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Factors affecting the regional haemodynamic responses to glyceryl trinitrate and molsidomine in conscious rats

K. Phillips, [1]S.M. Gardiner, P.A. Kemp & T. Bennett

Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 A series of experiments was performed in conscious, unrestrained, male, Long Evans rats, chronically instrumented for the measurement of regional haemodynamics.

2 Infusion of glyceryl trinitrate (GTN, 0.1 mg kg^{-1} min^{-1}, i.v.) for 10 min elicited tachycardia, but no sustained change in mean arterial blood pressure. Renal haemodynamics were unaffected, but there were reductions in hindquarters flow and vascular conductance together with substantial increases in flow and conductance in the mesenteric vascular bed.

3 In the presence of captopril (2 mg kg^{-1} bolus, and 1 mg kg^{-1} h^{-1} infusion, i.v.) GTN elicited significant hypotension and increases in renal blood flow and vascular conductance, indicating that activation of the renin-angiotension system opposed the dilator effects of GTN in this vascular bed. However, the mesenteric and hindquarters haemodynamic effects of GTN were not affected by captopril. In contrast, in the presence of enalaprilat (2 mg kg^{-1} bolus, and 1 mg kg^{-1} h^{-1} infusion, i.v.) there was significant enhancement of the mesenteric, as well as renal, haemodynamic effects of GTN. Hence, these results provide no evidence for the sulphydryl groups in captopril exerting a specific effect to enhance the haemodynamic actions of GTN in our experimental protocols.

4 Administration of molsidomine alone (1 mg kg^{-1}, i.v. bolus) elicited tachycardia and hypotension; there were no changes in mesenteric or hindquarters haemodynamics, but renal flow and vascular conductance fell. Thus, the hypotensive effect of molsidomine was probably due to a reduction in cardiac output, consequent upon venodilatation.

5 In the presence of captopril or enalaprilat, molsidomine evoked renal and mesenteric vasodilatations in association with hypotension, indicating that activation of the renin-angiotensin system contributed to the lack of vasodilator responses to administration of molsidomine alone. However, since the effects of enalaprilat were more marked than those of captopril (in spite of the dose of both drugs being supramaximal for inhibition of angiotensin-converting enzyme), other factors must have been involved.

6 In a separate experiment, pretreatment with the nitric oxide synthesis inhibitor, N^G-nitro-L-arginine methyl ester (1 mg kg^{-1} h^{-1}, i.v.), enhanced the mesenteric vasodilator effect of molsidomine. Collectively, these results are consistent with *in vitro* data showing that endogenous nitric oxide can inhibit the vasodilator effects of nitric oxide derived from molsidomine, and that the sulphydryl groups of captopril can protect endogenous nitric oxide from inactivation by oxygen-derived free radicals, thereby enhancing the inhibitory effect of endogenous nitric oxide on the vasodilator responses to exogenous nitric oxide derived from molsidomine (or GTN).

Keywords: Glyceryl trinitrate; molsidomine; haemodynamics; conscious rats; N^G-nitro-L-arginine methyl ester

Introduction

The demonstration that the major endothelium-derived relaxing factor is nitric oxide, synthesized from L-arginine (Palmer *et al.*, 1987; 1988), has not only opened up new areas of research (Moncada & Higgs, 1990), but has also put into context what was known already about the pharmacology of nitrovasodilators (Ignarro, 1989). Thus, the latter compounds exert their effects by virtue of releasing nitric oxide and, as with nitric oxide derived from L-arginine, the final common pathway for vasorelaxation is, probably, interaction between nitric oxide and the haem moiety of soluble guanylate cyclase, causing activation of the enzyme and hence increased production of guanosine 3′ : 5′-cyclic monophosphate (cyclic GMP) (Moncada *et al.*, 1988; Ignarro, 1989). The latter, by some means, causes reduction of free cytosolic levels of Ca^{2+} and, thereby, inhibition of the contractile process (see Kukovetz & Holzmann, 1990).

Although endogenous nitric oxide and organic nitrates have a common mode of action, some of the latter group of compounds are distinguished by the fact that tolerance to their effects develops, both *in vivo* and *in vitro* (see Katz, 1990, for review). While it is likely that the development of tolerance *in vivo* may partly be due to activation of counter-regulatory mechanisms, such as the renin-angiotensin system (RAS) (Katz, 1990), there is evidence that changes in the biochemical factors involved in the production of nitric oxide from some organic nitrates may also contribute to the development of tolerance (Ignarro, 1989; Katz, 1990). Thus, depletion of tissue sulphydryl groups retards the production of the unstable intermediate compounds through which nitric oxide is generated from some organic nitrates (Ignarro, 1989). Consistent with this proposal, supplementation with sources of sulphydryl groups has been shown to suppress tolerance to glyceryl trinitrate (GTN), for example (see Katz, 1990; Newman *et al.*, 1990). However, it should be noted that other studies have not confirmed these results (Gruetter & Lemke, 1983; Stewart *et al.*, 1988; Hogan *et al.*, 1989). Nonetheless, sulphydryl depletion as an explanation for organic nitrate tolerance has led to the proposal that sulphydryl-containing angiotensin-converting enzyme (ACE) inhibitors might be particularly useful in conjunction with organic nitrates, since they could combat both the biochemical and physiological bases of tolerance (Katz, 1990). Although *in vitro* studies (van Gilst *et al.*, 1987) have indicated that a sulphydryl-containing ACE inhibitor, but not a non-sulphydryl ACE inhibitor, prevents organic nitrate tolerance in the perfused coronary vascular bed of the rat, there have been no *in vivo* haemodynamic studies of this

[1] Author for correspondence.

phenomenon. Therefore, the first objective of the present work was to assess the regional haemodynamic effects of GTN in the absence and in the presence of captopril (a sulphydryl-containing ACE inhibitor) or enalaprilat (a non-sulphydryl ACE inhibitor).

The phenomenon of tolerance is not so apparent with compounds that produce nitric oxide spontaneously (Ignarro, 1989; Kukovetz & Holzman, 1990). Molsidomine is a drug which, following metabolic activation in the liver (Wilson *et al.*, 1986), produces nitric oxide by spontaneous decomposition (Noack & Feelish, 1989; Bohn & Schönafinger, 1989; Feelish *et al.*, 1989). Hence, comparison of the *in vivo* haemodynamic effects of molsidomine in the absence and presence of captopril or enalaprilat should allow delineation of the contribution of the RAS to the responses seen, and also the identification of any putative differences between the two ACE inhibitors under these circumstances. Therefore, this was the second objective of the present work.

There is evidence that the effects of exogenous nitric oxide derived from GTN (Moncada *et al.*, 1991) or from molsidomine (Lüscher *et al.*, 1989; Busse *et al.*, 1989; Flavahan & Vanhoutte, 1989) might be inhibited by endogenous nitric oxide, although the details of this phenomenon are not clear. Therefore, the third objective of the present work was to quantify the *in vivo* haemodynamic effects of GTN and of molsidomine in the absence and in the presence of N^G-nitro-L-arginine methyl ester (L-NAME; Moore *et al.*, 1990; Rees *et al.*, 1990), a potent inhibitor of the endogenous production of nitric oxide.

Methods

Male, Long Evans rats (350–450 g) were anaesthetized (sodium methohexitone, 60 mg kg^{-1}, i.p.) and had miniaturized, pulsed Doppler probes (Haywood *et al.*, 1981) implanted to monitor renal, mesenteric and hindquarters blood flows (Gardiner *et al.*, 1990a). Animals were given ampicillin (7 mg kg^{-1}, i.m.; Penbritin, Beechams) and left in their home cages for at least 7 days, by which time they were eating, drinking and behaving normally. Then, under brief anaesthesia (sodium methohexitone, 40 mg kg^{-1}, i.p.) an intra-arterial catheter (distal abdominal aorta via ventral caudal artery) and 3 intravenous catheters (right jugular vein) were implanted; the latter arrangement allowed separate catheters to be used for administration of GTN and molsidomine. The catheters were led subcutaneously to emerge at the back of the neck with the probe wires. The latter were soldered into a microconnector that was clamped into a harness fitted to the rat. A flexible spring was connected to the harness and the catheters ran through the spring which was supported by a counterbalanced lever. This arrangement allowed the animal free movement and permitted experiments to be carried out without disturbance (Gardiner *et al.*, 1990a). Animals were left in their home cages overnight before measurements were begun; the following experiments were then performed.

Experiment 1: Effects of glyceryl trinitrate in the absence and presence of captopril

Animals ($n = 8$) were given GTN by i.v. infusion (0.1 mg kg^{-1} min^{-1}, in a volume of 33 μl min^{-1}) over 10 min, since pilot experiments had shown this procedure produced clear-cut increases in mesenteric blood flow (see also Gardiner *et al.*, 1990b). At least 1 h after administration of GTN, a primed infusion of captopril (2 mg kg^{-1} bolus in 0.1 ml, 1 mg kg^{-1} h^{-1} at 0.3 ml h^{-1}; Muller *et al.*, 1990) was begun and, starting 1 h later, the GTN infusion was repeated.

Experiment 2: Effects of glyceryl trinitrate in the absence and presence of enalaprilat

In a separate group ($n = 8$) of rats, the above protocol was repeated except that enalaprilat was given instead of captopril, using the same dose regime.

Experiment 3: Effects of glyceryl trinitrate in the absence and presence of N^G-nitro-L-arginine methyl ester

A group of rats ($n = 8$), randomly selected from the 2 groups above, were re-challenged with GTN (dose as in experiment 1) at least 48 h after their previous exposure. After a delay of 1 h, an infusion of L-NAME (1 mg kg^{-1} h^{-1}, at 0.3 ml h^{-1}; Gardiner *et al.*, 1991) was begun, and 1 h later the animals were re-challenged with GTN.

Experiment 4: Effects of molsidomine in the absence and presence of captopril

The animals in this experiment were the same as those used in experiment 1 with each animal being randomized to receive molsidomine or GTN first, but in the same order in the absence and presence of captopril. Molsidomine was given as a bolus dose of 1 mg kg^{-1} (in 0.1 ml), and since pilot experiments had shown its effects were persistent, at least 2 h were allowed before any subsequent intervention was carried out. The protocol described in experiment 1 was followed.

Experiment 5: Effects of molsidomine in the absence and presence of enalaprilat

The animals in this experiment were those used in experiment 2, with random allocation to receive molsidomine or GTN first in the absence and presence of enalaprilat, at the dose used in experiment 2.

Experiment 6: Effects of molsidomine in the absence and presence of N^G-nitro-L-arginine methyl ester

The animals in this experiment were those studied in experiment 3, with random allocation to receive molsidomine or GTN first in the absence and presence of L-NAME (dose as in experiment 3).

Data analysis

Continuous recordings were made of phasic and mean systemic arterial blood pressure (MAP), instantaneous heart rate and mean renal, mesenteric and hindquarters Doppler shift signals; regional vascular conductances were calculated as (mean Doppler shift/MAP). Since all animals acted as their own controls, analysis was carried out on the raw data rather than percentage changes (Gardiner *et al.*, 1990a,b; 1991). Changes relative to baseline were assessed by Friedman's test (Theodorsson-Norheim, 1987). Comparisons between responses in the presence and absence of ACE inhibitors or L-NAME were carried out by use of Wilcoxon's ranks sums test applied to areas under or over curves. Wilcoxon's test was used also to compare baseline values in the same group of animals under different conditions; a P value <0.05 was taken as significant.

Drugs

Molsidomine (Sigma), captopril (Squibb, U.S.A.), enalaprilat (MSD, U.S.A.), and L-NAME hydrochloride (Sigma) were dissolved in isotonic NaCl (157 mmol l^{-1}). The pH of the captopril and enalaprilat solutions was adjusted to 7.0–7.4 with NaOH. GTN (Tridil, DuPont, U.S.A. provided as a 5 mg ml^{-1} stock solution) was diluted in isotonic NaCl.

Results

Vehicle administration had no consistent haemodynamic effects.

Experiment 1: Effects of glyceryl trinitrate in the absence and presence of captopril

Infusion of GTN alone produced tachycardia throughout the infusion period, and during the 5 min following infusion (Figure 1). However, there was no sustained reduction in MAP (Figure 1), although pulse pressure fell, and there was a very transient initial hypotension in most animals (data not shown). Renal blood flow and vascular conductance were unaffected by GTN, whereas hindquarters blood flow and vascular conductance showed significant reductions (Figure 1). In contrast, there were significant increases in mesenteric blood flow and vascular conductance (Figure 1).

Administration of captopril caused initial tachycardia, hypotension and increases in flow and conductance in the renal, mesenteric and hindquarters vascular beds. However, at the time of administration of GTN, only the renal blood flow and vascular conductance were increased relative to the resting values in the absence of captopril (Figure 1).

In the presence of captopril, GTN caused a slight, but significant, fall in MAP (AOC, 92 ± 24 units) and this effect was significantly different from the response to GTN in the absence of captopril (AOC, 31 ± 7 units) (Figure 1). In spite of this difference, the tachycardic response to GTN in the presence of captopril was not significantly different from the response to GTN alone (Figure 1). There was an increase in renal flow and vascular conductance in response to GTN in the presence of captopril (AUC, 17 ± 5 and 28 ± 7 units, respectively), and these effects were significantly different from the response to GTN alone (AUC, 7 ± 3 and 6 ± 3 units for renal flow and conductance, respectively) (Figure 1). However, the mesenteric and hindquarters haemodynamic responses to GTN were not different in the presence and absence of captopril (Figure 1).

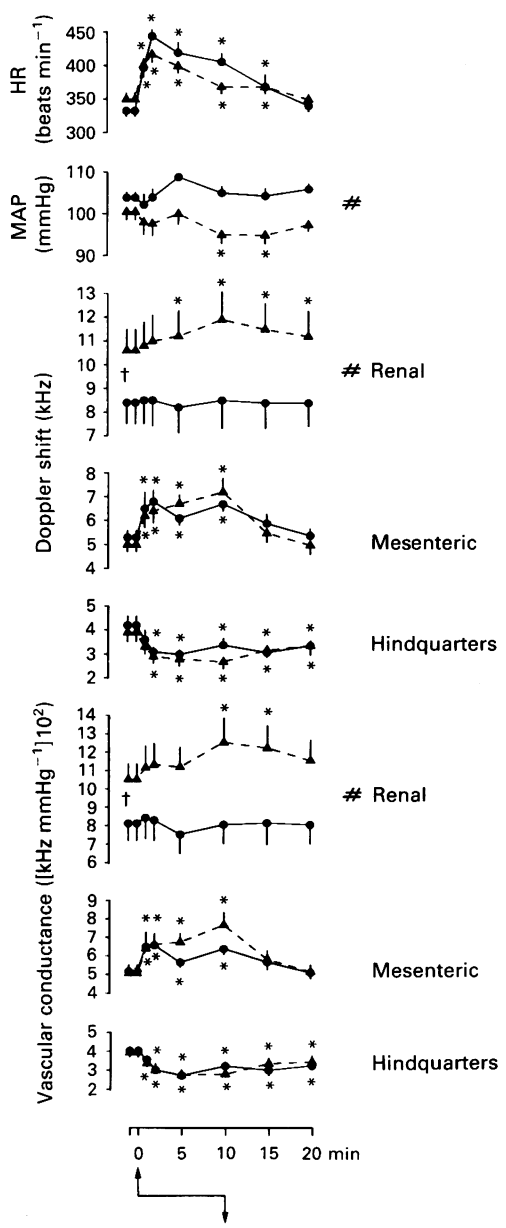

Figure 1 Cardiovascular responses to a 10 min infusion of glyceryl trinitrate at 0.1 mg kg^{-1} min^{-1} (between the arrows) in the same group (n = 8) of conscious, Long Evans rats in the absence (●) or in the presence (▲) of captopril (2 mg kg^{-1} bolus, and 1 mg kg^{-1} min^{-1}, infusion). Values are mean and bars show s.e.mean; where bars are missing they lie within the symbols. *P < 0.05 versus baseline; †P < 0.05 between resting values in the absence and presence of captopril; #P < 0.05 between responses in the absence and presence of captopril, based on areas under or over curves. MAP = mean systemic arterial blood pressure; HR = heart rate.

Experiment 2: Effects of glyceryl trinitrate in the absence and presence of enalaprilat

The responses to GTN alone were qualitatively similar to those in the group of animals in experiment 1 (Figure 2).

Administration of enalaprilat caused initial tachycardia, hypotension and increases in flow and vascular conductance in renal, mesenteric and hindquarters vascular beds. However, at the time GTN was administered, only the renal vascular bed showed increases in flow and conductance, but there was an increased mesenteric vascular conductance, in association with tachycardia and a reduction in MAP (Figure 2).

In the presence of enalaprilat, GTN caused tachycardia and a modest fall in MAP (AOC, 102 ± 26 units), but only the latter effect was different from the response to GTN alone (AOC, 43 ± 13 units) (Figure 2). There were increases in flow and vascular conductance in response to GTN in the renal and mesenteric vascular beds in the presence of enalaprilat (AUC, renal flow 21 ± 5 units; renal conductance 31 ± 8 units; mesenteric flow 50 ± 8 units; mesenteric conductance 65 ± 11 units), and all were greater than those to GTN alone (AUC, renal flow 10 ± 4 units; renal conductance 8 ± 3 units; mesenteric flow 40 ± 6 units; mesenteric conductance 37 ± 6 units) (Figure 2). Furthermore, the enhancement of the mesenteric responses to GTN was greater in the presence of enalaprilat than of captopril (AUC, mesenteric flow 26 ± 6 units; mesenteric conductance 31 ± 7 units) (Figures 1 and 2). Hindquarters haemodynamic changes following GTN were not significantly affected by enalaprilat (Figure 2).

Experiment 3: Effects of glyceryl trinitrate in the absence and presence of N^G-nitro-L-arginine methyl ester

The responses to GTN alone were as described above, although the changes in hindquarters haemodynamics were not significant in this experiment (Figure 3).

L-NAME caused bradycardia and an increase in MAP, accompanied by mesenteric and hindquarters vasoconstriction (Figure 3). Although the changes in heart rate and MAP in response to GTN in the presence of L-NAME were not significantly different from those in response to GTN alone, there was a significant hypotensive response to GTN in the presence of L-NAME (Figure 3). The renal, the mesenteric and the hindquarters responses to GTN in the absence and presence of L-NAME were not significantly different (Figure 3).

Experiment 4: Effects of molsidomine in the absence and presence of captopril

Injection of molsidomine alone caused tachycardia associated with a fall in MAP (Figure 4). Renal blood flow and vascular conductance were decreased but mesenteric and hindquarters haemodynamics were unchanged (Figure 4).

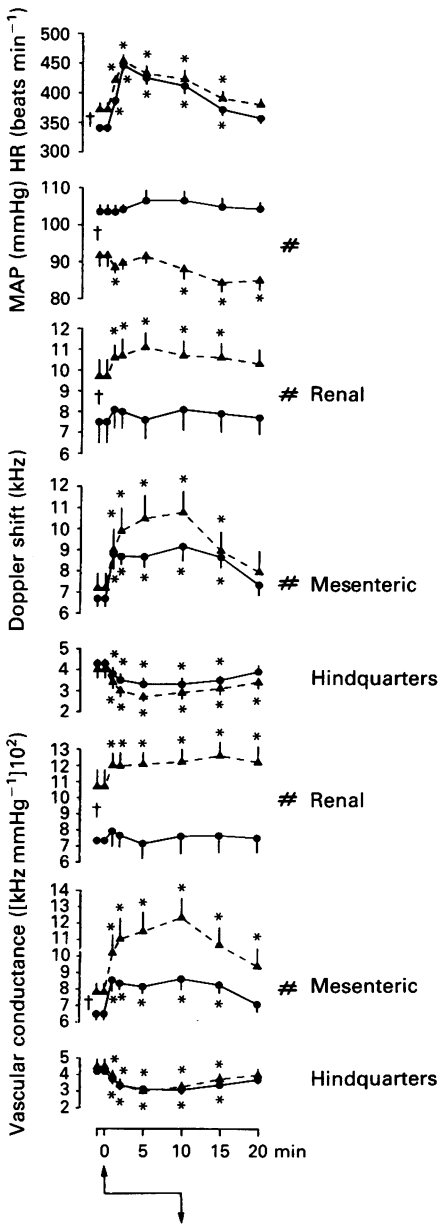

Figure 2 Cardiovascular responses to a 10 min infusion of glyceryl trinitrate at 0.1 mg kg^{-1} min^{-1} (between the arrows) in the same group (n = 8) of conscious, Long Evans rats in the absence (●) or in the presence (▲) of enalaprilat (2 mg kg^{-1} bolus, and 1 mg kg^{-1} min^{-1}, infusion). Values are mean and bars show s.e.mean; where bars are missing they lie within the symbols. *P < 0.05 versus baseline; †P < 0.05 between resting values in the absence and presence of enalaprilat; #P < 0.05 between responses in the absence and presence of enalaprilat, based on areas under or over curves. MAP = mean systemic arterial blood pressure; HR = heart rate.

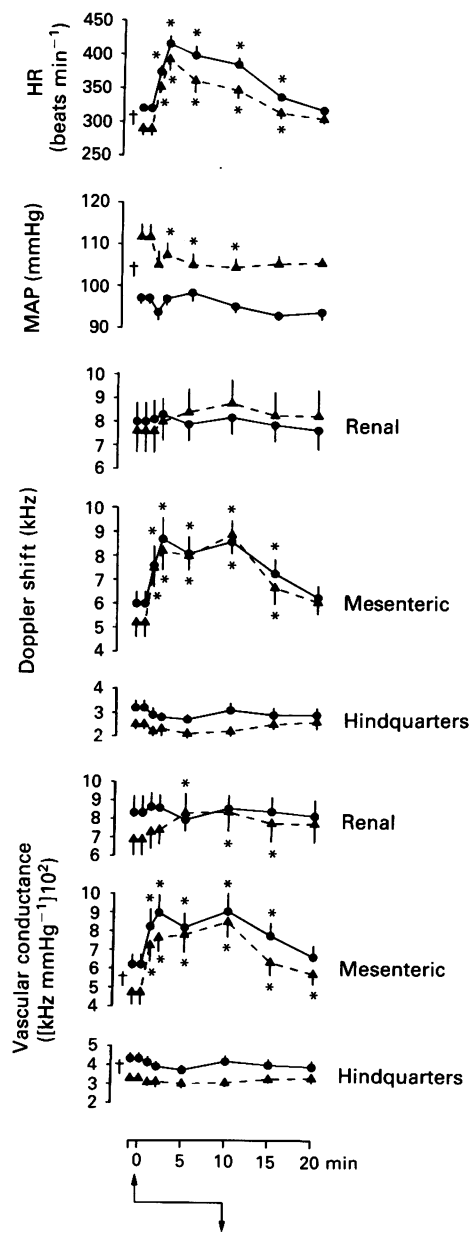

Figure 3 Cardiovascular responses to a 10 min infusion of glyceryl trinitrate at 0.1 mg kg^{-1} min^{-1} (between the arrows) in the same group (n = 8) of conscious, Long Evans rats in the absence (●) or in the presence (▲) of N^{G}-nitro-L-arginine methyl ester (L-NAME) (1 mg kg^{-1} min^{-1}, infusion). Values are mean and bars show s.e.mean; where bars are missing they lie within the symbols. *P < 0.05 versus baseline; †P < 0.05 between resting values in the absence and presence of L-NAME. MAP = mean systemic arterial blood pressure; HR = heart rate.

The animals in this experiment were those in experiment 1, hence, the effects of captopril were qualitatively similar to those described under experiment 1.

In the presence of captopril, the changes in heart rate and MAP in response to molsidomine were not different from those seen in response to molsidomine alone. However, there were increases in flow and vascular conductance in the mesenteric vascular bed (AUC, 33 ± 6 and 66 ± 8 units, respectively) and an increase in renal vascular conductance (AUC, 50 ± 17 units) that were significantly different from the changes evoked by molsidomine alone (AUC, mesenteric flow 3 ± 1 units; mesenteric conductance 17 ± 6 units; renal conductance 2 ± 1 units) (Figure 4). In contrast, even in the presence of captopril, molsidomine had no effect on hindquarters haemodynamics (Figure 4).

Experiment 5: Effects of molsidomine in the absence and presence of enalaprilat

The responses to molsidomine alone in this group of rats were qualitatively similar to those studied in experiment 4 (Figure 5).

The animals in this experiment were those in experiment 2, hence, the effects of enalaprilat were qualitatively similar to those described under experiment 2.

In the presence of enalaprilat, the hypotensive effect of molsidomine was clearly enhanced (molsidomine alone AOC, 426 ± 65 units; molsidomine in the presence of enalaprilat AOC, 1046 ± 154 units), although its tachycardic effect was unchanged (Figure 5). While renal blood flow fell (AOC, 49 ± 18 units), this change was less than that in response to molsidomine alone (AOC, 124 ± 24 units) (Figure 5), and in

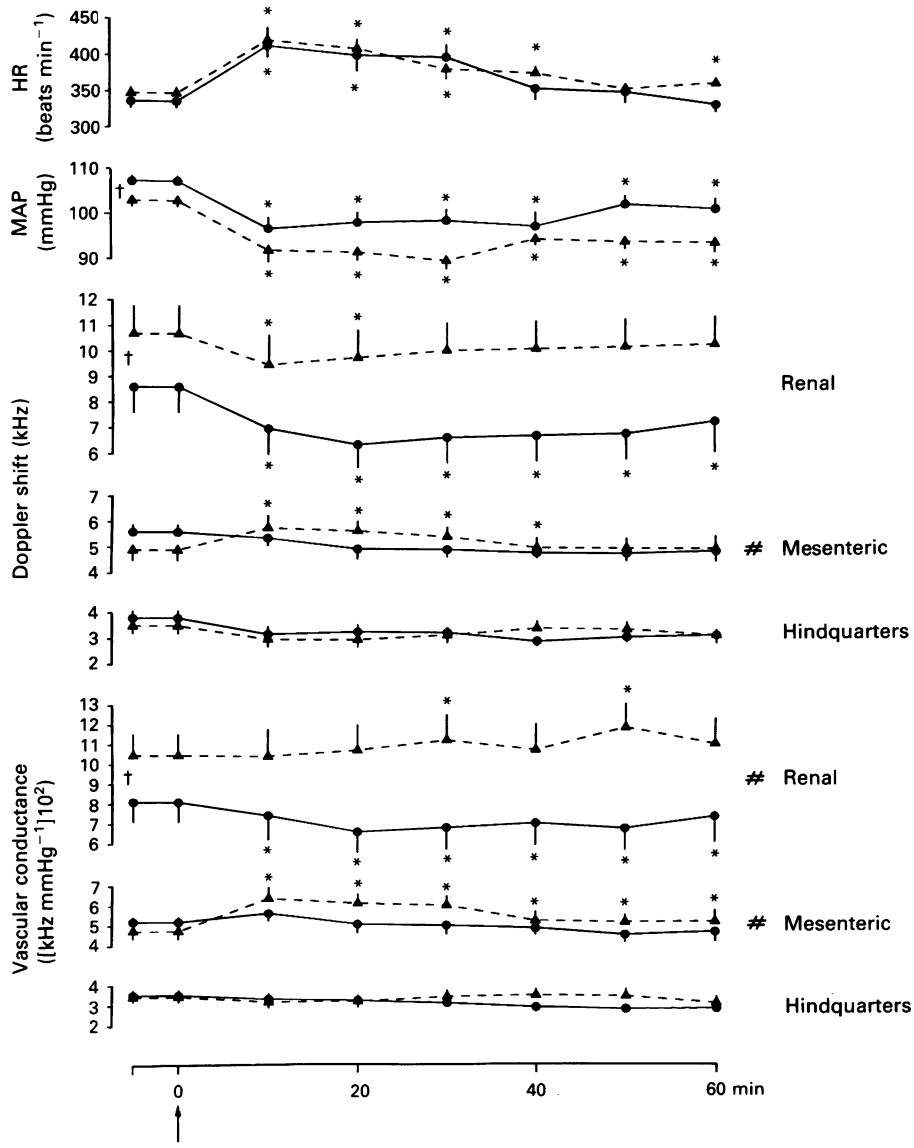

Figure 4 Cardiovascular responses to molsidomine (0.1 mg kg^{-1} bolus at arrow) in the same group (n = 8) of conscious, Long Evans rats in the absence (●) or in the presence (▲) of captopril (2 mg kg^{-1} bolus, and 1 mg kg^{-1} min^{-1}, infusion). Values are mean and bars show s.e.mean; where bars are missing they lie within the symbols. *P < 0.05 versus baseline; †P < 0.05 between resting values in the absence and presence of captopril; #P < 0.05 between responses in the absence and presence of captopril, based on areas under or over curves. MAP = mean systemic arterial blood pressure; HR = heart rate.

the presence of enalaprilat there was a renal vasodilatation in response to molsidomine (AUC, 100 ± 15 units), rather than a vasconstriction as seen with molsidomine alone (AOC, 96 ± 19 units) (Figure 5). Likewise, enalaprilat unmasked substantial increases in flow and vascular conductance in response to molsidomine in the mesenteric vascular bed (AUC, flow 62 ± 17 units; conductance 177 ± 35 units), and this effect was greater (P < 0.05) than that in the presence of captopril. As in captopril-treated rats, molsidomine had no effects on hindquarters haemodynamics in the presence of enalaprilat (Figure 5).

Experiment 6: Effects of molsidomine in the absence and presence of N^G-nitro-L-arginine methyl ester

The qualitative effects of molsidomine alone were as described above (Figure 6). The animals in this experiment were those in experiment 3, hence, the effects of L-NAME were as described under experiment 3.

In the presence of L-NAME the hypotensive effect of molsidomine was unchanged, although there was a slight enhancement of its tachycardic action (AUC, molsidomine alone 2988 ± 357 units; molsidomine + L-NAME 4481 ± 537 units) and renal vasconstrictor effects (AOC, molsidomine alone 66 ± 21 units; molsidomine + L-NAME 114 ± 27 units) (Figure 6). In contrast, there were increases in mesenteric blood flow and vascular conductance in response to molsidomine in the presence of L-NAME (AUC, flow 51 ± 15 units; conductance 69 ± 15 units), and these were significantly different from the changes in the absence of L-NAME (AUC, flow 19 ± 9 units; conductance 45 ± 8 units) (Figure 6). Molsidomine had no effects on hindquarters haemodynamics in the presence of L-NAME (Figure 6).

Discussion

The present work had three main objectives that can be restated in the form of the following questions:

Are the regional haemodynamic effects of GTN enhanced more by captopril (a sulphydryl-containing ACE-inhibitor) than by enalaprilat (an ACE-inhibitor lacking a sulphydryl group)? The answer to this question is no, at least under the

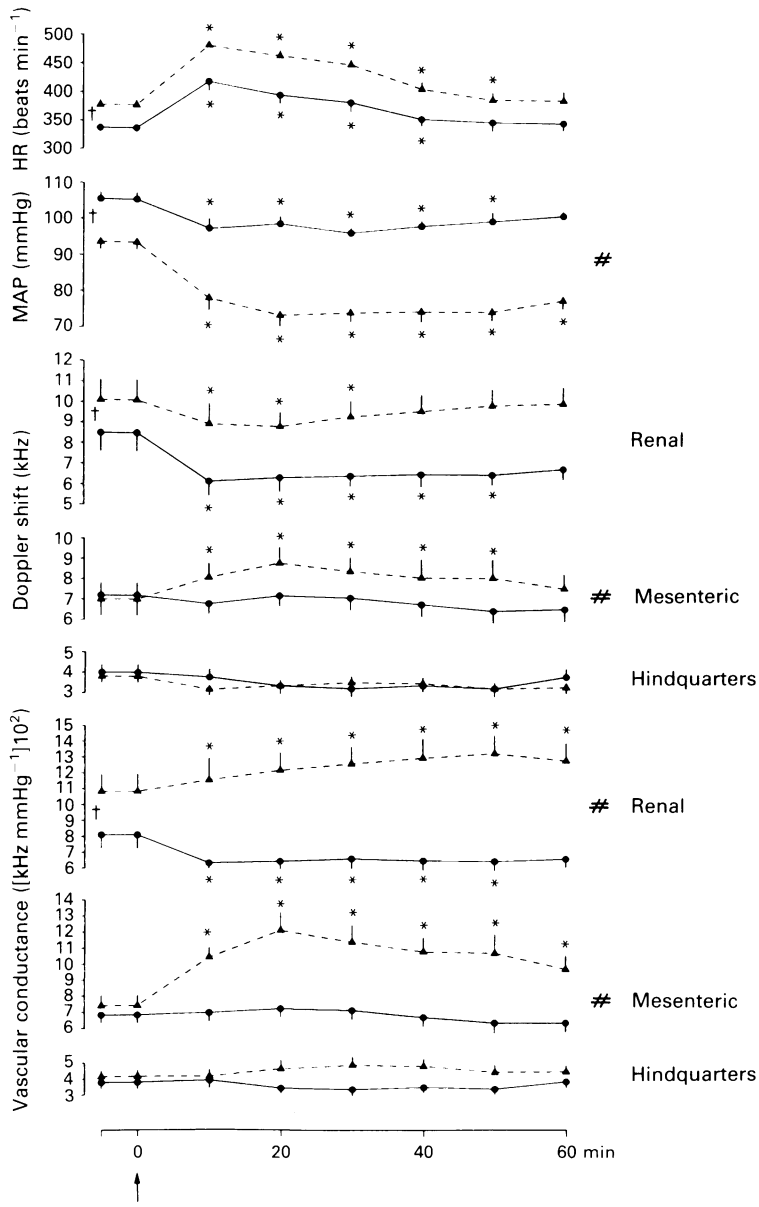

Figure 5 Cardiovascular responses to molsidomine (0.1 mg kg^{-1} bolus at arrow) in the same group (n = 8) of conscious, Long Evans rats in the absence (●) or in the presence (▲) of enalaprilat (2 mg kg^{-1} bolus and 1 mg kg^{-1} min^{-1}, infusion). Values are mean and bars show s.e.mean; where bars are missing they lie within the symbols. *$P < 0.05$ versus baseline; †$P < 0.05$ between resting values in the absence and presence of enalaprilat; #$P < 0.05$ between responses in the absence and presence of enalaprilat, based on areas under or over curves. MAP = mean systemic arterial blood pressure; HR = heart rate.

conditions of our experiment. While it could be argued that the exposure to GTN was too short to elicit tolerance, it is clear from the results of Stewart *et al.* (1988) that within 1 min of the onset of GTN infusion functional tolerance can develop. Moreover, it was apparent from the present results that both captopril and enalaprilat unmasked a renal vasodilator response to GTN, indicating that a component of 'tolerance' attributable to GTN-induced activation of the RAS was present. However, it was notable that, although there was a tendency for GTN to cause an enhanced mesenteric vasodilatation in the presence of captopril, this effect was not significant, whereas enalaprilat caused a clear augmentation of the mesenteric haemodynamic effects of GTN. Since both ACE inhibitors were given in doses that were supra-maximal for their effects on the RAS (Muller *et al.*, 1990) then a factor other than ACE inhibition must have been responsible for the difference between their effects on the mesenteric responses to GTN. There is some *in vitro* evidence for endogenous nitric oxide inhibiting responses to nitric oxide derived from GTN (Moncada *et al.*, 1991). If the sulphydryl groups of captopril act as selective scavengers of oxygen-derived free radicals (McMurray & Chopra, 1991) that inactivate nitric oxide (see Ignarro, 1989), then in the presence of captopril there could have been a greater inhibition by endogenous nitric oxide of the vasodilator response to nitric oxide derived from GTN. However, enalaprilat, lacking sulphydryl groups, and thus not acting to preserve endogenous nitric oxide, would not augment the inhibitory effects on the responses to nitric oxide derived from GTN. Inhibition of ACE with enalaprilat could thus remove the counter-regulatory vasconstrictor effects of the RAS and reveal a greater component of GTN-induced vasodilatation than seen in the presence of captopril. However, it is not clear why such an effect should not be apparent in the renal vascular bed.

As suggested elsewhere (Kiff *et al.*, 1991), the marked mesenteric vasodilator response to GTN in the absence of a hindquarters vasodilatation could have been due to differential biotransformation (Kawamoto *et al.*, 1990) of GTN to nitric oxide in the two vascular beds. However, the finding that neither captopril nor enalaprilat revealed a hindquarters vaso-

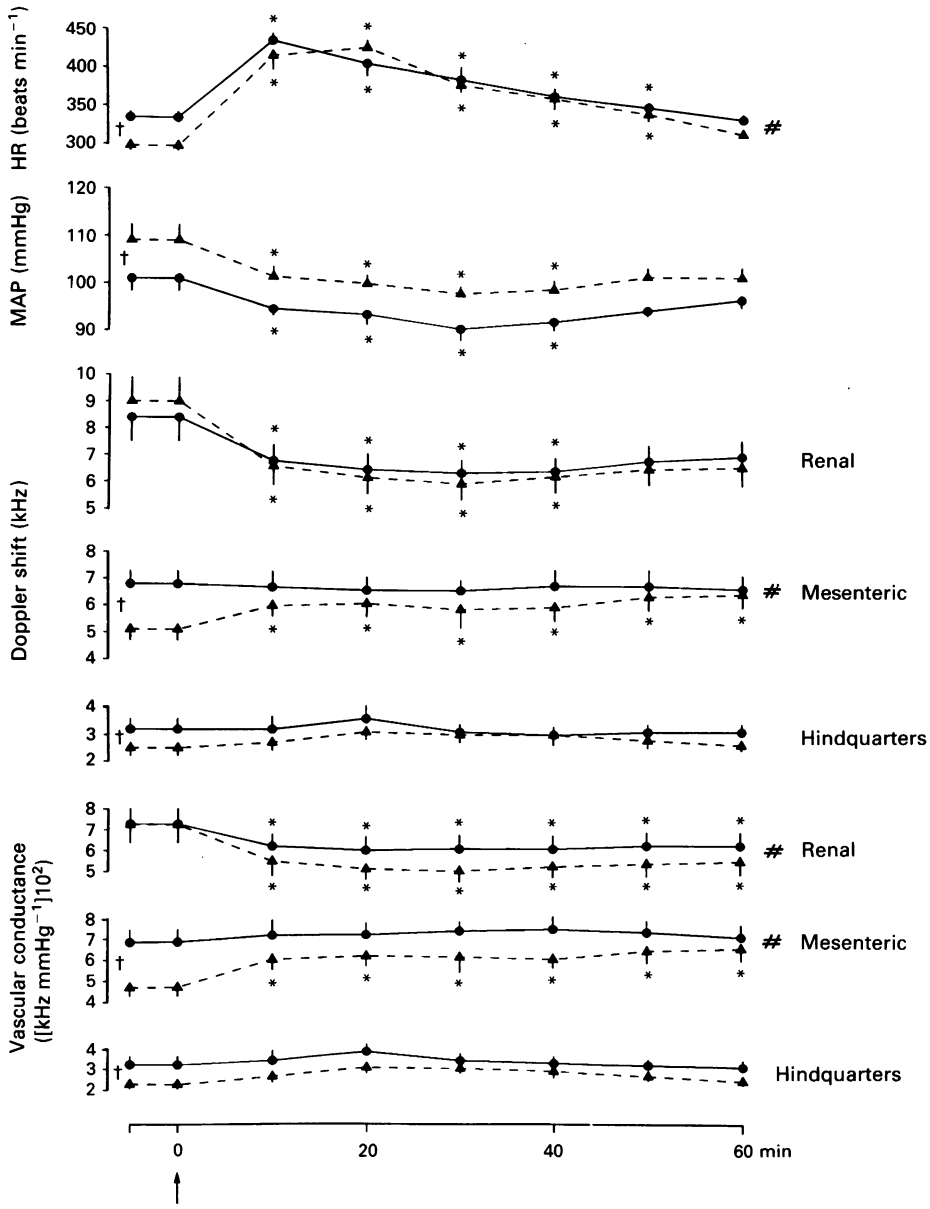

Figure 6 Cardiovascular responses to molsidomine (0.1 mg kg^{-1} bolus at arrow) in the same group (n = 8) of conscious, Long Evans rats in the absence (●) or in the presence (▲) of N^{G}-nitro-L-arginine methyl ester (L-NAME) (1 mg kg^{-1} min^{-1}, infusion). Values are mean and bars show s.e.mean; where bars are missing they lie within the symbols. *P < 0.05 versus baseline; †P < 0.05 between resting values in the absence and presence of L-NAME; #P < 0.05 between responses in the absence and presence of L-NAME, based on areas under or over curves. MAP = mean systemic arterial blood pressure; HR = heart rate.

dilator response to GTN indicates that the sulphydryl groups of captopril were not sufficient to promote a vasodilator effect of GTN in that vascular bed, and hence deficiency of sulphydryl groups may not have been the reason for the lack of a vasodilator response. Since the mesenteric vascular bed showed a substantial and maintained mesenteric vasodilator response to GTN it would be worthwhile in future experiments to determine if prolonged infusions of, or repeated dosing with, GTN produce tolerance in that vascular bed and, if so, whether or not captopril is more effective than enalaprilat in suppressing such tolerance.

What are the regional haemodynamic effects of molsidomine, and are they differentially affected by captopril or enalaprilat? Molsidomine caused hypotension and a tachycardia, presumably of reflex origin. It is notable that in the presence of molsidomine alone there were no changes in mesenteric or hindquarters haemodynamics, and there were reductions in renal blood flow and vascular conductance. Hence the hypotension was probably due to a fall in cardiac output, possibly resulting from venodilatation (Grund *et al.*, 1978). It is feasible that systemic generation of nitric oxide from molsidomine was responsible for this effect and consequent activation of counter-regulatory mechanisms over-rode any molsidomine-induced reductions in vascular conductances. The difference between GTN and molsidomine in this respect could have been due to local production of nitric oxide from GTN, especially in the mesenteric vascular bed, exerting a particularly potent effect at that site, compared to the less localized effects of nitric oxide generated spontaneously from molsidomine. However, it appears that molsidomine may be a more potent venodilator than GTN, as reported by Grund *et al.* (1978), since there was no indication that GTN caused a fall in cardiac output.

Captopril revealed renal and mesenteric vasodilator responses to molsidomine, consistent with activation of the RAS opposing its vasodilator effects, but enalaprilat had more marked effects than captopril on the mesenteric responses to molsidomine, possibly for the reasons discussed above in connection with GTN.

Are the regional haemodynamic effects of GTN or molsidomine affected by N^G-nitro-L-arginine methyl ester? Although L-NAME had haemodynamic effects consistent with suppression of endothelial nitric oxide production (Gardiner *et al.*, 1990a), it did not have a significant influence on the responses to GTN. However, there was a mesenteric vasodilator response to molsidomine in the presence of L-NAME that was not seen in its absence. Thus, these results are consistent with removal of a negative interaction between endogenous nitric oxide and that derived from molsidomine; the apparent lack of interaction between endogenous nitric oxide and that derived from GTN could have been due to the relative doses of L-NAME and GTN used (Moncada *et al.*, 1991).

In conclusion, it is clear that the nitrovasodilators, GTN and molsidomine, have different regional haemodynamic effects in conscious rats, although both elicit functional activation of the RAS. However, pretreatment with enalaprilat causes greater augmentation of the responses to GTN and molsidomine than does captopril. Hence, these results do not show a selective ability of the sulphydryl groups of captopril to enhance responses to GTN or molsidomine. Rather, they are consistent with an augmented inhibition of the responses to exogenous nitric oxide (derived from GTN or molsidomine) in the presence of captopril, possibly due to the sulphydryl groups of the latter protecting endogenous nitric oxide from inactivation by oxygen-derived free radicals.

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