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Effects of Opiate Antagonists on Social and Aggressive Behavior of Isolated Mice

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PUGLISI-ALLEGRA, S., A. OLIVERIO AND P. MANDEL. *Effects of opiate antagonists on social and aggressive behavior of isolated mice.* PHARMAC. BIOCHEM. BEHAV. 17(4) 691–694, 1982.—Opiate antagonists naloxone (1 and 1.5 mg/kg IP) and naltrexone (2.5 and 5 mg/kg IP) inhibit aggressive responses of DBA/2 isolated mice, while increasing the duration of some social activities such as sniff-body, sniff-nose and following. At the doses employed naloxone and naltrexone did not affect motor activity and self-grooming of paired mice. These findings are discussed in terms of the endogenous opioids system involvement in arousability, in the response of the organism to stressful events, in the motivational mechanisms which control social behavior and in the functioning of some neurotransmitter systems which are known to play an important role in the control of isolation-induced aggressive behavior.

Naloxone Naltrexone Isolation-induced aggressive behavior Social activities Mice

IN recent years several studies have indicated that endogenous opioids (endorphins and enkephalins) are involved in a number of behavioral patterns connected with emotionality. The effects of these compounds on motivation include primarily changes in pleasure and pain, the response of the organism to stress, social attachment, psychomotor activity, exploratory behavior and a number of learning paradigms [3, 4, 14, 20]. Some of these studies have shown that the endogenous opioids system modulates aversiveness in general and not only pain transmission or affective responses to pain. Such an action of endogenous opioids would consist in attenuating the aversiveness of a stressor [20], a fact which might have implications in human disturbed behavior [2, 23, 25]. It is clear that this modulating action of aversiveness may play an important role in motivational mechanisms which control the interaction between living organisms and social and non-social environment. On the basis of the experimental results concerning the role of endogenous opioids on social behavior and emotionality, it is conceivable that these compounds play a role in the control of aggressive behavior.

Isolation-induced agonistic behavior in mice is used as a tool in the study of neurochemical mechanisms underlying aggressive behavior [24]. It has been reported that prolonged isolation produces an increase of brain dopamine turnover, a slight decrease of brain noradrenaline turnover and a large decrease of brain serotonin turnover [24]. Recently, it has been shown that isolated aggressive mice are characterized by lower GABA levels in different brain areas in comparison with grouped mice [22]. Concerning the endogenous opioids system, in morphine withdrawal syndrome precipitated with naloxone, isolated rats show a reduction of jumping and diarrhea in comparison to grouped rats [1]. Furthermore, it is well known that social isolation in mice and rats affects the analgesic system. Breif periods of isolation can increase pain responsivity in young rats, at the same time decreasing the analgesic effects of morphine [13]. On the other hand, after a prolonged period of isolation, mice and rats exhibit an increase of morphine responsivity [8,10]. These effects have been interpreted in terms of opiate receptor proliferation or supersensitivity when environmental sources of opioid stimulation (i.e., social stimuli) have been absent for a prolonged period of time [8,20]. Thus, the social environment sustains a desirable level of endogenous opioid activity. It is possible that social environment stimulation affects also emotionality through endogenous opioid mechanisms. The purpose of this study was to investigate the effects of naloxone on isolation-induced aggressive behavior and sociability in mice. Since naloxone can also display opiate agonist activity, for example in humans (analgesic reactions), in rats (hot plate test) or in mice (acetic acid induced writhing) (for review see Sawynock *et al.* [21]), we investigated the effects of another opiate antagonist, naltrexone, on aggressive and social behavior of isolated mice.

METHOD

Male mice (Charles River, Italy) of DBA/2 strain, aged 11–12 weeks and weighing 21–24 g at the beginning of the experiment were used. They were individually housed (isolated) in opaque cages (27 × 21 × 13.5 cm) for 8 weeks. The mice were kept in a 12/12 hr light/dark cycle, given food and water ad lib and tested during the second half of the light period.

Aggressive responses and motor activity were assessed by means of a method previously described [19]. The latency of the first fighting episode, the number and the total time of fighting between the two mice were automatically recorded for a 10 min session. The motor activity of two interacting

mice was measured concomitantly to aggressive behavior. A total of eight pairs of naive mice for each experimental group were tested. Five behavioral items, sniff-body, sniff-nose, following, grooming-body which are commonly considered an expression of sociability [17] and self-grooming, were observed in both interacting mice and their duration (sec) was recorded by means of a keyboard connected to an electromechanic recorder (Esterline), according to Miczek [12].

Naloxone (HCl) and Naltrexone (HCl) (ENDO, Garden City, NY) were injected intraperitoneally (in a volume of 10 ml/kg) in a 0.9% saline 15 min before testing. Control mice were injected with saline at the same volume. Data were analyzed statistically by single-factor analysis of variance (ANOVA; independent). Mice injected with two doses of each drug were independently compared with saline injected mice. Additional analyses for individual between-group comparisons were carried out by employing the error term of the overall analysis of variance.

RESULTS

Single-factor ANOVA showed that naloxone (1 and 1.5 mg/kg) induced an increase of latency of the first fighting episode, $F(2,21)=3.48$, $p<0.05$, and a decrease in number, $F(2,21)=13.94$, $p<0.001$, and time, $F(2,21)=13.31$, $p<0.001$, of fighting (Fig. 1). Individual between-group comparisons showed that the dose of 1 mg/kg significantly decreased number ($p<0.001$) and time ($p<0.001$) of fighting and that the dose of 1.5 mg/kg increased latency of the first fighting episode ($p<0.05$) and decreased number ($p<0.001$) and time ($p<0.001$) of fighting. At the doses employed naloxone did not modify with statistical significance motor activity, $F(2,21)=0.99$, $p>0.05$. Moreover the decrease of aggressive responses accompanies an increase of social interactions measured by sniff-body, $F(2,21)=4.04$, $p<0.05$, sniff-nose, $F(2,21)=5.03$, $p<0.05$, and following, $F(2,21)=6.91$, $p<0.01$, while no difference in grooming-body, $F(2,21)=1.42$, $p>0.05$, was observed in our experimental conditions (Fig. 2). Individual between-group comparisons showed that the dose of 1 mg/kg significantly increased the duration of following ($p<0.05$) and that the dose of 1.5 mg/kg increased the duration of sniff-body ($p<0.05$), sniff-nose ($p<0.01$) and following ($p<0.01$).

No significant differences in self-grooming were observed between naloxone and saline injected mice, though naloxone injected mice showed a trend towards a decrease in this behavioral item.

It must be pointed out that preliminary experiments showed that naloxone at lower doses (0.05; 0.1; 0.5 mg/kg) induced a slight decrease of aggressive responses while no facilitating effects were evident.

Also naltrexone (2.5 and 5 mg/kg) induced an increase of latency of the first fighting episode, $F(2,21)=8.36$, $p<0.01$, and a decrease in number, $F(2,21)=12.44$, $p<0.001$, and time, $F(2,21)=11.33$, $p<0.001$, of fighting (Fig. 1). Individual between-group comparisons showed that both doses of naltrexone were effective in increasing latency of the first fighting episode (2.5 mg/kg=$p<0.05$; 5 mg/kg=$p<0.001$) and in decreasing number (2.5 mg/kg=$p<0.001$; 5 mg/kg=$p<0.001$) and time (2.5 mg/kg=$p<0.001$; 5 mg/kg=$p<0.001$) of fighting. At the doses employed naltrexone did not significantly affect motor activity in our experimental conditions, $F(2,21)=1.09$; $p>0.05$.

Moreover naltrexone produced an increase of sniff-body, $F(2,21)=5.25$; $p<0.05$, sniff-nose, $F(2,21)=10.29$, $p<0.001$, and following, $F(2,21)=6.58$, $p<0.01$, but not of grooming-body, $F(2,21)=1.42$, $p>0.05$. Individual between group comparisons showed that the dose of 2.5 mg/kg significantly increased the duration of sniff-body ($p<0.05$) and following ($p<0.01$) and that the dose of 5 mg/kg increased the duration of sniff-body ($p<0.01$), sniff-nose ($p<0.001$) and following ($p<0.05$) (Fig. 2).

Concerning self-grooming, saline injected mice did not differ significantly from mice injected with naltrexone, although also in this case drug injected mice showed a trend towards a decrease in this behavior.

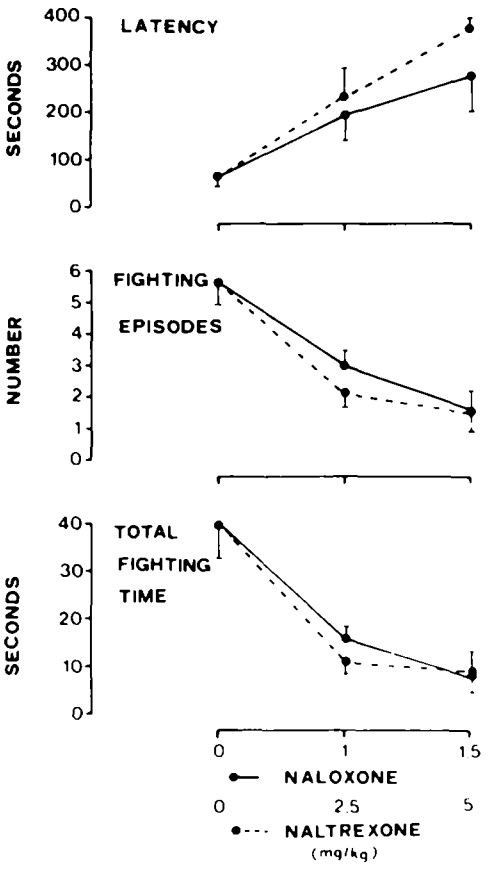

FIG. 1. Effects of naloxone and naltrexone on aggressive behavior of isolated mice. Aggressive responses (mean + S.E.) were expressed by the latency of the first fighting episode (Latency), the number (Number) and the total time of fighting episodes (Time) during a 10 min testing sessions. Those couples of mice that failed to fight during a ten minutes experimental session were assigned a maximum latency score of 600 sec.

DISCUSSION

These results, showing parallel effects of naloxone and naltrexone on aggressive and social behaviors, strongly suggest that such effects depend on the opiate antagonist action of both drugs. These effects on isolation-induced aggressive behavior could be explained in terms of different nervous and behavioral mechanisms.

First, the facilitating effects of naloxone on different be-

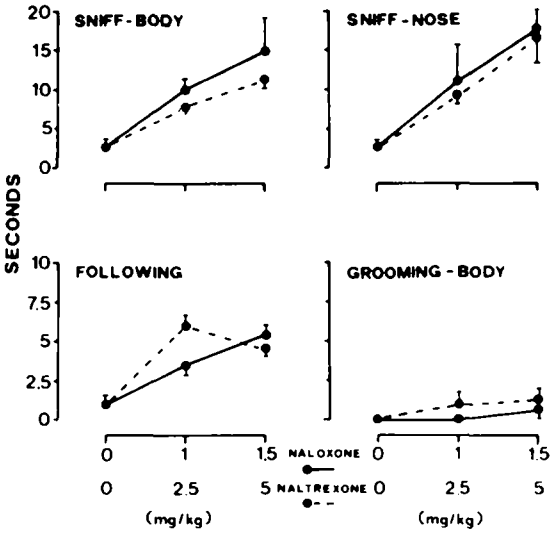

FIG. 2. Effects of naloxone and naltrexone on mean (±S.E.) duration (sec) of four social activities in isolated mice.

haviors ranging from copulatory behavior to social interactions have been interpreted in terms of its ability to block opiate induced inhibition of dopamine release in specific brain areas [14]. Thus naloxone (and naltrexone) by acting on the dopaminergic system may produce some behavioral changes such as stimulation of locomotor activity and stereotipies which would interfere with the expression of aggressive behavior. It must be pointed out that in our experimental conditions naloxone and naltrexone did not increase self-grooming. Moreover, the activity tests have shown that both opiate antagonists did not increase motor activity at the doses employed. On the other hand, it must be taken into account that it has recently been shown that morphine and δ-opiate agonists stimulate dopamine release in caudate nucleus [6] a brain structure which plays an important role in the control of motivational mechanisms underlying aggressive behavior [24].

Second, naloxone and naltrexone may produce in each opponent a high aversiveness towards the other one. This increase of reciprocal aversiveness would bring each opponent to avoid social contacts including aggressive interactions.

As we have seen, naloxone and naltrexone decrease aggressive responses at the same time increasing some behavioral patterns as sniff-body, sniff-nose, and following which are commonly considered expressions of sociability. This fact is not consistent with an hypothesis based on an aversiveness effect.

It is worth noting that these results are in part consistent with those of Panksepp *et al.* [15] who found a decrease of social interactions in rats injected with morphine and a trend towards an increase of social interactions following injections of naloxone.

On the other hand, it may be that in our experimental conditions each opponent is for the other one a stressor, which may provoke aggressive behavior. Opiate antagonists may interfere with endogenous opioids mechanisms involved in the response of the organism to stress and thus with the expression of aggressive behavior. It is worth noting that a number of studies have recently shown that the endogenous opioids system interacts with some hormones in pituitary in particular with adrenocorticotropin (ACTH) [9] which plays a basic role in the organism response to stress [3,4] and in social behavior [5].

Third, from a neurochemical and neuropharmacological point of view the hypothesis arises as to whether the effects of naloxone and naltrexone observed in our experimental conditions are mediated via opiate mechanisms, for instance by inhibiting a morphine-like molecule which may be involved in the control of the aggressive responses, or through mechanisms involving other neurotransmitter systems, whose metabolism, as it is well known, is strongly modified by social isolation [22,24].

Fourth, a final point is related to the site of action of opiates and opioids in the brain. It must be noted that our results may seem in contrast with some reports indicating the anti-aggression effects of morphine in isolated mice [7]. Concerning this, it must be pointed out that a number of studies have indicated that opiates and opioids may modulate different behavioral effects depending on their action in specific brain areas and their binding to different receptor sites [9,16]. Moreover some evidence exists which indicated that β-endorphin and its fragments may produce opposite behavioral effects [25]. On the other hand, it has recently been shown that opiate antagonist naloxone is effective at low systemic doses in improving memory consolidation and in potentiating shock-induced aggressive behavior, while it is ineffective at higher doses [11,18].

In the light of these results we can assume that the anti-aggression effects of morphine [7] and those of the opiate antagonists observed in the present research may depend on the involvement of different neurochemical mechanisms whose functioning results in the same effect, i.e., the inhibition of aggressive responses. Furthermore it may also be that both morphine and opiate antagonists displace a morphine-like molecule (opioid peptide) involved in the control of aggressive behavior, from its receptor site, thus antagonizing its action.

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