Pharmacology Biochemistry & Behavior, Vol. 23, pp. 871–876, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Mu and Kappa Opiate Receptor Involvement in Agonistic Behaviour in Mice

DAVID BENTON

Department of Psychology, University College, Swansea SA2 8PP, Wales, United Kingdom

BENTON, D. *Mu and kappa opiate receptor involvement in agonistic behaviour in mice.* PHARMACOL BIOCHEM BEHAV **23**(5) 871–876, 1985.—The influence of opioid drugs on agonistic behaviour is reviewed and an experiment is reported that examines the impact of U-50488 (a kappa agonist) and DAGO (a mu agonist) on the social encounters of male and female mice interacting with anosmic male partners. Although DAGO did not significantly influence inter-male social encounters, U-50488 decreased social investigation, increased timid/defensive behaviour and potently suppressed aggression. In contrast, U-50488 did not significantly influence the behaviour of timid female mice, whereas DAGO decreased social and timid/defensive behaviour. It was concluded that a kappa mechanism increases whereas a mu mechanism decreases submissive behaviour.

Aggression DAGO Kappa opiate receptor Mouse Mu opiate receptor Social interaction Submissiveness Social behaviour U-50488

OPIATES AND SOCIAL BEHAVIOUR

Panksepp [18] has reviewed the evidence that brain-opiates mediate social affect and social attachment. Morphine decreases and naloxone increases the distress displayed by the young of a number of species when they are separated from their mother. If opioid activity can fulfill social needs, then activation of these systems should reduce the tendency of animals to seek company. Low doses of morphine (likely to be acting via mu receptors) reduce the tendency of both socially-housed guinea pigs [11] and rats [19] to spend time close to other members of the same species.

Aggressive behaviour has also been reported to be influenced by opiate drugs. It has been known for many years that the acute administration of narcotic drugs suppresses aggression produced by many procedures and in several species. It is not surprising that morphine, a potent analgesic, in rats [14] and monkeys [8] blocks aggression induced by electric-shocks. However, morphine also reduces aggression in situations that do not involve pain: there are reports that it suppresses isolation-induced fighting in mice [7,12], mouse killing by rats [13] and the rage and irritability syndromes produced by lesioning the septum of the rat [5] and the hypothalamus of the cat [28]. In addicted animals, the withdrawal of morphine is associated with hyperirritability and spontaneously violent aggression. Thor *et al.* [26] reported that, in the rat, vigorous fighting begins three days after the withdrawal of morphine and lasts for 40–50 hours: the readministration of morphine suppresses this behaviour. This spontaneous aggression seems relatively specific to narcotics as it is not seen following the chronic administration of many other drugs.

It seems likely that these early studies on the influence of morphine on aggression reflect complex changes in brain chemistry. Rather than referring to opiate receptors, it has been hypothesised that aggression following morphine withdrawal reflects a supersensitivity of dopamine receptors; a role for cholinergic and serotonergic mechanisms has also been suggested [9]. The discovery of a range of endogenous peptides [16] that include at least eighteen that act via opiate receptors (including the enkephalins, endorphins and dynorphins), suggested the possibility that morphine may influence aggressive behaviour via these sites. Although the area is complex there seems general agreement that at least three opiate receptors exist within the brain, designated as mu, kappa and delta sites [22].

The analgesic influence of morphine is thought to be mediated via mu-receptors (hot-plate ED_{50}, 2.3 mg/kg). The analgesic dose of morphine should be contrasted with those used in the early study of aggression. Animals addicted to morphine may receive as much as 400 mg/kg a day, a dose that is certain to produce large and non-specific changes in brain chemistry. DaVanzo *et al.* [7] reported that the ED_{50}, for the inhibitory influence of morphine on isolation-induced aggression, was 21.2 mg/kg, again a dose unlikely to be acting specifically at opiate sites.

Recent trends have been:

(1) To use much smaller doses of morphine, likely to be acting more specifically at mu sites,

(2) To use drugs thought to act relatively specifically at different opiate sites,

(3) To examine the influence of the opioid antagonists on aggressive encounters.

These more recent approaches have produced results that are, at least superficially, inconsistent. Naloxone has usually [2, 15, 17, 21, 23], but not always [3,20], been found to decrease isolation-induced (offensive) and to increase shock-induced (defensive) aggression [21]. Based on the findings with naloxone, it might be expected that an opiate agonist would stimulate aggressive behaviour: an expectation that has not been met with either mu [4, 12, 20] or kappa agonists [4]. There have been no similar studies using delta agonists but the influence of the delta-antagonist ICI 154,129 has been

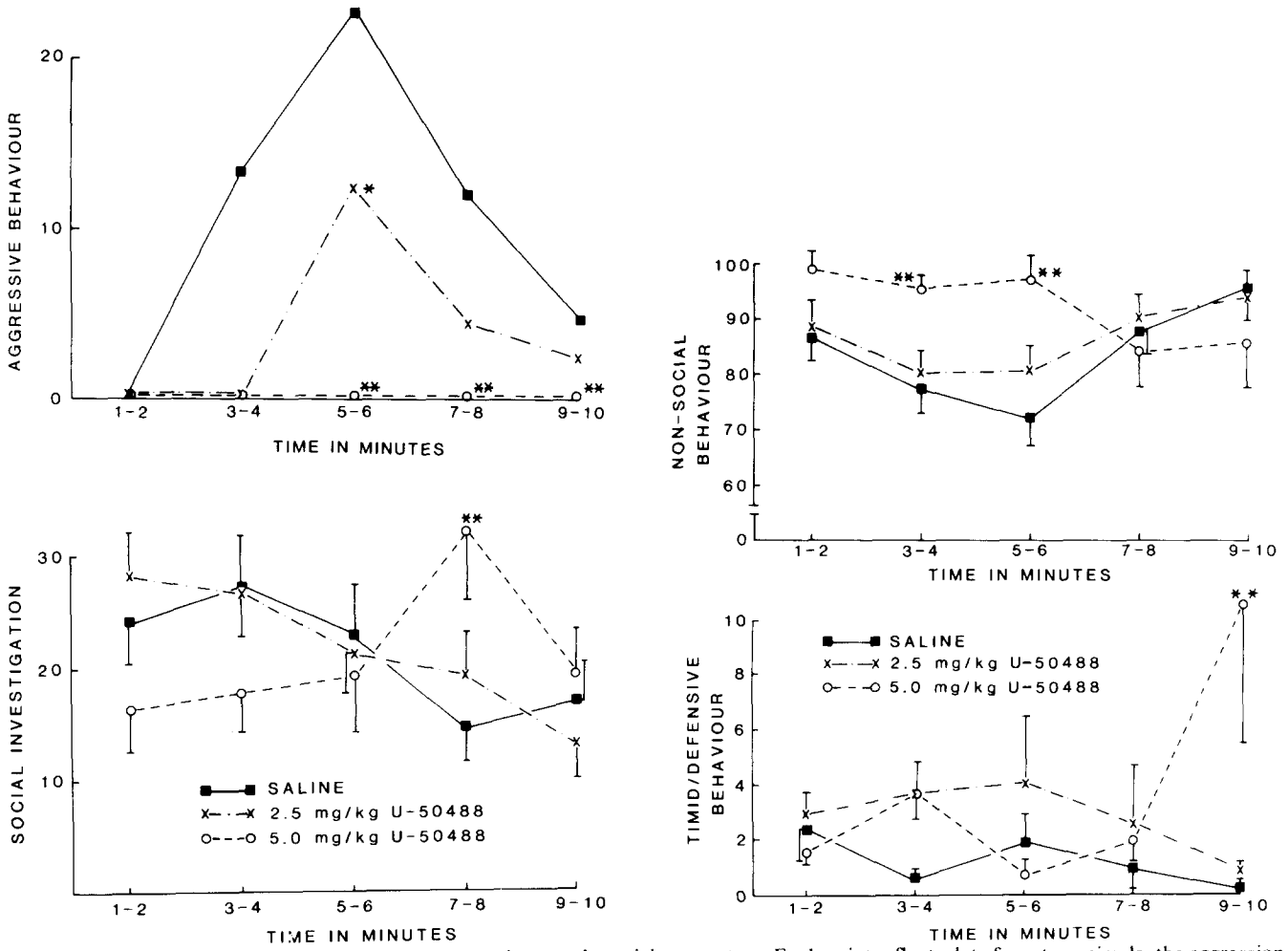

FIG. 1. The influence of the kappa agonist U-50488 on inter-male social encounters. Each point reflects data from ten animals, the aggression scores are medians and the remaining behaviour means ± S.E. For the aggressive behaviours * differs from control $p<0.05$, $^{**}p<0.005$. For other behaviours $^{*}p<0.05$, $^{**}p<0.01$.

interpreted as producing a decreased emotional reactivity that results in a slightly increased incidence of aggressive behaviour [3].

These data may be explained by emphasizing various methodological factors. A feature that seems to be of importance is whether the study has examined mice that are experienced in fighting, or naive to the test situation. For example, a study that examined "trained fighters" found that, in doses likely to be acting via mu sites, naloxone increased and morphine sulphate decreased the incidence of fighting [20]: data strongly indicating that mu sites specifically modulate circuits associated with murine aggressive behaviour. In contrast, those studies reporting an inhibitory effect of naloxone on fighting have used relatively large and thus non-specific doses and secondly have examined initial encounters for short periods of time.

Inevitably, when conspecifics meet, there is an initial uncertainty about relative status. Benton [4] concluded that during an initial encounter, when these uncertainties can be expected to predominate, mu-receptors do not seem to be involved. Consistent with this analysis is that most studies have failed to find that doses of naloxone below 1 mg/kg (doses below this will selectively block mu-receptors; high doses block mu, kappa, and delta sites) influence fighting during an initial encounter [3, 15, 21, 23]. The only exception [2] is a study that examined the interaction between isolated animals chosen to generate high levels of fighting; in this situation initial social investigation would be minimized and defensive mechanisms would be stimulated when the opponent fought back. The finding that low doses of morphine sulphate [4] do not influence aggressive or submissive behaviour during an initial encounter is also consistent with this analysis. The non-specific nature of high doses of naloxone makes the findings that doses of naloxone in excess of 1 mg/kg suppress fighting [2, 15, 17, 21, 23] difficult to interpret. Large doses of naloxone can be expected to block mu, kappa, and delta sites; each site may influence a different mechanism, having even opposite influences on a particular behaviour [3,4]. In addition, a high dose of naloxone may act as an opiate agonist and is known to influence GABA metabolism [25].

Adams [1] has argued that there are separate brain mechanisms controlling offensive, defensive and submissive behaviour. Benton [4] suggested that data obtained from the

study of initial encounters could be explained by the assumption that the mu-agonist morphine decreases, and the kappa-agonist tifluadom increases, timid/submissive behaviour. In aggressive isolated males the submissive mechanisms are not tonically active; thus morphine and low doses of naloxone (acting at mu sites) are without effect as the submissive mechanism is not active: in contrast the kappa agonist stimulated submissive behaviour. In isolated females, that display a lot of submissive behaviour, the kappa-mediated submissive mechanism seemed to be tonically active and the mu agonist decreased these types of behaviour [4].

COMPARISONS OF THE INFLUENCE OF U-50488 AND DAGO ON SOCIAL ENCOUNTERS IN MICE

Given the above evidence that suggests a role for opiate receptors in social behaviour, and more specifically the hypothesis that mu and kappa mechanisms have opposite influences on submissive behaviour, the present study has examined the impact of two novel compounds on social encounters. Handa *et al.*]10] synthesized analogues of Beta-Lipotropin 61–64 and produced a series of compounds, including DAGO, that are potent agonists at mu-receptors. U-50488 is a naloxone reversible analgesic that is thought to act selectively at the kappa receptor [27]. These drugs are compared in isolated male and female mice in interactions with anosmic male "standard opponents." These two groups of animals are strikingly different. Isolated females spend a great deal of time in timid/submissive activities, whilst isolated males display offensive and very little defensive or submissive behaviour.

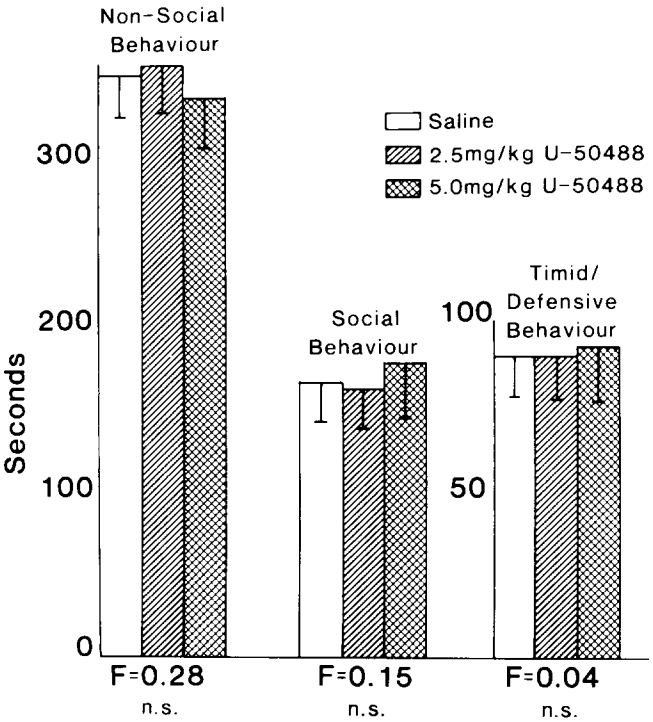

FIG. 2. The influence of the kappa agonist U-50488 on the social behaviour of female mice. Each point reflects data from ten animals; in no instance did the drug influence the behaviour recorded.

METHOD

Subjects

Alderley Park strain mice aged 65–75 days were used in these studies. They were bred in the Psychology Department of University College, Swansea, from stocks originally obtained from I. C. I. Ltd., Alderley Park, Macclesfield, U.K. Mice were weaned at 19–23 days of age and housed on a sawdust substrate in single sex groups of six animals, in opaque plastic cages measuring 30×12×11 cm. Animals were maintained at 21±1°C, under a reversed lighting schedule (lights on 2100–0900). Food and water were available ad lib, except during behavioural trials.

Male and female experimental mice were individually-housed for a period of 21 days before testing. Other male animals remained in their original groups until testing, and were used as "standard opponents"; they were rendered temporarily anosmic by applying, under ether anaesthesia, 25 μl of 4% zinc sulphate to the nasal tract, both three days and one day prior to social encounters. Anosmic animals are non-aggressive and spend little time in social investigation; thus the drug treated animals with which they interact can be expected to initiate most social encounters, and the results will thus more accurately reflect the influence of the drug treatment. Opponents were used only once.

Behavioural Trials

Thirty minutes before behavioural encounters, isolates were injected, subcutaneously, with either 0.9% saline, 2.5 or 5.0 mg/kg U-50488 (Trans-(+)-3,4-dichloro-N-methyl-N-(−(1-pyrrolidinyl) cyclohexyl) benzeneacetamide methane sulphonate hydrate [27]), 250 μg or 1 mg/kg DAGO (HTyr-D-Ala-Gly-N(Me)-Phe-NHCH CH OH; RX783006 [10]). The drugs and doses were chosen on the basis of relative selectivity for kappa and mu sites. Each animal received a single injection and one behavioural trial. Testing commenced one hour after the start of the dark phase of the illumination cycle and continued for a maximum of three hours, in order to minimize the influence of the known circadian fluctuations in endogenous opiates. The doses used, and the time scale, were chosen as preliminary experiments suggested that they did not cause sedation.

Thirty minutes after injection, a randomly-chosen, non-injected "standard opponent" marked with fur dye, was introduced into the home cage of the test animal. The social encounter was videotaped for ten minutes under white light (40 lux), with the wire-mesh lid of the cage being replaced with a transparent Perspex cover to facilitate observation. The resulting video record had a superimposed trace from an electronic timer. The video-tape was analyzed by allocating the behaviour of the drug-treated mouse to one of the following four categories.

(1) Non-social behaviour: digging, rearing, self-grooming, walking around and sniffing the arena.

(2) Social behaviour: approaching, following, grooming, sniffing the partner.

(3) Aggressive behaviour: sideways and upright offensive postures, chasing, lunging at and biting the opponent.

(4) Timid/defensive behaviour: sideways and upright defensive postures, avoiding or fleeing from the opponent.

Statistical Analysis

Where appropriate, the results were examined using a

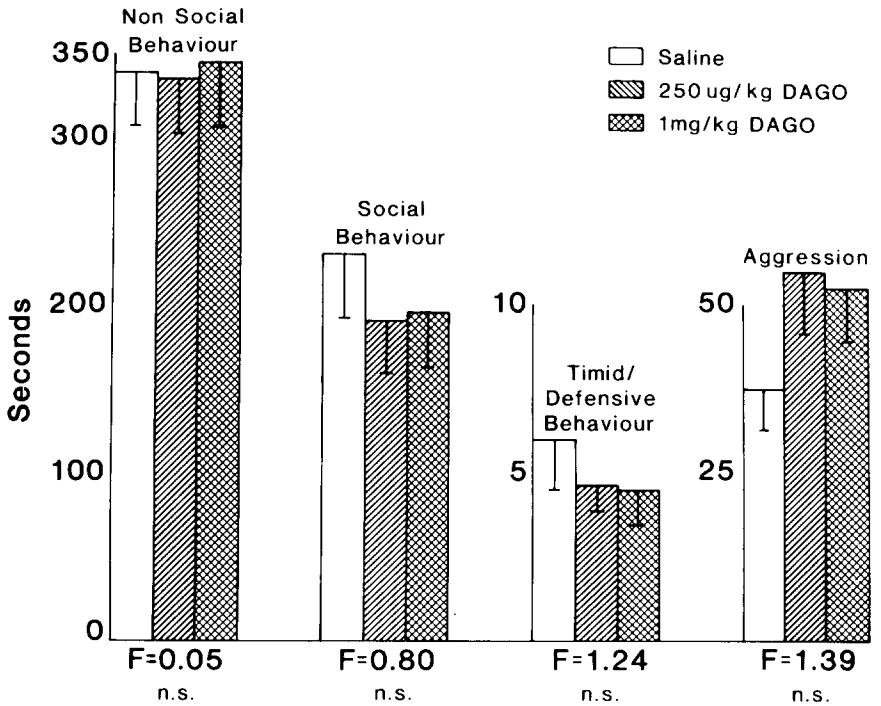

FIG. 3. The influence of the mu agonist DAGO on inter-male social encounters. Each point reflects data from ten animals; in no instance did the drug influence the behaviour recorded.

two-way analysis of variance: drug×time (five 2-min blocks) with repeated measured over the second factor. Significant interactions were examined using Dunnett's test to compare treatments with the control values. When little aggressive behaviour resulted the data were analysed using Kruskal-Wallis one-way analysis of variance.

RESULTS

U-50488

The behavioural influences of U-50488 on male mice are illustrated in Fig. 1. For non-social behaviour, a significant interaction between dose and time was significant, $F(8,108)=3.32$, $p<0.01$. Dunnett's test shows that mice treated with the higher dose spent significantly longer performing non-social behaviours during minutes 3–4, $t(3,135)=2.63$, $p<0.01$, and 5–6, $t(3,135)=3.73$, $p<0.001$. The time spent in social investigation again produced a significant interaction, $F(8,108)=2.54$, $p<0.05$, the higher dose of U-50844 resulted in higher scores during minutes 7–8, $t(3,135)=2.97$, $p<0.01$. A third interaction between the dose and time resulted from the analysis of timid/defensive behaviour, $F(8,108)=3.29$, $p<0.01$; during the last two minutes those treated with the higher dose spent significantly longer displaying these behaviours, $t(3,135)=4.26$, $p<0.01$. The most striking influence of this kappa agonist was on aggressive behaviours; the use of Kruskal-Wallis one-way analysis of variance showed that the scores differed during minutes 5–6 ($p<0.001$), 7–8 ($p<0.01$) and 9–10 ($p<0.05$). The higher dose resulted in particularly non-aggressive animals.

In contrast to the males, U-50488 had no influence on female mice (Fig. 2). The drug did not influence the time spent in social investigation, $F(2,27)=0.15$, n.s., non-social investigation, $F(2,27)=0.28$, n.s., or timid/defensive behaviour, $F(2,28)=0.04$, n.s. In no case was there a significant interaction between drug treatment and minutes of testing.

DAGO

The influence of DAGO on the social interaction of male mice is illustrated in Fig. 3. The drug did not influence the amount of non-social, $F(2,27)=0.05$, n.s., social, $F(2,27)=0.80$, n.s., timid-defensive behaviour, $F(2,27)=1.24$, n.s., or aggressive behaviour, $F(2,27)=1.39$, n.s. The social behaviour of female mice treated with DAGO is illustrated by Fig. 4. The examination of non-social behaviour produced an interaction between drug and minute of testing, $F(8,108)=2.29$, $p<0.05$. The use of Dunnett's test (all *df* 3,135) showed that the 250 μg/kg dose significantly increased these behaviours during minutes 1–2, $t=2.49$, $p<0.025$, 3–4, $t=2.0$, $p<0.05$, and 5–6, $t=3.65$, $p<0.005$; a similar profile resulted with the higher dose of DAGO, non-social behaviour was higher than the control values during minutes 1–2, $t=5.09$, $p<0.005$, 3–4, $t=3.21$, $p<0.005$, and 5–6, $t=2.31$, $p<0.025$. At the same time the drug influenced social behaviour, $F(2,27)=5.96$, $p<0.01$, an effect due to the higher dose that markedly reduced these behaviours throughout the period of test. Although the drug did not produce a significant influence on the timid/defensive scores, $F(2,27)=2.18$, n.s., the use of Dunnett's test showed that the lower dose significantly reduced these behaviours during minutes 1–2, $t(3,135)=2.62$, $p<0.01$, and 5–6, $t(3,135)=2.13$, $p<0.05$.

DISCUSSION

The present results can be explained if it is assumed that

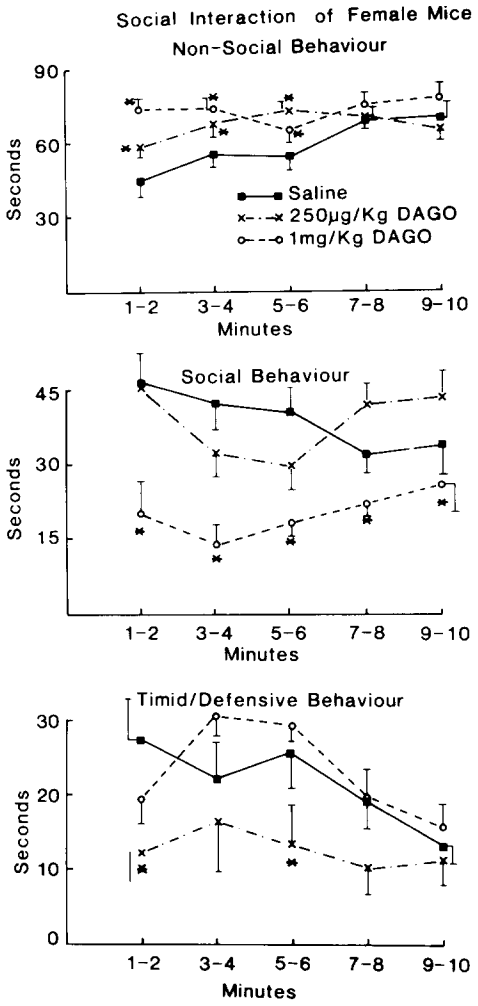

FIG. 4. The influence of the mu agonist DAGO on the social behaviour of female mice. Each point reflects data from ten animals; each point is a mean±S.E. *Differs from control at least with $p<0.05$.

U-50488 increases timid/submissive behaviour: in the male (Fig. 1) the initially less social and thus more non-social behaviour could reflect such a change. Only towards the end of the test period did social interaction increase in the drug treated animals and this was followed by high levels of timid/defensive and virtually no aggressive behaviour. The lack of effect of U-50488 on female behaviour could reflect a tonically active kappa system in animals that were already submissive. Given that kappa agonists are known to have sedative influences the possibility that the changes observed in males are merely secondary to this influence must be considered. However, this does not seem to be a valid explanation; the same doses of U-50488 did not produce any changes in the behaviour of females (Fig. 2); later in the test period the male animals spent more time in social exploration than the control animals; data not consistent with a sedative influence (Fig. 1). These results are similar to those that resulted from the use of tifluadom, a benzodiazepine derivative that lacks the typical pharmacological effects of minor tranquillizers, and acts selectively via opiate kappa-receptors [24]. Tifluadom increased the non-social and decreased social behaviour, while increasing timid/defensive activities in the male [4]. Similar to the negative results with U-50488 in the present study, tifluadom did not influence the behaviour of female mice.

The Benton *et al.* [4] study concluded that morphine sulphate, used in a dose that could be expected to act via mu receptors, decreased the likelihood that timid/defensive behaviour will be expressed. The data obtained using DAGO can be explained in a similar manner. Isolated male animals are predisposed to be aggressive and to display little timid/defensive behaviour: thus a drug that acts to further decrease these behaviours could be expected to have little influence; the result obtained (Fig. 3). The data obtained with the lower dose of DAGO in females (Fig. 4) are similar to those obtained with morphine [4], both drugs produced more non-social and less timid/defensive behaviours. It may well be that the higher dose of DAGO was acting in a similar way to morphine that in doses higher than those needed to stimulate mu sites, causes behavioural stimulation.

Several alternative explanations should be considered. It is logically possible that there are sex differences in the response to mu and kappa agonists. Given that there are marked differences in the tendency of male and female mice to act aggressively, it may be that the organizing influence of androgens during brain development has resulted in changes in the opioid mechanisms that modulate agonistic behaviour. If this is so then the changes are of a very specific nature as other opioid mechanisms, such as pain-perception and respiratory depression, are not differentially influenced. Another possibility is that the changes in social behaviour are only secondary to changes in emotionality: the data are consistent with mu agonists decreasing and kappa agonists increasing emotionality. The doses of drugs were chosen following initial studies in an open field (Benton, unpublished findings): all four drugs produced similar effects, a tendency in doses higher than those presently used to decrease ambulation and rearing; there was no reason to suggest that mu and kappa agonists differentially influence emotional responding. The finding that the drugs differentially influence males and females suggests that the mechanism of action is not simply via an opioid-mediated mechanism for social attachment as postulated by Panksepp [18].

In summary the most probable explanation that accounts for this and a previous study [4] is that mu and kappa opiate sites have opposite effects; the former decrease and the latter increase the likelihood of the expression of timid/submissive behaviour.

ACKNOWLEDGEMENTS

The gifts of U-50488 from Dr. P. F. Vonvoigtlander of The Upjohn Company, Kalamazoo, and DAGO from Dr. M. Rance of I. C. I. Pharmaceuticals, Alderly Edge, Chesire, England, are gratefully acknowledged.

REFERENCES

1. Adams, D. B. Brain mechanisms for offense, defense, and submission. *Behav Brain Sci* **2**: 201–241, 1979.

2. Benton, D. The long-term effects of naloxone, dibutyryl cyclic CMP and chlorpromazine on aggression in mice monitored by an automatic device. *Aggress Behav* **10**: 79–89, 1984.

3. Benton, D., S. Brain and P. F. Brain. Comparison of the influence of the opiate delta receptor antagonist ICI 154,129 and naloxone on social interaction and behaviour in an open field. *Neuropharmacology* **23:** 13–17, 1984.
4. Benton, D., R. Smoothy and P. F. Brain. Comparisons of the influence of morphine sulphate, morphine-3-glucuronide and tifluadom on social encounters in mice. *Physiol Behav* **35:** in press, 1985.
5. Bernard, P., J. Welch, J. Emberley and S. Fielding. The behavioral effects of morphine in rats with septal and hypothalamic lesions. In: *Drug Addiction*, vol 3, edited by M. D. Singh and H. Lal. Miami: Symposia Specialists, 1974, pp. 297–307.
6. Brain, P. F., R. Smoothy and D. Benton. An ethological analysis of the effects of tifluadom on social encounters in male albino mice. *Pharmacol Biochem Behav* **23:** in press, 1985.
7. DaVanzo, J. P., M. Daugherty, R. Ruckart and L. Kang. Pharmacological and biochemical studies in isolation-induced fighting mice. *Psychopharmacologia* **9:** 210–219, 1966.
8. Emley, G. S., R. R. Hutchinson and I. B. Brannan. Aggression: effects of acute and chronic morphine. *Mich Ment Hlth Res Bull* **4:** 23–26, 1970.
9. Gianutsos, G. and H. Lal. Narcotic analgesics and aggression. *Mod Prob Pharmacopsychol* **13:** 114–138, 1978.
10. Handa, B. A., A. C. Lane, J. A. H. Lord, B. A. Morgan, M. J. Rance and C. F. C. Smith. Analogues of B-LPH 61–64 possessing selective agonist activity at μ-opiate receptors. *Eur J Pharmacol* **70:** 531–540, 1981.
11. Herman, B. H. and J. Panksepp. Effects of morphine and naloxone on separation distress and approach attachment: evidence for opiate mediation of social affect. *Pharmacol Biochem Behav* **9:** 213–220, 1978.
12. Janssen, P. A. J., A. H. Jageeneau and C. J. E. Niemeggers. Effects of various drugs on isolation-induced fighting behaviours of male mice. *J Pharmacol Exp Ther* **129:** 471–475, 1960.
13. Janssen, P. A. J., C. J. E. Niemegeers et F. J. Verbruggen. A propos d'une methode d'investigation de substances susceptibles de modifier le comportement agressif inne du rat blanc vis-a-vis de la souris blanche. *Psychopharmacologia* **3:** 114–123, 1962.
14. Lal, H., G. Gianutsos and S. K. Puri. A comparison of narcotic analgesia with neuroleptics on behavioral measures of dopaminergic activity. *Life Sci* **17:** 29–34, 1975.
15. Lynch, W. C., L. Libby and H. F. Johnson. Naloxone inhibits intermale aggression in isolated mice. *Psychopharmacology (Berlin)* **79:** 370–371, 1983.
16. Martin, J. B. Neuroendocrinology and brain peptides. In: *Psychoneuroendocrine Dysfunction*, edited by N. S. Shah and A. G. Donald. New York: Plenum Press, 1984, pp. 15–40.
17. Olivier, B. and D. van Dalen. Social behaviour in rats and mice: an ethologically based model for differentiating psychoactive drugs. *Aggress Behav* **8:** 163–168, 1982.
18. Panksepp, J., B. H. Herman, T. Vilbeerg, P. Bishop and F. G. DeEskinazi. Endogenous opioids and social behavior. *Neurosci Biobehav Rev* **4:** 473–487, 1978.
19. Panksepp, J., N. Najam and F. Soares. Morphine reduced social cohesion in rats. *Pharmacol Biochem Behav* **11:** 131–134, 1979.
20. Poshivalov, V. P. Ethological analysis of neuropeptides and psychotropic drugs: effects on intraspecies aggression and sociability of isolated mice. *Aggress Behav* **8:** 355–369, 1982.
21. Puglisi-Allegra, S., A. Oliverio and P. Mandel. Effects of opiate antagonists on two kinds of aggressive behavior in mice. *Aggress Behav* **8:** 175–177, 1982.
22. Rance, M. J. Multiple opiate receptors—their occurrence and significance. *Clin Anaesthesiol* **1:** 183–199, 1983.
23. Rodgers, R. J. and C. A. Hendrie. Social conflict activates status-dependent endogenous analgesic or hyperalgesia mechanisms in male mice: effects of naloxone on nociception and behaviour. *Physiol Behav* **30:** 775–780, 1983.
24. Romer, D., H. H. Buscher, R. C. Hill, R. Mauer, T. J. Petcher, H. Zeugner, W. Benson, E. Finner, W. Milkowski and P. W. Thies. An opioid benzodiazepine. *Nature* **298:** 759–760, 1982.
25. Sawynok, J., C. Pinsky and F. S. LaBella. On the specificity of naloxone as an opiate antagonist. *Life Sci* **25:** 1621–1632, 1979.
26. Thor, D. H., D. L. Hoats and C. J. Thor. Morphine induced fighting and prior social experience. *Psychon Sci* **18:** 137–139, 1970.
27. Vonvoigtlander, P. F., R. A. Lahti and J. H. Ludens. U-50488: A selective and structurally novel non-mu (kappa) opioid agonist. *J Pharmacol Exp Ther* **224:** 7–12, 1983.
28. Wikler, A. Studies on the action of morphine on the central nervous system of cat. *J Pharmacol Exp Ther* **80:** 176–187, 1944.