Br. J. Pharmac. (1987), **91**, 421–429

Evidence that the mechanism of the inhibitory action of pinacidil in rat and guinea-pig smooth muscle differs from that of glyceryl trinitrate

Katharine M. Bray, D.T. Newgreen, R.C. Small, J.S. Southerton, [2]S.G. Taylor, [3]Sheila W. Weir & [1]A.H. Weston

Smooth Muscle Research Group, Department of Physiological Sciences, Medical School, University of Manchester, Manchester M13 9PT

1 The effects of pinacidil have been compared with those of glyceryl trinitrate (GTN) using the aorta and portal vein of the rat and the trachealis and taenia caeci of the guinea-pig.

2 In aorta, both pinacidil and GTN inhibited responses to noradrenaline and showed some selective inhibition of contractions to 20 mM K^+. Responses to 80 mM K^+ were little affected.

3 In trachealis, both pinacidil and GTN inhibited spontaneous tone and selectively relaxed spasms to 20 mM K^+. Responses to 80 mM K^+ were unaffected.

4 In portal vein, pinacidil completely inhibited spontaneous electrical and mechanical activity. GTN reduced the amplitude of tension waves and extracellularly-recorded discharges, but increased the frequency of spontaneous electrical and mechanical activity.

5 In portal vein, pinacidil inhibited contractions to noradrenaline and selectively inhibited responses to 20 mM K^+. GTN had little inhibitory effect on responses to either noradrenaline or K^+.

6 In portal veins loaded with ^{86}Rb as a K^+-marker, pinacidil significantly increased the ^{86}Rb efflux rate coefficient whilst GTN had no effect on ^{86}Rb exchange.

7 In taenia caeci, both pinacidil and GTN inhibited the spontaneous tone of the preparation. These inhibitory effects were not antagonized by apamin.

8 It is concluded that pinacidil and GTN do not share a common relaxant mechanism. Evidence has been obtained that pinacidil exerts its inhibitory effects by the opening of apamin-insensitive, ^{86}Rb-permeable K^+ channels.

Introduction

Pinacidil ((±)-N-cyano-4-pyridyl-N-1,2,2-trimethylpropylguanidine monohydrate) is one of a group of cyanoguanidine derivatives with antihypertensive properties in conscious dogs (Kawashima & Liang, 1985) and hypertensive patients (Carlsen *et al.*, 1981). *In vitro* experiments in a variety of vascular tissues have indicated that pinacidil has a direct arterial vasodilator effect (Arrigoni-Martelli *et al.*, 1980) but preliminary attempts to localize its site of action have been unsuccessful (Kaergaard Nielsen & Arrigoni-Martelli, 1981; Cohen & Colbert, 1986).

In the present study the inhibitory effects of pinacidil have been compared with those of glyceryl trinitrate (GTN) using a variety of smooth muscles from the rat and guinea-pig. The effects of these two drugs on spontaneous mechanical activity has been examined, together with their ability to modify contractions produced by noradrenaline or potassium. In this way it was hoped to characterize the inhibitory profile of pinacidil and to provide suggestions for further studies of the mode of action of this cyanoguanidine derivative.

[1]Author for correspondence.
[2]Present address: Beecham Pharmaceuticals, Biosciences Research Centre, Great Burgh, Epsom, Surrey, KT18 5XQ.
[3]Present address: Preclinical Research Department, Sandoz AG, CH-4002, Basle, Switzerland.

Methods

Portal veins and segments of aorta were obtained from male Wistar rats (300–400 g) which were killed by decapitation. Segments of trachea and taenia caeci were removed from guinea-pigs (300–500 g) of either sex which had been killed by stunning and bleeding. All animals were supplied by Manchester University Animal Unit.

Aorta

Each thoracic segment of aorta was cut into four rings approximately 0.5 cm long and each was opened along its longitudinal axis forming a flat sheet. A thread was attached to each of the longitudinally-cut edges using a small bent pin and the endothelium was mechanically removed using a cotton bud moistened with physiological salt solution (PSS). Each ring was then mounted for isometric tension recording under a resting tension of 1 g in a tissue bath containing PSS at 37°C, pH 7.4.

After 30 min equilibration in PSS the spasmogenic effects of noradrenaline or KCl were evaluated by constructing cumulative concentration-effect curves. The antispasmodic effects of GTN or pinacidil were then examined by allowing these drugs a 30 min equilibration period with the tissue, after which responses to noradrenaline or KCl were re-examined in the continuing presence of GTN or pinacidil. In some experiments, the inhibitory effects of GTN or pinacidil were tested by first exposing tissues to KCl (20 or 80 mM) or to noradrenaline (1 μM). When the maximal mechanical effects of these spasmogens had been obtained, the spasmolytic effects of GTN or pinacidil were investigated in the continuing presence of either KCl or noradrenaline using a cumulative protocol. Control tissues were treated similarly but were not exposed to pinacidil or GTN.

Portal vein

Portal veins, each approximately 2 cm in length were mounted for isometric tension recording under a resting tension of 0.5 g. Responses to noradrenaline were investigated using a sequential protocol. For KCl, cumulative concentration-effect experiments were carried out using a 3 min contact time for each KCl concentration. For both spasmogens, mechanical responses were quantified using integrators (Grass 7P10). Subtraction of the integrated mechanical activity obtained in the 2 min period before exposure to the spasmogen, from the value obtained during the first 2 min of exposure yielded an estimate of the response to the spasmogen.

In some experiments, the electrical activity of portal veins was recorded extracellularly using the perfused capillary method described by Jetley & Weston (1980). The resulting electrical and mechanical signals were each quantified with integrators using 2 min integration periods. Control tissues were treated similarly but were not exposed to pinacidil or GTN.

In the experiments in which ^{86}Rb was used as a marker for K^+, the technique of Hamilton *et al.* (1986) was broadly followed. Tissues were attached to a gassing manifold and loaded with $^{86}RbCl$ (1 μCi ml^{-1}) for 90 min at 37°C. The ^{86}Rb was then allowed to efflux from the tissues by transferring them to tubes each containing 5 ml PSS alone, using 2 min collection periods. After 7 such periods (14 min into the efflux) the tissues were exposed to pinacidil or GTN for the next 4 collection periods. For the final 3 collection periods, the tubes contained PSS alone. One ml aliquots of PSS were then counted for radioactivity and the efflux data expressed in terms of the rate coefficient (fractional loss of ^{86}Rb from the tissue standardized for a 1 min period, expressed as a percentage).

Taenia caeci

Segments of taenia caeci each approximately 2 cm long were removed and mounted in tissue baths under a 1 g load. Tissue length changes were recorded isotonically. After 45 min equilibration in PSS, each tissue was exposed to noradrenaline 0.8 μM for 1 min after which the preparations were washed repeatedly with PSS. This procedure optimized resting tone (Weir & Weston, 1986a).

After tone had been optimised, each tissue was exposed only to a single relaxant (either GTN or pinacidil or noradrenaline) using a cumulative concentration-response protocol. In some experiments, preparations were subsequently exposed to apamin 0.1 μM for 30 min and then re-challenged with GTN or pinacidil or noradrenaline in the continuing presence of apamin. The relaxation produced by papaverine 100 μM at the end of an experiment was used to define the position of zero tone against which the inhibitory effects of GTN, pinacidil and noradrenaline were assessed. Control tissues were treated similarly but were not exposed to pinacidil or GTN.

Trachea

Segments of trachea were set up for isometric recording as described by Foster *et al.* (1983), under an imposed tension of 1 g. After approximately 20 min, tissues were exposed to aminophylline 1 mM to define the position of zero tone. The aminophylline was washed from the tissues and when the tone subsequently became maximal, investigation of the action of GTN and pinacidil commenced.

The relaxant effects of GTN or pinacidil on spon-

taneous tone were first studied using a cumulative protocol, allowing 8 min for each relaxant concentration to achieve its maximum effect. Tissues were then divided into one of three groups: (1) control: tissues were exposed for a second time to either pinacidil or GTN; (2) 20 mM KCl test: tissues were exposed to KCl, 20 mM, and when the contraction had reached a plateau, pinacidil or GTN was applied cumulatively; (3) 80 mM KCl test: tissues were exposed to KCl, 80 mM and the effects of pinacidil or GTN examined as in (2).

Drugs and solutions/statistical analysis

The following substances were used: apamin (Sigma); glyceryl trinitrate (ICI), stock solution in 100% ethanol; (−)-noradrenaline bitartrate (Sigma), stock solution in 0.1 M HCl; papaverine (Sigma); (±)-pinacidil (Leo), stock solution in 70% v/v ethanol:distilled water; ^{86}RbCl (Amersham). Dilutions were performed using distilled water except in the case of GTN for which primary dilutions were performed using ethanol. The composition of the MOPS-buffered physiological salt solution (PSS) used is listed by Hamilton *et al.* (1986). When KCl was used as a spasmogen, the stated concentration excludes the KCl (5.9 mM) already present in the PSS.

The significance of differences between two means was assessed by use of a two-tailed unpaired Students *t* test.

Results

Effects on responses to noradrenaline

Pretreatment of rat aorta and portal vein with pinacidil (1–100 μM and 0.3–30 μM, respectively) produced a concentration-dependent inhibition of responses to noradrenaline (Figure 1). Responses of rat aorta to noradrenaline were also inhibited by GTN (0.003–0.3 μM). In contrast, however, the action of noradrenaline on portal vein was unaffected by GTN (0.3–3 μM) (Figure 1).

Extracellular recordings from portal vein showed that each spontaneous phasic tension wave was accompanied by a burst of electrical activity. On exposure to noradrenaline, the sustained increase in tension was associated with continuous fast electrical activity (Figure 2). Exposure to pinacidil (0.3–3 μM) inhibited spontaneous electrical and mechanical activity (Figure 2). Subsequent exposure to noradrenaline resulted in the re-appearance of electrical and mechanical activity, but at a lower level than in controls (Figure 2). GTN (0.01–1 μM) reduced the amplitude but increased the frequency of spontaneous tension waves. The amplitude of extracellularly-recorded electrical activity was also reduced. Concentrations of GTN > 1 μM had no further inhibitory effect on the spontaneous electrical and mechanical activity.

Effects on responses to KCl

In rat aorta, preincubation with pinacidil (1–100 μM) produced a concentration-dependent inhibition of responses to KCl (10–80 mM). There was some evidence in these experiments of a selective inhibitory action against low concentrations of KCl (10–20 mM) (Figure 3). This was more clearly seen when tissues were precontracted with KCl (20 or 80 mM) and the ability of pinacidil to relax the contraction was subsequently examined. In these experiments, the $-\log IC_{50}$ of pinacidil against 20 mM KCl was 6.1 ± 0.2 (mean ± s.e.mean, $n = 4$) whilst no significant inhibition was seen against an 80 mM KCl-induced contraction ($n = 4$).

In rat portal vein, preincubation with pinacidil (0.3–30 μM) produced an inhibition of responses to KCl (5–80 mM) qualitatively similar to that seen in rat aorta (Figure 3). The $-\log IC_{50}$ of pinacidil against an established 20 mM KCl contraction was 6.2 ± 0.1 (mean ± s.e.mean, $n = 4$) and no significant inhibition of responses to 80 mM KCl was detected ($n = 4$). In rat aorta, pretreatment with GTN (0.003–0.3 μM) inhibited responses to KCl (10–80 mM) but had no effect on similar responses to KCl in rat portal vein (Figure 3). In segments of aorta precontracted with KCl, GTN produced selective inhibition of responses to KCl 20 mM ($-\log IC_{50}$, 7.7 ± 0.3, mean ± s.e.mean, $n = 4$) but had no significant effect on responses to KCl 80 mM ($n = 4$).

In guinea-pig trachea precontracted with KCl (20 or 80 mM), both pinacidil and GTN were capable of completely inhibiting responses to KCl, 20 mM ($-\log IC_{50}$ values of 4.7 ± 0.2 and 6.2 ± 0.2, respectively; means ± s.e.mean, $n = 8$). Neither agent produced any significant inhibition of responses to KCl, 80 mM ($n = 8$).

Effects on spontaneous tone

In the guinea-pig trachea, both pinacidil (0.1–100 μM) and GTN (0.01–10 μM) produced full relaxation of the spontaneous tone of the preparations (Figure 4). In the guinea-pig taenia caeci, the relaxant effects of pinacidil and GTN were both relatively slow to develop and were compared with those of noradrenaline which produced a rapid inhibition of spontaneous tone. Both pinacidil and noradrenaline were capable of producing almost total relaxation of the tissue whilst GTN was only capable of generating approximately 50% relaxation (Figure 4). In the presence of apamin, 0.1 μM, the inhibitory effects of noradrenaline were abolished whilst the relaxations

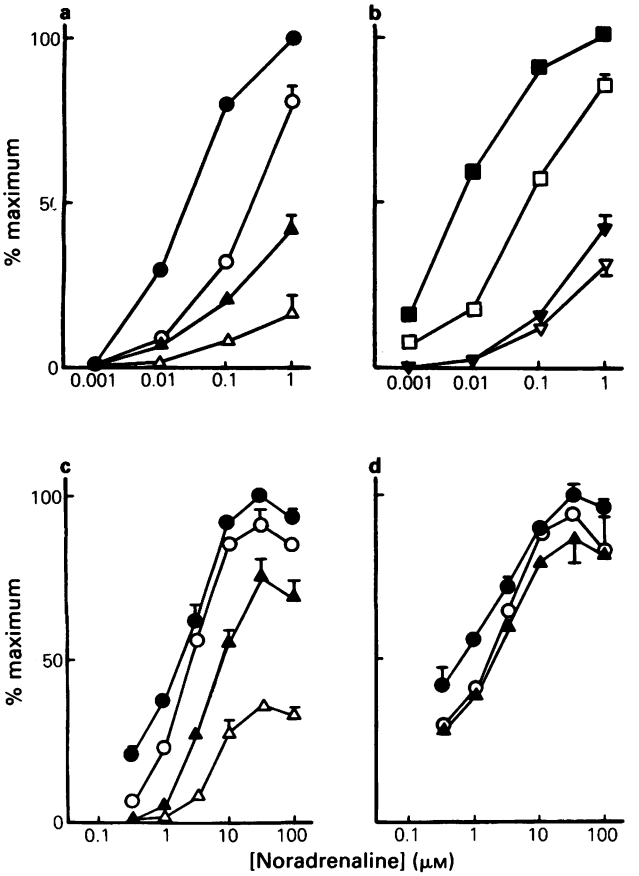

Figure 1 Effects of pinacidil and glyceryl trinitrate (GTN) on mechanical responses to noradrenaline in (a,b) rat aorta and (c,d) rat portal vein under isometric conditions. (a) Control responses (●); responses in the presence of pinacidil, 1 μM (○); 10 μM (▲); 100 μM (△). (b) Control responses (■); responses in the presence of GTN, 0.003 μM (□); 0.03 μM (▼); 0.3 μM (▽). (c) Control responses (●); responses in the presence of pinacidil, 0.3 μM (○); 3 μM (▲); 30 μM (△). (d) Control responses (●); responses in the presence of GTN, 0.03 μM (○); 3 μM (▲). Ordinate scales: % of the initial control maximum response to noradrenaline. Each point is the mean derived from 4 experiments; vertical lines show sample s.e.mean values. No significant changes in response to noradrenaline were detected in concurrent, time-matched control experiments.

produced by pinacidil and GTN were unaffected (Figure 4).

Effects on ^{86}Rb efflux

The similarity between the inhibitory effects of pinacidil on rat portal vein and those observed in an earlier study using BRL34915 (Hamilton *et al.*, 1986) prompted the ^{86}Rb efflux experiments. The average basal rate of ^{86}Rb efflux measured between the 14th and 22nd min of the efflux period was $1.34 \pm 0.06\%\ min^{-1}$ (mean ± s.e.mean, $n = 4$). In the presence of pinacidil, 10 μM, the ^{86}Rb efflux rate coefficient increased to $2.26 \pm 0.2\%\ min^{-1}$, whilst GTN had no significant effect on ^{86}Rb exchange (Figure 5). The effects of pinacidil were relatively slow in onset and the rate of ^{86}Rb efflux remained elevated even after pinacidil had been removed by washing.

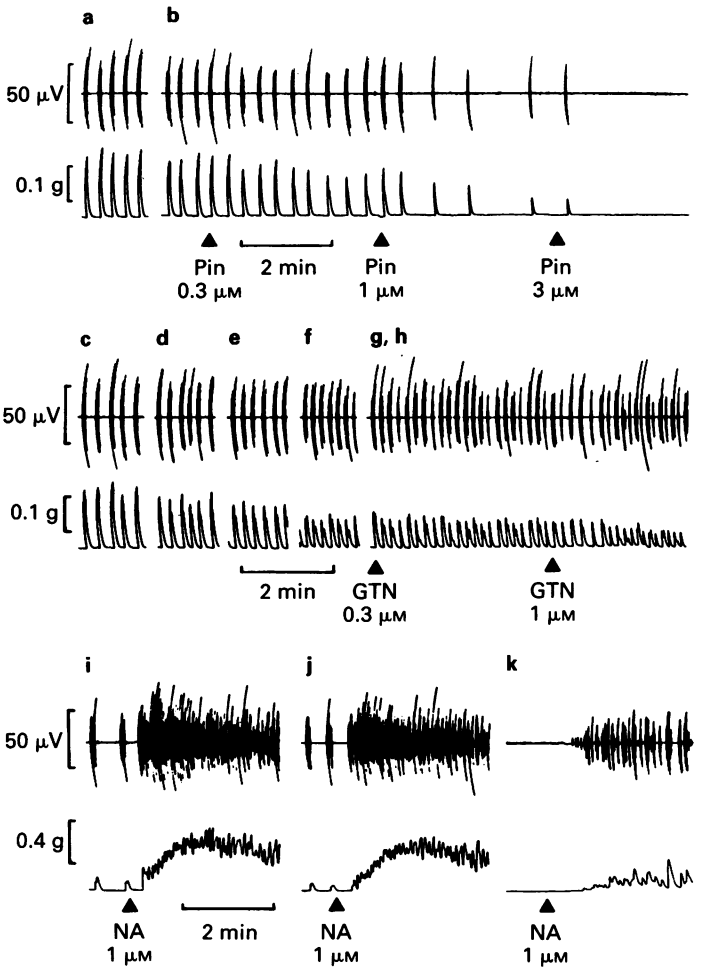

Figure 2 Effects of pinacidil (Pin) and glyceryl trinitrate (GTN) on extracellular electrical activity (upper traces) and isometric mechanical activity (lower traces) in rat portal vein. (a) Control activity and (b) effect of cumulative exposure to pinacidil at 4 min intervals. (c) Re-established control activity. (d–f) Effects after approximately 3 min cumulative exposure to GTN, 0.01, 0.03 and 0.1 μM, respectively. (g,h) Effects of cumulative exposure to GTN 0.3 μM and 1 μM, respectively. Further increase in the GTN concentration had no greater inhibitory effect. (i–k) Responses to noradrenaline (NA), 1 μM; (i) control, (j,k) after 30 min preincubation with GTN, 1 μM and pinacidil, 3 μM, respectively. Records (a–h) are from the same preparation; records (i–k) are from a different tissue.

Discussion

In the present study, the effects of pinacidil have been studied on a variety of smooth muscles with different properties. Rat aorta was chosen as an example of a tissue in which the guanylate cyclase system is well-developed (Murad, 1986). In contrast, this system is poorly represented in rat portal vein (Southerton & Taylor, unpublished), a tissue in which electrical spike generation accompanies phasic mechanical activity. Guinea-pig trachealis and taenia caeci were used because both tissues generate significant spontaneous tone and mechanisms by which this can be inhibited have recently been studied in some detail (Allen *et al.*, 1986a,b; Weir & Weston, 1986a). Furthermore, the actions of some relaxants on guinea-pig taenia caeci can be antagonized by apamin (Weir & Weston, 1986a).

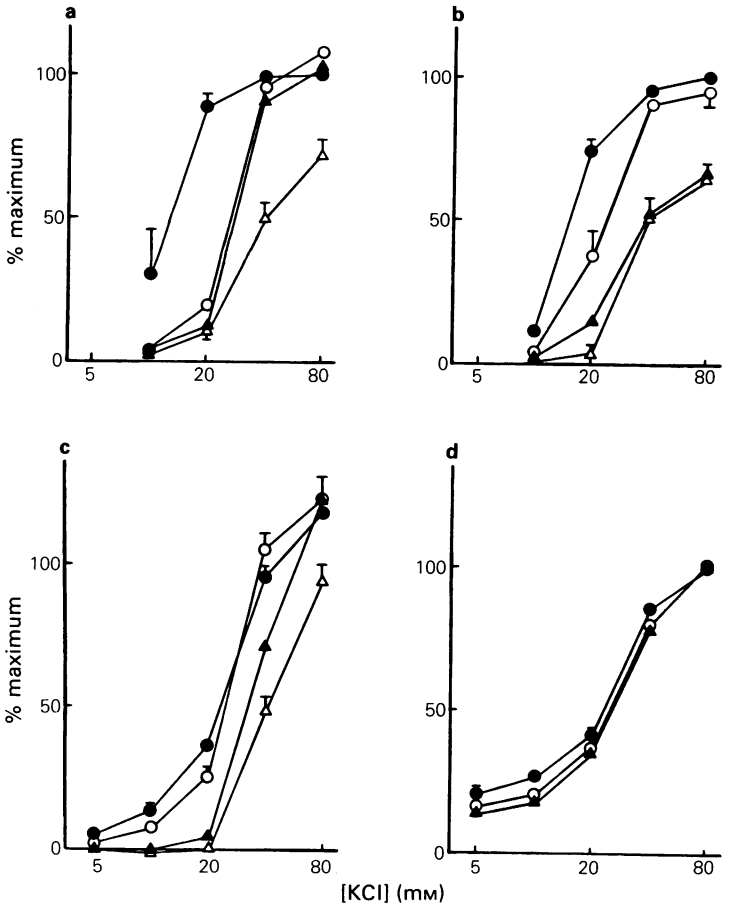

Figure 3 Effects of pinacidil and glyceryl trinitrate (GTN) on mechanical responses to KCl on (a,b) rat aorta and (c,d) rat portal vein under isometric conditions. (a) Control responses (●); responses in the presence of pinacidil, 1 μM (○); 10 μM (▲); 100 μM (△). (b) Control responses (●); responses in the presence of GTN, 0.003 μM (○); 0.03 μM (▲); 0.3 μM (△). (c) Control responses (●); responses in the presence of pinacidil, 0.3 μM (○); 3 μM (▲); 30 μM (△). (d) Control responses (●); responses in the presence of GTN, 0.3 μM (○); 3 μM (▲). Further increase in GTN concentration produced no greater inhibitory effect. Ordinate scales: % initial control maximum response to noradrenaline. Each point is the mean derived from 4 experiments; vertical lines show sample s.e.mean values. No significant changes in responses to noradrenaline were detected in concurrent, time-matched control experiments.

The inhibitory action of pinacidil was compared with that of GTN. Although the mode of action of this nitrovasodilator is not fully understood, much evidence suggests that GTN exerts its actions by stimulation of guanylate cyclase (Murad, 1986). Although not all data support this assumption, especially in non-vascular smooth muscle (Diamond, 1983), many directly-acting vasodilator drugs seem to exert a guanylate cyclase-stimulating action. It thus seemed reasonable to compare the actions of pinacidil with such a drug and GTN was selected because of its well-known vascular effects.

In most of the tissues studied, there were qualitative similarities between the inhibitory effects of pinacidil and GTN, confirming and extending the results of previous workers (Arrigoni-Martelli & Finucaine, 1985; Cohen & Colbert, 1986). However, there were marked quantitative differences in the potency of the two agents. In rat aorta, for example, GTN was more potent than pinacidil against noradrenaline-induced

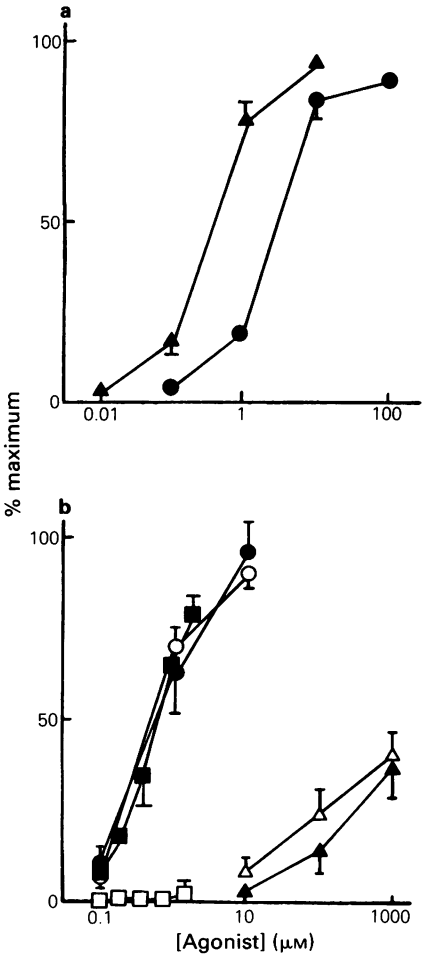

Figure 4 Effects of pinacidil, glyceryl trinitrate (GTN) and noradrenaline on spontaneous tone in (a) guinea-pig trachealis muscle under isometric conditions and (b) guinea-pig taenia caeci under isotonic conditions. (a) Relaxant responses to pinacidil (●) and GTN (▲) are expressed as a percentage of the maximum relaxation to aminophylline, 1 mM. (b) Relaxant responses in normal physiological salt solution to pinacidil (●), GTN (▲) and noradrenaline (■) and in the presence of apamin, 0.1 μM (equivalent open symbols) are expressed as a percentage of the maximum relaxation to papaverine, 100 μM. Each point is the mean derived from at least 6 experiments; vertical lines show sample s.e.mean values. No significant changes in responses to pinacidil, glyceryl trinitrate or noradrenaline were detected in concurrent, time-matched control experiments.

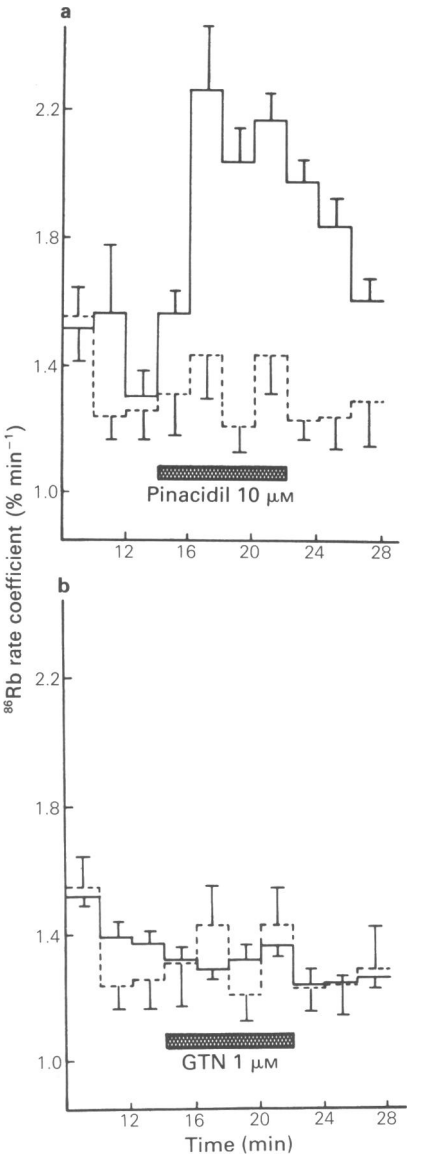

Figure 5 Effects of pinacidil and glyceryl trinitrate (GTN) (at hatched rectangles) on the efflux of ^{86}Rb from rat portal vein. (a) Effect of pinacidil, 10 μM (continuous line) and (b) effect of GTN, 1 μM (continuous line) compared with control, basal loss of ^{86}Rb (broken lines). Ordinate scales; ^{86}Rb efflux rate coefficient (% min^{-1}). Abscissa scales; time (min) after start of efflux period. Each point is the mean derived from 4 experiments; vertical bars show s.e.mean values.

tone whereas in guinea-pig taenia caeci the order of potency, measured against spontaneous tone, was reversed.

It had been hoped that the ability of pinacidil and GTN to inhibit responses to 20 mM and 80 mM KCl might allow any differences in their mode of action to be highlighted. Such a simple test easily allows calcium-entry blocking drugs to be distinguished from vasodilators with other modes of action (Hamilton *et al.*, 1986). However, although there were some differences between different tissues, both pinacidil and GTN were quite effective inhibitors of responses to 20 mM KCl and relatively ineffective when the stimulus was 80 mM KCl.

The key experiments which have allowed major differences between the mode of action of pinacidil and GTN to be characterized were performed using rat portal vein. In this tissue, GTN produced some changes in the pattern of spontaneous electrical and mechanical activity but had little effect on their total (integrated) values. In contrast, pinacidil produced complete abolition of spontaneous electrical and mechanical activity. Furthermore, GTN was essentially without effect on responses of the portal vein to either noradrenaline or KCl, whereas pinacidil was an effective inhibitor of the contractions produced by noradrenaline and 20 mM KCl. In veins rendered electrically quiescent by pinacidil, subsequent exposure to noradrenaline was associated with the reappearance of electrical spikes and a reduced level of mechanical activity. These actions of pinacidil are very similar to those of the novel amido-chromanol, BRL34915 (Hamilton *et al.*, 1986; Weir & Weston, 1986a,b) and prompted the experiments using ^{86}Rb as a K^+ marker. In these experiments pinacidil produced a marked increase in the ^{86}Rb efflux rate coefficient, whereas GTN was without effect.

Such results strongly suggest that the mode of action of pinacidil is quite different from that of the nitrovasodilator, GTN. They indicate that pinacidil is a K^+-channel opening drug which exerts its inhibitory effects by the production of a low resistance pathway in the smooth muscle cell membrane. This tends to hold the membrane potential close to the potassium equilibrium potential, thereby resisting the depolarizing actions of agonists. Further evidence in favour of this suggestion has recently been obtained (Southerton *et al.*, 1987). The nature of the K^+-channel opened by pinacidil is uncertain. However, the present study has shown that the inhibitory effects of this agent are not mediated via an apamin-sensitive K^+-channel.

This work was supported by the Mason Medical Foundation, The Royal Society and the SmithKline Foundation. The help and advice of Dr W.O. Godtfredsen and Dr Keith Willan (Leo) is gratefully acknowledged. The GTN was generously donated by ICI. K.M.B. is a Ciba-Geigy Research Student; D.T.N., J.S.S. and S.W.W. were supported by SERC Case Awards.

References

ALLEN, S.L., BOYLE, J.P., CORTIJO, J., FOSTER, R.W., MORGAN, G.P. & SMALL, R.C. (1986a). Electrical and mechanical effects of BRL34915 in guinea-pig isolated trachealis. *Br. J. Pharmac.*, **89**, 395–405.

ALLEN, S.L., CORTIJO, J., FOSTER, R.W., MORGAN, G.P., SMALL, R.C. & WESTON, A.H. (1986b). Mechanical and electrical aspects of the relaxant action of aminophylline in guinea-pig isolated trachealis. *Br. J. Pharmac.*, **88**, 473–483.

ARRIGONI-MARTELLI, E. & FINUCAINE, J. (1985). Pinacidil. In *New Drugs Annual; Cardiovascular Drugs*. ed. Scriabine, A., Vol. 3, pp. 133–151. New York: Raven Press.

ARRIGONI-MARTELLI, E., KAERGAARD NIELSEN, C., BANG OLSEN, V. & PETERSEN, H.J. (1980). N′-cyano-N-pyridyl-N′-1,2,2-trimethylpropylguanidine, monohydrate (1134): A new, potent vasodilator. *Experientia*, **36**, 445–447.

CARLSEN, J.E., KARDEL, T., HILDEN, T., TANGØ, M. & TRAP-JENSEN, J. (1981). Immediate central and peripheral haemodynamic effects of a new vasodilating agent Pinacidil (P1134) in hypertensive man. *Clin. Physiol.*, **1**, 375–384.

COHEN, M.L. & COLBERT, W.E. (1986). Comparison of the effects of pinacidil and its metabolite, pinacidil-N-oxide, in isolated smooth and cardiac muscle. *Drug Devel Res.*, **7**, 111–124.

DIAMOND, J. (1983). Lack of correlation between cyclic GMP elevation and relaxation of nonvascular smooth muscle by nitroglycerin, nitroprusside, hydroxylamine and sodium azide. *J. Pharmac. exp. Ther.*, **225**, 422–423.

FOSTER, R.W., SMALL, R.C. & WESTON, A.H. (1983). Evidence that the spasmogenic action of tetraethylammonium in guinea-pig trachealis is both direct and dependent on the cellular influx of calcium ion. *Br. J. Pharmac.*, **79**, 255–263.

HAMILTON, T.C., WEIR, S.W. & WESTON, A.H. (1986). Comparison of the effects of BRL34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br. J. Pharmac.*, **88**, 103–111.

JETLEY, M. & WESTON, A.H. (1980). Some effects of sodium nitroprusside, methoxyverapamil (D600) and nifedipine on rat portal vein. *Br. J. Pharmac.*, **68**, 311–319.

KAERGAARD NIELSEN, C. & ARRIGONI-MARTELLI, E. (1981). Effect of a new vasodilator, pinacidil (P1134) on potassium, noradrenaline and serotonin-induced contractions in rabbit vascular tissues. *Acta Pharmac. tox.*, **49**, 427–431.

KAWASHIMA, S. & LIANG, C-S. (1985). Systemic and coronary haemodynamic effects of pinacidil, a new

antihypertensive agent, in awake dogs: comparison with hydralazine. *J. Pharmac. exp. Ther.*, **232**, 369–375.

MURAD, F. (1986). Cyclic guanosine monophosphate as a mediator of vasodilation. *J. clin. Invest.*, **78**, 1–5.

SOUTHERTON, J.S., TAYLOR, S.G., WEIR, S.W. & WESTON, A.H. (1987). An investigation into the mechanism of action of pinacidil in rat blood vessels. *Br. J. Pharmac. Proc. Suppl.*, **90**, 126P.

WEIR, S.W. & WESTON, A.H. (1986a). Effect of apamin on responses to BRL34915, nicorandil and other relaxants in the guinea-pig taenia caeci. *Br. J. Pharmac.*, **88**, 113–120.

WEIR, S.W. & WESTON, A.H. (1986b). The effects of BRL34915 and nicorandil on electrical and mechanical activity and on ^{86}Rb efflux in rat blood vessels. *Br. J. Pharmac.*, **88**, 121–128.

(Received January 5, 1987.
Revised February 2, 1987.
Accepted March 5, 1987.)