An Analysis of Precipitated Withdrawal in Rats Acutely Dependent on Morphine

Krishnaswami RAMABADRAN*
Department of Pharmacology, Faculty of Medicine, National University of Singapore, Kent Ridge, Singapore 0511

Accepted December 24, 1984

Abstract—Acute dependence on a single dose of morphine was assessed in Wistar rats by observing the frequencies of occurrence of several signs of withdrawal precipitated by naloxone, diprenorphine, Mr2097, Mr1452 and Mr2266. Naloxone significantly precipitated urination, paw shakes, head shakes and chewing. Diprenorphine significantly precipitated urination and chewing. Mr2097 precipitated urination, head shakes, teeth chattering and chewing. The selective kappa antagonists Mr1452 and Mr2266 significantly precipitated only urination and teeth chattering. Signs of the precipitated withdrawal by Mr2097 were mediated by stereoselective opioid receptors, as the other diastereoisomer, Mr2097, did not precipitate them. Stereospecific opioid receptors were also involved in the induction of acute dependence, as naloxone precipitated withdrawal only in /-methadone-treated rats, but not in d-methadone treated rats. All the opioid antagonists produced at least some degree of “abstinoid” signs in morphine-free rats which might be caused by the blockade of endogenous opioids acting on / and/or K receptors. The signs of withdrawal precipitated by naloxone and Mr2097 might be primarily mediated by / receptors, those of diprenorphine by both / and K receptors, and those by Mr1452 and Mr2266 were likely to be selectively mediated by K receptors. The latter aspect was further supported by experiments showing that the novel K agonist U-50488H did not precipitate withdrawal. A low degree of precipitation of withdrawal by Mr1452 and Mr2266 and the absence of precipitation of abstinence by U-50488H might be related to either a lack or an existence of a low proportion of K receptors in rat brain. Further experiments using selective agonists and antagonists are needed to evaluate these findings.

The phenomenon of acute dependence on opioids has been studied in rodents (1–11), dogs (12–14), monkeys (15) and human subjects (16, 17). However, the stereoselectivity of opioid receptors and the different types of opioid receptors involved in the various signs of precipitated withdrawal in acutely dependent animals on morphine or other opioids have not been so far reported. Therefore, the present studies were carried out in rats to shed some light on the phenomenon of acute dependence by employing different classes of opioids to examine the stereoselectivity of opioid receptors and identify the different types of opioid receptors by behavioural pattern using different signs of precipitated abstinence, and the results obtained are reported in this paper.

Materials and Methods

Subjects were male Wistar rats (150–230 g; obtained from Laboratory Animal Center, National University of Singapore) that were given a single injection of morphine hydrochloride or NaCl (0.9% W/V=saline) 1.5 hr prior to the injection of NaCl or one of the following drugs: naloxone, diprenorphine, Mr2096, Mr2097, Mr1452, Mr2266, or U-50488H, according to the
procedures described earlier (8, 10, 11). The dose of morphine (15 mg/kg) and the time interval between morphine or methadone and the opioid antagonists (1.5 hr) were selected based on the maximum precipitated withdrawal from an earlier study (8). The diastereoisomers Mr2096 and Mr2097 (18) were used to test the stereoselectivity of the opioid receptors involved. Selective kappa (κ) antagonists, Mr1452 and Mr2266 (19, 20), and a novel kappa (κ) agonist, U-50488H (21, 22), were included to identify the participation of kappa opioid receptors in the precipitated abstinence signs. d-Methadone and /-methadone were employed to examine the stereospecificity of the opioid receptors involved in the induction of acute dependence. The various doses of drugs precipitating withdrawal used in this study were (expressed in mg/kg): naloxone (3, 10), diprenorphine (3, 10), Mr2096 and Mr2097 (10 each), Mr1452 (1, 3), Mr2266 (1, 3) and U-50488H (3, 10). The volume of injection was 0.5 ml/100 g. All drugs were injected sub-cutaneously (s.c.). Immediately after the injection of drugs precipitating withdrawal or NaCl or distilled water, the rats were placed individually on a raised platform (height, 25 cm; diameter, 23 cm) and observed for 30 min for various precipitated withdrawal signs such as jumping, urination, diarrhoea, paw shakes, head shakes, teeth chattering, chewing, ptosis and rearing. The number of rats exhibiting these withdrawal symptoms were observed and noted by experienced investigators who were not aware of drug treatment. Each rat was used only once. Six rats were observed at one time. The frequencies of occurrence of the signs of precipitated withdrawal was analyzed by Fisher's Exact Probability Test.

Drugs: Morphine hydrochloride (purchased from Government Pharmaceutical Laboratory, Singapore), /-methadone hydrochloride and d-methadone hydrochloride (gift from Eli Lilly, U.S.A.), Mr2096 {(-)-(N-[(S)-tetrahydrofurfuryl]-N-[((S)-tetrahydrofurfuryl)noroxymorphone hydrochloride, Mr2097 {(-)-(N-[(R)-tetrahydrofurfuryl]-N-[((R)-tetrahydrofurfuryl]-noroxymorphone hydrochloride, Mr1452 {(-)-5.9-di(-2-((3-furlylalkyl)-2'-hydroxy-6.7-benzomorphan) methanate sulphinate, Mr2266 {(-)-5.9α diethyl-2-(3-furlylalkyl)-2'-hydroxy 6.7-benzomorphan} (gifts from Dr. H. Merz, Boehringer Ingelheim, F.R.G.), U-50488H {trans(+)-3.4-dichloro-N-methyl-N(2-(1-pyridinidinyl)-cyclohexyl)-benzeneacetamide (gift from Dr. R.A. Lahti, Upjohn, U.S.A.)} and diprenorphine hydrochloride (gift from Dr. M.J. Rance, Reckitt & Colman, England). All compounds except Mr2266 and U-50488H were dissolved in 0.9% saline. Mr2266 and U-50488H were dissolved with a few drops of 0.1N HCl and made up with distilled water.

Results

The effects produced by naloxone (3 mg/kg) and diprenorphine (3 mg/kg) on the various signs of precipitated withdrawal in rats acutely dependent on morphine are shown in Table 1. In the saline-treated control group of rats, naloxone by itself produced some “abstinoid” signs, expressed as the number of rats exhibiting the signs out of the number of rats used for that group, urination (1/20), diarrhoea (1/20), head shakes (6/20), teeth chattering (9/20), chewing (6/20), rearing (5/20); diprenorphine (3 mg/kg) by itself produced diarrhoea (1/20) and rearing (9/20). Naloxone significantly (P<0.05) precipitated urination, paw shakes, head shakes and chewing, and non-significantly, jumping, teeth chattering and ptosis. The total absence of diarrhoea in the group of rats injected with morphine + naloxone might be due to the absence of development of acute dependence in the gut. This situation is in direct contrast with diarrhoea produced at the rate of 100% when naloxone is administered to a rat chronically treated with morphine. Diprenorphine significantly (P<0.05) precipitated urination, paw shakes, head shakes and chewing, and non-significantly, jumping, teeth chattering and ptosis. The total absence of diarrhoea in the group of rats injected with morphine + naloxone might be due to the absence of development of acute dependence in the gut. This situation is in direct contrast with diarrhoea produced at the rate of 100% when naloxone is administered to a rat chronically treated with morphine. Diprenorphine significantly (P<0.05) precipitated urination and chewing, and non-significantly, jumping, paw shakes, head shakes, teeth chattering and rearing.

Stereoselectivity of the opioid receptors involved in the various signs of abstinence in acutely dependent rats was analyzed by using a pair of diastereoisomers, Mr2096 and Mr2097, and the results are depicted in Table 2. The agonist Mr2096 did not produce any “abstinoid” sign in morphine naive rats and also did not precipitate withdrawal in
### Table 1. Precipitation of acute dependence by naloxone in rats

| Treatment                  | n  | Jumping | Urination | Diarrhoea | Paw shakes | Head shakes | Teeth chattering | Chewing | Ptosis | Rearing |
|----------------------------|----|---------|-----------|-----------|------------|-------------|-------------------|---------|--------|---------|
| NaCl + NaCl                | 20 | 0       | 0         | 0         | 3          | 3           | 5                 | 3       | 12     | 3       |
| NaCl + Naloxone            | 20 | 0       | 1         | 1         | 3          | 6           | 9                 | 6       | 12     | 5       |
| NaCl + Diprenorphine       | 20 | 0       | 0         | 1         | 3          | 3           | 5                 | 2       | 8      | 9       |
| Morphine + NaCl            | 20 | 0       | 0         | 0         | 0          | 0           | 0                 | 2       | 2      | 2       |
| Morphine + Naloxone        | 20 | 3       | 18         | 0         | 14         | 15         | 10                | 20      | 17     | 9       |
| Morphine + Diprenorphine   | 20 | 3       | 5          | 0         | 5          | 5           | 10                | 11      | 12     | 10      |

The time interval between morphine administration (15 mg/kg) (or NaCl) and naloxone (3 mg/kg) or diprenorphine (3 mg/kg) (or NaCl) was 1.5 hr. n = number of rats used in each group. The doses are expressed in terms of the respective salt. The figures indicate the number of rats exhibiting the respective sign. The superscripts \( ^a,^b,^c \) denote the statistical significance (P<0.05) between groups of rats as determined by Fisher's test; the effects were significant compared with \(^a\)NaCl plus NaCl, \(^b\)NaCl plus naloxone or diprenorphine and \(^c\)morphine plus NaCl, respectively. Similar results were obtained following naloxone (10 mg/kg) or diprenorphine (10 mg/kg) (not illustrated).

### Table 2. Stereoselectivity of opioid receptors involved in acute precipitated withdrawal in rats

| Treatment                  | n  | Jumping | Urination | Diarrhoea | Paw shakes | Head shakes | Teeth chattering | Chewing | Ptosis | Rearing |
|----------------------------|----|---------|-----------|-----------|------------|-------------|-------------------|---------|--------|---------|
| NaCl + NaCl                | 20 | 0       | 0         | 0         | 0          | 1           | 5                 | 3       | 15     | 4       |
| NaCl + Mr2096              | 20 | 0       | 0         | 0         | 0          | 1           | 1                 | 0       | 2      | 3       |
| NaCl + Mr2097              | 20 | 0       | 1         | 2         | 2          | 7           | 16                | 12      | 18     | 5       |
| Morphine + NaCl            | 20 | 0       | 0         | 0         | 0          | 0           | 0                 | 0       | 1      | 2       |
| Morphine + Mr2096          | 20 | 0       | 0         | 0         | 0          | 1           | 3                 | 2       | 4      | 1       |
| Morphine + Mr2097          | 20 | 2       | 9          | 3         | 4          | 15         | 17                | 20      | 20     | 7       |

The time interval between morphine administration (15 mg/kg) (or NaCl) and Mr2096 (10 mg/kg) or Mr2097 (10 mg/kg) (or NaCl) was 1.5 hr. The symbol "n" and the figures, as in Table 1. The superscripts \( ^a,^b,^c,^d \) denote the statistical significance (P<0.05) between groups of rats as determined by Fisher's test; the effects were significant compared with \(^a\)NaCl plus NaCl, \(^b\)NaCl plus Mr2097, \(^c\)morphine plus NaCl, and \(^d\)morphine plus Mr 2096, respectively.
| Treatment                  | n  | Jumping | Urination | Diarrhoea | Paw shakes | Head shakes | Teeth chattering | Chewing | Ptosis | Rearing |
|---------------------------|----|---------|-----------|-----------|------------|-------------|------------------|---------|--------|---------|
| NaCl+NaCl                 | 20 | 0       | 0         | 0         | 2          | 5           | 2                | 8       | 4      |         |
| NaCl+Naloxone             | 20 | 0       | 1         | 4         | 2          | 5           | 9                | 6       | 13     | 5       |
| d-Methadone+NaCl          | 20 | 2       | 1         | 2         | 2          | 6           | 3                | 9       | 8      |         |
| l-Methadone+NaCl          | 20 | 0       | 0         | 0         | 0          | 0           | 0                | 0       | 1      | 0       |
| d-Methadone+Naloxone      | 20 | 10  \(\text{a, b, c, d}\) | 16 \(\text{a, b, c, d}\) | 0         | 3          | 7  \(\text{e}\)  | 11  \(\text{e}\)  | 17  \(\text{a, b, c, d}\) | 19  \(\text{a, b, c, d}\) | 3      |
| l-Methadone+Naloxone      | 20 | 2       | 1         | 4         | 1          | 4           | 7  \(\text{e}\)  | 5       | 10     | 6       |

The time interval between d-methadone (5 mg/kg) or l-methadone (5 mg/kg) administration (or NaCl) and naloxone (10 mg/kg) (or NaCl) was 1.5 hr. The symbol "n" and figures as in Table 1. The dose of l-methadone was selected from a pilot study. All the rats treated with l-methadone plus NaCl showed profound catalepsy, muscular rigidity, immobility and respiratory depression which were not observed with d-methadone pretreated groups. The symptoms produced by l-methadone were reversed by naloxone. The superscripts \(\text{a, b, c, d}\) denote statistical significance (P<0.05) between groups of rats as determined by Fisher’s test; the effects of l-methadone plus naloxone were significant compared with \(\text{a}\) NaCl plus NaCl \(\text{b}\) NaCl plus naloxone \(\text{c}\) l-methadone plus NaCl, and \(\text{d}\) d-methadone plus naloxone, respectively.

---

| Treatment                  | n  | Jumping | Urination | Diarrhoea | Paw shakes | Head shakes | Teeth chattering | Chewing | Ptosis | Rearing |
|---------------------------|----|---------|-----------|-----------|------------|-------------|------------------|---------|--------|---------|
| NaCl+D.W                  | 20 | 0       | 0         | 0         | 0          | 2           | 0                | 10      | 1      |         |
| NaCl+Mr1452               | 20 | 3       | 0         | 5  \(\text{a}\) | 4          | 4           | 4                | 2       | 6      | 7  \(\text{a}\)  |
| NaCl+Mr2266               | 20 | 2       | 2         | 2         | 3          | 4           | 3                | 3       | 6      | 5       |
| Morphine+D.W.             | 20 | 0       | 0         | 0         | 0          | 0           | 0                | 0       | 2      | 3       |
| Morphine+Mr1452           | 20 | 4       | 8  \(\text{a, b, c}\) | 5  \(\text{e}\) | 3          | 4           | 11  \(\text{a, b, c}\) | 7  \(\text{e}\) | 10  \(\text{e}\) | 7  \(\text{a}\)  |
| Morphine+Mr2266           | 20 | 3       | 8  \(\text{a, b, c}\) | 6  \(\text{a, c}\) | 4          | 4           | 10  \(\text{a, b, c}\) | 7  \(\text{c}\) | 9  \(\text{c}\) | 9  \(\text{a, c}\) |

The time interval between morphine administration (15 mg/kg) (or NaCl) and Mr1452 (3 mg/kg) or Mr2266 (3 mg/kg) (or D.W.,=distilled water) was 1.5 hr. The symbol "n" and the figures, as in Table 1. The superscripts \(\text{a, b, c}\) indicate the statistical significance (P<0.05) between groups of rats as determined by Fisher’s test; the effects were significant compared with \(\text{a}\) NaCl plus NaCl \(\text{b}\) NaCl plus Mr1452 or Mr2266 and \(\text{c}\) morphine plus NaCl, respectively. Similar results were obtained following Mr1452 (1 mg/kg) or Mr2266 (1 mg/kg) (not illustrated).
Table 5. Absence of precipitated withdrawal by the kappa agonist U-50488H in rats acutely dependent on morphine

| Treatment                        | n  | Jumping | Urination | Diarrhoea | Paw shakes | Head shakes | Teeth chattering | Chewing | Ptosis | Rearing |
|----------------------------------|----|---------|-----------|-----------|------------|-------------|------------------|---------|--------|---------|
| NaCl + D.W.                      | 20 | 0       | 0         | 0         | 0          | 2           | 4                | 1       | 16     | 0       |
| NaCl + 3 mg/kg U-50488H          | 20 | 9a      | 0         | 5a        | 0          | 2           | 1                | 3       | 15     | 3       |
| NaCl + 10 mg/kg U-50488H         | 20 | 11a     | 3         | 6a        | 2          | 2           | 1                | 2       | 17     | 6a      |
| Morphine + D.W.                  | 20 | 0       | 0         | 0         | 0          | 0           | 0                | 0       | 3      | 7       |
| Morphine + 3 mg/kg U-50488H      | 20 | 1       | 1         | 0         | 0          | 0           | 1                | 4       | 6      | 4       |
| Morphine + 10 mg/kg U-50488H     | 20 | 0       | 4         | 0         | 0          | 0           | 0                | 0       | 7      | 1       |

The time interval between morphine (15 mg/kg) (or NaCl) administration and U-50488H (or D.W.=distilled water) was 1.5 hr. The symbol "n" and the figures as in Table 1. The superscript "a" indicate the statistical significance (P<0.05) between groups of rats as determined by Fisher’s test; the effects of NaCl+U-50488H were significant compared with "a" NaCl+distilled water.
morphine-dependent rats. On the contrary, Mr2097 by itself produced "abstinoid" signs such as urination (1/20), diarrhoea (2/20), paw shakes (2/20), head shakes (7/20, P<0.05 when compared to the NaCl+NaCl group), chewing (12/20, P<0.05 when compared to the NaCl+NaCl group), and rearing (5/20). Mr2097 significantly (P<0.05) precipitated withdrawal symptoms such as urination, head shakes, teeth chattering and chewing, and non-significantly, jumping, paw shakes and rearing. It is, therefore, conceivable that the "abstinoid" signs produced by Mr2097 in morphine-free rats and withdrawal symptoms precipitated by Mr2097 in rats acutely dependent on morphine were mediated through stereoselective opioid receptors.

The stereospecificity of opioid receptors involved in the production of acute dependence was also examined by using the stereoisomers d-methadone (5 mg/kg) and l-methadone (5 mg/kg) and precipitating the abstinence by a high dose of naltrexone (10 mg/kg). l-Methadone but not d-methadone induced profound catalepsy, muscular rigidity, respiratory depression and immobility which were reversed by naltrexone. Naltrexone significantly (P<0.05) precipitated jumping, urination, chewing and ptosis, and non-significantly, head shakes and teeth chattering in l-methadone-treated rats. Naltrexone did not precipitate these symptoms in d-methadone-treated rats (Table 3).

Mr1452 and Mr2266 are considered to be highly selective kappa receptor antagonists (Dr. H. Merz, personal communication; 19, 20). Therefore, it appeared interesting to test these compounds in acutely dependent rats to know more about the participation of kappa receptors in the various signs of precipitated withdrawal. The results obtained with these compounds are summarized in Table 4. Mr1452 (3 mg/kg) produced some "abstinoid" signs such as jumping (3/20), diarrhoea (5/20, P<0.05 when compared to the NaCl+distilled water group), paw shakes (4/20), head shakes (4/20), teeth chattering (4/20), chewing (2/20), and rearing (7/20, P<0.05 when compared to the NaCl+distilled water group). Mr2266 (3 mg/kg) produced jumping (2/20), urination (2/20), diarrhoea (2/20), paw shakes (3/20), head shakes (4/20), teeth chattering (3/20), chewing (3/20) and rearing (7/20, P<0.05 when compared to the NaCl+distilled water group). Mr1452 and Mr2266 significantly (P<0.05) precipitated urination and teeth chattering, and non-significantly, jumping and chewing in acutely morphine-dependent rats. Mr2266 non-significantly precipitated chewing and rearing.

In rats acutely dependent on morphine, the novel kappa agonist U-50488H (3, 10 mg/kg) (21, 22) did not precipitate withdrawal (Table 5). In contrast, however, in morphine-free rats, U-50488H by itself induced dose-dependent "abstinoid" signs such as jumping (9/20 at 3 mg/kg, P<0.05 when compared to the NaCl+distilled water group; 11/20 at 10 mg/kg, P<0.05 when compared to the NaCl+distilled water group), urination (3/20 at 10 mg/kg) and diarrhoea (5/20 at 3 mg/kg, P<0.05 when compared to the NaCl+distilled water group; 6/20 at 10 mg/kg, P<0.05 when compared to the NaCl+distilled water group), and rearing (3/20 at 3 mg/kg; 6/20 at 10 mg/kg, P<0.05 when compared to the NaCl+distilled water group). Thus, it seems likely that all these signs were mediated by kappa receptors.

Discussion
Some interesting findings were obtained in the present study. Firstly, the present technique of rendering rats dependent on a single dose of morphine might be of great use in laboratory studies of dependence on opioids as a rapid screening test. This method appears to have advantages over the pellet implantation or multiple injection schedules: (a) fabrication of pellets or reservoirs or slow release suspensions is not required, (b) rats can be made dependent on a single dose of morphine as low as 15 mg/kg, (c) there is no need to handle the rats several times a day as in the multiple injection method, and (d) the absence of lethality and poor health due to an inadequate consumption of water and food in multiple injection schedules. Apart from the above advantages, the withdrawal symptoms resulting from such acute dependence are quite similar to those observed after chronic administration. However, the
extrapolation of results obtained in rats acutely dependent on morphine to those observed in human subjects acutely dependent on morphine needs further study, along with determination of behavioural consequences.

Secondly, all the opioid antagonists reported in this study, naloxone, diprenorphine, Mr2097, Mr1452 and Mr2266, produced at least some degree of "abstinoid" signs in morphine-free rats, in confirmation of an earlier report (11). Even though the frequencies of occurrences were low with some of the antagonists, they cannot be simply excluded from consideration because withdrawal precipitated by them might be the outcome of "abstinoid" signs superimposed on real withdrawal effects. The "abstinoid" signs produced by opioid antagonists alone were often missed and not reported because in the studies on dependence, proper controls injected with only saline or opioid antagonists were not always included. The results of the present study thus indicate that while studying precipitated withdrawal, it is of critical importance to employ appropriate control groups. The "abstinoid" signs might be caused by the blockade of endogenous opioids acting on mu and/or kappa receptors. The "abstinoid" signs might also arise from blockade of opioid receptors by the antagonists if one accepts the view of the existence of physical dependence on physiologically released endogenous opioids (23).

Thirdly, naloxone is generally regarded as a selective antagonist at mu receptors (24) even though it might also have some competitive antagonistic properties at kappa receptors (25). Diprenorphine is considered as an almost universal antagonist at both mu and kappa receptors (24). Thus, the precipitation of withdrawal signs by naloxone such as jumping, urination, paw shakers, head shakes and chewing in acutely morphine-dependent rats and those such as jumping, urination, chewing and ptosis in l-methadone pre-treated rats were primarily mediated by mu receptors. The two signs, urination and chewing precipitated by diprenorphine were likely to be mediated by both mu and kappa receptors. The significant difference observed in jumping, pawshakes and head shakes between the group of morphine plus naloxone and that of l-methadone plus naloxone might be related to the degree of acute dependence developed by the respective opioid agonist and also might depend on the relative affinity of naloxone for those type of opioid receptors involved in the particular sign of withdrawal.

Fourthly, the "abstinoid" signs and the acute withdrawal syndrome precipitated by Mr2097 were mediated by stereoselective opioid receptors, as the other diastereoisomer Mr2096 did not produce them. Mr2096 did not precipitate withdrawal, possibly because of its agonistic properties (18) at mu receptors. The reverse phenomena was also found to be true in the acute dependence tests using the stereoisomers l-methadone and d-methadone. The induction of acute dependence as measured by precipitation of withdrawal by naloxone was observed only in l-methadone pre-treated rats, but not in those injected with d-methadone, indicating the stereospecificity of opioid receptors involved in this phenomenon. Apart from the withdrawal effects, the symptoms such as catalepsy, muscular rigidity, immobility and respiratory depression were also mediated by stereospecific opioid receptors as they were noticed only in l-methadone treated groups of rats and were reversed by naloxone.

Fifthly, some comments deserve to be mentioned regarding kappa opioid receptors mediating some "abstinoid" signs and precipitated withdrawal symptoms. The selective kappa receptor antagonists Mr1452 and Mr2266 precipitated urination and chewing. Additionally, in morphine-free rats, the novel kappa agonist U-50488H (21, 22) by itself produced jumping, urination, diarrhoea and rearing. Moreover, U-50488H did not antagonize the anti-diuresis in morphine-pretreated rats; conversely, the increase in urinary output by U-50488H was not prevented by morphine pre-treatment. On the contrary, the significant increase in the frequency of jumping response and diarrhoea produced by U-50488H was completely prevented by morphine pre-treatment. In this context, it should be remembered that
morphine was also shown to have some agonist activity at the kappa receptors (25–27). The reason for the production of jumping by U-50488H in morphine-free rats is not very clear at present, even though there is a possibility that it might be mediated through a different mechanism.

The anti-diuretic action observed in these experiments following morphine and /-methadone and the increased frequency of urination precipitated by naloxone in these rats were predominantly mediated by mu receptors, and the mechanism of anti-diuretic action was not dependent upon vasopressin release, because in Brattleboro rats that were homozygous for diabetes insipidus (where there is no vasopressin) morphine and /-methadone still decreased urination, and this effect was antagonized by naloxone, but not by quaternary naltrexone (28). The urination observed following either U-50488H or Mr1452 or Mr2266 are consistent with the hypothesis that substances which activate kappa receptors appear to increase urination by inhibiting the release of vasopressin from the neurohypophysis (29–33) and kappa receptors are intimately involved in the control of fluid balance (28, 32). Thus these data are compatible with the notion that the kappa receptor activators and mu agonists can be distinguished on the basis of their effects on urination; the former increases urination and the latter decreases urination (28). Also, high doses of naloxone are needed to achieve complete antagonism at kappa receptors.

Lastly, a low degree of precipitation, i.e., only two signs of withdrawal (urination and teeth chattering) by Mr1452 and Mr2266 and the absence of precipitation of withdrawal by U-50488H in rats acutely dependent on morphine might have at least some relation to a lack of kappa binding sites in rat brain (34, 35) or due to the existence of a low proportion of kappa binding sites in rat brain (36, 37). In agreement with all these data, recent evidence suggested that in rats, the degree of physical dependence and intensity of withdrawal produced by the endogenous substrate for kappa receptors, dynorphin(1–13), and its analogue, D-Ala²-dynorphin(1–11), was lower than that of the mu agonist morphine (38). Further elaborate experiments using different permutations and combinations of selective opioid agonists and antagonists are needed to extend these concepts.

Acknowledgements: Gifts of drugs by the pharmaceutical companies mentioned in the methods section are gratefully acknowledged. I am thankful to Miss Chua Mui Eng for her technical assistance in conducting these studies and to the Directorate of Medical Services, Ministry of Health of the Government of Singapore for permitting me to use controlled drugs in these studies. This research work was supported by a research grant (RP95/81: Functions of endorphins-multiple opioid receptors) from the National University of Singapore.

References

1 Kosersky, D.S., Harris, R.A. and Harris, L.S.: Naloxone-precipitated jumping activity in mice following the acute administration of morphine. Eur. J. Pharmacol. 26, 122–124 (1974)
2 Jacob, J.J.C., Barthelemy, C., Tremblay, E.C. and Colombel, M.-C.L.: Potential usefulness of single-dose acute dependence on and tolerance to morphine for the evaluation of narcotic antagonists. Adv. Biochem. Psychopharmacol. 8, 299–318 (1974)
3 Smits, S.E.: Quantitation of physical dependence in mice by naloxone-precipitated jumping after a single dose of morphine. Res. Commun. Chem. Pathol. Pharmacol. 10, 651–661 (1975)
4 Tremblay, E., Colombel, M.C. and Jacob, J.: Effets de la morphine et de la naloxone sur l'abstinence precipitee chez la souris en etat de dependance aigue. J. Pharmacol. 7, 103–114 (1976)
5 Wilely, J.N. and Downs, D.A.: Naloxone-precipitated jumping in mice pretreated with acute injections of opioids. Life Sci. 25, 797–802 (1979)
6 Ritzmann, R.F.: Opiate dependence following acute injections of morphine and naloxone: The assessment of various withdrawal signs. Pharmacol. Biochem. Behav. 14, 575–577 (1981)
7 Collier, H.O.J., Francis, D.L. and Schneider, C.: Modification of morphine withdrawal by drugs interacting with humoral mechanisms: Some contradictions and their interpretation. Nature 237, 220–223 (1972)
8 Tremblay, E., Colombel, M.C. and Jacob, J.: Precipitation et prevention de l'abstinence chez le rat et la souris en etat de dependance aigue: Comparaison de la naloxone, de la naltrexone et de la diprenorphine. Psychopharmacology
Acute Dependence on Morphine in Rats

9 Eisenberg, R. M.: Further studies on the acute dependence produced by morphine in opiate naive rats. Life Sci. 31, 1531–1540 (1982)

10 Ramabadran, K.: Nociceptive reactivity and precipitated abstinence in hypophysectomized rats. Japan. J. Pharmacol. 32, 751–755 (1982)

11 Ramabadran, K.: Naloxone precipitated abstinence in mice, rats, and gerbils acutely dependent on morphine. Life Sci. 33, Supp. I, 385–388 (1983)

12 Michaud, G. M. and Jacob, J. J. C.: Interactions between morphine and naloxone in the intact dog: a contribution to the problem of acute dependence. In 25th Annual Meeting of the Committee on Problems of Drug Dependence, Chapel Hill, N.C., p. 535–548 (1973)

13 Jacob, J. J. and Michaud, G. M.: Acute physical dependence in the waking dog after a single low dose of morphine. Psychol. Med. 4, 270–273 (1974)

14 Jacob, J. J.: Pharmacologie des antagonistes de la morphine. Rev. Médecine. No. 7, 431–444 (1975)

15 Krystal, J. H. and Redmond, D. E., Jr.: A preliminary description of acute physical dependence in the velvet monkey. Pharmacol. Biochem. Behav. 18, 289–291 (1983)

16 Jones, R. T.: Dependence in non-addict humans after a single dose of morphine. In Endogenous and Exogenous Opiate Agonists and Antagonists, Edited by Leong Way, E., p. 557–560, Pergamon Press, New York (1980)

17 Jones, R. T.: Caffeine enhances morphine dependence in humans. In Advances in Endogenous and Exogenous Opioids, Edited by Takagi, K. and Simon, E. J., p. 472–474, Kodansha Ltd., Tokyo (1981)

18 Merz, H., Stockhaus, K. and Wick, H.: Diastereoisomeric N-tetrahydrofururylnoroxymorphines with opioid agonist-antagonist properties. J. Med. Chem. 20, 844–846 (1977)

19 Merz, H.: Structural features of opioid kappa agonists and antagonists. In Quo Vadis-Analgiesia and Enkephalinases-Symposium on Kappa Receptors and Their Ligands, Edited by Boige grain, R., Cros, J., Morre, M., Muyard, J. P., and Roncucci, R., p. 295–306, Sanofi Recherche, Montpellier (1984)

20 Oka, T., Negishi, K., Suda, M., Sawa, A., Fujino, M. and Wakimasa, M.: Evidence that dynorphin (1–13) acts as an agonist on opioid \( \kappa \) receptors. Eur. J. Pharmacol. 77, 137–141 (1982)

21 Lahti, R. A., Von Voigtlander, P. F. and Barsuhn, C.: Properties of a selective kappa agonist, U-50488H. Life Sci. 31, 2257–2260 (1982)

22 VonVoigtlander, P. F., Lahti, R. A. and Ludens, J. H.: U-50488H: A selective and structurally novel non-mu(kappa) opioid agonist. J. Pharmacol. Exp. Ther. 224, 7–12 (1983)

23 Christie, M. J. and Chesher, G. B.: Physical dependence on physiologically released endogenous opiates. Life Sci. 30, 1173–1177 (1982)

24 Paterson, S. J., Robson, L. E. and Kosterlitz, H. W.: Classification of opioid receptors. Br. Med. Bull. 39, 31–36 (1983)

25 Martin, W. R.: Pharmacology of opioids. Pharmacol. Rev. 35, 283–323 (1984)

26 Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E. and Gilbert, P. E.: The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197, 517–532 (1976)

27 Gilbert, P. E. and Martin, W. R.: The effects of morphine- and nalorphine-like drugs in the non-dependent, morphine-dependent and cyclazocine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 198, 66–82 (1976)

28 Leander, J. D.: Further study of kappa opioids on increased urination. J. Pharmacol. Exp. Ther. 227, 35–41 (1983)

29 Miller, M.: Inhibition of ADH release in the rat by narcotic antagonists. Neuroendocrinology 19, 241–251 (1975)

30 Iversen, L. L., Iversen, S. D. and Bloom, F. E.: Opiate receptors influence vasopressin release from nerve terminals in rat neurohypophysis. Nature 284, 350–351 (1980)

31 Slizgi, G. R. and Ludens, J. H.: Studies on the nature and mechanism of the diuretic activity of the opioid analgesic ethylketocyclazocine. J. Pharmacol. Exp. Ther. 220, 585–601 (1982)

32 Leander, J. D.: A kappa opioid effect: increased urination in the rat J. Pharmacol. Exp. Ther. 224, 89–94 (1983)

33 Leander, J. D. and Zimmerman, D. M.: Picenadol, a mixed opioid agonist-antagonist without kappa opioid agonist activity in the rat urination test. Drug Dev. Res. 3, 483–488 (1983)

34 Hiller, J. M. and Simon, E. J.: \( ^{3}H \) Ethylketocyclazocine binding: lack of evidence for a separate \( \kappa \) receptor in the rat central nervous system. Eur. J. Pharmacol. 60, 389–390 (1979)

35 Hiller, J. M. and Simon, E. J.: Specific, high affinity \( ^{3}H \)-ethylketocyclazocine binding in the rat central nervous system: lack of evidence for \( \kappa \) receptors. J. Pharmacol. Exp. Ther. 214, 516–519 (1980)

36 Chang, K. J., Hazum, E. and Cuatrecasas, P.:...
Novel opiate binding sites selective for benzomorphan drugs. Proc. Natl. Acad. Sci. U.S.A. 78, 4141–4145 (1981)

37 Gillan, M.G.C. and Kosterlitz, H.W.: Spectrum of the μ-, δ- and κ-binding sites in homogenates of rat brain. Br. J. Pharmacol. 77, 461–469 (1982)

38 Khazan, N., Young, G.A. and Calligaro, D.: Self-administration of dynorphin(1–13) and D-Ala²-dynorphin(1–13) (kappa opioid agonists) in morphine (mu opioid agonist)-dependent rats. Life Sci. 33, Supp. I, 559–562 (1983)