pinacidil monohydrate (donated by Leo Pharmaceutical Products, Ballerup, Denmark) dissolved in HCl, phenylephrine hydrochloride, procaine hydrochloride

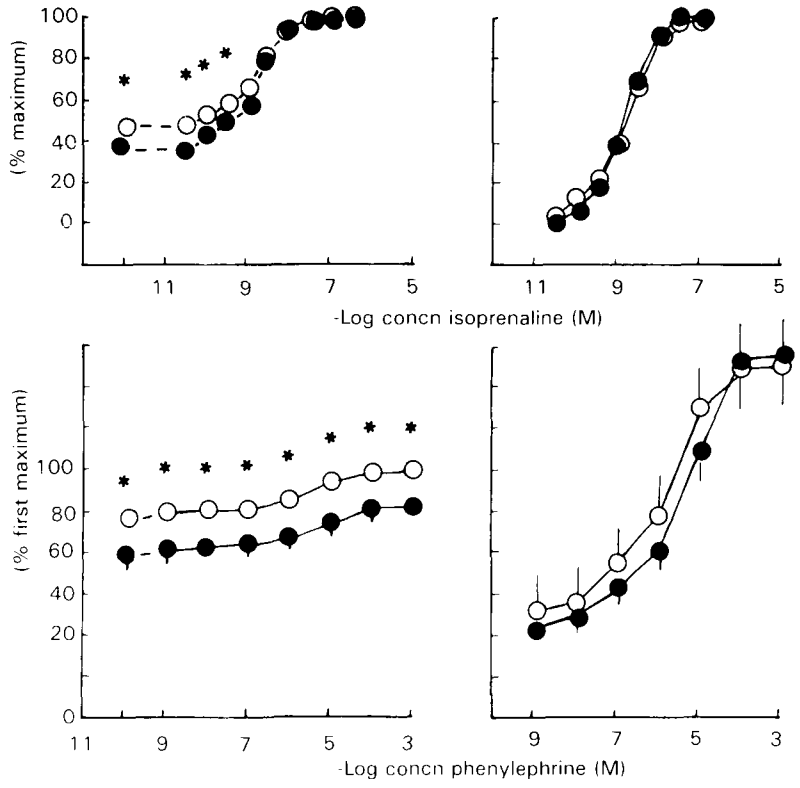

FIG. 1. Effect of pinacidil on the force responses of the rat left atria. Top left; responses to cardiac stimulation and isoprenaline. Top right; responses to isoprenaline. Bottom left; responses to cardiac stimulation and phenylephrine. Bottom right; responses to phenylephrine. Top experiment; responses in the absence (○) and presence (●) of pinacidil at 0·1 mM are calculated as a percentage of the maximum response and plotted against the log of the molar concentration of isoprenaline. Bottom experiment; responses in the absence (○) and presence (●) of pinacidil at 0·1 mM are calculated as a percentage of the first maximum and plotted against the log of the molar concentration of phenylephrine. Each value is the mean from 7–12 tissues, vertical lines show s.e.m. $*P < 0.05$.

(Serva, Heidelberg, Germany), (±)-cromakalim (donated by SmithKline Beecham, Worthing, UK) dissolved in ethanol, 4-aminopyridine, atropine sulphate, glibenclamide dissolved in ethanol, guanethidine sulphate, (−)-isoprenaline bitartrate and tetraethylammonium chloride (Sigma Chemicals Co., St Louis, MO).

Results and discussion

Direct muscle stimulation (4 Hz, 5 ms, 10 V) contracts the left atria and the force of contraction is increased by the cumulative addition of isoprenaline or phenylephrine. My first study using the present method with the left atria showed that the force responses to cardiac stimulation become successively smaller, whereas the maximal responses to isoprenaline become successively greater (Doggrell 1988).

Effects of cromakalim and pinacidil. Cromakalim at 5–50 μM and pinacidil at 0·1–10 μM had no effect on the cardiac stimulation response, the isoprenaline responses or the combined response to cardiac stimulation and isoprenaline (data not shown). Cromakalim at 50 μM in a vehicle of 0·2% ethanol and pinacidil at 0·1–10 μM had no effect on phenylephrine responses (data not shown). Pinacidil at 0·1 mM in a vehicle of 50 μM HCl attenuated the cardiac stimulation response, the combined submaximal responses to cardiac stimulation and isoprenaline, and the combined responses including the maximal responses to cardiac stimulation and phenylephrine (Fig. 1). This attenuating effect on the combined responses was solely due to the effect on cardiac stimulation alone, as the responses to isoprenaline and phenylephrine were not altered (Fig. 1). The vehicles of 0·2% ethanol or 50 μM HCl had no effect alone.

Effect of potassium-channel blockers. Glibenclamide blocks ATP-sensitive potassium channels. Most of the other available potassium-channel blockers are less selective. Thus, 4-aminopyridine blocks the transient outward and Na^+-activated potassium channel, and tetraethylammonium blocks the inward and delayed outward rectifiers and the Na^+-activated potassium channel. Procaine blocks the delayed outward rectifier and the Na^+-activated potassium channel and, in higher concentrations, also blocks sodium channels (for classification of channel blockers see Watson & Abbott (1992)).

In the present study glibenclamide at 1 and 10 μM in a vehicle of 0·007 and 0·07% ethanol, respectively, and 0·07% ethanol alone had no effect on the response of the rat left atria to stimulation. 4-Aminopyridine at 0·1, 0·3 and 1 mM augmented the cardiac stimulation response by 30, 23 and 33%, respectively. Procaine at 10 μM augmented the cardiac stimulation response by 13%, at 0·1 mM had no effect and at 0·3 mM attenuated the response by 19%. Tetraethylammonium at 1 mM had no effect and at 3 mM augmented the cardiac stimulation response by 17%.

Effect of pinacidil in the presence of potassium-channel blockers. The ability of pinacidil at 0·1 mM to attenuate the cardiac stimulation response was not altered by the presence of glibenclamide at 10 μM, 4-aminopyridine at 0·3 mM or procaine at 10 μM,

but was prevented by the presence of tetraethylammonium at 3 mM.

Previous studies have shown that pinacidil has a negative inotropic effect on cardiac preparations and that this negative inotropic effect is due to the ethanol vehicle on the rat right ventricle. The present study has demonstrated that a high concentration of pinacidil did have a negative inotropic effect on the rat left atria and that this was independent of the vehicle. This negative inotropic action of pinacidil caused a depression of the combined responses to cardiac stimulation and isoprenaline or phenylephrine. However, on the rat left atria, pinacidil did not modify the effects of isoprenaline and phenylephrine alone. This is in agreement with Ray & MacLeod (1990) who showed that pinacidil at 0·1 mM had no effect on the positive inotropic effects of isoprenaline or phenylephrine on the rabbit left atria. Ray & MacLeod (1990) also reported that very high concentrations of pinacidil antagonized the responses of the rabbit left atria to phenylephrine but not to isoprenaline.

Moderate concentrations of cromakalim and pinacidil (0·1–10 μM) open, and glibenclamide blocks, ATP-sensitive potassium channels (Cook & Quast 1990). In the present study, moderate concentrations of cromakalim, pinacidil and glibenclamide had no effect on the force responses of the rat left atria. Furthermore, the small negative inotropic effect of a high concentration of pinacidil was not blocked by glibenclamide and is probably independent of the opening of ATP-sensitive potassium channels. Thus it seems likely that under the experimental conditions used in the present study (e.g. normoxia, 37°C) the ATP-sensitive potassium channels do not modulate the contractility of the rat left atria.

Other potassium channels, however, do modulate the contractility of the rat left atria. Thus, 4-aminopyridine (which blocks the transient outward and Na^+-activated potassium channels) and tetraethylammonium (which blocks the inward and delayed outward rectifiers and the Na^+-activated potassium channels) augment the cardiac stimulation response. A low concentration of procaine also augmented the cardiac stimulation response, possibly by blocking the delayed outward rectifier or the Na^+-activated potassium channel, and at a high concentration, procaine attenuated the cardiac stimulation response, probably by predominantly blocking sodium channels. Of the potassium-channel blockers tested in the present study, only tetraethylammonium blocks the inward rectifying potassium channel and tetraethylammonium was also the only drug treatment to prevent the negative inotropic effect of pinacidil. Thus, it is possible that pinacidil is opening the inward rectifying potassium channel of the rat left atria to cause a negative inotropic effect. This possibility is supported by a previous report that high concentrations of pinacidil open the inward rectifying potassium channels in single ventricle cells from guinea-pig heart (Iijima & Taira 1987).

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