Pharmacology Biochemistry & Behavior, Vol. 23, pp. 883–887, 1985. 0091-3057/85 $3.00 + .00

Social Conflict Analgesia: Studies on Naloxone Antagonism and Morphine Cross-Tolerance in Male DBA/2 Mice

R. J. RODGERS AND JILL I. RANDALL

Pharmacoethology Laboratory, School of Studies in Psychology, University of Bradford Bradford BD7 1DP, U.K.

RODGERS, R. J. AND J. I. RANDALL. *Social conflict analgesia: Studies on naloxone antagonism and morphine cross-tolerance in male DBA/2 mice.* PHARMACOL BIOCHEM BEHAV **23**(5) 883–887, 1985. —It has recently been reported that male mice exhibit pronounced analgesia in response to attack from aggressive conspecifics. Although several studies indicate that this reaction can be blocked by opiate antagonist pretreatment, unequivocal evidence of opioid involvement is very much more limited. In the present study, the phenomenon of conflict analgesia has been studied in male DBA/2 intruder mice following exposure to a criterion level of attack from aggressive BKW residents. Our findings indicate that this analgesia is (1) blocked and reversed by naloxone (2) unaltered by methyl naloxone, except at high doses (75 mg/kg) and (3) fully cross-tolerant with morphine. This profile confirms and extends earlier findings with $B6AF_1$ mice, indicating that the opioid mediation of this biologically-relevant form of environmental analgesia is not strain specific.

Analgesia Social conflict Naloxone Methyl naloxone Morphine cross-tolerance Mice

IT is now well established that endogenous pain inhibitory systems can be activated by diverse environmental stimuli and that, depending upon stimulus context, opioid and/or non-opioid substrates are involved [18,21]. More recent studies on rodent social behaviour have indicated possible ecological relevance of such environmentally-induced analgesias [13]. For instance, male mice exhibit a pronounced analgesic reaction when subjected to attack from aggressive conspecifics [7,12]. Although this phenomenon of social conflict analgesia and, to a lesser extent, its sensitivity to opiate receptor blockade have since been reported in several mouse strains [6, 17, 19], detailed studies on opioid involvement have thus far been performed only in $B6AF_1$ mice [6, 7, 8].

Recent work in this laboratory [14] and elsewhere [6,17] has indicated that, in response to resident attack, intruder animals of the DBA/2 strain exhibit a strong analgesia which has a duration of 40–60 minutes. In view of major strain differences in response to both exogenous and endogenous opioids [1, 2, 17], the multiplicity of endogenous pain modulatory systems [18,21] and the inadequacy of naloxone antagonism alone in implicating opioid substrates [4,16], we now report a series of studies on the neurohumoral mediation social conflict analgesia in DBA/2 mice.

METHOD

Animals

Six to eight week old male DBA/2 mice (Bantin and Kingman, U.K.) and 15–20 week old male BKW mice (Bradford University colony) were used. The former (naive intruders) were housed in groups of 10/cage (45×28×13 cm) and the latter (experienced aggressive residents) in individual cages (33×15×13 cm). All animals were maintained in a temperature-controlled room (24±1°C), in which a 12 hr reversed light/dark cycle was operative. Food and water were freely available.

Analgesia and Behavioural Testing

All studies were conducted under dim red light (2×60 W) during the dark phase of the LD cycle. Analgesia was assessed by traditional (i.e., radiant heat method) tail-flick assay, with temperature adjusted to give control latencies (TFL) of approximately 4–5 seconds. A cut-off of 10 seconds was employed to prevent any possibility of tissue damage. All test animals (intruders) were given prior tail-flick experience to ensure stable baselines. In social conflict testing, DBA/2 intruders were individually placed into the home cage of an aggressive BKW resident, exposed to 35 attack bites and immediately removed. This fixed attack criterion was established in pilot studies as reliably inducing significant analgesia in the DBA/2 strain and was invariably reached within 5–10 minutes of the start of encounters.

Drugs

Naloxone hydrochloride (Endo Laboratories Inc, NY), methyl naloxone (Francopia, Paris) and morphine sulphate

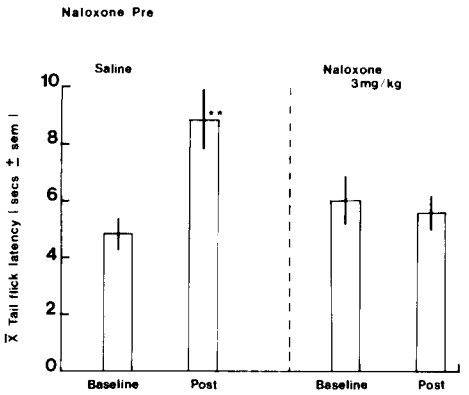

FIG. 1. Effect of naloxone hydrochloride (0–3 mg/kg, IP), administered prior to encounters, on social conflict analgesia in DBA/2 mice. $^{**}p<0.01$.

were used. All compounds were dissolved in physiological saline (0.9%) which, alone, served as vehicle control. Injections were performed intraperitoneally (IP) in a volume of 10 ml/kg and doses are expressed as the salts. In all studies, animals were randomly allocated to treatment conditions and tested in a counterbalanced order.

Statistical Analysis

All data were initially subjected to analysis of variance (ANOVA, 2 or 3 Factor; repeated measures), following which critical within-groups comparisons were performed by correlated t-test (2-tailed) using appropriate error variance terms from the ANOVAs.

RESULTS

Effects of Naloxone and Methyl Naloxone

Naloxone pretreatment. In several other strains, naloxone (0.3–10 mg/kg, IP) has been found to prevent the development of social conflict analgesia when administered *prior* to encounters [7, 12, 19]. In this study, DBA/2 mice were assigned to either saline (n=8) or 3 mg/kg naloxone hydrochloride (n=7) treatment conditions. Baseline TFLs were established prior to injection and, 10 minutes later, animals were exposed to criterion attack and immediately reassessed on the tail-flick assay. Results are summarized in Fig. 1. Although saline-pretreated mice displayed a pronounced analgesia, $t(12)=3.75$, $p<0.01$, naloxone pretreatment completely blocked this reaction ($t=0.43$, NS). Baseline latencies did not differ significantly between groups.

Naloxone post-treatment. Since naloxone has been reported to modify agonistic behaviour per se (for review: [13]), the antagonism of conflict analgesia by naloxone pretreatment may have been indirectly due to drug-induced changes during the actual encounter. To test this possibility, we next examined the effects of naloxone when administered *after* exposure to criterion attack. Mice (ns=5) were assigned to either saline or 3 mg/kg naloxone groups, assessed for basal TFL, subjected to criterion attack and immediately reassessed. Injections were then performed and, 10 minutes later, TFLs were determined for a third time. Results are summarized in Fig. 2. Analysis indicated that both groups displayed significant analgesia immediately after encounters

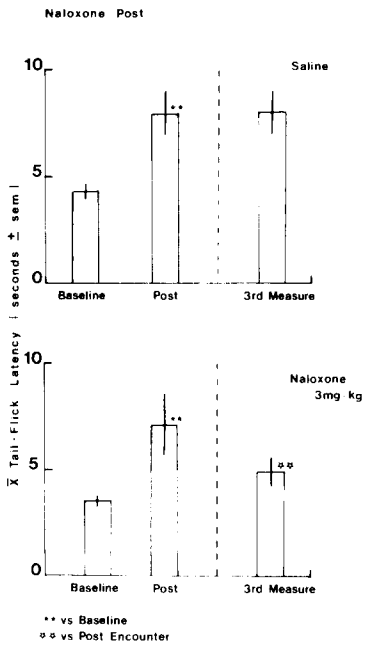

FIG. 2. Effect of naloxone hydrochloride (0–3 mg/kg, IP), administered immediately after encounters, on social conflict analgesia in DBA/2 mice. $^{**}p<0.01$ (vs. baseline), $^{\circ}p<0.02$ (vs. post-encounter).

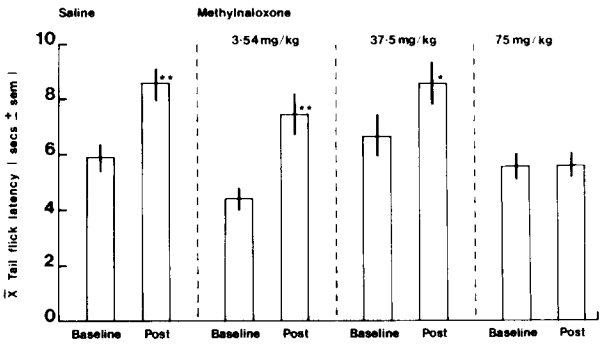

FIG. 3. Effects of methyl naloxone (0–75 mg/kg, IP) pretreatment on social conflict analgesia in DBA/2 mice. $^{*}p<0.05$, $^{**}p<0.001$.

($p<0.01$). However, whilst subsequent saline treatment did not alter this analgesia, $t(16)=0.03$, NS, naloxone post-treatment significantly reversed the reaction (vs. baseline, $t=1.83$, NS; vs. immediate post-encounter, $t=2.70$, $p<0.02$).

Methyl naloxone. To determine whether naloxone antagonism of conflict analgesia is of central or peripheral origin, we next studied the effects of its quarternary (methyl) derivative. As this analogue has been reported to have substantially lower receptor affinity than the parent drug in 'in vitro' assays [20], a wide dose range was employed. Animals were assigned to one of four treatment groups (ns=5–7): saline, 3.54, 37.5 or 75.0 mg/kg methyl naloxone. Basal TFLs were established prior to injection and, 10 minutes later, animals were exposed to criterion attack and immediately reassessed. Data are summarized in Fig. 3. Analysis indicated significant analgesia in animals receiving saline, $t(20)=6.89$, $p<0.001$, 3.54 mg/kg ($t=5.92$, $p<0.001$) and 37.5 mg/kg ($t=2.18$, $p<0.05$) methyl naloxone. However, no

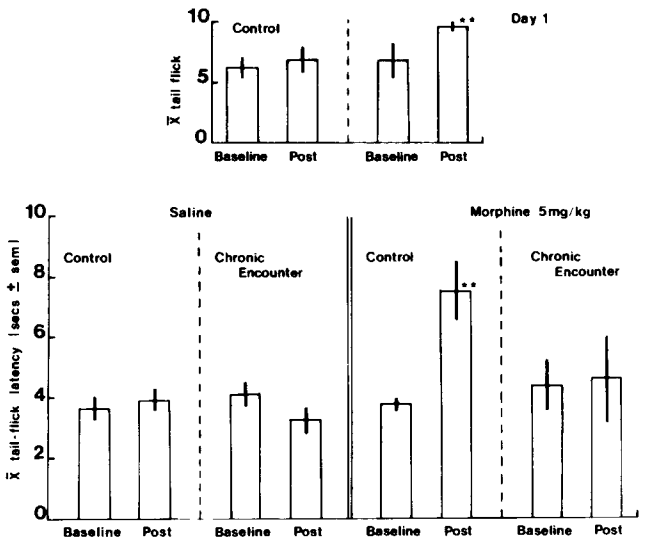

FIG. 4. Effect of chronic encounter experience (daily×7) on DBA/2 response to morphine challenge. The top panel (Day 1) illustrates the analgesic reaction to initial encounter (right) and response of control animals to repeated tail-flick testing (left). The bottom panel shows the reaction of control and chronic encounter groups to saline or morphine (5 mg/kg) challenge on Day 8. See text for full details. $^{**}p<0.01$.

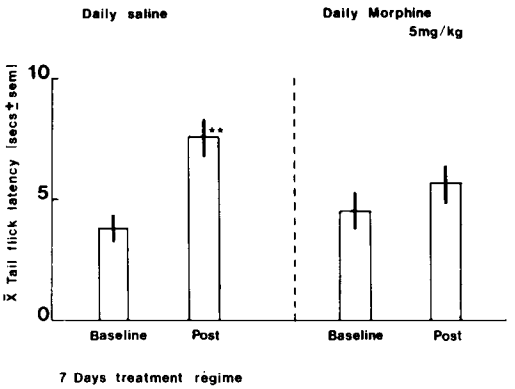

FIG. 5. Effect of chronic (daily×7) saline or morphine sulphate (5 mg/kg) treatment on DBA/2 response to conflict challenge on Day 8. See text for details. $^{**}p<0.01$.

analgesia was apparent in the 75 mg/kg methyl naloxone condition ($t=0.08$, NS).

Cross Tolerance With Morphine

Chronic conflict and analgesic response to morphine. In addition to naloxone antagonism, the demonstration of cross-tolerance with morphine is essential in establishing opioid involvement in physiological/behavioural processes [4,16]. In this study, DBA/2 intruders were assigned to two main conditions (n in each=10): a no-encounter control group and a chronic encounter group. On Day 1 of testing, basal TFLs were established for both groups, following which animals in the encounter condition were subjected to criterion attack and reassessed immediately; control animals were simply reassessed after identical inter-TFL intervals. Subsequently, encounter animals received a further 6 daily exposures to criterion attack whilst control animals were exposed to laboratory conditions only. No tail-flick testing was performed on these days. On Day 8, animals from each main condition were further assigned to saline (n=5) or 5 mg/kg morphine sulphate (n=5) groups. TFLs were determined prior to injection (baseline) and reassessed 30 minutes later. Data are summarized in Fig. 4. Analysis confirmed a significant Day 1 analgesia in the conflict group, $t(9)=3.45$, $p<0.01$. However, whilst control (no encounter) animals evidenced a pronounced analgesic reaction to morphine challenge on Day 8, $t(16)=6.12$, $p<0.01$, no such analgesia was detected in animals with a chronic encounter history ($t=0.46$, NS).

Chronic morphine and analgesic response to conflict. To determine whether full (i.e., bidirectional) cross-tolerance exists between conflict analgesia and morphine analgesia, the final study involved the converse procedure to that described above. DBA/2 intruders were allocated to two conditions (n in each=10): daily (×7) saline or daily (×7) morphine sulphate (5 mg/kg). Twenty-four hours after the last injection (i.e., on Day 8), all animals were assessed for basal TFLs, subjected to criterion attack and reassessed immediately. Results are summarized in Fig. 5. Analysis revealed a strong analgesic reaction to conflict in animals with a chronic saline history, $t(18)=3.51$, $p<0.01$, but the complete absence of such a response in the chronic morphine group ($t=1.06$, NS).

DISCUSSION

In contrast to several other strains (e.g., C57BL/6), DBA/2 mice are renowned for their sensitivity to the analgesic consequences of opiate manipulations, such as morphine administration [2] and immobilization stress [10]. More recently, this strain has also been reported to exhibit profound analgesia in response to 30–70 attack bites during intraspecific social encounters [6,17]. In this context, we have found that the analgesic reaction of DBA/2 intruder mice to relatively moderate attack (35 attack bites) has a duration of 40–60 minutes [12], confirming the findings of Miczek *et al.* [7] in $B6AF_1$ mice, another opiate-sensitive strain. Although the central opioid mediation of 'defeat' analgesia in the latter strain has been elegantly demonstrated [6, 7, 8], no detailed studies on the neurohumoral mediation of conflict analgesia in DBA/2 mice have thus far been performed. Such studies are essential in view of the multiplicity of endogenous analgesia mechanisms identified in recent years [18,21].

In this context, present results are entirely consistent with an opioid mediation of conflict analgesia in DBA/2 mice. Firstly, and in agreement with findings in BKW [12], $B6AF_1$ [7] and CF_1 [19] strains, conflict analgesia is blocked by naloxone (3 mg/kg) pretreatment. Secondly, and as has not previously been reported, post-treatment with naloxone *reverses* the analgesic consequences of attack. This finding is particularly important in view of the documented effects of opiate antagonists on social behaviour, *per se* [13]. More specifically, with naloxone pretreatment, it is impossible to dissociate direct and indirect effects of the antagonist on nociceptive mechanisms. However, naloxone *reversal* of conflict analgesia indicates that the pretreatment results are not an indirect consequence of drug-induced alterations in

social interaction during encounters. Thirdly, the effect of methyl naloxone (a peripherally-acting opiate antagonist) would suggest that the opioid substrate involved in conflict analgesia is of central, rather than peripheral, origin. However, these data are not as clear-cut as would have been desired. In selecting appropriate drug doses for this study, we took into account the substantially lower opiate receptor affinity [20] of the methyl compound, and earlier reports that it is devoid of central activity up to doses of at least 30 mg/kg [11]. Although our data indicate that conflict analgesia is unaffected by an equimolar dose (3.54 mg/kg) of methyl naloxone, there appeared to be partial antagonism at 37.5 mg/kg and an obvious complete antagonism at 75 mg/kg. It should, perhaps, be noted that the apparent partial antagonism profile may be an artefact related to the unusually high baseline in the 37.5 mg/kg group. Nevertheless, in view of these data, we subsequently examined the effects of methyl naloxone on responsivity to the hot-plate assay. In this unpublished study, naive DBA/2 mice were treated with 0, 3.54, 37.5 or 75.0 mg/kg methyl naloxone ten minutes prior to hot-plate testing (55±1°C). Jump latencies were significantly influenced by the drug, $F(3,33)=4.69$, $p<0.01$, a result attributable to a significant hyperalgesic effect of the 37.5 and 75.0 mg/kg doses. As Jacob and Ramabadran [5] have clearly demonstrated the central nature of opiate antagonist-induced hyperalgesia, it would appear that high doses of methyl naloxone do penetrate the blood-brain barrier to exert central effects. A similar conclusion has been reached concerning the inhibitory effects of 50 mg/kg methyl naloxone on fluid consumption in rats [3]. As such, present findings on the effects of this compound on conflict analgesia are not inconsistent with the postulated involvement of central opioid substrates. This conclusion is in agreement with that of Miczek *et al.* [7] who failed to antagonize 'defeat' analgesia in $B6AF_1$ mice with quarternary naltrexone.

Although naloxone antagonism is a necessary criterion in the demonstration of opioid involvement in physiological and behavioural processes, it is by no means sufficient [4,16]. It is now widely accepted that there ought to be evidence of morphine cross-tolerance. To this end, our final two studies on conflict analgesia confirm cross-tolerance both to and from morphine. Thus, mice rendered tolerant to morphine (7×5 mg/kg) fail to show an analgesic response to subsequent attack challenge, whilst mice rendered tolerant to the effects of attack (7 daily tests) fail to demonstrate an analgesic reaction to subsequent morphine challenge. These data are consistent with those reported for $B6AF_1$ mice [6,7], and provide convincing evidence that conflict analgesia in DBA/2 mice is an opioid-mediated phenomenon.

In conclusion, exposure to attack during social encounters precipitates a long-lasting (40–60 min) analgesia in DBA/2 mice, which is apparently mediated via central opioid mechanisms. Although the involvement of psychological factors in the development of conflict [12], or defeat [7], analgesia has previously been suggested, present data do not permit further conclusions on this issue. However, we have recently completed a series of studies in which 'display of defeat' (rather than 35 attack bites) was employed as the test criterion [15]. Under these conditions, DBA/2 mice show a short-lasting analgesia (less than 10 min) which is neither naloxone-sensitive nor cross-tolerant with morphine. As DBA/2 mice readily exhibit defeat early in encounters, it would appear that this acute non-opioid analgesia has not previously been detected because of the relatively long test periods normally employed (5–10 minutes). This finding also emphasizes that the demonstration of conflict analgesia *per se* does not necessarily imply activation of opioid mechanisms. To our knowledge, only cold-water swim has previously been reported to induce non-opioid analgesia in mice [9], although this may merely reflect the relatively infrequent use of mice in this field of research. Thus, in a manner analogous to rats [18,21], mice seem capable of displaying opioid and/or non-opioid analgesia depending upon the particular stimulus context. Studies are currently in progress to determine the temporal relationship between non-opioid and opioid analgesias in social conflict and to further clarify the behavioural correlates of each phenomenon.

ACKNOWLEDGEMENTS

The authors wish to thank Endo Laboratories Inc (New York) and Francopia (Paris) for the kind gifts of naloxone hydrochloride and methyl naloxone, respectively. This work was supported by the Medical Research Council.

REFERENCES

1. Alleva, E., C. Castellano and A. Oliverio. Effects of L- and D-amino acids on analgesia and locomotor activity of mice: their interaction with morphine. *Brain Res* **198:** 249–252, 1980.
2. Frischknecht, H.-R., B. Siegfried, G. Riggio and P. G. Waser. Inhibition of morphine analgesia and locomotor activity in strains of mice: a comparison of long-acting opiate antagonists. *Pharmacol Biochem Behav* **19:** 939–944, 1983.
3. Hemmer, R. C., G. A. Olson, A. J. Kastin, J. H. McLean and R. D. Olson. Effects of naloxone and its quarternary form on fluid consumption in rats. *Pharmacol Biochem Behav* **17:** 1287–1290, 1982.
4. Hill, R. G. The status of naloxone in the identification of pain control mechanisms. *Neurosci Lett* **21:** 217–222, 1981.
5. Jacob, J. J. C. and K. Ramabadran. Role of opiate receptors and endogenous ligands in nociception. *Pharmacol Ther* **14:** 177–196, 1981.
6. Miczek, K. A. and M. L. Thompson. Analgesia resulting from defeat in a social confrontation: the role of endogenous opioids in brain. In: *Modulation of Sensorimotor Activity During Altered Behavioural States*, edited by R. Bandler. New York: AR Liss, 1984, pp. 431–456.
7. Miczek, K. A., M. L. Thompson and L. Shuster. Opioid-like analgesia in defeated mice. *Science* **215:** 1520–1522, 1982.
8. Miczek, K. A., M. L. Thompson and L. Shuster. Naloxone injections into periaqueductal grey area and arcuate nucleus block analgesia in defeated mice. *Psychopharmacology (Berlin)*, in press, 1985.
9. O'Connor, P. and R. E. Chipkin. Comparisons between warm water and cold water swim stress in mice. *Life Sci* **35:** 631–639, 1984.
10. Puglisi-Allegra, S. and A. Oliverio. Social isolation: effects on pain threshold and stress-induced analgesia. *Pharmacol Biochem Bheav* **19:** 679–681, 1983.
11. Ramabadran, K., C. Suaudeau and J. J. C. Jacob. A comparison of some pharmacological effects of naloxone and N-methylnaloxone in mice. *Can J Physiol Pharmacol* **60:** 715–719, 1982.
12. Rodgers, R. J. and C. A. Hendrie. Social conflict activates status-dependent endogenous analgesic or hyperalgesic mechanisms in male mice: effects of naloxone on nociception and behaviour. *Physiol Behav* **30:** 775–780, 1983.

13. Rodgers, R. J. and C. A. Hendrie. On the role of endogenous opioid mechanisms in offense, defense and nociception. In: *Ethopharmacological Aggression Research*, edited by K. A. Miczek, M. R. Kruk and B. Olivier. New York: AR Liss, 1984, pp. 27–42.
14. Rodgers, R. J. and J. I. Randall. Strain differences in behaviourally-induced antinociception and morphine analgesia in male mice. *Br J Pharmacol* **84:** 105, 1985.
15. Rodgers, R. J. and J. I. Randall. Acute non-opioid analgesia in defeated male mice. Manuscript under revision. *Physiol Behav*, 1985.
16. Sawynok, J., C. Pinsky and F. S. LaBella. On the specificity of naloxone as an opiate antagonist. *Life Sci* **25:** 1621–1632, 1979.
17. Siegfried, B., H.-R. Frischknecht and P. G. Waser. Defeat, learned submissiveness and analgesia in mice: effect of genotype. *Behav Neural Biol* **42:** 91–97, 1984.
18. Terman, G. W., Y. Shavit, J. W. Lewis, J. T. Cannon and J. C. Liebeskind. Intrinsic mechanisms of pain inhibition: activation by stress. *Science* **226:** 1270–1277, 1984.
19. Teskey, G. C., M. Kavaliers and M. Hirst. Social conflict activates analgesic and ingestive behaviours in male mice. *Life Sci* **35:** 303–315, 1984.
20. Valentino, R. J., J. L. Katz, E. Medzihradsky and J. H. Woods. Receptor binding, antagonist and withdrawal precipitating properties of opiate antagonists. *Life Sci* **32:** 2887–2896, 1983.
21. Watkins, L. R. and D. J. Mayer. Organization of endogenous opiate and nonopiate pain control systems. *Science* **216:** 1185–1192, 1982.