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Regional haemodynamic effects of prolonged infusions of human α-calcitonin gene-related peptide in conscious, Long Evans rats

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1 Haemodynamic measurements were made in conscious, Long Evans rats chronically instrumented for the assessment of changes in regional blood flows (renal, mesenteric and hindquarters, or internal and common carotid) and systemic arterial blood pressure and heart rate, before, during and after 3 day infusions of vehicle or human α-calcitonin gene-related peptide (CGRP) (1.5 or 15 nmol $kg^{-1} h^{-1}$).

2 In animals with renal, mesenteric and hindquarters flow probes ($n = 8$), during the first day of infusion of human α-CGRP (1.5 nmol $kg^{-1} h^{-1}$) there was sustained tachycardia and hypotension, a sustained reduction in renal flow, a transient reduction in mesenteric flow and a relatively well-maintained increase in hindquarters flow. All these effects were significantly different from the changes seen in vehicle-infused rats ($n = 8$), but calculation of vascular conductances showed only the late mesenteric vasodilatation and the sustained hindquarters vasodilatation were different from the changes in vehicle-infused rats. However, by the second day of infusion and thereafter cardiovascular variables in the animals receiving vehicle and those receiving human α-CGRP were not different.

3 Nine animals instrumented with probes to monitor changes in internal and common carotid haemodynamics initially received human α-CGRP infused at a rate of 1.5 nmol $kg^{-1} h^{-1}$. Three of these animals still showed some response to the human α-CGRP (tachycardia, hypotension, hyperaemic vasodilatation) throughout the second day of infusion and hence were taken through the 3 day infusion protocol. When the infusion was stopped on the fourth day all these animals showed reversal of the effects of human α-CGRP. The other 6 animals in the original group showed complete desensitization to the effects of human α-CGRP by the end of the second day of infusion (as seen in the group instrumented with renal, mesenteric and hindquarter probes). Therefore, in order to assess the extent of desensitization, at the beginning of the third day the dose of human α-CGRP was increased to 15 nmol $kg^{-1} h^{-1}$. The resulting tachycardia, hypotension and hyperaemia in the carotid vascular beds were significant, (but no greater than the initial responses to human α-CGRP at 1.5 nmol $kg^{-1} h^{-1}$). These effects were maintained throughout the third infusion day, but there was some desensitization to the effects of this higher dose of human α-CGRP by the beginning of the fourth infusion day. However, when the infusion was stopped there was clear reversal of the effects of human α-CGRP.

4 The results indicate substantial inter-individual variation in the haemodynamic effects of prolonged infusions of human α-CGRP in conscious, Long Evans rats. However, since increasing the dose of human α-CGRP overcame the desensitization, it is feasible that, in the clinical setting, maintained increases in internal carotid blood flow could be achieved by individually-adjusted infusions of human α-CGRP.

Keywords: Human α-CGRP; regional haemodynamics; vasodilatation

Introduction

There is some evidence that prolonged infusions of human α-calcitonin gene-related peptide (CGRP) can cause sustained hypotension in conscious rats (Marshall *et al.*, 1987), although the associated tachycardia wanes. Several aspects of those studies are worth noting: (1) a very high dose of human α-CGRP was used, causing a fall in systemic arterial blood pressure that would be clinically unacceptable; (2) only female rats were studied; (3) although normotensive and hypertensive rats were used in the protocols involving infusions up to 24 h duration, only hypertensive animals were investigated when the effects of 3 day infusions were studied; (4) peptide delivery was achieved with an implanted osmotic minipump system in the experiments involving 3 day infusions; and (5) systemic arterial blood pressure and heart rate were the only cardiovascular variables measured. Subsequently we found that, in normotensive male rats, a 1 h infusion of rat α-CGRP at the same dose as used by Marshall *et al.* (1987) caused sustained hypotension, but the associated regional haemodynamic profile was not stable (Gardiner *et al.*, 1989a). Similar observations were made with 1 h infusions of lower doses of human α- and β-CGRP (Gardiner *et al.*, 1989b).

Putative clinical uses of human α-CGRP include the treatment of cerebral vasospasm (Gardiner *et al.*, 1990a; Johnston *et al.*, 1990) following subarachnoid haemorrhage, i.e. a situation which would require sustained administration of human α-CGRP with maintained effect. Therefore, the aims of the present work were: (1) to extend our previous observations and to determine if there were any progressive changes in the effects of human α-CGRP on renal, mesenteric and hindquarters haemodynamics and systemic arterial blood pressure when infusions were continued over 3 days, in normotensive rats; and (2) to assess the effects of prolonged infusions of human α-CGRP on carotid haemodynamics in normotensive rats.

Methods

All experiments were carried out on male, Long Evans rats (aged 3–4 months, 350–450 g). Under sodium methohexitone anaesthesia (60 mg kg^{-1}, i.p., supplemented as required), miniaturized, pulsed Doppler flow probes (Haywood *et al.*, 1981) were implanted around the left renal and superior mesenteric

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artery and the distal abdominal aorta (Gardiner et al., 1989a,b). In other animals probes were implanted around both common carotid arteries after the external carotid artery had been ligated on the left side to allow assessment of changes in ipsilateral internal carotid flow (Gardiner et al., 1990a). Following surgery all animals received ampicillin (7 mg kg^{-1}, i.m.; Penbritin, Beecham), and were returned to their home cages for 7–14 days. Thereafter, under brief anaesthesia (sodium methohexitone, 40 mg kg^{-1}, i.p.) intra-arterial (distal abdominal aorta via ventral caudal artery) and intravenous (right jugular vein) catheters were implanted and tunnelled subcutaneously to emerge at the back of the neck, together with the pulsed Doppler wires. The catheters were protected by a flexible, counter-balanced spring attached to a harness fitted to the rat and a connector to the pulsed Doppler lead was taped to this spring (Gardiner et al., 1988a,b; 1990a). Experiments began at least 24 h after catheter implantation.

Continuous intravenous infusions (0.3 ml h^{-1}) were given through a swivel system (Brown et al., 1976) that allowed animals free movement. One group of animals ($n = 8$) instrumented with renal, mesenteric and hindquarters probes received a 3 day infusion of isotonic saline containing 1% bovine serum albumin (BSA) (Sigma), this being the vehicle in which human α-CGRP was dissolved. Another group of rats ($n = 8$) with renal, mesenteric and hindquarters probes, and a further group of rats ($n = 9$) with internal carotid and common carotid probes were given infusions of human α-CGRP at a rate of 1.5 nmol kg^{-1} h^{-1}.

Infusions started at 07 h 00 min on day 1 after 30 min baseline recording. The infusion pumps were switched off at 07 h 00 min on day 4 and recordings continued for a further 5 h. In all experiments involving infusion of human α-CGRP the infusate was prepared fresh twice daily at 07 h 00 min and 16 h 00 min from aliquots of the same stock solution that was kept at −80°C (solution of 200 nmol ml^{-1}). Between 07 h 00 min and 16 h 00 min each day recordings were made of heart rate, mean arterial blood pressure and mean Doppler shift signals (Crystal Biotech VFI system). Changes (%) in mean Doppler shift signals were taken as changes in blood flow (Haywood et al., 1981; Gardiner et al., 1989a,b; 1990a) and % changes in vascular conductances were calculated from mean Doppler shift and mean arterial blood pressure signals (Gardiner et al., 1990b).

Experiments were organized so that animals with renal, mesenteric and hindquarters probes receiving vehicle or human α-CGRP infusions were run in tandem. Analysis of the results of this experiment showed there was marked tachyphylaxis to the effects of human α-CGRP (see Results). On the basis of this experiment the protocol involving assessment of the carotid haemodynamic effects of human α-CGRP was devised with the results being assessed during the experiment to detect the occurrence of tachyphylaxis. Of the 9 animals studied, 3 showed relatively sustained responses to human α-CGRP and went through the protocol described above. However, 6 animals showed marked tachyphylaxis during the second day of infusion (see Results) and so in these animals at 07 h 00 min on the third day of infusion the rate of administration of human α-CGRP was increased 10 fold (i.e. to 15 nmol kg^{-1} h^{-1}) in order to determine if there was complete desensitization to the peptide.

Data analysis

Within-group analysis was carried out by Friedman's test (Theodorsson-Norheim, 1987) and areas under or over curves (Gardiner et al., 1990b), were compared by the Mann-Whitney U test.

Peptides

Human α-CGRP (Lot Number ZD 949) (Celltech, U.K.) was used throughout the study. The peptide was dissolved in isotonic saline containing 1% BSA (Sigma).

Results

Cardiovascular effects of 3 day infusions of vehicle or human α-CGRP in rats with renal, mesenteric and hindquarters probes

During infusion of vehicle, heart rate and mean blood pressure showed no consistent changes (Figure 1). Renal blood flow showed some variation during the day, and hindquarters flow showed a downwards drift over the first day, but was relatively steady thereafter (Figure 1). However, mesenteric blood flow showed a clear rhythm with flow being highest in the morning at 07 h 00 min and falling subsequently to reach its lowest level at about 15 h 00 min. This variation we attribute to feeding-induced gastrointestinal hyperaemia. Rats are nocturnal and eat most of their daily intake when the lights are out (i.e. between 18 h 00 min and 06 h 00 min), and thus, when the recordings started at 06 h 30 min, the animals were post-prandial.

In spite of the variations during vehicle infusions there were substantial and significant differences between those and the changes seen during infusion of human α-CGRP on the first day (Figures 1 and 2). In the latter circumstance, initially, there was marked tachycardia, hypotension, reductions in renal and mesenteric flows and an increase in hindquarters flow (Figure 1). However, over the course of the day the reduction in mesenteric blood flow waned, so that, 9 h after the onset of infusion, mesenteric vascular conductance had changed from being below to being above baseline (Figure 2). There was a hindquarters hyperaemia and vasodilatation at this time, but the reduction in renal flow was not associated with a significant change in renal vascular conductance (Figures 1 and 2). During the second and third days of infusion and following the cessation of infusion, there were no significant differences between any variables in the vehicle and human α-CGRP-treated groups (Figures 1 and 2).

Examination of individual responses showed some quantitative variation, but similar patterns of change occurred in all animals studied with the exception of one animal that had relatively well-maintained responses during the 3 day infusion of human α-CGRP.

Cardiovascular effects of 3 day infusions of human α-CGRP in rats with internal and common carotid probes

In 3 animals there was a relatively sustained hypotensive response to human α-CGRP over the infusion period, although the tachycardia waned (Figure 3). However, while there were still substantial hypotensive effects and internal and common carotid vasodilatations on the second and third days of infusion, there was a clear reduction in response between the first and second days, during the time recordings were not made (i.e. between 16 h 00 min and 07 h 00 min the next day). Furthermore, at the beginning of the fourth day (i.e. just before the infusion pumps were switched off) there had been further, marked reductions in the effects of human α-CGRP, relative to the responses seen at the end of the third day (Figure 3).

In the other sub-group (6 animals), during the first day of infusion and at the beginning of the second day, the haemodynamic effects of human α-CGRP were the same as in the sub-group that showed a relatively maintained response to the peptide (see Figures 3 and 4). However, during the second infusion day (i.e. during the time that recordings were being made) there was desensitization to all the effects of human α-CGRP in these 6 animals. At the beginning of the third day the concentration of human α-CGRP in the infusate was increased 10 fold (i.e. to 15 nmol kg^{-1} h^{-1}) and all variables showed marked responses, but these effects were no greater than those seen at the start of infusion of the lower dose (Figure 4). The responses to human α-CGRP at 15 nmol kg^{-1} h^{-1} were maintained throughout the day, but by the beginning of the next day there was a clear, but incomplete

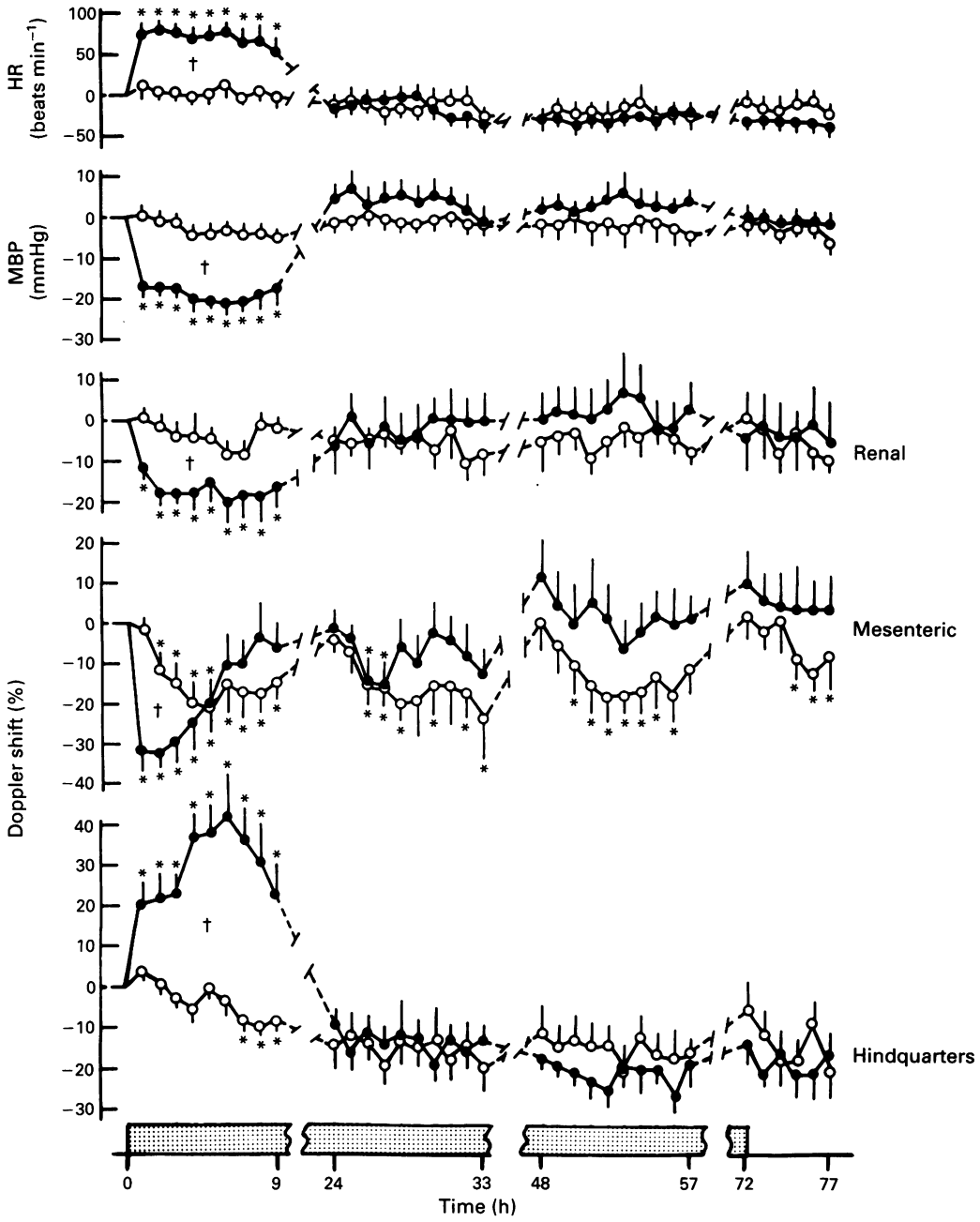

Figure 1 Cardiovascular changes during and after a 3 day infusion of human α-calcitonin gene-related peptide (α-CGRP, ●, 1.5 nmol kg^{-1} h^{-1}, $n = 8$) or vehicle (○, 0.3 ml h^{-1}, $n = 8$). Variables were recorded at 1 h intervals (averaged over 10 min) for 9 h each day (i.e. start 07 h 00 min, end 16 h 00 min). Values are mean and bars are s.e.mean. * $P < 0.05$ versus original baseline: † $P < 0.05$ for human α-CGRP versus vehicle (assessed from corresponding areas under or over curves). Infusion period indicated by shaded area on time axis. HR = heart rate; MBP = mean blood pressure.

desensitization to the effects of the peptide (Figure 4). Thus, human α-CGRP was still exerting measurable influences at the end of the infusion period and these were reversed when the infusion was stopped (Figure 4).

Discussion

In the present work marked desensitization has been shown to the haemodynamic effects of human α-CGRP when the peptide is infused over a period of 3 days in conscious, normotensive rats. There was some inter-individual variability but even the animals that showed the greatest sensitivity and most sustained responses to human α-CGRP administration demonstrated substantial tachyphylaxis by the end of the 3-day infusion period. These diminished responses were not due to the infusion system used delivering diminishing amounts of human α-CGRP over time since radioimmunoassay of the human α-CGRP concentration of the infusate showed no consistent change (unpublished observations).

The present results contrast with those of Marshall *et al.* (1987) who found that human α-CGRP administered at a nominal rate of 15 nmol kg^{-1} h^{-1} for 3 days caused sustained hypotension in female, spontaneously hypertensive rats. The differences between the observations and ours do not seem to be due to the dose of peptide administered, since, in the present study, when the infusion rate for human α-CGRP was increased to 15 nmol kg^{-1} h^{-1} (i.e. the rate used by Marshall *et al.*, 1987), tachyphylaxis was still observed. Furthermore, Marshall *et al.* (1987) administered human α-CGRP over 3 days from an implanted osmotic minipump and it is likely that the amount of human α-CGRP delivered by such a system is substantially less than calculated and not constant because of peptide degradation (unpublished observations).

It is feasible that the results obtained by Marshall *et al.*

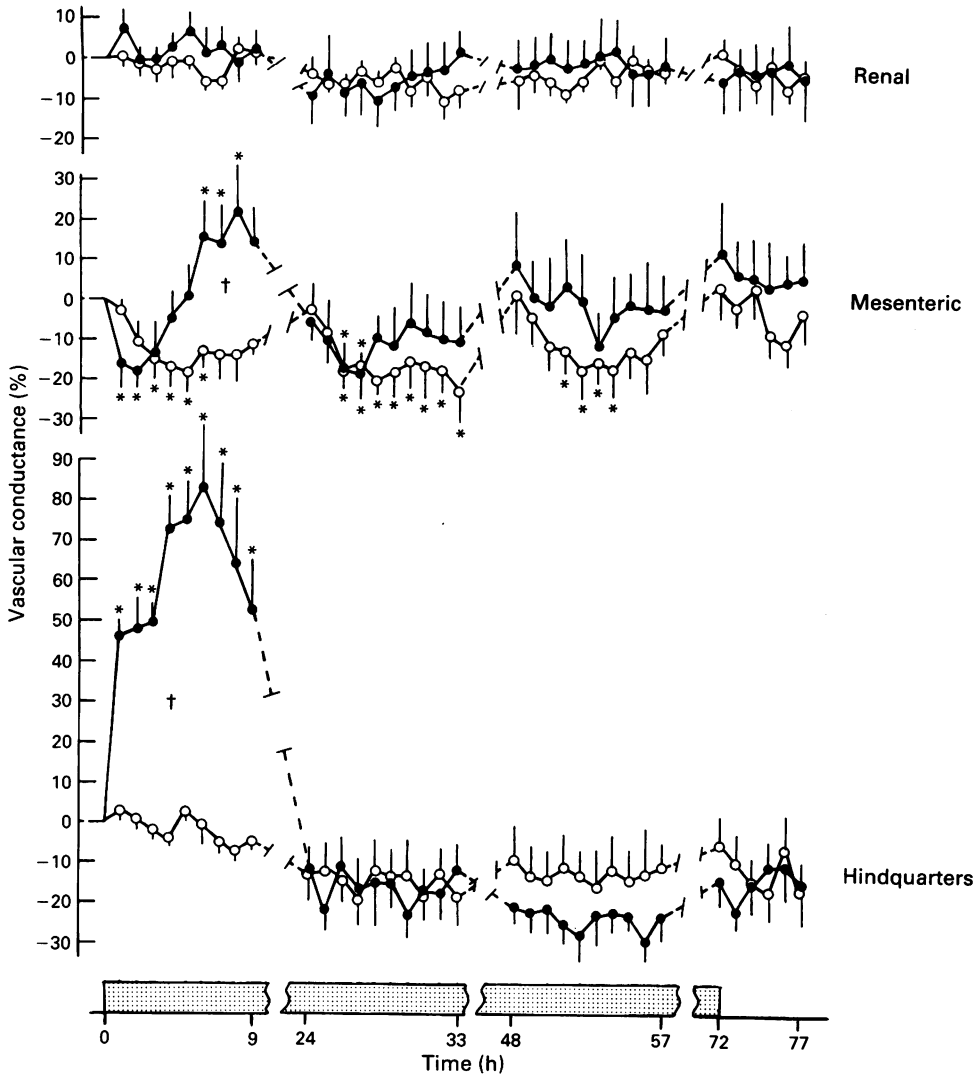

Figure 2 Changes in vascular conductance during infusion of human α-calcitonin gene-related peptide (α-CGRP, (●) or vehicle (○) calculated from the data in Figure 1. Values are mean and bars are s.e.mean. * $P < 0.05$ versus original baseline; † $P < 0.05$ for human α-CGRP versus vehicle (assessed from corresponding areas under or over curves). Infusion period indicated by shaded area on the time axis.

(1987) were due to the use of hypertensive animals, since it is well known that such animals are more sensitive to acute hypotensive stimuli (Takata & Hutchinson, 1983), possibly due to impaired baroreflexes (Thoren *et al.*, 1979; Verberne *et al.*, 1988; but see also Unger *et al.*, 1987). In addition, impaired baroreflexes might have contributed to a chronic failure of hypertensive rats to counteract the hypotensive effects of human α-CGRP. Thus, the apparent desensitization to human α-CGRP observed in normal rats in the present study could have been due to baroreflex-mediated recruitment of cardiovascular compensatory mechanisms in response to the hypotension caused by the peptide. However, this is not likely to be the full explanation, since tachyphylaxis was apparent also in the heart rate response to human α-CGRP, a component of which is reflex in origin (Sirén & Feuerstein, 1988). Furthermore, following cessation of human α-CGRP infusion there was no evidence of rebound effects that would have been expected if there was on-going stimulation of compensatory mechanisms such as the renin-angiotensin system (Bennett *et al.*, 1989). Hence, it appears more likely that the tachyphylaxis to the effects of prolonged infusions of human α-CGRP was due to receptor desensitization (i.e. loss of receptors or inhibition of the transducer mechanism, or both).

Han *et al.* (1990) have shown that exposure of the isolated mesenteric vasculature of the rat to rat CGRP at a concentration of 10^{-6} M for 2.5 h causes significant inhibition of vasodilator responses to rat CGRP at 10^{-8} M. However, they did not determine if higher doses of rat CGRP could overcome the desensitization. In the present work we found that increasing the infusion rate of human α-CGRP could re-establish the effects of the peptide. However, the response achieved was no greater than that seen with the initial, 10 fold lower dose of human α-CGRP and was considerably less than that achieved when naive rats were exposed to such a high dose of CGRP (Gardiner *et al.*, 1989a,b). Thus, the desensitization to the effects of human α-CGRP seen in the present work could have represented a shift in the dose-effect curve of the sort seen with competitive antagonism. In this context it is notable that human α-CGRP [8–37] can act as an antagonist of the *in vivo* haemodynamic actions of exogenous human α-CGRP (Gardiner *et al.*, 1990c). However, human α-CGRP [8–37] is not a likely candidate as an antagonist generated *in vivo* (Grèves *et al.*, 1989). Furthermore, since the N-terminal fragments of human α-CGRP show little affinity for CGRP binding sites (Dennis *et al.*, 1989), then human α-CGRP [28–37] might be considered as a candidate (Maton *et al.*, 1990; Chakder & Rattan, 1990), but we have found this peptide does not act as an antagonist of the *in vivo* haemodynamic effects of human α-CGRP (unpublished observations). Another possibility is that prolonged infusions of human α-CGRP induce peptidase activity that inactivates the peptide. Additional experiments are required to determine

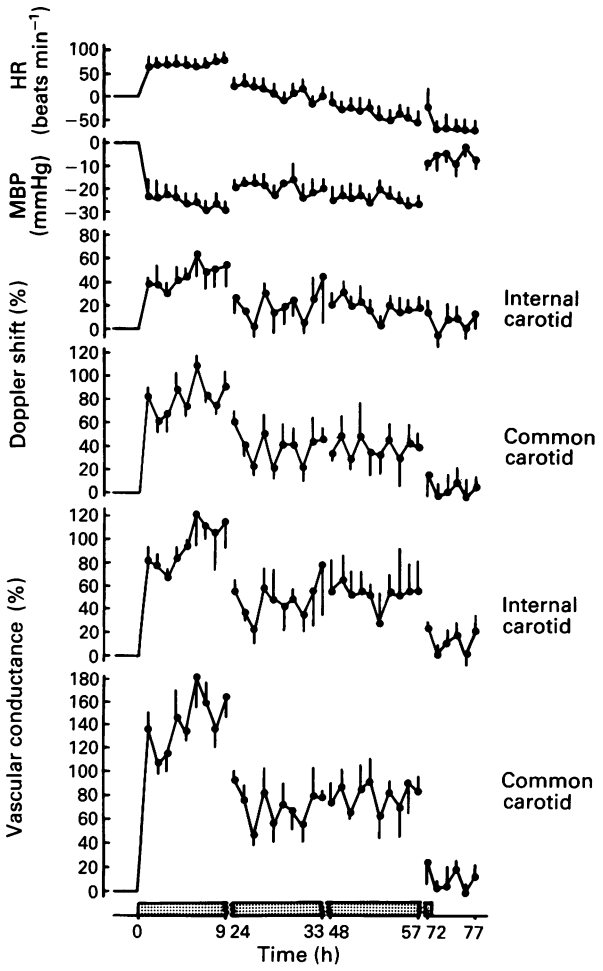

Figure 3 Cardiovascular changes during and after a 3-day infusion of human α-calcitonin gene-related peptide (α-CGRP, 1.5 nmol kg^{-1} h^{-1}) in conscious, Long Evans rats ($n = 3$). These animals showed a relatively well-maintained response to the peptide (compare Figure 4) but due to the small group size data were not analysed statistically. Infusion period indicated by shaded area on time axis. Values are mean and bars are s.e.mean. HR = heart rate; MBP = mean blood pressure.

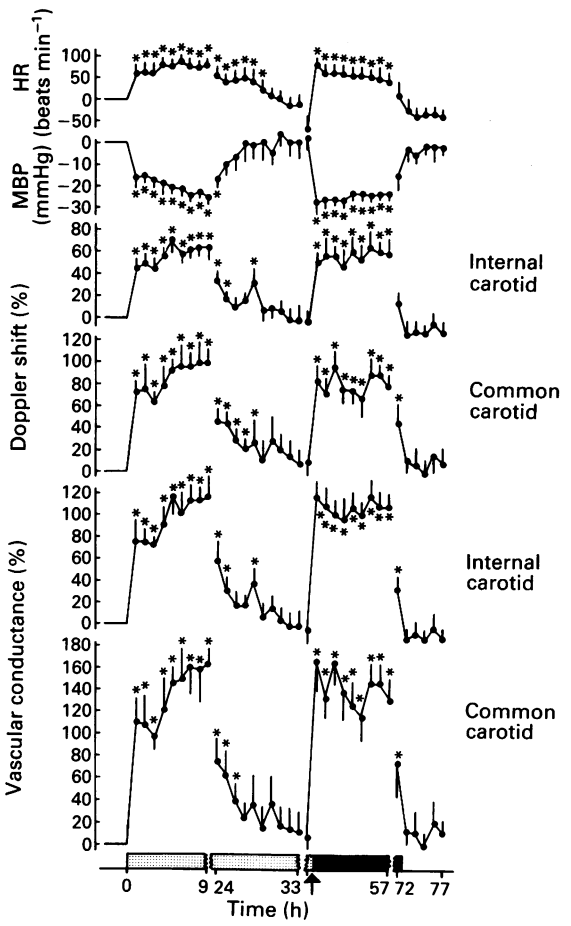

Figure 4 Cardiovascular changes during and after infusion of human α-calcitonin gene-related peptide (α-CGRP) in conscious, Long Evans rats ($n = 6$). Over the first 2 days human α-CGRP was infused at 1.5 nmol kg^{-1} h^{-1} and for the third day it was infused at 15 nmol kg^{-1} h^{-1} (onset at arrow). Values are mean and bars are s.e.mean; * $P < 0.05$ versus original baseline; infusion period indicated by shaded area on time axis. HR = heart rate; MBP = mean blood pressure.

if this phenomenon and/or receptor changes (see above) occur with prolonged administration of human α-CGRP.

At the outset of this study we thought it was possible that desensitization to the tachycardic, hypotensive and renal, mesenteric and hindquarters haemodynamic effects of human α-CGRP might not reflect what was happening in the carotid vascular bed, since the latter is the most sensitive to the vasodilator effects of exogenous human α-CGRP (Gardiner *et al.*, 1989a,b; 1990a). However, the present results have demonstrated that there can also be marked loss of the carotid vasodilator effects during prolonged infusions of human α-CGRP. If these findings can be extrapolated to the clinical setting it is likely that in order to achieve sustained effects of human α-CGRP on the carotid vasculature in patients, it would be necessary to infuse individually-adjusted and increasing amounts of the peptide.

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