EFFECT OF CYPROHEPTADINE ON MORPHINE ANALGESIA, TOLERANCE AND DEPENDENCE

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SUMMARY: The effect of cyproheptadine on morphine induced analgesia, tolerance and dependence was investigated in mice and rats. Analgesia was estimated by hot plate method. Pretreatment of mice with cyproheptadine (10 mg/kg s.c.) did not alter the ED_{50} of morphine analgesia, however, tolerance to the analgesic effect of morphine was reduced by cyproheptadine. Daily subcutaneous injection of morphine was reduced by cyproheptadine. Daily subcutaneous injection of morphine for one week significantly increased the ED_{50} of morphine from 3 (4.1-2.2) on first day of treatment to 6.68 (9.49-4.7) mg/kg on sixth day of treatment. However, daily co-administration of cyproheptadine significantly reduced the ED_{50} of morphine after 6 days of treatment to 5.1 (6.73-3.86) mg/kg. The effect of cyproheptadine on abstinence signs precipitated by naloxone in morphine dependent rats was examined. Dependence was produced by two daily s.c. injection of morphine starting with 2.5 mg/kg and doubling the dose each day up to a maximum of 40 mg/kg. The with drawl signs that were observed and recorded were umpping, teeth chattering, weight loss, wet dog shakes, diarrhea, ptosis, rhinorrhea and urination and wet dog shakes. However, jumping, teeth chattering, rhinorrhea and ptosis were not significantly affected by cyproheptadine. We conclude from these results that 5-HT plays a minor role in analgesia while 5-HT seems to partly contribute to the tolerance to and dependence on morphine.

Key Words: Cyproheptadine, morphine, analgesia, tolerance, dependence.

INTRODUCTION

A variety of experiments, mainly in rats and in mice indicated that monoaminergic pathways participate in the production of opiate analgesia and in the development of tolerance to opiate actions (36-38). The involvement of descending serotonergic pathways in morphine induced antinociception is suggested early by Yaksh and Tyce (38). They observed release of 5-HT in spinal cord after microinjection of morphine into the periaqueductal gray. Bero and Kuhn (3) demonstrated that the serotonin antagonist, cyproheptadine and the neurotoxin, 5,7 dihydroxytryptamine markedly reduced the antinociceptive action of morphine in rats. In addition, the antinociceptive action of morphine is potentiated by 5-HT reuptake inhibitors, cotalopram, clomipramine and amitriptyline (15, 27, 31). The serotonergic system is also strongly implicated in the development of tolerance to apiates (21). Ho and Takemori (10) suggested that the development of tolerance to the antinociceptive action of the Kappa opioid agonist, U-50, 488 H, was mediated by 5-HT system.

It has also been suggested that 5-HT was involved in the development and expression of morphine dependence (14, 22, 26, 28, 35). Administration of an agent that increases 5-HT transmission was found to block jumping of morphine abstinent rats (5, 26). The rate of 5-HT turnover in animals rendered dependent on morphine was found to be increased (1, 9,11,19) found that administration of the 5-HT precursor, tryptophan, accelerates the rate of development of dependence on morphine.

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Several studies claimed that administration of drugs that decreases brain 5-HT levels (i.e. p-chlorophenyl-alanine, atryptophan hydroxylase inhibitor) partially inhibits the development of morphine dependence (11,12, 28). Additionally, Neal and Sparber (22, 23) reported that mianserin and ketanserin were found to block or attenuate most of the signs associated with morphine withdrawal due to their antagonistic properties of 5-HT receptors.

In a previous study it was shown that cyproheptadine antagonized 5-HT receptors in the CNS and the periphery, in addition to its antihistaminic action (3, 30) and in view of the unsettled question about the involvement of 5-HT in morphine analgesia, tolerance and dependence, we decided to examine the effect of cyproheptadine on the above mentioned properties of morphine.

MATERIALS AND METHODS

Animals

Locally bred male Swiss-Webster mice weighing 15 to 20 g were used in experiments measuring antinociception and tolerance to morphine, while Sprague Dawley rats (150-200 g) were used for dependence experiments. All animals were supplied with food pellets (Benghazi Animal Food Factory) and tap water ad libitum and housed in a temperature controlled (22 ± 2°C) environment.

Evaluation of antinociception

The latency of hind paw licking in second (s) when the animal is placed on hot plate surface at 55°C was determined and taken as the nociceptive and point. The cut off time was 30 seconds the percent of maximum analgesia was calculated according to the method of Harris and Puison (8).

$$\text{The percent analgesia} = \frac{T_1 - To}{30 - To} \times 100.$$

When to is latency of control group, T_1 is latency after 30 min of morphine administration. Dose-response curved was plotted on probability paper and the ED_{50} of morphine and 95% confidence limits were estimated. The ED_{50} of morphine was estimated in animals pretreated with vehicle or cyproheptadine (10 mg/kg s.c.).

Assessment of tolerance

The same animals used for testing the antinociceptive action of morphine were injected daily by morphine (dose) and cyproheptadine (dose) for one week. The ED_{50} of morphine was estimated at the third day and sixth day of treatment.

Estimation of dependence

The animals were divided into three groups, the first group was used as a control group. The second and third groups were made dependent by chronic administration of morphine. The second and third groups were pretreated with vehicle and cyproheptadine (10 mg/kg s.c.) respectively, 45 min before precipitation of withdrawal by naloxone injection.

Dependence was induced in rats by daily administration of morphine for 7 days. Rats received two subcutaneous injections daily at 12 hr intervals. The dose of morphine on days one and two was 2.5 mg/kg. This dose of morphine was doubled everyday thereafter to reach a total daily dose of 40 mg/kg on day 6. On day 7, the animals received the last injection of morphine (30 mg/kg) and were tested for withdrawal 4 hr later. The abstinence syndrome was precipitated with an intraperitoneal injection of naloxone HCl 3 mg/kg. Before injection of naloxone, the animals were weighed and placed individually in plastic cages. Immediately after naloxone injection, each rat was observed for 30 minutes. The abstinence signs precipitated by naloxone consisted mainly of diarrhea, rhinorrhea, jumping, urination, paw shakes, wet dog shakes, teeth chattering and ptosis (22, 24). The number of rats exhibiting these withdrawal symptoms was recorded. Animals were weighed 4, 8 and 12 hours after naloxone injection. The weight loss for each animal was calculated as follow:

Weight loss = (weight before naloxone
= weight after naloxone)

RESULTS

Effect of cyproheptadine on the antinociceptive action of morphine

Cyproheptadine alone (10 mg/kg, s.c.) did not alter reaction times in the hot plate test. The latency to hind paw licking 30 min after s.c. administration of 10 mg/kg cyproheptadine was 10.2±1.1 while the latency in control animals (saline treated) was 9.2±0.9. Cyproheptadine (10 mg/kg, s.c.) did not change significantly the antinociceptive action of morphine. The ED_{50} value for morphine analgesia was 3 (4.1-2.2) mg/kg s.c. in saline injected mice, and was 3.2 (4.32-2.4) mg/kg s.c. in the cyproheptadine injected group (Table 1).

Table 1: Effect of cyproheptadine on the ED_{50} value of morphine analgesia in mice at the first, third and sixth day of treatment with morphine alone or morphine plus cyproheptadine.

	ED_{50} and 95% Confidence Limits (mg/kgs.c.)	
Day of Treatment	Morphine	Morphine plus cyproheptadine
First	3 (4.1 - 2.2)	3.2 (4.32 - 2.42)
Third	3.98 (5.97 - 2.65)	4.22 (6.75 - 2.64)
Sixth	6.68 (9.49 - 4.7)*	5.1 (6.73 - 3.8) **

* Significantly different from the ED_{50} value on the first day of treatment (P 0.05).

* * Significantly different from the ED_{50} value on the sixth day of treatment with morphine alone (P 0.05).

Effect of cyproheptadine on the development of tolerance to the antinociceptive action of morphine

Tolerance to the antinociceptive action of morphine was tested on the third and sixth day of treatment with morphine. The ED_{50} value of morphine analgesia increased about two fold by sixth day of morphine treatment compared to its value on the first day. Pretreatment with cyproheptadine partially blocked the development of tolerance to morphine analgesia. After 6 days of morphine treatment in both groups of animals, the ED_{50} value of morphine analgesia in cyproheptadine treated animals was 5.1 (6.73-3.86) mg/kg which is significantly less than the ED_{50} for the group given morphine alone (Table 1).

Effect of cyproheptadine on the withdrawal signs of morphine

The effects of naloxone (3 mg/kg i.p.) on rats chronically dependent on morphine are shown in Table 2. These precipitated withdrawal symptoms include urination, diarrhea, wet dog shakes, jumping, teeth chattering and ptosis. The frequencies of occurrence of most abstinence signs were high in morphine dependent rats (Table 2). However, jumping and rhinorrhea were much less frequent in these animals. Although the animals of the first group (morphine-free) exhibited teeth chattering, the frequency of occurrence was low. The differences between the proportion of animals exhibiting teeth chattering (and other abstinence signs) in group one (Control) and in group two (morphine dependent) were highly significant (p<0.01).

As shown in Table 2, pretreatment with cyproheptadine significantly reduced the number of animals showing withdrawal signs. Cyproheptadine significantly decreased the number of animals exhibiting wet-dog shakes diarrhea and urination. However, cyproheptadine failed to significantly alter the proportion of animals showing jumping, rhinorrhea, ptosis and teeth chattering.

Table 3: Mean±SE (gm) of weight loss in morphine dependent and nondependent rats after naloxone injection.

Group	4 HR	8 HR	12 HR
I	0.5 ± 0.05	0.5 ± 0.1	1.5 ± 0.1
II	7.0 ± 0.8	9.0 ± 0.9	15 ± 1.3
III	2.5 ± 0.3*	2.9 ± 0.4*	30 ± 0.3*

* Highly significantly different from group II (p 0.01).
I=Morphine-free rats
II=Morphine-dependent rats
III=Morphine-dependent rats pretreated with cyproheptadine.

The maximal loss of weight of animals in group two (morphine dependent) was 15±1.3 g (mean±SE) which was recorded 12 hr after naloxone injection. Severe diarrhea and urination might contributed to this acute loss of weight. However, the weights of animals in group one were not significantly changed after naloxone injection. Cyproheptadine significantly reduced the degree of precipitated weight loss (Table 3).

DISCUSSION

The result of this study demonstrated that cyproheptadine has no significant effect on the antinociceptive action of morphine nor on the response latency of control mice to hot plate. Contrary to our results, Bero and Kuhn (3) found that cyproheptadine (10 mg/kg) blocked the antinociceptive action of morphine in rats and did not affect the tail flick latency in normal rats. In our study, however,

Table 2: The number of animals exhibiting the following withdrawal signs during a 30 minutes period of observation following naloxone injection.

Group	N	Jumping	Diarrhea	Urination	Wet dog shakes	Teeth Chattering	Rhinorrhea	Ptosis
I	20	-	3	2	2	5	1	2
II	20	7	15	18	18	14	9	12
III	20	8	6*	4*	8*	11	7	10

* Significantly different from group II (P 0.05)
N=number of rats used in each group.
Group I - morphine-free rats
Group II - morphine-dependent rats.
Group III - morphine-dependent rats pretreated with cyproheptadine.

the tolerance to the antinociceptive action of morphine was reduced with repeated administration of cyproheptadine (10 mg/kg). The discrepancy between our results and those of Bero and Kuhn (3) about the effect of a single dose of cyproheptadine may be due to differences in animal species used (mice vs rats). The involvement of descending serotonergio pathways in morphine induced antinociception is supported by several reports. Wigdor and Wilcox (36) demonstrated that methysergide antagonized the antinociceptive action of morphine and failed by itself to significantly affect the tail flick response of rats to noxious heat. The antagonism of the antinociceptive action of other opioids by 5-HT antagonists was also reported. Methysergide, mianserin and ketanserin produced similar antagonistic effects to the antinociceptive action of the Kappa opioid agonist, U-50, 488 H (10, 23, 32, 33).

The results of this study may be important in understanding morphine tolerance. It proposes that serotonergic pathway plays an important part in the mechanism of development of tolerance to morphine analgesia. Consistent with this, it was reported that in tolerance, changes may occur in descending monoaminergic pathways rather than in opioid systems themselves (25). Opposite effects of the 5-HT1 and 5-HT2 receptor antagonists on the development of tolerance to Kappa opioid receptor agonist, U-50, 488 H has been demonstrated (10). Morever, Wigdor and Wiloox (36) have suggested that the noradrenergic pathway plays a role in development of tolerance to morphine.

The present study demonstrated that cyproheptadine blocked the loss of animal weight and attenuated some other signs of morphine withdrawal in morphine dependent rats. However, the administration of cyproheptadine did not significantly affect the frequencies of occurrence of jumping, rhinorrhea, ptosis and teeth chattering. Recent studies have shown that serotonergic systems appear to play a role in induction of withdrawal signs in opiate dependent animals and humans. Serotonin was first suggested to be involved in the development and expression of morphine dependence in 1963 (14, 35). A number of investigators reported that clonidine, inhibits the activity of central serotonergic neurons and the release of acetylcholine (6,18, 29) and thus it inhibits opiate-withdrawal signs. Also, it has been reported that clonidine could be acting as anti-withdrawal agent through inhibition of serotonergic neurotransmission by interaction with alpha2-adrenoceptors located on 5-HT neurons (7,20).

On the other hand, 5-hydroxytryptophan, a precursor of 5-HT, was found to have some beneficial effects on the abstinence syndrome of morphine and heroin addicts (2). Furthermore, Ramandini *et al.* (26) and Cervo, *et al.* (5) have shown that drugs which increase 5-HT transmission as fenfluramine or m-chlorophenylpiperazine inhibit naloxone-precipitated jumping in morphine dependent rats with little or no effect on other signs such as ptosis and diarrhea. They also reported that clonidine has the reverse action, it inhibits wet dog shakes and diarrhea while it has no effect on jumping. Recently, Berthold, *et al.* (4) have demonstrated that 5-HT receptor agonists attenuate the naloxone-induced jumping behavior in morphine-dependent mice. The finding that serotonin agonists could inhibit only the withdrawal jumping in opiate dependent animals may result from different population of 5-HT receptors with opposing interacting actions (16).

Our results demonstrate that cyproheptadine produces similar effects to clonidine. It inhibited diarrhea, urination and wet dog shakes and did not affect jumping of rats. Thus, our results corroborate the finding that serotonin antagonists inhibit some but not all signs of opiate withdrawal in morphine dependent rats. It thus seems reasonable to hypothesize that the development of tolerance to opiates and the expression of opiate withdrawal signs may be partially dependent on certain serotonergic mechanisms.

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