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Effects of glibenclamide on the regional haemodynamic actions of α-trinositol and its influence on responses to vasodilators in conscious rats

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1 In conscious rats, α-trinositol (D-*myo*-inositol-1, 2, 6 triphosphate; 5-80 mg kg^{-1} h^{-1} infusion) caused dose-dependent hypotension, tachycardia and hyperaemic dilatation in renal, mesenteric and hindquarters vascular beds. These effects were accompanied by inhibition of the renal vasodilator effects of acetylcholine (ACh), and of the mesenteric vasodilator effects of sodium nitroprusside (SNP) and, particularly, of levcromakalim (LCK).

2 In the light of the latter finding, in a second experiment, we assessed the influence of the K_{ATP} channel inhibitor, glibenclamide (20 mg kg^{-1}), on resting haemodynamics, on responses to ACh, bradykinin (BK), SNP and LCK, on the haemodynamic action of α-trinositol, and on the effects of the latter on responses to the vasodilators, over a period of 3 days.

3 In the presence of saline, glibenclamide caused a reproducible pressor effect, accompanied by renal, mesenteric, and hindquarters vasoconstrictions on all 3 experimental days; these effects were unrelated to changes in blood glucose. In the presence of glibenclamide, only the hindquarters vasodilator response to BK, and all the cardiovascular actions of LCK were inhibited.

4 On the first experimental day, the hindquarters vasodilator effect of α-trinositol was substantially inhibited by glibenclamide, the renal vasodilatation less so, and the mesenteric vasodilatation not at all. However, over the subsequent two days, the mesenteric vasodilator effect of α-trinositol became increasingly sensitive to glibenclamide.

5 In the presence of α-trinositol and glibenclamide, on the first experimental day, the inhibition of the renal vasodilator effect of ACh was no greater than with α-trinositol alone in the first experiment. Moreover, on the third experimental day, both before and after glibenclamide, the inhibition by α-trinositol of the renal vasodilator response to ACh was less than on the first experimental day. Similarly, the α-trinositol-induced inhibition of the mesenteric vasodilator effect of SNP, and of the hindquarters vasodilator action of BK, waned over the 3 experimental days. The inhibition of the cardiovascular effects of LCK were similar on all 3 experimental days, but no greater in the presence of α-trinositol and glibenclamide than with glibenclamide alone.

6 These results indicate that K_{ATP} channels are involved in the maintenance of resting vasodilator tone in renal, mesenteric and hindquarters vascular beds. However, although additional activation of K_{ATP} channels is responsible for all the haemodynamic effects of LCK, it contributes only to the hindquarters vasodilator action of BK and is not involved in any of the responses to ACh or SNP. The hindquarters, renal and mesenteric vasodilator effects of α-trinositol may involve (in the same rank order) activation of K_{ATP} channels, probably through an indirect mechanism. However, it is unlikely that direct or indirect interaction of α-trinositol with K_{ATP} channels explains the ability of the drug to inhibit the renal vasodilator action of ACh, or the mesenteric vasodilator effects of SNP or LCK.

Keywords: α-Trinositol; K_{ATP} channels; glibenclamide; vasodilators

Introduction

The inositol phosphate cascade plays a pivotal role in the control of intracellular Ca^{2+} levels in a variety of tissues (for review, see Berridge, 1993), including those of the cardiovascular system. However, relatively little is known of the pharmacology of inositol phosphates, although α-trinositol (D-*myo*-inositol-1,2,6 triphosphate; previously known as PP56) has been shown to act as a functional antagonist of the cardiovascular actions of exogenous neuropeptide Y (NPY) and ATP, *in vitro* and *in vivo* (Adamsson & Edvinsson, 1991; Sun *et al.*, 1991a,b; 1992; 1993; Wahlestedt *et al.*, 1992; Schweiler & Hjemdahl, 1993). Furthermore, α-trinositol has been found to exert anti-inflammatory, anti-aggregatory and anti-neuropathic actions in various animals models (Claxson *et al.*, 1990; Ruf *et al.*, 1991; Carrington *et al.*, 1993; Lund & Reed, 1994; Rodt *et al.*, 1994). Recently, we showed that α-trinositol had substantial hyperaemic vasodilator effects in conscious, chronically instrumented rats (Gardiner *et al.*, 1994). Although it is conceivable that this action was attributable to α-trinositol acting as a functional antagonist of endogenous NPY and ATP, other possibilities cannot be discounted. For example, it is feasible that the haemodynamic actions of α-trinositol were due to enhancement of endogenous vasodilator mechanisms, rather than, or in addition to, inhibition of vasoconstrictor processes. However, nothing is known about the influence of α-trinositol on vasodilator responses, so the first aim of the present work was to determine if α-trinositol influenced the regional haemodynamic effects of a series of vasodilator challenges in conscious, chronically instrumented rats. We chose to study the influence of α-trinositol on the responses to acetylcholine (ACh), bradykinin (BK), sodium nitroprusside

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(SNP), and the ATP-sensitive K^+1 (K_{ATP} channel) opener, levcromakalim (LCK), since these agents have different regional vasodilator effects that vary in their dependence on nitric oxide (NO; Gardiner *et al.*, 1990; 1991a,b; 1993). Surprisingly, the results of the first experiment showed that α-trinositol caused clear inhibition of the renal vasodilator response to ACh, and of the mesenteric vasodilator effects of SNP and LCK (see Results). Since there is increasing evidence for the involvement of K_{ATP} channels in the control of resting vascular tone, and in responses to vasodilators (Quayle & Standen, 1994; Nelson & Quayle, 1995), in a second experiment we assessed the influence of the K_{ATP} channel antagonist, glibenclamide (Schmid-Antomarchi *et al.*, 1987), on resting haemodynamics, on the responses to ACh, BK, SNP and LCK, and on the regional haemodynamic effects of α-trinositol and its influence on the responses to the vasodilators, in order to determine whether the effects of α-trinositol could be due to its interacting with K_{ATP} channels.

Methods

Experiments were carried out on male, Long Evans rats (350–450 g) bred in the Biomedical Services Unit, Nottingham University Medical School. All surgery was carried out under sodium methohexitone anaesthesia (Brietal, Lilly; 40–60 mg kg^{-1}, i.p., supplemented as required). All details of pulsed Doppler probe implantation and intravascular catheter implantation have been published (Gardiner *et al.*, 1990; 1991a, b). Experiments were not begun until at least 24 h after the final surgical intervention (catheter implantation).

Experiment 1: Responses to vasodilators in the presence of saline or α-trinositol

The protocol ran over 4 experimental days; on each day animals ($n=8$) received 3 min i.v. (right jugular vein) infusions of acetylcholine (ACh 10 μg kg^{-1} min^{-1}) bradykinin (BK, 20 μg kg^{-1} min^{-1}), sodium nitroprusside (SNP 3.75 μg kg^{-1} min^{-1}), and levcromakalim (LCK 5 μg kg^{-1} min^{-1}) in that order, separated by periods of 7 min to allow variables to return to baseline. These challenges were not randomized because the effects of SNP and, particularly, LCK were long-lasting. Furthermore, since the regional haemodynamic profiles of the vasodilators were different (see Results), it was not possible to match them for those effects, but the doses of the vasodilators chosen evoked integrated hypotensive responses that were not significantly different.

Three hours after the initial challenges with the vasodilators (Run 1), animals received, in random order, and on separate days, a primed i.v. infusion (as above) of α-trinositol (at doses of 0.1 mg kg^{-1} bolus, 5 mg kg^{-1} h^{-1} infusion (low dose), 0.5 mg kg^{-1} bolus, 20 mg kg^{-1} h^{-1} infusion (middle dose), or 2 mg kg^{-1} bolus, 80 mg kg^{-1} h^{-1} infusion (high dose) (bolus in 0.1 ml flushed in with 0.1 infusion solution, infusion at 0.4 ml h^{-1}) (Gardiner *et al.*, 1994), or a primed infusion of saline (NaCl, 154 mmol l^{-1}, 0.1 ml flushed in with 0.1 ml, and infused at 0.4 ml h^{-1}). Beginning 10 min (Run 2) and 3 h (Run 3) after the start of the primed infusion, animals were re-challenged with the 3 min infusions of ACh, BK, SNP and LCK.

Experiment 2: Effects of glibenclamide on responses to vasodilators before, and during infusion of saline or α-trinositol

Animals were studied on 3 consecutive days. On day 1, the rats received 3 min infusions of ACh, BK, SNP and LCK, as above.

Three hours after the administration of LCK, in one group of animals ($n=10$), an infusion of sterile isotonic saline was begun, and was continued throughout the experiment, i.e., for 3 days. Three h after the start of the saline infusion (i.e., 6 h after the vasodilator challenges), glibenclamide (20 mg kg^{-1}) (Moreau *et al.*, 1994), was given over 5 min. Beginning 10–15 min later, ACh, BK, SNP and LCK were administered as before. On the following 2 days, ACh, BK, SNP and LCK were administered between 07 h 00 min and 08 h 30 min, and again, 6 h later, 15 min after the administration of glibenclamide (as above).

A separate group of animals ($n=9$) was treated as above, but with α-trinositol (80 mg kg^{-1} h^{-1}) being infused instead of saline.

In both groups of animals, blood glucose was measured immediately before administration of glibenclamide, and immediately after the infusion of LCK after glibenclamide on each experimental day. Blood samples were obtained through the arterial catheter, and glucose concentration was measured with a glucose meter (Reflolux S, Boehringer). The catheters for infusions of ACh, BK, SNP and LCK were flushed with sterile saline before glibenclamide, whereas after glibenclamide administration, 5% dextrose was used to flush the catheters, to diminish the hypoglycaemic effect of glibenclamide (Moreau *et al.*, 1994).

Data analysis

Recordings were made of instantaneous heart rate, and phasic and mean blood pressures and Doppler shift signals. Under the conditions of our experiments the latter are indices of flow; vascular conductances were calculated by dividing mean Doppler shift by mean arterial blood pressure (Gardiner *et al.*, 1990; 1991a, b; 1993). Responses to ACh, BK, SNP and LCK were assessed from the integrated responses during the 3 min infusions (areas under or over curves, AUC or $AOC_{0-3\ min}$, respectively). Within-group comparisons were made by Friedman's test, and between group comparisons were made with the Mann-Whitney U test; a P value <0.05 was taken as significant.

Drugs and peptides

α-Trinositol was supplied by Perstorp Pharma, as the freeze-dried Na^+ salt in sealed glass vials. ACh chloride, SNP and glibenclamide were obtained from Sigma UK. LCK (BRL 38227; (−)-6-cyano-3, 4-dihydro-2, 2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-1-benzopyran-3-ol) was a gift from Smith-Kline Beecham UK, and BK was obtained from Bachem UK. ACh, SNP and BK were dissolved in sterile saline (in the case of BK, the saline contained 1% bovine serum albumin (Sigma) to prevent the peptide sticking to the catheter). LCK was dissolved in sterile water and diluted with sterile saline. α-Trinositol was dissolved at the appropriate concentration in sterile water. Glibenclamide was solubilized in 1% 2-hydroxypropyl-β-cyclodextrin.

Results

Experiment 1: Responses to vasodilators in the presence of saline or α-trinositol

At the beginning of each experimental run, resting values of heart rate, mean arterial pressure, and renal, mesenteric and hindquarters flows and vascular conductances were not significantly different (data not shown). α-Trinositol caused dose-related hypotension and tachycardia, accompanied by increases in renal, mesenteric and hindquarters flows and vascular conductances, confirming our previous report (Gardiner *et al.*, 1994) (data not shown).

Responses to ACh Before infusion of saline or α-trinositol, ACh evoked hypotension, tachycardia, and increases in renal flow and vascular conductance, but a decrease in mesenteric flow, although this was not accompanied by a significant change in vascular conductance (Figure 1). All these changes

were similar in all 4 experimental runs, but the slight hindquarters haemodynamic changes were variable (Figure 1).

During infusion of saline and the lowest and middle doses of α-trinositol, there were no significant changes in the responses to ACh (data not shown).

During infusion of the highest dose of α-trinositol, the hypotensive effect of ACh was inhibited although it was not different from the response seen following 3 h infusion of saline (Figure 1). The highest dose of α-trinositol also caused a significant inhibition of the ACh-induced increase in renal flow and vascular conductance, and the latter effect was different from that of saline (Figure 1). In addition, there was significant augmentation of the reduction in hindquarters flow and vascular conductance during infusion of ACh in the presence of the highest dose of α-trinositol, and the latter effect was different from that in the presence of saline (Figure 1).

Responses to BK Before infusion of saline or α-trinositol, BK evoked a variable hypotension, but clear tachycardia and increases in renal, mesenteric and hindquarters flows and vascular conductances that were reproducible in all 4 experimental runs (data not shown).

During infusion of saline and the lowest dose of α-trinositol, responses to BK were not changed significantly. However, during infusion of the middle and highest dose of α-trinositol, the renal vasodilator effect of BK was attenuated, but it was not significantly less than the corresponding change in the presence of saline (data not shown). Although the highest dose of α-trinositol caused reduction of the tachycardic response to BK, relative to that seen in the presence of saline, this effect was not significant compared to run 1; all other effects of BK showed no significant change (data not shown).

Responses to SNP Before infusion of saline or α-trinositol, SNP caused reproducible increases in heart rate accompanied by hypotension, mesenteric hyperaemia, and increases in renal, mesenteric and hindquarters vascular conductances (Figure 2).

During infusion of saline or the lowest dose of α-trinositol,

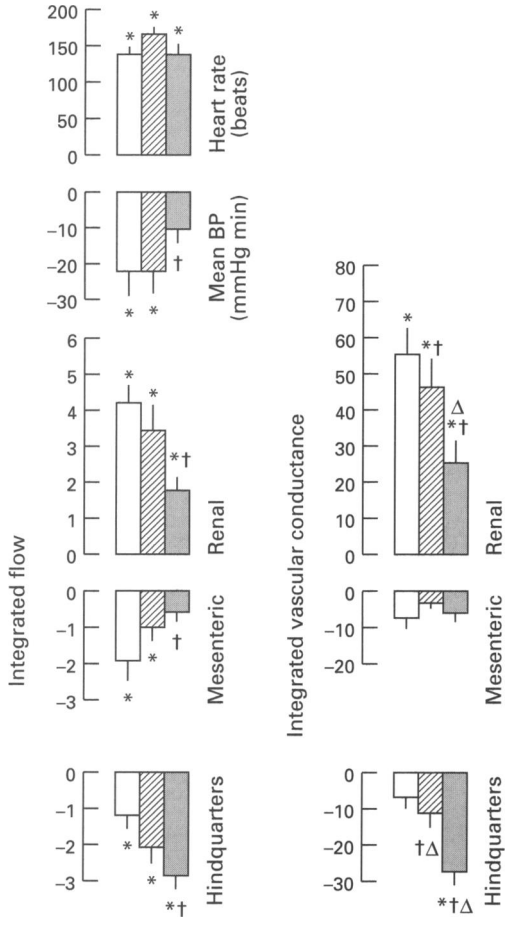


Figure 1 Integrated cardiovascular responses during 3 min infusions of acetylcholine (ACh) for each of the 3 experimental runs on the day on which animals received the highest dose of α-trinositol (2 mg kg^{-1} bolus, 80 mg kg^{-1} h^{-1} infusion); open columns, run 1 before infusion of α-trinositol; hatched columns, run 2 starting 10 min after onset of infusion of α-trinositol; stippled columns, run 3 starting 3 h after onset of infusion of α-trinositol. The tachycardia is shown as the area under the curve ($AUC_{0-3\,min}$) in total beats; the hypotension is shown as the area over the curve ($AOC_{0-3\,min}$) in mmHg min; the columns showing integrated flow changes are AUC or $AOC_{0-3\,min}$, as appropriate, and are given in units of kHz min; the columns showing integrated vascular conductance changes are AUC or $AOC_{0-3\,min}$, as appropriate, and are given in units of ([kHz mmHg^{-1}]10^{-3}) min. *$P<0.05$ for change relative to pre-ACh baseline; †$P<0.05$ for change versus change during corresponding run 1; $^{\Delta}P<0.05$ versus the corresponding change during saline infusion. Values are mean with s.e.mean; $n=8$.

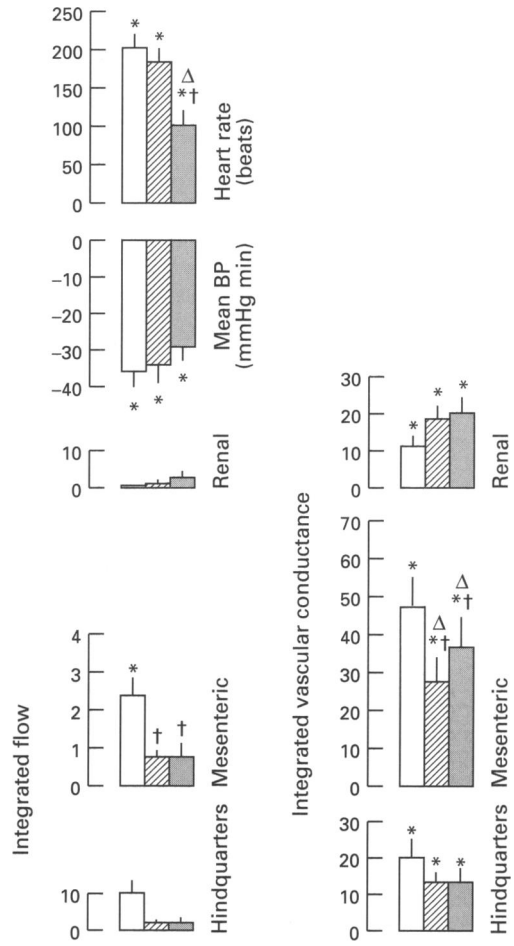


Figure 2 Integrated cardiovascular responses during 3 min infusions of sodium nitroprusside (SNP) for each of the 3 experimental runs on the day on which animals received the highest dose of α-trinositol (2 mg kg^{-1} bolus, 80 mg kg^{-1} h^{-1} infusion); open columns run 1 before infusion of α-trinositol; hatched columns, run 2 starting 10 min after onset of infusion of α-trinositol; stippled columns, run 3 starting 3 h after onset of infusion of α-trinositol. The tachycardia is shown as the area under the curve ($AUC_{0-3\,min}$) in total beats; the hypotension is shown as the area over the curve ($AOC_{0-3\,min}$) in mmHg min; the columns showing integrated flow changes are $AUC_{0-3\,min}$, and are given in units of kHz min; the columns showing integrated vascular conductance changes are $AUC_{0-3\,min}$, and are given in units of ([kHz mmHg^{-1}]10^{-3}) min. *$P<0.05$ for change relative to pre-SNP baseline; †$P<0.05$ for change versus change during corresponding run 1; $^{\Delta}P<0.05$ versus the corresponding change during saline infusion. Values are mean with s.e.mean; $n=8$.

responses to SNP were unchanged, but the middle dose of α-trinositol caused a slight inhibition of the mesenteric flow response (data not shown). However, during infusion of the highest dose of α-trinositol there was significant inhibition of the tachycardic and hyperaemic mesenteric vasodilator responses to SNP, and these changes were significantly different from those seen in the presence of saline (Figure 2).

Responses to LCK Before infusion of saline or α-trinositol, LCK caused reproducible tachycardia, hypotension, and increases in renal and mesenteric flows and renal, mesenteric and hindquarters vascular conductances (Figure 3).

During infusion of saline, there were no significant changes in the response to LCK. However, during infusion of α-trinositol there were dose-dependent reductions in the renal and mesenteric flow responses to LCK, and the highest dose of α-trinositol caused significant inhibition of the mesenteric vasodilatation and of the tachycardic effect of LCK, but not its hypotensive or hindquarters vasodilator actions (Figure 3).

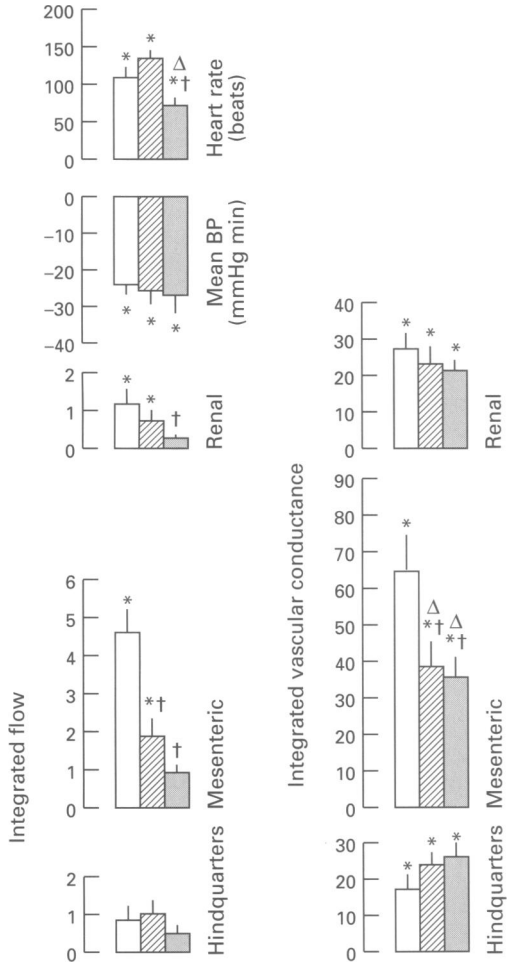


Figure 3 Integrated cardiovascular responses during 3 min infusions of levcromakalim (LCK) for each of the 3 experimental runs on the day on which animals received the highest dose of α-trinositol ($2\,mg\,kg^{-1}$ bolus, $80\,mg\,kg^{-1}\,h^{-1}$ infusion); open columns, run 1 before infusion of α-trinositol; hatched columns, run 2 starting 10 min after onset of infusion of α-trinositol; stippled columns, run 3 starting 3 h after onset of infusion of α-trinositol. The tachycardia is shown as the area under the curve ($AUC_{0-3\,min}$) in total beats; the hypotension is shown as the area over the curve ($AOC_{0-3\,min}$) in mmHg min; the blocks showing integrated vascular conductance changes are $AUC_{0-3\,min}$, and are given in units of kHz min; the blocks showing integrated vascular conductance changes are $AUC_{0-3\,min}$, and are given in units of ($[kHz\,mmHg^{-1}]10^{-3}$)min. $^{*}P<0.05$ for change relative to pre-LCK baseline; $^{\dagger}P<0.05$ for change versus change during corresponding run 1; $^{\Delta}P<0.05$ versus the corresponding change during saline infusion. Values are mean with s.e.mean; $n=8$.

Experiment 2: Effects of glibenclamide on responses to vasodilators before and during infusion of saline or α-trinositol

There were no significant differences between the initial resting cardiovascular variables in the group that subsequently received saline infusion, compared to the group that received α-trinositol (Figure 4).

Effects of glibenclamide in the absence and presence of α-trinositol There were no significant changes during infusion of saline on the first experimental day, whereas α-trinositol caused a slight tachycardia and hypotension, and marked increases in renal, mesenteric and hindquarters flows and vascular conductances (Figure 4).

On the first experimental day, in the presence of saline, glibenclamide had a pressor effect, accompanied by bradycardia, and reductions in renal, mesenteric and hindquarters flows and vascular conductances (Figure 4). In the presence of α-trinositol, glibenclamide had a pressor and bradycardic effect, accompanied by reductions in renal and hindquarters flows and vascular conductances; however, mesenteric flow increased, and there was no significant change in mesenteric vascular conductance (Figure 4). While the pressor, bradycardic and renal haemodynamic effects of glibenclamide were similar in the presence of saline or α-trinositol, the mesenteric vasoconstriction was less, and the hindquarters vasoconstriction was greater, in the presence of α-trinositol. The effect of glibenclamide on blood glucose in the two groups was similar (Table 1).

At the beginning of the second experimental day both groups of animals still had slightly elevated blood pressures; moreover, the α-trinositol-induced hindquarters hyperaemia was still inhibited and blood glucose was still low (Table 1). These effects are consistent with a persistent action of glibenclamide, although signs of this were not seen in the renal vascular bed (Figure 4). All animals showed hyperphagia, consistent with the relative hypoglycaemia, and this may have contributed to the significant mesenteric hyperaemia seen in the saline-infused group (Figure 4). The haemodynamic effects of glibenclamide were similar in the two groups on the second experimental day, and this action was not accompanied by a reduction in blood glucose (Table 1).

At the beginning of the third experimental day, blood pressures, heart rates, and blood glucose levels (Table 1) were normal in both groups, there were no signs of any persistent cardiovascular influence of glibenclamide, and the vasodilator effects of α-trinositol were again clearly apparent (Figure 4), although the increase in hindquarters vascular conductance was still significantly attenuated (Figure 4). On the third experimental day, responses to glibenclamide were generally as before, although there was a larger mesenteric vasoconstriction in the animals infused with α-trinositol compared to the response on the first experimental day (Figure 4). Glibenclamide had no effect on blood glucose on day 3 (Table 1).

Responses to ACh At the beginning of the first experimental day, responses to ACh were similar in the two groups (Figure 4). In the presence of saline and glibenclamide, the hypotensive effect of ACh was enhanced, but there were no significant changes in its renal haemodynamic actions (Figure 4). However, in the presence of α-trinositol and glibenclamide, the hypotensive effect of ACh was not enhanced (and was significantly less than that in the absence of α-trinositol), and the renal hyperaemic vasodilator effect of ACh was abolished (Figure 4).

On the second experimental day, in the presence of saline, the effects of ACh were as on day 1, before and after administration of glibenclamide. In the group infused with α-trino-

sitol, the renal vascular effects of ACh were markedly inhibited, both before and after administration of glibenclamide (Figure 4).

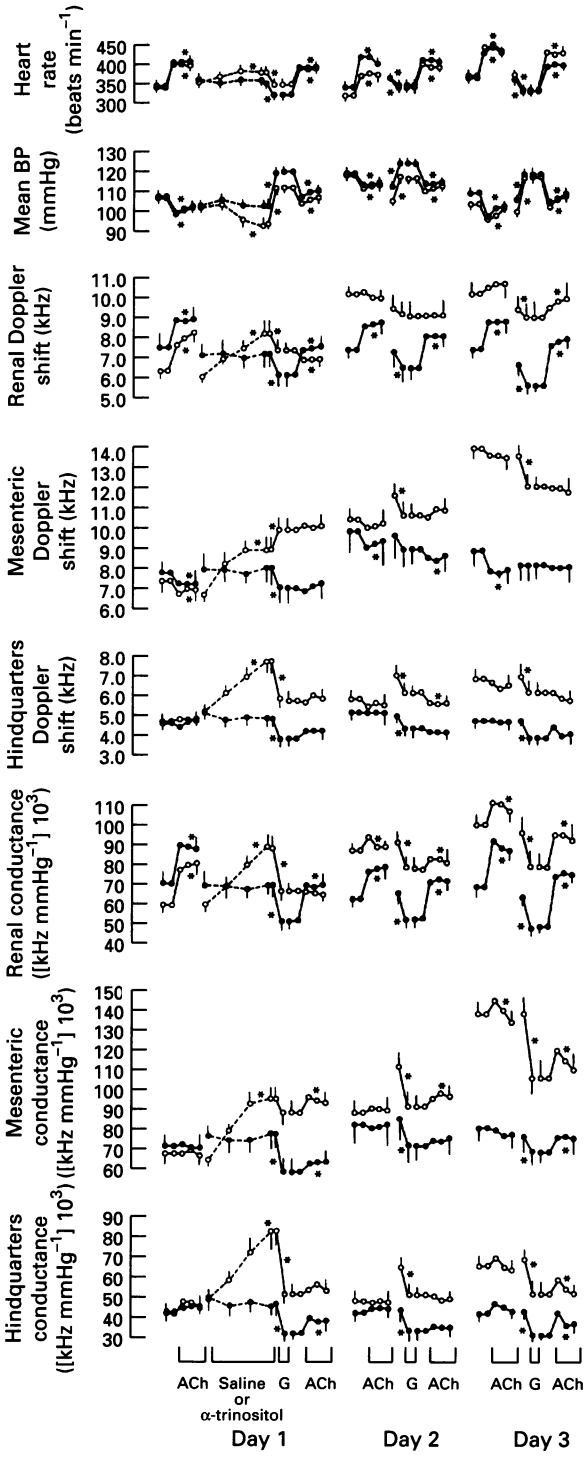


Figure 4 Cardiovascular variables on three consecutive experimental days in two groups of conscious, Long Evans rats. On day 1, a 3 min infusion of acetylcholine (ACh; 10 $\mu g\,kg^{-1}\,min^{-1}$) was given before the start of an infusion of saline ($n=10$; ●) or α-trinositol ($n=9$; ○); 3 h later, glibenclamide (G, 20 $mg\,kg^{-1}$) was administered, and 10 min later, the 3 min infusion of ACh was repeated. Throughout the experiment, saline or α-trinositol infusion was continued, and on each subsequent experimental day, animals were re-challenged with 3 min infusions of ACh, before and after glibenclamide administration. Values are mean, with s.e.mean (the majority have been omitted for clarity). *$P<0.05$ for change judged from area under or over curves. Differences between responses on different experimental days, or between groups, are given in the text.

On the third experimental day, the group receiving saline showed reproducible tachycardic, hypotensive and renal hyperaemic vasodilator responses to ACh before and after administration of glibenclamide. In both conditions, the group receiving α-trinositol showed some recovery of the renal vasodilator effect of ACh, but the response was still attenuated (Figure 4).

Responses to BK At the beginning of the first experimental day, responses to BK were similar in the two groups (Figure 5). In the presence of glibenclamide, both groups showed an enhanced hypotensive, and attenuated hindquarters vasodilator, effect of BK, but otherwise the response to BK was maintained. On the third experimental day the hindquarters vasodilator effect of BK was not attenuated in the animals infused with α-trinositol (Figure 5).

Responses to SNP At the beginning of the first experimental day, responses to SNP in the two experimental groups were similar (Figure 6). However, in the presence of α-trinositol and glibenclamide, there was significant attenuation of the hypotensive, tachycardic and mesenteric vasodilator effects of SNP; such effects were not seen in the saline-infused group (Figure 6). On the subsequent experimental days, responses to SNP in the two groups were, once again, similar, and unaffected by glibenclamide (Figure 6).

Responses to LCK At the beginning of the first experimental day, responses to LCK were similar in the two groups (Figure 7). These responses were inhibited similarly in the two groups after administration of glibenclamide, and this pattern was reproduced on the second and third experimental days (Figure 7).

Discussion

In the present work, effects of α-trinositol on resting cardiovascular status and on responses to vasodilators were seen. It is feasible that the former influenced the latter, but this cannot be the only explanation of the findings, since the influence of α-trinositol varied depending on the vasodilator challenge.

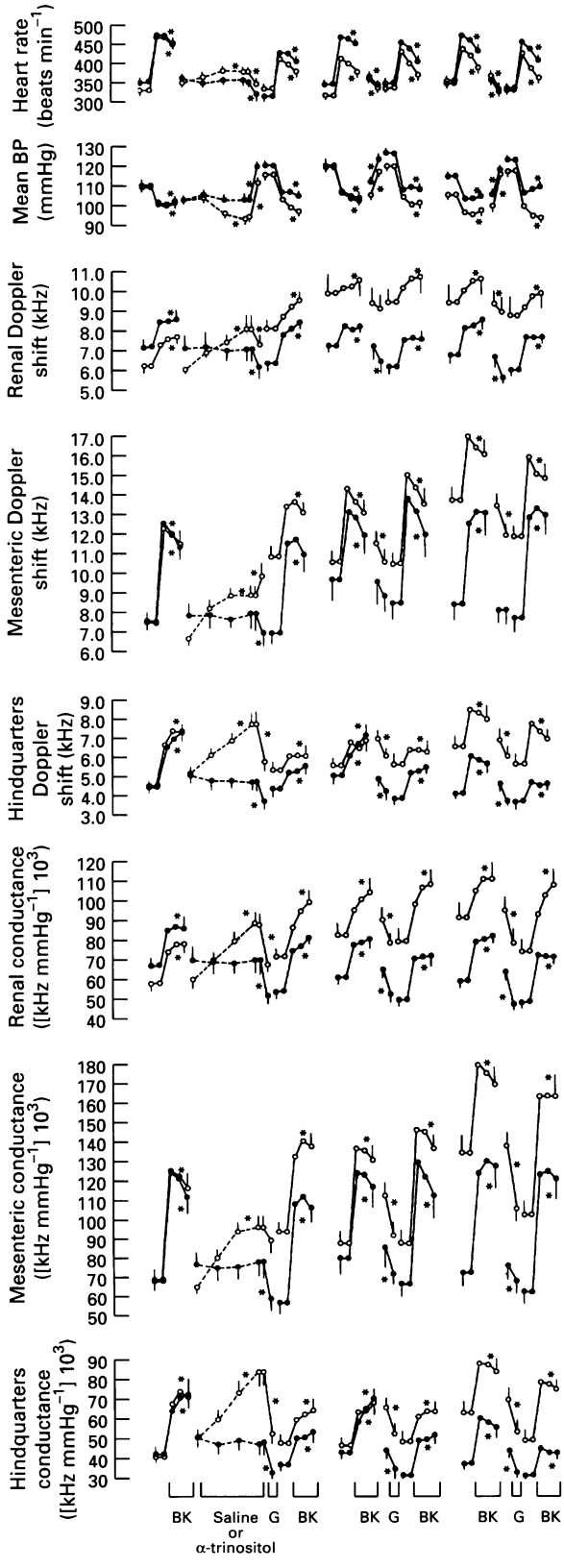
Effects of glibenclamide and α-trinositol on baseline cardiovascular status

In animals infused with saline, glibenclamide had a significant pressor effect, accompanied by bradycardia and reductions in renal, mesenteric and hindquarters vascular conductances on all three experimental days. Since blood glucose fell on day 1, was low and did not change on day 2, and was normal and unaffected by glibenclamide on day 3, it is unlikely that the haemodynamic effects of the latter were dependent on changes in blood glucose. Indeed, hypoglycaemia (induced by insulin) is accompanied by hindquarters vasodilatation, rather than the vasoconstriction seen with glibenclamide (Bennett *et al.*, 1989). Hence, a more straightforward explanation is that the action of glibenclamide was due to its inhibitory effect on vascular K_{ATP} channels; the present results indicate that such an action caused similar vasoconstriction in renal, mesenteric and hindquarters vascular beds. Our findings are thus consistent with some of the recent observations of Moreau *et al.* (1994), but those workers did not observe a pressor response to glibenclamide. Indeed, most previous studies have failed to detect any pressor or other cardiovascular effects of glibenclamide (e.g., Cavero *et al.*, 1989; see McPherson, 1993, for review); we have no explanation for these disparities. Although we used 2-hydroxypropyl-β-cyclodextrin as a vehicle for glibenclamide, infusion of that substance alone has no haemodynamic action (unpublished observations). In confirmation of previous findings (Gardiner *et al.*, 1994) α-trinositol caused significant, albeit modest, hypotension and tachycardia, but marked hyperaemic dilatation in renal, mesenteric and hindquarter

Table 1 Blood glucose levels (mmol l^{-1}) before and after glibenclamide (G) injection on three consecutive experimental days in rats infused with saline ($n=10$) or α-trinositol ($n=9$)

	Day 1		*Day 2*		*Day 3*	
	Before G	*After G*	*Before G*	*After G*	*Before G*	*After G*
Saline	5.7±0.2	2.2±0.2*	2.8±0.2	4.4±0.3	6.8±0.3	7.8±0.7
α-Trinositol	5.3±0.2	2.5±0.3*	2.5±0.2	2.7±0.4	6.9±0.6	7.7±0.4

Values are mean±s.e.mean; *$P<0.05$ versus before G value (Wilcoxon test).



vascular beds. While these effects could have been due to augmentation of endogenous vasodilator mechanisms, they were not accompanied by enhanced responses to vasodilator challenges (see below).

The results of our second experiment, showed that on the first experimental day, the marked hindquarters vasodilator action of α-trinositol was substantially inhibited by glibenclamide. In contrast, glibenclamide had no significant effect on the α-trinositol-induced mesenteric vasodilatation, and the renal vasoconstrictor response to glibenclamide was similar to that seen in saline-infused animals, and constant throughout the experiment. Thus, it seems that after 3 h infusion of α-trinositol, the hindquarters vasodilatation was largely dependent on activation of K_{ATP} channels. However, since in all vascular beds in the presence of glibenclamide, flows and conductances were still higher in α-trinositol-infused rats, than in saline-infused rats, additional vasodilator actions of α-trinositol must have been involved, albeit to different extents.

The hindquarters vasoconstrictor response to glibenclamide in the animals receiving α-trinositol occurred on each of the three experimental days, although the degree of vasoconstriction varied, due to the fact that the hindquarters hyperaemic effect of α-trinositol never fully recovered after the first administration of glibenclamide. Since the level to which hindquarters vascular conductance fell was the same on each day, these findings indicate that the hindquarters vasodilator effect of α-trinositol was largely dependent on K_{ATP} channels, and their involvement and that of other mechanisms was relatively constant. This picture contrasts with that in the renal vascular bed where the vasoconstrictor response to glibenclamide was similar on all three experimental days, but there was an increasing difference between the flows and between the vascular conductances in saline- and α-trinositol-infused rats in the presence of glibenclamide. This finding is consistent with a constant contribution from K_{ATP} channels, and an increasing component of the α-trinositol-induced renal vasodilatation being due to other mechanisms. The mesenteric vascular bed was different again, in that the incremental vasodilator effect of α-trinositol over the three experimental days was accompanied by an increasing vasoconstrictor response to glibenclamide. This finding indicates an incremental contribution of K_{ATP} channels to the mesenteric vasodilator effect of α-trinositol, with a relatively constant contribution from other mechanisms.

Of course, an apparent involvement of K_{ATP} channels in the haemodynamic action of α-trinositol does not mean the latter

Figure 5 Cardiovascular variables on three consecutive experimental days in two groups of conscious, Long Evans rats. On day 1, a 3 min infusion of bradykinin (BK, 20 μg kg^{-1} min^{-1}) was given before the onset of an infusion of saline ($n=10$; ●) or α-trinositol ($n=9$; ○); 3 h later, glibenclamide (G, 20 mg kg^{-1}) was administered, and 15 min later, the 3 min infusion of BK was repeated. Throughout the experiment, saline or α-trinositol infusion was continued, and on each subsequent experimental day, animals were re-challenged with 3 min infusions of BK, before and after glibenclamide administration. Values are mean, with s.e.mean (the majority have been omitted for clarity). *$P<0.05$ for change judged from area under or over curves. Differences between responses on different experimental days, or between groups, are given in the text.

directly interacts with the former. Thus, an increase in flow caused by α-trinositol could be responsible for activating endothelial, and/or vascular smooth muscle K_{ATP} channels (see Revest & Abbott, 1992). However, the current findings in-

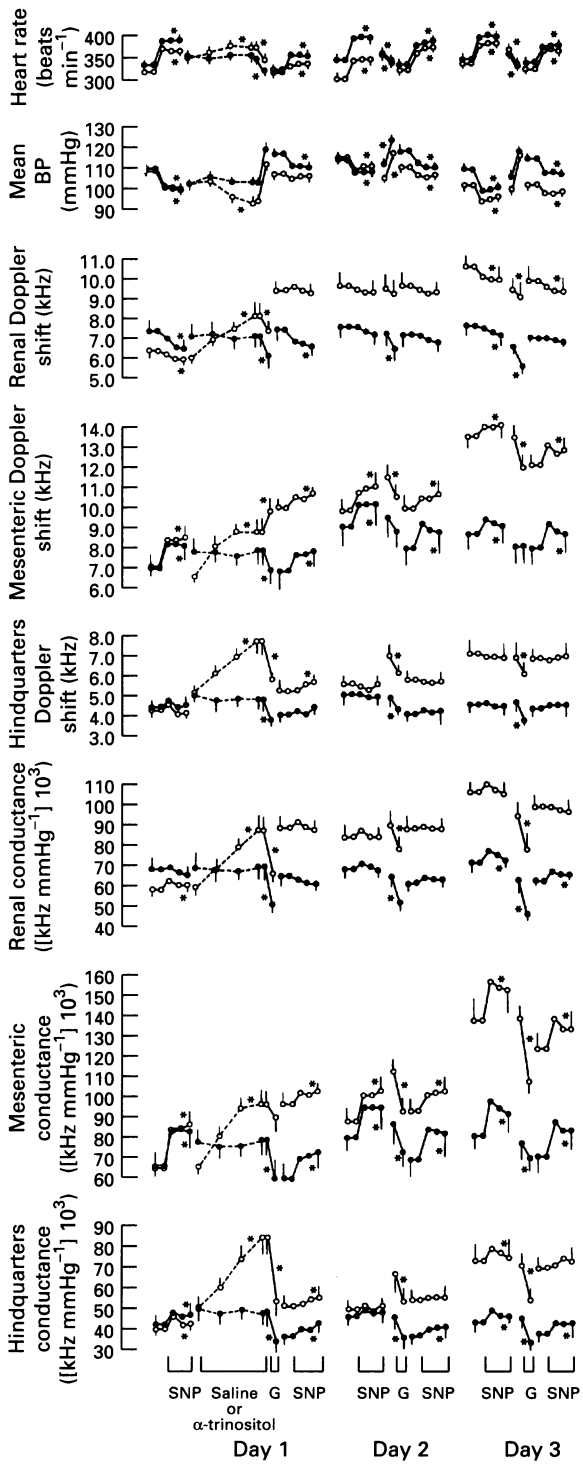


Figure 6 Cardiovascular variables on three consecutive experimental days in two groups of conscious, Long Evans rats. On day 1, a 3 min infusion of sodium nitroprusside (SNP, 3.75 μg kg^{-1} min^{-1}) was given before the start of an infusion of saline ($n=10$; ●) or α-trinositol ($n=9$; ○); 3 h later, glibenclamide (G, 20 mg kg^{-1}) was administered, and 15 min later, the 3 min infusion of SNP was repeated. Throughout the experiment, saline or α-trinositol infusion was continued, and on each subsequent experimental day, animals were re-challenged with 3 min infusions of SNP, before and after glibenclamide administration. Values are mean, with s.e.mean (the majority have been omitted for clarity). *$P<0.05$ for change judged from area under or over curves. Differences between responses on different experimental days, or between groups, are given in the test.

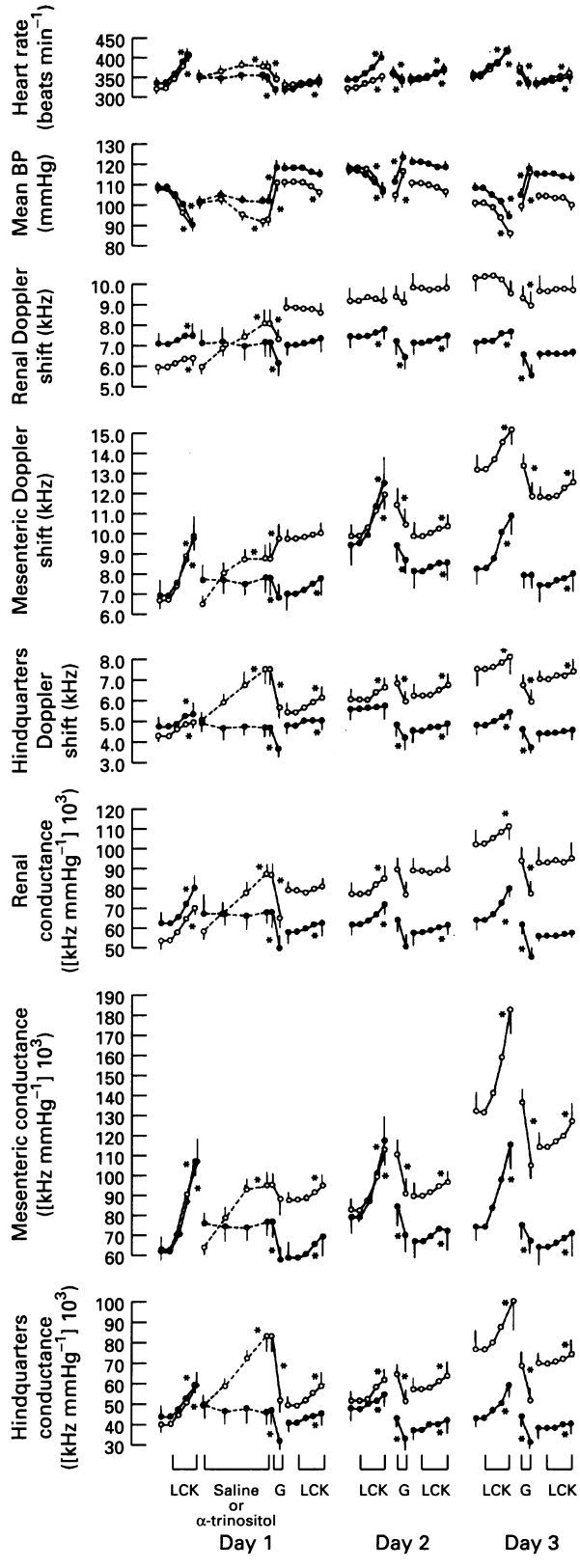


Figure 7 Cardiovascular variables on three consecutive experimental days in two groups of conscious, Long Evans rats. On day 1, a 3 min infusion of levcromakalim (LCK, 5 μg kg^{-1} min^{-1}) was given before the start of an infusion of saline ($n=10$; ●) or α-trinositol ($n=9$; ○); 3 h later, glibenclamide (G, 20 mg kg^{-1}) was administered, and 15 min later, the 3 min infusion of LCK was repeated. Throughout the experiment, saline or α-trinositol infusion was continued, and on each subsequent experimental day, animals were re-challenged with 3 min infusions of LCK, before and after glibenclamide administration. Values are mean, with s.e.mean (the majority have been omitted for clarity). *$P<0.05$ for change judged from area under or over curves. Differences between responses on different experimental days, or between groups, are given in the text.

dicate that any such phenomenon is not expressed uniformly in renal, mesenteric and hindquarters vascular beds. Considering that the K_{ATP} channel opener, LCK, had most marked dilator effects in the mesenteric vascular bed (see also Gardiner *et al.*, 1991b), it is intriguing that glibenclamide caused the least inhibition of the vasodilator influence of α-trinositol at that site.

Effects of α-trinositol on responses to ACh

In the first experiment, α-trinositol, at the highest dose, reduced the renal vasodilator, and augmented the hindquarters vasoconstrictor, effects of ACh. However, ACh caused the most marked increases in renal flow and vascular conductance of all the vasodilator agents used, and hence this is an instance in which the change in baseline status evoked by α-trinositol infusion could have been responsible for its apparent inhibitory effects on renal vasodilator responses to ACh (but see below).

In the second experiment, the haemodynamic effects of ACh in animals infused with saline were not inhibited by glibenclamide, indicating a lack of involvement of K_{ATP} channels, as found in other studies (Jackson, 1993). Hence, the abolition of the renal hyperaemic vasodilator effect of ACh in animals receiving α-trinositol, or α-trinositol and glibenclamide must be attributed to α-trinositol acting by some means other than through an influence on K_{ATP} channels. One possibility is that α-trinositol has antagonist effects on muscarinic receptors. However, this seems unlikely because the hypotensive effect of ACh was only slightly inhibited by α-trinositol. Moreover, with continued infusion of α-trinositol, responses to ACh recovered, such that by the third experimental day, its hypotensive and renal hyperaemic vasodilator effects were re-established. For this reason, it is also unlikely that the inhibition seen on the first experimental day was due to the change in baseline (see above).

Effects of α-trinositol on responses to BK

In the first experiment, the tendency for α-trinositol to inhibit renal and hindquarters vasodilator effects of BK could have been due to the changes in baseline caused by α-trinositol, although the fact that there was no difference between the middle and the highest doses of α-trinositol in that regard makes this unlikely, because of the dose-dependency of the influence of α-trinositol on resting cardiovascular variables.

In the second experiment, responses to BK were generally unaffected by glibenclamide, in the absence or presence of α-trinositol, except that there was inhibition of the hindquarters hyperaemic vasodilator response to BK. Since this response is partly dependent upon release of adrenal catecholamines causing activation of β_2-adrenoceptors (Gardiner *et al.*, 1992), it is possible that β-adrenoceptor-mediated hindquarters vasodilatation involves K_{ATP} channels, as seen in the isolated mesenteric vascular bed (Randall & McCulloch, 1995) and other systems (Jackson, 1993).

Effects of α-trinositol on responses to SNP

In the first experiment, the highest dose of α-trinositol caused clear inhibition of the tachycardic and mesenteric hyperaemic vasodilator effects of SNP. These effects were not due to the baseline changes caused by α-trinositol, since in its presence the peak heart rate during infusion of SNP was 432 ± 7 beats min^{-1}, whereas the peak rate during infusion of BK was 485 ± 4, i.e., heart rate was capable of rising by at least a further 50 beats min^{-1} than seen during infusion of SNP, and the peak mesenteric vascular conductance during infusion of SNP was 111 ± 13 [kHz $mmHg^{-1}$]10^3, whereas the peak mesenteric vascular conductance during infusion of BK was 149 ± 17 [kHz $mmHg^{-1}$]10^3, i.e., the response during SNP was not the maximum possible.

Since, in the second experiment, glibenclamide had no effect on responses to SNP in animals infused with saline, it appears unlikely that K_{ATP} channels were involved, consistent with previous findings (Fulton *et al.*, 1994). Hence, the clear inhibition of the hypotensive and mesenteric vasodilator responses to SNP in the presence of α-trinositol, or α-trinositol and glibenclamide, on the first experimental day must have been due to the involvement of mechanisms other than K_{ATP} channels. Since the vasodilator actions of SNP depend on release of NO (Ahlner *et al.*, 1991), it may be that interference with the effects of the latter, by α-trinositol, was responsible for attenuation of the actions of SNP. If NO release from SNP activates vasodilator mechanisms in the same way as endogenous NO released by ACh, then inhibition of the renal vasodilator action of ACh, and of the mesenteric vasodilator action of SNP by α-trinositol could be due to the same process. This proposal is consistent with the finding that, as for the renal vasodilator effect of ACh, the mesenteric vasodilator action of SNP recovered over the subsequent two experimental days, in spite of continued infusion of α-trinositol.

Effects of α-trinositol on responses to LCK

In the first experiment, the most striking action of α-trinositol was a dose-dependent inhibition of the hyperaemic effects of LCK in the mesenteric vascular bed, accompanied by inhibition of vasodilatation at the highest dose of α-trinositol. The inhibitory influence of α-trinositol was apparent on the first exposure to LCK in the presence of α-trinositol but, because of the experimental design, this was about 40 min after the onset of α-trinositol infusion. Hence, we cannot delineate the rate of onset of the effect of α-trinositol from the present findings, although there was a tendency for the inhibitory action of the highest dose of α-trinositol to be slightly greater after 210 min, than after 40 min infusion.

It is notable that the inhibitory influence of α-trinositol on the mesenteric vasodilator response to LCK was not accompanied by a change in the hypotensive effect of the latter. One possible explanation is that the hypotensive response to LCK was due to a fall in cardiac output as a result of a negative inotropic and/or venodilator action that was not influenced by α-trinositol. However, current evidence indicates LCK is devoid of such actions (Cavero *et al.*, 1989; Hamilton *et al.*, 1993). It is clear that α-trinositol was exerting selective effects on the mesenteric vasodilator responses to LCK, and these were apparent also with the middle dose of α-trinositol.

In the second experiment on the first experimental day, in the presence of α-trinositol and glibenclamide, the inhibition of the cardiovascular responses to LCK was similar to that in the presence of saline and glibenclamide. Thus, it is clear the inhibitory effect of α-trinositol on the mesenteric vasodilator action of LCK (see above) was not additive with that of glibenclamide. Moreover, the inhibitory effect of α-trinositol on the mesenteric vasodilator action of LCK was not apparent on the second and third experimental days, prior to administration of glibenclamide. These results indicate that α-trinositol does not act as a straightforward antagonist or agonist of K_{ATP} channels.

In summary, it appears that the inhibitory effects of α-trinositol on the renal vasodilator responses to ACh, or the mesenteric vasodilator responses to SNP or LCK, are not maintained during continuous infusion of α-trinositol, and are not seen with glibenclamide; hence these actions are not likely to be due to α-trinositol interacting directly with K_{ATP} channels. These findings indicate that any involvement of K_{ATP} channels in the hyperaemic vasodilator responses to α-trinositol may be a secondary effect triggered by the haemodynamic influence of α-trinositol. Whether or not the haemodynamic actions of α-trinositol involve its ability to modulate β_1 integrin function (Rodt *et al.*, 1994) remains to be determined.

References

ADAMSSON, M. & EDVINSSON, L. (1991). Blockade of neuropeptide Y-induced potentiation of noradrenaline-evoked vasoconstriction by D-myo-inositol-1,2,6-trisphosphate (PP56) in rabbit femoral arteries. *Neuropeptides*, **19,** 13–16.

AHLNER, J., ANDERSSON, R.G.G., TORFGARD, K. & AXELSSON, K.L. (1991). Organic nitrate esters: clinical use and mechanisms of actions. *Pharmacol. Rev.*, **43,** 351–423.

BENNETT, T., COMPTON, A.M. & GARDINER, S.M. (1989). Regional haemodynamic effects of hypoglycaemia in conscious Long Evans rats. *J. Physiol.*, **416,** 57P.

BERRIDGE, M.J. (1993). Inositol trisphosphate and calcium signalling. *Nature*, **361,** 315–325.

CARRINGTON, A.L., CALCUTT, N.A., ETTLINGER, C.B., GUSTAFSSON, T. & TOMLINSON, D.R. (1993). Effects of treatment with myoinositol or its 1,2,6-trisphosphate (PP56) on nerve conduction in streptozotocin-diabetes. *Eur. J. Pharmacol.*, **237,** 257–263.

CAVERO, I., MONDOT, S. & MESTRE, M. (1989). Vasorelaxant effects of cromakalim in rats are mediated by glibenclamide-sensitive potassium channels. *J. Pharmacol. Exp. Ther.*, **248,** 1261–1268.

CLAXSON, A., MORRIS, C., BLAKE, D., SIREN, M., HALLIWELL, B., GUSTAFSSON, T., LOFKVIST, B. & BERGELIN, I. (1990). The anti-inflammatory effects of D-myo-inositol-1,2,6-trisphosphate (PP56) on animal models of inflammation. *Agents Actions*, **29,** 68–70.

FULTON, D., MCGIFF, J.C. & QUILLEY, J. (1994). Role of K^+ channels in the vasodilator response to bradykinin in the rat heart. *Br. J. Pharmacol.*, **113,** 954–958.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990). Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester. *Br. J. Pharmacol.*, **101,** 632–639.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991a). Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to acetylcholine, 5'-N-ethylcarboxamidoadenosine or salbutamol in conscious rats. *Br. J. Pharmacol.*, **103,** 1725–1732.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991b). Effect of N^G-nitro-L-arginine methyl ester on vasodilator responses to adrenaline or BRL 38227 in conscious rats. *Br. J. Pharmacol.*, **104,** 731–737.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1993). Effects of chronic treatment with nitric oxide synthase inhibitors on regional haemodynamic responses to vasodilators in conscious Brattleboro rats. *Br. J. Pharmacol.*, **109,** 222–228.

GARDINER, S.M., KEMP, P.A., BENNETT, T., BOSE, C., FOULKES, R. & HUGHES, B. (1992). Involvement of β_2-adrenoceptors in the regional haemodynamic responses to bradykinin in conscious rats. *Br. J. Pharmacol.*, **105,** 839–848.

GARDINER, S.M., KEMP, P.A., FALLGREN, B. & BENNETT, T. (1994). Effects of chronic infusions of α-trinositol on regional and cardiac haemodynamics in conscious rats. *Br. J. Pharmacol.*, **113,** 129–136.

HAMILTON, T.C., BEERAHEE, A., MOEN, J.S., PRICE, R.K., RAMJI, J.V. & CLAPHAM, J.C. (1993). Levcromakalim. *Cardiovasc. Drug Rev.*, **11,** 199–222.

JACKSON, W.F. (1993). Arteriolar tone is determined by activity of ATP-sensitive potassium channels. *Am. J. Physiol.*, **265,** H1797–H1803.

LUND, T. & REED, R.K. (1994). Alpha-trinositol inhibits edema generation and albumin extravasation in thermally injured skin. *J. Trauma*, **36,** 761–765.

MCPHERSON, G.A. (1993). Current trends in the study of potassium channel openers. *Gen. Pharmacol.*, **24,** 275–281.

MOREAU, R., KOMEICHI, H., KIRSTETTER, P., YANG, S., AUPETIT-FAISANT, B., CAILMAIL, S. & LEBREC, D. (1994). Effects of glibenclamide on systemic and splanchnic haemodynamics in conscious rats. *Br. J. Pharmacol.*, **112,** 649–653.

NELSON, M.T. & QUAYLE, J.M. (1995). Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.*, **268,** C799–C822.

QUAYLE, J.M. & STANDEN, N.B. (1994). K_{ATP} channels in vascular smooth muscle. *Cardiovasc. Res.*, **28,** 797–804.

RANDALL, M.D. & MCCULLOCH, A.I. (1995). The involvement of ATP-sensitive potassium channels in β-adrenoceptor-mediated vasorelaxation in the isolated rat mesenteric arterial bed. *Br. J. Pharmacol.*, **115,** 607–612.

REVEST, P.A. & ABBOTT, N.J. (1992). Membrane ion channels of endothelial cells. *Trends Pharmacol. Sci.*, **13,** 404–407.

RODT, S.A., REED, R.K., LJUNGSTROM, M., GUSTAFSSON, T.O. & RUBIN, K. (1994). The anti-inflammatory agent α-trinositol exerts its edema-preventing effect through modulation of β_1 integrin function. *Circ. Res.*, **75,** 942–948.

RUF, J.C., CIAVATI, M., GUSTAFFSSON, T. & RENAUD, S. (1991). Effects of PP-56 and vitamin E on platelet hyperaggregability, fatty acid abnormalities, and clinical manifestations in streptozocin-induced diabetic rats. *Diabetes*, **40,** 233–239.

SCHMID-ANTOMARCHI, H., DE WEILLE, J., FOSSET, M. & LAZDUNSKI, M. (1987). The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K^+ channel in insulin-secreting cells. *J. Biol. Chem.*, **262,** 15840–15844.

SCHWIELER, J.H. & HJEMDAHL, P. (1993). D-myo-inositol-1,2,6-trisphosphate (PP56) antagonizes nonadrenergic sympathetic vasoconstriction: possible involvement of neuropeptide Y. *J. Cardiovasc. Pharmacol.*, **21,** 347–352.

SUN, X.Y., DAHLOF, C., EDVINSSON, L. & HEDNER, T. (1991a). D-myo-inositol-1,2,6-trisphosphate is a selective antagonist of neuropeptide Y-induced pressor responses in the pithed rat. *Eur. J. Pharmacol.*, **204,** 281–286.

SUN, X.Y., EDVINSSON, L. & HEDNER, T. (1991b). Neuropeptide Y-induced pressor responses in spontaneously hypertensive and Wistar-Kyoto rats antagonized by D-myo-inositol-1,2,6-trisphosphate (PP56). *J. Hypertens.*, **9,** S344–S345.

SUN, X.Y., EDVINSSON, L. & HEDNER, T. (1992). Effects of D-myo-inositol-1,2,6-trisphosphate on neuropeptide Y-induced potentiation of various vasoconstrictor agents in the rat. *J. Pharmacol. Exp. Ther.*, **261,** 1147–1152.

SUN, X.Y., EDVINSSON, L. & HEDNER, T. (1993). Cardiovascular effects of alpha-trinositol in spontaneously hypertensive and normotensive Wistar-Kyoto rats. *J. Hypertens.*, **11,** 935–943.

WAHLESTEDT, C., REIS, D.J., YOO, H., ADAMSSON, M., ANDERSSON, D. & EDVINSSON, L. (1992). A novel inositol phosphate selectivity inhibits vasoconstriction evoked by the sympathetic co-transmitters neuropeptide Y (NPY) and adenosine triphosphate (ATP). *Neurosci. Lett.*, **143,** 123–126.

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