Pharmacology Biochemistry & Behavior, Vol. 14, pp. 299–303. Printed in the U.S.A.

Social Crowding Enhances Aversiveness of Naloxone in Rats[1]

C. W. T. PILCHER AND SARAH M. JONES

Department of Pharmacology, Faculty of Medicine, Kuwait University, Kuwait

Received 20 June 1980

PILCHER, C. W. T. AND S. M. JONES. *Social crowding enhances aversiveness of naloxone in rats.* PHARMAC. BIOCHEM. BEHAV. 14(3) 299–303, 1981.—If endorphins mediate various behavioural states including reactions to stress and social affect, then experimental manipulation of such states should alter the stimulus properties of opioid antagonists. The results of experiments in which the aversiveness of naloxone was increased by chronic environmental stress produced by rearing and maintaining male rats under conditions of severe social crowding, support this theoretical proposition. All rats acquired aversions to flavoured solutions that had been presented for 15 min immediately prior to a systemic injection of naloxone. The degree of the conditioned aversion was greater in rats maintained in crowded conditions. Transferring rats from crowded conditions to individual accommodation 3 weeks before the start of aversive conditioning did not attenuate the increased aversiveness of the antagonist. It is suggested that social interaction under conditions of chronic crowding induces a prolonged increase in the level of tonic activity in endorphinergic systems.

Endorphins   Social interaction   Crowding stress   Conditioned taste aversion

THERE is evidence that endorphins mediate various behavioural states including pain, pleasure and reactions to stress. Endorphinergic function is implicated when narcotic antagonists, which competitively replace opioid agonists at specific receptor sites, have effects *in vivo* in the absence of exogenous narcotic agonists. However, the most widely used antagonist, naloxone, has other actions and interpretations invoking involvement of endorphins must be made with caution. Results of studies on taste aversions conditioned with naloxone [15] or enantiomorphic pairs of opiate antagonists [20] and self-administration of enkephalins [3] have together implicated endorphins in reward-aversion mechanisms. It has also been reported that stress-induced endorphin release modifies nociceptive responding [1] in a manner compatible with known analgesic actions of opioids. If endorphinergic systems have a role in nociception and reactions to stress, then experimental manipulation of these states might be expected to alter the stimulus properties of opioid antagonists. Conversely, alterations in the stimulus properties of such antagonists following stressful and noxious experience would be suggestive of endorphinergic involvement in these processes. In an earlier study in which acute, footshock-induced stress was imposed on rats, we were unable to alter the aversiveness of naloxone [16]. This failure could have been due to the low shock-levels that we were obliged to use and/or the brevity of the period of stress.

Endorphins have also been postulated to mediate social affect, and it has been speculated that an animal's normal social contacts may chronically raise levels of tonic activity in central endorphinergic systems [14]. We therefore reasoned that the stimulus properties of naloxone might be modified by chronic environmental stress produced by rearing and maintaining rats under conditions of severe social crowding. This communication reports the results of preliminary experiments conducted to test this theoretical proposition.

METHOD

Animals

Male, hooded rats bred in the Faculty of Medicine, University of Kuwait, were used. Throughout the experiments all animals were kept in rooms maintained at about 22°C with a regular 12-hour light cycle imposed by fluorescent lighting. From weaning, at 21 days of age, until 3 weeks before the commencement of taste aversion conditioning, lights were on in the cycle from 06.00 hr to 18.00 hr. Thereafter the animals were housed in a reverse light cycle with lights on from 21.00 hr to 09.00 hr.

Drugs

Naloxone hydrochloride (Endo Laboratories) was dissolved in isotonic saline and injected intraperitoneally in a volume of 1.0 ml/kg body weight.

Solutions with 'sweet' or synthetic 'chicken' flavours were similar to those used in previous studies [4]. Sweet flavour consisted of sodium saccharin (0.1% w/v) and chicken flavour of monosodium glutamate (40 mM) and

[1]Naloxone was generously donated by Endo Laboratories, Inc. A breeding stock of hooded rats was the kind gift of Professor P. L. Broadhurst, University of Birmingham, England.

[2]Send reprint requests to Dr. C. W. T. Pilcher, Department of Pharmacology, Faculty of Medicine, P.O. Box 24923, Kuwait, Arabian Gulf.

Copyright © 1981 ANKHO International Inc.—0091-3057/81/030299-05$01.00/0

sodium chloride (120 mM), all dissolved in distilled water. All chemicals were from B.D.H. Chemicals, Poole, England.

Taste Conditioning Procedure

The principles and full details of the method using two-flavour discrimination for conditioning taste aversions have been described previously [4]. Rats were adapted to a regimen of daily access to distilled water for 15 min in the morning (at 10.00 hr) and 45 min in the afternoon (at 15.00 hr). All rats remained on this schedule for 12 days before the first presentation of flavours. Then, on days 1 and 3 of a four-day cycle, which was repeated three times, all rats were presented with one of the two flavoured solutions for 15 min instead of water in the morning session (single-stimulus tests). Immediately after the solutions were removed the animals were injected with either naloxone at 3.2 mg/kg body weight or saline. For half of the rats chicken flavour was repeatedly paired with naloxone and sweet with saline, whilst for the other half of the animals the flavour-injection pairings were reversed in order to balance out any effects of unconditioned palatabilities of the flavours. After four flavour-drug and four flavour-saline pairings, injections were stopped. Two days later, drug- and saline-paired flavours were presented simultaneously for 15 min (two-stimulus test). On the following day the positions of the two flavours were reversed to balance out side preference. Only the mean flavour intakes for these two tests were plotted in Figs. 1 and 2.

Aversion indices [4] for the single-stimulus tests were calculated as the rate of decline of flavour intake (ml/trial) and were analysed by two-factor analyses of variance using repeated measures [21] and t-tests. For the two-stimulus tests, the amount of naloxone-paired flavour consumed by each rat was calculated as a percentage of its total fluid intake. These percentage scores were subjected to arc-sine transformation [21] and t-tests carried out to determine whether the mean scores differed significantly from 50%.

Housing Conditions

In the following experiments, standard colony cages (38×25×18 cm and 56×38×18 cm) were used throughout. Floor space was restricted when necessary by placing an aluminium plate vertically across the cage. All floor-space allocations were decided on a purely arbitrary basis. The designation 'non-crowded' was applied to housing densities equal to, as in experiment 2, or one half of, as in experiment 1, those routinely used in the colony. Densities four- to eight-fold higher were designated as 'crowded'.

In the first experiment 20 rats were taken randomly from the colony at weaning and divided into two equal groups. One group (n=10), designated crowded, was housed in a single cage providing 50 cm^2 of floor space per animal and the second group (non-crowded, n=10) in a similar cage allowing 215 cm^2 per animal. When the mean body weight for all rats reached 90 g, the crowded group was rehoused in a single cage at 80 cm^2 per animal whilst 9 of the non-crowded rats were distributed equally as 3 groups among 3 cages, providing 710 cm^2 per animal, for the duration of the experiment. The floor space allocation for the crowded rats in the single cage was increased to 95 cm^2 when their group mean weight reached 130 g and was restricted to that for the remainder of the experiment. The animals were then maintained in a reverse light cycle (lights on 21.00 hr to 09.00 hr) for 3 weeks before aversive conditioning began. All rats were transferred to individual cages (23×25×23 cm) before each drinking session and returned to their colony cages immediately afterwards. Because of the counter-balancing design only 8 in each group were used in the experiment.

Modified housing conditions were used in the second experiment. At weaning, 20 rats to be designated as crowded were accommodated at 10 per cage allowing 50 cm^2 per animal. On reaching 100 g they were transferred to cages providing 95 cm^2. Twenty animals in the non-crowded group were housed 10 per cage at weaning and provided with 80 cm^2. On reaching 100 g they were rehoused 6 per cage and their floor space allocation was increased to 355 cm^2. Three weeks before the start of aversive conditioning 16 rats were randomly selected from each group and housed singly in cages (23×25×23 cm) in the same reverse light cycle as in experiment 1. However, the animals were maintained individually in these cages throughout the remainder of the experiment.

TABLE 1

AVERSIVENESS OF NALOXONE IN DIFFERENTIALLY HOUSED RATS

		Single-stimulus Test Mean Aversion Index*	Two-stimulus Test†
Experiment 1			
Crowded	(n=8)	−4.81 ± 0.86‡	2.2 ± 2.1
Non-crowded	(n=8)	−2.21 ± 0.23	4.1 ± 1.7
Experiment 2			
Crowded	(n=16)	−4.26 ± 0.3 ¶	11.0 ± 7.6
Non-crowded	(n=16)	−2.49 ± 0.68	24.3 ± 7.1

*Rate of decline of flavour intake (ml/trial).
†Indicates drug-paired flavour intake as % total fluid intake.
‡$p<0.02$, ¶$p<0.01$; significance levels for differences between crowded and non-crowded groups.

RESULTS

The mean intakes of drug- and saline-paired flavours for both crowded and non-crowded rats in the first experiment are shown in Fig. 1. It will be seen that, in the single-stimulus tests, mean naloxone-paired flavour intake declined relative to saline-paired flavour intake in both crowded, $F(1,14)=4.63$, $p<0.05$, and non-crowded, $F(1,14)=9.79$, $p<0.01$, rats. However, after only two pairings of flavour with naloxone, the mean intake in the crowded group had fallen to 16% of that in trial 1, whereas in the non-crowded animals it fell to 47% and even after further pairing did not decline below 40%. The mean aversion indices (Table 1) of the two groups differed reliably ($t=2.94$; $d.f.$ 14; $p<0.02$), indicating that naloxone aversiveness was significantly greater in crowded rats. Saline-paired intakes in the crowded group showed a slight fall in the initial trials but a comparison of the slopes for crowded and non-crowded rats did not indicate any significant difference in control intakes between the two groups ($t=0.34$; $d.f.$ 14; $p>0.1$). In the two-stimulus tests drug-paired flavour consumption was significantly less than 50% of total fluid intake, confirming the aversiveness of naloxone in crowded and non-crowded rats. The values for the two groups, however, did not differ significantly.

FIG. 1. The effects of severe crowding on the development of aversions to flavours paired with systemic injections of naloxone at 3.2 mg/kg. Rats were housed in crowded or non-crowded conditions from weaning and throughout the entire experiment. ●——● naloxone-paired flavours; ○——○ saline-paired flavours; n=8 for crowded and non-crowded groups.

FIG. 2. The effect of earlier, prolonged crowding on the development of aversions to flavours paired with systemic injections of naloxone at 3.2 mg/kg. Rats were housed in crowded or non-crowded conditions from weaning to 3 weeks before the commencement of aversive conditioning. At that point and throughout the remainder of the experiment they were housed individually. ●——● naloxone-paired flavours; ○——○ saline-paired flavours; n=16 for crowded and non-crowded rats.

In the second experiment, comparison of the aversion indices (see Table 1) again indicated that naloxone was significantly more aversive in crowded than in non-crowded rats ($t=3.5$; *d.f.* 30; $p<0.01$). The results of the single- and two-stimulus tests for this experiment are presented in Fig. 2. Non-crowded rats significantly reduced their intake of drug-paired flavours compared with saline-paired flavours, $F(1,30)=4.26$, $p<0.05$, but it will be seen that crowded rats reduced their drug- and saline-paired flavour intakes at similar rates. Thus in the single-stimulus tests there was a complete lack of discrimination between drug-paired and saline-paired flavours and control intakes differed significantly between crowded and non-crowded groups ($t=3.92$; *d.f.* 30; $p<0.001$). Although the drug-paired flavour intake in the two-stimulus tests was significantly below 50% ($t=2.53$; *d.f.* 15; $p<0.05$) and therefore demonstrated an acquired conditioned aversion, the total intakes in these tests were unusually low. More commonly, total intakes in the two-stimulus tests approximate to intakes of control flavours during the single-stimulus trials, as indicated in Figs. 1 and 2 and previous reports [4,20].

DISCUSSION

The above results provide confirmation of earlier reports [5,15] that naloxone is an aversive stimulus in opiate-free rats. Significant taste aversions were conditioned in all the experimental situations but the results of the single-stimulus tests in the second experiment present difficulties in interpretation. Rats in the crowded group reduced their intakes of both drug- and saline-paired flavours at equal rates, and it can be argued that the processes that led to lower control flavour baselines may have predisposed these animals to a more rapid aversive conditioning. An increased sensitivity of these unidentified processes, rather than an increased sensitivity to naloxone, could therefore have been the cause for the differences between the groups. The implied naloxone-independent nature of the processes suggests that intakes of flavours paired with saline should decline over trials in the absence of naloxone treatment. Such an effect is considered unlikely and more plausible explanations are: the naloxone-induced conditioned aversion generalised to all novel tastes, or the animals were unable to discriminate effectively between the flavours when they were presented singly on every fourth day, or a combination of both factors. The significant difference in intakes in the two-stimulus tests indicates that discrimination was possible when the two flavours were available simultaneously. As stress is known to impair discrimination performance, the second of the explanations may be the most likely and it is possible that isolation following crowding, as occurred in the second experiment only, was more stressful than continuous crowding.

It is clear from the experiments described above that social environment modifies the stimulus properties of naloxone in such a way that the antagonist is more aversive in rats that have been maintained in crowded conditions. Naloxone has pharmacological actions other than those arising from blockade of opioid receptors and it has been emphasised that caution must be exercised when invoking endogenous opioids as mediators of a response [18]. Using enantiomorphic pairs of other opiate antagonists we have previously obtained evidence that the aversive effects of naloxone are due to actions at opioid receptors [20]. More recently we reported that we were unable to condition taste aversions with either picrotoxin or bicuculline at high but subconvulsive doses [17]. Both of these drugs are more potent inhibitors of GABA-ergic systems than naloxone [8], thus excluding the possibility that naloxone exerts its aversive effect primarily by an antagonist action at the GABA receptor. It further seems unlikely that the aversiveness of naloxone is due to direct agonistic action on dopaminergic neurones since bicuculline and picrotoxin block aversive conditioning with the opioid antagonist but not with apomorphine or amphetamine (Pilcher and Jones, in preparation). We have therefore interpreted the results reported here as indicating that social interaction in rats chronically raises activity in endogenous opioid systems.

Such an interpretation is compatible with a variety of previous findings. Firstly, it has been demonstrated that separation distress produced by social isolation of the young of several species can be potentiated by naloxone and alleviated by opiates and opioid peptides [10, 13, 14]. These findings have led to the suggestion that social interaction "may chronically activate" endorphinergic systems [13]. Certainly an elevated tonic activity brought about by social crowding could accommodate the notion of the development of endorphin tolerance and offer an explanation for the reported reduction in the analgesic potency of morphine in communally housed animals [7,11]. Tolerance and dependence have been considered by many to be expressions of the same neural process [12] and raised levels of endorphin release resulting from chronic social interaction may therefore lead to a condition resembling dependence on exogenous opiates. Our present results are consistent with this suggestion and the concept of a crowding-induced condition resembling dependence is strengthened by our previous finding that the aversiveness of naloxone is greater in opiate-dependent rats than in those that are opiate-free [15]. Moreover, enhanced tonic activity in endorphinergic systems resulting from social interaction could account for the observation that rats housed in groups self-administer less morphine than animals kept in isolation [2].

In a previous study [16] we failed to modify the aversiveness of naloxone by using brief, low-level footshock as an acute stressor and this contrasts with the results obtained here with the chronic stress resulting from prolonged, severe crowding. A feature of the theoretical mechanisms mediating adaptation to stress is the assumption that a previous history of stress alters the physiological "set" of the hypothalamic-pituitary-adrenal control system [19,22]. More recently it has been proposed that endogenous opioids have a role in the control of stress-induced corticosteroid secretion in mice [9]. It is possible therefore that an endorphinergic mechanism is responsible for regulating the "set" of the hypothalamic-pituitary-adrenal system. The long-term requirement for such an adaptive change could be met by the plasticity of endorphinergic function. In the experiments reported here, enhanced tonic activity persisted for at least one month after depriving the animals of their accustomed social interaction; in other studies with exogenous opiates, tolerance, a prime example of plasticity, has been reported to persist for a year after cessation of morphine administration in rats [6].

REFERENCES

1. Akil, H., J. Madden, R. L. Patrick and J. D. Barchas. Stress-induced increase in endogenous opiate peptides: concurrent analgesia and its partial reversal by naloxone. In: *Opiates and Endogenous Opioid Peptides*, edited by H. W. Kosterlitz. Amsterdam: Elsevier, 1976, pp. 63–70.
2. Alexander, B. K., R. B. Coambs and P. F. Hadaway. The effect of housing and gender on morphine self-administration in rats. *Psychopharmacology* **58:** 175–179, 1978.
3. Belluzzi, J. D. and L. Stein. Enkephalin may mediate euphoria and drive-reduction reward. *Nature* **266:** 556–558, 1977.
4. Booth, D. A., G. D. D'Mello, C. W. T. Pilcher and I. P. Stolerman. Comparative potencies of amphetamine, fenfluramine and related compounds in taste aversion experiments in rats. *Br. J. Pharmac.* **61:** 669–677, 1977.
5. LeBlanc, A. E. and H. Cappell. Antagonism of morphine-induced aversive conditioning by naloxone. *Pharmac. Biochem. Behav.* **3:** 185–188, 1975.
6. Cochin, J. and C. Kornetsky. Development and loss of tolerance to morphine in the rat after single and multiple injections. *J. Pharmac. exp. Ther.* **145:** 1–10, 1964.
7. DeFeudis, F. V., P. A. DeFeudis and E. Somoza. Altered analgesic response to morphine in differentially housed mice. *Psychopharmacology* **49:** 117–118, 1976.
8. Dingledine, R., L. L. Iversen and E. Breuker. Naloxone as a GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies. *Eur. J. Pharmac.* **47:** 19–27, 1978.
9. Gibson, A., M. Ginsburg, M. Hall and S. L. Hart. The effects of opiate receptor agonists and antagonists on the stress-induced secretion of corticosterone in mice. *Br. J. Pharmac.* **65:** 139–146, 1979.
10. Herman, B. H. and J. Panksepp. Effects of morphine and naloxone on separation distress and approach attachment: evidence for opiate mediation of social affect. *Pharmac. Biochem. Behav.* **9:** 213–220, 1978.
11. Kostowski, W., A. Czlonkowski, W. Rewerski and T. Peichocki. Morphine action in grouped and isolated rats and mice. *Psychopharmacology* **53:** 191, 1977.
12. Martin, W. R. and J. W. Sloan. Neuropharmacology and neurochemistry of subjective effects, analgesia, tolerance and dependence produced by narcotic analgesics. In: *Drug Addiction*, edited by W. R. Martin. Berlin, Heidelberg: Springer Verlag, 1977, pp. 43–158.
13. Panksepp, J., B. Herman, R. Connor, P. Bishop and J. P. Scott. The biology of social attachments: opiates alleviate separation distress. *Biol. Psychiat.* **13:** 607–618, 1978.
14. Panksepp, J., T. Vilberg, N. J. Bean, D. H. Coy and A. J. Kastin. Reduction of distress vocalization in chicks by opiate-like peptides. *Brain Res. Bull.* **3:** 663–667, 1978.
15. Pilcher, C. W. T. and I. P. Stolerman. Recent approaches to assessing opiate dependence in rats. In: *Opiates and Endogenous Opioid Peptides*, edited by H. W. Kosterlitz. Amsterdam: Elsevier, 1976, pp. 327–334.
16. Pilcher, C. W. T., I. P. Stolerman and G. D. D'Mello. Aversive effects of narcotic antagonists in rats. In: *Characteristics and Functions of Opioids*, edited by J. M. Van Ree and L. Terenius. Amsterdam: Elsevier, 1978, pp. 437–438.
17. Pilcher, C. W. T., S. M. Jones and I. P. Stolerman. Possible GABA involvement in the mediation of narcotic antagonist-induced aversions. In: *Endogenous and Exogenous Opiate Agonists and Antagonists*, edited by E. L. Way. New York: Pergamon, 1979, pp. 451–454.
18. Sawynok, J., C. Pinsky and F. S. LaBella. Mini-review on the specificity of naloxone as an opiate antagonist. *Life Sci.* **25:** 1621–1632, 1979.
19. Schapiro, S., E. Geller and S. Eiduson. Corticoid response to stress in the steroid-inhibited rat. *Proc. Soc. exp. Biol. Med.* **109:** 935–937, 1962.
20. Stolerman, I. P., C. W. T. Pilcher and G. D. D'Mello. Stereospecific aversive property of narcotic antagonists in morphine-free rats. *Life Sci.* **22:** 1755–1762, 1978.
21. Winer, B. J. *Statistical Principles in Experimental Design*. London: McGraw-Hill, 1971.
22. Yates, F. E. and J. Urqhart. Control of plasma concentrations of adrenocortical hormones. *Physiol. Rev.* **42:** 359–433, 1962.