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Effect of Adenosine Receptor Agonists and Antagonists on the Expression of Opiate Withdrawal in Rats

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SALEM, A. AND W. HOPE. *Effect of adenosine receptor agonists and antagonists on the expression of opiate withdrawal in rats.* PHRMACOL BIOCHEM BEHAV **57**(4) 671–679, 1997.—The effects of the selective A_1 adenosine receptor agonist N^6-cyclopentyladenosine (CPA) and the selective A_{2a} agonist 2-[p-(2-carboxethyl)phenylethyl-ethylamino]-5′-ethylcarboxamidoadenosine (CGS 21680) (each at 0.03, 0.1 and 0.3 mg/kg, SC) as well as the selective A_1 adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), non-selective antagonists 3-isobutyl-1-methylxanthine (IBMX), aminophylline, 3,7-dimethyl-1-propargyl-xanthine (DMPX) and 8(p-sulfophenyl)-theophylline (8-SPT) were investigated (each at 5, 10 and 30 mg/kg, SC) for their ability to alter the naloxone-precipitated opiate withdrawal syndrome in morphine-dependent rats. Effects of CPA and CGS 21680 on opiate withdrawal in the presence of aminophylline were also investigated. Both CPA and CGS 21680, caused a significant reduction in the incidence of body shakes, teeth chatter and paw shakes and decreased the amount of faecal matter produced. DPCPX, IBMX, DMPX, 8-SPT and aminophylline significantly increased the incidence of jumps and decreased the amount of faecal matter produced. The incidence of body shakes was significantly increased by DMPX, 8-SPT and IBMX. Neither CPA nor CGS 21680 were able to reverse the significant increase in the incidence of jumps caused by aminophylline. These data suggest that there is a role for endogenous adenosine in the modulation of the opiate abstinence syndrome and both A_1 and A_{2a} adenosine receptors are involved in this phenomenon. © 1997 Elsevier Science Inc.

Adenosine receptor agonists Adenosine receptor antagonists Morphine dependence
Naloxone-precipitated withdrawal

SINCE the initial observation that theophylline reduced the analgesic effect of morphine in mice (27) evidence in support for a role of adenosine in the acute actions of opioids has been accumulating (46). This evidence can be seen to follow several different lines. Firstly, both adenosine agonists and morphine when administered to laboratory animals have similar effects, including sedation and antinociceptive activity (1,2,19,29, 41,48). As well, drugs with antagonist activity at adenosine receptors have been shown to block many of the effects of morphine, including its antinociceptive effects (16,33,52). The antinociceptive effects of adenosine analogues, however, are not blocked by the opioid antagonist naloxone (1,16), suggesting that adenosine receptor activation is the final step by which actions of morphine are produced. Lastly, morphine-induced antinociception is potentiated by adenosine analogues and inhibitors of adenosine uptake (1,17).

On the basis of this and similar findings, it has been suggested that the effects of morphine are mediated at least partially indirectly via the release of adenosine (16,50,51,62). In order to provide direct evidence in confirmation of such an hypothesis, it is necessary to demonstrate that release of adenosine from (presumably) intracellular sites occurs in response to the administration of morphine. Whilst such experiments have been carried out in brain slices of different brain regions (32,49) the most clear-cut and convincing data implicating adenosine in the effects of morphine has been obtained in the spinal cord, where a significant increases in the mean basal adenosine levels in response to morphine given intra-

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cerebroventricular in a dose known to produce an antinociceptive response have been demonstrated (51,53).

Given that the acute effects of morphine are mediated at least in part by release of endogenous adenosine, it is to be expected that changes in this interaction would also be observed following chronic morphine treatment. Thus the effects of drugs which act via adenosine receptors could be altered during opiate dependence and any physiological process which is mediated by adenosine could also be modified. For example, it has been shown that extracellular levels of adenosine within the central nervous system are in the micromolar range (25,61,63) and an inhibitory "tone" maintained by adenosine has been postulated (30). Whether such a "tone" is still present during opiate dependence and any potential involvement of adenosine as an endogenous regulator of the opiate abstinence syndrome is not known. There is, however, evidence to support a role for adenosine during withdrawal based on the observation that nonselective adenosine receptor agonists and inhibitors of adenosine uptake depress the opiate withdrawal syndrome in mice (1,57) and rats (18,43). Whilst there are several reports in the literature which describe the effects of adenosine antagonists, usually caffeine or theophylline, on the development of opiate tolerance and dependence, the data presented is conflicting. Matsuda (42), found that simultaneous administration of caffeine and morphine inhibited the development of opiate tolerance in rats but not in monkeys, whilst Ho et al. (28), reported that tolerance and dependence were potentiated when theophylline was administered to mice 2h prior to implantation of morphine pellets then at a daily dose of 100 mg/kg over 3 days. Administration of caffeine, theophylline or IBMX just prior to naloxone challenge in morphine-dependent rats was reported to exacerbate the opiate abstinence syndrome (10,11,21,43) whilst Brailowsky et al. (7) showed that administration of theophylline over four days with morphine pellet implantation on day two of treatment decreased the development of dependence in mice.

The conflicting results in these studies can possibly be explained in terms of the different treatment schedules which were used; in fact it appears that the development of dependence was reduced when theophylline or caffeine were given either with or prior to induction of dependence whilst treatment of animals which were already morphine-dependent with methylxanthines resulted in exacerbation of the abstinence syndrome. Furthermore, it was not possible to determine the adenosine receptor subtype involved as none of the drugs available at that time showed any marked receptor selectivity. Adenosine receptors were originally classified into two subtypes based on changes in cAMP levels. A_1 receptors were linked to inhibition of adenylate cyclase and subsequent decreases in cAMP content whilst A_{2a} receptors elevated cAMP via stimulation of adenylate cyclase (39). More recently, subtypes of both adenosine A_1 and adenosine A_2 receptors have been identified and a third class of receptor, the adenosine A_3 receptor, postulated (12,15,23,24). At this stage, receptor identification is carried out largely on the basis of agonist affinities as there are very few antagonists with the required degree of selectivity available.

The aim of the present study was to assess more accurately the effects of adenosine receptor agonist and antagonists, particularly the contribution of subtype of adenosine receptors, on the expression of the opiate withdrawal syndrome. The compounds chosen were the A_1 selective adenosine receptor agonist N^6-cyclopentyladenosine (CPA), the A_{2a} selective adenosine receptor agonist 2-[p-(2-carboxethyl)phenylethylethylamino]-5′-ethylcarboxamido- adenosine (CGS 21680), the A_1 selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and the non-selective antagonists, 3-isobutyl-1-methylxanthine (IBMX), aminophylline, 3,7-dimethyl-1-propargylxanthine (DMPX) and 8(p-sulfophenyl)-theophylline (8-SPT).

METHODS

Animals

Experiments were carried out in female Hooded Wistar rats (250–300 g). Animals were randomly divided into 26 equal groups. They were housed individually in North Kent Plastics breeding cages with sawdust bedding. Standard laboratory chow and tap water was available ad lib. The room in which animals were housed was maintained at 18–20°C on a 12L:12D cycle.

Induction of Dependence

Morphine base was formulated into an emulsion (saline: liquid paraffin: arlacel A, 8:6:1). Animals were injected SC in the scruff of the neck with a total of 250 mg/kg morphine in a volume of 10 ml/kg. Half the dose was administered on the morning of the first day and the remainder on the morning of the second day. Withdrawal was induced 24h after the last dose of emulsion.

Assessment of Withdrawal Behaviour

At the end of the 48 h treatment period rats were weighed, injected IP with 3 mg/kg naloxone and placed into clear perspex observation boxes (20 × 20 × 30 cm) lined with preweighed paper towelling to allow collection of faecal matter. The frequency of jumps, body shakes, teeth chatter and paw shakes was recorded over a 20 min observation period immediately following naloxone challenge. Data were expressed as the mean frequency of each sign (± SEM) for each animal over the 20 min observation period. Assessment was carried out on two rats simultaneously. The presence or absence of other signs (eg. ptosis, chromodacryorrhea, abnormal posture, writhing and irritability to touch) were also noted for each animal. For some experimental groups, data were also calculated as the number of animals per group displaying each specific sign (quantal assessment). At the end of the observation period rats were removed from the observation boxes, reweighed and weight loss during withdrawal calculated. Paper towelling was also weighed and faecal output over the 20 min calculated.

Effects of Adenosine Receptor Agonists on Withdrawal

In this experimental group, rats were injected SC with 0.03, 0.1 or 0.3 mg/kg CPA or CGS 21680 10 min prior to naloxone challenge. Only one dose of one drug was used for each animal. Control experiments were carried using the vehicle in which the adenosine agonists were administered. Withdrawal was then assessed as described previously.

Effects of Adenosine Receptor Antagonists on Withdrawal Behaviour

In these experiments the effects of a series of methylxanthines (aminophylline, DMPX, IBMX, 8-SPT or DPCPX) on the expression of opiate withdrawal were determined. The methylxanthines (5, 10 or 30 mg/kg) were administered SC into the scruff of the neck 1h before challenge with naloxone. Withdrawal was then assessed as described previously. Only

one drug at one dose was used in each rat. Vehicle controls were carried out in a separate group of morphine-dependent animals.

Effects of CPA or CGS 21680 on Withdrawal in the Presence of Aminophylline

Following the induction of morphine dependence, three groups of rats were given 30 mg/kg aminophylline dissolved in saline via SC injection. After 50 min, one group of aminophylline- treated rats was given (SC) 0.3 mg/kg CPA, another was given 0.3 mg/kg CGS 21680 and the third group was given vehicle. Naloxone was administered (IP) 10 min later and assessment of withdrawal behaviour was carried out as previously described.

Statistics

The effects of each dose of treatment drugs on behavioural signs of withdrawal were compared to vehicle controls using a Mann-Whitney U test. The number of animals per group displaying specific withdrawal signs were compared to vehicle controls using Chi-square analysis. Dunnett's t test was used to assess the effects of each dose of treatment drug on faecal output. $p < 0.05$ was used as an indicator of significant difference.

Drugs

DMPX, 8-SPT, DPCPX, IBMX, CPA, CGS 21680 (Research Biochemicals Inc.), aminophylline, naloxone, arlacel A (Sigma, USA), morphine hydrochloride (Macfarlane Smith), light liquid paraffin oil (BDH). Aminophylline was dissolved in 0.9 % w/v NaCl to the required concentrations. DMPX, IBMX, 8-SPT and DPCPX were mixed to the required concentrations with 20% w/v gum acacia in saline (0.9 % w/v NaCl). CPA and CGS 21680 were dissolved in 1:1 ethanol:saline to give 1 mg/ml solution and then diluted to the required concentration in saline. All drugs were administered at 0.1 ml per 100g body weight. Control rats were treated with the appropriate vehicle.

RESULTS

Effects of Adenosine Receptor Agonists on Withdrawal

The adenosine A_1-selective agonist CPA significantly reduced the frequencies of body shakes, teeth chatter and paw shakes at the two highest doses used (0.1 and 0.3 mg/kg). Neither the A_1-selective agonist CPA nor the A_{2a}-selective agonist CGS 21680 had any significant effect on the frequency of jumping. CGS 21680 significantly reduced the frequencies of paw shakes at the two highest doses used (0.1 and 0.3 mg/kg) and body shakes at 0.3 mg/kg. Teeth chatter was displayed at a significantly lower frequency in animals treated with 0.03 and 0.3 mg/kg CGS 21680, whilst no significant effect was observed at 0.1 mg/kg. These data are summarised in Figures 1 and 2.

Other withdrawal signs that were noted included ptosis, chromodacryorrhea, abnormal posture, and writhing. Further consideration of the quantal responses suggests that there could be a dose-related incidence of withdrawal behaviour. Thus, of those analysed as graded responses, there was a negative relationship between the number of animals within a group displaying a given withdrawal response (eg. body shakes, teeth chatter and paw shakes) and the total dose of CPA or CGS 21680 to which the animals were exposed. It is of interest that some signs which were not seen in all animals treated with the vehicle (eg. chromodacryorrhea and abnormal posture) were seen in a larger proportion of those animals treated with CPA (0.1 and 0.3 mg/kg) or CGS 21680 (0.3 mg/kg).

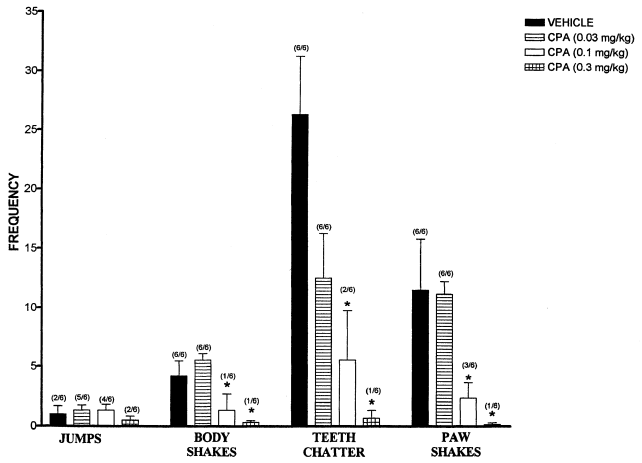

FIG. 1. Effect of CPA (0.03, 0.1 or 0.3 mg/kg, SC) 10 min prior to challenge with 3 mg/kg naloxone on the frequency of jumps, body shakes, paw shakes and teeth chatter in morphine-dependent animals. Each column represents the mean frequency (± SEM, n = 6) of each sign per rat and the number of animals within a group displaying the named withdrawal signs are shown in parenthesis. Asterisks indicate that the frequency of the behavioural sign observed in CPA pretreated animals was significantly different ($p <$ 0.05, n = 6) from that observed in morphine-dependent animals treated with the vehicle.

Effects of Adenosine Receptor Agonists on Amount of Faecal Matter Deposited Following Naloxone Challenge

During the 20 min observation period, the mean amount of faecal matter produced by the vehicle-treated morphine-

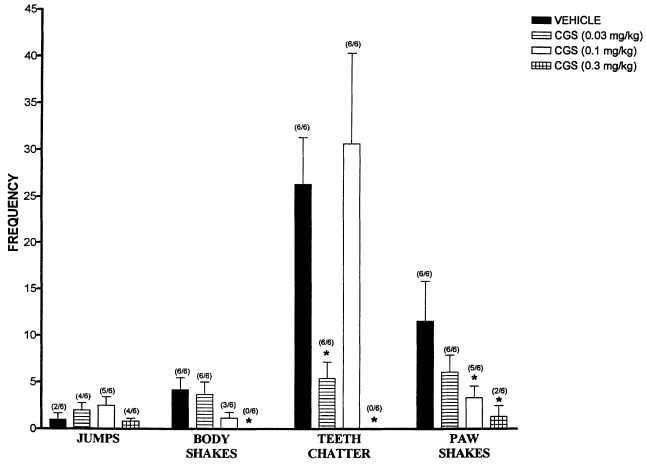

FIG. 2. Effect of CGS 21680 (0.03, 0.1 or 0.3 mg/kg, SC) 10 min prior to challenge with 3 mg/kg naloxone on the frequency of jumps, body shakes, paw shakes and teeth chatter in morphine-dependent animals. Each column represents the mean frequency (± SEM, n = 6) of each sign per rat and the number of animals within a group displaying the named withdrawal signs are shown in parenthesis. Asterisks indicate that the frequency of the behavioural sign observed in CGS 21680 pretreated animals was significantly different ($p <$ 0.05, n = 6) from that observed in morphine-dependent animals treated with the vehicle.

dependent rats was 4.265 ± 0.825 (g; ± SEM, $n = 6$). The quantity of faecal matter produced during withdrawal was significantly reduced by 0.1 and 0.3 mg/kg CPA (Figure 3). CGS 21680, however, had no significant effect on the quantity of faecal matter produced.

Effects of Adenosine Receptor Antagonists on Withdrawal Behaviour

A significant increase in the incidence of jumping was observed in rats treated with IBMX (10 and 30 mg/kg), aminophylline (30 mg/kg), DMPX (10 and 30 mg/kg), 8-SPT (10 mg/kg) and DPCPX (30 mg/kg). IBMX- and aminophylline-treated animals showed a positive relationship between the number of animals within a group displaying jumps and the total dose of the methylxanthine to which the animals were exposed. A significant increase in the incidence of body shakes was observed with IBMX (5 mg/kg), DMPX (30 mg/kg) and 8-SPT (5 and 10 mg/kg) (Figures 4a and 4b). None of the methylxanthines tested produced statistically significant increases in the incidence of paw shakes or teeth chattering. Aminophylline (30 mg/kg), however, significantly reduced the incidence of teeth chattering.

Animals treated with methylxanthines were observed to exhibit a characteristic jumping which resembled that referred to by Blasig et al. (5) as flying. This was described as vigorous jumps out of the observation box without stopping on the edge of the box or aimless jumps from the edge of the box. The number of animals per group showing the incidence of flying increased with the high doses (10 and 30 mg/kg) of aminophylline and DPCPX. A similar relationship was also observed in the number of animals exhibiting chromodacryorrhea and the dose of aminophylline, IBMX and DPCPX used.

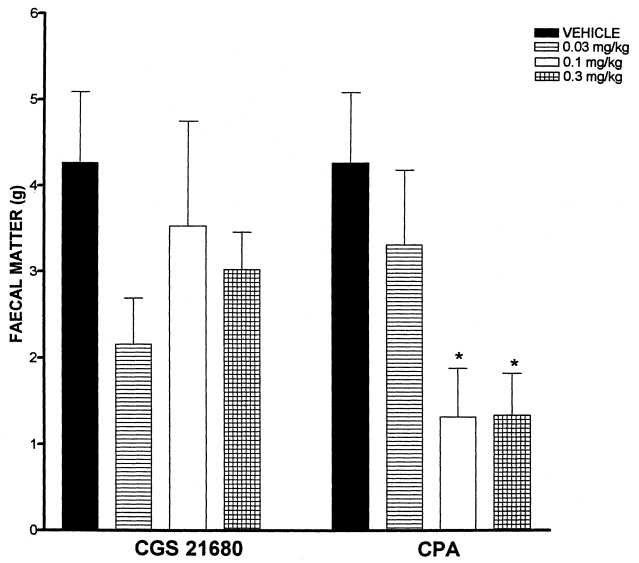

FIG. 3. Effects of CPA and CGS 21680 (0.03, 0.1 or 0.3 mg/kg, SC) 10 min prior to challenge with 3 mg/kg naloxone on the mean faecal matter (g; ± SEM, $n = 6$) deposited by morphine-dependent animals in the 20 min observation periods following challenge with naloxone. Asterisks indicate that the mean faecal matter deposited by CPA or CGS 21680 pretreated animals was significantly different ($p < 0.05$, $n = 6$) from that of the morphine-dependent animals pretreated with the vehicle.

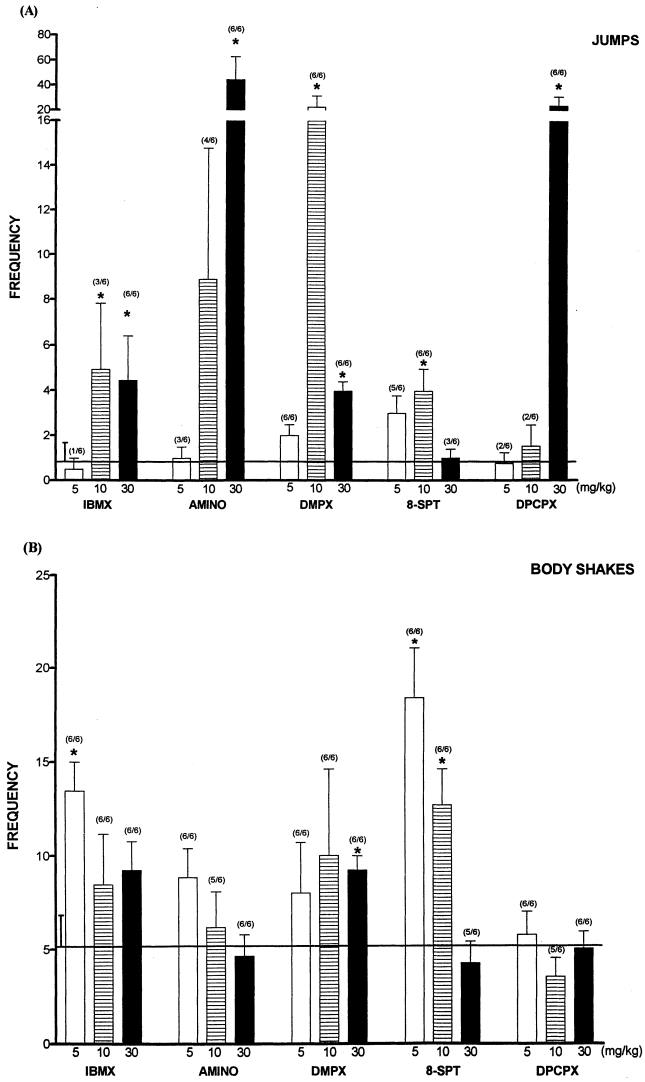

FIG. 4a and b. Effect of pretreatment with IBMX, aminophylline (AMINO), DMPX, 8-SPT and DPCPX (5, 10 or 30 mg/kg, SC), 60 min prior to challenge with 3 mg/kg naloxone on the frequency of jumps and body shakes in morphine-dependent animals. Each column represents the mean frequency (± SEM, $n = 6$) of each sign per rat and the number of animals within a group displaying the named withdrawal signs are shown in parenthesis. The horizontal line shows the mean frequency (± SEM, $n = 6$) of the named behaviour observed per rat in morphine-dependent animals injected SC with the vehicle and challenged with naloxone. Asterisks indicate that the frequency of the behavioural sign observed in methylxanthine-pretreated animals was significantly different from that observed in morphine-dependent animals treated with the vehicle ($p < 0.05$, $n = 6$).

Effects of Adenosine Receptor Antagonists on Amount of Faecal Matter Deposited Following Naloxone Challenge

During the 20 min observation period, the mean amount of faecal matter produced by the vehicle-treated morphine-dependent rats was 5.69 ± 0.30 (g; ± SEM, $n = 6$). Prior treatment with IBMX (10 and 30 mg/kg), aminophylline (5 and 10 mg/kg), DMPX (10 mg/kg), 8-SPT (5,10 and 30 mg/kg) and DPCPX (10 mg/kg) caused a significant reduction in the amount of faecal matter produced (Figure 5).

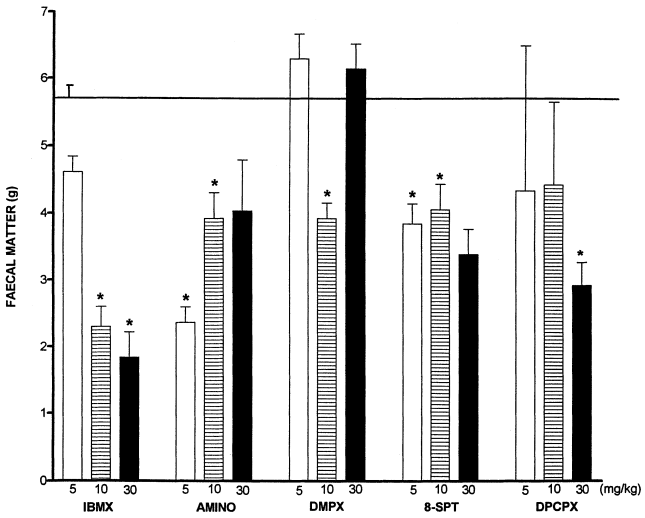

FIG. 5. Effect of pretreatment with IBMX, aminophylline (AMINO), DMPX, 8-SPT and DPCPX (5, 10 or 30 mg/kg, SC), 60 min prior to challenge with 3 mg/kg naloxone on the mean faecal matter (g; ± SEM, $n = 6$) deposited by morphine-dependent animals in the 20 min observation periods following challenge with naloxone. The horizontal line shows the mean faecal matter deposited by morphine-dependent animals injected SC with the vehicle (g; ± SEM, $n = 6$). Asterisks indicate that the mean faecal matter deposited by methylxanthine-pretreated animals was significantly different from that of the morphine-dependent animals treated with the vehicle ($p < 0.05, n = 6$).

Effects of CPA and CGS 21680 on Withdrawal in the Presence of Aminophylline

In rats treated with aminophylline (30 mg/kg) followed by 0.3 mg/kg CPA or CGS 21680, the incidence of jumping was still significantly higher than in vehicle-treated rats. The number of animals showing flying behaviour, however, was significantly reduced by both agonists. Aminophylline did not alter the reduction in body shakes caused by both agonists nor was there any change in the effects of CGS 21680 on paw shakes. Aminophylline, however, was able to prevent the reduction in teeth chatter caused by both agonists (Figure 6).

Although the quantity of faecal matter produced by rats treated with aminophylline and 0.3 mg/kg CGS 21680 was similar to that produced by rats treated with aminophylline alone, 0.3 mg/kg CPA caused a significant reduction in the faecal output during the withdrawal period (Figure 7).

DISCUSSION

The results described here have shown that compounds which have affinity for adenosine receptors produce changes in the frequency of some of the behavioural signs of opiate withdrawal and in the quantity of faecal matter produced. In general, whilst both A_1 and A_{2a} selective adenosine receptor agonists decreased the incidence of most withdrawal behaviours, non-selective adenosine receptor antagonists tended to increase the incidence of some behaviours but the effects were not as clear-cut. Jumping, a behaviour never observed in opiate-naive rats and only seen at a low frequency in vehicle treated morphine-dependent rats following naloxone challenge, was not changed by either the A_1 nor the A_{2a} selective agonist which probably indicates that neither A_1 nor A_{2a} adenosine receptors are involved in the expression of this sign.

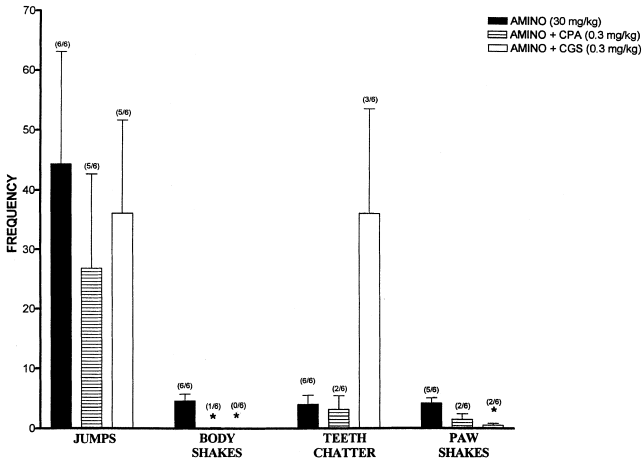

FIG. 6. Effects of CPA (0.3 mg/kg, SC) and CGS 21680 (0.3 mg/kg, SC) on withdrawal in the presence of aminophylline (30 mg/kg) on the frequency of jumps, body shakes, paw shakes and teeth chatter in morphine-dependent animals. Each column represents the mean frequency (± SEM, $n = 6$) of each sign per rat and the number of animals within a group displaying the named withdrawal signs are shown in parenthesis. Asterisks indicate that the frequency of the behavioural sign observed in adenosine analogue and aminophylline pretreated animals was significantly different from that observed in morphine-dependent animals treated with vehicle and aminophylline ($p < 0.05, n = 6$).

This is in contrast with previous work carried out in our laboratory (18), which found that the selective A_1 adenosine receptor agonist N^6-[(R)-1-methyl-2-phenylethyl] adenosine (R-PIA) and the non-selective agonist 5′-N-ethylcarboxamidoadenosine both reduced the frequency of withdrawal jumping at the highest dose used (0.3 mg/kg). With this dose, how-

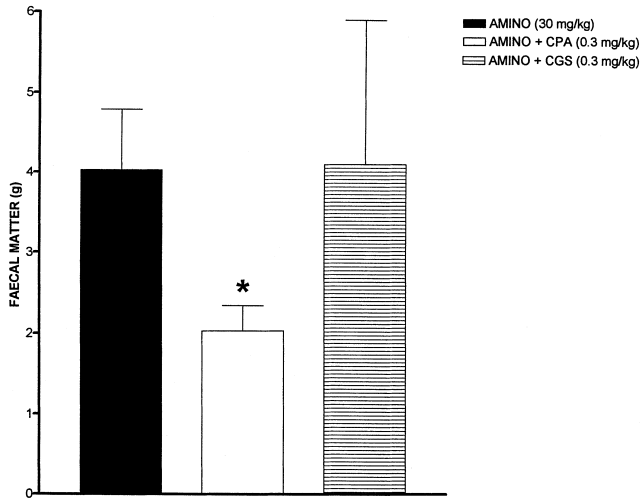

FIG. 7. Effects of CPA (0.3 mg/kg, SC) and CGS 21680 (0.3 mg/kg, SC) on withdrawal in the presence of aminophylline (30 mg/kg) on the mean faecal matter deposited (g; ± SEM, $n = 6$) in opiate-dependent animals. Asterisks indicate that the mean faecal matter deposited by morphine-dependent animals pretreated with vehicle and aminophylline was significantly different ($p < 0.05, n = 6$) from that of the morphine-dependent animals pretreated with the adenosine receptor agonist and aminophylline.

ever, both of these compounds were also associated with a large decrease in motor-activity and muscle tone. Other workers also found that high doses of adenosine receptor agonists given parenterally produce a decrease in locomotor activity (19) and muscle relaxation (3). It is therefore likely that the reduction in jumping which we observed in earlier experiments was due to these actions of the drugs rather than via a direct effect on adenosine receptors. A similar mechanism has also been suggested by Kaplan and Sears (35) who found that lower doses of adenosine agonists R-PIA and CGS 21680 did not affect the frequency of withdrawal jumps in morphine-dependent mice.

Whilst all four non-selective adenosine receptor antagonists had no significant effect on the frequency of jumping at doses of 5 mg/kg, this behaviour was significantly increased by IBMX, DMPX and 8-SPT at doses of 10 mg/kg and, with the exception of 8-SPT, 30 mg/kg. In addition, all animals in each group of six displayed this sign whilst only two of the six morphine-dependent rats treated with the vehicle did so. The selective adenosine A_1 receptor antagonist DPCPX was inactive at doses of 5 and 10 mg/kg, with the incidence of jumping being at control levels in both treatment groups and only at 30 mg/kg was a significant increase seen. An unexpected observation was the ability of 8-SPT to significantly increase withdrawal jumps in morphine-dependent animals. 8-SPT is a polar analogue of theophylline that is reported to have a poor ability to cross the blood-brain barrier (13). It was not detected in rat brain following IP administration at doses as high as 50 mg/kg but antagonized the cardiovascular actions of peripherally administered adenosine analogues when given at less than half this dose (20). It has been reported to lack any locomotor activity in mice (36) and a similar lack of locomotor effect was noted in our experiments. The fact that in our experiments 8-SPT increased jumping, a centrally mediated sign of opiate withdrawal (6,59), is difficult to explain. It should be noted, however, that the increase produced was not as profound as that of the other adenosine receptor antagonists.

An increase in the frequency of jumping following administration of caffeine, theophylline or IBMX to morphine-dependent rats has been noted by other workers (10,11,21,43) who interpreted these data to indicate that the opiate abstinence syndrome was exacerbated by these compounds. It is arguable, however, that increased frequency of only one sign of opiate withdrawal indicates an exacerbation of the entire withdrawal syndrome and there is no explanation given for the particular emphasis placed on this sign in any of these studies. Also, the doses of the methylxanthines used were high, which makes it more likely that effects other than adenosine receptor blockade would be present. In fact, based on the data presented in this paper, it could be argued that adenosine receptors are not involved in modulating withdrawal jumping. This is indicated by both the lack of effect of either adenosine receptor agonist and their inability to reduce the increase in jumping caused by aminophylline. At the doses used, neither CPA nor CGS 21680 showed behavioural depression or muscle relaxing activity but behavioural activation was noted in rats treated with non-selective methylxanthines, an effect which has been well established by other workers (47,48,55). It is of interest to note that the dose-response effect on locomotor activity of DMPX in opiate-naive mice (47) has great similarity to the dose-response effect on withdrawal jumps of DMPX observed in our experiments. In both studies, DMPX produced a biphasic increase in locomotor activity and withdrawal jumps, the lowest and highest doses (5 and 30 mg/kg) producing minimum increases whilst the intermediate dose (10 mg/kg) produced a maximal effect. Although the mechanisms of action of this biphasic increase remains to be determined, it appears that the ability of DMPX to stimulate locomotor activity correlates with its ability to increase withdrawal jumps. Thus the effects of the adenosine antagonists on jumping could therefore be due to their behavioural stimulant properties rather than any direct effect on the withdrawal syndrome. It has been noted that morphine-dependent rats treated with amphetamine just prior to naloxone challenge frequently show increased jumping (Hope, W. unpublished data) which suggests that a relationship between frequency of withdrawal jumping and degree of alertness is valid in this species.

One behaviour which was noted in association with a high levels of jumping was flying, which consisted of vigorous leaps from the rim of observation box. This sign was observed only in animals treated with methylxanthines. Whilst flying was only noted as being present or absent for each animal, both CPA and CGS 21680 significantly reduced the number of animals showing this sign following pretreament with aminophylline (30 mg/kg). Thus, although it is unlikely that adenosine receptors are involved in expression of jumping, there is a possibility that flying is a behaviour which is distinct from jumping and that adenosine receptor agonists and antagonists are able to alter the frequency of this sign.

Body shakes, another sign of opiate withdrawal which is not seen in opiate-naive rats, was significantly decreased by the two adenosine receptor agonists and significantly increased by the non-selective adenosine receptor antagonists IBMX, DMPX and 8-SPT. The fact that 8-SPT, a peripherally acting adenosine receptor antagonist (13), was the only compound which significantly increased body shakes at 5 and 10 mg/kg is indicative that this sign could be peripherally mediated as well as centrally (58). DMPX, which shows a small degree of selectivity for adenosine A_2 receptors in binding studies (9) and is 10-fold more potent as an A_2 antagonist in its behavioural effects (47) also increased the frequency of this behavioural sign but DPCPX which is approximately 700-fold selective for the A_1 receptor subtype (37, 40), was inactive at any dose used. The lack of effect of DPCPX is interesting given that the A_1 adenosine receptor agonist CPA (38) effectively suppressed body shakes, and perhaps rules out A_1 receptor involvement. Since the A_{2a} adenosine receptor agonist CGS 21680 (12) and DMPX decreased and increased this behaviour respectively, it is possible that these effects are mediated through adenosine A_2 receptors. Whilst it would be interesting to test the effects of an antagonist with a higher degree of selectivity for the A_2 receptors such as 8-(3-Chlorostyryl) caffeine (31), this was not possible as only very recently has such a compound become commercially available. Further experiments will be carried out when this drug is available in our laboratory. Aminophylline pretreament did not alter the inhibitory effects of either agonist on the incidence of body shakes. Since only the highest dose of the agonists was tested, it is possible that this lack of effect was due to the fact that the agonists were able to overcome the blockade of receptors by aminophylline.

Teeth chatter was significantly reduced by 0.1 and 0.3 mg/kg CPA. The effects of CGS 21680, however, were more difficult to interpret. Whilst at 0.03 mg/kg a significant reduction was observed and 0.3 mg/kg abolished this sign, at 0.1 mg/kg there was no significant difference from control values, with neither the frequency nor the number of rats showing the behaviour being changed. It is difficult to explain why this lack of effect of CGS 21680 at 0.1 mg/kg should occur. Based on data from these experiments, however, it is not possible to de-

termine the precise involvement of adenosine receptors in expression of this behavioural sign. Teeth chatter and paw shakes were not increased by the adenosine receptor antagonists. In fact, the frequency of teeth chatter was significantly decreased by 30 mg/kg aminophylline. This dose of aminophylline was, however, associated with the greatest increase in frequency of jumping. Animals which displayed a high incidence of jumping tended to carry out this behaviour to the exclusion of all other behavioural signs of withdrawal; in some instances rats jumped as many as fifty times over the 20 min. observation period. Since teeth chatter and paw shakes tended to occur during times of relative inactivity, it is not surprising that the frequency of these signs were not increased or that in the one dose of aminophylline in which a decrease was noted in the frequency of teeth chatter, a very high number of jumps were recorded. It is also possible that the lack of any significant effect on body shakes of any of the tested doses of aminophylline can be explained at least in part by the large increase in the frequency of jumping caused by this methylxanthine.

The finding that the quantity of faecal matter produced during the 20 min observation period was significantly reduced by the two highest doses of CPA but not altered by CGS 21680 indicates that the receptors on the rat gastrointestinal tract which are involved in this effect are of adenosine A_1 subtype. Since other workers have observed that adensoine receptors in the gastrointestinal tract are of the A_1 subtype (26) and that endogenous adenosine inhibits defecation via A_1 receptors (56), the decrease in faecal output caused by the A_1 agonist CPA is not surprising. In addition, the observation that CGS 21680 was without effect on this parameter makes it appear likely that selectivity of the two agonists for A_1 and A_{2a} adenosine receptors is maintained at the doses used in these experiments. The fact that the quantity of faecal matter produced was significantly reduced by all the methylxanthines tested is difficult to explain in terms of effect on adenosine receptors. Since the behavioural signs of opiate withdrawal are, however, due to predominantly central mechanisms whilst withdrawal diarrhoea is mediated via a direct effect on gastrointestinal motility and fluid secretion (4,8) changes at the level of the central nervous system may not necessarily result in changes to this largely peripheral component of the abstinence syndrome. It should also be noted that Blasig et al. (5) demonstrated that withdrawal behaviours were interrelated in such a way that as the incidence of jumping increased the production of faecal matter decreased. This finding could well account for the methylxanthine-induced decrease in faecal matter described in our results.

One of the factors which could affect the interpretation of results in these experiments is the change in adenosine receptors which has been noted to occur in association with morphine dependence. Work carried out in our laboratory has shown that cardiovascular responses to adenosine A_1 receptor agonists are decreased and the ability of DPCPX to block the hypotensive response to adenosine is reduced whilst the sensitivity of the cardivascular system to adenosine A_2 receptor agonists is increased in morphine-dependent rats (60). Whilst these changes in A_1 receptors appear to be occurring to a large extent in the nucleus of the Tractus Solitarius, the A_2 receptors mediating the cardiovascular effects of adenosine are located peripherally (White, P. J., personal communication) and it is not possible from these experiments to determine whether centrally located adenosine A_2 receptors are also altered. This has been investigated by Kaplan et al. (34) who used receptor binding studies to investigate central adenosine receptors. These workers found that whilst A_{2a} sites in striatal homogenates from morphine-dependent mice were not changed, cortical A_1 receptor sites were up-regulated and suggested that endogenous adenosine could modulate the opiate abstinence syndrome. Tao and Liu (54), however, found that rats made dependent to morphine were less sensitive to the antinociceptive effects of the A_1 agonist CPA and that spinal A_1 receptors were down-regulated whilst cortical A_1 receptors were not changed. In terms of our results, a down-regulation of A_1 receptors (possibly coupled with an increased response to adenosine A_{2a} agonists) could explain why there was very little difference in the ability of CPA and CGS 21680 to inhibit the expression of the opiate abstinence syndrome and also the lack of effect of DPCPX except at a very high dose.

Given that both selective adenosine receptor agonists and nonselective adenosine receptor antagonists altered the frequencies of some of the signs of opiate abstinence it can be suggested, in agreement with Kaplan et al. (34), that endogenous adenosine acts as a modulator of the expression of withdrawal behaviour and possibly both A_1 and A_{2a} adenosine receptors are involved in this phenomenon. It is therefore possible that adensoine agonists may have a role in the treatment of the opiate abstinence syndrome. Whilst the fact that adenosine is an important modulator of neuronal activity suggests that such changes in adenosine receptors may also have important functional consequences for the central nervous system functions, the way in which the receptor population is altered and the physiological consequences of these changes is not yet known and further experiments will be required for their elucidation.

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