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Differential effects of (±)-dobutamine and human α-CGRP on cardiac and on regional haemodynamics in conscious Long Evans rats

[1]S.M. Gardiner, A.M. Compton, P.A. Kemp, T. Bennett, *B. Hughes & *R. Foulkes

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH and *Pharmacology Department, Celltech Ltd, Slough, SL1 4EN

1 Comparisons were made of the full haemodynamic profiles of the known cardiostimulant, (±)-dobutamine, and the putative inotropic peptide, human α-calcitonin gene-related peptide (human α-CGRP), in conscious, chronically-instrumented Long Evans rats. Both substances were administered continuously i.v. for 60 min at two doses ((±)-dobutamine, 2 and 10 μmol kg^{-1} h^{-1}; human α-CGRP, 0.15 and 1.5 nmol kg^{-1} h^{-1}).

2 In spite of their similar (small) effects on mean arterial blood pressure, the low doses of (±)-dobutamine and human α-CGRP influenced cardiac haemodynamics differently. Thus, (±)-dobutamine caused an increase in cardiac index (due to a tachycardia), accompanied by rises in peak aortic flow, maximum rate of rise of aortic flow (dF/dt_{max}) and total peripheral conductance. However, the latter waned during the infusion, and after the infusion there was a significant systemic vasoconstriction and reductions in peak aortic flow, dF/dt_{max} and stroke index. Such 'off' effects following dobutamine infusion have not been described previously. The infusion of the lower dose of human α-CGRP caused only a transient fall in central venous pressure.

3 The rise in total peripheral conductance during infusion of the lower dose of (±)-dobutamine was associated with increases in hindquarters and common and internal carotid vascular conductances. The fall in total peripheral conductance after infusion was associated with renal vasoconstriction. Although there was no significant change in total peripheral conductance during the infusion of the lower dose of human α-CGRP there were hindquarters and carotid vasodilatations together with mesenteric vasoconstriction.

4 Infusion of the higher dose of (±)-dobutamine had greater effects than the lower dose on all cardiac haemodynamic variables and additionally, increased stroke index. However, the negative cardiac haemodynamic effects following the offset of infusion were also enhanced in association with marked renal and mesenteric vasoconstrictions. While infusion of the higher dose of human α-CGRP increased cardiac index, peak aortic flow, dF/dt_{max} and total peripheral conductance, stroke index fell together with central venous pressure.

5 (±)-Dobutamine caused greater cardiostimulation and increases in hindquarters blood flow than did human α-CGRP. However, the latter at the higher dose caused substantially greater common and internal carotid hyperaemia than did (±)-dobutamine, possibly indicating a selective and additional effect of human α-CGRP on cranial blood flow. Furthermore, there were no adverse cardiovascular effects following infusion of human α-CGRP.

Keywords: (±)-Dobutamine; human α-CGRP; regional haemodynamics; cardiac function

Introduction

In vivo studies in conscious dogs (Wang *et al.*, 1989) and man (Gennari *et al.*, 1989; 1990) indicate that human α-calcitonin gene-related peptide (human α-CGRP) can increase cardiac output. However, results of experiments in rats are less clear-cut, possibly because of limitations with the methodology used. Thus, there have been two studies (DiPette *et al.*, 1987; Ando *et al.*, 1990) using the radiolabelled microsphere technique in acutely prepared rats (3 h post surgery), in which single measurements of cardiac output were made at a fixed time point after bolus injections of CGRP. Using this approach DiPette *et al.* (1987) observed a small increase, while Ando *et al.* (1990) found no change, in cardiac output after administration of rat and human α-CGRP, respectively. In contrast, Sirén & Feuerstein (1988) measured cardiac output intermittently with the thermodilution technique in conscious rats and reported an increase of 95 ± 16 ml min^{-1} kg^{-1} in cardiac index in response to a bolus dose of 1.0 nmol kg^{-1} rat CGRP, but found doses of 0.1 and 10 nmol kg^{-1} were without significant effect. However, Lappe *et al.* (1987) recorded cardiac output continuously in conscious rats (chronically instrumented with electromagnetic flow probes on their ascending aortae), and observed that incremental bolus doses of rat CGRP (over the range 66–1320 pmol kg^{-1}) caused dose-related (maximum 28 ± 2%) increases in cardiac output.

Hence, to date, results are conflicting and there have been no studies on the profile of cardiac haemodynamic effects of sustained infusions of rat or human α-CGRP in chronically instrumented rats. Furthermore, none of the experiments performed so far have compared either rat or human α-CGRP to an established drug, such as (±)-dobutamine, which is used clinically for its ability to improve cardiac function (Ruffolo, 1987; Marcus *et al.*, 1987). The primary aim of the present work was, therefore, to measure the cardiac haemodynamic effects of intravenous infusions of two doses of human α-CGRP and to compare them with the effects of infusions of (±)-dobutamine in the same animals. Our initial intention was to compare doses of human α-CGRP and (±)-dobutamine with equal cardiotonic effects. However, from preliminary experiments it became clear that human α-CGRP only had slight effects on cardiac index over a wide dose range. Furthermore, the profiles of the haemodynamic effects of human α-CGRP and (±)-dobutamine were so different (see Results) that it was not possible to find doses that were

[1] Author for correspondence.

matched for their influences on all variables. Therefore, the lower doses of the substances were chosen to have similar, initial, but small, effects on mean arterial blood pressure, since this variable impinges on cardiac function (O'Rourke, 1982). The higher doses were chosen, likewise, to have similar initial hypotensive effects; in addition, the higher dose of human α-CGRP and the lower dose of (±)-dobutamine happened to be matched for their effects on total peripheral conductance.

Although (±)-dobutamine is used clinically and is administered by intravenous infusion (Ruffolo, 1987), when we came to devise the protocols for the present work we discovered that surprisingly little is known about the full haemodynamic profile of this drug during prolonged constant infusion either in man (Ruffolo, 1987; Leier *et al.*, 1977; 1978; Sonnenblick *et al.*, 1979) or in experimental animals (Liang & Hood, 1979). Thus, while a considerable literature on (±)-dobutamine exists, in large part it relates to bolus injections, brief infusions or incremental infusions of the drug (e.g. Vatner *et al.*, 1974; Schoemaker, 1989; Dorszewski *et al.*, 1990) often in anaesthetized or pithed animals (e.g. Robie & Goldberg, 1975; Ruffolo & Yaden, 1983; Ruffolo & Morgan, 1984; Maccarone *et al.*, 1984; Ruffolo & Messick, 1985a,b; Biro *et al.*, 1988). Furthermore, only incomplete data are available regarding the cardiovascular changes following the end of (±)-dobutamine infusion (Leier *et al.*, 1977; 1978). However, this period is of interest because dobutamine increases plasma renin activity (Jaski & Peters, 1988) and hence activation of the renin-angiotensin system could contribute to its post-infusion haemodynamic profile. Therefore, another objective of the present work was to delineate the extent to which changes in cardiac haemodynamics elicited by (±)-dobutamine were associated with regional vascular changes during and after infusion. To this end we monitored, continuously, renal, mesenteric, hindquarters, common carotid and internal carotid haemodynamics before, during and after infusion of (±)-dobutamine at the two dose levels.

In previous studies we have described some regional haemodynamic changes during and after 60 min infusions of human α-CGRP in Wistar rats (Gardiner *et al.*, 1989). However, in the present study in Long Evans rats it was important to monitor the full regional haemodynamic profile of human α-CGRP in the same animals that received (±)-dobutamine and to do this in a randomized, cross-over fashion, in order that the comparison of the cardiac haemodynamic effects of (±)-dobutamine and human α-CGRP was against a defined background of regional haemodynamic changes for the two substances.

Methods

Male, Long Evans rats (350–450 g) were used in all studies. Surgery was carried out under anaesthesia (sodium methohexitone, 60 mg kg^{-1} i.p., supplemented as required).

Animals with ascending thoracic aortic flow probes

Through a right intercostal (third and fourth rib space) incision an electromagnetic flow probe (Skalar MDL 1401, Delft, Netherlands) was placed around the ascending thoracic aorta (Smith & Hutchins, 1979; Smits *et al.*, 1982; Gardiner *et al.*, 1990c) of the rat.

Animals with renal, mesenteric and hindquarters flow probes

Through a midline, abdominal incision, pulsed Doppler probes (Haywood *et al.*, 1981) were sutured around the left renal and superior mesenteric arteries and the distal abdominal aorta, below the level of the ileocaecal artery, to monitor flow to the hindquarters of the rat (Gardiner *et al.*, 1989; 1990c).

Animals with common carotid and internal carotid flow probes

Through a midline, ventral, cervical incision both common carotid arteries were exposed and gently separated from the vagosympathetic nerve trunks. On the right side a probe was sutured around the common carotid artery, but on the left side the external carotid artery was ligated before placement of the probe on the common carotid artery. This arrangement permitted assessment of changes in total common carotid flow (i.e. external plus internal carotid flow) on the right side, together with changes in internal carotid flow alone on the left side (Gardiner *et al.*, 1990b).

In all animals the probe connections were exteriorised at the back of the neck. Following surgery, rats were given ampicillin (7 mg kg^{-1}, i.m.) and returned to their home cages for at least 7 days, with free access to food and water. Between 7 and 14 days after probe implantation animals were briefly re-anaesthetized (sodium methohexitone, 40 mg kg^{-1}, i.p.) and had intravascular catheters implanted (1 catheter in the abdominal aorta via the ventral caudal artery and 2 catheters in the right jugular vein). In animals with thoracic aortic probes, a third venous catheter was constructed of a short length (3 cm) of small bore polyethylene tubing (i.d. 0.28 mm; o.d. 0.61 mm) connected to a longer length (120 cm) of more rigid, wider bore, nylon tubing (i.d. 0.58 mm; o.d. 1.02 mm) and its tip was positioned close to the junction of the superior vena cava with the right atrium to permit recording of central venous pressure (Gardiner *et al.*, 1990c).

Catheters were tunnelled subcutaneously to emerge at the back of the neck. The catheters ran through a flexible spring connected to a harness worn by the rat and the linking cable to the flow probes was taped to the spring. The venous catheter that was to be used to monitor central venous pressure was connected to a slow infusion pump (SRI) through a fluid-filled swivel (Brown *et al.*, 1976). Isotonic saline (NaCl 157 mmol l^{-1}) was infused (0.3 ml h^{-1}) through this catheter to ensure it remained patent until recordings were started (Gardiner *et al.*, 1990c). Animals were allowed to recover in their home cages overnight before experiments were begun.

All protocols involved a 30 min baseline recording period followed by a 60 min infusion period and a 60 min post-infusion period. Animals receiving no infusion or vehicle infusion over this time showed no consistent cardiovascular changes. From preliminary experiments we found that human α-CGRP and (±)-dobutamine infused at 0.15 nmol kg^{-1} h^{-1} and 2 μmol kg^{-1} h^{-1}, respectively caused similar, slight, initial falls in mean systemic arterial blood pressure, whereas human α-CGRP and (±)-dobutamine at 1.5 nmol kg^{-1} h^{-1} and 10 μmol kg^{-1} h^{-1}, respectively caused similar, sustained reductions in mean systemic arterial blood pressure. Therefore, in the full experimental protocols animals were randomized to receive human α-CGRP or (±)-dobutamine on the first day and the other substance on the second day. The lower dose infusion was always given before the higher dose infusion, with the infusions being separated by at least 2.5 h.

In those experiments involving assessment of cardiac haemodynamics, the intra-arterial blood pressure, thoracic aortic flow and central venous pressure signals were fed into a Tandon 386 microcomputer interfaced with a custom-built haemodynamics microprocessor (University of Limburg, Department of Instrument Services). The latter provided mean cardiac output, peak aortic flow, maxium rate of rise of aortic flow (dF/dt_{max}), total peripheral conductance, stroke volume and mean central venous pressure (Gardiner *et al.*, 1990c). Subsequent analysis provided group mean values for the changes in these variables (relative to baseline). Mean cardiac output and stroke volume were factored by body weight to give cardiac index and stroke index.

In those experiments in which regional haemodynamic changes were assessed, continuous recordings of mean and phasic arterial blood pressure and Doppler shift signals (VFI system, Crystal Biotech, Holliston, U.S.A.) were made,

together with instantaneous heart rate (all on a Gould ES 1000 system). At selected time points, changes in regional blood flows and vascular conductances were calculated (Gardiner et al., 1990c).

Drugs

Infusions were given in isotonic saline (containing 1% bovine serum albumin for human α-CGRP) at a rate of 0.3 ml h^{-1}. Human α-CGRP was obtained from Celltech Ltd (Lot No ZD 949) while (±)-dobutamine hydrochloride (Eli Lilly Ltd; Dobutrex) was obtained from University Hospital Pharmacy (Nottingham). Solutions of human α-CGRP and (±)-dobutamine were prepared immediately before use.

Data analysis

Within group changes were analysed by Friedman's test (Theodorsson-Norheim, 1987); between group differences (i.e human α-CGRP versus (±)-dobutamine) were assessed by Wilcoxon's ranks sums test applied to areas under or over curves (Gardiner et al., 1990c). A P value <0.05 was taken as significant.

Results

Low dose infusions

Cardiac haemodynamics (±)-Dobutamine (2 μmol kg^{-1} h^{-1}) caused a transient, small fall in mean arterial blood pressure, but there were sustained increases in heart rate, cardiac index, peak aortic flow and dF/dt_{max}. Although total peripheral conductance was elevated throughout the infusion period, by 60 min the magnitude of the vasodilatation was only about half that at 5 min (Figure 1). Stroke index and central venous pressure showed no significant changes (Figure 1). Following infusion there were transient undershoots in peak aortic flow, dF/dt_{max}, total peripheral conductance and stroke index (Figure 1).

Human α-CGRP (0.15 nmol kg^{-1} h^{-1}) caused slight hypotension but produced no significant tachycardia (Figure 1). Furthermore, there were no significant changes in cardiac index, peak aortic flow, dF/dt_{max}, total peripheral conductance or stroke index (Figure 1), although central venous pressure showed an early, transient fall (Figure 1).

During infusion of (±)-dobutamine the overall changes in heart rate, cardiac index, peak aortic flow, dF/dt_{max} and total peripheral conductance (over the first 30 min of infusion) were greater ($P < 0.05$ for areas under curves) than seen during infusion of human α-CGRP. There was no significant difference between the overall changes in mean arterial blood pressure. Table 1 summarizes the maximum changes in cardiovascular variables during infusion of human α-CGRP and (±)-dobutamine.

Table 1 Mean maximum changes (% of baseline) in cardiovascular variables during infusions of human α-calcitonin gene-related peptide (α-CGRP) or (±)-dobutamine in the same conscious, Long Evans rats

	(±)-Dobutamine (μmol kg^{-1} h^{-1})		Human α-CGRP (nmol kg^{-1} h^{-1})	
	2	10	0.15	1.5
Heart rate	19	22	3	18
Mean arterial blood pressure	−6	−18	−9	−20
Cardiac index	23	43	3	9
Peak aortic flow	20	31	3	9
dF/dt_{max}	45	53	5	16
Total peripheral conductance	31	58	11	35
Stroke index	5	19	−3	−10
Central venous pressure	11	18	−23	−27

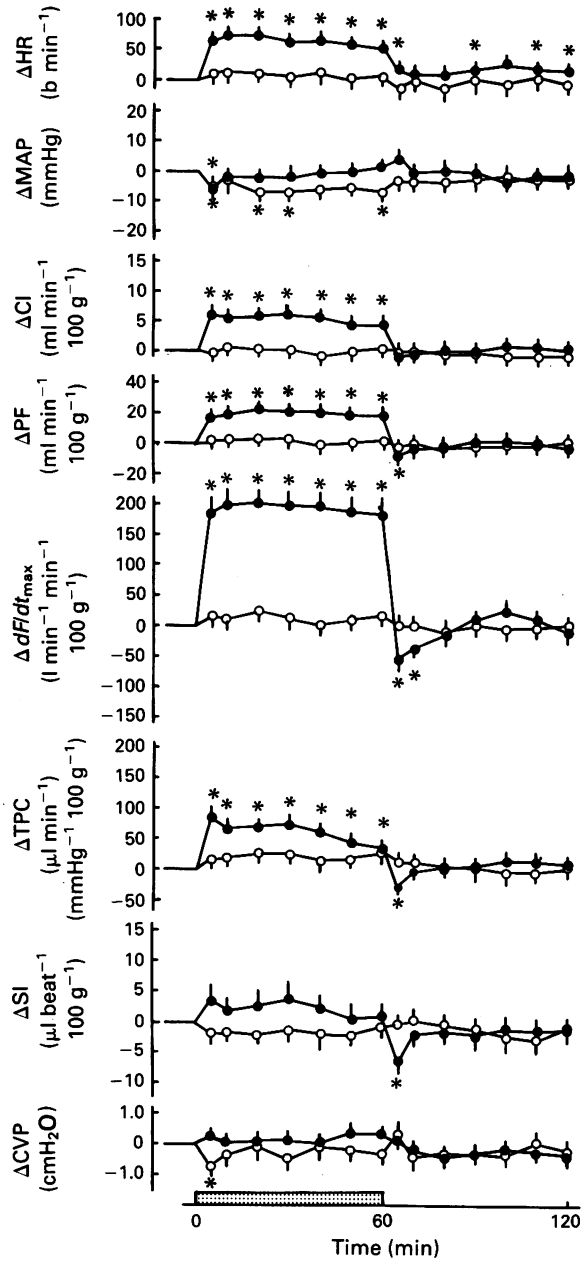

Figure 1 Cardiovascular changes (Δ) during and after a 60 min infusion of human α-calcitonin gene-related peptide (α-CGRP, ○) (0.15 nmol kg^{-1} h^{-1}) or (±)-dobutamine (●) (2 μmol kg^{-1} h^{-1}) in conscious, Long Evans rats ($n = 9$), chronically instrumented with intravascular catheters and ascending thoracic aortic electromagnetic flow probes. Values are mean and the bars show s.e.mean; * $P < 0.05$ versus baseline (Friedman's test). Statistics for comparisons between human α-CGRP and (±)-dobutamine are given in the text. HR = heart rate; MAP = mean arterial blood pressure; CI = cardiac index; PF = peak thoracic aortic flow; dF/dt_{max} = maximum rate of rise of thoracic aortic flow; TPC = total peripheral conductance; SI = stroke index; CVP = central venous pressure.

Regional haemodynamics

Renal, mesenteric and hindquarters haemodynamics. In the animals with renal, mesenteric and hindquarters probes the changes in mean arterial blood pressure and heart rate were not different from those above, during or after (±)-dobutamine infusion (2 μmol kg^{-1} h^{-1}). However, in this group the small overshoot in mean arterial blood pressure after the infusion was stopped did reach significance (Figure 2). There were no significant changes in renal or mesenteric blood flows

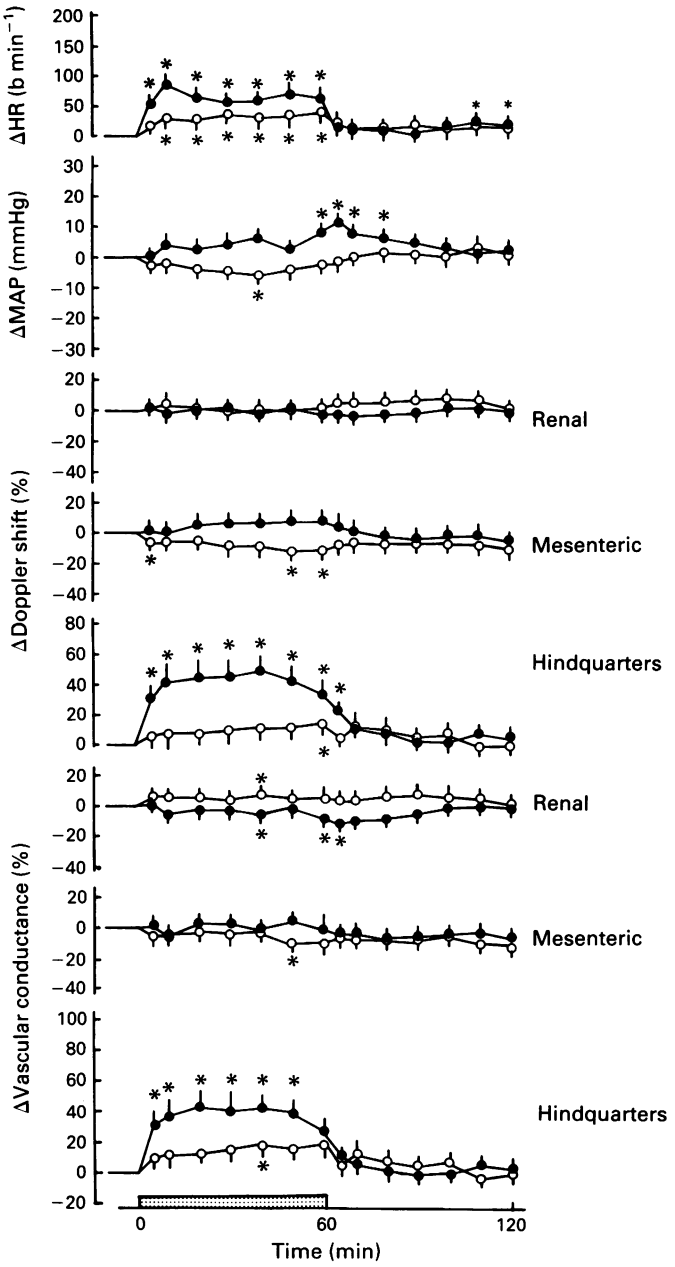

Figure 2 Cardiovascular changes during and after a 60 min infusion of human α-calcitonin gene-related peptide (α-CGRP, ○) (0.15 nmol kg^{-1} h^{-1}) or (±)-dobutamine (●) (2 μmol kg^{-1} h^{-1}) in conscious, Long Evans rats ($n = 8$), chronically instrumented with intravascular catheters and renal, mesenteric and hindquarters pulsed Doppler flow probes. Values are mean and bars are s.e.mean; * $P < 0.05$ versus baseline (Friedman's test). Statistics for comparisons between human α-CGRP and (±)-dobutamine are given in the text. Abbreviations as in Figure 1.

during infusion, but there was a marked hindquarters hyperaemia (Figure 2). There was a slight renal vasoconstriction during, and a transient renal vasoconstriction after, (±)-dobutamine infusion. The marked hindquarters vasodilator effect of (±)-dobutamine faded towards the end of the infusion period (Figure 2).

Infusion of human α-CGRP (0.15 nmol kg^{-1} h^{-1}) caused slight tachycardia and hypotension (Figure 2). There was no change in renal flow, but a modest decrease in mesenteric flow and an increase in hindquarters flow (Figure 2). These changes were associated with slight renal and hindquarters vasodilatations and mesenteric vasoconstriction (Figure 2). Following infusion all variables were not different from baseline.

The overall changes in heart rate, mesenteric and hindquarters flows, and hindquarters vascular conductance during infusion of (±)-dobutamine were significantly different from those during infusion of human α-CGRP ($P < 0.05$ for areas under or over curves). There was no significant difference between the overall changes in mean arterial blood pressure or renal haemodynamics or mesenteric vascular conductance. Table 2 summarizes the maximum changes in cardiovascular variables during infusion of human α-CGRP and (±)-dobutamine.

Table 2 Mean maximum changes (% of baseline) in cardiovascular variables during infusions of human α-calcitonin gene-related peptide (α-CGRP) or (±)-dobutamine in the same conscious, Long Evans rats

	(±)-Dobutamine (μmol kg^{-1} h^{-1})		*Human α-CGRP* (nmol kg^{-1} h^{-1})	
	2	10	0.15	1.5
Heart rate	19	41	12	31
Mean arterial blood pressure	5	−12	−6	−22
Renal flow	−2	−18	4	−12
Mesenteric flow	7	−8	−14	−26
Hindquarters flow	49	66	15	35
Renal conductance	−9	−15	7	13
Mesenteric conductance	−5	10	−11	−7
Hindquarters conductance	42	81	18	68

Common and internal carotid haemodynamics. In animals with probes monitoring common and internal carotid blood flows, infusion of (±)-dobutamine (2 μmol kg^{-1} h^{-1}) caused tachycardia and transient hypotension (Figure 3) not different from those in the groups described above. There was a slight overshoot in mean blood pressure when the infusion was stopped (Figure 3). In addition, there were marked increases in common and internal carotid blood flows that persisted for 30 min after cessation of (±)-dobutamine infusion (Figure 3).

Infusion of human α-CGRP (0.15 nmol kg^{-1} h^{-1}) caused modest tachycardia and slight hypotension (Figure 3). There was no significant increase in internal carotid blood flow, but common carotid flow increased slightly. Internal and common carotid vascular conductances increased during infusion, but returned to baseline when the infusion was stopped (Figure 3).

The overall changes in heart rate and internal and common carotid blood flows during infusion of (±)-dobutamine were greater than those seen during infusion of human α-CGRP ($P < 0.05$ for areas under curves), but the overall changes in mean arterial blood pressure and in the internal and common carotid vascular conductances were not different during the two infusions. Table 3 summarizes the maximum changes in cardiovascular variables during infusion of human α-CGRP and (±)-dobutamine.

Table 3 Mean maximum changes (% of baseline) in cardiovascular variables during infusions of human α-calcitonin gene-related peptide (α-CGRP) or (±)-dobutamine in the same conscious, Long Evans rats

	(±)-Dobutamine (μmol kg^{-1} h^{-1})		*Human α-CGRP* (nmol kg^{-1} h^{-1})	
	2	10	0.15	1.5
Heart rate	35	45	7	24
Mean arterial blood pressure	5	−23	−7	−22
Internal carotid flow	40	33	15	47
Common carotid flow	51	35	18	86
Internal carotid conductance	37	46	20	89
Common carotid conductance	48	48	28	139

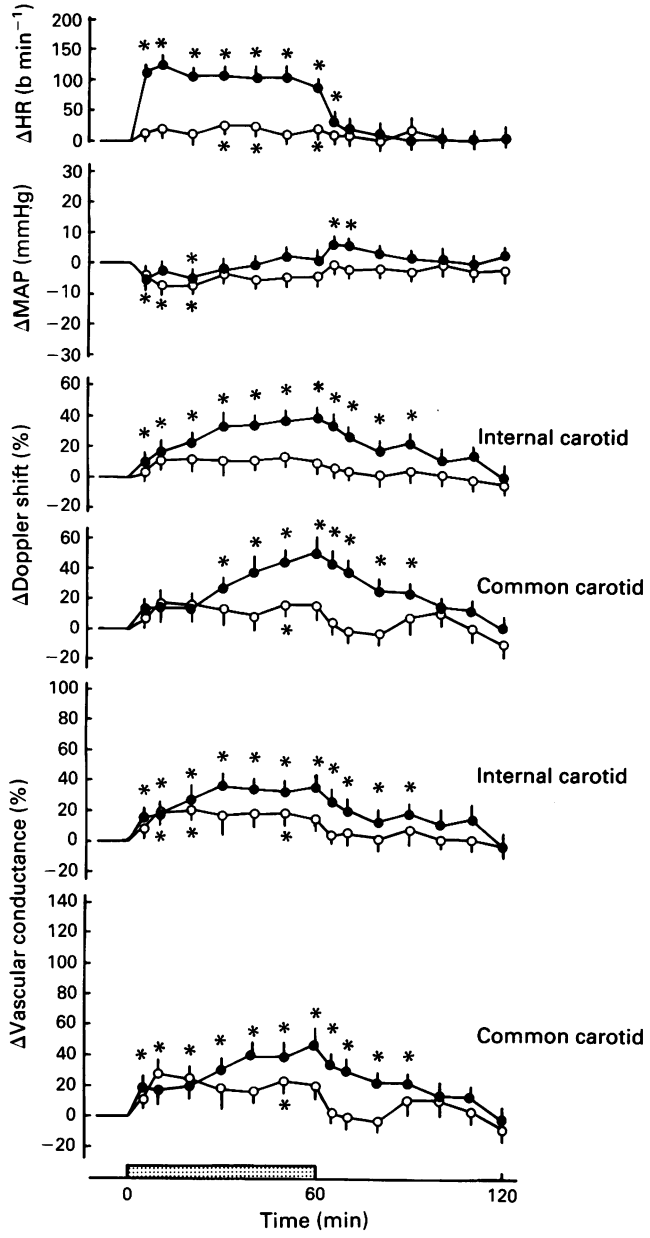

Figure 3 Cardiovascular changes during and after a 60 min infusion of human α-calcitonin gene-related peptide (α-CGRP, ○) (0.15 nmol kg^{-1} h^{-1}) or (±)-dobutamine (●) (2 μmol kg^{-1} h^{-1}) in conscious, Long Evans rats ($n = 8$), chronically instrumented with intravascular catheters and internal and common carotid pulsed Doppler flow probes. Values are mean and bars are s.e.mean; * $P < 0.05$ versus baseline (Friedman's test). Statistics for comparisons between human α-CGRP and (±)-dobutamine are given in the text. Abbreviations as in Figure 1.

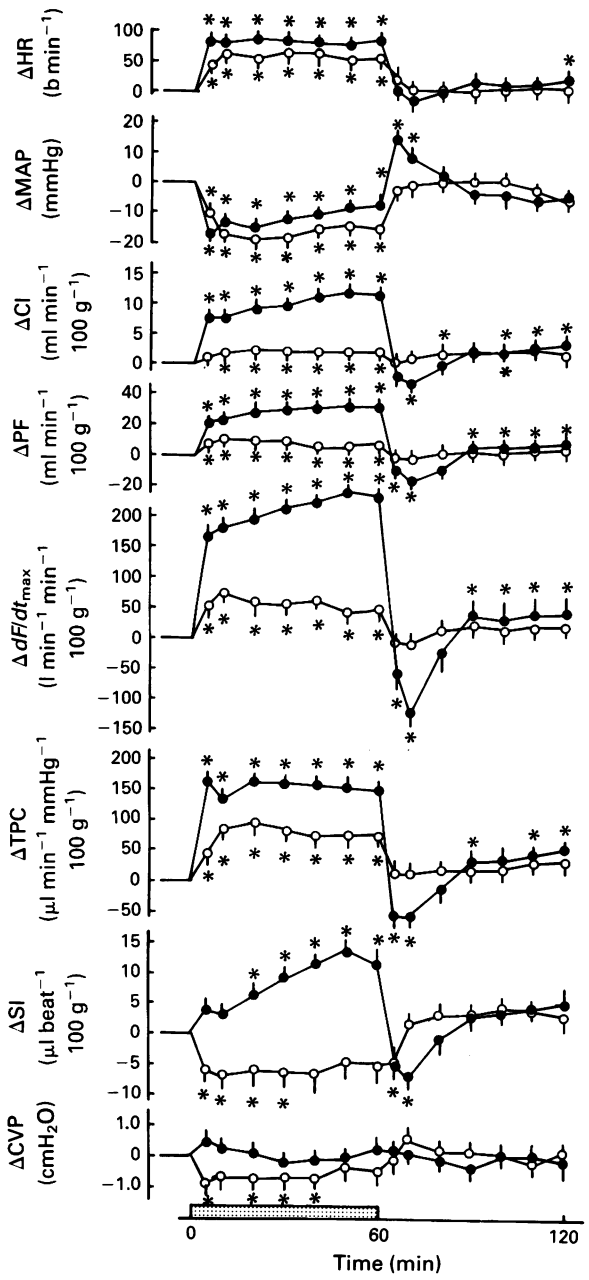

Figure 4 Cardiovascular changes during and after a 60 min infusion of human α-calcitonin gene-related peptide (α-CGRP, ○) (1.5 nmol kg^{-1} h^{-1}) or (±)-dobutamine (●) (10 μmol kg^{-1} h^{-1}) in conscious, Long Evans rats ($n = 9$), chronically instrumented with intravascular catheters and ascending thoracic aortic electromagnetic flow probes. Values are mean and bars are s.e.mean; * $P < 0.05$ versus baseline (Friedman's test). Statistics for comparisons between human α-CGRP and (±)-dobutamine are given in the text. Abbreviations as in Figure 1.

High dose infusions

Cardiac haemodynamics During infusion of (±)-dobutamine (10 μmol kg^{-1} h^{-1}) hypotension was accompanied by sustained tachycardia and a maintained increase in total peripheral conductance. However, there were progressive increases in cardiac index, peak aortic flow, dF/dt_{max}, and stroke index; there was no significant change in central venous pressure (Figure 4). After the end of the infusion there was a transient overshoot in mean arterial blood pressure accompanied by reductions in cardiac index, peak aortic flow, dF/dt_{max} and stroke index; these events coincided with a fall in total peripheral conductance (Figure 4). Thereafter, total peripheral conductance rose above baseline and blood pressure fell; at this time there were increases in cardiac index, peak aortic flow and dF/dt_{max} (Figure 4).

Infusion of human α-CGRP (1.5 nmol kg^{-1} h^{-1}) caused hypotension accompanied by sustained increases in heart rate, cardiac index, peak aortic flow, dF/dt_{max} and total peripheral conductance (Figure 4). However, there were reductions in stroke index and central venous pressure (Figure 6). Within 5 min of the end of human α-CGRP infusion all variables were back to baseline levels (Figure 4).

During and after infusion of (±)-dobutamine the overall changes in cardiac index, peak aortic flow, dF/dt_{max}, total peripheral conductance and stroke index were greater ($P < 0.05$ for areas under curves) than the changes seen during infusion of human α-CGRP (Figure 4). Although the overall changes in mean arterial blood pressure or in heart rate during infusion of human (±)-dobutamine and α-CGRP were not significantly

different, there was a significant difference in the overall changes in mean arterial blood pressure following the infusions (Figure 4). Table 1 summarizes the maxium changes in cardiovascular variables during infusion of human α-CGRP and (±)-dobutamine.

Regional haemodynamics

Renal, mesenteric and hindquarters haemodynamics In animals with renal, mesenteric and hindquarters probes the changes in heart rate and mean arterial blood pressure during and after (±)-dobutamine (10 μmol kg^{-1} h^{-1}) infusion (Figure 5) were as described above in animals with thoracic aortic flow pobes. During the infusion period, renal blood flow was reduced while there was a hindquarters hyperaemia, but no significant change in mesenteric blood flow (Figure 5). There

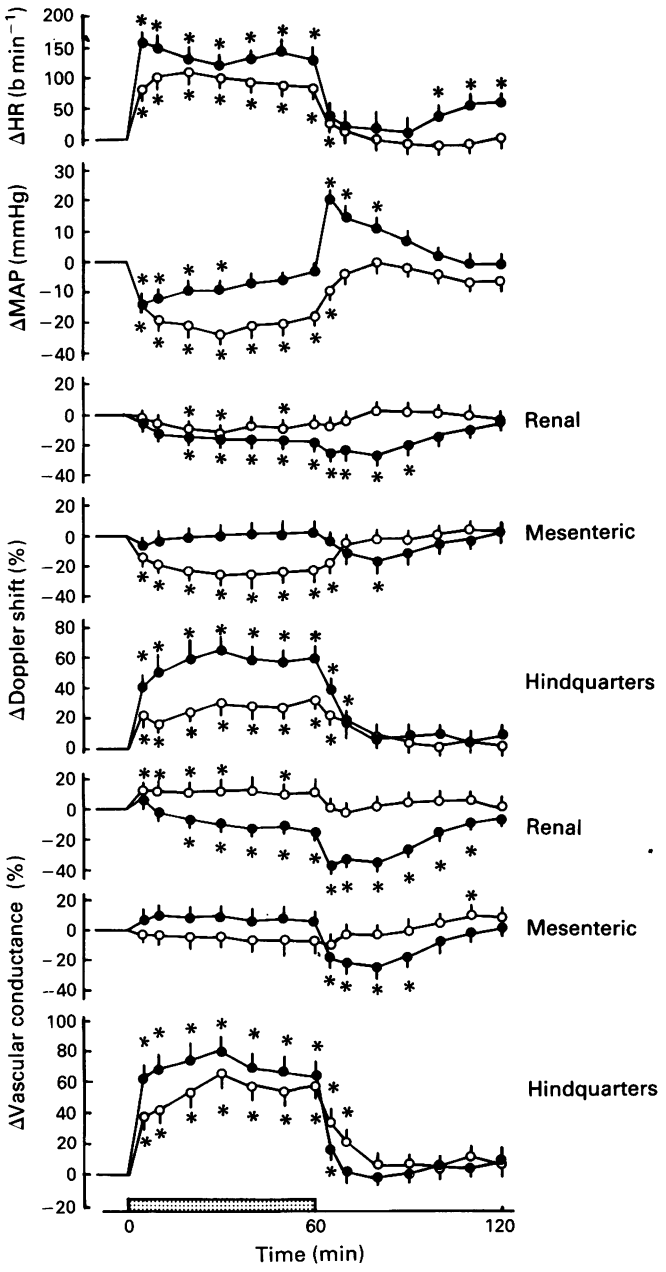

Figure 5 Cardiovascular changes during and after a 60 min infusion of human α-calcitonin gene-related peptide (α-CGRP, ○) (1.5 nmol kg^{-1} h^{-1}) or (±)-dobutamine (●) (10 μmol kg^{-1} h^{-1}) in conscious, Long Evans rats ($n = 8$), chronically instrumented with intravascular catheters and renal, mesenteric and hindquarters pulsed Doppler flow probes. Values are mean and bars are s.e.mean; * $P < 0.05$ versus baseline (Friedman's test). Statistics for comparisons between human α-CGRP and (±)-dobutamine are given in the text. Abbreviations as in Figure 1.

was renal vasoconstriction and hindquarters vasodilatation; when the infusion was stopped further renal vasoconstriction occurred, together with mesenteric vasoconstriction (Figure 5).

The changes in heart rate and mean arterial blood pressure during and after infusion of human α-CGRP (1.5 nmol kg^{-1} h^{-1}) were not different from those described above in animals with thoracic aortic flow probes. During infusion of human α-CGRP there were reductions in renal and mesenteric flows, together with an increase in hindquarters blood flow (Figure 5). There were renal and hindquarters vasodilatations but no change in mesenteric vascular conductance, although there was an upwards drift in the latter following infusion (Figure 5).

During infusion of (±)-dobutamine, the overall changes in heart rate, mean blood pressure, mesenteric and hindquarters flows and renal vascular conductance were different ($P < 0.05$ for areas under or over curves) from those seen during human α-CGRP infusion. Furthermore, the overall changes in mean blood pressure and renal and mesenteric vascular conductances were different ($P < 0.05$ for areas under or over curves) following infusion of human α-CGRP and (±)-dobutamine (Figure 5). Table 2 summarizes the maximum changes in cardiovascular variables during infusion of human α-CGRP and (±)-dobutamine.

Common and internal carotid haemodynamics In animals with common and internal carotid flow probes, the changes in heart rate and mean arterial blood pressure during infusion of (±)-dobutamine (10 μmol kg^{-1} h^{-1}) were similar to those in the corresponding groups above. There were increases in internal and common carotid blood flows and vascular conductances (Figure 6). Following infusion there was an overshoot in mean arterial blood pressure, but carotid haemodynamics remained above baseline levels at that time (Figure 6).

Infusion of human α-CGRP (1.5 nmol kg^{-1} h^{-1}) caused tachycardia, hypotension and increases in internal and common carotid blood flows and vascular conductances (Figure 6). The changes in carotid haemodynamics were still significant for about 10 min after the infusion was stopped (Figure 6).

During infusion of (±)-dobutamine the overall changes in carotid flows and vascular conductances were less, but the tachycardia was greater ($P < 0.05$ for areas under curves) than during infusion of human α-CGRP. Table 3 summarizes the maximum changes in cardiovascular variables during infusion of human α-CGRP and (±)-dobutamine.

Discussion

The major objective of the present work was to compare the full haemodynamic profiles of infusions of human α-CGRP and (±)-dobutamine at two dose levels. As explained in the Introduction, our initial intention of matching the doses of human α-CGRP and (±)-dobutamine for their cardiotonic influences was confounded by the relative lack of effect of human α-CGRP on cardiac index. Therefore, since cardiac performance is affected by systemic arterial blood pressure (O'Rourke, 1982), we matched the doses of human α-CGRP and (±)-dobutamine for their initial effects on this variable. As it turned out, the higher dose of human α-CGRP and the lower dose of (±)-dobutamine had similar effects on total peripheral conductance but otherwise generally had dissimilar actions.

The lower dose infusion of human α-CGRP did not cause any increase in cardiac function. Hence the slight increases in flows through hindquarters and carotid vascular beds under these conditions must have been met by the reduced flow through the mesenteric vascular bed (and, possibly, through other, unmeasured, regions). With the higher dose of human α-CGRP there were increases in cardiac index and indices of contractility (peak flow and dF/dt_{max}) and, thus, a positive ino-

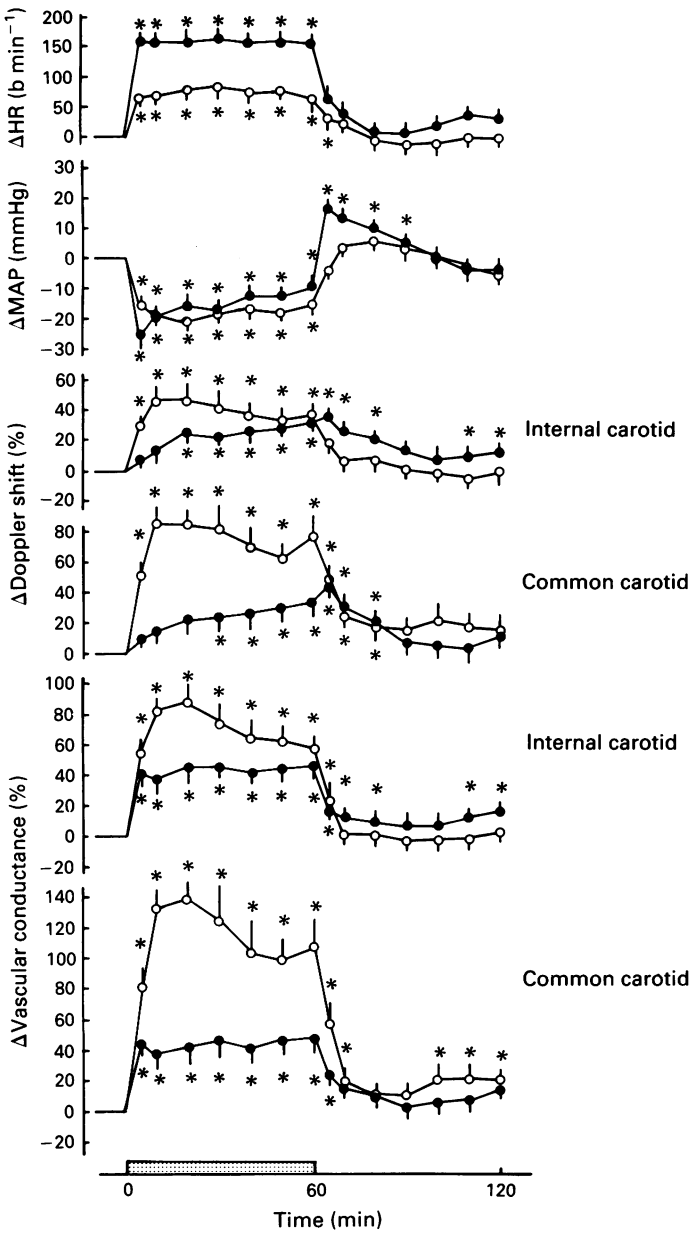

Figure 6 Cardiovascular changes during and after a 60 min infusion of human α-calcitonin gene-related peptide (α-CGRP, ○) (1.5 nmol kg^{-1} h^{-1}) or (±)-dobutamine (●) (10 μmol kg^{-1} h^{-1}) in conscious, Long Evans rats ($n = 8$), chronically instrumented with intravascular catheters and internal and common carotid pulsed Doppler flow probes. Values are mean and bars are s.e.mean; * $P < 0.05$ versus baseline (Friedman's test). Statistics for comparisons between human α-CGRP and (±)-dobutamine are given in the text. Abbreviations as in Figure 1.

tropic effect of human α-CGRP, together with redirection of blood flow from the mesenteric vascular bed, could have contributed to the increase in carotid and hindquarters blood flows. Since there was a significant reduction in afterload it is feasible this contributed to the increase in cardiac index. However, there was a clear-cut reduction in stroke index, possibly resulting from reduced cardiac filling, since central venous pressure fell. Thus, it is feasible that any putative positive inotropic effects of human α-CGRP did not result in more marked increases in cardiac index because of a reduction in venous return. It is likely the latter was due to a venodilator action of human α-CGRP (Törnebrandt *et al.*, 1987; Crossman *et al.*, 1987; but see Al-Kazwini *et al.*, 1987; Marshall *et al.*, 1988). The present results, therefore, provide some explanation for the conflicting data in the literature regarding the cardiac haemodynamic effects of CGRP (DiPette *et al.*, 1987; Lappe *et al.*, 1987; Sirén & Feuerstein, 1988; Ando *et al.*, 1990) (see Introduction), since it is apparent that the effects of the peptide depend on the dose used. Moreover, it may be that the cardiac haemodynamic responses to bolus injections of CGRP would give a less clear picture of the interrelated changes that were seen in the present study, particularly with the infusion of the higher dose of human α-CGRP.

The lower dose of (±)-dobutamine had potent, positive chronotropic and inotropic effects, together with marked hindquarters and carotid vasodilator actions. Although the vasodilatations were similar to those seen with the lower dose of human α-CGRP, the hyperaemias in these vascular beds were greater than those observed following the lower dose of human α-CGRP, probably because they were supported by the increase in cardiac index. This proposal is consistent with the finding that superior mesenteric blood flow was not reduced under these conditions. The results indicate that, in the conscious rat, (±)-dobutamine may have substantial β_1- (i.e. cardiac) and β_2- (i.e. hindquarters, Gardiner & Bennett, 1988) adrenoceptor-stimulating properties, although reflex responses to the increased cardiac output may have contributed to changes in regional vascular conductance (Ruffolo & Messick, 1985b; Ruffolo, 1987).

The present results, showing that the infusion of the lower dose of (±)-dobutamine (i.e. 2 μmol kg^{-1} h^{-1}) caused an increase in cardiac index that was primarily due to a tachycardia rather than an increase in stroke index are the opposite of the findings of Ruffolo & Messick (1985b), but they studied the responses to bolus doses of (±)-dobutamine (0.01 μmol kg^{-1}) in anaesthetized rats. In general, our results are compatible with those of Schoemaker (1989), although she found that (±)-dobutamine (4–40 μmol kg^{-1} h^{-1}) caused no significant increase in stroke volume at any dose level, whereas our higher dose infusion of (±)-dobutamine (i.e. 10 μmol kg^{-1} h^{-1}) caused a substantial rise in stroke index. However, under these conditions there was a large increase in total peripheral conductance and hence mean arterial blood pressure fell. This finding contrasts with those of the majority of previous studies in which administration of (±)-dobutamine caused no change (Vatner *et al.*, 1974, boluses 1.6–8.0 μmol kg^{-1}, infusions 1.6 μmol kg^{-1} h^{-1}; Robie & Goldberg, 1975, 0.4–6.4 μmol kg^{-1} h^{-1}; Leier *et al.*, 1977, 1978, 0.5–3.0 μmol kg^{-1} h^{-1}; Liang & Hood, 1979, 2–8 μmol kg^{-1} h^{-1}; Dorszewski *et al.*, 1990, 0.2–0.8 μmol kg^{-1} h^{-1}) or an increase (Ruffolo & Messick, 1985b) in mean arterial blood pressure. However, these differences are likely to be due to differences in administered dose, since Biro *et al.* (1988), Schoemaker (1989) and Dorszewski *et al.* (1990) using doses of (±)-dobutamine (6, 12 and 4 μmol kg^{-1} h^{-1}, respectively) similar to those employed here, also observed hypotension.

The increase in total peripheral conductance seen in response to infusion of (±)-dobutamine was found to be associated with marked hindquarters and common and internal carotid vasodilatations. Under the same conditions, mesenteric and renal vascular conductances were either unchanged or, in the case of the latter during the higher dose infusion, reduced. While a relatively selective hindquarters vasodilator effect of (±)-dobutamine has been described previously (Vatner *et al.*, 1974; Robie & Goldberg, 1975; Liang & Hood, 1979) this has not been observed invariably (Drexler *et al.*, 1987; Biro *et al.*, 1988), even in studies that did demonstrate a cerebral vasodilator response to (±)-dobutamine (Drexler *et al.*, 1987).

Although there was a greater hindquarters hyperaemia and a better maintenance of mesenteric blood flow during infusion of (±)-dobutamine than of human α-CGRP at either dose level, carotid hyperaemia was greater during infusion of the latter at the higher dose. This could indicate an additional, selective, carotid vasodilator effect of human α-CGRP compared to (±)-dobutamine. It was also apparent that renal blood flow during infusion, and renal and mesenteric flows

after infusion were more compromised when (±)-dobutamine was administered than when human α-CGRP was given. While there is evidence that administration of CGRP activates the renin-angiotensin system (Kurtz *et al.*, 1988; Gardiner *et al.*, 1990a) the present results are most likely explained by (±)-dobutamine having a much more potent direct action in this regard, since it is relatively devoid of pre-junctional actions on noradrenaline release (Marcus *et al.*, 1987; Fischer *et al.*, 1989). It seems likely that activation of neurohumoral vasoconstrictor mechanisms, such as the renin-angiotensin system (Jaski & Peters, 1988), was responsible for the apparent desensitization to the vasodilator effects of (±)-dobutamine during infusions of the lower dose (see Figures 1 and 2) since there was no evidence for down-regulation of cardiac effects, even during infusion of the higher dose of (±)-dobutamine. The initial reduction in cardiac function following the end of (±)-dobutamine infusion was probably secondary to the increased afterload (i.e. decreases in renal and mesenteric vascular conductances and overshoot in mean blood pressure), since, subsequently, mean blood pressure fell as total peripheral conductance increased and cardiac function rose again above baseline. It is likely this series of events was due to a relatively more rapid offset of the direct vasodilator effects of (±)-dobutamine than of the vasoconstrictor effects of an activated renin-angiotensin system. Such deleterious cardiovascular effects have not been described previously following prolonged infusion of (±)-dobutamine in clinical studies (Leier *et al.*, 1977; 1978), but in those investigations the first measurements were made 30 min after the end of infusion in patients with congestive heart failure who were being treated with other drugs at the time.

In conclusion, the present results in conscious rats indicate that, in doses matched for their initial effects on mean arterial blood pressure, (±)-dobutamine has a more marked influence on cardiac function than does human α-CGRP. The increase in cardiac index caused by the latter is attenuated by a reduction in stroke index, resulting from a fall in central venous pressure, and probably due to venodilatation. Nonetheless, human α-CGRP can produce more potent carotid hyperaemias than (±)-dobutamine and, furthermore, does not trigger the substantial overshoot in blood pressure, and associated renal or mesenteric vasoconstrictions, or the reduction in cardiac function seen following (±)-dobutamine administration, at least at the highest doses used here.

References

AL-KAZWINI, S.J., CRAIG, R.K., HOLMAN, J.J. & MARSHALL, I. (1987). Comparison of the relaxation of rat arteries and veins by human α-CGRP, sodium nitroprusside and acetylcholine. *Br. J. Pharmacol.*, **90**, 220P.

ANDO, K., PEGRAM, B.L. & FROHLICH, E.D. (1990). Hemodynamic effects of calcitonin gene-related peptide in spontaneously hypertensive rats. *Am. J. Physiol.*, **258**, R425–R429.

BIRO, G.P., DOUGLAS, J.R., KEON, W.J. & TAICHMAN, G.C. (1988). Changes in regional blood flow distribution induced by infusions of dopexamine hydrochloride or dobutamine in anaesthetized dogs. *Am. J. Cardiol.*, **62**, 30c–36c.

BROWN, Z.W., AMIT, Z. & WEEKS, J.R. (1976). Simple flow-thru swivel for infusions into unrestrained animals. *Pharmacol. Biochem. Behav.*, **5**, 363–365.

CROSSMAN, D., McEWAN, J., MacDERMOT, J., MacINTYRE, I. & DOLLERY, C.T. (1987). Human calcitonin gene-related peptide activates adenylate cyclase and releases prostacyclin from human umbilical vein endothelial cells. *Br. J. Pharmacol.*, **92**, 695–701.

DiPETTE, D.J., SCHWARZENBERGER, K., KERR, N. & HOLLAND, O.B. (1987). Systemic and regional hemodynamic effects of calcitonin gene-related peptide. *Hypertension*, **9**, Suppl. III, III-142–III-146.

DORSZWESKI, A., MÜLLER-BECKMAN, B., KLING, L. & SPONER, G. (1990). Haemodynamic profile of an inhibitor of phosphodiesterase III, adibendan (BM 14.478): comparison with nitroprusside and dobutamine in conscious dogs. *Br. J. Pharmacol.*, **101**, 689–690.

DREXLER, H., HÖING, S., FAUD, F., WOLLSCHLÄGER, H. & JUST, H.J. (1987). Central and regional vascular hemodynamics following intravenous milrinone in the conscious rat: Comparison with dobutamine. *J. Cardiovasc. Pharmacol.*, **9**, 563–569.

FISCHER, P., BURGER, A., GRAEFE, K.H. & TRENDELENBURG, U. (1989). The effect of (±)-dobutamine on adrenergic nerve endings. *Naunyn Schmiedebergs Arch. Pharmacol.*, **339**, 79–84.

GARDINER, S.M. & BENNETT, T. (1988). Regional hemodynamic responses to adrenoceptor antagonism in conscious rats. *Am. J. Physiol.*, **225**, H813–H824.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989). Regional haemodynamic effects of human α- and β-calcitonin gene-related peptide in conscious rats. *Br. J. Pharmacol.*, **98**, 1225–1232.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1990a). Renal vasodilator response to captopril in the presence of calcitonin gene-related peptide. In *Diuretics III, Chemistry, Pharmacology, and Clinical Applications*, ed. Puschett, J.B. & Greenberg, A. pp. 279–281. New York: Elsevier.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., KEMP, P.A. & NEY, U. (1990b). Synergistic internal carotid vasodilator effects of human α-calcitonin gene-related peptide and nimodipine in conscious rats. *Br. J. Pharmacol.*, **99**, 830–834.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990c). Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. *Br. J. Pharmacol.*, **101**, 625–631.

GENNARI, C., NAMI, R., AGNUSDEI, D., BIANCHINI, C. & PAVESE, G. (1989). Acute cardiovascular and renal effects of human calcitonin gene-related peptide. *Am. J. Hypertens.*, **2**, 45S–49S.

GENNARI, C., NAMI, R., AGNUSDEI, D. & FISCHER, J.A. (1990). Improved cardiac performance with human calcitonin gene-related peptide in patients with congestive heart failure. *Cardiovasc. Res.*, **24**, 239–241.

HAYWOOD, J.R., SHAFFER, R., FASTENOW, C., FINK, G.D. & BRODY, M.J. (1981). Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. *Am. J. Physiol.*, **241**, H273–H278.

JASKI, B.E. & PETERS, C. (1988). Inotropic, vascular and neuroendocrine effects of dopexamine hydrochloride and comparison with dobutamine. *Am. J. Cardiol.*, **62**, 63c–67c.

KURTZ, A., MUFF, R., BORN, W., LUNDBERG, J.M., MILLBERG, B.I., GNÄDINGER, M.P., UEHLINGER, D.E., WEIDMANN, P., HOKFELT, T. & FISCHER, J.A. (1988). Calcitonin gene-related peptide is a stimulator of renin secretion. *J. Clin. Invest.*, **82**, 538–543.

LAPPE, R.W., SLIVJAK, M.J., TODT, J.A. & WENDT, R.L. (1987). Hemodynamic effects of calcitonin gene-related peptide in conscious rats. *Regul. Pept.*, **19**, 307–312.

LEIER, C.V., HEBAN, P.T., HUSS, P., BUSH, C.A. & LEWIS, R.P. (1978). Comparative systemic and regional hemodynamic effects of dopamine and dobutamine in patients with cardiomyopathic heart failure. *Circulation*, **58**, 466–475.

LEIER, C.V., WEBEL, J. & BUSH, C.A. (1977). The cardiovascular effects of the continuous infusion of dobutamine in patients with severe cardiac failure. *Circulation*, **56**, 468–472.

LIANG, C.S. & HOOD, W.B. (1989). Dobutamine infusion in conscious dogs with and without autonomic nervous system inhibition: effects on systemic hemodynamics, regional blood flows and cardiac metabolism. *J. Pharmacol. Exp. Ther.*, **211**, 698–705.

MACCARONE, C., MALTA, E. & RAPER, C. (1984). β-Adrenoceptor selectivity of dobutamine: *in vivo* and *in vitro* studies. *J. Cardiovasc. Pharmacol.*, **6**, 132–141.

MARCUS, F.I., OPIE, L.H. & SONNENBLICK, E.H. (1987). Digitalis, sympathomimetics and inotropic-dilators. In *Drugs for the Heart* ed. Opie, L.H. 2nd edition, pp. 101–103. Florida: Grune and Stratton.

MARSHALL, I., AL-KAZWINI, S.J., HOLMAN, J.J. & CRAIG, R.K. (1988). Human α-calcitonin gene-related peptide (CGRP) is a potent vasodilator in human mesenteric vasculature. *Br. J. Clin. Pharmacol.*, **26**, 691–695.

O'ROURKE, M.F. (1982). Vascular impedance in studies of arterial and cardiac function. *Physiol. Rev.*, **62**, 570–623.

ROBIE, N.W. & GOLDBERG, L.I. (1975). Comparative systemic and regional hemodynamic effects of dopamine and dobutamine. *Am. Heart J.*, **90**, 340–345.

RUFFOLO, R.R. (1987). Review: the pharmacology of dobutamine. *Am. J. Med. Sci.*, **294**, 244–248.

RUFFOLO, R.R. & MESSICK, K. (1985a). Inotropic selectivity of dobutamine enantiomers in the pithed rat. *J. Pharmacol. Exp. Ther.*, **235**, 344–348.

RUFFOLO, R.R. & MESSICK, K. (1985b). Systemic hemodynamic effects of dopamine, (±)-dobutamine and the (+)- and (−)-enantiomers of dobutamine in anaesthetized normotensive rats. *Eur. J. Pharmacol.*, **109**, 173–181.

RUFFOLO, R.R. & MORGAN, E.L. (1984). Interaction of the novel inotropic agent, ASL-7022, with alpha and beta adrenoceptors in the cardiovascular system of the pithed rat: comparison with dobutamine and dopamine. *J. Pharmacol. Exp. Ther.*, **229**, 364–371.

RUFFOLO, R.R. & YADEN, E.L. (1983). Vascular effects of the steroisomers of dobutamine. *J. Pharmacol. Exp. Ther.*, **224**, 46–50.

SCHOEMAKER, R. (1989). Experimental heart failure in rats. *Ph.D. Thesis, University of Limburg, Netherlands.*

SIRÉN, A.-L. & FEUERSTEIN, G. (1988). Cardiovascular effects of rat calcitonin gene-related peptide in the conscious rat. *J. Pharmacol. Exp. Ther.*, **247**, 69–78.

SMITH, T.L. & HUTCHINS, P.M. (1979). Central hemodynamics in the developmental stage of spontaneous hypertension in the unanaesthetized rat. *Hypertension*, **1**, 508–517.

SMITS, J.F.M., COLEMAN, T.G., SMITH, T.L., KASBERGEN, C.M., VAN ESSEN, H. & STRUYKER-BOUDIER, H.A.J. (1982). Antihypertensive effect of propranolol in conscious spontaneously hypertensive rats: central hemodynamics, plasma volume, and renal function during β-blockade with propranolol. *J. Cardiovasc. Pharmacol.*, **4**, 903–914.

SONNENBLICK, E.H., FRISHMAN, W.H. & LEJEMTEL, T.H. (1979). Dobutamine: a new synthetic cardio-active sympathetic amine. *New Engl. J. Med.*, **300**, 17–22.

THEODORSSON-NORHEIM, E. (1987). Friedman and Quade tests: BASIC computer program to perform non-parametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comput. Biol. Med.*, **17**, 85–99.

TÖRNEBRANDT, K., NOBIN, A. & OWMAN, C. (1987). Contractile and dilatory action of neuropeptides on isolated human mesenteric blood vessels. *Peptides* **8**, 251–256.

VATNER, S.F., MCRITCHIE, R.J. & BRAUNWALD, E. (1974). Effects of dobutamine on left ventricular performance, coronary dynamics, and distribution of cardiac output in conscious dogs. *J. Clin. Invest.*, **53**, 1265–1273.

WANG, B.C., BIE, P., LEADLEY, J.R. & GOETZ, K.L. (1989). Cardiovascular effects of calcitonin gene-related peptide in conscious dogs. *Am. J. Physiol.*, **257**, R726–R731.

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