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Systemic administration of β-endorphin: Potent hypotensive effect involving a serotonergic pathway

(blood pressure/naloxone/*p*-chlorophenylalanine/serotonin antagonists)

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ABSTRACT **In normal adult rats anesthetized with urethane, intravenous injections of β-endorphin (30–150 μg kg^{-1}) induced a transient fall of blood pressure followed by a small hypertension and a prolonged hypotension. Prior administration of naloxone completely blocked these effects, whereas naloxone, given 1 hr after β-endorphin, did not reverse the prolonged depressor phase of the opioid peptide. The effects of β-endorphin on the arterial blood pressure were greatly reduced in animals pretreated with *p*-chlorophenylalanine, a specific depletor of serotonin. Moreover, in rats pretreated with potent serotonin antagonists such as cyproheptadine, mianserin, and metergoline, β-endorphin did not produce a significant hypotension. Furthermore, the depressor effect of β-endorphin was potentiated by fluoxetine, a specific serotonin uptake inhibitor. These observations suggest the participation of a serotonergic pathway in the action of β-endorphin on the arterial blood pressure.**

Since its isolation and identification from the pituitary glands of different species (1–4), β-endorphin, an untriakontapeptide corresponding to residues 61–91 of β-lipotropin (5), has been found to have potent analgesic (2, 6, 7) and hypothermic (8) effects when injected intraventricularly. However, peripheral injections of large doses (8–20 mg kg^{-1}) of β-endorphin produced much smaller analgesic effects (8, 9), probably because β-endorphin does not easily cross the blood–brain barrier. Recently, it was shown that β-endorphin, injected in the cisterna magna of dogs, has central cardiovascular effects resulting in a small transient increase and prolonged decrease of the arterial blood pressure (BP) (10). The present work was undertaken to study the effects of β-endorphin on the arterial BP of rats by the use of a systemic approach. We report here that β-endorphin given intravenously has potent hypotensive effects at doses similar to those injected intracisternally (10). Because serotonin (5-hydroxytryptamine, 5-HT) was reported to be involved in the analgesic action of β-endorphin (11) and to have important depressor effects (12–14), we undertook additional studies in order to investigate its possible role in mediating the action of β-endorphin on BP.

EXPERIMENTAL PROCEDURE

β-Endorphin, with a sequence corresponding to the camel hormone (1), was synthesized by using the solid-phase technique as previously described (15). Naloxone·HCl was purchased from Endo Laboratories. DL-*p*-Chlorophenylalanine (*p*Cl-Phe) was purchased from Sigma. Fluoxetine (Lilly 110140) was obtained through the courtesy of R. S. Hosley, Eli Lilly. Cyproheptadine and mianserin were kindly provided, respectively, by W. E. Dorian, Merck Frosst Laboratories, Pointe Claire, PQ, and R. M. Koenig, Organon, West Orange, NJ. Metergoline was generously supplied by C. Praga, Farmitalia, Milan, Italy. Male Wistar rats (Canadian Breeding Farm & Laboratories, St. Constant, PQ) weighing 275–325 g were housed two to a cage in soundproof quarters maintained at 25 ± 0.5°C and 55% relative humidity, on a day–night lighting cycle of 12 hr of light and 12 hr of darkness. They were given Purina rat chow and water ad lib. One week after reception, the animals were used for BP recordings.

BP Recording. Rats were anesthetized with urethane (1 g kg^{-1}, subcutaneous). The trachea was intubated and BP was recorded on a Narcophysiograph or Grass polygraph with a catheter introduced in the carotid artery and connected to a pressure transducer (E & M Instruments or, when indicated, Statham P23). Drugs were injected directly in the jugular vein in a volume of 0.1 ml followed by 0.2 ml of saline. The initial BP, prior to the injection of β-endorphin, was considered as control and the pressor response was expressed as percentage of control. The results are expressed as mean ± SEM of six to eight determinations. Statistical significance was calculated with a Student *t* test; $P < 0.05$ was chosen as the level of significance.

Depletion of Endogenous 5-HT. Groups of six rats received 100 mg kg^{-1} of *p*Cl-Phe intraperitoneally for three consecutive days. Control rats were similarly pretreated with saline. Twenty-four hours after the last injection of *p*Cl-Phe, animals were prepared as described above and BP was recorded.

RESULTS

Effects of Intravenous Injections of β-Endorphin on BP. A typical response to intravenous (i.v.) injection of β-endorphin is shown in Fig. 1. Such injection of the opioid peptide caused an immediate fall of BP (a) followed by a small hypertension (b) and a prolonged hypotension (c). In some animals, the late hypotension (c) lasted for more than 90 min, whereas in others BP returned more rapidly to normal values. All the animals tested displayed the initial fall of BP in various degrees, whereas, out of 29 rats, 15 did not show the hypertensive phase b.

Pretreatment of rats with naloxone·HCl (60 μg kg^{-1}), 4 min before the injection of β-endorphin, completely blocked the effects of the opioid peptide (Fig. 2*B*), demonstrating the specificity of action of β-endorphin and the involvement of an opiate receptor (16). However, when the same dose of naloxone was given 60 min after the injection of β-endorphin, the late hypotension observed was not reversed (Fig. 2*A*). These results suggest that changes in BP are not directly caused by β-endorphin itself but are more likely consecutive to its action on some mediator(s).

Fig. 3 illustrates the dose-related inhibition of BP measured 2 min after i.v. injection of β-endorphin. In normal rats, BP was lowered to 77.4% of the control 2 min after the administration

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Abbreviations: BP, blood pressure; 5-HT, serotonin (5-hydroxytryptamine); *p*Cl-Phe, *p*-chlorophenylalanine; i.v., intravenous.

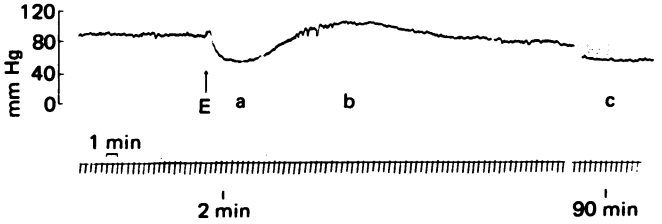

FIG. 1. Typical effect of i.v. injection of β-endorphin on BP, recorded on a Narcophysiograph. E, β-endorphin, 150 μg kg^{-1}, i.v.; a, initial fall of BP; b, small hypertensive effect; c, late hypotensive effect. One mm Hg = 133 Pa.

of β-endorphin at 45 μg kg^{-1}, a maximal inhibition of 30% (70% of control) being obtained with a dose of 75 μg kg^{-1}. Thus, the potency of β-endorphin, administered intravenously, is similar or even higher to that reported upon direct injection of the opioid peptide in the cisterna magna of dogs (10). In addition, our results indicate that β-endorphin has profound effects on BP, at doses 1/200th to 1/300th those reported to produce analgesia (8, 9) when administered by the systemic route.

Depletion of Endogenous 5-HT. The effects of β-endorphin on BP are rather complex and can be compared to the well-known depressor effects of 5-HT in rats (12, 13, 14). Because 5-HT was reported to be involved in the analgesic response to β-endorphin (11), we investigated the effects of β-endorphin in rats pretreated with *p*Cl-Phe, a specific depletor of 5-HT (17). By blocking the biosynthesis of 5-HT at the tryptophan hydroxylation step, *p*Cl-Phe was reported to decrease the levels of 5-HT to less than 10% in the brain (17, 18) and 40% in the blood (17), while norepinephrine and dopamine concentrations remain relatively unchanged (17–19). Rats pretreated for three consecutive days with *p*Cl-Phe (100 mg kg^{-1}) showed no or very small change of BP following i.v. injections of 75 and 150 μg kg^{-1} β-endorphin (Fig. 3 and Table 1).

Effect of 5-HT Antagonists. In order to further study the possible involvement of 5-HT in the depressor response to β-endorphin, we also investigated the effects of various 5-HT antagonists on β-endorphin-induced hypotension. Cyproheptadine in the dose range of 0.1–1 mg kg^{-1} was reported to be a potent antagonist of the pressor effect of 5-HT in the dog while having little or no adrenergic blocking activity (20). Mianserin was also reported to antagonize the hypertensive, coronary vasodilator and venoconstrictor actions of 5-HT (21) at doses much lower than those required to antagonize the effects of epinephrine and norepinephrine. Finally, metergoline was found to be a selective 5-HT antagonist in the rat brain (22–25) and appeared to be the most suitable antiserotonergic agent (26). Table 1 shows that pretreatment of animals with cyproheptadine (0.8 mg kg^{-1}), mianserin (0.1 mg kg^{-1}), and metergoline (10 mg kg^{-1}) blocked the early depressor response to β-endorphin (150 μg kg^{-1}), their BP being decreased, respectively, to 89.5%, 90.8%, and 94.7% of the control compared with 77.1% in untreated animals.

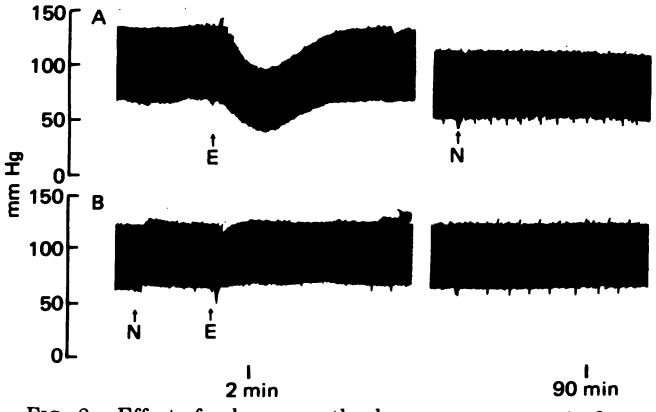

FIG. 2. Effect of naloxone on the depressor responses to β-endorphin. BP was recorded on a Grass polygraph. E, β-endorphin, 150 μg kg^{-1}, i.v. Naloxone (N, 60 μg kg^{-1}, i.v.) was injected 60 min after β-endorphin (*A*) or 4 min before β-endorphin (*B*).

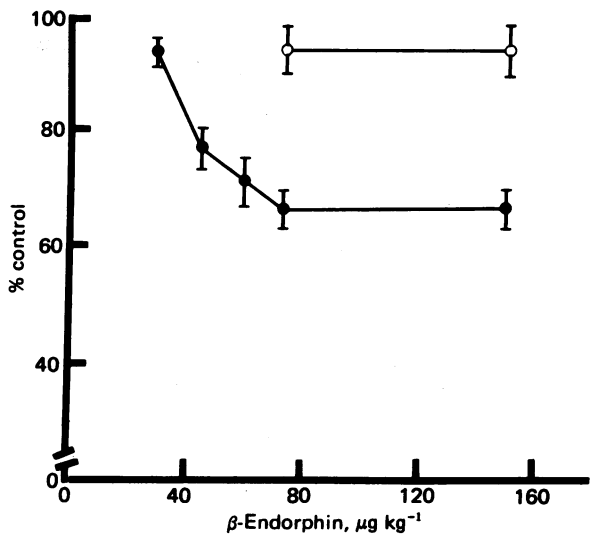

FIG. 3. Dose-related inhibition of BP 2 min after i.v. injection of increasing amounts of β-endorphin. Results are expressed as percentage of control ± SEM. ●, Normal rats; ○, rats treated with *p*Cl-Phe, 100 mg kg^{-1}, intraperitoneally, for 3 consecutive days.

Effect of a 5-HT Uptake Inhibitor. Our data indicate that depletion of endogenous 5-HT by *p*Cl-Phe or blockade of 5-HT receptors by specific antagonists blocks the hypotensive effect of β-endorphin. Conversely, increase of 5-HT availability at the receptor site by blockade of its reuptake would be expected to potentiate the action of β-endorphin. Fluoxetine is a potent inhibitor of 5-HT reuptake in the brain (27) and platelets (28)

Table 1. Effects of 5-HT depletor, antagonists, and uptake inhibitor on the initial depressor effect of β-endorphins

Drug*	Animals	BP, % control†
Depletor		
None	4	77.1 ± 2.6
*p*Cl-Phe	6	92.4 ± 1.7
Antagonists		
None	4	77.1 ± 2.6
Cyproheptadine, 0.8 mg kg^{-1}	8	89.5 ± 0.2
Mianserin, 0.1 mg kg^{-1}	8	90.8 ± 1.4
Metergoline, 10 mg kg^{-1}	9	94.7 ± 1.5
Uptake inhibitor		
None	4	79.4 ± 2.9
Fluoxetine, 10 mg kg^{-1}	6	63.2 ± 3.8

* *p*Cl-Phe was administered as described in *Experimental Procedure*. Cyproheptadine and mianserin were given intravenously 15 min and 30 min, respectively, before β-endorphin. Metergoline and fluoxetine were injected intraperitoneally 1 hr and 2 hr, respectively, before β-endorphin.

† Values represent mean ± SEM. BP was recorded 2 min after the i.v. injection of β-endorphin at 150 μg kg^{-1}.

while being essentially devoid of effect on norepinephrine reuptake (27, 29). Table 1 shows that the depressor effect of β-endorphin (150 μg kg^{-1}) is potentiated by pretreatment of rats with fluoxetine (10 mg kg^{-1}), their BP being decreased to 63.2% of control compared with 79.4% in untreated animals. It is worth mentioning that two out of nine rats died after the injection of β-endorphin, indicating the supersensitivity of fluoxetine-treated rats to β-endorphin.

DISCUSSION

Previous studies have shown that morphine-like compounds induce a decrease in the sympathetic tone and an increase in the vagal tone leading to hypotension (30, 31). More recently, β-endorphin was found to have central cardiovascular effects when injected in the cisterna magna of dogs (10), and it was suggested that opioid peptides may be involved in central cardiovascular control. The present work shows that systemic administration of β-endorphin in rats at doses similar to those applied centrally (10) also has profound effects on arterial BP.

Our finding that *p*Cl-Phe, a specific depletor of 5-HT (17), blocks these effects supports a role for 5-HT in the action of β-endorphin on BP. Earlier reports point out the participation of 5-HT in the central analgesic action of morphine (32–34) and β-endorphin (11). Our studies also reveal striking similarities between the effects of 5-HT (13, 14) and those of β-endorphin on the arterial BP, namely, an initial fall in BP (a) followed by a short pressor phase (b) and a prolonged depressor phase (c) (Fig. 1). Moreover, blockade of 5-HT receptors by potent antagonists such as cyproheptadine, mianserin, and metergoline greatly reduces the first depressor effect of the opioid peptide, bringing additional support for the participation of 5-HT in the action of β-endorphin on BP. The fact that fluoxetine, a selective inhibitor of 5-HT uptake (27, 28), potentiates this depressor effect also speaks for this assumption.

The observations herein reported indicate that circulating β-endorphin may regulate the systemic BP and that a serotonergic mechanism is instrumental in this regulation. However, the participation of other mediator(s) cannot be ruled out and further studies are necessary to elucidate the mechanism of action of β-endorphin.

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