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HYPOTHALAMIC OPIOID μ-RECEPTORS REGULATE DISCRETE HEMODYNAMIC FUNCTIONS IN THE CONSCIOUS RAT

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Summary—The effect of the selective μ-opioid agonist D-Ala[2]-Me-Phe[4]-Gly-ol[5]-enkephalin (DAGO), injected into the medial preoptic nucleus of hypothalamus, on cardiac output and regional blood flow was studied in the conscious rat and the effect of DAGO on renal sympathetic nerve activity and renal blood flow was studied in anesthetized rats. In conscious rats, injections of DAGO (1 or 10 nmol) into the preoptic nucleus increased the blood pressure in a dose-related manner. The maximum rises of mean arterial pressure and pulse pressure after the larger dose were $+23 \pm 5$ mmHg (mean ± SEM, $P < 0.01$) and $+17 \pm 3$ mmHg ($P < 0.01$), respectively. A small dose (0.1 nmol) increased heart rate ($+47 \pm 13$ bpm, $P < 0.05$); the 1 nmol dose produced bradycardia (-39 ± 11 bpm, $P < 0.05$), while the 10 nmol dose initially decreased heart rate (-68 ± 15 bpm ($P < 0.01$) and then gradually increased heart rate to a maximum of $+74 \pm 13$ bpm, ($P < 0.01$). A long-lasting increase in cardiac output was also elicited by DAGO, with maximum changes after 1 and 10 nmol of $+14 \pm 6\%$ and $+22 \pm 7\%$ ($P < 0.01$), respectively. Blood flow in the hindquarters increased after DAGO but the mesenteric and renal blood flow decreased in a dose-related manner. Significant responses in hindquarter and mesenteric blood flow after DAGO were independent of systemic hemodynamic responses at the dose of 0.1 nmol. The vascular resistance in the hindquarters significantly decreased after a small dose of DAGO while the larger doses dose-dependently increased mesenteric and renal vascular resistance. A crucial role of the sympathetic nervous system in the hemodynamic effects of DAGO was demonstrated: (1) by the profound activation of renal sympathetic nerve activity after injections of DAGO (1 nmol/100 nl) into the preoptic nucleus, (2) by blockade of the pressor, tachycardic and regional hemodynamic effects of DAGO (1 nmol) by the ganglion blocker chlorisondamine (5 mg/kg i.v.). The results suggest that the pressor effect of DAGO in preoptic nucleus is due primarily to an increase in cardiac output. The differential changes in blood flow in organs further suggest that the opioid μ-receptors in the preoptic nucleus might be involved in the integration of peripheral blood flow in the hypothalamus during affective behavior.

Key words—D-Ala[2]-Me-Phe[4]-Gly-ol[5]-enkephalin (DAGO), chlorisondamine, blood pressure, heart rate, cardiac output, regional blood flow, sympathetic nerve activity.

Opioid peptides and opiate receptors are present in hypothalamic nuclei and opioid peptide containing connections have been demonstrated from the hypothalamus to other cardiovascular nuclei (parabrachial nuclei, nucleus tractus solitarius) and to the preganglionic sympathetic nerve roots in the intermediolateral cell column of the spinal cord (Khachaturian, Lewis, Schafer and Watson, 1985; Mansour, Khatchaturian, Lewis, Akil and Watson, 1988; Pasternak and Wood, 1986). Opioid peptides induce a complex pattern of cardiovascular changes, which has been attributed to multiple opiod receptors: μ- and δ-receptors mediating the pressor and kappa-receptors mediating the depressor responses to centrally-administered opioids (Feuerstein, 1985; Feuerstein and Sirén, 1987). Previous studies in this laboratory have shown that the selective μ-opiate-receptor agonist, D-Ala[2]-Me-Phe[4]-Gly-ol[5]-enkephalin (DAGO) (Handa, Lane, Lord, Morgan, Rance and Smith, 1981) increases blood pressure and causes a biphasic heart rate response (tachycardia at small doses but bradycardia, followed by tachycardia, at large doses) upon injections into the ventricles of the brain or into the medial preoptic-anterior hypothalamic area of the conscious rat (Pfeiffer, Feuerstein, Faden and Kopin, 1982; Pfeiffer, Feuerstein, Kopin and Faden, 1983a; Pfeiffer, Feuerstein, Zerbe, Faden and Kopin, 1983b). In the pentobarbital anesthetized rat, small (pmol) doses of DAGO, injected into the medial preoptic nucleus of hypothalamus, increased heart rate while larger doses produced hypotension and tachycardia (Faden and Feuerstein, 1983). Other investigators discribed pressor and tachycardic responses to DAGO, injected into the paraventricular nucleus in conscious rats (Kiritsy-Roy, Appel, Bobbitt and Van Loon, 1986). The cardiovascular effects of stimulation of μ-opioid receptors in the hypothalamic nuclei have been argued to be due to activation of the sympatho-adrenomedullary system, since concomitantly with their peak effect on blood

pressure and heart rate, centrally-injected selective μ-agonists, like DAGO and dermorphin induce rises of catecholamines in plasma (Kiritsy-Roy *et al.*, 1986; Appel Kiritsy-Roy and Van Loon, 1986; Appel and Van Loon, 1986; Sirén, Paakkari, Goldstein and Feuerstein, 1989). Moreover, the pressor and tachycardic effects of DAGO were attenuated or even reversed in adrenal demedulated rats, treated with the sympathetic blocker bretylium (Pfeiffer *et al.*, 1982). The bradycardic and hypotensive response to centrally injected DAGO, on the other hand, have been related to its strong respiratory depressant action and to an activation of vagal pathways (Faden and Feuerstein, 1983; Pfeiffer *et al.*, 1983b; Hassen, Feuerstein and Faden, 1984). Though the effects of μ-opioid agonists on blood pressure and heart rate are well characterized, little is known about the hemodynamic mechanisms underlying these actions or their effects on discrete redistribution of blood flow in peripheral organs. Since the preoptic nucleus of the hypothalamus might be an important site for the integration of the hemodynamic component of the stress-evoked "defense reaction" (Yardley and Hilton, 1986) and the μ-opioid agonists are known to induce potent systemic hemodynamic responses in this site, it was of interest to further explore the role of μ-opioid receptors in the preoptic nucleus, in the control of cardiac output and redistribution of blood flow to discrete organs. The role of the autonomic nervous system in the systemic and regional hemodynamic responses to DAGO was also studied by treating the rats with the ganglion blocker chlorisondamine prior to administration of DAGO. In order to directly demonstrate sympathetic activation, the effect of DAGO on the activity of efferent renal sympathetic nerves was examined in anesthetized rats and the changes in renal activity in nerves were correlated to the changes in blood flow *in vivo*, by simultaneously recording renal sympathetic nerve activity and renal blood flow.

METHODS

Male Sprague–Dawley rats (260–340 g) were used in all experiments. After the surgical operations, the rats were housed individually in plastic cages (21 × 27 × 16 cm, $H \times L \times H$) with food and water *ad libitum*.

Injections into the brain

The rats were anesthetized wtih an intramuscular injection of ketamine-acepromazine, 0.13 ml/100 g of a solution of ketamine (100 mg/ml) and acepromazine (1 mg/ml) and placed in a stereotaxic device (DKI, California). A stainless steel guide cannula was inserted through the skull and fixed with glue (Eastman 910 adhesive). Coordinates for the injections into the medial preoptic nucleus of the hypothalamus were measured from the bregma: AP = 0.2 mm and L = 0.6 mm. For intracerebroventricular injections, the coordinates from the bregma were: AP = 0.8 mm, L = 1.2 mm, V = 7.5 mm. On the day of the experiment, injection of 0.9% (w/v) NaCl (saline) or DAGO was made by means of a premeasured 30 g-cannula, inserted into the preoptic nucleus (V = 7.7 mm from dura level) through the guide cannula. The injection cannula was then connected by a polyethylene tube to a Hamilton microliter syringe and a volume of 100 nl or 1 μl of the control or solution of drug was injected over a period of 30 sec. The proper position of the injection cannula was confirmed microscopically after the experiments, according to the brain atlas of König and Klippel (1967).

Measurement of cardiac output

The effect of DAGO on cardiac output and total peripheral resistance was investigated according to the following protocol: the rats were anesthetized with ketamine-acepromazine and PE-50 tube inserted into the femoral arteries. These catheters were tunnelled beneath the skin of the back and exited at the back of the neck, as described above. Then, an incision was made at the midline of the neck from the cricoid to the clavicle and a PE-50 tube was inserted into the right atrium, through the external jugular vein. Then, the left common carotid artery was exposed, ligated and a thermistor probe (MX2-780-33 Model THMP f# 1.5, Teflon reusable, Columbus Instruments, Ohio) was advanced through the carotid into the ascending aorta (placement above the aortic valve was confirmed in each animal at the end of the experiment and by the shape of the dilution curve before the probe was finally sutured to the neck muscles). The jugular vein catheter and the thermistor probe was tunnelled under the skin to the back of the neck. All lines were secured by a soft spring wire, throughout the cage. Twenty-four hr after surgery, the arterial line was connected to a blood pressure transducer (Narco RP1500i) and the continuous recording of blood pressure (systolic, diastolic, mean) and heart rate was carried out by the Narcotrace 80 computerized dynograph. The cardiac output was measured by a thermodilution technique, as the thermistor was attached to the computerized Cardiomax II (CMx2-780-k with the microprobe option R, Columbus Instruments, Ohio). The dead-space of the venous line was first flushed with 0.2 ml of saline at room temperature (22°C); after a brief period of stabilization (10 sec to assure normal core temperature), an additional injection of 0.05 ml normal saline (22°C) was rapidly made using a 1 ml syringe. Cardiac output was recorded in the following way: a control period of 15 min included 2–3 measurements of cardiac output to test for consistency and placement of the probe and also to get control values for mean arterial pressure and heart rate. The time on the automatic data collection system was started and data points were taken immediately before and 5, 10, 15, 30, 45, 60, 90 and

120 min after the injection of drug. Total peripheral resistance was calculated by dividing the mean arterial pressure by the cardiac output; values of cardiac output and total peripheral resistance were further indexed per unit of weight (kg).

Measurement of blood flow with the directional pulsed Doppler technique

Another set of rats was used to study the effect of DAGO on regional blood flow and vascular resistance by the directional pulsed Doppler technique. The Doppler method is preferred to the microsphere or electromagnetic flow technique, because it allows continuous measurements of blood flow and vascular resistance in the conscious, freely moving rat. The animals were anesthetized with ketamine-acepromazine and miniaturized Doppler flow probes (Valpey Fisher, Massachusetts) were implanted around the abdominal aorta, superior mesenteric artery and the left renal artery, according to the method described by Haywood, Shaffer, Fastenow, Fink and Brody (1981). Briefly, a midline laparotomy was made and 4 mm lengths of a lower abdominal aorta, below the left renal artery, the superior mesenteric artery and the left renal artery were carefully isolated, with the aid of a dissecting microscope. Minaturized Doppler flow probes were then sutured around each vessel. The wire leads were tunnelled beneath the skin and exteriorized at the nape of the neck, where they were soldered to a connector plug, which was fixed to the skull of the animal with small screws and dental acrylic cement. The animals were allowed to recover for at least 7 days from the surgery. Twenty-four hr before the experiment, the animal was anesthetized with halothane (2% in oxygen) and arterial and venous catheters (PE50) were introduced into the femoral vessels. The catheters were led beneath the skin to exit at the neck and secured in place with an adhesive collar.

On the day of the experiment, the rat was connected to the flow-probe connectors and the connector line was suspended from the top of the home cage to allow freedom of movement during the experiment. Regional blood flow was measured with a directional pulsed Doppler flowmeter (Model # 545C-3, University of Iowa Bioengineering Facility, Iowa). The arterial line was attached to a pressure transducer (as above) and the blood pressure, heart rate and regional blood flows were continuously recorded on the Narco dynograph. Vascular resistance was calculated by dividing the mean arterial pressure by blood velocity (Doppler shift in kilohertz). Changes in blood flow and vascular resistance are expressed as a percentage of control values. In some animals, the vascular reactivity to exogenously administered catecholamines was tested by an injection of epinephrine 0.3–1 μg/kg) and norepinephrine 0.3–1 μg/kg), given intravenously, before the central injections. The responses to these reference substances were in accord with previously published data (Faber, Barron, Bonham, Lappe, Muirhead and Brody, 1984; Sirén, Lake and Feuerstein, 1988).

Recording of activity in renal sympathetic nerve in anesthetized rats

To further support the evidence that stimulation of μ-opioid receptors in the preoptic nucleus activates the sympathetic nervous system, the effect of DAGO in renal sympathetic nerve activity and blood flow was studied in anesthetized rats. In these experiments, the animals were first anesthetized with halothane (2% in oxygen) and arterial and venous catheters (PE50) were inserted into the femoral vessles. Thereafter, the anesthesia was continued with an intravenous infusion of chloralose (100 mg/kg) and the trachea cannulated. The animal was artificially respirated with oxygen during the experiment. From an incision in the left flank, a branch of the renal nerve was traced to the renal artery, separated from the fat and connective tissue and placed on a bipolar platinum hook electrode (Cooner Wire Co., Chatsworth, California). A Doppler flow probe was placed around the renal artery, distal from electrode and the renal blood flow was continuously recorded. The tissue was protected by a pool of warm mineral oil. The nerve signal was amplified ($\times$ 10,000–50,000) and filtered (low 30, high 3000 Hz) with a Grass P511 Bandpass amplifier (Grass Instrument Co., Quincy, Massachusetts) and the amplified and filtered signal was channelled to a Tektronix 5113 oscilloscope (Tektronix Inc., Beaverton, Oregon) for visual evaluation, to an audio amplifier/sound speaker for auditory evaluation and to a rectifying voltage integrator (Narco 730M). The rectified neurogram and integrated nerve signal were displayed on the Narcotrace physiograph. The quality of the nerve signal and blood flow was assessed by examining the magnitude of change in recorded activity of the nerve, during baroreceptor loading and unloading, with an intravenous injection of norepinephrine (1 μg/kg) and sodium nitroprusside (3 μg/kg). The efferent nature of the renal nerve fibers was further verified by complete silence of nerve activity, after injection of hexamethone (20 mg/kg, i.v.). At the end of the experiment, the renal nerve was cut and the zero activity was measured from the distal nerve stump. This remaining activity was substracted from the previously recorded activity of the nerve.

Drugs used

D-Ala^2-Me-Phe^4-Gly-ol^5-Enkephalin (DAGO) (Peninsula, Louisiana) was dissolved in saline and injected into the preoptic nucleus at the dose of 0.1, 1 or 10 nmol/rat. The injection volume in the experiments on blood flow and nerve activity was 100 nl and in the cardiac output studies it was 1 μl. L-Epinephrine bitartrate and *l*-norepinephrine hydrochloride (Sigma, Missouri), sodium nitroprusside

(Roche) and chlorisondamine hydrochloride (Ciba–Geigy) were dissolved in saline and injected intravenously in a volume of 25 μl/100 g body weight.

Statistical analysis of the data

Data in the text and figures are represented as means ± SEM for the indicated number of rats. Analysis of variance with repeated measures (MANOVA, CSS complete Statistical System, Stat-Soft, 1987) and one-way analysis at variance (ANOVA), followed by Student–Newman–Keul test, were used for statistical analysis of the data. A significant difference was accepted at $P < 0.05$.

RESULTS

Effect of DAGO on blood pressure and heart rate (Figs 1 and 2)

Injection of DAGO (1 or 10 nmol) into the preoptic nucleus dose-dependently increased the blood pressure. The maximum rises of mean arterial pressure, after the 1 and 10 nmol doses, were +14 ± 4 mmHg ($P < 0.01$) and +26 ± 4 mmHg ($P < 0.01$), respectively. The changes in systolic, diastolic and pulse pressure after the 1 nmol dose were +19 ± 6 mmHg, +9 ± 5 mmHg and +10 ± 3 mmHg ($P < 0.05$), respectively. The corresponding changes after the 10 nmol dose were +34 ± 8 mm Hg, +16 ± 5 mmHg and +17 ± 3 mmHg ($P < 0.05$), respectively. The pressor response to DAGO became apparent in 3 min after the injection of the drug and reached a maximum 10 min after the injection, but the blood pressure remained elevated for more than an hour after the administration of DAGO. The smallest dose of 0.1 nmol of DAGO had no significant effect on blood pressure.

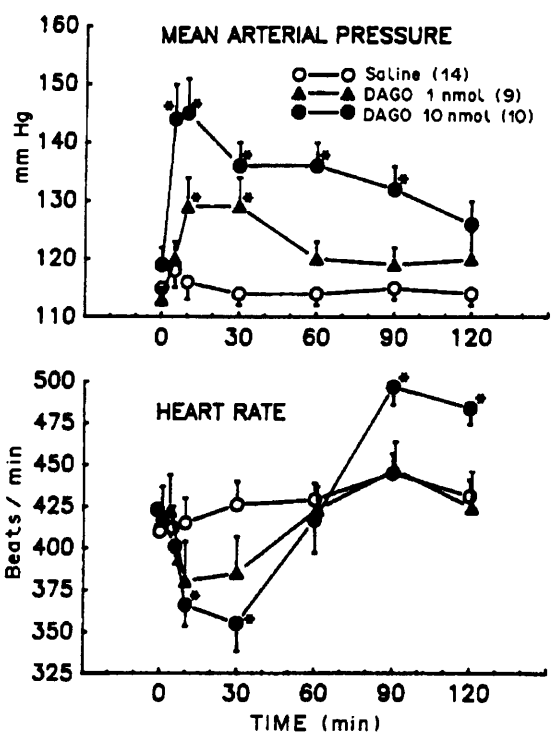

Fig. 1. Effect of DAGO, injected into the preoptic nucleus of the hypothalamus, on mean arterial pressure and heart rate in the conscious rat. Values represent means ± SEM. Asterisks denote statistical significance vs saline group by Student–Newman–Keul test; *$P < 0.05$. The time–response effect at both dose levels was significant when assessed by two-way MANOVA with repeated measures; the F and P-values for the mean arterial pressure were $F = 4.78354$, $P = 0.00000$ (1 nmol vs saline) and $F = 7.73935$, $P = 0.00000$ (10 nmol vs saline), $F = 2.96753$, $P = 0.00458$ (1 nmol vs 10 nmol). The corresponding values for the heart rate were $F = 2.11526$, $P = 0.03653$ (1 nmol vs saline), $F = 8.34186$, $P = 0.00000$ (10 nmol vs saline) and $F = 3.44125$, $P = 0.00151$ (1 nmol vs 10 nmol). The number of animals in each group is given in parentheses.

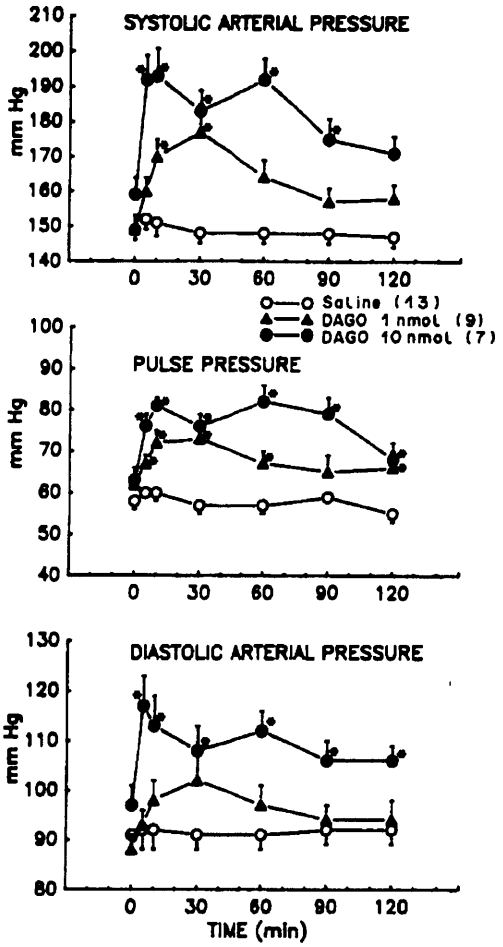

Fig. 2. Effect of DAGO, injected into the preoptic nucleus, on systolic arterial pressure, pulse pressure and diastolic pressure in the conscious rat. Values represent means ± SEM. Asterisks denote statistical significance vs saline group by Student–Newman–Keul test; *$P < 0.05$. The time–response effect at both dose levels was significant when assessed by two-way MANOVA with repeated measures; the F and P-values for the systolic arterial pressure were $F = 6.15946$, $P = 0.00000$ (1 nmol vs saline) and $F = 6.29410$, $P = 0.00000$ (10 nmol vs saline), $F = 2.2159$, $P = 0.03096$ (1 nmol vs 10 nmol). The corresponding values for the pulse pressure were $F = 2.35000$, $P = 0.02023$ (1 nmol vs saline), $F = 5.55830$, $P = 0.00000$ (10 nmol vs saline) and $F = 2.13208$, $P = 0.03798$ (1 nmol vs 10 nmol); and for the diastolic pressure $F = 3.03660$, $P = 0.00378$ (1 nmol vs saline, $F = 3.47959$, $P = 0.00144$ (10 nmol vs saline), the effect at 1 nmol was not significant as compared to the effect at 10 nmol ($F = 1.81552$, $P = 0.08089$). The number of animals in each group is given in parentheses.

The injection of DAGO into the preoptic nucleus induced a biphasic heart rate response: immediately after the 0.1 nmol dose (1–5 min), the heart rate initially decreased slightly (-10 ± 4 bpm), followed by a significant tachycardic response ($+47 \pm 13$ bpm, $P < 0.05$) at 30 min after the injection. The 1 nmol dose decreased heart rate (-39 ± 11 bpm, $P < 0.05$) only, while the 10 nmol dose produced bradycardia (-68 ± 15 bpm, $P < 0.05$, at 10 nmol) about 30 min after injection of the drug and at 45–60 min tachycardia ($+74 \pm 13$ bpm, $P < 0.05$), which subsided in 120 min. Saline (100 nl or 1 μl) had no significant effect on blood pressure or heart rate.

Intracerebroventricular injection of DAGO at the 10 nmol dose induced a transient increase in blood pressure, with a maximum of $+15 \pm 4$ mm Hg ($P < 0.05$) 5 min after the injection (Table 1). The pressor response was succeeded by a decrease in blood pressure reaching a maximum (-23 ± 5 mm Hg, $P < 0.05$) 30 min after intraventricular injection of DAGO. After intraventricular injection, DAGO 10 nmol did not decrease heart rate but produced a tachycardic response which reached a maximum of $+74 \pm 10$ bpm ($P < 0.05$) at 20 min after the injection.

Effect of DAGO on cardiac output and total peripheral resistance (Fig. 3)

Doses of 1 nmol and 10 nmol of DAGO produced a dose-related increase in cardiac output. The maximum rises in cardiac output were $+41 \pm 17$ ml/min/kg ($P < 0.05$) and $+98 \pm 28$ ml/min/kg ($P < 0.01$), respectively. The maximum effect after the 10 nmol dose was achieved 10 min after the injection, but the cardiac output remained significantly elevated for 90 min after administration of DAGO. The increase in cardiac output after the 1 nmol dose subsided in 60 min. The effect of DAGO on total peripheral resistance was not significant, compared to the saline-control, though the total peripheral resistance slightly increased 5 min after injection of the 10 nmol dose. Saline, injected into the preoptic nucleus in a volume of 1 μl, had no effect on these hemodynamic variables.

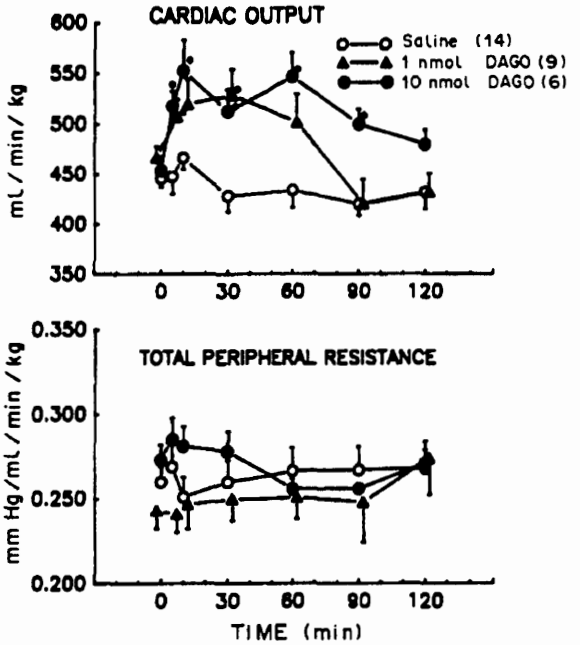

Fig. 3. Effect of DAGO, injected into the preoptic nucleus, on cardiac output and total peripheral resistance in the conscious rat. Values represent means ± SEM. Asterisks denote statistical significance vs saline group by Student–Newman–Keul test: $*P < 0.05$. The time–response effect at both dose levels was significant when assessed by one-way MANOVA with repeated measures; $F = 4.47991$, $P = 0.00140$ (1 nmol) and $F = 4.66638$, $P = 0.00212$ (10 nmol). The effect of saline was not significant ($F = 1.44786$, $P = 0.20783$). The number of animals in each group is given in parentheses.

Effect of DAGO on regional blood flow and vascular resistance (Fig. 4)

Blood flow in hindquarters. The small dose (0.1 nmol) of DAGO, which had no effect on systemic blood pressure variables, significantly increased blood flow in the hindquarters, due to a decrease in vascular resistance in the hindquarters. The larger doses also significantly increased blood flow to the hindquarter, but had no effect on regional vascular resistance. The blood flow in the hindquarters was still significantly increased 30 min after the 1 nmol dose.

Mesenteric blood flow. The blood flow in the mesenteric artery was decreased even by the smallest

Table 1. Effect of DAGO on mean arterial pressure and heart rate in conscious rats after intracerebroventricular (i.c.v.) administration. Values indicate actual values of mean arterial pressure (MAP) and heart rate (HR) after intraventricular injection of DAGO (10 nmol/10 μl) or saline (10 μl)

		Minutes after intraventricular injection			
Group	Baseline	5	10	20	30
Saline (i.c.v.)					
MAP (mmHg)	131 ± 6	131 ± 7	130 ± 6	128 ± 6	129 ± 6
HR (bpm)	320 ± 21	345 ± 21	333 ± 22	347 ± 20	340 ± 21
DAGO 10 nmol (i.c.v.)					
MAP (mmHg)	130 ± 6	145 ± 9	140 ± 8	122 ± 4	108 ± 8
HR (bpm)	390 ± 11	428 ± 18	443 ± 6*	464 ± 9*	461 ± 9*

Values indicate mean ± SEM, $N = 6$, MAP = mean arterial pressure, HR = heart rate, bpm = beats per min. Asterisks indicate statistical difference between the two groups by Student–Newman–Keul test ($*P < 0.05$). The time–response effect, as compared to saline treated group, was significant when assessed by two-way MANOVA with repeated measures; the F and P-values for MAP were $F = 8.15396$ and $P = 0.00017$ and for the heart rate $F = 6.95822$, $P = 0.00041$.

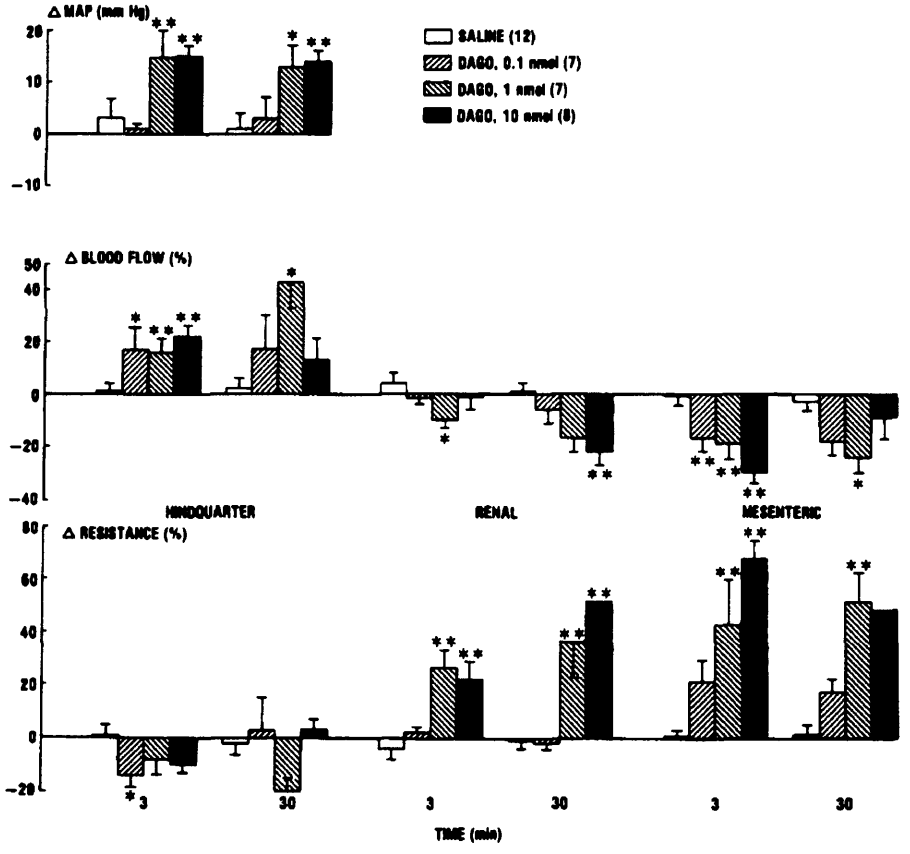

Fig. 4. Effect of DAGO, injected into the preoptic nucleus, on regional blood flow and vascular resistance in the conscious rat. Values (means ± SEM) represent changes 3 and 30 min after injection of DAGO or saline. Asterisks denote statistical significance vs saline group by Student–Newman–Keul test; $*P < 0.05$, $**P < 0.01$. The number of animals in each group is given in parentheses.

dose of DAGO, the larger doses decreased mesenteric blood flow in a dose-related manner. The reduction in mesenteric blood flow was accompanied by an increase in vascular resistance. After the 1 nmol dose, the mesenteric vascular resistance was still significantly increased, 30 min after the injection.

Renal blood flow. In the artery, DAGO (1 and 10 nmol) induced a gradual decrease in the blood flow and increase in the vascular resistance. The maximum decrease in blood flow became apparent 30 min after the administration of DAGO and subsided in 120 min. Similarly, DAGO induced a long-lasting increase in vascular resistance with a maximum at 30 min, but the renal resistance remained high during the whole observation period of 120 min.

Microinjections of saline (100 nl) into the preoptic nucleus had no effect on the blood flow or vascular resistance in any of the blood vessels studied.

Influence of chlorisondamine

The ganglion blocker chlorisondamine (5 mg/kg i.v.) significantly decreased mean arterial pressure and increased heart rate. The renal blood flow was significantly less in chlorisondamine-treated rats, while in mesenteric and hindquarter vascular beds, the flow was maintained due to a decrease in vascular resistance (Table 2).

Chlorisondamine totally blocked the pressor response to DAGO (1 nmol) in the preoptic nucleus (Fig. 5). The initial bradycardia produced by DAGO (1 nmol) was not affected by chlorisondamine (23 ± 3 bpm in control vs -16 ± 8 bpm in chlorisondamine treated group), while the late tachycardic responses to DAGO were significantly attenuated ($+108 \pm 23$ bpm in control vs $+40 \pm 20$ bpm in chlorisondamine, $P < 0.05$). The increase in hindquarter blood flow by DAGO (1 nmol) in the preoptic nucleus was blocked by chlorisondamine, as

Table 2. Resting levels of systemic and regional hemodynamic variables before administration of DAGO in saline- or chlorisondamine (5 mg/kg i.v.)-treated rats

	Control	Chlorisondamine
MAP (mmHg)	117 ± 5	79 ± 5**
HR (bpm)	367 ± 21	310 ± 6*
Blood flow (kHz)		
HQ	5.2 ± 0.4	6.1 ± 1.3
R	8.4 ± 0.4	4.8 ± 0.9*
M	4.4 ± 0.5	4.6 ± 0.4
Resistance (mmHg/kHz)		
HQ	28 ± 4	15 ± 3*
R	20 ± 8	21 ± 5
M	30 ± 4	18 ± 1*

Values indicate mean ± SEM, $N = 7$, MAP = mean arterial pressure, HR = heart rate, bpm = beats per minute. HQ = hindquarter, R = renal, M = mesenteric. Asterisks indicate statistical difference between the two groups by Student–Newman–Keul test ($*P < 0.05$, $**P < 0.01$).

was also the vasoconstrictor responses in renal and mesenteric arteries (Fig. 5).

Effect of DAGO on sympathetic nerve activity in anesthetized rats (Fig. 6)

In a separate group of artificially ventilated rats, anesthetized with chloralose, injection of DAGO (1 nmol/100 nl) into preoptic nucleus induced a biphasic change in the renal nerve activity. Initially, the activity in the renal nerve tended to decrease during the first 5 min after injection of drug. This initial sympathoinhibition was then replaced by an increase in activity 10 min after injection of DAGO. However, no significant changes were observed in renal blood flow or systemic pressure, whereas the heart rate increased concomitantly with the increase in activity in the renal nerve.

DISCUSSION

Injections of DAGO (1 or 10 nmol/rat), a selective μ-opiate receptor agonist (Handa *et al.*, 1981), into the preoptic nucleus of the hypothalamus elicited dose-related rises of blood pressure, accompanied by a biphasic heart rate response in the conscious rat. These results are in agreement with the previous findings that injections of selective μ-agonists, such as DAGO and dermorphin, into the preoptic nucleus

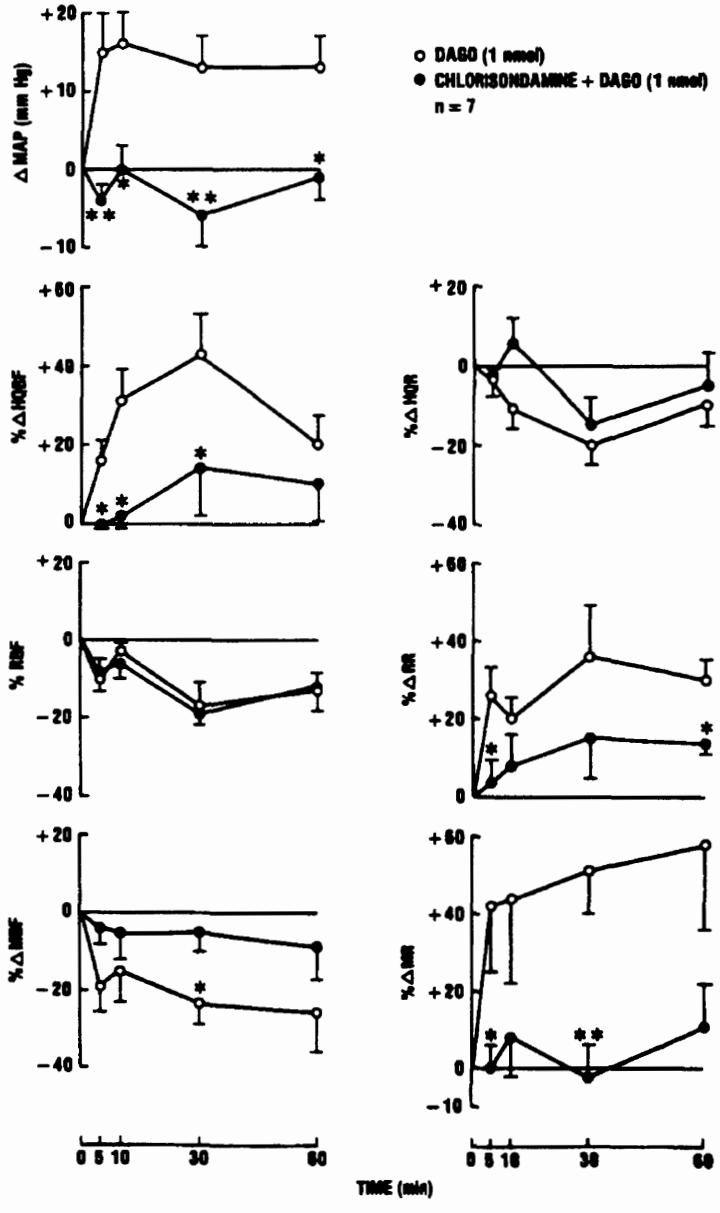

Fig. 5. Influence of chlorisondamine on the DAGO-induced hemodynamic responses in the conscious rat. Chlorisondamine was injected intravenously 20 min before injection of DAGO (1 nmol) into the preoptic nucleus. The values represent means ± SEM. Asterisks denote statistical significance between the two groups by Student–Newman–Keul test; $*P < 0.05$, $**P < 0.01$. MAP = mean arterial pressure, HQBF = hindquarter blood flow, HQR = hindquarter resistance, MBF = mesenteric blood flow, MR = mesenteric resistance, RBF = renal blood flow, RR = renal resistance.

or into the anterior hypothalamic nucleus, as well as paraventricular hypothalamic nucleus, increased blood pressure and, depending on the dose, caused tachycardia or bradycardia in rodents (Diz, Vitale and Jacobowitz, 1984; Pfeiffer *et al.*, 1982, 1983a, b; Feuerstein, Zerbe and Faden, 1983; Kiritsy-Roy *et al.*, 1986), whereas injections of these μ-opioids into the dorsomedial or posterior hypothalamus had no effect (Diz *et al.*, 1984). In anesthetized rats, comparable doses of DAGO, injected into the anterior hypothalamic/preoptic region, decreased the blood pressure and induced tachycardia (Faden and Feuerstein, 1983). Pressor and tachycardic responses in the conscious animals, but cardiovascular depression in anesthetized animals, are also observed after systemic or central administration of morphine or enkephalins (Bolme, Fuxe, Agnati, Bradley and Smythies, 1978; Schaz, Stock, Simon, Schlör, Unger, Rockhold and Ganten, 1980; Laubie and Schmitt, 1981).

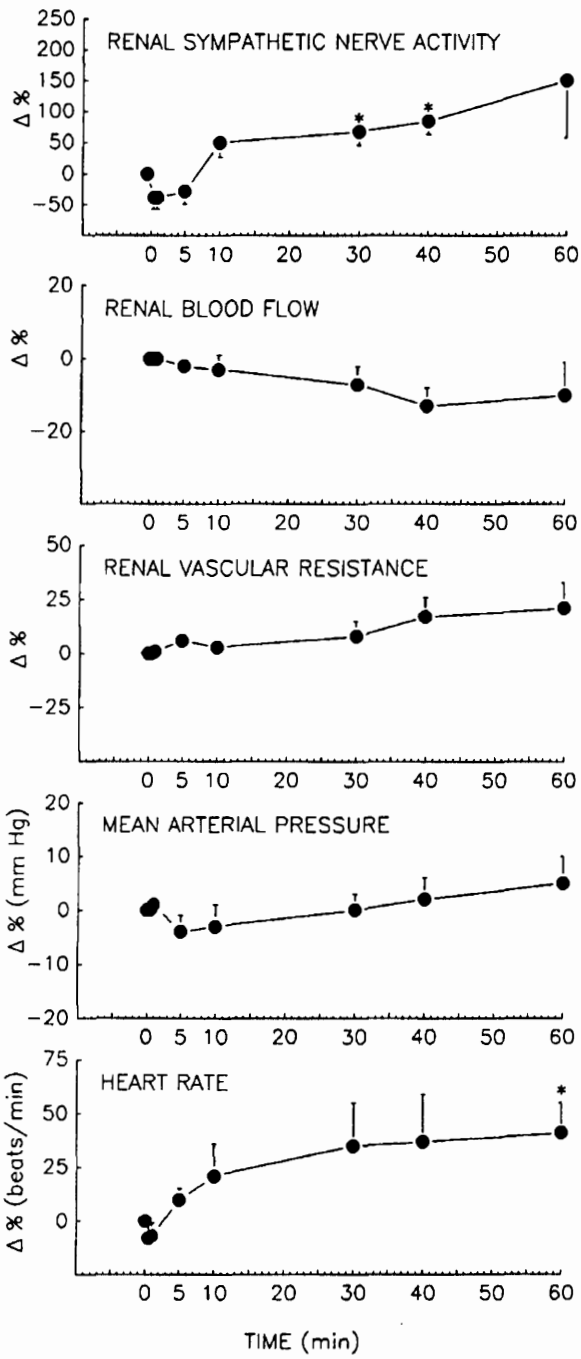

Fig. 6. Effect of DAGO, injected into the preoptic nucleus, on activity in renal sympathetic nerves activity, mean arterial pressure, heart rate, renal blood flow and vascular resistance in the chloralose-anesthetized rat. Values (means ± SEM) represent changes after injection of DAGO. Asterisks denote statistical significance from baseline by Student–Newman–Keul test; $^*P < 0.05$.

Since the volume of injection, used in the present experiment (1 μl) was fairly large, the effects described in the present report might have been due to activation of other neighboring nuclei, in addition to the preoptic nucleus. However, multiple reasons suggested that the hemodynamic changes, elicited by DAGO, were due to an action restricted within the region of the preoptic nucleus. Firstly, studies using tritiated enkephalin have shown that around 90% of the injected radioactivity, after 1 μl injections of D-ala^2-D-leu-enkephalin (DADL) into preoptic nucleus was confined to an area with a radius of 1 mm from the site of injection (Feuerstein *et al.*, 1982). Moreover, injections of a 1 μl volume of DADL produced increases in blood pressure and heart rate in the preoptic nucleus, whereas injections into the periventricular preoptic nucleus (with an AP coordinate 1 mm anterior from the coordinate of the preoptic nucleus), resulted in hypotension and bradycardia (Feuerstein and Faden, 1982). In the present study, injections of DAGO at all doses produced identical responses when injected into the preoptic nucleus regardless of the injection volume, whereas intracerebroventricular injections of the 10 nmol dose of DAGO produced a qualitatively different heart rate response (tachycardia only) and a transient and moderate pressor response, which was succeeded by a hypotensive effect 20 min after the intraventricular injection, while the same dose of DAGO in the preoptic nucleus produced profound pressor effect and a bradycardic–tachycardic response. Therefore, it seems likely that activation of neurons in the preoptic nucleus, rather than wide-spread stimulation of hypothalamic nuclei, accounted for the effects of DAGO after injection into the preoptic nucleus.

The tachycardic and pressor responses to centrally-injected DAGO are likely to be due to an activation of the sympathetic nervous system, since these responses were effectively blocked by the ganglion blocker, chlorisondamine. Earlier studies have shown that, concomitantly with its peak cardiovascular effects, the μ-opioid agonists DAGO and dermorphin induced rises of epinephrine and norepinephrine in plasma in the conscious rat (Kiritsy-Roy *et al.*, 1986; Appel *et al.*, 1986; Sirén *et al.*, 1989). Moreover, these cardiovascular changes were partly attenuated in adrenal demedulated animals and even reversed when these rats were further treated with bretylium (Pfeiffer *et al.*, 1983b). Tachycardia seems also to be the

primary effect of DAGO in anesthetized rats, since extremely small doses of DAGO, which have no effect on blood pressure, cause acceleration of the heart (Faden and Feuerstein, 1983), whereas the hypotensive and bradycardic effects, seen after large doses, are in part due to the potent respiratory depressant action of this agent (Pfeiffer *et al.*, 1982; Hassen *et al.*, 1984), though a vagal component may also contribute (Pfeiffer *et al.*, 1983b; Laubie and Schmitt, 1981).

In the present study, DAGO injected into the preoptic nucleus increased cardiac output. The rise in cardiac output induced by DAGO became apparent, concomitantly with the peak pressor response, while it had no significant effect on total peripheral resistance. Thus, the hypertensive effect of DAGO seems to be mediated by its action on the cardiac output. Since the increase in cardiac index was accompanied by bradycardia, a positive inotropic effect and/or increased venous return to the heart may underlie the improvement in cardiac index, produced by DAGO. The lack of effect of DAGO on peripheral resistance, on the other hand, may be due to its opposite action on hindquarter vs renal and mesenteric blood flow and vascular resistance. In the hindquarter, DAGO dose-dependently increased the blood flow and vascular resistance, but in the mesenteric and renal vasculature it reduced the blood flow and raised the vascular resistance in a dose-related manner.

The pattern of changes in blood flow in organs (an increase in hindquarters but decreases in mesenteric and renal vascular beds), induced by DAGO, suggests an activation of the sympatho-adrenomedullary system. Indeed, these effects were attenuated by the ganglion blocker chlorisondamine, indicating that these effects were mediated by activation of sympathetic nerves and/or adrenal medulla. These findings are supported by the earlier studies (Pfeiffer *et al.*, 1983b) that intrahypothalamic injections of DAGO (7.5 nmol) increased epinephrine and norepinephrine in plasma by 17 and 5 fold, respectively. Moreover, the present study provided direct evidence for activation of sympathetic nerves; activity in the renal nerve was increased over 150% by injection of DAGO into the preoptic nucleus, even at doses which did not significantly alter blood pressure or renal blood flow.

However, the increase in activity in the renal nerve was preceded by a brief initial decrease. This inhibition/stimulation pattern of changes in activity agree with the typical heart rate response to μ-opioid agonists; bradycardia followed by tachycardia (Pfeiffer *et al.*, 1983a, b; Sirén *et al.*, 1989). In a recent study, another μ-opioid agonist, morphine, was shown to induce a transient decrease in activity in the renal nerve after intravenous administration in chloralose-anesthetized rats (Delle, Thorén, Skarphedinsson, Hoffman, Carlsson and Ricksten, 1990). The decrease in activity was not only completely abolished but replaced by a profound increase in activity in vagotomized rats and was therefore suggested to be mediated by a reflex activation of vagal afferents (Delle *et al.*, 1990), which was previously shown to underlie the sympathoinhibitory and hypotensive effects of the enkephalin-analog, D-ala^2-met^2-enkephalinamide (DAME) after injections into the right atrium (Willette, Krieger and Sapru, 1982). The bradycardic effect of centrally-administered DAGO was also reduced by atropine methyl nitrate in an earlier study (Pfeiffer *et al.*, 1983b), as well as the cardiorespiratory depressant effect of μ-opioid agonists (Pfeiffer *et al.*, 1982; Hassen *et al.*, 1984). Thus, the μ-opioid agonists seem to produce activation of both sympathetic and vagal outflow.

The biphasic pattern of cardiovascular responses to DAGO and other μ-opioid agonists might also concur with the hypothesis that μ-opioid effects are mediated by two subclasses of opioid receptors: μ_1-receptors mediating respiratory stimulatory and analgesic responses and μ_2-receptors mediating inhibitory and depressant responses (Pasternak and Wood, 1986; Paakkari, Paakkari, Sirén and Feuerstein, 1990). A recent study from this laboratory (Paakkari *et al.*, 1990) implied that the μ_1-receptors mediate respiratory stimulation, while activation of μ_2-receptors was responsible for the respiratory depressant effect of the μ-agonist dermorphin. It would be tempting to speculate that stimulation of multiple subclasses of μ-opioid receptors could also explain the biphasic nature of the cardiovascular responses to DAGO.

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REFERENCES

Appel N. M., Kiritsy-Roy J. A. and Van Loon G. R. (1986) μ Opioid receptors at discrete hypothalamic and brainstem sites mediate opioid peptide induced increases in central sympathetic outflow. *Brain Res.* **378:** 8–20.

Appel N. M. and Van Loon G. R. (1986) β-Endorphin-induced stimulation of central sympathetic outflow: Inhibitory modulation by central noradrenergic neurons. *J. Pharmac. exp. Ther.* **237:** 695–701.

Bolme P., Fuxe K., Agnati F. L., Bradley R. and Smythies J. (1978) Cardiovascular effects of morphine and opioid peptides following intracisternal administration of chloralose-anesthetized rats. *Eur. J. Pharmac.* **48:** 319–324.

Delle M., Thorén P., Skarphedinsson J. O., Hoffman P., Carlsson S. and Ricksten S.-E. (1990) Differential responses of renal and adrenal sympathetic activity to

intravenous morphine administration in anesthetized rats. *J. Pharmac. exp. Ther.* **253:** 655–660.

Diz D. I., Vitale J. and Jacobowitz D. M. (1984) Increases in heart rate and blood pressure by injections of dermorphin into discrete hypothalamic sites. *Brain Res.* **294:** 47–57.

Faber J. E., Barron K. W., Bonham A. C., Lappe R., Muirhead E. E. and Brody M. J. (1984) Regional hemodynamic effects of antihypertensive renomedullary lipids in conscious rat. *Hypertension* **6:** 494–502.

Faden A. I. and Feuerstein G. (1983) Hypothalamic regulation of the cardiovascular and respiratory systems: Role of specific opiate receptors. *Br. J. Pharmac.* **79:** 997–1002.

Feuerstein G. (1985) The opioid system and central cardiovascular control: analysis of controversies. *Peptides* **6:** suppl 12, 51–56.

Feuerstein G. and Faden A. I. (1982) Differential cardiovascular effects of mu, delta and kappa opiate agonists at discrete hypothalamic sites in the anesthetized rat. *Life Sci.* **31:** 2197–2200.

Feuerstein G., Zerbe M. D. and Faden A. I. (1983) Opiate receptors and cardio-vascular control in conscious SHR and WKY rats. *Hypertension* **5:** 663–671.

Feuerstein G. and Sirén A.-L. (1987) Opioid peptides: A role in hypertension? *Hypertension* **9:** 561–565.

Handa B. K., Lane A. C., Lord J. A. H., Morgan B. A., Rance M. J. and Smith C. F. C. (1981) Analogs of beta-LPH 61-64 possessing selective agonist activity at μ-opiate receptors. *Eur. J. Pharmac.* **70:** 531–540.

Hassen A. H., Feuerstein G. and Faden A. I. (1984) Selective cardiorespiratory effects mediated by μ-opioid receptors in the nucleus ambiguus. *Neuropharmacology* **23:** 407–415.

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

Khachaturian H., Lewis M. E., Schafer M. K. H. and Watson S. J. (1985) Anatomy of the CNS opioid systems. *TINS* **8:** 111–119.

Kiritsy-Roy J. A., Appel N. M., Bobbitt F. G. and Van Loon G. R. (1986) Effects of μ-opioid receptor stimulation in the hypothalamic paraventricular nucleus on basal and stress-induced catecholamine secretion and cardiovascular responses. *J. Pharmac. exp. Ther.* **239:** 814–822.

König J. F. R. and Klippel R. A. (1967) *The Rat Brain (A Stereotaxic Atlas).* Williams and Wilkins, Baltimore, Maryland.

Laubie M. and Schmitt H. (1981) Sites of the vagally mediated bradycardia induced by morphine-like agents and opioid peptides. In: *Central Nervous Mechanisms in Hypertension* (Buckley J. J. and Ferrario C. M. Eds), pp. 235–248. Raven Press, New York.

Mansour A., Khatchaturian H., Lewis M. E., Akil H. and Watson S. J. (1988) Anatomy of CNS opioid receptors. *Trends Neurosci.* **11:** 308–314.

Paakkari P., Paakari I., Sirén A.-L. and Feuerstein G. (1990) Respiratory and locomotor stimulation by low doses of dermorphin, a mu, receptor-mediated effect. *J. Pharmac exp. Ther.* **252:** 235–240.

Pasternak G. W. and Wood P. J. (1986) Minireview: Multiple mu opiate receptors. *Life Sci.* **38:** 1889–1898.

Pfeiffer A., Feuerstein G., Faden A. I. and Kopin I. J. (1982) Evidence for an involvement of mu- but not delta- or kappa-opiate receptors in sympathetically and parasympathetically mediated cardiovascular responses to opiates upon anterior hypothalamic injection. *Life Sci.* **31:** 1279–1282.

Pfeiffer A., Feuerstein G., Kopin I. J. and Faden A. I. (1983a) Cardiovascular and respiratory effects of mu-, delta- and kappa-opiate agonists microinjected into the anterior hypothalamic brain area of awake rats. *J. Pharmac. exp. Ther.* **225:** 735–741.

Pfeiffer A., Feuerstein G., Zerbe R. L., Faden A. I. and Kopin I. J. (1983b) Mu-receptors mediate opioid cardiovascular effects at anterior hypothalamic sites through sympatho-adrenomedullary and parasympathetic pathways. *Endocrinology* **113:** 929–938.

Schaz K., Stock G., Simon W., Schlör K. H., Unger T., Rockhold R. and Ganten D. (1980) Enkephalin effects on blood pressure, heart rate and baroreceptor reflex. *Hypertension* **2:** 395–407.

Sirén A.-L., Lake C. R. and Feuerstein G. (1988) Hemodynamic and neural mechanisms of action of thyrotropin releasing hormone in the rat. *Circ. Res.* **62:** 139–154.

Sirén A.-L., Paakkari P., Goldstein D. S. and Feuerstein G. (1989) Mechanisms of central hemodynamic and sympathetic regulation by mu opioid receptors: Effects of dermorphin in the conscious rat. *J. Pharmac. exp. Ther.* **248:** 596–604.

Willette R. N., Krieger A. J. and Sapru N. H. (1982) Blood pressure and splanchnic nerve activity are reduced by a vagally mediated opioid action. *J. Cardiovasc. Pharmac.* **4:** 1006–1011.

Yardley C. P. and Hilton S. M. (1986) The hypothalamic and brainstem areas from which the cardiovascular and behavioral components of the defense reaction are elicited in the rat. *J. Aut. Nerv. Syst.* **15:** 227–244.