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Cardiovascular Effects of Rat Calcitonin Gene-Related Peptide in the Conscious Rat[1]

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ABSTRACT

The role of rat calcitonin gene-related peptide (CGRP), a recently characterized vasoactive neuropeptide, in cardiovascular regulation was studied in the conscious rat. Mean arterial pressure (MAP), heart rate, cardiac output (thermodilution technique) and regional blood flow (directional pulsed Doppler velocimetry) were monitored after i.v. or i.c.v. administration of CGRP. Systemic administration of CGRP (0.1–10 nmol/kg i.v.) decreased MAP and increased heart rate in a dose-related manner. Cardiac output increased (+95 ± 16 ml/min/kg, $P < .01$) after the 1-nmol/kg dose. At the lower or higher doses, CGRP produced no consistent changes in cardiac output. Total peripheral resistance was decreased significantly at the doses of 1 and 10 nmol/kg of CGRP. The CGRP i.v. doses of 1 and 10 nmol/kg increased mesenteric and hindquarter blood flow to a maximum of +23 ± 7 and +30 ± 6%, respectively ($P < .01$). An increase in renal blood flow (+19 ± 6%, $P < .05$) and a decrease in renal resistance (−15 ± 4%, $P < .05$) were produced by the 0.1-nmol/kg dose of CGRP which had no effect on MAP; higher doses of CGRP tended to decrease renal blood flow. The resistance in all vascular beds was decreased by the CGRP doses of 1 and 10 nmol/kg. The maximum decreases in mesenteric, renal and hindquarter vascular resistance after the 10-nmol/kg dose were −53 ± 3, −42 ± 5 and −48 ± 4%, respectively ($P < .01$). The hypotensive and vasodilator responses to CGRP i.v. were significantly magnified, and the tachycardia produced by CGRP was attenuated in the sinoaortic denervated rats. Atropine (muscarinic blockers), propranolol (*beta* adrenoceptor blocker), cimetidine and pyrilamine (histamine H_1 and H_2 blockers), indomethacin (prostaglandin synthesis inhibitor), BN52021 (platelet activating factor antagonist) or a substance P antagonist had no effect on the cardiovascular responses elicited by systemic CGRP. CGRP, i.c.v. (0.1–10 nmol/kg), induced a modest tachycardia in both intact and sinoaortic denervated rats, but was devoid of any other cardiovascular effects. The results indicate that CGRP is a potent vasodilator of mesenteric, renal and hindquarter skeletal muscle blood vessels in the conscious rat. The hypotensive and vasodilator actions of circulating CGRP are likely to be mediated by direct peripheral interaction with CGRP receptors on vascular smooth muscle, whereas its tachycardic effect seems to involve reflex activation of the sympathetic nervous system.

CGRP is a neuropeptide formed from alternative RNA slicing processes during calcitonin gene expression in rat and human tissues (Amara *et al.*, 1982; Rosenfeld *et al.*, 1983; Tschopp *et al.*, 1985). CGRP immunoreactivity, frequently colocalized with SP, has been demonstrated in blood vessel periadventitial nerves, heart, brain and spinal cord of both rats and humans (Amara *et al.*, 1983; Golzman and Mitchell, 1985; Mulderry *et al.*, 1985; Rosenfeld *et al.*, 1983; Tschopp *et al.*, 1985, Lundberg *et al.*, 1985; Goodman and Iversen, 1986). CGRP can be detected in the systemic circulation (Wang *et al.*, 1988; Zaidi *et al.*, 1985), and the plasma levels of CGRP are increased in endotoxemia (Wang *et al.*, 1988) or after sensory nerve injury (Zaidi *et al.*, 1985).

Exogenously administered, rat or human CGRP induces vasodilation in human and rabbit skin (Brain *et al.*, 1985; Brain and Williams, 1985). In various isolated vascular tissue preparations (human pial arteries, feline and rat mesenteric arteries or rat aortic strips) CGRP produces vasodilation (Hanko *et al.*, 1985; Marshall *et al.*, 1986a; Brain *et al.*, 1985). Intravenous administration of CGRP produces strong dose-related hypotensive and tachycardic responses both in the rat (Fisher *et al.*, 1983; DiPette *et al.*, 1987; Lundberg *et al.*, 1985; Marshall *et al.*, 1986b) and in human subjects (Franco-Cereceda *et al.*, 1987). When injected directly into coronary circulation of the intact pig, CGRP was shown to produce coronary vasodilation (Ezra *et al.*, 1987). In a recent study in the rat, the effects of CGRP on blood flow (microsphere technique, DiPette *et al.*, 1987) were

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ABBREVIATIONS: CGRP, calcitonin gene-related peptide; SP, substance P; SAD, sinoaortic denervated; TPR, total peripheral resistance; MAP, mean arterial pressure; Paf, platelet activating factor; ANOVA, analysis of variance; TPRI, total peripheral resistance index.

monitored 2 min after CGRP administration, at a time point when the responses were already compensated or reflexly modified. No attempt has yet been made to investigate the potential role of cardiac function in systemic or regional hemodynamic responses to CGRP or were the putative mediators of CGRP effects on specific blood vessels studied.

The present report presents our investigation on the systemic and regional hemodynamic actions of CGRP in the intact conscious rat by utilizing the thermodilution technique for cardiac output monitoring, and the pulsed directional Doppler velocimetry to monitor continuous blood flow in the chronically instrumented rat. To examine the role of the central nervous system in the cardiovascular responses to CGRP, the peptide was administered i.c.v. both to intact rats and to SAD rats which completely lack the baroreceptor reflexes. The role of other vasoactive substances which might share some similarities to CGRP or mediate its effects on the microcirculation were studied by using specific blockers or synthesis inhibitors.

Materials and Methods

Male Sprague-Dawley rats (300–360 g) were purchased from Taconic Farms (Germantown, NY) and housed at 22°C with a 12 hr/12 hr light/dark cycle. After surgical operations the rats were housed individually in plastic cages (21 × 27 × 16 cm, width × length × height) with food and water *ad libitum*.

Measurement of cardiac output. The cardiac output was measured by thermodilution technique as described previously in detail (Osborn *et al.*, 1986; Sirén *et al.*, 1986, 1988; Unger *et al.*, 1983; Walker, 1986). The principle of this technique is to produce a change in heat content of blood at one point of the circulation (right atrium) and detect the resultant change in temperature at a point downstream (ascending aorta). Although the cardiac output values obtained by thermodilution tend to be somewhat higher than those determined by electromagnetic flowmetry (Lappe *et al.*, 1985; Osborn *et al.*, 1986) or by radioactive microsphere method (Ishise *et al.*, 1980); Flaim and Zelis, 1980), the thermodilution method offers many advantages, including 1) the possibility to do repeated cardiac output recordings in a conscious chronically instrumented rat and 2) in contrast to Doppler velocimetry (Werber *et al.*, 1984) or electromagnetic flowmetry (Lappe *et al.*, 1985), thermodilution does not require the extensive open chest surgery with high mortality and prolonged postoperative recovery.

Each rat to undergo cardiac output measurements was anesthetized with an i.m. injection of ketamine (130 mg/kg) and acepromazine (1.3 mg/kg) and a PE-50 catheter was inserted into the left femoral artery. The catheters were tunneled under the skin and exited at the nape of the neck. An incision was made at the midline of the neck from the cricoid to the clavicle, and a PE-50 catheter was inserted into the right atrium *via* the external jugular vein. The left common carotid artery was exposed and ligated, and a thermistor (MX2-780-33 model THMP f 1.5, Teflon reusable, Columbus Instruments, OH) was advanced through the carotid into the ascending aorta. Placement above the aortic valve was confirmed in each animal by the shape of the dilution curve before the probe was finally sutured to the neck muscles and again at the end of experiment. The jugular vein catheter and the thermistor leads were tunneled under the skin to the nape of the neck. All catheters and the probe wire were secured by a soft spring wire attached to the animal's neck using an adhesive collar. Twenty-four hours after the surgery, the arterial line was connected to a pressure transducer (Narco Bio-Systems model RP 1500i) attached to a strain gauge coupler (Narco Bio-Systems type 7032). Blood pressure (mean, systolic, diastolic and pulse) and heart rate were recorded continuously on a Narcotrace 80 computerized physiograph and sampled automatically at 30- to 60-sec intervals by a Northstar-Hazeltine computer.

To obtain measurements of cardiac output, the aortic thermistor was attached to the computerized Cardiomax II (CMX2-780-k with microprobe option R, Columbus Instruments). The dead space of the venous line was first flushed with 50 μl of 0.9% (w/v) NaCl (saline) at room temperature (22°C); after a brief stabilization period an additional injection of 200 μl normal saline (22°C) was injected rapidly into the right atrium using a 1-ml syringe, and the blood temperature monitored by the aortic thermistor. The thermodilution curve representing the change in aortic temperature was then calculated by the Cardiomax II. A control period of 15 min included two or three cardiac output recordings to test for consistency and placement of the probe. During this time period, control values for blood pressure and heart rate also were collected. The timer for automatic data collection was started and data points for blood pressure, heart rate and cardiac output were collected immediately before and 2, 5, 15, 30 and 45 min after CGRP injection into the jugular catheter. TPR was calculated by dividing the mean arterial pressure by the cardiac output; values of cardiac output and TPR were further indexed per unit of weight (kilograms). Core temperature was monitored by the aortic thermoprobe before each cardiac output measurement.

Measurement of organ blood flow. Regional blood flow in hindquarter, renal and mesenteric arteries was monitored in a separate group of rats by using the directional pulsed Doppler technique. Although not allowing for quantitative blood flow monitoring, this method is superior to other available techniques, as 1) it can be used chronically in conscious animals, 2) it allows continuous on-line recording of blood flow and 3) it can detect instantaneous, transient changes in blood flow within seconds after drug administration. Haywood and co-workers (1981) demonstrated that the velocity signals recorded from the Doppler flow probes are directly and reliably proportional to changes in true volume flow measured by electromagnetic flowmetry.

Each rat to undergo regional hemodynamic measurements was anesthetized with ketamine-acepromazine, and a guide cannula for i.c.v. injections was placed on the skull as described below. A midline laparotomy was then made, and the left renal and superior mesenteric arteries and lower abdominal aorta above its bifurcation were isolated carefully under a dissecting microscope. Doppler flow probes (Valpey-Fisher, Hopkinton, MA) were then sutured loosely around each vessel as described earlier (Haywood *et al.*, 1981; Sirén *et al.*, 1988). The insulated wire leads were fixed to the back muscles, tunneled under the back skin to exit at the neck and soldered to a receptacle which was then attached to the skull using small screws and dental acrylic. The animals were allowed to recover from the surgery for 7 days. Twenty-four hours before the experiment the rat was reanesthetized with halothane (2% in oxygen) and femoral artery and vein catheterized with PE-50 tubing. The catheters were tunneled under the back skin, exited at the nape of the neck and secured by a soft spring wire as described above.

On the day of the experiment, the arterial catheter was connected to a pressure transducer (Narco) and blood pressure and heart rate were recorded continuously on the Narcotrace 80 physiograph. A cable connecting the blood flow receptacle and the Doppler flowmeter (University of Iowa, Bioengineering Facility, model no. 545C-4) was attached to the animal and the mean blood flow recorded continuously on the physiograph. Vascular resistance was calculated by dividing the MAP by blood velocity (Doppler shift in kilohertz) as described earlier (Haywood *et al.*, 1981; Sirén *et al.*, 1988). Changes in blood flow and vascular resistance are expressed as a percentage of control values.

Intracerebroventricular Injections. Rats were anesthetized with ketamine-acepromazine (see above) and placed on a stereotaxic device (David Kopf Instruments, Tujunga, CA). A stainless-steel guide cannula was inserted through the skull and fixed with glue (Eastman 910 Adhesive). Coordinates for the injections into the right lateral brain ventricle (i.c.v.) were measured from the bregma: AP, −0.8 mm and L, 1.2 mm. After the placement of the i.c.v. cannula, implantation of Doppler flow probes and femoral catheters was done as described in detail in the previous section (measurement of organ blood flow). On the day of the experiment, injections of saline or CGRP were made using a premeasured 30-g (7.5 mm) cannula inserted into the ventricular space through the guide cannula. The injection cannula was then

connected *via* polyethylene tubing to a Hamilton microliter syringe, and a volume of 10 μl of the control or drug solution was injected over a period of 30 sec. The proper position of the i.c.v. cannula was determined at the end of each experiment by an injection of dye (methylene blue) into the cerebral ventricles.

Experimental Protocols

Dose-response studies. The effect of CGRP on systemic and regional hemodynamics was studied by injections of increasing doses of CGRP (0.1–10 nmol/kg) i.v. or i.c.v. at 30- to 60-min intervals. The volume for i.v. injections was 150 μl; for i.c.v. injections, 10 μl. Before commencement of CGRP administration each animal received an injection of saline i.v. (150 μl) or i.c.v. (10 μl). The reactivity of the blood flow recordings was first tested in each animal by i.v. injections of epinephrine (0.1, 0.3 and 1 μg/kg) and norepinephrine (0.1, 0.3 and 1 μg/kg).

Sinoaortic denervation. In a separate group of rats ($n = 8$), SAD was performed according to the method described by Krieger (1964). One week before the experiments, the rats were anesthetized with ketamine-acepromazine (see above) and given an injection of atropine (0.4 mg/rat i.p.). A midventral neck incision was made and both of the carotid sinuses were exposed. The superior laryngeal nerve and cervical sympathetic chains were cut. Nerve segments of 0.5 cm were removed to prevent the possibility of reconnection; all connective tissue and nerves were stripped from internal, external and common carotid arteries and the occipital and thyroid arteries along 0.5 cm of their length extending from the carotid bifurcation region. In addition, the vessels were painted with 10% phenol, and special attention was denoted to avoid damage to the vagus nerve or other nearby structures. In addition to the SAD surgery, guide cannulas for i.c.v. injections and Doppler flow probes were implanted as described above. Twenty-four hours before the experiment, the animals were anesthetized with halothane (2% in oxygen), and PE-catheters inserted into the left femoral artery and vein as described above. The adequacy of the baroreceptor deafferentation procedure was assessed before any interventions by evaluating the maximal heart rate changes to phenylephrine (1 and 3 μg/kg i.v.). For all studies, only SAD animals exhibiting a decline in heart rate of less than 15 beats/min were selected. The drug injections i.v. and i.c.v. were made as described in the previous section (dose-response studies).

Pretreatment studies. In a separate group of rats the effects of various treatments on the hemodynamic responses to CGRP were tested. The treatments and doses of the drugs are given in table 1. Atropine, BN 52021, cimetidine + pyrilamine, indomethacin and propranolol were injected i.v. 15 to 20 min before i.v. administration of CGRP at the doses of 1 or 10 nmol/kg; the SP-antagonist, [D-Pro^2-D-Phe^7-D-Trp^9]-SP was administered i.v. 5 min before CGRP. To assure that the selected doses of the antagonists are sufficient to block the respective receptors, all these drugs were tested against their respective agonists. Thus, atropine (2 mg/kg) abolished the hemodynamic effects of acetylcholine (0.3–10 μg/kg i.v.); BN 52021 (15 mg/kg) blocked the hypotensive effect of Paf (0.3, 1 nmol/kg i.v.); cimetidine (5 mg/kg) and pyrilamine (2 mg/kg) abolished the vasomotor responses to histamine (0.3–10 μg/kg i.v.); propranolol (2 mg/kg) blocked the hindquarter vasodilator response to epinephrine (0.3, 1 μg/kg) and the tachycardia elicited by isoprenaline (0.1, 0.3 μg/kg); the SP-antagonist PPT-SP (0.3 mg/kg i.v.) attenuated partially the cardiovascular effects of SP (10, 100 nmol/kg). SP effects on both intestinal and vascular smooth muscle are attenuated by this antagonist (Pernow, 1983). Indomethacin at the selected dose effectively blocks the prostaglandin synthesis (Flower, 1974).

TABLE 1

Treatments and doses of the drugs used to modify the hemodynamic responses to rat CGRP i.v. in the conscious rat

PG, prostaglandin; PPT-SP, [D-Pro^2-D-Phe^7-D-Trp^9]-SP.

Pharmacological Action Receptor/Mediator	Drug	Dose, I.V. mg/kg	Time of Treatment min
Beta-adrenoceptors			
Blocker	Propranolol	2	20
Muscarinic receptors			
Blocker	Atropine	2	20
Histamine receptors			
Blockers	H_1: pyrilamine	2	15
	H_2: cimetidine	5	15
Paf receptors			
Blocker	BN 52021	15	20
SP receptors			
Blocker	PPT-SP	0.3	5
PG cyclooxygenase			
Inhibitor	Indomethacin	5	15

Drugs used. The following drugs were used: atropine sulfate (American Hospital Supply Co., McGaw Park, IL), CGRP (rat, Bachem, Torrance, CA), cimetidine hydrochloride (Sigma Chemical Co., St. Louis, MO), *l*-epinephrine (Sigma), indomethacin (Sigma), *l*-norepinephrine (Sigma), phenylephrine (Sigma), *dl*-propranolol (Sigma), pyrilamine (Smith Kline and French Laboratories, Philadelphia, PA), SP (Peninsula Laboratories, San Carlos, CA), [D-Pro^2-D-Phe^7-D-Trp^9]-SP (Sigma) and BN 52021 [9H1,7a-(epoxymethano)-1H,6aH-cyclopenta [c]furo[2,3-b]furo-[3′,2′,3,4]cyclopenta[1,2-d] furan-5,9,12-(4H)trione, 3-tert-butylhexahydro-4,-7b, -11hydroxy-8 methyl]. BN 52021 was kindly provided in a pure powder form by Dr. P. Braquet (IHB Research Laboratories, Paris, France). BN 52021 was first dissolved in 50% dimethyl sulfoxide, the pH adjusted to 7 to 8 and diluted further with saline (0.9% NaCl w/v). Indomethacin was dissolved in 0.3 M Na_2CO_3. All the other drugs were dissolved in saline. The peptides were aliquoted, stored at −20°C, and thawed only once before administration.

Statistical analysis of the data. Data in text and figures are mean ± S.E. for the given number of rats. ANOVA with repeated measures (BMD P2V, Dixon and Brown, 1979), and ANOVA with Student-Newman-Keul's test were used for statistical analysis of the data.

Results

Hemodynamic Effects of i.v. Administered Rat CGRP

Effects on blood pressure and heart rate. The base-line values of MAP and heart rate were not different in saline- or CGRP-treated rats (table 2). CGRP doses of 1 and 10 nmol/kg decreased MAP and increased heart rate in a dose-related manner (fig. 1). At the 1-nmol/kg dose, the maximum decrease in blood pressure became apparent in 1 to 2 min after the injection and subsided in 15 to 30 min. The maximum increase in heart rate was achieved 2 min after the CGRP and subsided in 15 min. The 10-nmol/kg dose induced sustained hypotensive and tachycardiac responses which fully receded in 60 min.

Effects on cardiac index and TPRI. The 1-nmol/kg dose of CGRP induced a sustained increase in cardiac index (fig. 2). The maximum effect (+95 ± 16 ml/min/kg, $P < .01$) became apparent in 5 to 10 min after the injection but in three of seven animals the cardiac index was elevated significantly 60 min after the 1-nmol/kg dose. Thus, the base-line cardiac index before the 10-nmol/kg dose was significantly higher than in the saline-treated group (table 2). The 10-nmol/kg dose tended to decrease cardiac index of six of seven rats, including animals with normal base-line cardiac index (fig. 2).

The hypotensive doses of CGRP (1 and 10 nmol/kg) decreased TPRI (fig. 2). The maximum reduction in TPRI was reached 2 to 5 min after the injections but the TPRI remained low over the 60-min observation period. The effect of CGRP on TPRI was dose-related in spite of the significantly lower base-line TPRI before the 10-nmol/kg dose (0.228 ± 0.02

TABLE 2

Base-line values of hemodynamic parameters before administration of saline or rat CGRP i.v. in conscious rats

Values indicate mean ± S.E. HR, heart rate; CI, cardiac index.

	n	MAP	HR	CI	TPRI
		mm Hg	bpm	ml/min/kg	mm Hg/ml/min/kg
SALINE, i.v.	10	110 ± 3	389 ± 10	401 ± 14	0.29 ± 0.02
CGRP, 0.1 nmol/kg	8	111 ± 4	403 ± 11	396 ± 15	0.28 ± 0.02
CGRP, 1 nmol/kg	8	107 ± 4	392 ± 10	443 ± 18	0.25 ± 0.01
CGRP, 10 nmol/kg	8	106 ± 5	406 ± 14	488** ± 24	0.23* ± 0.02

Asterisks denote statistical significance *vs.* saline (Student-Newman-Keul's test): * $P < .05$; ** $P < .01$.

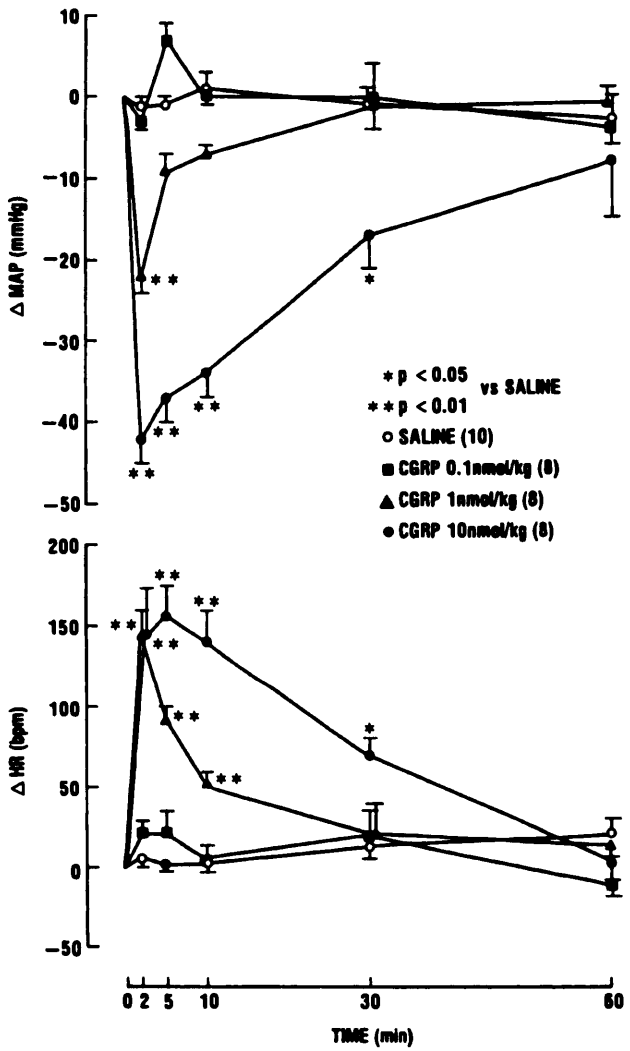

Fig. 1. Effect of rat CGRP i.v. on MAP and heart rate (HR) in the conscious rat. Vertical bars indicate S.E. Number of rats in each group is given in parenthesis. Asterisks denote statistical significance *vs.* saline, i.v. by Student-Newman-Keul's test.

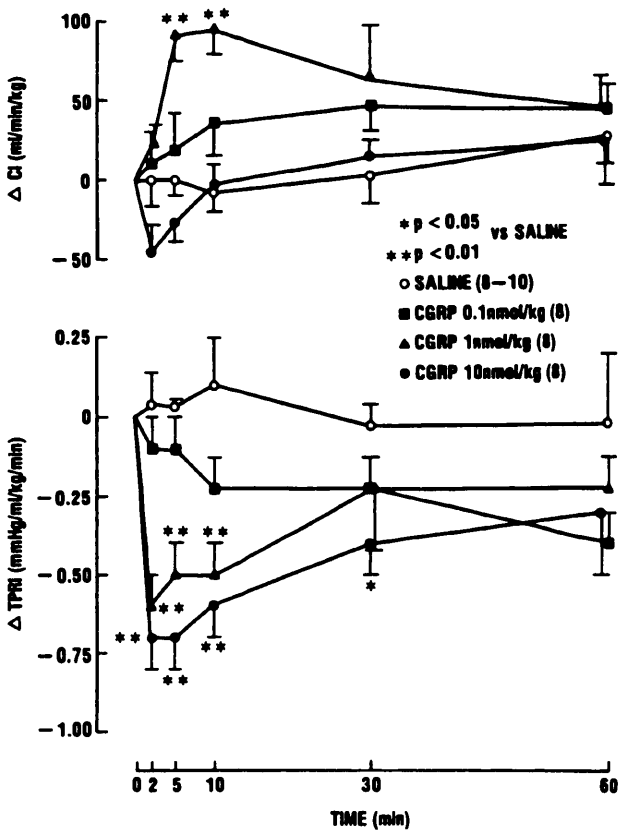

Fig. 2. Effect of rat CGRP i.v. on cardiac index (CI) and TPRI in the conscious rat. Vertical bars indicate S.E. Number of rats in each group is given in parenthesis. Asterisks denote statistical significance *vs.* saline, i.v. by Student-Newman-Keul's test.

mmHg/ml/min/kg *vs.* 0.29 ± 0.02 mm Hg/ml/min/kg in saline-treated animals, $P < .05$).

Effects on regional blood flow. Figures 3 and 4 demonstrate typical recordings of the effect of CGRP on arterial pressure and organ blood flow in the conscious rat. At the 0.1-nmol/kg dose CGRP i.v. produced an increase in renal blood flow (+19 ± 6%, $P < .05$) whereas the higher doses produced variable effects on renal flow. The maximum effect after the 0.1-nmol/kg dose was achieved 1 min after the injection and the effect subsided in 3 min. At this dose CGRP i.v. had no effect on systemic arterial pressure or mesenteric and hindquarter blood flow (fig. 3).

The hindquarter blood flow was increased by the CGRP doses of 1 and 10 nmol/kg by +17 ± 5 and +27 ± 8% ($P < .05$ *vs.* control), respectively (fig. 4). The peak effect was reached 1 min after each injection and subsided in 5 min after the 1-nmol/kg dose. After the 10-nmol/kg dose, the hindquarter blood flow was increased significantly (+20 ± 5%, $P < .05$) 10 min after the CGRP injection.

In the mesenteric artery, CGRP doses of 1 and 10 nmol/kg first increased and then decreased flow (fig. 4). After the 1-nmol/kg dose, the peak increase (+30 ± 8%, $P < .01$ *vs.* control) in mesenteric blood flow became apparent in 30 sec after CGRP administration and was followed 2 min later by a sustained

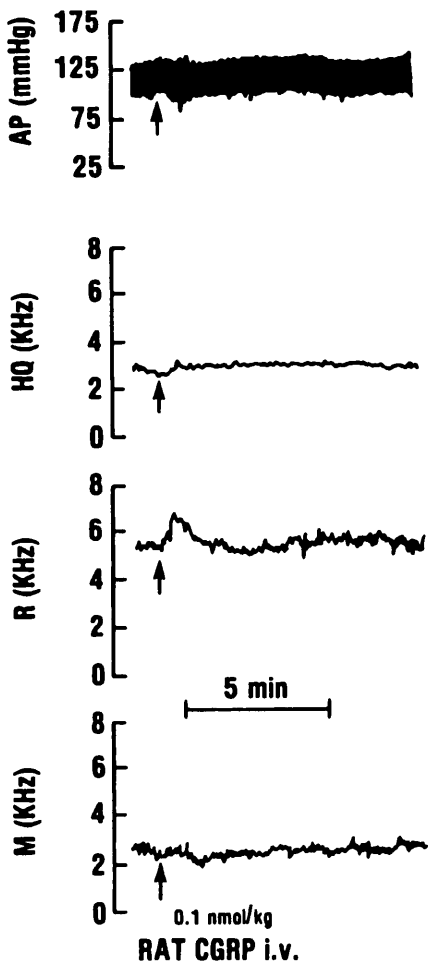

Fig. 3. A representative chart recording of the effect of rat CGRP (0.1 nmol/kg i.v.) on arterial pressure (AP) and blood flow (BF) in the conscious rat. HQ, hindquarter; R, renal; M, mesenteric.

decrease (−27 ± 6%, P < .05 *vs.* control) in flow which subsided in 30 min. The corresponding changes in mesenteric flow after the 10-nmol/kg dose were +66 ± 11% (P < .01 *vs.* control) and −19 ± 7% (P < .05 *vs.* control), respectively.

All the hemodynamic responses produced by the 10-nmol/kg dose of CGRP were significantly more potent (P < .01) than those elicited by the same dose of SP (fig. 4). At the 10-nmol/kg dose, SP had no significant effect on arterial pressure or heart rate but induced vasodilator responses in each vascular bed. The maximum increases in blood flow in the renal, hindquarter and mesenteric vessels were +10 ± 2, +21 ± 4 (P < .01) and +24 ± 4% (P < .01), respectively. The corresponding changes in vascular resistance were −17 ± 2 (P < .01), −22 ± 4 (P < .01) and −23 ± 4% (P < .01). The 100-nmol/kg dose of SP decreased mean arterial pressure (−19 ± 6 mmHg, P < .01) and increased heart rate (+67 ± 18 bpm, P < .05) with regional hemodynamic changes similar to the 10-nmol/kg dose. At lower doses SP had no significant effect on any of the hemodynamic variables.

Effects of regional vascular resistance. At the 1- and 10-nmol/kg doses, CGRP i.v. decreased vascular resistance in the hindquarter, renal and mesenteric blood vessels (fig. 5). The lowest dose (0.1 nmol/kg) produced a significant decrease in renal resistance (−15 ± 4%, P < .05) but had no effect on vascular resistance in the other vascular beds (fig. 5). The maximum changes in vascular resistance became apparent simultaneously with the peak blood flow 30 sec to 2 min after the drug injection. In the mesenteric and renal vasculature, the vasodilation subsided within 10 min after the 10-nmol/kg dose whereas the hindquarter resistance was still decreased (−25 ± 4%, P < .05) 30 min after the highest dose of CGRP. Although the mesenteric blood flow was reduced after the initial increase in blood flow, mesenteric vascular resistance was never significantly increased.

Role of other mediators in the hemodynamic effects of CGRP. The various treatments tested to block hemodynamic responses to CGRP i.v. are shown in table 1. None of these treatments had any significant effect on the base-line values of cardiovascular variables before CGRP administration. The *beta* adrenoceptor blocker propranolol attenuated the tachycardia produced by CGRP but had no effect on the hypotensive and vasodilator responses to CGRP (table 3). All the other treatments failed to significantly alter the hemodynamic responses produced by CGRP i.v. (table 3).

Effects of CGRP after sinoaortic denervation. The base-line MAP (145 ± 6 mm Hg) and heart rate (492 ± 8 bpm) in SAD rats were significantly (P < .01) higher than in intact rats (112 ± 3 mm Hg and 381 ± 16 bpm, respectively), whereas there was no significant differences in the base-line blood flow or vascular resistance between these groups. In SAD rats CGRP i.v. (0.1–1 nmol/kg) produced hypotension with no significant effect on the heart rate. The depressor responses to CGRP doses of 0.1 and 1 nmol/kg were significantly magnified in SAD rats compared to the responses in intact animals (fig. 6). The tachycardic effect of the 1-nmol/kg dose was attenuated in SAD rats compared to intact rats (fig. 6). At the lower doses CGRP i.v. had no significant effect on heart rate in either intact or baroreceptor-denervated animals.

The vasodilator responses of all three vascular beds to the 1-nmol/kg dose of CGRP i.v. were significantly enhanced in SAD rats compared to intact rats (fig. 7). In the mesenteric artery, even the 0.1-nmol/kg dose produced a significant decrease in vascular resistance in the SAD rats.

Effects of centrally administered CGRP. The effects of i.c.v. administration of CGRP (0.1–10 nmol/kg) on hemodynamic variables are summarized in table 4. The 10-nmol/kg dose of CGRP i.c.v. produced a moderate increase in heart rate in intact rats but had no effect on blood pressure or organ blood flow. In SAD rats, CGRP (i.c.v. doses of 1 and 10 nmol/kg) elicited tachycardia. After baroreceptor deafferentation, the blood pressure tended to increase by CGRP i.c.v. (0.1–10 nmol/kg) but the effect did not reach statistical significance when compared to vehicle injection.

Discussion

The hypotensive and tachycardic effects of CGRP i.v. have been described in several species including humans (DiPette *et al.*, 1987; Fisher *et al.*, 1983; Franco-Cereceda *et al.*, 1987; Lappe *et al.*, 1987; Marshall *et al.*, 1986b). Previous studies have suggested that the hypotensive action of CGRP is due to generalized vasodilation. Thus, intradermal injections of human or rat CGRP of less than picomolar doses produced sustained local vasodilation in the rabbit ear or human skin (Brain *et al.*, 1985; Brain and Williams, 1986) and elicited

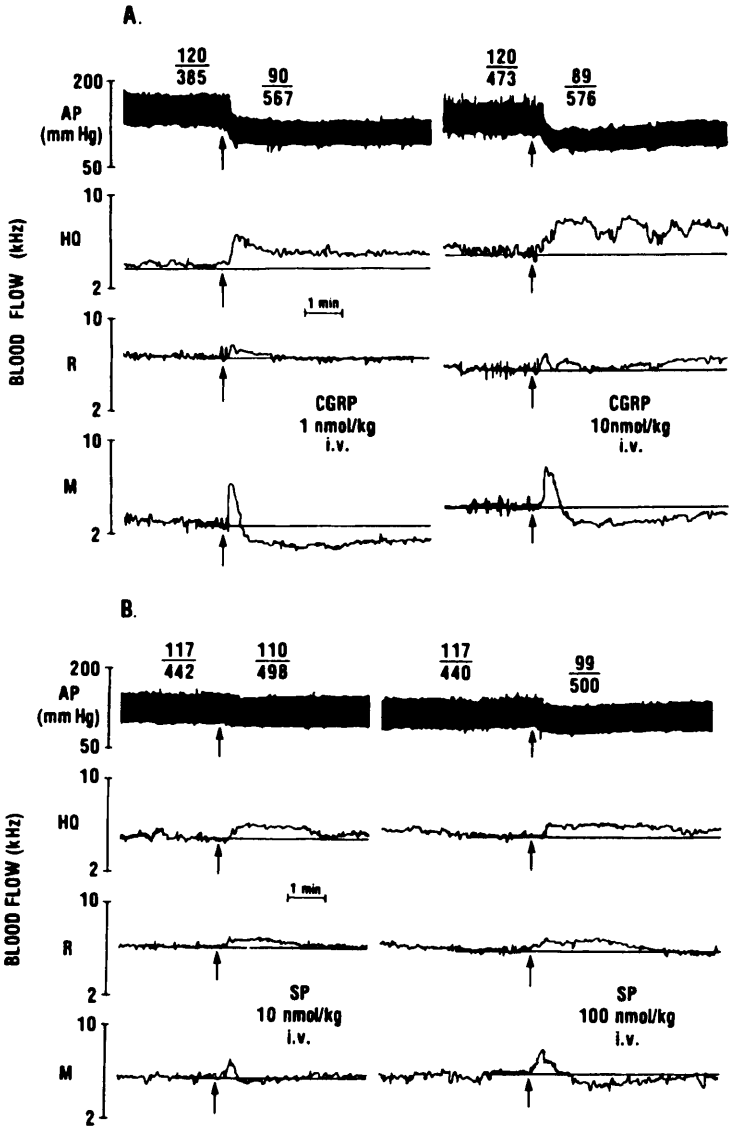

Fig. 4. A representative chart recording of the effect of rat CGRP (A) and SP (B) on arterial pressure (AP) and blood flow (BF) in the conscious rat. HQ, hindquarter; R, renal; M, mesenteric. Base-line and peak values of MAP and heart rate are marked on the top the AP tracing.

relaxation of vascular smooth muscle *in vitro* (Brain *et al.*, 1985; Hanko *et al.*, 1985; Marshall *et al.*, 1986a). The present study provides direct evidence that the hypotension produced by CGRP *in vivo* is due to reduced total peripheral resistance and sustained vasodilation in hindquarter, mesenteric and renal vasculature.

In the mesenteric artery, CGRP produced a biphasic blood flow response: a transient increase in flow followed by a sustained reduction. However, the mesenteric resistance was always decreased after CGRP. The mesenteric vasodilation preceded the hypotensive response indicating that the decrease in mesenteric resistance probably contributed to the hypotension. CGRP was reported to produce splanchnic vasodilation in the conscious rat in recent studies by DiPette *et al.* (1987) and Lappe *et al.* (1987). The splenic vessels of the rat, however, were shown to respond to CGRP injections with vasoconstriction (DiPette *et al.*, 1987). Thus, the regional vasomotor responses to CGRP even in a restricted area such as the splanchnic region might be very diverse. The blood vessels in the gastrointestinal tract have been demonstrated to be lavishly innervated with CGRP-immunoreactive fibers (Mulderry *et al.*, 1985). The highest accumulation of these nerve fibers were found in the adventitia and muscle layers of the superior mesenteric artery (Mulderry *et al.*, 1985). Although the CGRP receptor distribution in the peripheral blood vessels is not known at the present time, it appears that high levels of CGRP immunoreactivity in the gastrointestinal blood vessels are well in accordance with the responsiveness of vasculature to the vasodilator action of CGRP *in vivo*.

The lowest dose of CGRP (0.1 nmol/kg) produced a selective increase in renal blood flow with no effect on systemic blood pressure or blood flow in the other vascular beds. At higher doses, the renal flow tended to decrease due to the fall in systemic pressure. The reduction in arterial pressure always exceeded the decrease in renal blood flow and the renal vascular resistance was reduced consistently by all doses of CGRP. In this regard our results contradict the findings of a recent study in which CGRP had no effect on renal resistance and a decrease in renal flow paralleled the systemic hypotension produced by similar doses (0.2 and 6 nmol/kg) of CGRP (DiPette *et al.*,

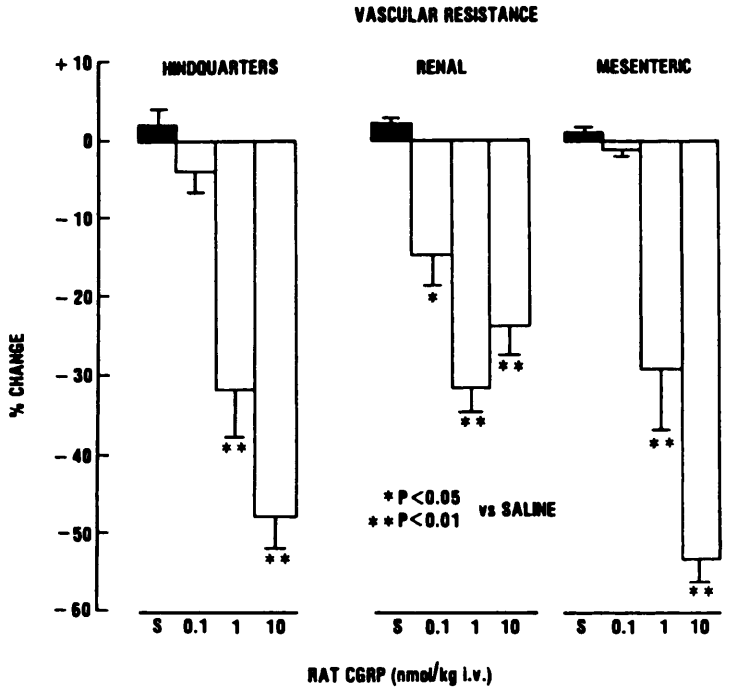

Fig. 5. Effect of rat CGRP i.v. on regional vascular resistance in the conscious rat. Vertical bars indicate S.E. Each group comprised six rats. Asterisks denote statistical significance *vs.* saline, i.v. by Student-Newman-Keul's test.

TABLE 3

Influence of atropine, Paf-antagonist BN 52021, cimetidine + pyrilamine, indomethacin, propranolol or the SP-antagonist PPT-SP on the hemodynamic responses to rat CGRP (10 nmol/kg/i.v.) in conscious rats

Values (mean ± S.E.) indicate maximum changes 2 to 5 min after GCRP. HR, heart rate; BF, blood flow; VR, vascular resistance; HQ, hindquarter; R, renal; M, mesenteric; CIM, cimetidine; PYRIL, pyrilamine; PPT-SP, [D-Pro^2-o-Phe^7-o-Trp^9]-SP.

Treatment	*n*	ΔMAP	ΔHR	Δ%BF			Δ%VR		
				HQ	R	M	HQ	R	M
CGRP	16	−59	+120	+30	+5	+66	−48	−42	−53
		±5	±23	±6	±6	±11	±4	±5	±3
CGRP after	8	−54	+108	+25	+18	+60	−52	−45	−59
atropine		±9	±26	±13	±6	±16	±9	±9	±8
CGRP after	7	−57	+83	+25	+3	+27	−61	−43	−37
BN 52021		±10	±15	±9	±9	±10	±4	±10	±4
CGRP after	9	−41	+106	±51	+21	+42	−55	−46	−35
CIM + PYRIL		±3	±16	±15	±7	±13	±5	±4	±14
CGRP after	7	−62	+94	+28	+9	+30	−63	−53	−56
indomethacin		±5	±23	±12	±11	±12	±4	±8	±7
CGRP after	6	−64	+42**	+34	+18	+33	−64	−46	−66
propranolol		±8	±20	±8	±10	±10	±4	±9	±8
CGRP after	7	−52	+83	+42	+16	+55	−58	−43	−50
PPT-SP		±5	±23	±10	±7	±11	±5	±8	±5

1987). In the study of DiPette *et al.* (1987), the blood flow was monitored only at one time point, 3 min after the CGRP administration, and the initial renal vasodilation most probably was missed. Thus, our data underscore the usefulness of the Doppler velocimetry in monitoring instantaneous and transient vasomotor responses which otherwise might not be detected.

CGRP i.v. produced vasodilator responses also in the hindquarter (mainly skeletal muscle) blood vessels. This finding is in accordance with the recent reports which demonstrate vasodilator responses in skeletal muscle blood vessels in the conscious rat (DiPette *et al.*, 1987; Lappe *et al.*, 1987). In these previous studies, the time-response relationship of the vasodilator response was not examined. Our present study revealed a sustained hindquarter vasodilation and increase in hindquarter blood flow at times at which hypotension already counteracted the vasodilation induced increase in the renal and mesenteric blood flow.

The hypotensive and vasodilator effects of the 1-nmol/kg dose of CGRP were accompanied by a strong tachycardia and an increase in cardiac output. At the highest dose the cardiac output tended to decrease in spite of the profound positive chronotropic response and decreased cardiac afterload. This may be due to the decrease in venous return caused by the extreme peripheral vasodilation and reduction in total peripheral resistance. A direct cardiac depressor effect is not likely as CGRP has been shown to increase cardiac frequency and contractility in the isolated rat heart (Asimakis *et al.*, 1987; Lundberg *et al.*, 1985; Marshall *et al.*, 1986b; Saito *et al.*, 1986), and to elicit coronary vasodilation in the pig or rat (Asimakis *et al.*, 1987; Ezra *et al.*, 1987; Holman *et al.*, 1986). The stimulation

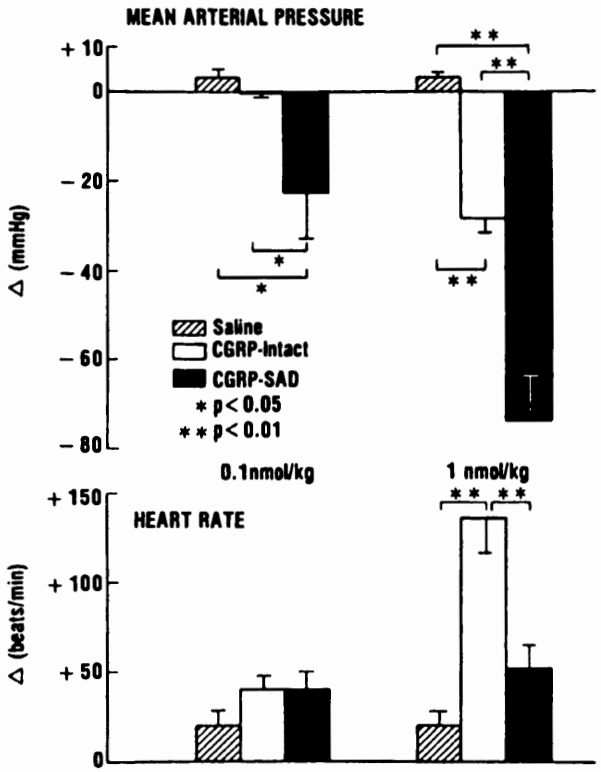

Fig. 6. Effect of rat CGRP i.v. on MAP and heart rate in SAD and intact rats. Values (mean ± S.E.) indicate maximum responses 30 sec to 2 min after CGRP injections. Each group comprised six rats. Asterisks denote statistical significance by Student-Newman-Keul's test.

of sympathetic outflow by CGRP in humans (Franco-Cereceda *et al.*, 1987) also contradicts a negative inotropic action.

The hypotension and splanchnic vasodilator responses to CGRP i.v. were significantly enhanced in SAD rats compared to intact animals. These results indicate clearly that CGRP produces hypotension by directly relaxing the peripheral resistance vessels rather than by interfering with the baroreflex circuits. In fact, the failure of the 0.1-nmol/kg dose to elicit changes in arterial pressure was probably due to the cardiovascular stimulation caused by baroreceptor activation inasmuch as removal of the baroreflex mechanisms resulted in a strong depressor response even at this low dose of CGRP. Typical for a peripherally acting vasodilator agent, the tachycardic effect of CGRP was reduced after baroreceptor deafferentation. Although the higher resting level of heart rate in SAD rats compared to intact animals might have contributed to the reduced tachycardic response, the primary reason for the attenuation of CGRP-induced tachycardia was obviously the lack of baroreceptor stimulation, as heart rate can be increased markedly in SAD rats by other stimuli such as central injections of thyrotropin releasing hormone (Sirén *et al.*, 1988).

A reflex activation of the sympathetic nervous system as a mechanism for the tachycardiac action of CGRP i.v. is supported also by the attenuation of the tachycardia by the *beta*-adrenoceptor blocker, propranolol. However, a central nervous system activation of sympathetic outflow probably contributed to the cardiac acceleration as CGRP also produced a moderate increase in heart rate after i.c.v. administration. This assumption is supported further by the studies showing increases in plasma norepinephrine after i.c.v. administration of CGRP in the rat (Fisher *et al.*, 1983). A tendency for tachycardia was also found after the 0.1-nmol/kg dose which had no effect on arterial pressure. CGRP i.v. was still capable of producing hypotensive and tachycardic responses in the pithed rat which completely lacks the central nervous system (Haass and Skofitissch, 1985). CGRP increased frequency and contractility of isolated heart muscle (Lundberg *et al.*, 1985; Marshall *et al.*, 1986b; Saito *et al.*, 1986). Thus, a direct cardiac action might also contribute to the tachycardia produced by CGRP.

The hypotensive and vasodilator effects of CGRP were not influenced by drugs which block *beta* adrenergic, muscarinic, histamine, SP or Paf receptors or the synthesis of prostaglandins. CGRP was shown to potentiate the plasma extravasation and skin edema produced by other proinflammatory mediators such as bradykinin, leukotriene B_4, Paf or SP (Brain and Williams, 1985) but the vasorelaxing effect of CGRP was never blocked by receptor blockers or synthesis inhibitors of other mediators including acetylcholine, histamine, Paf or prostaglandins (Brain *et al.*, 1985; Marshall *et al.*, 1986ab). Our data are in accord with and extend these observations which indicate clearly that the vascular actions of CGRP i.v. are mediated by a direct action on CGRP receptors.

The potency of CGRP to induce hypotensive and vasodilator responses in the conscious rat was at least 10-fold that of SP. The same was also true of the ability of the peptides to produce tachycardia. Previous studies have shown that SP is one of the most potent vasodilator mediators (Pernow, 1983). In a pre-

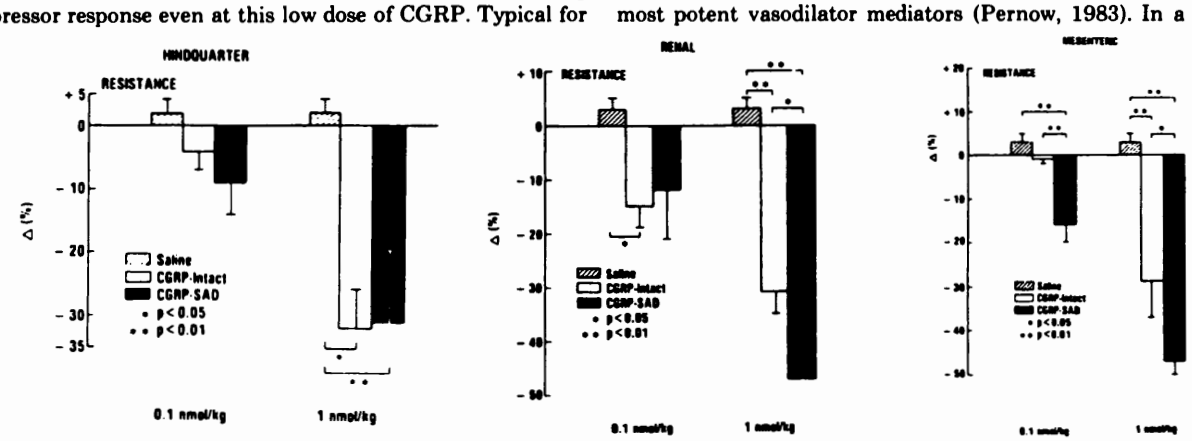

Fig. 7. Effect of rat CGRP i.v. on regional vascular resistance in SAD and intact rats. Values (mean ± S.E.) indicate maximum responses 30 sec to 2 min after CGRP injections. Each group comprised six to eight rats. Asterisks denote statistical significance by Student-Newman-Keul's test.

TABLE 4

Maximum hemodynamic responses to CGRP i.c.v. in conscious intact and SAD rats

HR, heart rate; BF, blood flow; VR, vascular resistance; HQ, hindquarter; R, renal; M, mesenteric.

	ΔMAP	ΔHR	ΔBF (%)			ΔVR (%)		
			HQ	R	M	HQ	R	M
Intact								
Saline, i.c.v.	+5	−7	+6	0	+2	+1	+2	−1
	±4	±16	±7	±2	±2	±5	±3	±4
0.1 nmol/kg i.c.v.	+3	−11	+8	−9	+10	−5	+14	−3
	±3	±8	±4	±4	±8	±4	±5	±5
1 nmol/kg i.c.v.	+7	+33	+6	+2	−6	−4	+1	+15
	±7	±16	±4	±1	±4	±3	±2	±9
10 nmol/kg i.c.v.	+7	+78	+15	−8	−5	−10	+15	+11
	±2	±37*	±5	±4	±5	±4	±6	±4
SAD								
Saline, i.c.v.	+5	−28	−2	0	0	0	−3	+8
	±3	±9	±2	±0	±2	±5	±4	±8
0.1 nmol/kg i.c.v.	+18	−11	+13	−8	−10	−7	+12	+28
	±4	±20	±8	±4	±5	±6	±8	±9
1 nmol/kg i.c.v.	+14	+36**	+19	−7	−11	−13	+16	+24
	±5	±9	±5	±4	±4	±5	±5	±11
10 nmol/kg i.c.v.	+19	+52**	+7	−2	−4	+7	+48	+20
	±8	±10	±5	±5	±2	±9	±12	±8

* $P < .05$; ** $P < .01$ *vs.* saline (Student-Newman-Keuls' test).

vious study in the pithed rat, CGRP was 10-fold more potent to produce hypotension than SP but had less than one-third of the potency of SP to cause plasma extravasation (Haass and Skofitisch, 1985). CGRP was more potent than SP to induce dilation in the rabbit microvasculature (Öhlén *et al.*, 1987). In the rat skin, SP was shown to potentiate CGRP-induced edema (Brain and Williams, 1985). CGRP and SP are colocalized in the perivascular nerves of the vascular tissue (Lundberg *et al.*, 1985, Uddman *et al.*, 1986; Öhlén *et al.*, 1987). These data taken together suggest that participation of both CGRP and SP in vascular responses to sensory nerve stimulation. CGRP and SP are probably acting in an additive manner and the effects of CGRP seem to be independent of SP release as the SP antagonist failed to modify any of the hemodynamic changes produced by CGRP. Our data are supported by the recent observations that the effects of SP but not those of CGRP were blocked by the SP-antagonist, spantide, in the guinea pig arterioles (Uddman *et al.*, 1986). The lack of a CGRP antagonist, however, makes it difficult to evaluate the role of CGRP in vascular responses to SP.

Administration of CGRP into the cerebral ventricles elicited a modest tachycardic response but was devoid of any other hemodynamic actions in the conscious rat. Our data contradict the findings by Fisher *et al.* ((1983) which showed marked increases in heart rate and plasma epinephrine content together with a moderate pressor response after i.c.v. injections of equal doses of CGRP in the conscious rat. Mild tachycardia (+60 bpm) and a slight hypertensive response (+10 mm Hg) was also found after local application of CGRP into the central amygdaloid nucleus of the halothane-anesthetized rat (Nguyen *et al.*, 1986) but these findings are difficult to interpret due to the extremely slow onset of the responses (the effects became apparent 30 to 40 min after CGRP administration) and the relatively high injection volume (200 nl). There is no clear explanation for the discrepancy between our data and the earlier findings (Fisher *et al.*, 1983). Technical problems such as a misplacement of the i.c.v. cannula were not the reason for the lack of effect of CGRP in our study, as the injection into the ventricular space was confirmed after each experiment with an injection of dye into the cannula. In addition to CGRP, every rat was tested with the centrally active agent, thyrotropin releasing hormone which consistently produced a strong pressor/tachycardic response (Sirén *et al.*, 1988). CGRP was diluted fresh each day and was capable of producing the full scope of cardiovascular responses after systemic (i.v.) administration at lower doses than those used in the i.c.v.

In conclusion, the present study shows that systemic administration of CGRP decreases arterial pressure by reducing vascular resistance in mesenteric, renal and hindquarter skeletal muscle blood vessels. The hypotensive and vasodilator actions of CGRP i.v. are most likely mediated by a direct action on peripheral vasculature, whereas the tachycardic response seems to be dependent primarily upon reflex activation of the sympathetic outflow. In conjunction with the earlier neuroanatomical data, our findings suggest a role for CGRP as a local regulator of peripheral vascular tone and regional blood flow but unlike previous reports do not support a role for CGRP in the central cardiovascular control. The strong vasodilator potency of CGRP may warrant a therapeutic role for this peptide in clinical situations in which the selective regional vasodilation and increase in cardiac output are desired (*e.g.* congestive heart failure). These hypotheses, however, await further investigation.

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References

Amara, S. G., Jonas, V., Rosenfeld, M. G., Ong, E. S., and Evans, R. M.: Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature (Lond.) **298:** 240–244, 1982.

Asimakis, G. K., DiPette, D. J., Conti, V. R., Holland, O. B. and Zwischenberger, J. B.: Hemodynamic action of calcitonin gene-related peptide in the isolated rat heart. Life Sci. **41:** 597–603, 1987.

Brain, S. D. and Williams, T. J.: Inflammatory edema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. Br. J. Pharmacol. **86:** 855–860, 1985.

Brain, S. D., Williams, T. J., Tippins, J. R., Morris, H. R. and MacIntyre, I.: Calcitonin gene-related peptide is a potent vasodilator. Nature (Lond.) **313:** 54–56, 1985.

DiPette, D. J., Schwarzenberger, K., Kerr, N. and Holland, O. B.: Systemic and regional hemodynamic effects of calcitonin gene-related peptide. Hypertens. **9:** Suppl. III, III142–III146, 1987.

Dixon, W. J. and Brown, M. B.: Biomedical Computer Programs. P-Series, University of California Press, Los Angeles, 1979.

Ezra, D., Laurindo, F. R. M., Goldstein, D. S., Goldstein, R. E., and Feuerstein, G.: Calcitonin gene-related peptide: A potent modulator of coronary flow. Eur. J. Pharmacol. **137:** 101–105, 1987.

Fisher, L. A., Kikkawa, D. O., Rivier, J. E., Amara, S. G., Evans, R. M., Rosenfeld, M. G., Vale, W. W. and Brown, M. R.: Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide. Nature (Lond.) **305:** 534–536, 1983.

Flaim, S. F. and Zelis, R. F.: Regional distribution of cardiac output in conscious rats at rest and during exercise. Chest **78:** (Suppl.) 187–192, 1980.

Flower, R. J.: Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. **26:** 33–67, 1974.

Franco-Cereceda, A., Gennari, C., Nami, R., Agnusdei, D., Pernow, J., Lundberg, J. M. and Fisher, J. A.: Cardiovascular effects of calcitonin gene-related peptides I and II in man. Circ. Res. **60:** 393–397, 1987.

Golzman, D. and Mitchell, J.: Interaction of calcitonin and calcitonin gene-related peptide at receptor sites in target tissues. Science (Wash. DC) **227:** 1343–1345, 1985.

Goodman, E. C. and Iversen, L. L.: Calcitonin gene-related peptide: Novel neuropeptide. Life. Sic. **38:** 2169–2178, 1986.

Haass, M. and Skofitisch, G.: Cardiovascular effects of calcitonin gene-related peptide in the pithed rat: Comparison with substance P. Life Sci. **37:** 2085–2090, 1985.

Hanko, J., Hardebo, J. E., Kåhrström, J., Owman, C. and Sundler, F.: Calcitonin gene-related is present in mammalian cerebroventricular nerve fibres and dilates pial and peripheral arteries. Neurosci. Lett. **57,** 91–95, 1985.

Haywood, J. R., Shaffer, R. A., Fastenow, C., Fink, G. D. and Brody, M. J.: Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. Am. J. Physiol. **241:** H273–H278, 1981.

HOLMAN, J. J., CRAIG, R. K. AND MARSHALL, I.: Human α- and β-CGRP and rat α-CGRP are coronary vasodilators in the rat. Peptides 7: 231–235, 1986.
ISHISE, S., PEGRAM, B. L., YAMAMOTO, J., KITAMURA, Y. AND FRÖLICH, E. D.: Reference sample microsphere method: Cardiac output and blood flows in conscious rat. Am. J. Physiol. 239: H443–H449, 1980.
KRIEGER, E. M.: Neurogenic hypertension in the rat. Circ. Res. 15, 511–521, 1964.
LAPPE, R. W., SMITS, J. F. M., TODT, J. A., DEBETS, J. J. M. AND WENDT, R. L.: Failure of atriopeptin II to cause arterial vasodilation in the conscious rat. Circ. Res. 56: 606–612, 1985.
LAPPE, R. W., TODT, J. A. AND WENDT, R. L.: Regional vasodilator actions of calcitonin gene-related peptide in conscious SHR. Peptides 8: 747–749, 1987.
LUNDBERG, J. M., FRANCO-CERECEDA, A., HUA, X., HÖKFELT, T. AND FISHER, J. A.: Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. Eur. J. Pharmacol. 108: 315–319, 1985.
MARSHALL, I., AL-KAZWINI, S. J., HOLMAN, J. J., AND CRAIG, R. K.: Human and rat α-CGRP but not calcitonin cause mesenteric vasodilation in rats. Eur. J. Pharmacol. 123: 217–222, 1986a.
MARSHALL, I., AL-KAZWINI, S. J., ROBERTS, P. M., SHEPPERSON, N. B., ADAMS, M. AND CRAIG, R. K.: Cardiovascular effects of human and rat CGRP compared in the rat and other species. Eur. J. Pharmacol. 123: 207–216, 1986b.
MULDERRY, P. K., GHATEI, M. A., RODRIGO, J., ALLEN, J. M., ROSENFELD, M. G., POLA K, J. M. AND BLOOM, S. R.: Calcitonin gene-related peptide in cardiovascular tissues of the rat. Neuroscience 14: 947–954, 1985.
NGUYEN, K. Q., SILLS, M. A. AND JACOBOWITZ, M. A.: Cardiovascular effect produced by microinjections of calcitonin gene-related peptide into the rat central amygdaloid nucleus. Peptides 7: 337–339, 1986.
ÖHLÉN, A., LINDBOM, L., STAINES, W., HÖKFELT, T., CUELLO, A. C., FISHER, J. A. AND HEDQVIST, P.: Substance P and calcitonin gene-related peptide: Immunohistochemical localization and microvascular effects in rabbit skeletal muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. 336: 87–93, 1987.
OSBORN, J. W., BARBER, B. J., QUILLEN, E. W., ABRAM, R. J. AND COWLEY, A. W.: Chronic measurement of cardiac output in unanesthetized rats using miniature thermocouplers. Am. J. Physiol. 251: H1365–H1372, 1986.
PERNOW, B.: Substance P. Pharmacol. Rev. 35: 85–141, 1983.
ROSENFELD, M. G., MERMOD, J.-J., AMARA, S. G.: SWANSON, L. W., SAWCHENKO, P. E., RIVIER, J., VALD, W. W. AND EVANS, R. M.: Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature (Lond.) 304: 129–135, 1983.
SAITO, A., KIMURA, S. AND GOTO, G.: Calcitonin gene-related peptides as potential neurotransmitter in guinea pig right atrium. Am. J. Physiol. 250: H693–H698, 1986.
SIRÉN, A.-L., LAKE, C. R. AND FEUERSTEIN, G.: Hemodynamic and neural mechanisms of action of thyrotropin releasing hormone in the rat. Circ. Res. 62: 139–154, 1988.
SIRÉN, A.-L., POWELL, E. AND FEUERSTEIN, G.: Thyrotropin releasing hormone in hypovolemia: A hemodynamic evaluation in the rat. Am. J. Physiol. 250: H1093–H1101, 1986.
TSCHOPP, F. A., HENKE, H., PETERMANN, J. B., TOBLER, P. H., JANZER, R., HÖKFELT, T., LUNDBERG, J. M., CUELLO, C. AND FISHER, J. A.: Calcitonin gene-related peptide and its binding sites in the human central nervous system and pituitary. Proc. Natl. Acad. Sci. U.S.A. 82: 248–252, 1985.
UDDMAN, R., EDVINSSON, L., EKBLAD, E., HÅKANSON, R. AND SUNDLER, F.: Calcitonin gene-related peptide (CGRP): Perivascular distribution and vasodilatory effects. Reg. Pept. 15: 1–23, 1986.
UNGER, T., BECKER, H., PETTY, M., DEMMERT, G., SCHNEIDER, B., GANTEN, D. AND LANG, R. E.: Differential effects of central angiotensin II and substance P on sympathetic nerve activity in conscious rats. Circ. Res. 56: 563–575, 1983.
WALKER, B. R.: Evidence for a vasodilatory effect of vasopressin in the conscious rat. Am. J. Physiol. 251: H34–H39, 1986.
WANG, X., JONES, S. B., ZHOU, Z., HAN, C. AND FISCUS, R. R.: Calcitonin gene-related peptide (CGRP) levels in plasma are greatly elevated during endotoxic shock in the rat. Circ. Shock 24: 242, 1988.
WERBER, A. H., BRYAN, W. J., AND FINK, G. D.: Hemodynamic and neural mechanisms of acute neurogenic hypertension in the rat. Am. J. Physiol. 247: H991–H998, 1984.
ZAIDI, M., BEVIS, P. J. R., GIRGIS, S. I., LYNCH, C., STEVENSON, J. C. AND MACINTYRE, I.: Circulatory CGRP from perivascular nerves. Eur. J. Pharmacol. 117: 183–284, 1985.

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