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The Effects of Racemic, (+)- and (−)-Pinacidil on the Membrane Potential of the Rat Aorta

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Abstract

The endothelium-intact and -denuded rat aorta is hyperpolarized by racemic and (−)-pinacidil, probably by opening ATP-dependent potassium channels. (+)-Pinacidil caused depolarization of the endothelium-intact and -denuded rat aorta.

The depolarization induced by 20 mM KCl in the endothelium-intact rat aorta was reversed by racemic and (−)-, but not by (+)-pinacidil. On the endothelium-intact rat aorta, isoprenaline produced hyperpolarization and ICI 118 551 (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) had no effect alone but prevented isoprenaline from causing hyperpolarization. The hyperpolarization induced by isoprenaline was reversed by racemic and (+)-, but not by (−)-pinacidil. Glibenclamide depolarized the endothelium-intact rat aorta and prevented the hyperpolarizing action of racemic pinacidil and (−)-pinacidil. (+)-Pinacidil prevented the hyperpolarizing action of (−)-pinacidil. Glibenclamide is probably preventing the hyperpolarization associated with opening of the ATP-dependent potassium channel by blocking this channel.

Several mechanisms may underlie the depolarizing action of (+)-pinacidil, including blocking of ATP-dependent potassium channels.

Pinacidil has anti-spasmogenic activity on smooth muscle which is thought to originate from the opening of ATP-dependent potassium ion channels which hyperpolarizes the membrane, closing voltage-sensitive calcium channels and reducing the influx of calcium, itself the immediate cause of contraction (Hamilton & Weston 1989). Electrophysiological studies with smooth muscle have demonstrated the hyperpolarizing action of pinacidil (Southerton et al 1988).

Pinacidil has an asymmetric carbon and, therefore, has (+)- and (−)-isomers. The present report describes the effects of racemic, (+)- and (−)-pinacidil on the membrane potential of the endothelium-intact and -denuded rat aorta and demonstrates a hyperpolarizing action with (−)-pinacidil but a depolarizing action with (+)-pinacidil. Also, the report further characterizes these hyperpolarizing and depolarizing actions on the endothelium-intact rat aorta. A preliminary account of this data has been presented to the British Pharmacological Society (Doggrell & Bishop 1992).

Materials and Methods

General

Male Wistar rats were killed by stunning followed by cervical dislocation. The thoracic aorta was removed, placed in Krebs solution and cleared of fat. Experiments were performed in Krebs solution (composition in mM: NaCl 116, KCl 5·4, $CaCl_2$ 2·5 $MgCl_2$, 1·2 NaH_2PO_4, 1·2, $NaHCO_3$ 22·0 and glucose 11·2), bubbled with 95% O_2–5% CO_2 at 37°C. Data are expressed as mean ± s.e.m. Tests of significance were made by analysis of variance and Student's *t*-test as appropriate, and were considered significantly different when $P < 0.05$. In some experiments the endothelium was removed from the aorta by gentle rubbing of the intimal surface with a pair of forceps.

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Membrane potentials

An endothelium-intact or -denuded rat aorta was cut along the longitudinal axis to form a flat sheet approximately 0·5 cm in length. The aorta was pinned luminal side uppermost in a recording bath with $CaCl_2$-containing Krebs solution flowing at a rate of approximately 2 mL min^{-1}. No attempt was made to record changes in length or in tension. Tissues were equilibrated for 30 min and then the resting membrane potential of at least 10 cells was determined. Microelectrodes filled with 2 M KCl were used and impalements using standard techniques were made through the internal elastic lamina. Membrane potential was recorded only if the penetration of the cell was abrupt and the creep following penetration was less than ± 4 mV in 45 s. Measurements of the membrane potential were made following deliberate electrode withdrawal after 45 s. The endothelium-intact or -denuded aorta was then equilibrated for 30 min in the presence of Krebs solution containing racemic, (+)- or (−)-pinacidil, KCl, ICI 118 551, isoprenaline, glibenclamide or ethanol before the determination of the membrane potentials of a further 10 cells or more. Some experiments were continued to determine whether the effects of (+)- and (−)-pinacidil were reversible in endothelium-intact tissues. Thus tissues were washed by overflow for 30 min and the membrane potential of a further 10 cells or more was determined. Other experiments were continued to study the effects of racemic, (+)- or (−)-pinacidil in the presence of other drugs, racemic, (+)- or

(−)-pinacidil being added to the drug-containing Krebs solution and equilibrated for 30 min before the determination of the membrane potential of at least 10 cells.

Solutions and drugs

The drugs used were: racemic, (+)- and (−)-pinacidil, all donated by Leo Pharmaceuticals, Ballerup, Denmark; ICI 118 551, erythro- (±)-7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol, donated by ICI Ltd, Manchester, UK; glibenclamide and (−)-isoprenaline bitartrate (Sigma Chemical Co., St Louis, MO). Racemic, (+)- and (−)-pinacidil were dissolved in hydrochloric acid, glibenclamide was dissolved in ethanol, and (−)-isoprenaline and ICI 118 551 were dissolved in distilled water.

Results and Discussion

Effects of racemic, (+)- and (−)-pinacidil on the endothelium-intact rat aorta

The resting membrane potential of the endothelium-intact rat aorta was -61 ± 1 mV (120 determinations of the membrane potential from the aortae of 12 rats) and was not changed by racemic pinacidil at 1 μM, -59 ± 2 mV, but was hyperpolarized by racemic pinacidil at 10 μM to -65 ± 2 mV ($P < 0.05$). (+)-Pinacidil at 1 μM had no effect (data not shown) but at 10 μM depolarized the endothelium-intact rat aorta by 16 mV to -45 ± 2 mV ($P < 0.05$). (−)-Pinacidil at 1 and 10 μM hyperpolarized the endothelium-intact rat aorta by 7 and 10 mV to -68 ± 1 ($P < 0.05$) and -71 ± 1 mV ($P < 0.05$), respectively. The depolarizing action of (+)-pinacidil at 10 μM and the hyperpolarizing action of (−)-pinacidil at 10 μM was completely reversed by washing the tissue for 30 min in drug-free Krebs (n = 4 for each isomer, data not shown).

Racemic, (+)- and (−)-pinacidil at 10 μM were in a vehicle of 0·5 mM HCl. HCl at 0·5 mM had no effect alone on the membrane potential (data not shown).

Effects of racemic, (+)- and (−)-pinacidil on the endothelium-denuded rat aorta

Qualitatively, the effects of racemic, (+)- and (−)-pinacidil were not altered by removing the endothelium from the rat aorta. The resting membrane potential of the endothelium-denuded rat aorta was -46 ± 1 mV and was not changed by racemic pinacidil at 1 μM, -45 ± 2 mV, but was hyperpolarized by racemic pinacidil at 10 μM to -55 ± 2 mV ($P < 0.05$). (+)-Pinacidil at 1 μM had no effect (n = 4, data not shown) but at 10 μM depolarized the endothelium-denuded rat aorta by 6 mV to -40 ± 2 mV ($P < 0.05$). (−)-Pinacidil at 1 and 10 μM hyperpolarized the endothelium-denuded rat aorta by 9 and 15 mV to -55 ± 1 ($P < 0.05$) and -61 ± 1 mV ($P < 0.05$), respectively.

The effects of pinacidil in the presence of depolarization with KCl

The endothelium-intact rat aorta was depolarized in the presence of 20 mM KCl by 36 mV (difference between mean potential in the absence and presence of KCl, $P < 0.05$). In the presence of KCl, racemic and (−)-pinacidil, but not (+)-pinacidil, reversed the depolarization of the rat aorta (Table 1). Thus, in the presence of KCl, there were reversals of 17 and 25 mV by racemic pinacidil at 1 and 10 μM and of 12 and 23 mV by (−)-pinacidil at 1 and 10 μM, respectively.

The effects of isoprenaline and ICI 118 551, alone and together

The endothelium-intact rat aorta was hyperpolarized by isoprenaline at 1 μM by 9 mV from an untreated membrane potential of -61 ± 2 mV to an isoprenaline-treated membrane potential of -70 ± 2 mV ($P < 0.05$). ICI 118 551, a selective β_2-adrenoceptor antagonist (O'Donnell & Wanstall 1985), at 0·1 μM had no effect on the membrane potential of the endothelium-intact rat aorta (data not shown). In the presence of ICI 118 551 at 0·1 μM, isoprenaline at 1 μM had no effect on the membrane potential (data not shown).

The effects of pinacidil in the presence of hyperpolarization with isoprenaline

In the presence of isoprenaline-induced hyperpolarization, racemic and (+)-pinacidil, but not (−)-pinacidil, reversed this hyperpolarization of the endothelium-intact aorta (Table 1). The effects of racemic and (+)-pinacidil were maximal at 1 μM as the reversals at 1 and 10 μM were not significantly different. Thus, in the presence of isoprenaline, racemic pinacidil at 1 and 10 μM reversed the hyperpolarization by 25 and 24 mV and (+)-pinacidil at 1 and 10 μM reversed it by 22 and 23 mV, respectively.

The effects of pinacidil in the presence of depolarization with glibenclamide

Glibenclamide blocks ATP-dependent potassium channels in arterial smooth muscle (Standen et al 1989). In the present study, glibenclamide at 1 μM depolarized the rat endothelium-intact aorta by 15 mV (Table 2). The ability of isoprenaline at 1 μM to hyperpolarize the rat aorta was maintained in the presence of glibenclamide at 1 μM, the mean membrane potential being −47 mV in the presence of glibenclamide and −66 mV in the presence of glibenclamide and isoprenaline (Table 2).

Pretreatment with glibenclamide at 1 μM prevented the hyperpolarizing actions of racemic pinacidil at 10 μM and of (−)-pinacidil at 1 μM (Table 2). However, pretreatment with glibenclamide in the presence of isoprenaline, did not prevent (+)-pinacidil at 1 and 10 μM from depolarizing the aorta by 20 and 19 mV, respectively.

The effect of (−)-pinacidil in the presence of (+)-pinacidil

The ability of (−)-pinacidil at 1 μM to hyperpolarize the rat aorta was abolished by pretreatment with (+)-pinacidil at 1 μM (Table 2).

Racemic and (−)-pinacidil (Southerton et al 1988) and isoprenaline (Asano et al 1982) relax the rat aorta. The present study shows that these agents also hyperpolarize the rat aorta. It is generally considered that pinacidil opens ATP-dependent potassium channels to cause hyperpolarization that indirectly closes voltage-dependent calcium channels to produce vasodilation (Southerton et al 1988; Hamilton & Weston 1989). The isoprenaline-induced hyperpolarization and relaxation of the rat aorta are mediated by β_2-adrenoceptors, as both events are blocked by ICI 118 551, a selective β_2-adrenoceptor blocker (hyperpolarization, this study; relaxation, Doggrell unpublished observation). The details of the mechanism linking the activation of β_2-adrenoceptors to hyperpolarization and to relaxation of the rat aorta are not known at present.

Table 1. Effect of pinacidil on the membrane potential of the rat aorta.

	Membrane potential (mV)	
	Depolarization with 20 mM KCl	Hyperpolarization with 1 μM isoprenaline
Control	−23 + 1 (40)*	−69 ± 1 (60)*
(±)-Pinacidil 1 μM	−40 ± 2 (40)+	−44 ± 1 (60)+
(±)-Pinacidil 10 μM	−48 ± 3 (40)+	−45 ± 1 (60)+
Control	−21 ± 1 (40)*	−70 ± 2 (30)*
(+)-Pinacidil 1 μM	−22 ± 1 (40)	−48 ± 2 (30)+
(+)-Pinacidil 10 μM	−22 ± 1 (40)	−47 ± 2 (30)+
Control	−22 ± 1 (40)*	−69 ± 1 (40)*
(−)-Pinacidil 1 μM	−35 ± 1 (40)+	−69 ± 1 (40)
(−)-Pinacidil 10 μM	−45 ± 1 (40)+	−68 ± 1 (40)

The membrane potential in the absence of drugs was −59 ± 1 mV (270). Each table value is the mean ± s.e.m. from aortas of three, four or six rats; number in parenthesis shows the number of determinations. *$P < 0.05$ by Student's t-test compared with experiments in the absence of drugs, +$P < 0.05$ by Student's t-test compared with experiments in the presence of KCl or isoprenaline as appropriate.

The present study confirms that the endothelium-denuded rat aorta has a lower resting membrane potential than the endothelium-intact rat aorta (Southerton et al 1988), possibly because removal of the endothelium is associated with the removal of basally released endothelium derived hyperpolarizing factors. The present study also shows that the hyperpolarizing actions of racemic and (−)-pinacidil are smooth-muscle mediated as they persist in endothelium-denuded rat aorta.

The most interesting finding of the present study was that (+)-pinacidil depolarized the rat aorta. We have also recently demonstrated that BRL 38226, the (+)-3*R*,4*S*-isomer of cromakalim, depolarizes the rat aorta (Bishop & Doggrell 1994). This depolarizing action of (+)-pinacidil was mediated by the aorta smooth muscle as it was observed in the endothelium-denuded preparation. (+)-Pinacidil also reversed the hyperpolarization caused by isoprenaline.

Table 2. Effect of glibenclamide or (+)-pinacidil pretreatment on the actions of racemic and (−)-pinacidil.

Control	Membrane potential (mV)
	−60 ± 2 (30)
Glibenclamide 1 μM	−45 ± 2 (30)*
Glibenclamide 1 μM	−47 ± 2 (30)
Glibenclamide 1 μM, isoprenaline 1 μM	−66 ± 2 (30)*
Glibenclamide 1 μM	−45 ± 2 (30)
Glibenclamide 1 μM, (±)-pinacidil 10 μM	−45 ± 2 (30)
Glibenclamide 1 μM	−44 ± 1 (30)
Glibenclamide 1 μM, (−)-pinacidil 1 μM	−45 ± 1 (30)
Glibenclamide 1 μM, isoprenaline 1 μM	−66 ± 2 (30)
Glibenclamide 1 μM, isoprenaline 1 μM, (+)-pinacidil 1 μM	−46 ± 2 (30)*
Glibenclamide 1 μM, isoprenaline 1 μM, (+)-pinacidil 10 μM	−47 ± 2 (30)*
(+)-Pinacidil 10 μM	−38 ± 2 (30)
(+)-Pinacidil 10 μM, (−)-pinacidil 1 μM	−38 ± 2 (30)

Each value is the mean ± s.e.m. from the aortas of three or four rats; number in parenthesis shows the numbers of determinations. *$P < 0.05$ by Student's t-test from own control.

A number of mechanisms could underlie the ability of (+)-pinacidil to depolarize the rat aorta. Thus noradrenaline stimulates α_1-adrenoceptor and 5-hydroxytryptamine stimulates 5-HT_2 receptors to depolarize the rat aorta (Doggrell et al 1989). Glibenclamide, a well characterized blocker of the vascular ATP-dependent potassium channel (Standen et al 1989), also depolarizes the rat aorta (this study). It is possible that (+)-pinacidil is acting as a blocker of ATP-dependent potassium channels to depolarize the rat aorta. The only evidence for this is that (+)-pinacidil and glibenclamide both prevent (−)-pinacidil, the ATP-dependent potassium-channel opener, from causing hyperpolarization. Further experimentation will be required to test whether (+)-pinacidil is a blocker of the ATP-dependent potassium channel.

Racemic pinacidil at 1 μM had no effect and at 10 μM caused a small hyperpolarization of the endothelium-intact or -denuded rat aorta. Most racemic mixtures are an equal mix of the (+)- and (−)-isomers. Consequently, the effect of racemic pinacidil will be a balance between the depolarizing action of (+)-pinacidil and the hyperpolarizing action of (−)-pinacidil.

The effect of racemic pinacidil is also dependent on the starting membrane potential. Thus, racemic pinacidil alone at 1 μM had no effect on the membrane potential of the endothelium-intact rat aorta, but in the presence of a large depolarization with KCl, reversed the depolarization and in the presence of hyperpolarization (with isoprenaline) reversed the hyperpolarization.

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