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Effects of pinacidil on arterial and venous resistances and mean circulatory filling pressure in rats

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1 The effects of the potassium channel opener, pinacidil, on mean arterial pressure (MAP), mean circulatory filling pressure (MCFP), total peripheral resistance (TPR), cardiac output (CO) and resistance to venous return (R_V) were studied in rats.

2 In pentobarbitone-anaesthetized rats given mecamylamine (ganglionic blocker, 3.7 $\mu g\ kg^{-1}$) and noradrenaline (1.5 $\mu g\ kg^{-1}\ min^{-1}$) to suppress autonomic reflexes, pinacidil (60 and 180 $\mu g\ kg^{-1} min^{-1}$), relative to the vehicle, reduced MAP and TPR in a dose-dependent manner but did not significantly alter CO, MCFP or R_V.

3 Pinacidil (10-300 $\mu g\ kg^{-1} min^{-1}$) caused similar increases in MCFP, an inverse index of venous compliance, and similar dose-dependent reductions in mean arterial pressure (MAP) in conscious, intact rats and rats infused with the ganglionic blocker, hexamethonium (150 $\mu g\ kg^{-1}\ min^{-1}$). In rats with vasomotor tone elevated by the infusion of noradrenaline (1.5 $\mu g\ kg^{-1}\ min^{-1}$), pinacidil caused markedly greater depressor responses but did not significantly alter MCFP.

4 Our results show that pinacidil is an efficacious vasodilator of arterial resistance blood vessels but has little venodilator activity.

Keywords: Pinacidil; K channel openers; mean circulatory filling pressure (MCFP); cardiac output, total peripheral resistance, venous resistance, venous compliance; capacitance vessels

Introduction

Potassium channel (ATP-sensitive K^+ channel) openers, such as pinacidil and cromakalim, are believed to cause vasodilatation primarily by the opening of plasmalemmal K^+ channels, leading to hyperpolarization and vascular relaxation (see, Quast, 1992; 1993). Other actions of the K^+-channel openers, however, may also contribute to the vasorelaxation effects of these drugs and these may include the opening of ATP-insensitive K^+-channels (see de Weille, 1992), dephosphorylation of the delayed rectifier channels, K_V, into K_{ATP} channels as well as effects unrelated to the activity of K^+-channels (see Edwards & Weston, 1994). While it is clear that the K^+-channel openers lower blood pressure via peripheral vasodilatation (see, Ahnfelt, 1988), there is no published information on the effects of these drugs on total body venous tone. *In vitro* studies show that the K^+-channel activator, pinacidil, increases K^+ conductance of arterial and venous smooth muscle cells (Hermsmeyer, 1988), and increases K^+ efflux (Southerton *et al.*, 1988) and relaxes (e.g. Toda *et al.*, 1985; Cook *et al.*, 1988; Longman *et al.*, 1988; Steinberg *et al.*, 1988; Weston *et al.*, 1988; Edwards *et al.*, 1991; Zografos *et al.*, 1992; Cai *et al.*, 1994) arterial as well as venous smooth muscle preparations.

The primary aim of this study was to investigate the effects of pinacidil on mean circulatory filling pressure (MCFP), an index of body venous tone (Tanbrizchi & Pang, 1992; Rothe 1993; Pang, 1994). MCFP is the systemic pressure that would develop after circulatory arrest and the instantaneous equilibration of arterial and venous pressures. In our study, MCFP was estimated from central venous pressure measured within 5 s of circulatory arrest induced by the inflation of a balloon implanted in the right atrium. The effects of pinacidil on venous and arterial resistances were also measured concurrently in anaesthetized rats to allow a comparison of the dilator effects of this drug on small arterial and venous resistance vessels (Wang *et al.*, 1995). Since anaesthesia and surgical stress could affect the equilibration of central venous pressure and portal venous pressure, thereby diminishing pressure transmission from the splanchnic capacitance vessels to the inferior vena cava where central venous pressure was to be measured (Tabrizchi *et al.*, 1993), MCFP measurements were also made in conscious, unrestrained intact rats as well as rats given a ganglionic blocker to suppress hypotension-induced reflex increase in sympathetic tone to the capacitance vessels.

Methods

Surgery

Male Sprague-Dawley rats (400-500 g) were anaesthetized with pentobarbitone (60 mg kg^{-1}) or halothane (2% in air). Body temperature was maintained at 37°C via a rectal thermometer and a heating pad connected to a Thermistemp Temperature Controller (Model 71; Yellow Spring Instrument Co. Inc. Ohio). A polyethylene cannula (PE50) was inserted into the left iliac artery, for the recordings of mean arterial pressure (MAP), by a pressure transducer (P23DB, Gould Statham, CA, U.S.A.), and heart rate (HR), which was derived electronically from the upstroke of the arterial pulse pressure by a tachograph (Grass, Model 7P4G). PE50 cannulae were also inserted into the right iliac vein, for the administration of vehicle or drugs, and the inferior vena cava *via* the left iliac vein, for the measurement of central venous pressure (CVP) by another pressure transducer (P23DB, Gould Statham). A saline-filled, balloon-tipped catheter was inserted into the right atrium *via* the right external jugular vein. The proper location of the atrial balloon was tested by injecting saline into the balloon and obtaining a simultaneous decrease of MAP to 20-25 mmHg and an increase in CVP within 5 s of circulatory arrest. MAP, HR and CVP were continuously monitored and recorded by a Grass Polygraph (Model RPS 7C8).

In pentobarbitone-anaesthetized rats, additional cannulae were inserted via the right carotid artery into the left ventricle

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for the injection of radioactively-labelled microspheres, and into the right iliac artery for blood withdrawal, as described in detail elsewhere (Pang, 1983; Wang *et al.*, 1995). These rats were given 30 min to stabilize before MAP, MCFP and cardiac output (CO) measurements were made.

In halothane-anaesthetized rats, all cannulae were tunnelled s.c. along the back, exteriorized at the back of the neck and secured. Bupivacaine (local anaesthetic, 0.25% solution) and cicatrin (antibiotic) were topically applied to the surgical wounds to alleviate pain and prevent infection, respectively. Halothane was withdrawn after the completion of surgery which took about 30 min. The rats were in the upright posture within 10 min of removing halothane and were used 24 h later. The conscious rats were allowed to wander freely in a small cage. The method for measuring MCFP in rats has been described in detail (Pang & Tabrizchi, 1986; see Tabrizchi & Pang, 1992; Wang *et al.*, 1995). Briefly, steady-state readings of MAP and CVP were noted at 4 to 5 s after circulatory arrest via inflation of the implanted balloon. To avoid rapid equilibration of arterial and venous pressures during circulatory arrest, the arterial pressure contributed by the small amount of trapped arterial blood was corrected by the following equation: MCFP = VPP + 1/60 (FAP-VPP), where FAP and VPP represent the final arterial pressure and venous plateau pressure, respectively, obtained within 5 s of circulatory arrest, and 1/60 represents the ratio of arterial to venous compliance.

Microspheres studies

A well-stirred suspension (200 μl) containing 20,000–25,000 microspheres (15 μm diameter), labelled with ^{57}Co (Du Pont Canada Inc., Ontario, Canada), was injected and flushed over 10 s into the left ventricle in the control period and 10 min after the i.v. bolus injection of a drug or vehicle. At 10 s before the injection of each set of microspheres, blood was withdrawn (Harvard infusion/withdrawal pump) from the iliac arterial cannula into a heparinized syringe at 0.35 ml min^{-1} for 45 s. The blood removed was slowly injected back to the rats immediately after the counting of radioactivity at 80–160 kev using a 1185 Series Dual Channel Automatic Gamma Counter (Nuclear-Chicago, Illinois, U.S.A.) with a 3 inch NaI crystal.

Experimental protocol

Pentobarbitone-anaesthetized rats Rats were divided into 2 groups ($n = 6$ each). At 20 min after a baseline measurement of CO followed by MCFP, both groups received i.v. bolus injections of mecamylamine (3.7 μmol kg^{-1}) followed by i.v. infusion of noradrenaline (1.5 μg kg^{-1} min^{-1}) at 12 min later. After another 12 min, one group of rats was infused with pinacidil (60 and 180 kg^{-1} min^{-1}) for 12 min each dose, whereas the other received infusion of equivalent volumes of the vehicle (20% ethanol in normal saline). CO followed by MCFP measurements were made 10 min after the injection or infusion of a drug or vehicle, which was at the plateau phase of response to the drugs, in all cases. The dose of mecamylamine used was found to block ganglionic transmission effectively for more than 2 h (Wang & Pang, 1991).

Conscious rats Three groups received a continuous infusion of either vehicle (normal saline, $n = 6$), hexamethonium (150 μg kg^{-1} min^{-1}, $n = 6$) or noradrenaline (1.5 μg kg^{-1} min^{-1}, $n = 4$), followed 12 min later by the infusion of pinacidil (10, 20, 40, 80, 160, 320 μg kg^{-1} min^{-1}) at dose-intervals of 12 min per dose. A fourth group (time-control, $n = 6$) received infusions of equal volumes of vehicle (20% ethanol in saline). MCFP measurements were made in the baseline conditions as well as 10 min after the administration of a drug or an equivalent volume of vehicle. The dose of hexamethonium used reduced reflex tachycardiac response to i.v bolus injection of acetylcholine by >50% throughout the course of the experiment (Waite *et al.*, 1988).

Drugs

Pinacidil was a gift from Eli Lilly & Co. (IN, U.S.A.). Mecamylamine hydrochloride and noradrenaline bitartrate were obtained from Sigma Chemical Co. (MO, U.S.A.). Hexamethonium bromide was from K & K Lab. (CA, U.S.A.). All drugs were dissolved in normal saline (0.9% NaCl) except for pinacidil, which was dissolved in 20% ethanol in normal saline. Cicatrin powder was from Burroughs Wellcome Inc. (Que., Canada) and Bupivacaine HCl solution was from Sanofi Winthrop (Ontario, Canada).

Calculations and statistics analysis

CO, total peripheral resistance (TPR) and venous resistance (R_V) were calculated as follows:

$$\text{CO (ml min}^{-1}) = \frac{\text{rate of withdrawal of blood (ml min}^{-1}) \times \text{total injected c.p.m.}}{\text{c.p.m. in withdrawn blood}}$$

$$\text{TPR (mmHg min ml}^{-1}) = \frac{\text{BP (mmHg)}}{\text{CO (ml min}^{-1})}$$

$$R_v \text{ (mmHg min ml}^{-1}) = \frac{\text{MCFP (mmHg)} - \text{CVP (mmHg)}}{\text{CO (ml min}^{-1})}$$

Due to the technical difficulty of making concurrent measurements of right atrial pressure and MCFP in small animals, CVP rather than right atrial pressure was used to estimate pressure gradient to venous return (MCFP-right atrial pressure). This is legitimate since mean CVP is nearly identical to mean right atrial pressure (see Rothe, 1993).

All results were analysed by the analysis of variance/covariance followed by Duncan's multiple range test, with $P < 0.05$ selected as the criterion for statistical significance. Profile/trend analysis was used to compare dose-dependency of responses using the statistical package, SYSTAT v. 5.03 (SYSTAT Inc., IL, U.S.A.).

Results

Anaesthetized rats

The injection of mecamylamine reduced MAP, HR, CO and MCFP in both the pinacidil and control (vehicle) groups, but did not significantly alter TPR or R_V in either of the two groups (Figure 1). The infusion of noradrenaline did not significantly alter TPR but increased MAP, HR, CO, R_V and MCFP in both pinacidil and control groups (Figure 1).

During the continuous infusion of noradrenaline, the vehicle did not significantly alter any measured parameters but tended to reduce MCFP slightly with the passage of time. The infusion of pinacidil dose-dependently reduced MAP and TPR but did not significantly alter HR, CO, R_V or MCFP (Figure 1). As with the vehicle in the time-control group, there were slight but insignificant reductions in MCFP with pinacidil. The small declines in MCFP with pinacidil and the vehicle were probably due to the loss of venomotor response to infused noradrenaline with time.

Conscious, unrestrained rats

Baseline values of MAP, HR and MCFP among the four groups of conscious rats were not significantly different from each other and they ranged from mean group values of 110 to 121 mmHg, 380 to 417 beats min^{-1} and 5.6 to 5.9 mmHg, respectively. The infusion of hexamethonium reduced MAP significantly from 117 ± 3 to 102 ± 3 mmHg, HR insignificantly from 417 ± 11 to 400 ± 15 beats min^{-1} and MCFP significantly

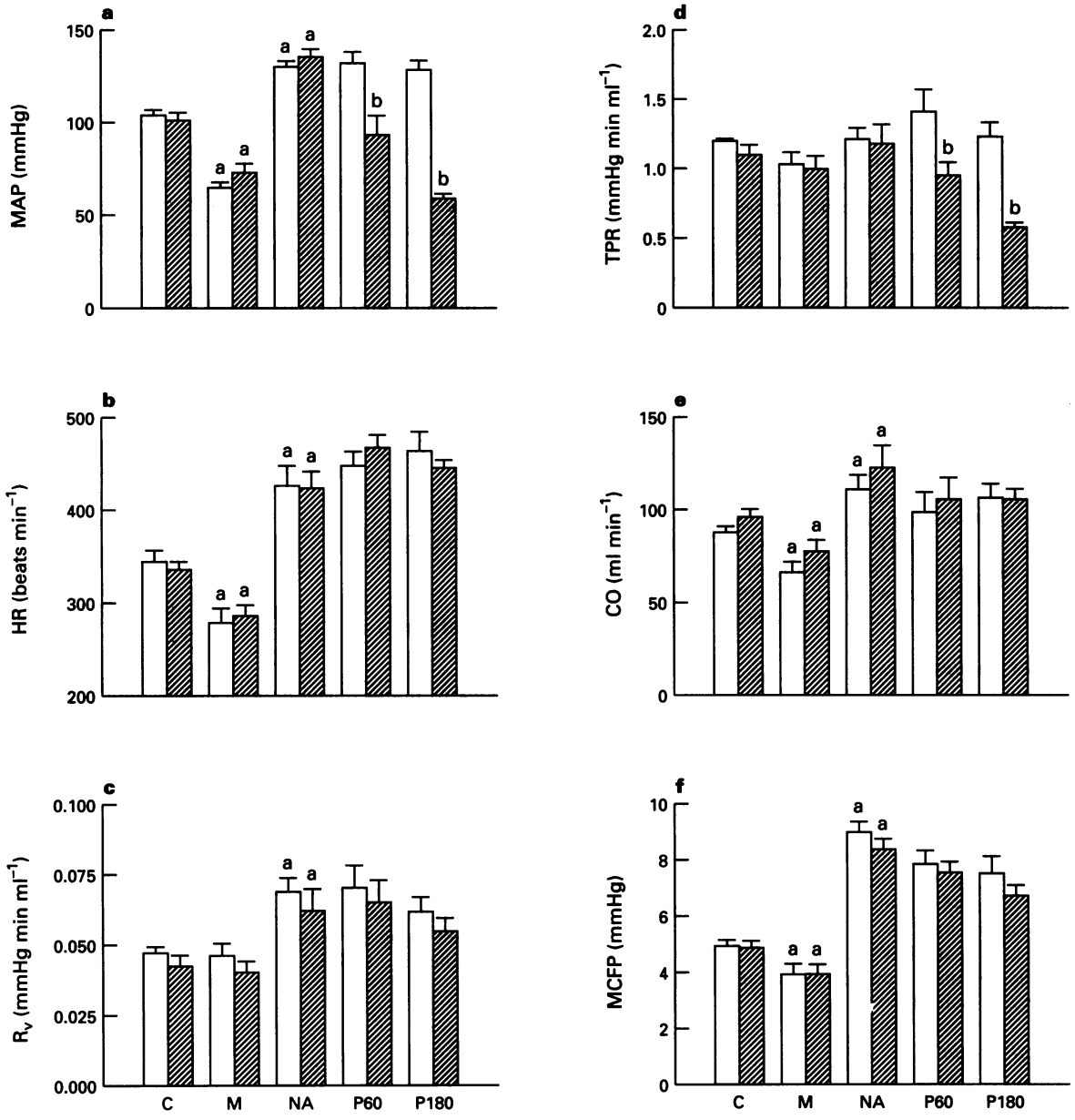

Figure 1 Effects (mean ± s.e.mean) of i.v. infusion of a low (60 $\mu g\,kg^{-1}\,min^{-1}$, P60) and a high (180 $\mu g\,kg^{-1}\,min^{-1}$, P180) dose of pinacidil ($n=6$) and equivalent volumes of vehicle (20% ethanol in 0.9% NaCl solution, C, $n=6$) on mean arterial pressure (MAP), total peripheral resistance (TPR), heart rate (HR), cardiac output (CO), venous resistance (R_V) and mean circulatory filling pressure (MCFP) in pentobarbitone-anaesthetized rats given mecamylamine (3.7 $\mu g\,kg^{-1}$ M) followed by a continuous infusion of noradrenaline (1.5 $\mu g\,kg^{-1}\,min^{-1}$, NA). [a] Significantly different ($P<0.05$) from the corresponding baseline readings prior to any drug treatment. [b] Significantly different ($P<0.05$) from the corresponding pre-drug readings obtained after pretreatment with both mecamylamine and noradrenaline.

from 5.8 ± 0.1 to 5.3 ± 0.2 mmHg. The infusion of noradrenaline increased MAP from 121 ± 1 to 154 ± 5 mmHg, MCFP from 5.6 ± 0.2 to 7.2 ± 0.4 mmHg but did not significantly alter HR which was 390 ± 21 beats min^{-1} before and 385 ± 23 beats min^{-1} after, the infusion of noradrenaline.

The vehicle did not significantly alter MAP, HR or MCFP during the 100 min observation period (Figure 2). Curve analysis shows that pinacidil dose-dependently reduced MAP, but increased HR and MCFP in intact rats (Figure 2). In hexamethonium-treated rats, pinacidil also caused similar dose-dependent reductions in MAP and increases in MCFP as in intact rats but did not increase HR (Figure 2). Curve analysis also shows that in rats treated with noradrenaline, pinacidil caused markedly greater reductions in MAP than it did in either intact or hexamethonium-treated rats, similar increases in HR as it did in intact rats, but did not alter MCFP relative to pre-drug MCFP value prior to the infusion of pinacidil.

Discussion

Our results in pentobarbitone-anaesthetized rats showed that pinacidil reduced MAP via peripheral vasodilatation, as TPR and MAP were both reduced in dose-related manner and CO was unchanged. Pinacidil caused vasodilatation in arterial resistance vessels, but not venous capacitance or venous resistance vessels, since the drug altered neither MCFP nor R_V, respectively. These results are consistent with the interpretation that pinacidil has negligible venodilator actions, as the drug was examined under conditions that favour venodilatation via suppression of autonomic reflex with mecamylamine and elevation of venomotor tone with the infusion of noradrenaline (see Tabrizchi & Pang, 1992; Pang, 1994).

Since a combination of anaesthesia and surgical stress may interfere with the equilibration of CVP and portal venous pressure, thereby reducing pressure transmission from the splanchnic venous bed into the inferior cava (Tabrizchi *et al.*,

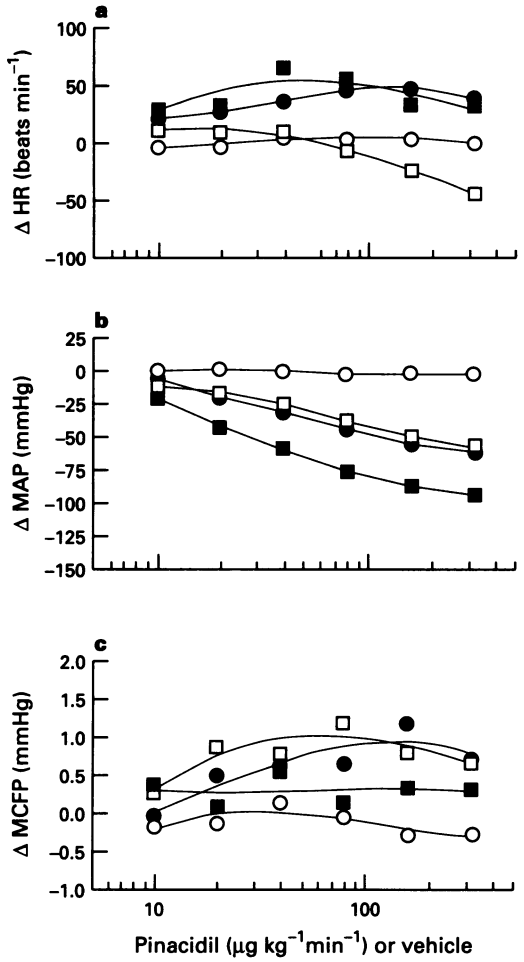

Figure 2 Effects of (means) of i.v. infusion of pinacidil on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in three groups of conscious rats: intact rats (●, $n=6$), rats infused with hexamethonium (□, 150 μg kg^{-1} min^{-1}, $n=6$) and rats continuously infused with noradrenaline (■, 1.5 μg kg^{-1} min^{-1}, $n=4$). Effects of i.v. infusions of the pinacidil vehicle (○, equivalent volumes of 20% ethanol in 0.9% NaCl solution) on the above parameters in conscious control rats ($n=6$) are also shown. S.e.mean was omitted to avoid overlapping of lines. See text for significant differences among curves.

1993), the effects of pinacidil on MCFP was also measured in three groups of conscious, unrestrained rats. In intact as well as hexamethonium-treated rats, pinacidil reduced MAP and increased MCFP. These results are supportive of the lack of venodilator activity of pinacidil. Pinacidil increased HR in intact rats but not in hexamethonium-treated rats, suggesting that the tachycardiac response to pinacidil was mediated via the modulation of autonomic reflex activity. In rats infused with noradrenaline, pinacidil caused markedly greater depressor response than it did in intact rats or hexamethonium-treated rats. This is consistent with the well-known observation of enhanced depressor response to a vasodilator drug in an animal with high vasomotor tone. HR was similarly increased by pinacidil in noradrenaline-treated rats as in intact rats not receiving noradrenaline. Pinacidil, however, did not significantly reduce MCFP even under conditions of elevated venomotor tone. These results are again supportive of pinacidil's lack of venodilator activity. Over the years, we have examined the effects of various classes of venodilator and venoconstrictor agents on MCFP in conscious rats (see Tabrizchi & Pang, 1992; Pang, 1994). A comparison of these MCFP studies which were conducted under similar experimental conditions shows that pinacidil has less venodilator activity than does sodium nitroprusside (D'Oyley *et al.*, 1989), nitroglycerin (D'Oyley *et al.*, 1989), verapamil (Waite *et al.*, 1988), calcitonin gene-related peptide (Abdelrahman & Pang, 1992), hexamethonium (D'Oyley & Pang, 1990), prazosin (D'Oyley & Pang, 1990), rauwolscine (D'Oyley & Pang, 1990), or isoprenaline (Abdelrahman & Pang, 1990).

To summarize, pinacidil dose-dependently reduced MAP and TPR but did not alter CO, R_V or MCFP of pentobarbitone-anaesthetized, areflex rats with vasomotor tone elevated by the infusion of noradrenaline. These results show that pinacidil vasodilates arterial resistance vessels, but not venous resistance or venous capacitance vessels. Pinacidil also did not alter MCFP in conscious rats which are reflex-intact or ganglion-blocked, or conscious rats with vasomotor tone elevated by the infusion of noradrenaline.

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