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Mechanisms of Central Hemodynamic and Sympathetic Regulation by *Mu* Opioid Receptors: Effects of Dermorphin in the Conscious Rat[1]

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ABSTRACT

The effects of i.c.v. administered dermorphin, a highly selective μ-opioid agonist, on cardiac function and renal, mesenteric and hindquarter blood flow were studied in conscious rats. Core temperature, blood gases, arterial plasma levels of norepinephrine, epinephrine, dopamine, 3,4-dihydroxyphenylalanine and dihydroxyphenylacetic acid (DOPAC) also were examined. Cardiac output was measured using a thermodilution technique and regional blood flows using directional pulsed Doppler velocimetry. Dermorphin, at doses of 0.1–100 nmol/kg, increased blood pressure and hindquarter blood flow, renal and mesenteric resistance, and core temperature. Higher doses (1–5 μmol/kg) caused respiratory depression, acidosis, and shock despite profound sympatho-adrenomedullary stimulation. Circulating levels of catecholamines were significantly increased at the dermorphin doses of 0.1–100 nmol/kg. At the 100 nmol/kg dose, plasma levels of epinephrine, norepinephrine, the dopamine metabolite dihydroxyphenylacetic acid and the catecholamine precursor 3,4,-dihydroxyphenylalanine were increased by 2–15-fold. The data indicate that *mu* opioid receptor stimulation exerts potent effects on cardiorespiratory functions, activates the sympathoadrenomedullary system and produces a pattern of blood flow changes consistent with the stress-induced "defense" response (skeletal muscle vasodilation and splanchnic vasoconstriction). Excessive *mu* opioid receptor stimulation leads to shock due to respiratory and hemodynamic collapse.

The heptapeptide dermorphin has a primary amino acid sequence that differs from any known natural opioid peptide in that it contains D-alanine (Montecucchi *et al.*, 1981; de Castiglione and Rossi, 1985). It nevertheless is a potent and long-acting opioid (Broccardo *et al.* 1981; Stevens and Yaksh, 1986). In intact animals dermorphin produces analgesia and affects neuroendocrine, respiratory and cardiovascular variables (for review see Feuerstein, 1986). In *in vitro* studies dermorphin, like morphine, has a distinct pattern of actions consistent with a *mu* receptor agonist (Glaser *et al.*, 1981; Westphal *et al.*, 1985; Krumins 1987). The affinity of dermorphin binding to *mu* sites in the rat brain was almost three times greater than that of D-Ala^2, MePhe^4, Gly-ol^5-enkephalin (DAGO; Krumins, 1987), which previously was considered to be the most potent and selective *mu* opioid agonist (Handa *et al.*, 1981, Kosterlitz *et al.*, 1980, Pfeiffer *et al.*, 1982).

Due to resistance of dermorphin to plasma and tissue peptidases, it also is the most toxic natural opioid peptide known (de Castiglione and Rossi, 1985; Negri and Improta, 1984). We reported that minute amounts of dermorphin administered into brain parenchyma or the cerebroventricular system caused severe respiratory depression and hypotension despite profound activation of the sympatho-adrenomedullary system (Feuerstein and Faden, 1983b). The mechanism of dermorphin-induced cardiovascular collapse is not fully understood, since none of the previous studies examined cardiac function or regional hemodynamics. The unique selectivity of dermorphin for the *mu* opioid receptors also provides an opportunity to assess the effects of the *mu* opioid receptor stimulation on autonomic functions.

In the present study we report the effects of dermorphin on

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ABBREVIATIONS: MAP, mean arterial pressure; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; CI, cardiac index; TPRI, total peripheral resistance index; VR, vascular resistance; NE, norepinephrine; EPI, epinephrine; DA, dopamine; DOPA, dihydroxyphenylalanine; DOPAC, dihydroxyphenylacetic acid; DAGO, D-Ala^2,MePhe^4,Gly-ol^5 enkephalin.

discrete organ blood flow and cardiac function in conjunction with respiratory, thermoregulatory, and sympatho-adrenomedullary responses. All studies were conducted in conscious freely moving rats, since anesthesia can obscure the central actions of opiates on the cardiovascular system (Feuerstein, 1985; Hassen and Feuerstein, 1987; Kayaalp and Kaymakcalan, 1966).

Materials and Methods

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

Measurement of cardiac output. Rats were anesthetized with ketamine (130 mg/kg i.m.) and acepromazine (1.3 mg/kg i.m.) and placed in a stereotaxic device (David Kopt Instruments, Tujunga, CA). A stainless steel guide cannula for i.c.v. injections was placed on the skull (coordinates from bregma: AP=−0.8 mm, L=1.2 mm) and fixed with glue. PE-50 catheters were then inserted into femoral vessels. 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, Columbus, 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 and before the probe was finally sutured to the neck muscles and at the end of experiment. The jugular vein catheter and the thermistor were tunneled under the skin to the nape of the neck. All catheters and 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) coupled to a strain gauge coupler (Narco Bio-Systems type 7032). Blood pressure (mean, systolic, diastolic, pulse) and heart rate were continuously recorded on a Narcotrace 80 computerized physiograph and sampled automatically every 30–60 sec by a Northstar-Hazeltine computer.

Cardiac output was measured by thermodilution technique as described (Sirén *et al.*, 1988). In brief, the thermistor was attached to the computerized Cardiomax II (CMX2-780-k with microprobe option R, Columbus Instruments, Columbus, OH). The dead space of the venous line was first flushed with 50 μl of 0.9% (wt/vol) NaCl (saline) at room temperature (22°C); after a brief stabilization period an additional injection of 200 μl normal saline (22°C) was rapidly injected using a 1-ml syringe. A control period of 15 min included two or three cardiac output recordings to test for consistency and placement of the probe. During this period control values for blood pressure and heart rate also were collected. The timer for automatic data collection was started and data points collected immediately before and 2, 5, 15, 30 and 45 min after dermorphin injection. Total peripheral resistance 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. Core temperature was monitored by the aortic thermoprobe before each cardiac output measurement.

Increasing doses of dermorphin were injected i.c.v. in 5 μl saline slowly over 30 sec by means of a premeasured 30 gauge cannula (7.5 mm), and the cardiovascular index were monitored for 45 min for each dose. The proper position of the i.c.v. cannula was ascertained at the end of the experiment by an injection of dye (methylene blue, 5 μl) into the ventricular space.

Measurement of organ blood flow. The directional pulsed Doppler velocimetry method was selected to measure organ blood flow in hindquarter, renal and mesenteric arteries. Though it does not allow quantitative blood flow monitoring, this method is superior compared with other available techniques, since 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 above. A midline laparotomy was then made, and the left renal and superior mesenteric arteries and lower abdominal aorta above its bifurcation were carefully isolated under a dissecting microscope. Doppler flow probes (Valpey-Fisher, Hopkinton, MA) were then loosely sutured 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 that 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 were 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 continuously recorded on the Narcotrace 80 physiograph. A cable connecting the blood flow receptacle and the Doppler flowmeter (University of Iowa, Bioengineering Facility, model 545C-4) was attached to the animal, and the mean blood flow was continuously recorded on the physiograph. Vascular resistance was calculated by dividing the mean arterial pressure by blood velocity (Doppler shift in kilohertz) as described (Haywood *et al.*, 1981; Sirén *et al.*, 1988). Changes in blood flow and vascular resistance are expressed as a percent of control values.

Increasing doses of dermorphin were injected i.c.v. in 5 μl saline over 30 sec and all indexes followed continuously for 30–45 min for each dose.

Assay of blood pCO_2, pO_2 pH. Blood samples (0.2 ml) were withdrawn from the arterial catheter 30 min after injection of dermorphin (1, 100 or 1000 nmol/kg). An equal volume of fresh rat blood was used to replace the amount withdrawn. Blood pCO_2, pO_2 and pH were assayed using a Corning pH-blood gas analyzer (model 165/2, Corning Instruments, Corning, NY).

Assay of plasma catecholamines. Blood samples (0.8 ml) were withdrawn from the arterial catheter 30 min after dermorphin administration (0.1, 1 and 100 nmol/kg). The blood withdrawn was replaced with an equal volume of fresh rat blood. Blood specimens were collected in chilled test tubes, centrifuged (Beckman microfuge B) for 1 min, and the plasma removed and rapidly frozen on dry ice. Epinephrine (EPI), norepinephrine (NE), dopamine (DA), catecholamine precursor 3,4-dihydroxyphenylalanine (DOPA), and the dopamine metabolite dihydroxyphenylacetic acid (DOPAC) were separated by alumina extraction and assayed by high performance liquid chromatography with electrochemical detection (Goldstein *et al.*, 1985; Eisenhofer *et al.*, 1986).

Dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) was purchased from Sigma (St. Louis, MO), and naloxone was kindly provided by DuPont Pharmaceuticals (Wilmington, DE). Both drugs were dissolved in 0.9% NaCl (saline).

Statistical analysis. Data are presented as means ± S.E. for the given number of rats. One-way analysis of variance followed by Dunnett's test, Student-Newman Keul's test or the Kruskal-Wallis test (Theodorsson-Norheim, 1986) was used for statistical analysis as appropriate. A significant difference was defined by $P < .05$.

Results

Effect of dermorphin on blood pressure and heart rate. The baseline values for hemodynamic variables before dermor-

phin administration were not statistically different from the control values before saline injection (table 1). Dermorphin i.c.v. produced a bell-shaped response of mean arterial pressure (MAP) as a function of dose (fig. 1). The doses of 0.1–100 nmol/kg increased MAP, whereas higher doses (1–5 μmol/kg) produced smaller pressor responses or even decreased MAP (fig. 1). Dermorphin at doses of 10 nmol/kg to 1 μmol/kg significantly increased systolic arterial pressure (SAP) and slightly increased diastolic arterial pressure (DAP). At 5 μmol/kg, dermorphin decreased DAP (fig. 2). Pulse pressure therefore increased after i.c.v. dermorphin with maximal pressor responses reached at 10 and 100 nmol/kg (fig. 1). The maximum increases in MAP and pulse pressure were achieved 5 min after the dermorphin administration and subsided in 30–45 min.

Heart rate first decreased and then slowly increased after dermorphin (1–100 nmol/kg). The maximum bradycardic response was reached 5–15 min after drug injection; tachycardia was maximal 30–45 min after injection. The peak bradycardic effects are shown in figure 1. The maximum tachycardic response after the 1 nmol/kg dose was 54±10 bpm ($P<.01$ *versus* saline). The corresponding responses after the 10 and 100 nmol/kg doses were 64±13 bpm ($P<.01$ *versus* saline) and 105±15 bpm ($P<.01$ *versus* saline). High doses of dermorphin (1–5 μmol/kg) induced only tachycardia (fig. 1).

Effect of dermorphin on cardiac output and TPRI. Dermorphin i.c.v. increased cardiac index (CI) at the doses of 1 nmol/kg to 1 μmol/kg (fig. 3). The maximum increase in CI (106±14 ml/min/kg, $P<.01$) was achieved 15 min after the 1 μmol/kg dose. The CI was still significantly elevated 30 min after the 10 nmol/kg to 1 μmol/kg doses of dermorphin. Total peripheral resistance index (TPRI) tended to increase shortly after the 10 nmol/kg dose; at high doses (1–5 μmol/kg) dermorphin decreased TPRI (fig. 3).

Effect of dermorphin on organ blood flow. At the 0.1 nmol/kg to 1 μmol/kg doses, dermorphin i.c.v. induced a significant increase in hindquarter blood flow; at higher doses a later decrease in hindquarter blood flow was found, and the highest dose induced only a fall in hindquarter blood flow (fig. 4). The blood flow in mesenteric artery significantly decreased after the 10 nmol/kg and 1 μmol/kg doses of dermorphin, and the other doses had no significant effect on mesenteric blood flow (fig. 4). Blood flow in the renal artery decreased in a dose-related manner at the 10 nmol/kg to 5 μmol/kg doses of dermorphin (fig. 4).

TABLE 1

Base-line levels of hemodynamic variables before saline or dermorphin administration

PP, pulse pressure; BF, blood flow; HR, heart rate. Values are means ± S.E. *n*, number of rats in each group.

Variable	*n*	Saline	Dermorphin
MAP (mmHg)	17	114 ± 2	112 ± 2
SAP (mmHg)	17	149 ± 3	152 ± 3
DAP (mmHg)	17	92 ± 3	88 ± 5
PP (mmHg)	17	57 ± 3	58 ± 3
HR (bpm)	17	403 ± 10	397 ± 13
CI (ml/min/kg)	17	421 ± 10	425 ± 12
TPRI (mmHg/ml/min/kg)	17	0.27 ± 0.01	0.27 ± 0.01
Blood flow (kHz)			
Hindquarter	14	4.6 ± 1	5.0 ± 1
Renal	14	5.0 ± 1	5.5 ± 1
Mesenteric	14	4.0 ± 1	4.0 ± 1
Vascular resistance (mmHg/kHz)			
Hindquarter	14	39 ± 8	35 ± 6
Renal	14	35 ± 7	24 ± 4
Mesenteric	14	36 ± 5	39 ± 4

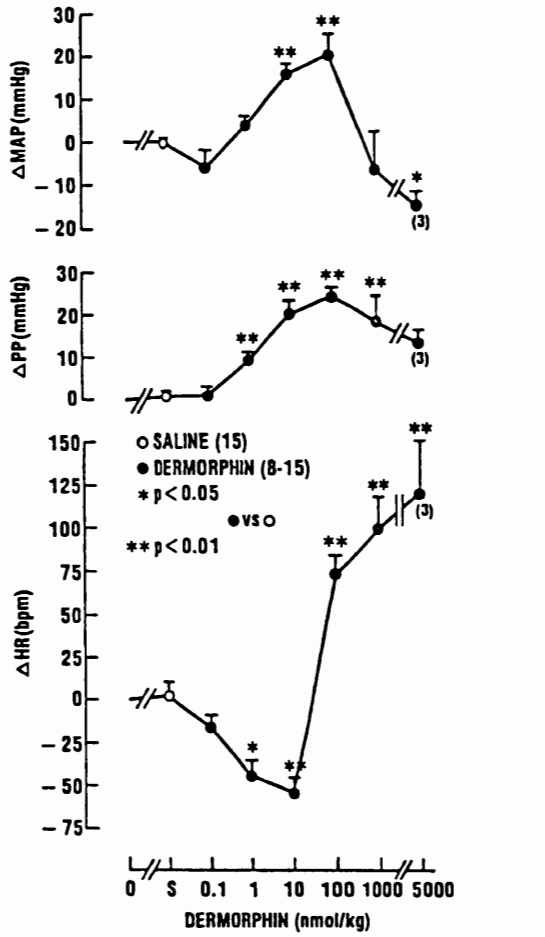

Fig. 1. Effect of i.c.v. dermorphin on MAP, pulse pressure (PP) and heart rate (HR) in the conscious rat. Values are means ± S.E. and represent maximum changes after saline (open circles) or dermorphin (closed circles). Number of rats in each group is in parenthesis. Asterisks, statistical significance versus saline by Dunnett's test.

Effect of dermorphin on regional vascular resistance. The hindquarter vascular resistance decreased after the 0.1 nmol/kg dose of dermorphin (−24±5%, $P<.05$); the 10 and 100 nmol/kg doses also decreased hindquarter vascular resistance initially but thereafter produced a longer-lasting hindquarter vasoconstriction (47±18%, $P<.01$) (fig. 5). The 5 μmol/kg dose of dermorphin increased hindquarter vascular resistance (fig. 5).

The renal vascular resistance increased dose-dependently after dermorphin administration (fig. 5). The maximum vasoconstrictor response (138±36%, $P<.01$) was reached 10 min after the 100 nmol/kg dose, and the vascular resistance was still significantly elevated 30 min after this dose.

Between doses of 10 and 100 nmol/kg dermorphin induced mesenteric vasoconstriction (fig. 5). The 10 nmol/kg dose gradually increased mesenteric vascular resistance up to a maximum of 113±24% ($P<.01$) 30 min after the injection. At the 100 nmol/kg dose the maximum increase in mesenteric vascular resistance (100±20%, $P<.01$) became apparent 2 min after dermorphin and subsided in 20 min.

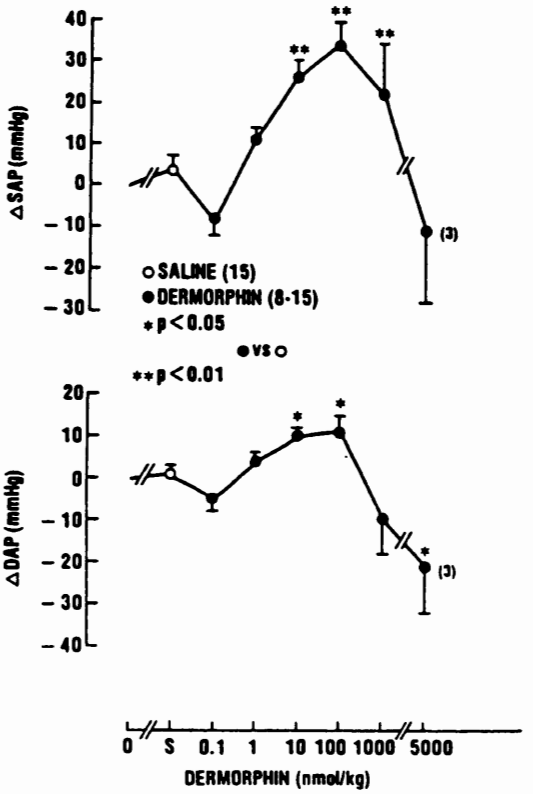

Fig. 2. Effect of i.c.v. dermorphin on SAP and DAP in the conscious rat. Values are means ± S.E. and represent maximum changes after saline (open circles) or dermorphin (closed circles). Number of rats in each group is in parentheses. Asterisks, statistical significance versus saline by Dunnett's test.

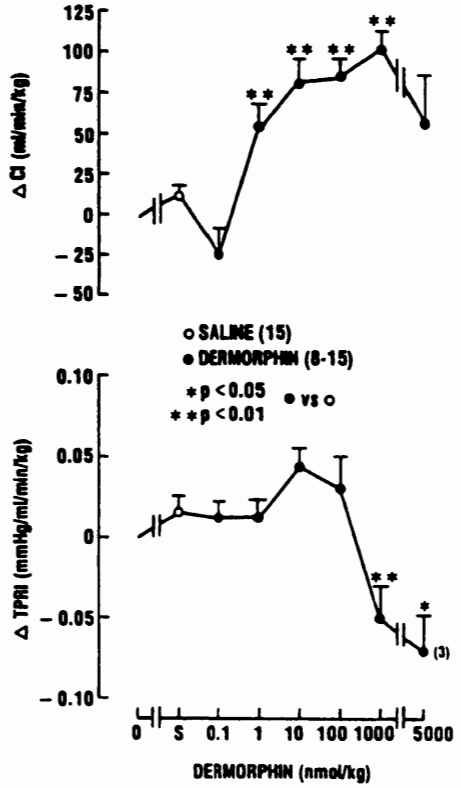

Fig. 3. Effect of i.c.v. dermorphin on CI and TPRI in the conscious rat. Values are means ± S.E. and represent maximum changes after saline (open circles) or dermorphin (closed circles). Number of rats in each group is in parentheses. Asterisks, statistical significance versus saline by Dunnett's test.

Effect of dermorphin on plasma concentration of NE, EPI, DA, DOPA and DOPAC. As shown in figure 6 groups to be treated with saline or dermorphin had similar baseline levels of EPI, and of NE, with base-line catecholamine values in agreement with those of previous studies (Pfeiffer *et al.*, 1983a,b; Feuerstein and Faden, 1983a,b). Dermorphin at doses of 0.1–100 nmol/kg increased plasma EPI and NE in a dose-related manner (fig. 6). At 0.1 nmol/kg plasma EPI increased by almost 4-fold and plasma NE by approximately 50%. The 1 nmol/kg dose increased plasma EPI by 7-fold and NE by 1.5-fold. The 100 nmol/kg dose increased plasma EPI by 15-fold and NE by 8-fold. At this dose DA was elevated 2-fold (from 129±15 to 334±39 pg/ml, n=5, P<.01); the DA metabolite DOPAC was increased by over 3-fold (from 315±41 to 998± 121 pg/ml, n=5, P<.01) and DOPA by 2-fold (from 899±58 to 1669±167 pg/ml, n=5, P<.05).

The changes in plasma NE induced by dermorphin were in correlation with its pressor (r=0.972, P=.05) and renal constrictor (r=0.995, P=.01) effects. There was also a significant correlation between the increase in plasma EPI and the tachycardic response to dermorphin (r=0.957, P=.05).

Effect of dermorphin on core temperature. Core temperature increased at dermorphin doses of 0.1 nmol/kg to 1 μmol/kg. The increment in core temperature was dose-dependent to a maximum change of approximately 2°C (fig. 7). After the highest dose (5 μmol/kg) the temperature tended to decline back to base line level (fig. 7).

Effect of dermorphin on blood gases. The blood pH, pO_2 and pCO_2 were all in a normal range after the 1 nmol/kg dose of dermorphin but with a slight but significant decrease in pO_2 (table 2). The higher doses, 100 nmol/kg and 1 μmol/kg, produced severe respiratory acidosis of similar magnitude (table 2).

Effect of naloxone on hemodynamic responses produced by dermorphin. In a separate set of rats, the effects of a single dose of dermorphin (10 nmol/kg) on the cardiovascular system were monitored after treatment with saline or naloxone (fig. 8). In this experiment dermorphin was injected i.c.v. in rats treated with naloxone (5 mg/kg i.v.) 20 min before dermorphin administration. Naloxone totally blocked the pressor response, reversed the bradycardia and attenuated the increase in cardiac output produced by the 10 nmol/kg dose of dermorphin.

The potency of naloxone (5–20 mg/kg i.v.) to reverse the changes in blood pressure, heart rate and organ blood flow produced by high doses of dermorphin (1–5 μmol/kg) was studied in 11 additional rats. Three of 11 rats were still alive 24 hr after dermorphin administration; the remaining eight animals died within 10 min after the dermorphin injection despite the i.v. injection of naloxone (10–20 mg/kg) 2–10 min after dermorphin. The animals that ultimately died despite naloxone treatment experienced further decreases in renal and mesenteric blood flow and increases in vascular resistance after naloxone administration (table 3). In the surviving animals naloxone (5–10 mg/kg i.v.) significantly increased blood flow

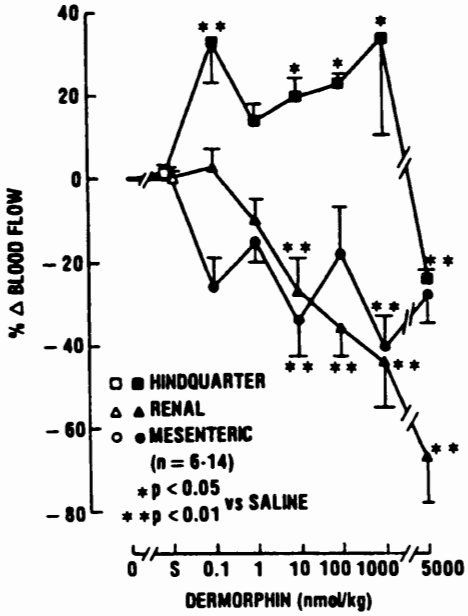

Fig. 4. Effect of i.c.v. dermorphin on organ blood flow in hindquarter, renal and mesenteric vessels of the conscious rat. Values are means ± S.E. and represent maximum changes after saline (open symbols) or dermorphin (closed symbols); *n*, number of rats. Asterisks, statistical significance versus saline by Dunnett's test.

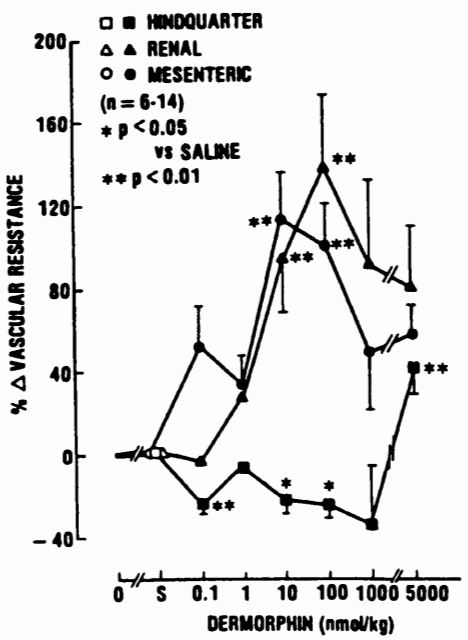

Fig. 5. Effect of i.c.v. dermorphin on vascular resistance in hindquarter, renal and mesenteric vessels of the conscious rat. Values are means ± S.E. and represent maximum changes after saline (open symbols) or dermorphin (closed symbols); *n*, number of rats. Asterisks, statistical significance versus saline by Dunnett's test.

and decreased vascular resistance in the renal and mesenteric circulation but did not affect blood pressure, heart rate or hindquarter blood flow and resistance (table 3). Figure 9 shows an authentic chart recording demonstrating the influence of

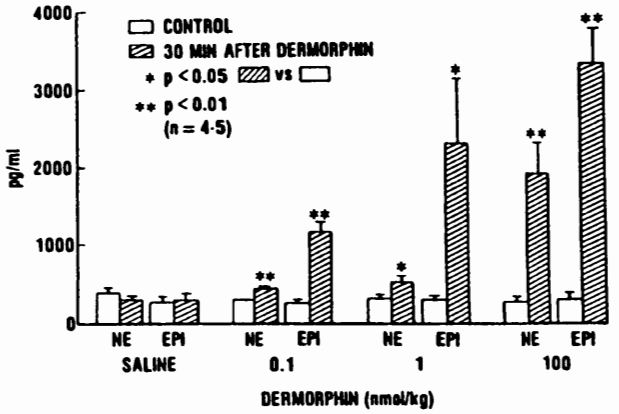

Fig. 6. Effect of i.c.v. dermorphin on plasma EPI and NE in the conscious rat. Control values (open bars) and values 30 min after saline or dermorphin (hatched bars) are given as means ± S.E.; *n*, number of rats. Asterisks, statistical significance from control by Kruskal-Wallis test.

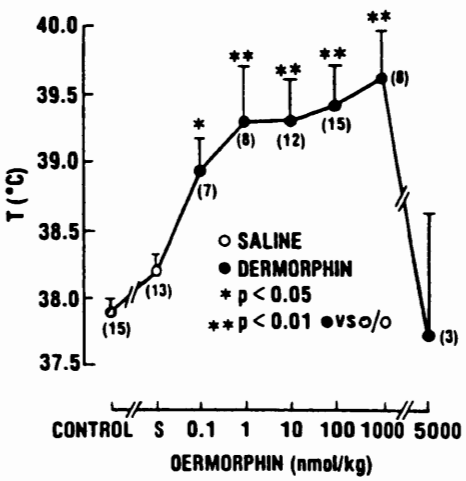

Fig. 7. Effect of i.c.v. dermorphin on body temperature (T) in the conscious rat. Control values (hatched circle) and maximal values after saline (open circle) or dermorphin (closed circles) are given as means ± S.E. Number of rats in each group is in parentheses. Asterisks, statistical significance versus saline and control by Student-Newman-Keul's test.

TABLE 2

Effect of i.c.v. dermorphin on blood gases in the conscious rat

Values are mean ± S.E. at control and 30 min after dermorphin are given; *n*, number of rats.

Group	*n*	Blood pH	pCO_2	pO_2
			mmHg	
Control	18	7.36 ± .01	35 ± 1	97 ± 1
Dermorphin				
1 nmol/kg	7	7.31 ± .01	33 ± 1	89 ± 3**
100 nmol/kg	7	7.13 ± .03**	58 ± 5**	42 ± 4**
1 μmol/kg	4	7.19 ± .04**	70 ± 6**	38 ± 3**

** P < .01 by Student-Newman-Keul's test.

naloxone on dermorphin-induced cardiovascular derangements in both these situations.

Discussion

The present results confirm and extend previous reports that dermorphin is an extremely potent agent affecting key hemodynamic and autonomic functions. Cardiovascular effects of

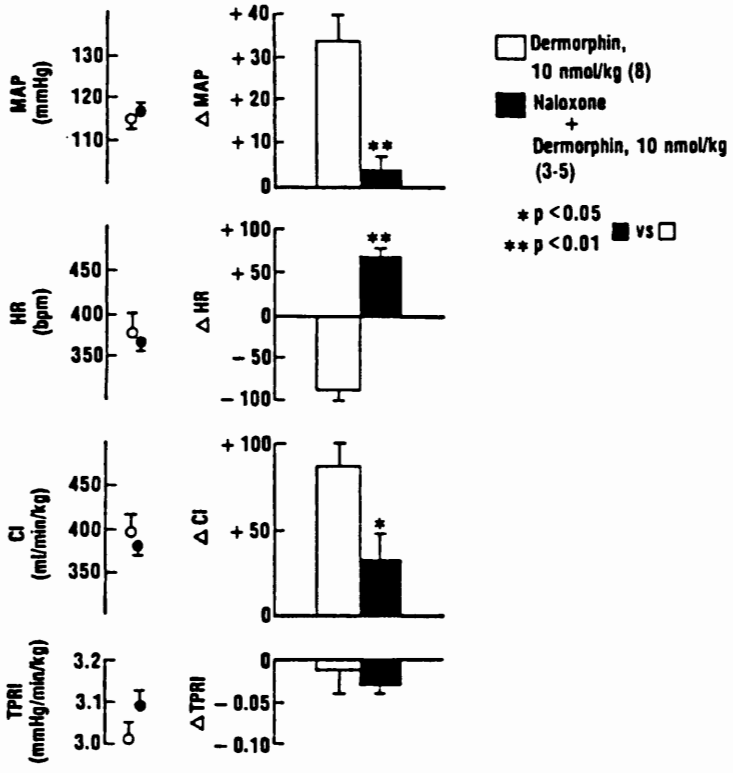

Fig. 8. Influence of naloxone on the hemodynamic effects of dermorphin in the conscious rat. Naloxone (5 mg/kg) was injected into the jugular vein 20 min before i.c.v administration of a single dose of dermorphin (10 nmol/kg). Control values and peak changes after dermorphin administration are presented as mean ± S.E.; *n*, number of rats. Asterisks, statistical significance between naloxone and saline treated group by Student-Newman-Keul's test. HR, heart rate.

TABLE 3

Effect of naloxone on hemodynamic variables after dermorphin administration

Naloxone (5–10 mg/kg) was injected i.v. 2–10 min after dermorphin (1–5 × 10^{-6} mol/kg i.c.v.). Values are means ± S.E. Changes are calculated from control for dermorphin and from the level immediately before naloxone injection for naloxone. Changes in blood flow and resistance are given as percent values.

	Control	Dermorphin	Naloxone		
			1 min	3 min	30 min[a]
Rats died (*n* = 8)					
MAP (mmHg)	138 ± 8	−10 ± 15	−8 ± 15	−34 ± 26	
HR (bpm)	380 ± 24	−125 ± 31**	−25 ± 40	17 ± 49	
Blood flow* (kHz)					
Hindquarter	5.7 ± 0.9	−84 ± 9**	−95 ± 109	−17.85	
Renal	7.0 ± 0.9	−55 ± 13**	24 ± 46	−66 ± 22*	
Mesenteric	4.8 ± 0.4	−82 ± 7**	140 ± 165	−91 ± 8	
Resistance (mmHg/kHz)					
Hindquarter	32 ± 7	670 ± 179**	63 ± 66	−8 ± 33	
Renal	27 ± 8	276 ± 148**	51 ± 84	1844 ± 719*	
Mesenteric	39 ± 9	509 ± 181**	8 ± 62	104 ± 51*	
Rats recuperated (*n* = 3)					
MAP (mmHg)	136 ± 8	−31 ± 12	19 ± 12	33 ± 16	13 ± 10
HR (bpm)	391 ± 36	34 ± 60	8 ± 41	41 ± 35	−32 ± 16
Blood flow * (kHz)					
Hindquarter	6.7 ± 0.5	−14 ± 22	42 ± 34	38 ± 43	28 ± 38
Renal	5.2 ± 0.7	−65 ± 16**	159 ± 22**	156 ± 76**	216 ± 183**
Mesenteric	6.6 ± 0.9	−62 ± 9	174 ± 110*	225 ± 194*	211 ± 179*
Resistance (mmHg/kHz)					
Hindquarter	20 ± 3	4 ± 24	−13 ± 9	4 ± 18	−12 ± 24
Renal	27 ± 5	100 ± 33**	−48 ± 7*	−40 ± 13	−47 ± 21
Mesenteric	22 ± 4	126 ± 43**	−34 ± 44	−36 ± 34	−49 ± 19

[a] Rats in first group died within 10 min.
* P < .05.
** P < .01.

dermorphin were biphasic, similar to the responses reported by Pfeiffer *et al.* (1983a,b) using the selective *mu* opioid agonist DAGO (Handa *et al.*, 1981). In the present study increases in SAP, pulse pressure and CI were produced by low doses of dermorphin, and high doses produced a depressor response due to a decline in TPRI. In a previous study from this laboratory dermorphin i.c.v. at similar doses (0.3–150 nmol/kg) produced increments of SAP and DAP similar to those found in the present study (Feuerstein and Faden, 1983a,b), with a dose-related bradycardia. In the present study bradycardia was pro-

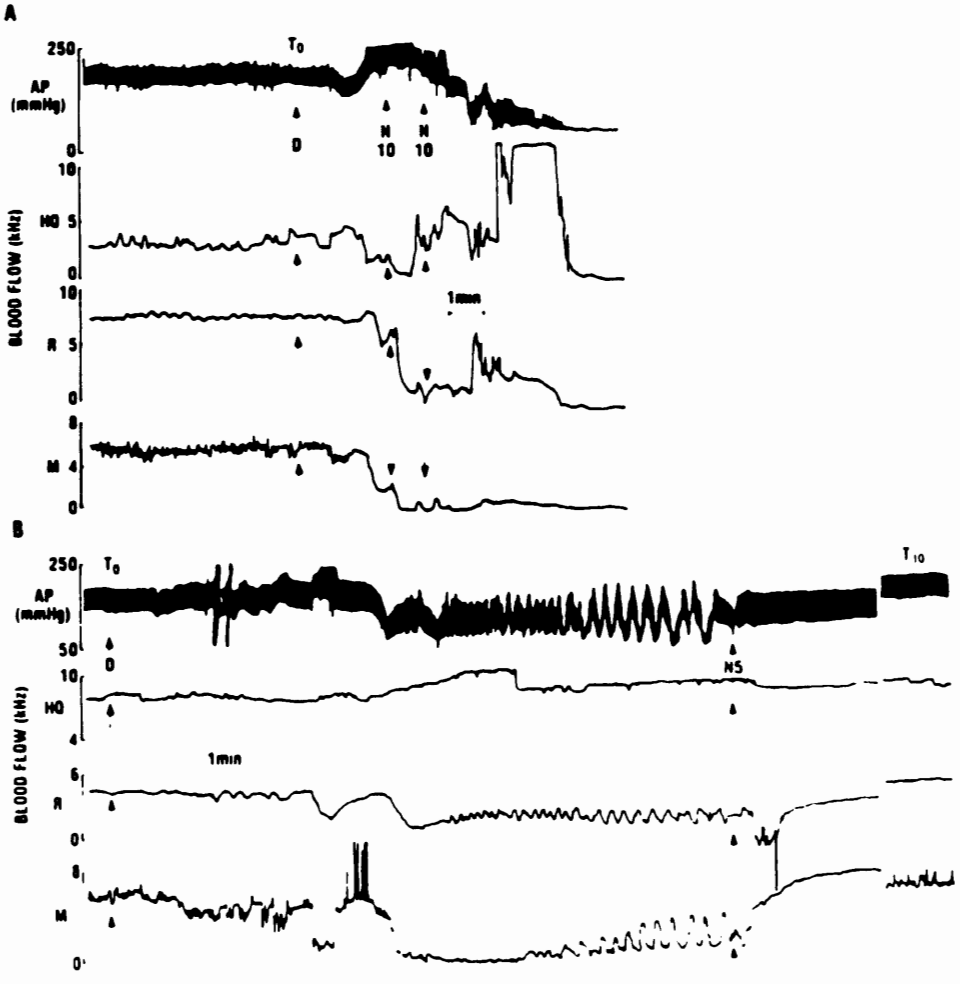

Fig. 9. Original chart recordings demonstrating the effect of naloxone on the hemodynamic responses to a high i.c.v. dose of dermorphin (1 μmol/kg) in the conscious rat. Panel A, rat that did not survive; panel B, example of the beneficial effect of naloxone (N) after dermorphin (D). AP, arterial pressure; HQ, hindquarter; R, renal, M, mesenteric. N = naloxone, T_0, T_{10} denote time points before/after dermorphin treatment.

duced by low doses of dermorphin, whereas at higher doses its hemodynamic responses included a marked tachycardia. Other investigators reported increases in heart rate and, to a lesser degree, blood pressure after local administration of dermorphin (40 pmol) into the preoptic-anterior hypothalamic region of halothane-anesthetized rats (Diz *et al.*, 1984, Diz and Jacobowitz, 1984). Both bradycardic and tachycardic responses to centrally injected dermorphin can be explained by stimulation of *mu* opioid receptors, since stimulation of the central *mu* opioid receptors with agonists such as DAGO, morphiceptin and morphine typically induces a biphasic heart rate response, bradycardia followed by tachycardia in the rat (see Feuerstein and Sirén, 1987a,b).

Regional hemodynamic responses to low doses of dermorphin included increases in renal and mesenteric vascular resistance and consequently progressive decreases in blood flow. An opposite response, vasodilation, was clearly seen in the hindquarter skeletal muscle. This pattern of regional hemodynamic changes was reported in studies using i.c.v. or intraparenchymal injections of the selective *mu* agonist DAGO in the conscious rat (Feuerstein and Sirén, 1987b; Sirén and Feurerstein, 1987). After the higher doses of dermorphin, the vasoconstriction diminished, possibly due to the development of acidosis, which is known to cause vasodilation and decrease the sensitivity of vascular smooth muscle cells to catecholamines (Astrup 1968; Ford *et al.*, 1968).

The increases in blood pressure, mesenteric and renal vascular resistance and hindquarter skeletal muscle vasodilation are consistent with activation of the sympatho-adrenomedullary system. This suggestion is supported by the increased circulating levels of all catecholamines. Similar results have been reported showing large increments in circulating levels of EPI and NE after i.c.v. administration of dermorphin (Feuerstein and Faden 1983; Diz and Jacobowitz 1984) and other *mu* opioid agonists (Pfeiffer *et al.*, 1983a,b; Kiritsy-Roy *et al.*, 1986; Appel *et al.*, 1986). Other studies from our laboratory have revealed direct activation of renal sympathetic nerve activity by centrally administered DAGO in the rat (Sirén and Feuerstein, 1987). In the present study a significant increase in plasma EPI and NE was observed even after the lowest dose of dermorphin (0.1 nmol/kg) with increases in EPI predominating. This dose had no effect on systemic hemodynamic variables but induced a vasodilation in hindquarter vasculature, a pattern that could be attributed to circulating EPI. Since plasma catecholamines were markedly elevated at doses of dermorphin that did not produce consistent respiratory changes, sympatho-adrenomedullary activation probably was not secondary to

respiratory acidosis and seemed to be a primary mechanism determining the hemodynamic responses of dermorphin.

The *mu* opioid agonists when administered centrally are known to increase plasma levels of NE and EPI (Pfeiffer et al., 1983a,b; Feuerstein and Faden, 1982a,b; Kiritsy-Roy *et al.*, 1986). In the present study, levels of DA, DOPA and DOPAC also increased significantly after dermorphin administration at high doses. The elevated levels of DOPAC, a key metabolite of DA, and DA could have resulted from release of both from noradrenergic nerves or the adrenal medulla. Consistent with enhanced synthesis and release of catecholamines, levels of DOPA, the precursor of the catecholamines and the immediate product of the rate-limiting step in catecholamine biosynthesis, approximately doubled. Increased activity of tyrosine hydroxylase, the rate-limiting step in DOPA production, is known to occur during intense sympatho-adrenomedullary stimulation (Kopin, 1985), and circulating DOPA appears to be derived substantially from sympathetic nerve endings (Goldstein *et al.*, 1987).

The pronounced increase in the sympatho-adrenomedullary activity could have been the cause for the increase in cardiac output that was maintained even after the high doses of dermorphin. Additional factors that might contribute to the increase in CI are a) increase in heart rate, b) increase in preload due to catecholamine mediated venoconstriction and c) increase in cardiac contractility mediated by the increase in sympathetic activity and circulating EPI. At the high doses of dermorphin, respiratory acidosis could have counteracted the effects of sympathetic stimulation leading eventually to circulatory collapse.

The high levels of plasma NE and especially of EPI probably also contributed to the effects of dermorphin on the hindquarter, mesenteric and renal circulation. Whereas NE is a constrictor in most vascular beds, EPI causes vasodilation in skeletal muscle (Berecek and Brody, 1982; Sirén *et al.*, 1988). Thus, the differential effects of EPI and NE on hindquarter vasculature, but the synergistic effect on the renal and mesenteric vessels can explain the pronounced constriction of the renal and mesenteric vasculature and the dilator response of the hindquarter blood vessels at low doses of dermorphin. Similar hemodynamic and sympathetic responses to those produced by the *mu* opioid agonist dermorphin can also be elicited by electrical or chemical stimulation of the hypothalamic and periaqueductal brain nuclei (Berecek and Brody, 1982; Yardley and Hilton, 1986; Stoddard *et al.*, 1987; Sirén and Feuerstein, 1987). These brain areas are important for the integration of the stress-induced autonomic and behavioral defense responses (Yardley and Hilton, 1986). Since *mu* opioid receptors are abundantly present in these areas (Quirion *et al.*, 1983), the present results lead to the hypothesis that *mu* opioid stimulation in these brain areas may be related to the neuroendocrine and hemodynamic changes associated with stress responses.

In animals exposed to high (micromole) doses of dermorphin, cardiovascular collapse developed within a few minutes. This shock state was characterized by almost complete cessation of blood flow to the peripheral organs despite normal systemic arterial pressure. Attempts to reverse the shock by large doses of naloxone failed in most animals. Only in the few animals where the hemodynamic changes produced by dermorphin were moderate did naloxone improve the cardiovascular status as indicated by substantial improvement of the circulation in the splanchnic area.

The use of the aortic thermistor also disclosed the effect of *mu* opioid receptor stimulation in increasing body temperature, which was not observed in our previous study (Feuerstein and Faden, 1983b) in which a rectal temperature probe was used. Since the body temperature was increased even after the lowest dose that had no behavioral effects, the hyperthermic effect of dermorphin was not a result of the increased muscle tone and catalepsy characteristic of high doses of dermorphin (Improta and Guglietta, 1985; Paakkari and Feuerstein, 1988). The *mu* opioid receptors have been suggested to mediate the hyperthermic action of morphine and other opioids, since these effects are naloxone reversible (Clark and Clark, 1980). However, the thermoregulatory responses to opiates show large variation depending on animal species, ambient temperature and the presence of restraint (for review, see Clark 1981).

In summary, the results indicate that stimulation of *mu* opioid receptors produces a redistribution of organ blood flow most likely resulting from activation of the sympatho-adrenomedullary system. Cardiovascular collapse and shock produced by high doses of dermorphin probably are due to severe vasoconstriction leading to organ ischemia that is confounded by respiratory depression and especially hypoxemia. An interesting new observation made in this study is the unique pattern of hemodynamic responses to *mu* opioid stimulation similar to the stress-induced defense response; these similarities may be due to the same final common pathway of sympatho-adrenomedullary activation. Further studies are needed to evaluate whether endogenous *mu* opioid peptides can mediate the cardiovascular responses to stress.

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