Regional haemodynamic effects of μ-, δ-, and κ-opioid agonists microinjected into the hypothalamic paraventricular nuclei of conscious, unrestrained rats

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1   The cardiovascular effects of bilateral injection into the hypothalamic paraventricular nuclei of selective μ-, δ-, and κ-opioid receptor agonists were investigated in conscious, unrestrained Wistar Kyoto rats, chronically instrumented with pulsed Doppler flow probes for measurement of regional haemodynamics.

2   The selective μ-agonist [D-Ala^2,MePhe^4,Gly^{5}ol]enkephalin (DAMGO), injected bilaterally into the hypothalamic paraventricular nuclei (0.01 – 1.0 nmol), caused increases in blood pressure, tachycardias, vasoconstriction in renal and superior mesenteric vascular beds and substantial vasodilatation in the hindquarter vascular bed.

3   The administration of increasing doses (0.01 – 5.0 nmol) of the selective δ-agonist [D-Phe^{2,5}]enkephalin (DPDPE) or the selective κ-agonist, U50488H into the paraventricular nuclei (PVN) had no significant effect on blood pressure, heart rate, or regional haemodynamics.

4   Together, the present results are further evidence of a role for opioid peptides, especially acting at μ-receptors in the PVN, in the central regulation of the cardiovascular system, whereas a role for opioid peptides, acting at δ- and κ-receptors in the PVN, seems less obvious from the present results.

Keywords: Regional haemodynamics; hypothalamic paraventricular nuclei; blood pressure; heart rate, opioid agonists

Introduction

There is accumulating evidence to suggest a role for endogenous opioid peptides in central cardiovascular control (Holaday, 1983; Feuerstein, 1985; Feuerstein & Sirèn, 1987; Sirèn & Feuerstein, 1992). Mainly, opioid peptides and opiate receptors have been found in specific brain nuclei, with an established role in the regulation of cardiovascular activities (Atweh & Kuhar, 1977; Hokfelt et al., 1977; Fallon & Leslie, 1986; Mansour et al., 1988; Desjardins et al., 1990), and potent cardiovascular effects have been reported following central administration of opioid peptides (Hassen et al., 1983; Pfeiffer et al., 1983a,b; Appel et al., 1986; Kiritsy-Roy et al., 1986; Marson et al., 1989a,b; May et al., 1989; Sirèn et al., 1989; Jin & Rockhold, 1991; Sirèn & Feuerstein, 1991). However, pharmacological studies with opioid ligands have revealed a complex pattern of cardiovascular responses, which has been attributed to the multiple opioid receptors, the type of opioid ligand and its selectivity toward specific opioid receptor, the state of consciousness of the experimental animals, the site(s) of injection and dosage, species, and experimental conditions (i.e., stressed versus resting animals) (Holaday, 1983; Feuerstein, 1985).

The existence of at least three subtypes of opioid receptors, namely, μ-, δ-, and κ-receptors, in the mammalian central nervous system has now been generally accepted (Paterson et al., 1983; Martin, 1984; Goldstein & Naidu, 1989; Reisine & Bell, 1993). Although the functional implications of different opioid receptor subtypes is still difficult to establish, pharmacological studies have shown that activation of different opioid receptor subtypes may have different functional consequences. Unfortunately, some of the opioid ligands used in previous studies are relatively nonselective and have significant affinities for more than one subtype of receptor (Goldstein & James, 1984; James & Goldstein, 1984; Mulder et al., 1989), which makes it difficult to distinguish receptor-specific cardiovascular activity. However, the development of enkephalin analogues

with more selective affinities toward each opioid receptor subtype permits further examination of the role of specific opioid receptors in cardiovascular regulation.

Previous studies have demonstrated that i.c.v. or intracisternal injection of the highly selective μ-opioid receptor agonist [D-Ala^2,MePhe^4,Gly^5-ol] enkephalin (DAMGO) (Handa et al., 1981) in conscious animals causes a large increase in blood pressure, a biphasic heart rate response (bradycardia followed by tachycardia) and increases in renal sympathetic nerve activity and plasma catecholamine (Pfeiffer et al., 1982; 1983b; Appel et al., 1986; Marson et al., 1989a,b: May et al., 1989; Matsumura et al., 1992). However, the cardiovascular responses to i.c.v. or intracisternal administration of opioid peptides may reveal little about the discrete functions of the opioid peptides in specific cardiovascular nuclei, as these approaches do not permit accurate localization of the responsible population of neurones. Indeed, activation of opioid receptors in different nuclei in the same region of the brain can induce opposite cardiovascular effects (Morilak et al., 1990; Drolet et al., 1991). The presence of multiple opioid receptors in the brain and their heterogeneous distribution in various brain nuclei (Goodman et al., 1980; Mansour et al., 1988; May et al., 1989; Desjardins et al., 1990) suggest that the cardiovascular effects of centrally administered opioids are highly dependent on the receptor selectivity and the site of drug administration. Therefore, in the present study, we characterized the cardiovascular responses to microinjection of selective opioid receptor agonists into a discrete brain area, the hypothalamus paraventricular nuclei (PVN).

There is considerable evidence to indicate that the PVN play an important role in integrating autonomic control of the cardiovascular system (Sawchencko & Swanson, 1982a,b; Swanson & Sawchencko, 1983). Neuroanatomical and electrophysiological studies have demonstrated that the PVN are reciprocally connected to a number of brain areas thought to be important in cardiovascular regulation (Caverson et al., 1983; Swanson & Sawchencko, 1983; Yamashita et al., 1984; Kannan & Yamashita, 1985; Luiten et al., 1985; Strack et al.,

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1989a,b) and important cardiovascular effects have been reported following electrical or chemical stimulation of neurones in PVN (Cireillo & Calaresu, 1980; Jin & Rockhold, 1989; Kannan et al., 1989; Martin et al., 1991; Martin & Haywood, 1992). The PVN have numerous enkephalin-containing neurones and opioid binding sites for μ-, δ-, and κ-receptors (Sar et al., 1979; Goodman et al., 1980; Sawchenko & Swanson, 1982a; Wamsley, 1983; Fallon & Leslie, 1986; Desjardins et al., 1990). Previous studies have shown that microinjection of the selective μ-opioid agonist DAMGO into the PVN produces dose-related increases in blood pressure, heart rate, and plasma levels of catecholamine in conscious rats (Appel et al., 1986; Kiritsy-Roy et al., 1986). Although the effects of μ-opioid receptor activation on blood pressure and heart rate are well known, little is known about the regional haemodynamic mechanisms underlying these actions, and even less is known about the cardiovascular effects resulting from stimulation of PVN δ- and κ-opioid receptors. The study of regional haemodynamics is of major importance, given that a central injection of opioid agonist (or any drug) may fail to produce changes in either blood pressure or heart rate while producing major but opposite changes in vascular resistance in different vascular beds.

The present study was undertaken to investigate the regional haemodynamic effects produced by bilateral injection into the PVN of opioid agonists known to have a high degree of subtype selectivity. Thus, blood pressure, heart rate, and regional haemodynamic responses to PVN administration of DAMGO have been compared with the cardiovascular responses to the δ-opioid receptor agonist [D-Pen^{2,5}]enkephalin (DPDPE) (Mosberg et al., 1983; Gulya et al., 1986; Goldstein & Naidu, 1989) and the κ-opioid receptor agonist U50488H (Von Voigtlander et al., 1983). We used rats that were conscious and unrestrained to avoid interference from anaesthetic agents (Van Loon, 1984).

Methods

Male Wistar Kyoto rats (250–300 g; from Charles River) were anaesthetized with a mixture of ketamine-xylazine (100 and 10 mg kg^{-1}, respectively, i.p., supplemented as required) and then positioned in a stereotaxic frame with the incisor bar set at 3.3 mm below the interaural line. The skull was exposed and cleaned, and two 23-gauge stainless steel guide cannulae targeted 2 mm dorsal to the PVN were obliquely implanted (angle of 10° relative to the vertical) according to the following coordinates: 1.90 mm caudal and ± 1.75 mm lateral to the bregma and 6.3 mm ventral to the surface of the skull. The cannulae were secured to the skull with screws and dental cement. Patency of the guide cannulae was ensured by inserting 31-gauge stainless steel stylets fashioned to extend 0.5 mm beyond the end of the 23-gauge guides and maintained in place with a piece of silastic tubing. The reflected muscles and skin were replaced and sutured. After surgery the animals were treated with ampicillin (Polyflex, Ayerst, 7 mg kg^{-1}, i.m.) and flunixin (Banamine, Schering, 1 mg kg^{-1}, i.m.), housed in individual cages, and allowed to recover.

At least 7 days later, the rats were re-anaesthetized with a mixture of ketamine-xylazine (100 and 10 mg kg^{-1}, respectively, i.p., supplemented as required) and had pulsed Doppler flow probes implanted around the left renal and superior mesenteric arteries and the lower abdominal aorta, as described previously (Haywood et al., 1981; Bachelard et al., 1992; 1994). After operation, the rats were given i.m. injections of ampicillin (7 mg kg^{-1}) and flunixin (1 mg kg^{-1}) and allowed to recover for at least 7 days. After this period, the rats were re-anaesthetized with a mixture of ketamine-xytazine (100 and 10 mg kg^{-1}, respectively, i.p., supplemented as required). The leads of the implanted probes were soldered to a six-way microconnector (Microtech Inc.), which was connected to a pulsed Doppler monitoring system (VF-1 mainframe, Crystal Biotech) to check the quality of the signals. Any animal not

showing good-quality signals (signal : noise ratio >20 : 1) from all three probes was rejected from the study. Those that met this criterion had one catheter implanted in the right jugular vein (for drug administration) and one in the distal abdominal aorta via the femoral artery (for measurement of blood pressure and heart rate). The catheters were tunnelled subcutaneously to emerge at the same point as the Doppler probe wires. The microconnector, soldered to the Doppler probe wires, was clamped in a custom-made harness worn by the rat, and the catheters were passed through a flexible, protecting spring attached to the harness. Experiments were begun following an overnight recovery period of 18 to 20 h.

Continuous recordings were made of heart rate, phasic and mean blood pressures, and phasic and mean Doppler shift signals from renal, mesenteric, and hindquarters probes using a pulsed Doppler monitoring system (Crystal Biotech, Holliston, U.S.A.), modified to operate with a pulse repetition frequency of 125 kHz (Gardiner et al., 1990). It has been shown that % change in the Doppler shift signal is a reliable index of change in blood flow (Haywood et al., 1981; Wright et al., 1987). At selected time points (averaged over 20 s) heart rate, mean blood pressure, and mean Doppler shifts were measured and related to the predrug baseline (absolute changes for the former two variables, percentages for the Doppler shifts). In addition, regional vascular conductances were calculated by dividing the appropriate mean Doppler shift by the mean arterial blood pressure. Before every experiment, baseline measurements were made over a 30 min period. The rats were allowed free access to food and water for the duration of the experiment.

Bilateral injections were made directly into the PVN of undisturbed, conscious, freely moving rats through 31-gauge stainless steel injectors that extended 2 mm beyond the previously implanted guide cannulae. The injectors were connected via polyethylene tubing to two Hamilton microsyringes (5 μl) and inserted into the guide cannulae without handling the rats. The animals were allowed to settle following this procedure so that remote injections could be made while they were undisturbed. All solutions for microinjections were freshly prepared. The injection volume was 0.2 μl, delivered by hand simultaneously into both sides for 1 min.

At the end of the experiments, all animals received an injection of 0.2 μl of India ink to mark the placement of the cannula tip. The placements of the microinjection sites were verified histologically in serial coronal sections (40 μm, cut on a freezing microtome), mounted on glass slides, and stained with neutral red (Figure 1). Of a total of 30 rats, six were rejected from the study because one or both cannulae tips were not within the PVN. An animal was considered successfully injected when both cannulae tips were shown to be in the PVN or within 0.5 mm of the PVN according to the atlas of Paxinos & Watson (1986). No apparent anatomical associated differences were observed in individual cardiovascular responses to microinjections of DAMGO, DPDPE, or U50488H within this area. A schematic map showing the mean of sites of injection within the PVN and the sites where failures were observed is illustrated in Figure 1b.

Experimental protocols

Cardiovascular responses to PVN injections of opiate agonists The rats were used on four consecutive days, during which they received eight randomized bilateral injections of DAMGO, DPDPE, or U50488H into the PVN at doses ranging from 0.01 to 1 nmol (for DAMGO) and 0.01 to 5 nmol (for DPDPE and U50488H) per microinjection site. The agonists were dissolved in artificial cerebrospinal fluid (aCSF), pH 7.4 (vehicle), which served as the control injection. On a single day, no rat received more than two bilateral injections, separated by at least 180 min, by which time all monitored variables had returned to control levels. On the first day, agonist injection into each rat was preceded by an injection of vehicle. The composition of the aCSF (in mM) was: NaCl 125,

$NaHCO_3$ 27, KCl 2.5, NaH_2PO_4 0.5, Na_2HPO_4 1.2, Na_2SO_4 0.5, $CaCl_2$ 1.0, $MgCl_2$ 1.0 and glucose, 5.0. Cardiovascular variables were recorded for 60 min following each injection.

Cardiovascular responses to i.v. injections of DAMGO   In this experiment, a separate group of rats ($n = 14$) received i.v. bolus injections of agonist vehicle (0.1 ml) and DAMGO (2 nmol), in that order, to determine whether the effects of DAMGO were due to leakage into the periphery. Injections were separated by at least 180 min. Measurements were made before, during, and 15 min after i.v. injections of vehicle or DAMGO.

Drugs

The drugs used were DAMGO ([D-Ala^2,$MePhe^4$,Gly^5-ol]enkephalin; Bachem), DPDPE ([D-$Pen^{2,5}$]enkephalin; BACHEM), and U50488H (*trans*-($\pm$)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methane sulphonate; RBI).

Data analysis

Values are expressed as the mean $\pm$ s.e. mean; n is the number of observations. Results were analysed for statistical sig-
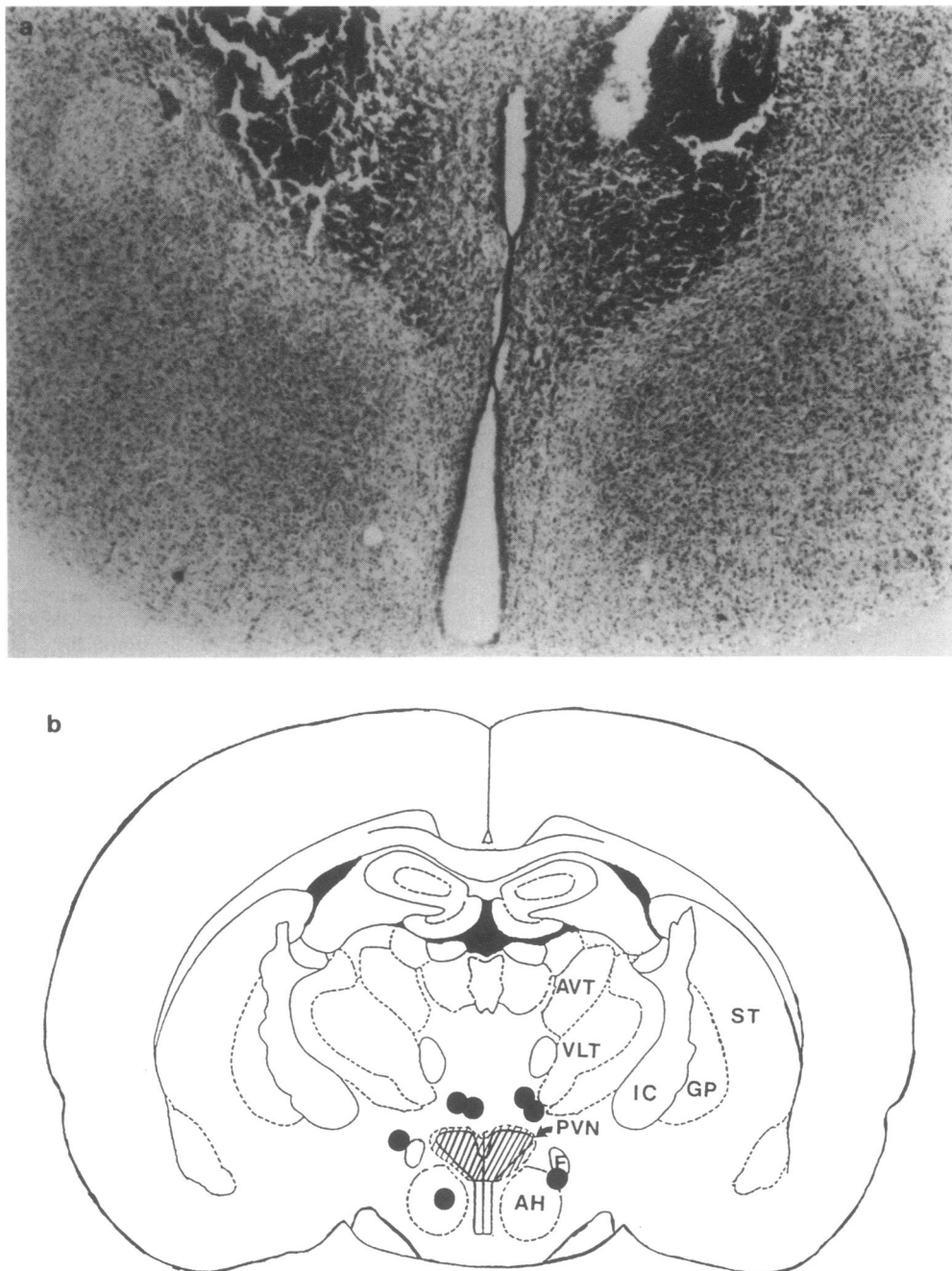

Figure 1   Representative micrograph (a) showing the location of the injection sites in the paraventricular nuclei (PVN) of the hypothalamus. In (b) is the appropriate diagram modified from Paxinos & Watson (1986) showing the mean of sites of injection (shaded area) within the PVN and the sites where failures were observed (●). A rat was considered successfully injected when both cannulae tips were shown to be in the PVN or within 0.5 mm of the PVN. Incisor bar: 3.3 mm below the interaural line; $AP = 1.9$ mm posterior to the bregma; $L = \pm 1.75$ mm relative to bregma; $V = 8.3$ mm ventral to the surface of the skull. AH, anterior hypothalamic area; AVT, anteroventral thalamic nucleus; F, fornix; GP, globus pallidus; IC, internal capsule; ST, striatum; VLT, ventrolateral thalamic nucleus.

Table 1   Baseline values for heart rate, blood pressure and regional vascular conductance in conscious, unrestrained Wistar Kyoto rats ($n = 18$)

Heart rate (beats min^{-1})	Mean blood pressure (mmHg)	Doppler shift (kHz)			Vascular conductance (kHz $mmHg^{-1}$)10^3		
		Renal	Mesenteric	Hindquarters	Renal	Mensenteric	Hindquarters
345 ± 10	108 ± 3	4.1 ± 0.4	11.0 ± 7.3	2.6 ± 1.0	38.2 ± 3.4	103.9 ± 6.6	24.5 ± 2.1

Values are mean ± s.e.mean; $n =$ number of animals.

nificance by an analysis of variance (ANOVA) with repeated measures using the Macintosh Statview 4 programme. *Post-hoc* comparisons were made using Fisher's test. A P value < 0.05 was taken to indicate a significant difference.

Results

Haemodynamic responses to PVN injection of DAMGO in conscious, unrestrained rats

The baseline values (prior to any drug administration) for cardiovascular variables are listed in Table 1. A control bilateral injection of aCSF (0.2 μl) into the PVN had no significant effect on any measured or calculated variables (Figures 2–5). Figures 2 and 3 show that the lowest dose of DAMGO bilaterally injected into the PVN (0.01 nmol on each side) caused a significant increase in hindquarter flow (significant at 2–4 min), but had no significant effect on blood pressure, heart rate, or renal and superior mesenteric flows. Increases in hindquarter vascular conductance (significant at 2–4 min) ocurred, but no significant change was seen in renal and superior mesenteric vascular conductances. However, the PVN bilateral injection of a higher dose of DAMGO (0.1 nmol on each side) elicted a long-lasting increase in blood pressure (significant at 10–60 min) accompanied by tachycardia (significant at 10–60 min) and increases in hindquarter flow (significant at 2–60 min), but no significant change was seen in renal and superior mesenteric flows compared with measurements following aCSF (Figure 2). The maximum rise in mean arterial blood pressure, after the 0.1 nmol dose, was $+18 \pm 3$ mmHg and was reached 15 min after the injection of DAMGO, but blood pressure remained elevated for 1 h after the administration of DAMGO (0.1 nmol). The maximum rise in heart rate was $+100 \pm 19$ b.p.m. and was reached 15 min after the injection of DAMGO, subsiding in 60 min. The maximum increase in hindquarter flow was $+72 \pm 18\%$ and was achieved 15 min after the injection, but the hindquarter flow remained significantly elevated for 1 h after administration of DAMGO. These responses were associated with falls in renal (significant at 30 min) and superior mesenteric (significant at 5–30 min) vascular conductances and a long-lasting increase in hindquarter (significant at 2–4 and 15–60 min) vascular conductance (Figure 3). The maximum decreases in renal ($-12 \pm 4\%$) and superior mesenteric ($-20 \pm 4\%$) vascular conductances were observed 30 min after administration of DAMGO (0.1 nmol), and the maximum increase in hindquarter vascular conductance ($+48 \pm 15\%$) was observed 15 min after the administration of DAMGO, but it remained high during the entire 60 min observation period.

Bilateral injection of the highest dose of DAMGO tested (1 nmol on each side) into the PVN produced cardiovascular
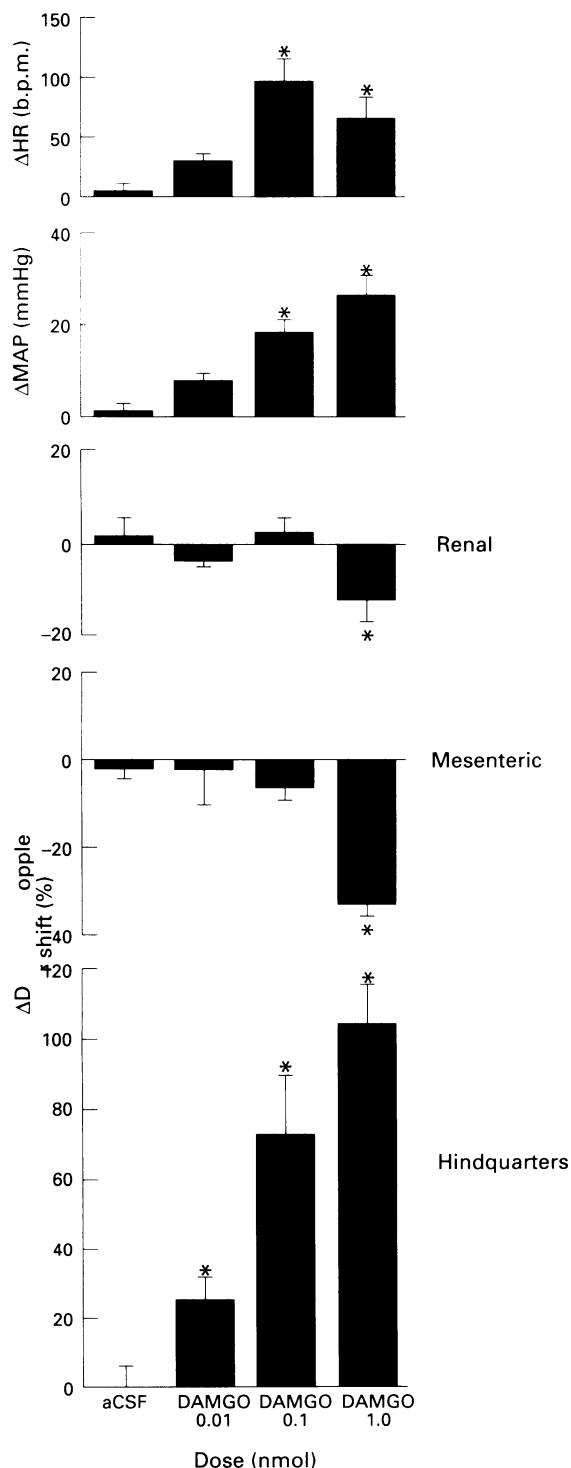
Figure 2 Maximum cardiovascular changes elicited by bilateral microinjection of artificial CSF (aCSF; $n = 14$) or [D-Ala^2,Me-Phe^4,Gly^5-ol]enkephalin (DAMGO) at a dose of 0.01 nmol ($n = 14$), 0.1 nmol ($n = 12$), and 1.0 nmol ($n = 14$) on each side into the paraventricular nuclei of conscious, unrestrained Wistar Kyoto rats.

Comparisons are made between vehicle (aCSF) evoked responses and those to DAMGO. Columns are mean with s.e.mean. *$P < 0.05$ for DAMGO-injected group versus aCSF-injected group, analysis of variance followed by Fisher's test.

effects characterized by a long-lasting increase in blood pressure (significant at 3–60 min) and increases in heart rate (significant at 4 and 15–60 min) (Figures 2 and 4). The maximum rise in blood pressure was $+26\pm4$ mmHg, achieved 45 min after the injection of DAMGO (1 nmol); the tachycardic response reached a maximum of $+66\pm21$ b.p.m. 30 min after the injection. However, both effects remained significantly elevated during the entire 60 min observation period. Furthermore, substantial falls were noted in renal (significant at 5–30) and superior mesenteric (significant at 2–60 min) flows, whereas hindquarter flow increased (significant at 2–60-min) (Figures 2 and 4). The maximum decrease in renal ($-12\pm4\%$) flow was observed 15 min after the administration of DAMGO; the maximum decreases in superior mesenteric ($-33\pm3\%$) and maximum increase in hindquarters ($+104\pm11\%$) flows were reached 30 min after the injection of DAMGO. These blood flow effects in mesenteric and hindquarter vascular beds were still significant during the entire 60 min observation period. The cardiovascular responses to DAMGO (1 nmol) were associated with falls in renal (significant at 2–45 min) and superior mesenteric (significant at 3–60 min) vascular conductances and increases in hindquarter (significant at 2–60 min) vascular conductance (Figures 3 and 5). The maximum decreases in renal ($-30\pm4\%$) and superior mesenteric ($-45\pm3\%$) vascular conductances were observed 45 min after the administration of DAMGO, whereas the maximum increase in hindquarter ($+73\pm8\%$) vascular conductance was reached 30 min after the injection of DAMGO. However, the mesenteric vasoconstrictor and hindquarter vasodilator effects remained significant during the entire 60 min observation period.

It is unlikely that the pressor responses induced by DAMGO injection into the PVN were due to changes in regional blood flow and vascular conductance secondary to increased motor activity. Indeed, compared with baseline controls, PVN injection of DAMGO (0.01, 0.1, and 1.0 nmol) produced no significant locomotor activity over the 60 min observation period. Instead, at all doses of DAMGO tested, the rats dis-
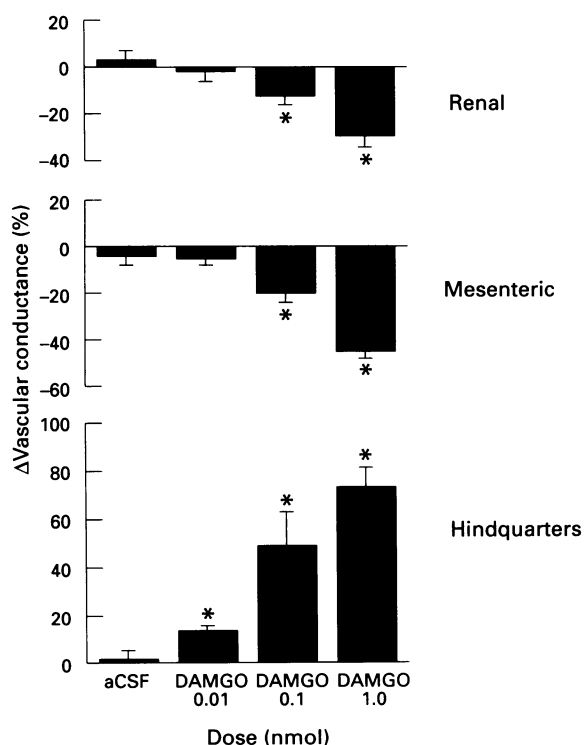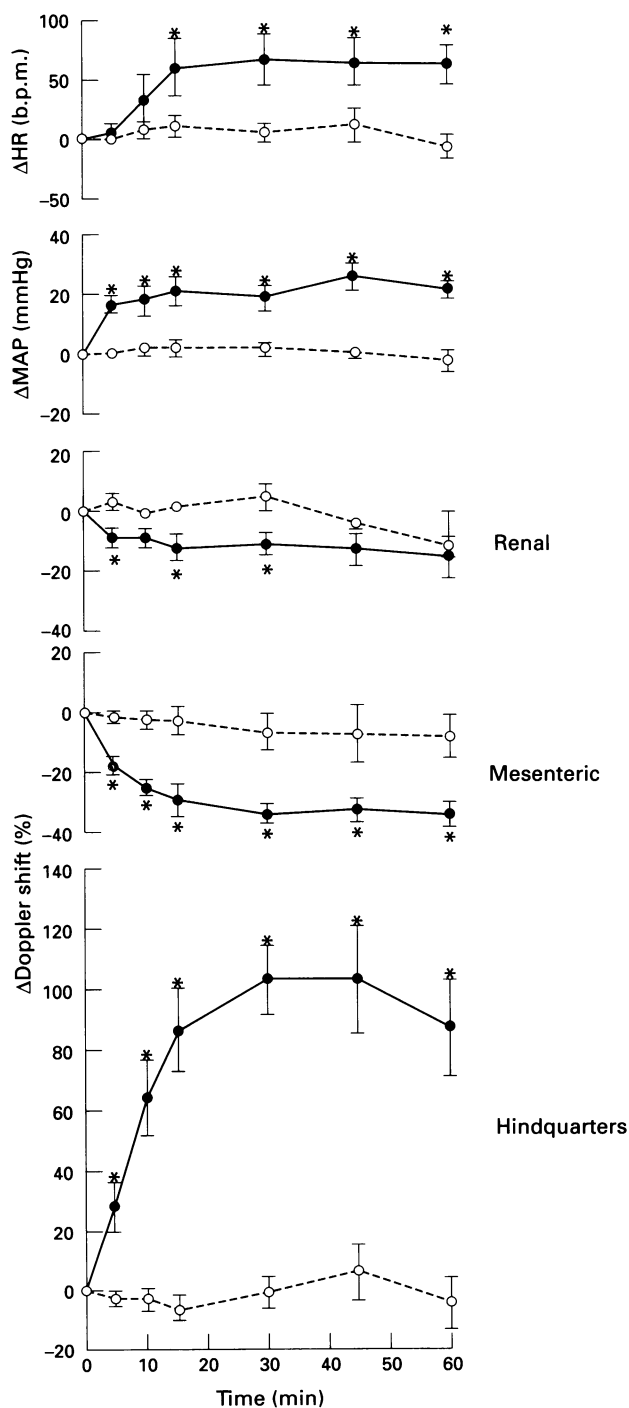

Figure 3 Maximum changes in renal, superior mesenteric and hindquarter vascular conductances produced by bilateral microinjection of artificial CSF (aCSF; $n=14$) or [D-Ala^2,MePhe^4,Gly^5-ol]enkephalin (DAMGO) at a dose of 0.01 nmol ($n=14$), 0.1 nmol ($n=12$) and 1.0 nmol ($n=14$) on each side into the paraventricular nuclei of conscious, unrestrained Wistar Kyoto rats. These data were derived from the data shown in Figure 2. Comparisons are made between vehicle (aCSF) evoked responses and those to DAMGO. Vertical lines represent s.e.mean. $*P<0.05$ for DAMGO-injected group versus aCSF-injected group, analysis of variance followed by Fisher's test.

Figure 4 Cardiovascular changes elicited by bilateral administration of 1 nmol of [D-Ala^2,MePhe^4,Gly^5-ol]enkephalin (DAMGO) on each side (●, $n=14$) of aCSF (○, $n=14$) into the paraventricular nuclei of conscious, unrestrained Wistar Kyoto rats. Values are mean with s.e. mean shown by vertical lines. $*P<0.05$ for DAMGO-injected group versus aCSF-injected group, analysis of variance followed by Fisher's test. BP, blood pressure; HR, heart rate; b.p.m. beats per minute.

played signs of sedation; that is, they remained motionless and piloerected in a corner of the test cage, showing little response to external stimuli.

Haemodynamic responses to PVN injection of DPDPE in conscious, unrestrained rats

The effects of bilateral injection of the δ-opiate agonist, DPDPE at the doses of 0.01 ($n = 6$), 0.1 ($n = 14$), 1.0 ($n = 16$), or
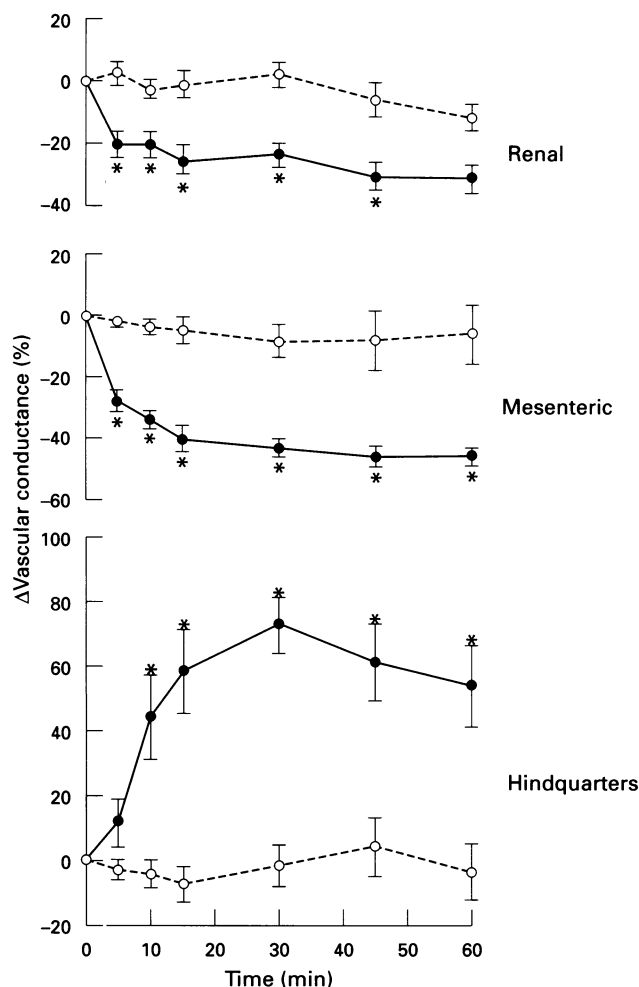

Figure 5 Changes in regional vascular conductances elicited by bilateral administration of 1 nmol of [D-Ala^2,MePhe^4,Gly^5-ol]enkephalin (DAMGO) on each side (●, $n = 14$) or aCSF (○, $n = 14$) into the paraventricular nuclei of conscious, unrestrained Wistar Kyoto rats. These data were derived from the data shown in Figure 4. Values are mean with s.e. mean shown by vertical lines. *$P < 0.05$ for DAMGO-injected group versus aCSF-injected group, analysis of variance followed by Fisher's test.

5.0 nmol ($n = 9$) into the PVN (on each side) were not significant compared with the effects of aCSF injection (data not illustrated).

Haemodynamic responses to U50488H injected into the paraventricular nuclei

The effects of bilateral injection of the κ-opiate agonist, U50488H at the doses of 0.01 ($n = 12$), 0.1 ($n = 11$), 1.0 ($n = 11$) or 5.0 nmol ($n = 8$) into the PVN (on each side) were not significantly different from the effects of aCSF (data not illustrated).

Haemodynamic responses to i.v. injection of DAMGO

The effects of i.v. injection of DAMGO (2 nmol, 0.1 ml) were not significantly different from those of saline (NaCl, 0.9%, 0.1 ml) (Table 2).

Discussion

This study focused on the regional haemodynamic responses to PVN administration of specific opioid-receptor agonists in conscious, unrestrained rats. Our results confirm the previous observation that bilateral administration into the PVN of the highly selective and potent μ-opioid receptor agonist, DAMGO, induced a dose-related increase in blood pressure in conscious, freely moving rats (Appel et al., 1986; Kiritsy-Roy et al., 1986). The present study elucidates the peripheral mechanisms of these changes. Thus, bilateral injection of DAMGO into the PVN caused increases in blood pressure accompanied by marked, dose-related decreases in renal and superior mesenteric vascular conductances and increases in hindquarter vascular conductance. Moreover, tachycardia occurred but was less clearly dose-related. The increases in heart rate elicited by the doses of 0.1 and 1.0 nmol of DAMGO in the PVN were not significantly different, although the magnitude of the tachycardic response tended to be smaller at the highest dose tested (1 nmol). This latter result might be due to a direct effect on vagal outflow or to a baroreceptor-mediated reflex response to the rise in blood pressure. Therefore, if incremental doses of DAMGO injected into PVN cause increasing sympathoadrenal activation (see later), a baroreflex-mediated increase in vagal tone might increasingly counteract the effects of the sympathoadrenal stimulation on the heart and thus obscure any dose-relatedness of the latter; this cannot be determined from the present study. However, previous studies have demonstrated that high doses of DAMGO activate parasympathetic outflow when unilaterally injected into the PVN (Kiritsy-Roy et al., 1986) or into the anterior hypothalamus (Pfeiffer et al., 1983a,b).

Intravenous injections of DAMGO (2 nmol) do not cause cardiovascular effects. Thus, the present results were clearly not due to leakage of DAMGO from its central site of injection into the periphery. Moreover, injection of aCSF (0.2 μl) into the PVN had no cardiovascular effects, indicating that dis-

Table 2 Cardiovascular changes produced by intravenous injection of saline (0.1ml) or [D-Ala^2, MePhe^4, Gly^5-ol] enkephalin (DAMGO, 2 mol) in conscious, unrestrained Wistar Kyoto rats

	ΔHR (beats min^{-1})	ΔMAP (mmHg)	$\Delta Doppler\ shift\ (\%)$			$\Delta Vasuclar\ conductance\ (\%)$		
			Renal	Mesenteric	Hindquarters	Renal	Mesenteric	Hindquarters
Saline ($n = 6$)	14 ± 9	3 ± 3	-1.3 ± 2.0	-4.3 ± 4.6	22.3 ± 12.7	-4.2 ± 1.9	-6.5 ± 6.1	17.8 ± 9.4
DAMGO 2.0 nmol ($= 11$)	6 ± 11	1 ± 5	1.5 ± 4.1	-5.8 ± 1.6	38.1 ± 8.8	0.2 ± 5.1	-7.0 ± 3.4	39.2 ± 14.5

Values are mean $\pm$ s.e.mean; $n =$ number of animals.

placement of the tissue by the volume injected was not a cause of the observed effects. However, we cannot exclude the possibility that the injections could have diffused to regions surrounding the PVN, considering the volume of injection (0.2 μl) we used. Therefore, the effects described in the present paper may have been due to activation of other neighbouring nuclei besides the PVN.

The cardiovascular responses following bilateral injection of DAMGO in the PVN were probably due to an activation of the sympathoadrenomedullary axis. The pattern of changes in regional blood flows (an increase in hindquarter but decreases in the superior mesenteric and renal vascular beds) induced by DAMGO suggests activation of the central sympathetic outflow to the adrenal medulla and sympathetic nerve terminals. The factors underlying the enormous hindquarter vasodilator responses to DAMGO might be due to relatively selective activation of β_2-adrenoceptor-mediated vasodilator mechanisms. This possibility is supported by previous studies showing that central administration of β-endorphin, DAMGO or dermorphin in rats produced a naloxone-reversible increase in plasma catecholamine concentration (Van Loon et al., 1981; Pfeiffer et al., 1983b; Appel et al., 1986; Kiritsy-Roy et al., 1986; Sirén et al., 1989), an increase in sympathetic outflow in peripheral postganglionic sympathetic nerves (Sirén & Feuerstein, 1991), and blockade of the cardiovascular responses by adrenergic neuronal blocking drugs (Jin & Rockhold, 1991; Sirén & Feuerstein, 1991; Pfeiffer et al., 1983b). Plasma adrenaline was far more sensitive to the effects of DAMGO in the PVN, suggesting that the treatment produced a relatively selective activation of the adrenal medulla (Kiritsy-Roy et al., 1986). Moreover, the vascular bed of the hindquarter is particularly well endowed with β_2-adrenoceptors, which mediate vasodilatation (Gardiner & Bennett, 1988). Thus, if DAMGO injected into the PVN causes sympathoadrenal activation, it is feasible that the increase in hindquarter flow and vascular conductance in response to PVN injection of DAMGO was due to relatively selective activation of β_2-adrenoceptor-mediated vasodilator mechanisms, but this cannot be determined from this study.

In the present study, we found no significant cardiovascular effects of PVN administration in increasing doses (0.01 – 5.0 nmol) of the highly selective δ-opioid receptor agonist DPDPE (Mosberg et al., 1983; Gulya et al., 1986; Goldstein & Naidu, 1989). However, a previous study showed that i.c.v. injection of DAMGO or DPDPE in conscious rats produced dose-related increases in sympathoadrenal outflow and blood pressure, but only DAMGO (5 nmol) caused significant changes in heart rate: an atropine-sensitive bradycardia (Marson et al., 1989a,b). These effects were antagonized by the i.c.v. injection of a μ-selective dose of naloxone (Gordon, 1986; Marson et al., 1989b), but these responses were not reversed by the δ-selective antagonist ICI 174,864 (Marson et al., 1989a,b). Moreover, the concentration of DPDPE used in that study to produce cardiovascular effects was far higher than ours (125 nmol). Therefore, considering the relatively high dose of DPDPE used, and because the δ-antagonist ICI 174,864 was without effect, it was suggested that both DAMGO and

DPDPE act on a μ-type brain opioid receptor to modulate cardiovascular responses (Marson et al., 1989a,b). In another study carried out in conscious rabbits, i.c.v. administration of increasing doses (0.01 – 1.0 nmol kg^{-1}) of DPDPE was associated with increases in blood pressure and heart rate, but not with any change in plasma catecholamine (May et al., 1989). Furthermore, the δ-agonist increased blood pressure more than 10 times less potently than DAMGO (May et al., 1989). However, differences between those results and our present findings might be explained by the different site of administration as well as by the different species used.

In the present study, we found no cardiovascular effects following PVN administration of U50488H, a purported highly selective κ-opioid receptor agonist (Von Voigtlander et al., 1983). These results agree with those of Pfeiffer et al. (1982, 1983a) showing that microinjection of a κ-agonist (MR 2034) into the anterior hypothalamic and septal brain regions of conscious rats had no effect on cardiovascular parameters. Similarly, May et al. (1989) reported that i.c.v. injection of the highly selective κ-agonist U69593 and dynorphin A(1-13) in conscious rabbits had no cardiovascular effect. However, in conscious rats, dynorphin A(1-13) produced a transient dose-related pressor effect after i.c.v. administration (Glatt et al., 1987). The difference from the present findings may result from the higher doses used, the different site of administration, or the selectivity of the opioid agonists used. Although dynorphin A(1-13) is known to have actions on κ-receptors, other studies would be required to address whether the cardiovascular responses reported in that study were due to κ-receptor activation (Goldstein & James, 1984; James & Goldstein, 1984; Mulder et al., 1989; Rochford et al., 1991).

In conclusion, by using compounds that are highly selective for the various opioid receptor subtypes, we demonstrated that in conscious rats only DAMGO, a highly selective μ-receptor agonist, injected into the PVN, produced significant cardiovascular effects. Thus, bilateral microinjection of DAMGO into the PVN exerts tachycardia and a hypertensive effect through marked vasoconstrictor actions in the renal and superior mesenteric vascular bed and a substantial vasodilatation in the hindquarter vascular bed. According to previous studies, the cardiovascular responses to DAMGO are probably due to activation of the sympathoadrenomedullary axis. On the other hand, we observed no significant cardiovascular effects after central administration of selective δ- and κ-opioid receptor agonists into the PVN. Together with the specific haemodynamic effects of DAMGO, the present results are further evidence of a role for opioid peptides and μ-opioid receptors in the central regulation of cardiovascular function, whereas the involvement of PVN δ- and κ-receptors in cardiovascular regulation are less obvious from the present findings.

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