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Research Article

ROLE OF VARIOUS OPIOID RECEPTORS IN OPIOID DEPENDENCE AND WITHDRAWAL SYNDROME

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ABSTRACT
Although dependence liability of opioids is widely recognized, the relative involvement of different opioid receptors mediating physical dependence is not completely known. The present study was aimed to explore the role of various types of receptors in opioid dependence and withdrawal. Mice were rendered dependent on opioids such as morphine, pentazocine, buprenorphine and ketamine which have differential agonistic activities on μ, κ, δ, and σ receptors. Withdrawal was precipitated by giving an opioid antagonist, naloxone. The magnitude of withdrawal was evaluated by scoring different withdrawal signs within the next 30 min following naloxone challenge. The results indicated the involvement of both μ and κ opioid receptor in the development of opioid dependence and withdrawal. The results also exclude the possibility of σ receptor involvement in the process. However, with the data obtained in the study, it is not possible to comment on the involvement of δ receptors in opioid withdrawal syndrome.
KEYWORDS: Morphine dependence, Opioid withdrawal, Pentazocine, Buprenorphine, Ketamine

INTRODUCTION

Opioids, the most effective drugs for the management of pain, are associated with the problems of dependence and tolerance limiting its clinical use.[1] If these problems are dissociated somehow, opioid use can again become popular. There is an indication that it is possible to prevent tolerance and psychological dependence by a better understanding of opioid receptors through which the opioid actions are mediated.[2,3]

There are three principal classes of opioid receptors viz. μ (mu), κ (kappa) and δ (delta) receptors.[4] Although up to seventeen opioid receptors have been reported including ε (epsilon),[5] ζ (zeta)[6], λ (lambda) [7] and nociceptin receptor[8]. σ (sigma) receptor, although earlier considered an opioid receptor, is no longer considered to be in this family as it is not activated by endogenous opioids. Several reviews on opioid receptors have been published.[9-17] Many studies provide evidence for the existence of subtypes of these receptors. Development of selective receptor ligands and recent cloning of each receptor have contributed greatly to our increasing knowledge of the pharmacological profile of each opioid receptor type.[2]

In general, opioids acting at μ receptors produce a high degree of dependence, as evidenced by the appearance of severe distressing physical symptoms called the withdrawal or abstinence syndrome. However, the differential involvement of other receptors viz. δ, κ and σ receptors in the development of withdrawal syndrome is not completely known. Therefore, the present study is conducted to explore the role of various opioid receptors in the genesis of opioid dependence and withdrawal syndrome.

MATERIALS & METHODS

Animals

Male albino Swiss mice weighing 20-25 g were housed 5 per cage at room temperature under a standard light/dark cycle with free access to food and water. All the animals were acclimatized to the laboratory conditions for at least 2 days prior to the initiation of any experiment. Each animal was used for only one experiment. The experiments were performed between 9 A.M. and 5 P.M.

Drugs

Since we wanted to investigate the relative importance of different opioid receptors in causation of opioid withdrawal syndrome, various opioid agonists viz. morphine, pentazocine, buprenorphine and ketamine were used which have differential agonistic activity on different subtypes of opioid receptors. Morphine sulphate was procured through the official agencies of government of India. All other drugs were supplied by Aldrich Sigma Co. (Bangalore, India). The drugs were dissolved in normal saline (0.9%).

Experimental procedure

The experiments were conducted after getting permission from the institutional ethics committee of the college. Mice were rendered dependent on opioids by subcutaneous (s.c.) injections of the opioids administered for eight days (Eight day dependence studies).[18-19] Also acute dependence studies were conducted according to the method described elsewhere.[20-21]

Eight day dependence studies

Various opioid agonists such as morphine, pentazocine, buprenorphine and ketamine were injected twice a day for eight days in gradually increasing doses. On the ninth day, withdrawal was precipitated by giving an opioid antagonist, naloxone (10 mg/kg s.c.). The magnitude of withdrawal was evaluated by scoring different withdrawal signs within the next 30 min following naloxone challenge. A positive jumping response, where a mouse jumps more than four times during the observation period,[20] was assigned a score of 4, hyperactivity response a score of 3, diarrhea a score of 2 and urination a score 1.

Acute dependence studies

The mice received a single high dose of the opioids and withdrawal reaction was precipitated 4 hours later through a subcutaneous injection of naloxone (10 mg/kg). The withdrawal signs were observed for the next 30 minutes and scoring of the signs was done as that in the eight day dependence studies.

Statistical analysis

Median scores of withdrawal were calculated for each group of 5 mice in the study. The significance of difference between the withdrawal scores of two groups was calculated by nonparametric statistical analysis by employing Mann-Whitney U test.[22] The difference between values was considered significant at $P < 0.05$.

RESULTS

Eight day dependence studies

Mice were rendered dependent on morphine by gradually increasing doses of 10, 20, 30, 50, 60, 70, 80, and 100 mg s.c. two times a day for eight days. On the ninth day, withdrawal was precipitated by injecting naloxone (10 mg/kg s.c.). Stereotype jumping and hyperactivity was observed in 80% animals, defecation and urination in 90% animals and in 10% no sign of withdrawal was observed. The median score in this group was 10. Pentazocine was also given in doses of 10, 20, 30, 50, 60, 70, 80, and 100 mg/kg s.c. two times a day and the withdrawal was induced on ninth day by naloxone (10 mg/kg s.c.). Stereotype jumping was observed in 40%, hyperactivity in 80%, defecation in 100% and urination in 80% of animals. The median score was 6 ($P > 0.05$ compared to morphine). Similarly, buprenorphine was given in comparable doses of 0.25, 0.5, 1.0, 1.25, 2.25, 2.5, 3.0, and 4.0 mg/kg s.c. two times a day. Withdrawal signs after naloxone (10 mg/kg s.c.) were hyperactivity in 80%, defecation in 100% and urination in 60% of mice. None of the animals exhibited stereotype jumping. The median score was 6 and it was significant ($P < 0.05$) as compared to morphine due to lack of jumping in this group in contrast to pentazocine treated group.

Ketamine was also given in gradually increasing comparable doses i.e. 5, 10, 15, 20, 30, 35, 40, and 50 mg/kg for eight days. Naloxone (10 mg/kg) induced withdrawal signs comprised of hyperactivity and defecation in 60%, urination in 40% and jumping in none of the animals. The median score was 5 ($P < 0.05$ compared to morphine). (Figure 1)

Acute dependence studies

Morphine in a dose of 125 mg/kg followed 4 hours later by naloxone 10 mg/kg was found to induce a full-fledged withdrawal syndrome in 80 to 100% mice. All animals exhibited signs of withdrawal like urination and defecation while 80% animals exhibited hyperactivity and stereotype jumping. The median score was 10.

Pentazocine, when given in a comparable dose of 120 mg/kg s.c. followed 4 hours later by naloxone 10 mg/kg, induced urination and defecation in 100% animals, hyperactivity in 60% animals and stereotype jumping in 20% animals. The median score in this group was 6 ($P > 0.05$ compared to morphine group taken as control).

Buprenorphine in a comparable dose of 2 mg/kg s.c. and later 10 mg/kg s.c. of naloxone could induce urination and defecation only. While none of the mice exhibited hyperactivity and stereotype jumping. Median score was found to be 3 ($P < 0.01$ compared to morphine).

Ketamine given in a dose of 40 mg/kg s.c. (comparable to morphine) followed by naloxone (10 mg/kg s.c.) could induce urination and defecation in 40% animals only. While the rest of the animals did not exhibit any sign of withdrawal. And the median score was 0 ($P < 0.01$ compared to morphine). (Figure 2)

DISCUSSION

Although cloning of the three major types of opioid receptors, μ, κ, and δ, and development of their selective ligands have greatly contributed to our knowledge of the pharmacological profile of each receptor, the differential involvement of these receptors in the opioid dependence and withdrawal is still not very clear. Both μ and δ receptors have been implicated in the development of dependence.[23]

However, since most μ and δ agonists have effects at both receptors and dependence is produced usually with very high doses, it becomes difficult to ascribe definite role to δ receptor because in these doses excessive μ

receptors activation always takes place.[24] As far as κ receptors involvement is concerned, the reports are conflicting.[25] The status of σ receptors involvement is also not clear. As we intended to evaluate the relative importance of different opioid receptors in causation of opioid withdrawal syndrome, various opioid agonists viz. morphine, pentazocine, buprenorphine and ketamine were used which have differential agonistic activities on different subtypes of opioid receptors.

Various workers have used multiple i.p. or s.c. injections of morphine in increasing doses to induce dependence in the rats.[18,19] We in our study produced eight day treatment induced dependence by various opioids in gradually increasing doses and withdrawal was precipitated by naloxone (10 mg/ kg s.c.) on the ninth day. Morphine in a dose starting from 10 mg/kg and then gradually increasing up to 100 mg/ kg was shown to produce a withdrawal syndrome with a median score of 10 on the ninth day when given naloxone challenge. Pentazocine, when given in doses starting from 10 mg/ kg and then increasing up to 100 mg/ kg s.c. produced withdrawal syndrome with a median score 6 ($P > 0.05$). Similarly, buprenorphine when given in gradually increasing comparable doses produced a withdrawal syndrome of median score 6 ($P < 0.05$). Here it has become significant because of the fact that jumping was not observed in this group (in contrast to the pentazocine group). Ketamine also in comparable doses induced a withdrawal of median score 5 ($P < 0.05$). The results indicate the involvement of both μ and κ opioid receptor as pentazocine which is an antagonist of μ opioid receptor and agonist of κ and σ receptors could induce withdrawal syndromes but ketamine which acts as agonist on both σ and phencyclidine receptors could not induce withdrawal signs. The most logical conclusion is that pentazocine induced withdrawal is due to activation of κ opioid receptors. These results also exclude the possibility of involvement of σ opioid receptor in the process. σ receptors are shown to be responsible for psychomimetic effects, dysphoria, and stress-induced depression. They are no longer considered opioid receptors, but rather the target sites for phencyclidine (PCP) and its analogs.[26]

In the second series of experiments, acute dependence was induced by the method of Yano & Takemori and Abdelhamid *et al*.[20,21] We have observed that morphine (125 mg/kg s.c.) followed 4 hours later by naloxone (10 mg/kg s.c.) produced a full-fledged withdrawal syndrome with a median score of 10.

Pentazocine, an agonist at κ and σ receptors and antagonist at μ receptors, was found to induce a withdrawal syndrome of median score 6 ($P > 0.05$). Buprenorphine, a partial agonist at μ receptor, was found to produce withdrawal syndrome of median score 3 ($P < 0.01$). Ketamine, an agonist of σ opioid receptors and phencyclidine receptor, could not produce full-fledged withdrawal symptoms, the median score was 0 ($P < 0.01$). The results of acute dependence studies also suggest that activation of μ and κ receptors appear to play an important role in the development of opioid withdrawal.

Some earlier studies also provide evidence to support substantial role of μ opioid receptor in the development of physical dependence on morphine. The knockout mice with deleted μ opioid receptors display no expression of naloxone-induced withdrawal symptoms including jumping.[27] β-funaltrexamine, a selective μ receptor antagonist, has been shown to inhibit the development of physical dependence on morphine in rats.[28,29]

There have been a couple of studies supporting the involvement of κ opioid receptors in dependence. For example, a κ-antagonist, nor-binaltorphimine, aggravates the morphine withdrawal[30] while a κ-agonist, dynorphin A, has been reported to suppress morphine withdrawal symptoms.[31] We concur with a study indicating that central κ-opioid receptors may play a major role in the development of butorphanol dependence in rats.[32]

However, with the data obtained in the study, it is not possible to comment on the involvement of δ receptors in opioid withdrawal syndrome.

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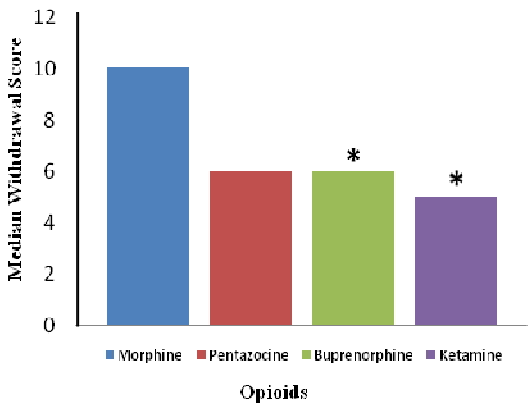

Figure 1: Naloxone precipitated withdrawal following eight day dependence by opioids. * P < 0.05 as compared to morphine.

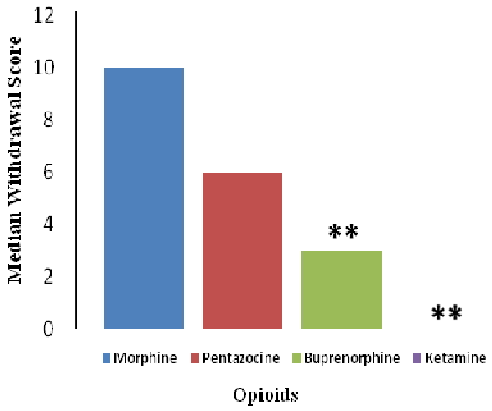

Figure 2: Naloxone precipitated withdrawal following acute dependence by opioids. ** $p < 0.01$ as compared to morphine.

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