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CENTRAL CARDIOVASCULAR EFFECTS OF NARCOTIC ANALGESICS AND ENKEPHALINS IN RATS

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1 The cardiovascular effects of morphine, fentanyl, [D-Ala^2]-met-enkephalinamide were analyzed after intracisternal injection in anaesthetized rats. Pa_{O_2} was measured as an index of respiratory function.

2 At low doses in spontaneously breathing rats, morphine, fentanyl and [D-Ala^2]-met-enkephalinamide induced a pressor response with slight tachycardia and no significant change in Pa_{O_2}.

3 The pressor response appeared to be due to activation of opiate receptors and mediated through the sympathetic nervous system.

4 High doses of morphine and [D-Ala^2]-met-enkephalinamide induced a biphasic effect with a secondary hypotension associated with bradycardia in spontaneously breathing rats. A marked reduction in Pa_{O_2} was found during the depressor phase.

5 High doses of [D-Ala^2]-met-enkephalinamide produced only a pressor response in artificially-ventilated rats with no signs of secondary hypotension.

6 Our data support the idea that morphinomimetic agents are centrally pressor at low doses in the rat. The respiratory depression observed with high doses may be the cause of hypotension.

Introduction

The presence of both enkephalins and opiate receptors in the nucleus tractus solitarii (NTS), nucleus ambiguus and nucleus dorsalis vagus suggests that enkephalins may play a role in central cardiovascular regulation (Atweh & Kuhar, 1977; Hökfelt, Elde, Johanson, Terenius & Stein, 1977). A biphasic blood pressure response has been reported after central injection of morphinomimetic agents in ventilated dogs (Laubie, Schmitt, Vincent & Remond, 1977). In spontaneously breathing rats, Bolme, Fuxe, Agnati, Bradley & Smythies (1978) observed that met-enkephalin administered into the cisterna magna caused a pressor response. By contrast, the enkephalin analogue [D-Ala^2]-met-enkephalinamide produced only a depressor response. The inconsistency of these results has led us to reinvestigate the effects of narcotic analgesics and enkephalins in the rat, particularly taking into account the variations in blood pressure which may be induced by respiratory changes.

Methods

Normotensive naïve male Wistar rats, weighing 300–350 g, were anaesthetized with pentobarbitone (50 mg/kg) administered intraperitoneally. The left carotid artery was cannulated in order to measure blood pressure and heart rate. The right jugular vein was also cannulated for intravenous injections. Blood pressure was recorded by means of a RP 1500 pressure transducer connected to a Narco MK III physiograph. A biotachometer coupler was used to integrate heart rate from the phasic blood pressure. Rectal temperature was monitored and maintained at 38 ± 0.2 C using an infrared lamp. Rats were placed in a Kopf stereotaxic apparatus with the head inclined at an angle of 20 degrees. The neck muscles were incised and deflected in order to expose the occipito-atloid membrane; a portion of the occipital bone was removed and the dura mater was exposed. A needle (o.d. 0.3 mm) connected to a microsyringe was introduced into the cisterna magna. Drugs were administered in a volume of 5 µl over a 2 min period. Doses are expressed in mol contained in this volume. A single injection was performed for each animal. Rats were normally breathing spontaneously. However, [D-Ala^2]-met-enkephalinamide was also studied in rats ventilated by a Braun pump (Type RA 0254). The Pa_{O_2} of carotid blood samples (0.15 ml) was measured by means of Radiometer Copenhagen AME

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1C. Blockade of the autonomic nervous system was performed by bilateral adrenalectomy and the injection, at 20 min intervals, of pentamethonium (20 mg/kg).

Drugs used were morphine (morphine chlorhydrate, Assistance Publique, Paris), fentanyl (fentanyl citrate, Lebrun), met-enkephalin (by the courtesy of Dr Vincent, Servier Laboratories), [D-Ala^2]-met-enkephalinamide (Peninsula), naloxone (American Cyanamid Company), diprenorphine (Reckitt & Colman) and pentamethonium (Delagrange). Stock solutions were made up in distilled water and were kept in plastic tubes at −25°C.

Statistical analyses were performed by means of Student's *t*-test for comparison of paired values. Pre-drug levels of blood pressure, heart rate and Pa_{O_2} were compared to levels obtained after drug injection. All values were expressed as mean ± s.e. mean.

Results

Cardiovascular effects of [D-Ala^2]-met-enkephalinamide and met-enkephalin administered intracisternally in spontaneously breathing rats

The effects of [D-Ala^2]-met-enkephalinamide studied in 23 rats are summarized in Table 1 and Figure 1. Injection of 10^{-12} mol of [D-Ala^2]-met-enkephalinamide did not significantly modify blood pressure whereas 10^{-11} mol caused a pressor effect in some cases. Hypertension became frequent at 10^{-10} mol (Figure 2) and was constantly observed at 10^{-9} mol. A dose of 10^{-8} mol produced a biphasic change, also shown in Figure 2.

Elevation of blood pressure was associated with a significant tachycardia (+37 ± 10 beats/min, $P < 0.01$, pre-drug level 390 ± 15 beats/min). A non-significant reduction in heart rate was observed during the fall in blood pressure (−20 ± 15 beats/min).

By contrast, met-enkephalin was without effect on blood pressure even at doses of 10^{-8} mol.

Cardiovascular effects of morphine and fentanyl administered intracisternally in spontaneously breathing rats

The effects of morphine injection in 18 rats are shown in Table 2 and Figure 1. At 10^{-9} mol no change in blood pressure was observed; at 10^{-8} mol a rise in blood pressure was followed in 3 out of 9 cases by a depressor effect. This biphasic response was more frequent after administration of 10^{-7} mol of morphine, occurring in 5 out of 6 cases.

Fentanyl at a dose of 0.75×10^{-9} mol induced only a rise in blood pressure as shown in Table 2.

The elevation in blood pressure induced by morphine and fentanyl was accompanied by a non-significant tachycardia (+13 ± 8 beats/min and +7 ± 6 beats/min respectively; pre-drug level 408 ± 12 beats/min and 429 ± 13 beats/min respectively). Similarly, a non-significant reduction in heart rate was found during the depressor phase (−14 ± 10 beats/min) when it occurred.

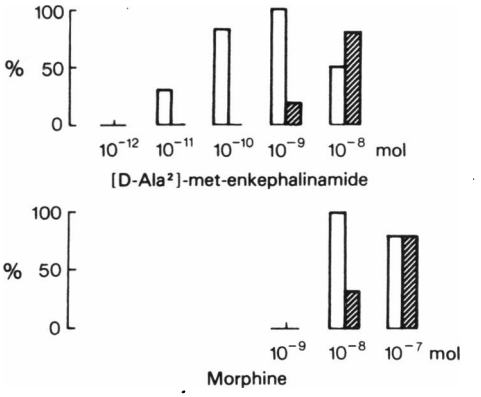

Figure 1 Effects of [D-Ala^2]-met-enkephalinamide and morphine on mean arterial blood pressure following intracisternal injection in spontaneously breathing rats. Results are expressed as % of rats which showed a blood pressor (open columns) or/and depressor (hatched columns) effect.

Cardiovascular effects of intravenously injected morphine and [D-Ala^2]-met-enkephalinamide

Morphine at doses of 10^{-8} and 10^{-7} mol induced a rapid and short-lasting fall in blood pressure. [D-Ala^2]-met-enkephalinamide at 10^{-9} mol did not change blood pressure but at 10^{-8} mol lowered blood pressure 2 min after the injection for 30 min. No pressor response was observed.

Pa_{O_2} variations in spontaneously breathing rats

In the control period, Pa_{O_2} was 95 ± 2 mmHg (10 animals). During the pressor response following central injection of drugs, Pa_{O_2} was not significantly changed, 86 ± 3 mmHg ($n = 8$). However, at the beginning of the depressor phase Pa_{O_2} was significantly diminished: 59 ± 3 mm Hg ($n = 12$; $P < 0.02$).

Cardiovascular effects of [D-Ala^2]-met-enkephalinamide injected intracisternally in artificially ventilated rats

The effects of [D-Ala^2]-met-enkephalinamide at a dose of 10^{-8} mol studied in 6 artificially ventilated

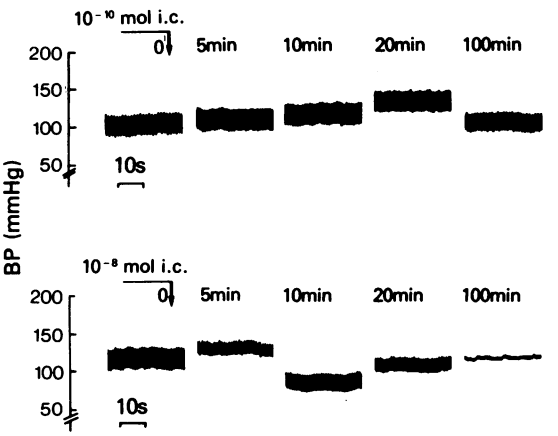

Figure 2 Monophasic pressor effect and biphasic effect of [D-Ala²]-met-enkephalinamide at low dose (10^{-10} mol) (a) and high dose (10^{-8} mol) (b) injected intracisternally (i.c.) in anaesthetized spontaneously breathing rats.

rats are summarized in Table 3. The only effect was a pressor response. No secondary hypotension, such as was observed in spontaneously breathing rats, occurred.

The elevation of blood pressure was associated with a significant tachycardia ($+49 \pm 9$ beats/min, $P < 0.01$, pre-drug level 340 ± 20 beats/min).

Effects of antagonists of opioid compounds

Naloxone injected intracisternally (10^{-7} mol) antagonized the cardiovascular effects of morphine (10^{-8} mol) administered by the same route 15 min later (5 experiments). Diprenorphine (10^{-7} mol), a more potent antagonist at enkephalin receptors (Childers, Creese, Snowman & Snyder, 1979), similarly abolished the blood pressure response to 10^{-8} mol of [D-Ala²]-met-enkephalinamide (5 experiments).

Blockade of the autonomic nervous system

Bilateral adrenalectomy was performed and pentamethonium was injected intraperitoneally in order to

Table 1 Effect on blood pressure of [D-Ala²]-met-enkephalinamide injected intracisternally in anaesthetized spontaneously breathing rats

[D-*Ala²*]-*met-enkephalinamide* (in 5 μl)	n	Latency of response (min)	Maximal effect (min)	Duration (min)	Mean Blood pressure (mmHg)
10^{-12} mol					
Control period	4	—	—	—	108 ± 3
Initial pressor effect	0	—	—	—	—
Secondary depressor effect	0	—	—	—	—
10^{-11} mol					
Control period	3	—	—	—	105 ± 5
Initial pressor effect	1	2	25	90	120
Secondary depressor effect	0	—	—	—	—
10^{-10} mol					
Control period	5	—	—	—	101 ± 1
Initial pressor effect	4	1–5	15–40	80–100	134 ± 6*
Secondary depressor effect	0	—	—	—	—
10^{-9} mol					
Control period	5	—	—	—	96 ± 4
Initial pressor effect	5	1–5	5–25	30–120	125 ± 8*
Secondary depressor effect	1	35	37	15	70
10^{-8} mol					
Control period	6	—	—	—	110 ± 4
Initial pressor effect	3	1–2	4–6	2–4	130 ± 9*
Secondary depressor effect	5	5–8	10–12	20	92 ± 5*

This table gives the number of rats showing pressor and depressor responses in each experimental group with the total number of rats indicated in the control period; both effects can be observed successively in the same rat. A single injection of drug was administered to each animal. Blood pressure values are expressed as mean ± s.e. mean.
*$P < 0.05$.

block the autonomic nervous system. In these animals, intracisternal injection of morphine (10^{-8} and 10^{-7} mol) no longer induced a rise in blood pressure (4 experiments). Similarly, intracisternal injection of [D-Ala^2]-met-enkephalinamide (10^{-9} mol) did not alter blood pressure (5 experiments).

Discussion

In the present study, central cardiovascular effects of morphinomimetic agents have been investigated in anaesthetized rats since marked behavioural changes occurred in conscious animals. Under these conditions a pressor response followed intracisternal injection of morphine, fentanyl and [D-Ala^2]-met-enkephalinamide at low doses. By contrast, a biphasic effect on blood pressure was observed with higher doses. Since morphine-like agents are known to produce respiratory depression (Teschemacher, Bläsig & Kromer, 1976; Denavit-Saubié, Champagnat & Zieglgänsberger, 1978), Pa_{O_2} was measured as an index of oxygenation. At the beginning of the depressor phase we observed a significant decrease in Pa_{O_2}.

Table 2 Effect on blood pressure of morphine and fentanyl intracisternally in anaesthetized spontaneously breathing rats

Drug (in 5 μl)	n	*Latency of response* (min)	*Maximal effect* (min)	*Duration* (min)	*Mean blood pressure* (mmHg)
Morphine					
10^{-9} mol					
Control period	4	—	—	—	115 ± 5
Initial pressor effect	0	—	—	—	—
Secondary depressor effect	0	—	—	—	—
10^{-8} mol					
Control period	9	—	—	—	118 ± 4
Initial pressor effect	9	3–5	15–30	50–80	148 ± 4*
Secondary depressor effect	3	60	60–65	10–20	94 ± 5*
10^{-7} mol					
Control period	6	—	—	—	124 ± 4
Initial pressor effect	5	3–7	10–20	15–40	150 ± 5*
Secondary depressor effect	5	20–30	30–35	10–20	96 ± 4*
Fentanyl					
0.75×10^{-9} mol					
Control period	3	—	—	—	125 ± 6
Initial pressor effect	3	1–2	8–12	20–30	156 ± 3*
Secondary depressor effect	0	—	—	—	—

This table gives the number of rats showing pressor and depressor responses in each experimental group with total number of rats indicated in the control period. Both effects can be observed successively in the same rat. A single injection of drug was administered to each animal. Blood pressure values are expressed as mean ± s.e. mean.
* $P < 0.05$.

Table 3 Effects on blood pressure of [D-Ala^2]-met-enkephalinamide injected intracisternally in anaesthetized, artificially ventilated rats

[D-Ala^2]-met-enkephalinamide (in 5 μl)	n	*Latency of response* (min)	*Maximal effect* (min)	*Duration* (min)	*Mean blood pressure* (mmHg)
10^{-8} mol					
Control period	6	—	—	—	106 ± 5
Initial pressor effect	6	2–10	5–90	40–180	142 ± 4*
Secondary depressor effect	0	—	—	—	—

Blood pressure values are expressed as mean ± s.e. mean
* $P < 0.01$.

Both respiratory depression and hypotension have also been described in cats after local application of met-enkephalin to the ventral surface of the brain stem (Florez & Mediavilla, 1977). This association suggests that the hypotension could be due to hypoxemia. In order to investigate this hypothesis we performed experiments in rats in which the Pa_{O_2} was kept constant by artificial ventilation and found that in those circumstances, [D-Ala^2]-met-enkephalinamide showed only a pressor effect. These results demonstrate clearly that the hypotension in the rat is associated with respiratory depression. It seems therefore that the effects of morphine-like drugs may depend on species differences, since in artificially ventilated dogs the hypotensive effect is predominant (Laubie, Schmitt, Canellas, Roquebert & Demichel, 1974; Laubie *et al.*, 1977).

Our data support the idea that morphinomimetic agents have a central pressor action in the rat. However, met-enkephalin did not change blood pressure in our experiments. This result may be explained by its rapid metabolism (Pert, Bowie, Fong & Chang, 1976) and doses higher than those used in the present study are necessary to produce an increase in blood pressure (Bolme *et al.*, 1978; Simon, Schaz, Ganten, Stock, Schlör & Ganten, 1978). By contrast, [D-Ala^2]-met-enkephalinamide, which is metabolized slowly (Walker, Berntson & Sandman, 1977), consistently caused a pressor effect at low doses. The pressor response to morphine-like agents after intracisternal administration is not secondary to a leakage into the systemic circulation since intravenous administration of morphine and [D-Ala^2]-met-enkephalinamide induced a transient depressor response. This centrally mediated hypertension was related to activation of sympathetic tone as it was abolished by bilateral adrenalectomy and pentamethonium administration. Finally, using specific antagonists we have demonstrated that central opiate receptors were activated.

Morphinomimetic agents inhibit *in vitro* the release of catecholamines via an action occurring presynaptically (Arbilla & Langer, 1978). Noradrenaline causes blood pressure to fall when locally applied to the NTS (De Jong, 1974). It is therefore possible that the hypertensive effect of opioids is a consequence of a reduction of noradrenaline release in the medullary cardiovascular centres.

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