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Implication of Endogenous Opioid System in the Learned Helplessness Model of Depression

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TEJEDOR-REAL, P., J. A. MICO, R. MALDONADO, B. P. ROQUES AND J. GIBERT-RAHOLA. *Implication of endogenous opioid system in the learned helplessness model of depression.* PHARMACOL BIOCHEM BEHAV **52**(1) 145–152, 1995. – The involvement of opioid system on the learned helplessness model of depression was investigated. Animals preexposed to inescapable shocks were treated with either Met-enkephalin, Leu-enkephalin, morphine, imipramine, naloxone, RB 38A (a mixed inhibitor of enkephalin degrading enzymes), or RB 38B (a selective inhibitor of neutral endopeptidase EC 3.4.24.11). Stimulation of opioid system by either opioid agonists or enkephalin catabolism inhibitors reversed the escape deficit induced by shock pretreatment. In contrast, administration of naloxone potentiated the effect of inescapable shocks. Imipramine reduced the number of escape failures in this test, and this effect was antagonized by naloxone. These results point to the involvement of the endogenous opioid system in this model of depression.

Opioid system Learned helplessness Depression Met-enkephalin Leu-enkephalin Morphine Imipramine Enkephalin-catabolism inhibitors

THE EUPHOROGENIC and anxiolytic properties of opioids and endorphins raise the possibility that a dysfunctioning endorphinergic system may cause the pathogenesis of endogenous depression. Indeed, since the work of Kraepelin (31), the opium cure has been recommended for the treatment of depressed patients. In 1977, Kline et al. (30) were the first to perform clinical trials in different types of psychiatric disorders (schizophrenia, depression, and neuroses) by use of β-endorphin infusion, and they observed an antidepressant effect. Further studies also support this hypothesis. Thus, β-endorphin has been associated with some specific clinical symptoms of depression (10), whereas buprenorphine exhibited antidepressant properties in depressed patients who did not respond to electroconvulsive therapy (17). Ciclazocyne, a mixed agonist-antagonist opioid, exerted a strong antidepressant effect on depressed patients, but it was not used in clinic because of its psychotomimetic action (20). Electroconvulsive therapy, one of the most effective treatments for severe depression, has also revealed interesting findings. Thus, this treatment increased the level of β-endorphin in plasma (16, 27,38), and naloxone was able to reverse the effect of electroconvulsive therapy on depressed patients (3).

The possible role of endogenous opioids in depression is supported by various neurochemical and neurobehavioral findings. Endogenous enkephalins are closely involved in the behavioral reinforcement system (4,13,46). Thus, both enkephalins and opioid receptors are localized in brain areas involved in mood and stress responses (3,54). Furthermore, interactions between opioid and dopaminergic systems have been widely reported [i.e., enkephalinergic and dopaminergic neurons are colocated in several brain areas implicated in reward processes (41)].

Evidence shows that antidepressant drugs have an influence on opioid systems. Thus, Reisine and Soubrié (42) found a specific loss of receptors sites for opioid binding in the cortex, with no change in affinity, after chronic administration

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of desipramine. Tricyclic antidepressants have also been reported to inhibit naltrexone (28) and naloxone (6) binding in brain. Moreover, chronic treatment with amitriptyline and amoxapine increased the level of endogenous enkephalins in spinal cord and several brain areas (25). On the other hand, opiates are also able to modify the effect of antidepressants. Thus, methadone displaces imipramine from specific binding sites (12), and naloxone reverses the analgesic effects of some antidepressant drugs (6,18).

Taken into account the monoaminergic theory of depression (45,53,57-59), the efficacy of tricyclic antidepressants is thought to stem from inhibition of the uptake of biogenic amines, such as noradrenaline and serotonin (24). Opiate compounds are also able to induce this inhibitory effect. Thus, methadone and morphine inhibit noradrenaline reuptake, whereas methadone inhibits both noradrenaline and serotonin reuptake (9).

In consideration of these findings, the present study was undertaken to investigate the involvement of the endogenous opioid system in an experimental model of depression. Recently, the number of behavioral paradigms proposed as animal models of depression has drastically increased (56,60). Learned helplessness (47) is now widely employed as a model of depression with high validity criteria (60). In this paradigm, exposure to an uncontrolled aversive stimulus leads to a decreased ability to escape future aversive situations. The degree to which organisms can exert control over outside events has a strong impact on behavioral and physiologic functioning. Helplessness effects are considered to be those that stem from the uncontrollability of events (i.e., beyond the organism's control) rather than from the events per se. Uncontrollable and aversive events induce cognitive (29), motivational (15), and emotional deficits (34). In addition, at a neurochemical level, uncontrollable but not controllable aversive events lead to disturbances in cholinergic, noradrenergic, dopaminergic, and GABAergic systems (1,40,48,55). This model is highly sensitive to antidepressant drugs (48,49) and is being used increasingly in investigating the neurobiology of depressive illness.

Initial attempts to train animals to become helpless were only partially successful as a significant proportion of rats do not respond in this way after inescapable shocks. In previous studies we found that rats expressing high emotivity in open field test were more susceptible to acquire the learned helplessness paradigm (51). Consequently, in a preliminary experiment, animals were preselected by using open-field test. Only rats showing a high emotivity in this test were used to acquire learned helplessness. We investigated in the preselected rats the effects induced by the administration of Met-enkephalin, Leu-enkephalin, morphine, imipramine, naloxone, RB 38A (a mixed inhibitor of enkephalin degrading enzymes), or RB 38B [a selective inhibitor of neutral endopeptidase E.C. 3.4.24.11 (NEP)] during a helplessness test, on this experimental model of depression.

METHOD

Animals

Male Wistar rats (Central Animal Service of the University of Cádiz, Cádiz, Spain) weighing 200–250 g at the beginning of each experiment, were used. They were individually housed and exposed to a 12L : 12D cycle in a room at constant temperature (21 ± 1°C) and humidity. Food and water were available ad lib. Behavioral tests and care of the animals were in accordance with ethical guidelines recommended by Zimmerman (62).

Apparatus

The open field used to select emotive animals was 50 cm high and 1 m in diam. The walls and the floor were made of a white material. The floor was divided into three concentric circles; the outer and the medium ones, but not the inner one, were divided into 12 squares 6 cm in diam. The open field was placed in a dark and soundproof room and was brightly illuminated with a 100-W light source located 60 cm above the apparatus.

Inescapable foot-shocks were delivered in Plexiglas chambers (walls and covers 20 × 10 × 10 cm) with a stainless-steel grid floor consisting of rods spaced 1.5 cm apart.

Escape and avoidance training was evaluated in automated two-way shuttle-boxes (52 × 22 × 29 cm). Each shuttle-box was divided into two equally sized compartments by a partition with a gate (7 × 7 cm) that provided access to the adjacent compartment. The floors were stainless-steel grids (1.5 cm apart). Static load-cells connected to the grid floor monitored escape and avoidance responses and the position of the animal on any given trial. Both apparatuses were provided with a scrambler.

Surgery

Animals receiving drugs that do not cross the blood-brain barrier were anesthetized with equitesin (5 mg/kg), and polyethylene cannulae were stereotactically implanted in the lateral ventricle. The cannulae were implanted at the following coordinates with respect to the bregma (A = +0.2; L = +1.5; V = −4 from the skull) (39). Correct location was checked by inflow of saline because of the negative pressure in the ventricle. The cannulae were secured to the skull with stainless-steel screws and dental cement.

Treatment Groups

The different pharmacologic treatments were designed to increase or decrease the activity of the opioid system. Animal groups received at random an acute treatment during the 3 days of escape and avoidance test, according to one of the following protocols: Met-enkephalin (25 or 50 μg/day, ICV), Leu-enkephalin (25 or 50 μg/day, ICV), RB 38A (a mixed inhibitor of enkephalin catabolism) (6 μg/day, ICV), RB 38B (30 μg/day, ICV), morphine (0.5, 1, or 2 mg/kg per day, SC; 1, 2, or 4 mg/kg per day, SC; or 2, 4, or 8 mg/kg per day, SC). Control rats were given saline. The doses of morphine were increased from the 1st day on to avoid tolerance.

In a further experiment, four groups of animals were treated according to one of the following protocols: imipramine (24 mg/kg per day, IP), naloxone (2.5 mg/kg per day, SC), imipramine (24 mg/kg per day, IP) + naloxone (2.5 mg/kg per day, SC) and saline.

Drug Administration

Solutions were prepared in distilled water. Enkephalins and enkephalin catabolism inhibitors were administered ICV in a volume of 10 μl, 15 min before each shuttle-box session. Morphine and naloxone were administered SC in a volume of 0.2 ml/100 g body wt., 15 min before each shuttle-box session. Imipramine was administered IP in a volume of 0.5 ml/100 g body wt.

Procedure

Emotive rat selection. In previous experiments we found that rats with a high emotivity level were more susceptible to learned helplessness (51). Before each experiment, all rats were individually subjected to two open-field sessions of 5 min duration each, one per day on 2 consecutive days. The number of boluses excreted was recorded and the rats were selected according to the average number in both sessions. Animals with moderate or low defecation rates were eliminated. The average number of boluses excreted in the selected animals was about seven (the individual number in each animal was over five). The method was performed as described previously (51). The defecation rate in an unfamiliar situation has been reported to be directly related to the emotivity level (7).

Inescapable shock pretreatment. We delivered 60 scrambled, randomized, inescapable electric foot-shocks (1 mA, 15 s duration, intershock interval 5–80 s) (AC, 50 Hz) to the grid floor. Inescapable foot-shock pretreatment was performed in the morning.

Conditioned avoidance training. To evaluate escape and avoidance performance, avoidance training was commenced 48 h after the inescapable shock pretreatment. Animals were placed singly in the shuttle-box and subjected to 30 avoidance trials, with 30 s between trials. During the first 3 s of each trial, a light signal was presented (CS) allowing animals to avoid shock (21). If no avoidance response occurred within this period, a 1 mA shock lasting 3 s was applied via the grid floor. If there was no escape response within this period, shock and light were terminated. The response required of the rat, either avoidance or escape, was to cross the gate into the other compartment of the shuttle-box. An escape failure was when the rat failed to cross into the other compartment during shock delivery. Avoidance sessions were performed for 3 consecutive days in the morning, and both the number of escape failures and the intertrial interval (ITI) activity (number of intersignal crossing) were recorded.

Statistical Analysis

Values are expressed as mean number of escape failures ± SEM recorded over 30 trials during each shuttle-box session. Results were statistically evaluated using the Kruskal-Wallis H test. Post hoc comparisons between treatment groups were made using the Mann-Whitney U-test. The level of significant was $p < 0.05$. Spearman's rank correlation test was used to compare the escape failure number and ITI activity of each animal.

RESULTS

Effects Induced by Imipramine and Their Antagonism by Naloxone

In previous experiments (52), we showed that imipramine reduced the helplessness induced by inescapable shocks. In the present study, a new factor in the learned helplessness paradigm was introduced: the preselection in open field of high emotive rats. Consequently, in a first step we validated this new protocol by studying the effects induced after imipramine administration. Kruskal-Wallis analysis showed a significant treatment effect during the three shuttle-box sessions [first session: $\chi^2(3) = 30.99$, $p < 0.0001$; second session: $\chi^2(3) = 31.07$, $p < 0.0001$; third session: $\chi^2(3) = 28.88$, $p < 0.0001$]. Imipramine-treated animals showed significantly fewer escape failures during the three shuttle-box sessions compared with control animals [first session: $U = 154$ (39–27), $p < 0.001$; second session: $U = 219$ (38–25), $p < 0.001$; third session: $U = 202$ (39–24), $p < 0.001$]. Naloxone antagonized the effect of imipramine, because animals treated with both drugs exhibited a similar number of escape failures to that of control animals, and made more escape failures than did the group receiving imipramine alone. The differences between both groups were statistically significant during the three shuttle-box sessions (first session: $U = 63$ (27–10), $p < 0.02$; second session: $U = 38$ (25–10), $p < 0.002$; third session: $U = 50$ (24–10), $p < 0.001$]. Naloxone by itself facilitated the induction of learned helplessness, because treated animals preexposed to inescapable shocks exhibited more escape failures than did control animals; the difference between both groups was statistically significant during the second and third sessions (second session: $U = 180$ (38–19), $p < 0.01$; third session: $U = 246$ (39–20), $p < 0.05$) (Fig. 1).

Effects Induced by Leu-Enkephalin and Met-Enkephalin

Kruskal-Wallis analysis revealed a significant dose effect in animals receiving Met-enkephalin during the first [$\chi^2(2) = 6.25$, $p < 0.05$] and second [$\chi^2(2) = 6.45$, $p < 0.05$] shuttle-box session. Post hoc comparisons showed that Met-enkephalin at the dose of 50 μg significantly reduced the number of escape failures. Saline-treated animals exhibited greater escape and avoidance deficits when tested for subsequent responding in the shuttle-box, and the differences between enkephalin and saline groups were significant only during the first and second shuttle-box sessions (first session: $U = 25$ (11–12), $p < 0.05$; second session: $U = 28$ (11–12), $p < 0.05$]. Lower doses of Met-enkephalin failed to improve the performance of animals pretreated with inescapable foot-shocks (Fig. 2). A significant dose effect was observed using Kruskal-Wallis analysis in animals receiving Leu-enkephalin in the first shuttle-box session [$\chi^2(2) = 6.55$, $p < 0.05$]. Post hoc comparisons revealed that the administration of Leu-enkephalin at the dose of 50 μg

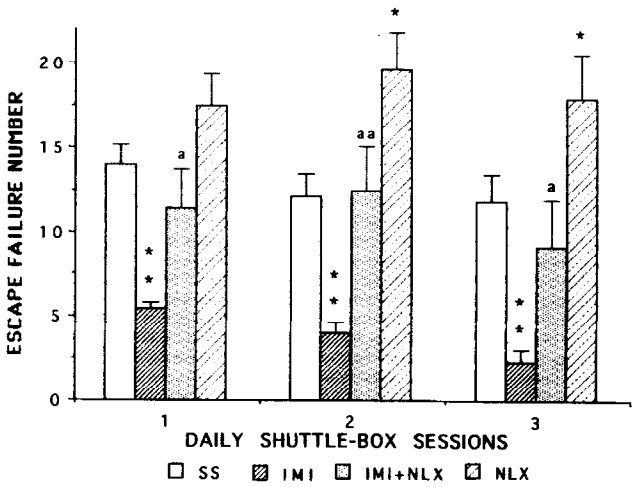

FIG. 1. Effect of naloxone on learned helplessness and imipramine-induced reversal of escape failures produced by inescapable shock treatment. Data are the mean number of escape failures during the three daily shuttle-box sessions in animals treated with saline ($n = 38$), imipramine ($n = 25$) (IMI) (24 mg/kg per day), naloxone ($n = 19$) (NLX) (2.5 mg/kg per day), or imipramine + NLX ($n = 10$) (24 and 2.5 mg/kg per day, respectively). The bars represent the SEM. *$p < 0.05$, **$p < 0.01$ vs. control animals. a, $p < 0.05$; aa, $p < 0.01$ vs. imipramine-treated animals (Mann-Whitney U test).

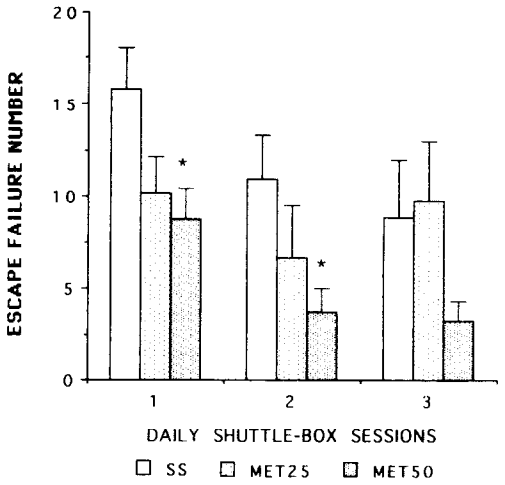

FIG. 2. Mean number of escape failures during the 30 trials of the three daily shuttle-box sessions in control rats (n = 11) and Met-enkephalin-treated rats (25 or 50 μg) (n = 12) after inescapable shock pretreatment. The bars represent the SEM. *$p < 0.05$ vs. control animals (Mann-Whitney U test).

also produced an antidepressant-like effect. Animals receiving Leu-enkephalin exhibited significantly fewer escape failures than did control rats during the first and second shuttle-box sessions (first session: U = 83 (17-18), $p < 0.05$; second session: U = 94 (17-19), $p < 0.05$]. Lower doses of this drug had no significant effect (Fig. 3).

Effects Induced by Morphine

Kruskal-Wallis analysis revealed a significant dose effect in animals receiving morphine during the three daily test session [first session: $\chi^2(3) = 11.40$, $p < 0.01$; second session: $\chi^2 = 12.47$, $p < 0.01$; third session: $\chi^2 = 18.37$, $p < 0.001$]. Morphine treatment influenced performance in escape and avoidance sessions. Morphine also affected the performance of the experimental animals, which made significantly fewer escape failures in the shock escape paradigm compared with control animals during the three shuttle-box sessions after treatment with either 0.5-1-2 mg/kg [first session: U = 75.5 (22-12), $p < 0.05$; second session: U = 63.5 (22-11), $p < 0.05$; third session: U = 56.5 (21-11), $p < 0.02$], 1-2-4 mg/kg [first session: U = 58 (22-10), $p < 0.05$; second session: U = 47.5 (22-10), $p < 0.02$; third session: U = 50 (21-10), $p < 0.05$], or 2-4-8 mg/kg [first session: U = 36 (22-10), $p < 0.01$; second session: U = 41 (22-10), $p < 0.01$; third session: U = 62 (21-10), NS] of this drug (Fig. 4).

Effects Induced by Inhibitors of the Enkephalin Catabolism

Animals treated with the complete enkephalin catabolism inhibitor RB 38A exhibited a statistically significant reduction in the number of escape failures during the three daily test sessions as compared with the saline control group [first session: U = 13.5 (10-8), $p < 0.05$; second session: U = 13 (8-10), $p < 0.05$; third session: U = 14 (10-9), $p < 0.05$) (Fig. 5).

Administration of the selective inhibitor of NEP, RB 38B, to animals preexposed to inescapable shocks also reduced the number of escape failures during the three shuttle-box sessions, but a statistical significance was only observed in the third session [U = 48 (13-14), $p < 0.05$] (Fig. 6). These findings are in agreement with the results obtained with both RB 38A and RB 38B in a previous study (52).

Locomotor Activity During ITI

It is well known that morphine and enkephalins administration modify locomotor activity in normal rats. To determine whether these changes participate in the effects observed after morphine or enkephalin administration, we investigated

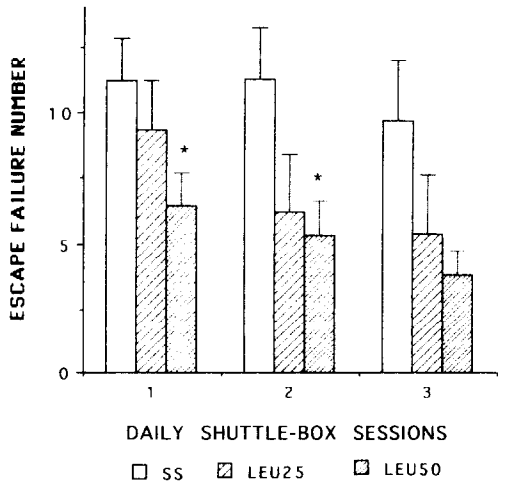

FIG. 3. Mean number of escape failures during the 30 trials of the three daily shuttle-box sessions in control rats (n = 17) and Leu-enkephalin-treated rats (25 or 50 μg) (n = 18) after inescapable shock pretreatment. The bars represent the SEM. *$p < 0.05$ vs. control animals (Mann-Whitney U test).

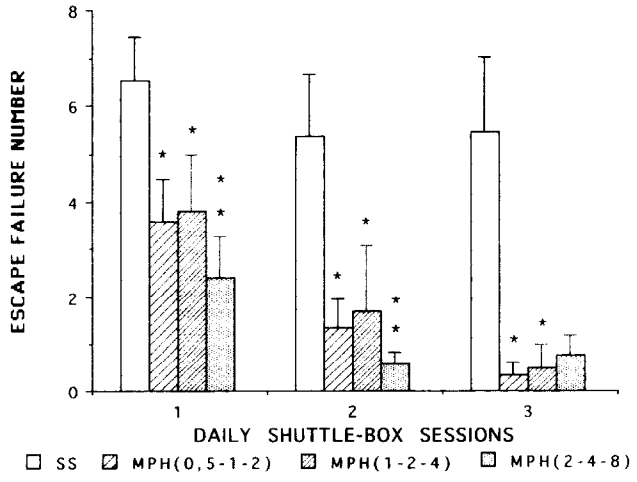

FIG. 4. Mean number of escape failures during the three daily shuttle-box sessions in animals treated with saline (n = 22) or with an increasing doses of morphine (0.5-1-2 mg/kg) (n = 12), (1-2-4 mg/kg) (n = 12), (2-4-8 mg/kg) (n = 12), from the first shuttle-box session onward to avoid tolerance, after inescapable shock pretreatment. The bars represent the SEM. *$p < 0.05$; **$p < 0.01$ vs. control animals (Mann-Whitney U test)

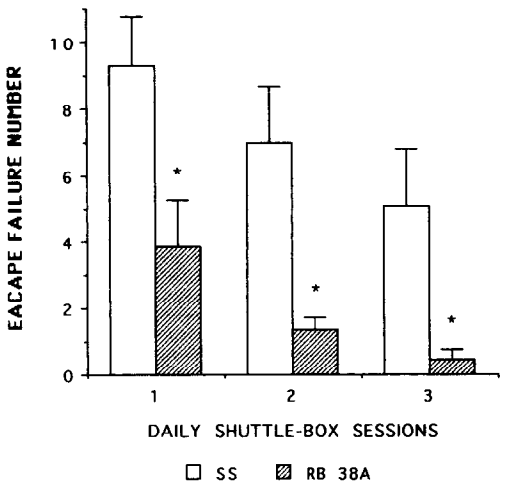

FIG. 5. Mean number of escape failures during the three daily shuttle-box sessions in rats treated with saline or RB 38A (6 μg, ICV) after inescapable shock pretreatment (n = 10–18 rats/group). The bars represent the SEM. *$p < 0.05$ vs. control group (Mann–Whitney U test).

locomotor activity during ITI. Analysis of ITI activity showed that the three morphine-treated groups and the control one displayed a similar motor activity during the first avoidance session. This activity increased during the second and third shuttle-box sessions in groups receiving morphine (Table 1). However, not all animals that exhibited reverse of escape deficits showed a high ITI activity. Therefore, a Spearman's rank correlation was calculated. A negative correlation between escape failure number and ITI activity was observed (higher activity was associated with improve performance), but this relationship was not significant except for the highest dose during the third session. However, this group of animals exhibited the highest number of escape failures during the same session (Table 2).

Animals treated with either Met-enkephalin or Leu-enkephalin also exhibited a higher ITI activity average than control groups (Table 1). Consequently, the possible relationship between ITI activity and the number of escape failures was studied in both experiments to determine whether higher ITI activity was related to improved performance. Spearman's rank correlation showed that there was no significant relationship between ITI activity and the number of escape failure in animals receiving Met-enkephalin or Leu-enkephalin (Table 2).

DISCUSSION

The involvement of the endogenous opioid system in the development of learned helplessness in the rat was investigated by administration of different opioid agonists, antagonists, and enkephalin-degrading enzyme inhibitors after sessions of inescapable shock. Various studies have previously investigated the neurochemical mechanisms involved in the learned helplessness model of depression (60). Our results point to endogenous opioid peptides as a possible neurochemical basis for this model. Thus, stimulation of the opioid system either by exogenous administration of Met- or Leu-enkephalin or morphine, or by treatment with enkephalin-degrading enzyme inhibitors, RB 38A or RB 38B (61), which raise levels of endogenous opioids (43), reversed the escape deficit after inescapable shock pretreatment. This hypothesis was also supported by the ability of naloxone to potentiate the effect of uncontrollable stress. Moreover, we found in a previous study that naloxone antagonizes the effect of enkephalin catabolism inhibitors on learned helplessness (52).

Previous results obtained by others reported that opioid antagonists are able to reverse learned helplessness in some specific experimental conditions. Thus, the previous administration of naltrexone to inescapable shocks prevented subsequent shuttle-performance deficits (33). Furthermore, naloxone prevented predicted learned helplessness in animals unable to escape when administered before exposure to the initial stress, but did not revert this deficit when administered immediately before the shuttle-box test (26). In this context, it was difficult to arrive at a conclusion that clarifies the role of opioids in learned helplessness.

Tricyclic antidepressants such as imipramine, which inhibit noradrenaline uptake, also interfere (directly or indirectly) with a variety of other neurotransmitter systems including the opioid system (6). The interaction of tricyclic antidepressants with the opioid receptors have been reported to participate in their analgesic effect, but also seems to account for their antidepressant action (36). Thus, in the present study, naloxone inhibited the effect of imipramine in the learned helplessness paradigm, providing a strong support for the opioid mediation of this antidepressant effect. Naloxone has also been found to antagonize the effect of tricyclic antidepressants in behavioral models of depression involving a despair dimension such as the forced swimming test (14,19). The selective inhibitor of NEP, thiorphan (44), and bestatin, a nonspecific aminopeptidase inhibitor, were also effective in this behavioral despair test (5). Furthermore, the ability of opioid agonists to prevent escape failures in the present study is not without resemblance to the action of antidepressants. It has been reported, for example, that tricyclic antidepressant reduces the number of escape failures in rats preexposed to inescapable shocks (36,52).

Activation of enkephalinergic systems might prove beneficial in the treatment of endogenous depression, but this effect

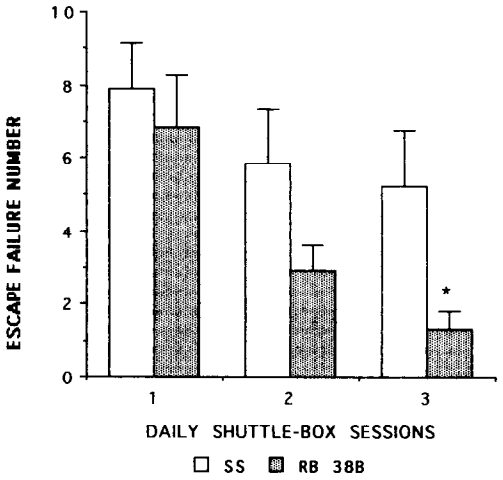

FIG. 6. Mean number of escape failures during the three shuttle-box sessions in rats treated with saline or RB 38B (30 μg, ICV) after inescapable shock pretreatment (n = 13–14 rats/group). The bars represent the SEM *$p < 0.05$ vs. control rats (Mann–Whitney U test).

TABLE 1

INTERTRIAL INTERVAL ACTIVITY (MEAN ± SEM) OF MORPHINE-, MET-ENKEPHALIN-, OR LEU-ENKEPHALIN-TREATED ANIMALS COMPARED TO CONTROL ANIMALS

Treatment	First Session	Second Session	Third Session
Morphine			
SS	10.00 ± 1.43	12.50 ± 2.24	15.08 ± 2.23
0.5-1-2 mg/kg	10.33 ± 1.35	18.27 ± 3.74	49.00 ± 13.13
	NS	NS	U = 56.5 (21-11), $p < 0.02$
1-2-4 mg/kg	10.50 ± 3.00	36.90 ± 8.89	73.50 ± 17.30
	NS	U = 47.5 (22-11), $p < 0.02$	U = 33.5 (21-10), $p < 0.01$
2-4-8 mg/kg	11.00 ± 1.79	35.50 ± 8.46	78.70 ± 17.92
	NS	U = 51 (22-10), $p < 0.02$	U = 34.5 (21-10), $p < 0.01$
Met-enkephalin			
SS	7.33 ± 1.59	7.41 ± 1.99	9.83 ± 2.77
50 μg	9.54 ± 1.48	9.70 ± 2.61	12.20 ± 2.73
	NS	NS	NS
Leu-enkephalin			
SS	5.42 ± 0.91	6.15 ± 1.18	8.78 ± 2.30
50 μg	8.94 ± 1.26	8.68 ± 1.93	15.64 ± 2.79
	U = 93.5 (19-17), $p < 0.05$	NS	U = 93 (19-17), $p < 0.05$

Data were derived using the Mann-Whitney U test.

could be direct or via an influence on the noradrenergic, dopaminergic, or serotoninergic systems that are also implicated in the neurobiology of depression (57). In this respect, it is interesting that enkephalinergic, noradrenergic, dopaminergic, and serotoninergic neurones are colocated in several brain areas (22,32,41). The participation of monoaminergic systems in the antidepressant response induced by opioid compounds in the present study needs further clarification and is currently being explored.

The opioid compounds used in this study were not selective of any type of opioid receptor. Enkephalins, which have been proposed as the endogenous ligands for δ-receptor, elicit an affinity for δ-receptors only 10- or 20-fold higher than for μ-receptors [see (50)]. However, several findings suggest that the effect induced by opioids on the learned helplessness paradigm could be mediated through the activation of δ-receptors. Thus, the antidepressant effects induced by endogenous enkephalins, protected from their catabolism by RB 101, a systemically active complete inhibitor of enkephalin catabolism, were selective suppressed by a low dose of the selective δ-

TABLE 2

RELATIONSHIP BETWEEN ITI ACTIVITY AND ESCAPE FAILURE NUMBER IN CONTROL AND TREATED ANIMALS WITH MORPHINE, MET-ENKEPHALIN, OR LEU-ENKEPHALIN

Treatment	First Session	Second Session	Third Session
Morphine			
0.5-1-2 mg/kg	r = −0.108; df 22	r = −0.510; df 11	r = 0.013; df 12
	NS	NS	NS
1-2-4 mg/kg	r = 0.016; df 10	r = 0.441; df 10	r = −0.522; df 10
	NS	NS	NS
2-4-8 mg/kg	r = −0.446; df 10	r = −0.468; df 10	r = −0.667; df 10
	NS	NS	$p < 0.03$
SS	r = −0.108; df 22	r = −0.237; df 22	r = −0.304; df 21
	NS	NS	NS
Met-enkephalin			
50 μg	r = −0.06; df 11	r = −0.39; df 11	r = 0.24; df 10
	NS	NS	NS
SS	r = −0.64; df 12	r = −0.46; df 12	r = −0.79; df 12
	$p < 0.02$	NS	$p < 0.002$
Leu-enkephalin			
50 μg	r = 0.03; df 17	r = −0.44; df 17	r = −0.23; df 17
	NS	NS	NS
SS	r = −0.403; df 19	r = −0.51; df 19	r = −0.75; df 19
	NS	$p < 0.02$	$p < 0.001$

Data were derived using Spearman's rank correlation.

antagonist, naltrindole (2). This hypothesis is also supported by previous studies showing the preferential involvement of δ-receptors in the behavioral responses induced by kelatorphan microinjected into the mesolimbic and nigrostriatal pathways of rats (8,11,35) or administered ICV to mice (37).

In conclusion, these results indicate that exogenous opioids and endogenous enkephalins, protected from their catabolism by peptidase inhibitors, induced antidepressant-like effects on the learned-helplessness paradigm, supporting the implication of endogenous opioids in the etiology of depressive disorders. These behavioral observations suggest a possible future use of opioid compounds in the management of depression.

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