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MIF-1 Does Not Act Like Naloxone in Antagonizing the Cardiovascular Activity of Leucine-Enkephalin in the Conscious Dog

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SANDER, G. E., A. J. KASTIN AND T. D. GILES. *MIF-1 does not act like naloxone in antagonizing the cardiovascular activity of leucine-enkephalin in the conscious dog.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1301–1303, 1982.—MIF-1 (Pro-Leu-Gly-NH_2), a hypothalamic tripeptide, has been demonstrated to simulate naloxone in antagonizing the effects of opioid peptides in a number of experimental systems including enkephalin-induced analgesia in the tail-flick assay, β-endorphin induced hypothermia and hypomotility, deprivation-induced drinking, and analgesia in goldfish. MIF-1, however, has no effect upon the activity of enkephalins in the mouse vas deferens or enkephalin binding in the rat striatum. We have studied the interactions of MIF-1 with Leu^5-enkephalin (Leu^5-ENK) in the conscious, chronically instrumented dog. Although naloxone inhibits both the elevations of heart rate and blood pressure produced by IV Leu^5-ENK in the conscious state and the depressions in these variables produced by Leu^5-ENK after pentobarbital anesthesia, MIF-1 has no effect upon the Leu^5-ENK response in either state. However, both naloxone and MIF-1 seem to raise mean arterial pressure in the conscious dog. These results indicate that MIF-1 does not act like naloxone in antagonizing the peripheral effects of Leu^5-ENK and lend further support to the existence of mechanistic differences among opiate-mediated behavior, analgesia, and cardiovascular activity.

MIF-1 Naloxone Leu-Enkephalin

THE hypothalamic peptide MIF-1 (Pro-Leu-Gly-NH_2) exhibites a range of behavioral and pharmacologic activity after central and peripheral administration. It was used in the initial demonstration of the direct central nervous system (CNS) activity of a brain peptide independent of the pituitary [7,12]. In rats, MIF-1 potentiates the behavioral effects of dopa, inhibits oxotremorine-induced tremors, and reduces deserpidine-induced sedation [7].

MIF-1 alters opiate actions in several systems. Both the facilitation [19,21] and blockade [2,22] of opiate tolerance by MIF-1 have been described. It has been suggested that MIF-1 might represent a class of naturally occurring opiate antagonists with varying activities in independent situations [8], and has been studied in experimental conditions in which the opiate antagonist naloxone is active. MIF-1 blocks the analgesic effects of the enkephalins and morphine in the mouse tail-flick assay, but does not antagonize the actions of enkephalins in the mouse vas deferens assay or reduce food intake in VMH-lesioned rats, models in which naloxone is highly antagonistic [8]. MIF-1 displays actions similar to naloxone in analgesia in goldfish [5], hypothermia and hypomotility induced by beta-endorphin [23], deprivation-induced drinking [11] and, to a much lesser extent, in the guinea pig ileum [4,9]. MIF-1 did not compete with either ^{3}H-D-Ala^2-D-Leu^5-ENK or ^{3}H-naloxone in rat striatum, suggesting that the differential actions of MIF-1 on opiate systems may occur at a later point in the receptor-mediated chain of events such as guanylate nucleotide metabolism [6,18], or indirectly through an MIF-1 receptor [10].

In the conscious dog, intravenously administered Leu^5-ENK elevates heart rate (HR) and mean systemic arterial pressure (MAP) in a dose-dependent fashion [14]. After pentobarbital anesthesia, Leu^5-ENK produces hypotension and slight bradycardia [15]. The purpose of the present experiments was to compare the actions of MIF-1 with those of naloxone in the conscious, chronically instrumented dog model.

METHOD

Animal Model

Microfilaria-free adult mongrel dogs were anesthetized with intravenous pentobarbital (20 mg/kg). An anterior neck incision was made and polyethylene catheters were introduced into the proximal aorta via the common carotid artery and the right atrium via the external jugular vein. Catheters were tunnelled subcutaneously and brought out at the nape of the neck; they were flushed with sodium heparin solution as necessary to maintain patency. Experiments in the anesthetized state were performed immediately afterwards. Dogs were then allowed to recover for a minimum of 24 hr before performing experiments in the conscious state.

During the experiment, they were placed in an adjustable sling frame. Systolic, diastolic, and mean systemic arterial pressures were recorded using Statham P23Db pressure transducers and an Electronics for Medicine VR-6 physiograph. Heart rate was determined from a continuously recorded limb lead electrocardiogram.

TABLE 1

CHANGES IN HEART RATE AND MEAN ARTERIAL PRESSURE INDUCED BY LEU^5-ENK AND MIF-1 IN CONSCIOUS AND ANESTHETIZED DOGS

	Conscious Dogs			Anesthetized Dogs	
Order of Injection	n	HR	MAP	n	HR	MAP
Control	6	7± 3	3±1	8	− 6±4	− 5±2
Leu^5-ENK	6	43± 9*	18±3*	8	−13±5	−32±4*
MIF-1	6	11± 5	10±3	7	− 8±4	− 4±2
Leu^5-ENK	4	35±10*	19±6			
Leu^5-ENK and MIF-1	3	39± 5*	13±7	8	− 9±5	−22±3*
Leu^5-ENK	3	40± 5*	16±4*	7	− 9±5	−19±2*

Data are expressed as mean±S.E.M. for the absolute change in heart rate (HR) in beats/min and mean arterial pressure (MAP) in mm Hg.
*$p<0.05$ relative to control.

Peptides were dissolved in diluent solution (0.01 M acetic acid in 0.9% saline) in a 1 mg/ml concentration and administered into the venous catheter, which was immediately flushed with 5 ml of 0.9% NaCl. A bolus dose of the diluent was always given initially as the matched control for the responses in that experiment, and was consistently devoid of significant physiological effect. An experimental sequence was performed one time in each dog. Maximal heart rate and mean arterial pressure changes occurred within 120 sec of administration of enkephalin. When Leu^5-ENK and MIF-1 were administered together, the peptides were mixed together in a syringe and given as a single bolus. In a separate series of experiments, naloxone (1 mg/kg) was dissolved in diluent and infused over 60 sec; its activity was reported relative to that of the diluent infusion.

Materials

Leu^5-ENK was purchased from Calbiochem-Behring (La Jolla, CA), and sodium heparin and pentobarbital from Abbott Labs (North Chicago, IL). Naloxone was provided by Endo Labs (Wilmington, DE).

Statistical Methods

All data are expressed as mean±S.E.M., with heart rates or absolute changes in rate expressed as beats/min and arterial blood pressures or absolute changes in mmHg. Duncan's New Multiple Range Test was used to compare the treatments. Student's paired t-test was used to evaluate the activity of naloxone relative to diluent.

RESULTS

MIF-1 Interactions with Leu^5-ENK in the Conscious Dog

In the conscious dog given diluent the slight elevations of HR from 116±13 to 124±13 and MAP from 104±8 to 107±8 (n=6) were not statistically significant. Leu^5-ENK (35 μg/kg) elevated HR from 125±30 to 168±25 ($p<0.05$) and MAP from 108±9 to 126±9 ($p<0.05$, n=6). MIF-1 (35 μg/kg) had little effect on HR, 115±17 to 126±15, but elevated MAP from 91±7 to 101±6 (n=6). Five minutes after MIF-1, Leu^5-ENK (35 μg/kg) increased HR from 126±16 to 169±6 ($p<0.05$) and MAP from 119±13 to 141±9 ($p<0.05$, n=3). Leu^5-ENK and MIF-1 administered together raised HR from 110±5 to 149±6 ($p<0.05$) and MAP from 128±15 to 141±9 (n=3). Leu^5-ENK injected 5 min after the MIF-1:Leu^5-ENK mixture significantly elevated HR from 112±5 to 152±2 ($p<0.05$) and MAP from 123±11 to 139±8 ($p<0.05$, n=3). In two experiments, Leu^5-ENK was again administered 30 min after MIF-1, and again a full Leu^5-ENK response was noted. These data are shown in Table 1 as the absolute change in HR and MAP occurring after administration of peptide. MIF-1, either alone or together with Leu^5-ENK, failed to alter any of the Leu^5-ENK responses.

MIF-1 Interactions with Leu^5-ENK after Pentobarbital Anesthesia

In the pentobarbital anesthetized dog given diluent, the reductions of HR from 152±12 to 147±8 and MAP from 131±6 to 127±6 (n=8) were not statistically significant. Leu^5-ENK lowered HR from 165±6 to 153±8 and MAP from 131±7 to 99±6 ($p<0.05$, n=8). MIF-1 was essentially devoid of activity in this system, changing HR from 165±8 to 157±9 and MAP from 131±8 to 127±8 (n=7). The Leu^5-ENK:MIF-1 combination decreased HR from 162±9 to 153±10 and MAP from 130±8 to 107±6 ($p<0.05$, n=8). The final dose of Leu^5-ENK lowered HR from 160±8 to 151±11 and MAP from 133±7 to 114±6 ($p<0.05$, n=7). In no case were the HR changes to Leu^5-ENK different after MIF-1 than without it. All subsequent Leu^5-ENK injections significantly lowered MAP ($p<0.05$), but to less extent than did the initial dose; otherwise, Leu^5-ENK responses were unaffected by MIF-1 (Table 1).

Effect of Naloxone

Fifteen conscious dogs were given naloxone (1 mg/kg) intravenously over 60 sec. HR rose from 106±10 to 120±9, and MAP from 99±5 to 108±5 ($p<0.05$). By comparison, the MAP after MIF-1 went from 91±7 to 101±6, an increase that by Student's t-test was significant ($p<0.05$).

DISCUSSION

In the conscious, chronically instrumented dog, Leu^5-ENK elevates HR, respiratory rate, and MAP in a dose-dependent fashion; this response is abolished by pretreatment with intravenous naloxone at a dose of 1 mg/kg [14].

Similarly, naloxone inhibits the increases in HR and MAP induced by Leu^5-ENK in the conscious rat [17]. Naloxone also inhibits the vasodepressor response to Leu^5-ENK in dogs after pentobarbital anesthesia [15].

The results show that MIF-1 does not alter the response to Leu^5-ENK whether administered before or with the enkephalin in the conscious dog. In the anesthetized dog, MIF-1 also has minimal effect. Although the reduction in MAP after the final dose of Leu^5-ENK is less than after the initial dose, it remains a significant reduction relative to control and is not different from the Leu^5-ENK:MIF-1 response. This reduced depressor response may result from a lighter level of pentobarbital anesthesia at this later time during the experiment.

Intravenously administered naloxone has been reported to elevate blood pressure in conscious dogs [20] and in awake human beings [3]. Our results in unanesthetized dogs are in accord with these findings. The mechanism of this increase in pressure is unclear, but may reflect an effect on the central nervous system [1]. The apparent elevation of MAP after intravenous MIF-1 in the conscious dog does parallel naloxone in this regard and has been observed previously after oral administration in dogs [13].

In the intact dog, however, MIF-1 does not act like naloxone in antagonizing the cardiovascular responses to the enkephalins. This observation is consistent with the failure of MIF-1 to inhibit enkephalins in the mouse vas deferens [8], a predominantly δ receptor assay. The cardiovascular activity of the enkephalins in the conscious dog appears generally correlated with δ-receptor affinity, although these responses cannot be fully explained by *in vitro* μ and δ receptor models [16]. This dissimilarity of MIF-1 and naloxone in moderating cardiovascular activity may be contrasted with the similarity of these agents in such analgesia models as the mouse tail-flick assay [8] to lend further support to the existence of mechanistic differences among opiate-mediated behavior, analgesia, and cardiovascular activity [7,14].

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