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BRIEF COMMUNICATION

Behavioral Activation by Enkephalins in Mice

R. J. KATZ, BERNARD J. CARROLL AND GIULIO BALDRIGHI

Mental Health Research Institute, Department of Psychiatry, University of Michigan, Ann Arbor, MI 48109

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KATZ, R. J., B. J. CARROLL AND G. BALDRIGHI. *Behavioral activation by enkephalins in mice.* PHARMAC. BIOCHEM. BEHAV. 8(4) 493–496, 1978. – Intraventricular injection of long lasting enkephalin analogues (D–Ala^2 Leu and Met enkephalin-amides) produced a sustained elevation of psychomotor activity in mice. The motor syndromes were characterized by continual stereotyped activity and were reversed by naloxone pretreatment. Naloxone administered to a separate group of mice reduced the initial activation seen after exposure to a novel environment. The present findings suggest one or more endogenous opiates normally facilitates behavioral excitation in mice.

Activity Enkephalin Naloxone Opiate Psychomotor

MORPHINE and its related narcotic analgesic drugs produce a variety of behavioral effects. In most species the response to opiates is behavioral sedation and somnolence. Some well-known exceptions occur, however, particularly in rats (at low dosages only), cats, mice, horses and some humans [2, 6, 7]. In these species excitement and increased motor activity are observed after morphine injections. This phenomenon can be studied most easily in mice, which display a highly stereotyped running response, Straub tails, a virtually complete absence of rearing activity, and a profound oblivion to external objects [1,2].

Several endogenous peptides with opiate-like activity (enkephalins) have recently been identified [3, 4, 8, 10, 13, 14, 15]. Methionine enkephalin can produce analgesia, tolerance and dependence [1, 3, 14]. Leucine enkephalin is self-administered strongly by animals which also self-administer morphine [12].

Several recent reports suggest that enkephalins and enkephalin analogues may facilitate the behavioral activation induced by other psychoactive compounds, or themselves induce a state of increased motor activity [5, 9, 10, 15]. Since reports noting a direct effect of enkephalins upon activity have generally reported these changes as incidental observations with limited statistical description (e.g., [10,15]), we examined the ability of enkephalins to produce the behavioral activation typically caused by morphine in mice. To maximize behavioral effects, we used analogues which are known to have extended biological and behavioral activity [3,5]. Enkephalin analogues were injected directly into the cerebral ventricles.

METHOD AND PROCEDURE

Ninety-seven adult male Swiss-Webster mice, weighing 25–35 g (Charles River, Portage, Michigan) were maintained on standard ad lib diets (Wayne Lab Blocks) and a 12/12 light-dark cycle. A ventricular cannula of our own design was implanted under sodium pentobarbital anaesthesia. The cannula consisted of a 23 ga stainless steel needle with an indwelling obturator and was aimed at the third ventricle. The needle was attached to the skull with acrylic dental cement reinforced by three stainless steel screws. During the week after surgery each mouse was placed in the testing cage for five two-hour sessions to allow habituation. During drug-administration sessions each mouse was placed in the testing cage for one hour of initial habituation and baseline activity recording before the drug was injected. One additional group of 12 mice was not habituated, and was used to assess the effects of naloxone upon initial motor activity. Motor activity was recorded with four field-sensitive platforms (Stoelting, Chicago, SA 1566, 1666, 1583, 1454) calibrated to within 5% of each other. Mice were placed individually in polypropylene cages (Scientific Products Series 70, 51 × 41 × 22 cm) which rested directly on the recording platforms. Fresh pine chip bedding was placed in each cage. The activity of each mouse was recorded in 10 min intervals for 120 min after drug administration. The vehicle used for all injections was Ringer-Locke solution. Injections were performed manually with a Hamilton micro-syringe, using a fixed volume of 10 μl. This procedure took less than 30 sec to complete. We tested the D–Alanine^2 analogues of both methionine- and leucine-enkephalinamides (Peninsula Laboratiores, San

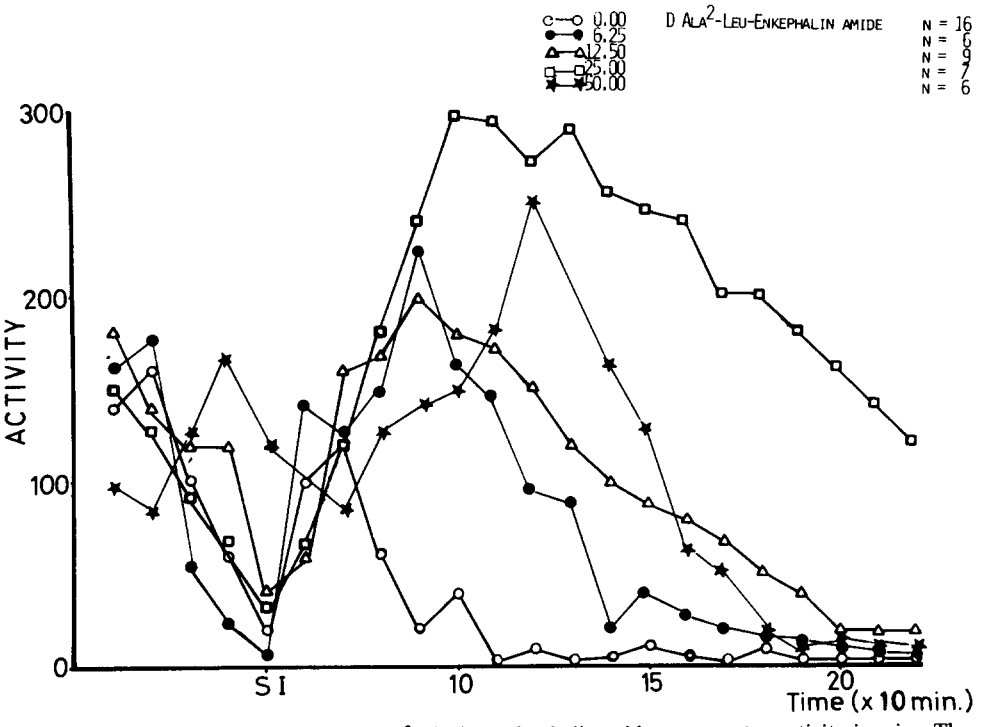

FIG. 1. Effects of intraventricular D–Ala^2-leucine enkephalinamide upon motor activity in mice. The median activity of each group at each time point is displayed.

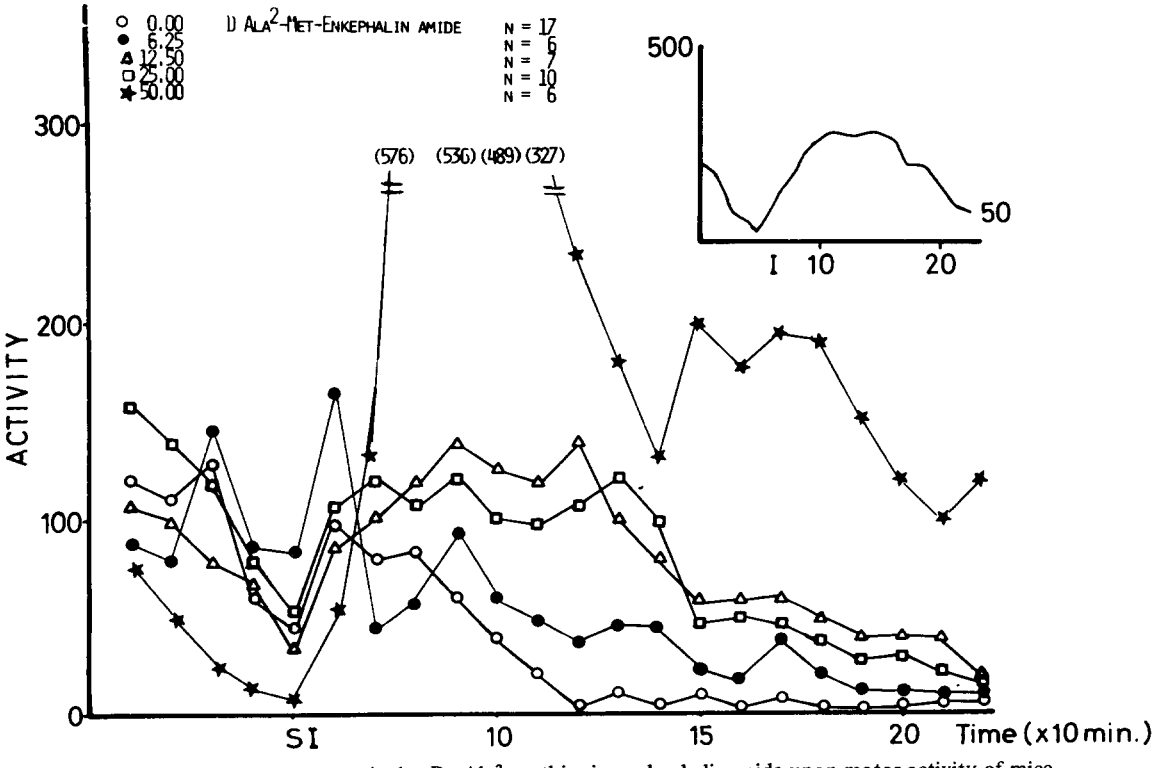

FIG. 2. Effects of intraventricular D–Ala^2-methionine enkephalinamide upon motor activity of mice. The median activity of each group at each time point is displayed. Insert: Normal response of mice to intraperitoneal morphine 50 mg/kg.

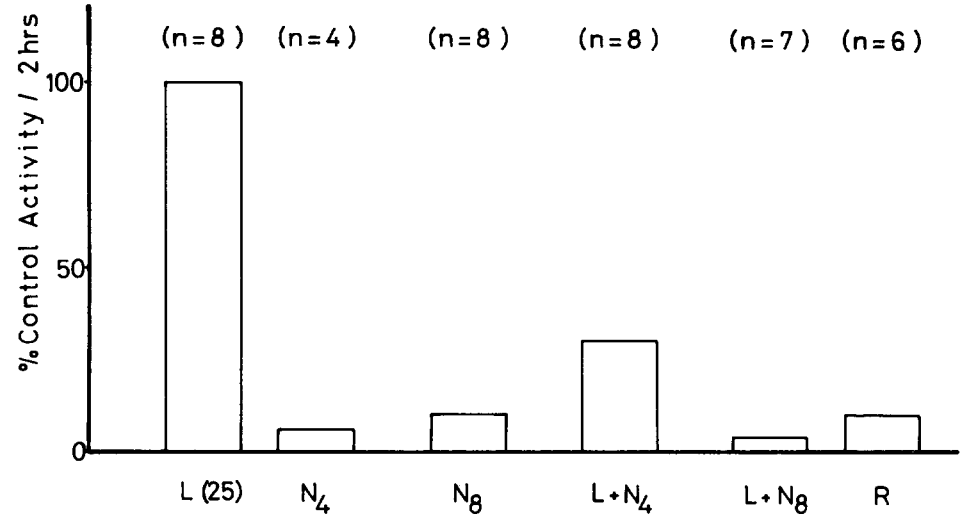

FIG. 3. Effects of Naloxone (4 and 8 mg/kg) on activity response of mice to intraventricular D–Ala^2-leucine enkephlinamide 25 μg (L25). N = Naloxone. R = Ringer-Locke solution. All scores are displayed as median percent of L25 response.

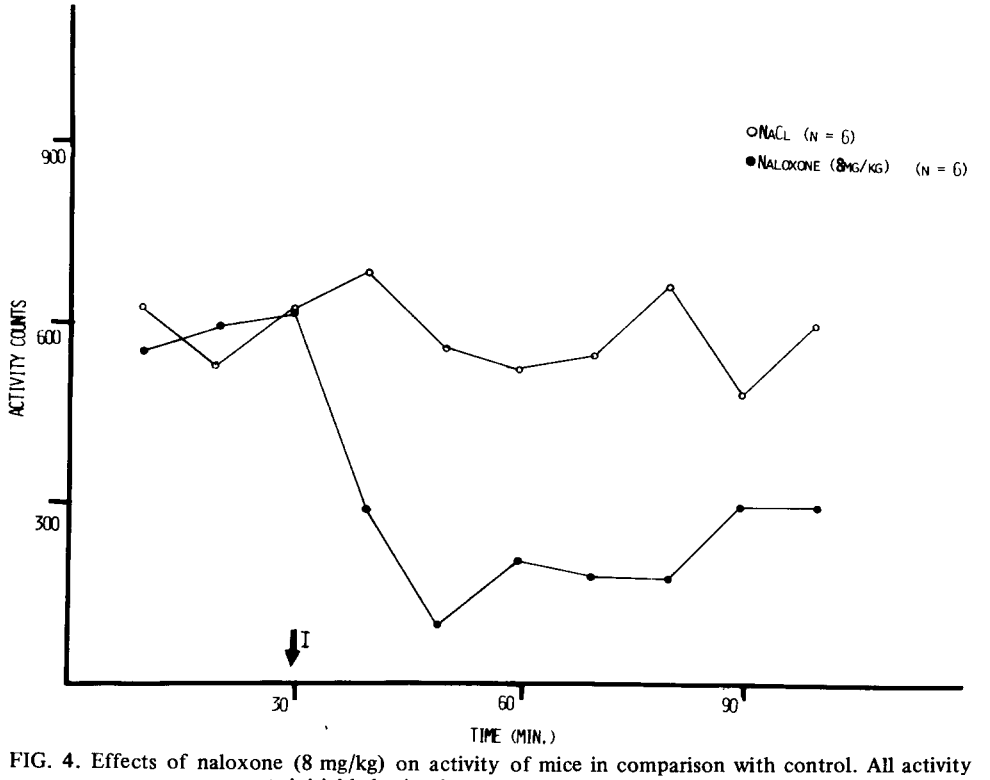

FIG. 4. Effects of naloxone (8 mg/kg) on activity of mice in comparison with control. All activity represents initial behavioral response to the recording apparatus.

Carlos, California, No. 8613, 8619), each at four doses: 6.25, 12.5, 25.0 and 50.0 μg. These compounds were used within 48 hr of preparation, and were stored in teflon coated containers at 4.0°C until injection. These compounds occupy morphine receptors but have a longer duration of action than endogenous enkephalins since they are less rapidly catabolized [3]. For some experiments naloxone (0, 4 or 8 mg/kg) was injected IP (10 ml/kg) either by itself or 15 min before 25 μg of D–Alanine^2 leucine enkephalinamide or vehicle.

RESULTS

The results are shown in Figs. 1–4. Figure 1 presents the effects of the leu-analogue, while Fig. 2 presents met-analogue effects. Both enkephalin analogues produced hyperactivity. The leu-analogue was more potent than the met-analogue at all but the highest dose. Both drugs produced dose-related increases in behavior, although leu-showed a relative decline at the highest dose. Two way repeated measures analysis of variance indicated significant

effects of drugs, $F_{leu}(4,41) = 9.97$, $F_{met}(4,39) = 13.76$; $p<0.01$, time, $F_{leu}(21,861) = 4.76$, $F_{met}(21,819) = 22.7$, $p<0.001$ and interaction, $F_{leu}(84,861) = 3.89$, $F_{met}(84,819) = 9.93$, $p<0.001$. By visual observation, both enkephalin analogues produced a stereotyped running syndrome which closely resembled morphine running [6]. Figure 3 indicates that, under conditions of extensive habituation (i.e., low or no normal activity), naloxone was without effect on spontaneous motor activity, and 2) naloxone prevented the running response to intraventricular leucine enkephalin analogue (U = 4,3, respectively, for 4 and 8 mg/kg naloxone, $p<0.01$ – Mann Whitney U test) [10].

On the other hand, this cannot be taken as an indication that naloxone is without effect. Under conditions of normally high activity (initial cage exposure prior to habituation), naloxone (8 mg/kg) produced a significant reduction in motor activity in comparison with a vehicle injected control group which was matched for activity at minute 30. This decline was significant, $F(1,10) = 6.79$ (drug), $F(10,90) = 1.77$ (time), $F(9,90) = 8.72$ (interaction), $p<0.05$, >0.05, <0.01, respectively).

DISCUSSION

Our findings suggest enkephalin analogues contribute to the activation responses of mice. The results with naloxone further suggest the involvement of an opiate receptor in our effects. The results with naloxone on initially active animals also suggest the involvement of some opiate-like compound in the control of behavioral activation. The present model of opiate induced activation represents a highly specific and quantifiable action of enkephalins which may prove useful in the further psychopharmacological analysis of the actions of endogenous opiates and their relationships with neural and behavioral systems.

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