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Effect of Adenosine Analogues on the Expression of Opiate Withdrawal in Rats

TIM DIONYSSOPOULOS, WENDY HOPE AND IAN M. COUPAR[1]

Unit of Addictive Drug Research, School of Pharmacology, Victorian College of Pharmacy, Parkville 3052, Australia

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DIONYSSOPOULOS, T., W. HOPE AND I. M. COUPAR. *Effect of adenosine analogues on the expression of opiate withdrawal in rats.* PHARMACOL BIOCHEM BEHAV **42**(2) 201-206, 1992.—The aim of this study was to test whether convergent dependence occurs in vivo. The adenosine A_1 receptor agonist N^6-[(*R*)-1-methyl-2-phenylethyl]adenosine (R-PIA), the A_2 agonist 2-(phenylamino)adenosine (CV-1808), the nonselective A_1, A_2 agonist (adenosine-5′-ethylcarboxamide (NECA), and the α_2-adrenoceptor agonist clonidine were screened (each at 30, 100, and 300 μg/kg, SC) for their ability to alter naloxine-precipitated withdrawal signs in morphine-dependent rats. The results indicate that there is convergent dependence involving opioid and adenosine A_1 receptors on those effects expressed by withdrawal diarrhoea, paw-shakes, teeth-chattering, body-shakes, and jumping. Further, dependence expressed by body-shakes involves convergence involving A_1 receptors, as well as α_2-adrenoceptors; while A_1 receptors are involved in dependence expressed by jumping, stimulation of α_2-adrenoceptors augments this sign. Adenosine analogues may be of clinical value for detoxification of opiate addicts.

Adenosine agonists Opiate dependence Opiate withdrawal

THE mechanisms underlying opiate tolerance and dependence are highly complex and still remain poorly understood. Of the systems affected, the most studied is adenylate cyclase of opiate-sensitive neuroblastoma cells in which the opiate receptors are negatively coupled to the enzyme (3,30,43). These cells adapt to continued exposure with opiates by increasing the activity of adenylate cyclase. Consequently, overproduction of cyclic 3′5′ adenosine monophosphate (cyclic 3′,5′AMP) occurs on withdrawal of the opiate. The same effect has been reported to occur in normal brain cells and it has been suggested that the ability of adenylate cyclase to hypertrophy is the underlying mechanism explaining tolerance to and dependence upon opiates (2,19,30,31,39).

There is also good evidence that adenosine is involved in regulating adenylate cyclase activity. For instance, methyl xanthines such as theophylline and caffeine when administered to naive rats produce a series of behavioural disturbances that resemble those of opiate withdrawal (17,24). This "quasimorphine abstinence syndrome" (QMAS) may be due to stimulation of adenylate cyclase since cyclic 3′5′ AMP itself increases the response (16). Further, the effect of methyl xanthines occurs at doses that block adenosine receptors (21,40,50). The taxonomy of these receptors is partly based on their coupling to adenylate cyclase. In general, adenosine receptors that inhibit the enzyme are ascribed A_1 and those stimulating it are the A_2 type [see (38) for review]. Hence, the QMAS can be explained on the basis that methylxanthines block endogenous adenosine at A_1 receptors, resulting in elevated levels of cyclic 3′5′ AMP.

In addition, α_2-adrenoceptors are also negatively linked to adenylate cyclase (11,25) and occupation by the α_2-adrenoceptor agonist clonidine reduces some of the opiate withdrawal signs in rats (47,48) and opiate addicts.

Collier and Tucker (20) used the term "convergent dependence" to describe dependence where more than one receptor appears to control adenylate cyclase. This phenomenon is seen in the isolated guinea pig ileum, which becomes dependent on normorphine, clonidine, and adenosine (14,15,20).

Consequently, the aim of the following study was to determine whether there is also a functional association between opiate and adenosine receptors in vivo. If so, then adenosine agonists should modify the signs of morphine withdrawal. In particular, A_1 receptor agonists should substitute for morphine and hence be of potential value in the treatment of opiate addiction. We assessed the effects of the A_1 agonist N^6-[(*R*)-1-methyl-2-phenylethyl]adenosine (R-PIA), the mixed A_1 and A_2 agonist adenosine-5′-ethylcarboxamide (NECA), and the A_2 selective agonist 2-(phenylamino)adenosine (CV-

[1] Requests for reprints should be addressed to Ian M. Coupar, Unit of Addictive Drug Research, School of Pharmacology, Victorian College of Pharmacy, 381 Royal Parade, Parkville 3052, Australia.

1808) (9) in comparison to the α_2-adrenoceptor agonist clonidine on various withdrawal signs.

METHOD

Animals

Ninety-eight male and female Hooded Wistar rats (230–300 g) were randomly divided into 14 equal groups. They were individually housed in North Kent Plastics Breeding Cages with sawdust bedding. Each animal was provided with tap-water ad lib and food in the form of Clark King ARM cubes. The room in which animals were housed was maintained at 18–20°C on a 12 L : 12 D cycle.

Induction of Dependence

Morphine base was formulated into an emulsion (saline : liquid paraffin : arlacel 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 48 h after administering the first dose of morphine.

Withdrawal

Each morphine-treated animal was injected SC with either the test drug (30, 100, or 300 μg/kg) or the appropriate vehicle. Treatments were coded and randomized to eliminate observer bias. Abrupt withdrawal was then induced 20 min later by administering naloxone (10 mg/kg, IP).

Diarrhoea

Each rat was transferred to a Perspex observation box (20 × 20 cm width, 30 cm height) immediately following naloxone injection. The floor of each box was lined with pre-weighed paper towelling to allow collection of wet and dry faecal matter, which was weighed 20 min after administering naloxone. Results are expressed as the weight in g of faecal material defecated per 100 g body weight in 20 min.

Behaviour

Animals were also observed for 20 min during the collection of faeces. Two Perspex boxes were used, which allowed the observer to score the behaviours of two animals simultaneously. The quantified signs of withdrawal were jumping (all feet off the floor), body-shakes (wet-dog shakes), paw-shakes, and teeth-chattering. The incidence of these behaviours was measured for each animal and results expressed as increases or decreases compared to the incidence in the appropriate control group (100%).

Locomotor Activity

Separate groups of morphine-naive rats were injected SC with 300 μg/kg test drug or the appropriate vehicle as control. After 20 min, animals were placed individually into a BRS/LVE light beam activity meter for a further 20 min. The activity score of each animal was recorded and results expressed as the percentage reduction in activity compared to the relevant vehicle-treated group.

Drugs

Drugs used were clonidine (Boehringer Ingelheim), morphine (Macfarlane Smith), naloxone (Sigma), R-PIA, NECA and CV-1808 (Research Biochemicals Inc.). All drugs except CV-1808 were dissolved in distilled water to give a stock concentration of 1 mg/ml. Further dilutions were made using 0.9% w/v saline to give the required doses, which were administered at 0.1 ml per 100 g body weight. CV-1808 was dissolved in 1 : 1 ethanol : saline solution to give 1 mg/ml, which was further diluted in saline to give the required doses.

Statistics

The effects of individual doses of the treatment drugs on the amount of faecal matter was compared to controls using Dunnett's *t*-test. The effect of treatments on behaviours were compared to controls using the Mann–Whitney *U*-test.

RESULTS

Diarrhoea

R-PIA and NECA caused a dose-related reduction in the total amount of faecal matter. The inhibitory effects of these drugs became statistically significant at 100 and 300 μg/kg. CV-1808 also reduced faecal output, but the effect was not dose related since the reduction was statistically significant at 100 but not 300 μg/kg.

Clonidine did not have any significant effect on the total amount of faeces produced at any of the doses used. There was, however, a significant reduction in the quantity of dry faeces produced when animals were treated prior to withdrawal with 100 and 300 μg/kg clonidine (control = 1 ± 0.1, 100 μg/kg clonidine = 0.3 ± 0.6, 300 μg/kg clonidine = 0.5 ± 0.2 g/100 g, $n = 7$ each group, $p < 0.05$ compared to control). The above results are shown in Fig. 1.

Behaviours

R-PIA produced dose-related reductions in the incidence of all noted behavioural withdrawal signs. It was most effective against paw-shakes, body-shakes, and teeth-chattering, where significant reductions were achieved following 100 μg/kg. The incidence of jumping was not significantly affected by 100 μg/kg although 300 μg/kg abolished this sign. None of the behaviours were significantly affected by 30 μg/kg.

NECA was more potent than R-PIA at inhibiting paw-shakes, body-shakes, and teeth-chattering. This is shown, for instance, by significant reductions in paw-shakes and teeth-chattering by 30 μg/kg and abolition of body-shakes by 100 μg/kg NECA. The profile of activity for NECA against jumps was similar to R-PIA.

CV-1808 did not effect the behaviours except at 30 μg/kg, which produced a statistically significant reduction in the incidence of teeth-chattering.

Clonidine had no significant effect on the incidence of paw-shakes or teeth-chattering but it caused a significant reduction in the number of body-shakes at 300 μg/kg. Interestingly, this dose (300 μg/kg) caused a striking increase in the incidence of jumping (Fig. 2).

Locomotor Activity

All drugs (300 μg/kg each) caused a large reduction in locomotor activity compared to vehicle-treated animals. The values were R-PIA 85% ($n = 4$), NECA 97% ($n = 4$), CV-1808 52% ($n = 3$), and clonidine 82% ($n = 4$).

DISCUSSION

The intensity of opiate withdrawal signs reflects the degree of dependence. This is in turn affected by the dose of opiate

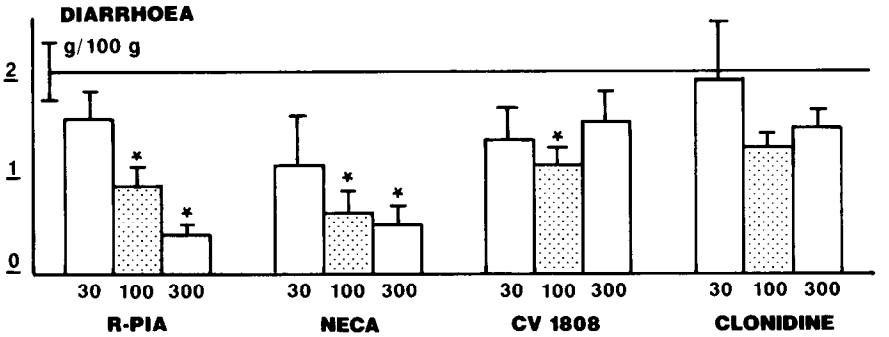

FIG. 1. Effect of adenosine agonists and clonidine on naloxone-precipitated withdrawal diarrhoea. Values are of the total weight of faeces produced by the different groups of animals adjusted to amount per 100 g body weight. The upper continuous line represents the amount of faecal material produced by the morphine/naloxone-treated control group (saline instead of R-PIA, NECA, and clonidine). The control value of diarrhoea for CV-1808 (vehicle 1:1 saline:ethanol, SC) was 2.3 ± 0.4 g/100 g. This value has been set at the saline control value and the values for the three different doses of CV-1808 have been adjusted accordingly for clarity and ease of comparison. Columns show the effect of increasing doses (low, medium, high) of the test drugs while bars are the SEM. Asterisks indicate that the mean is significantly different to the control mean ($p < 0.05$, $n = 7$ all groups, Dunnett's t-test). The middle and high doses of clonidine caused a significant reduction in the amount of dry faeces (not shown; see the Results Section).

administered, the time for which treatment continues, and the antagonist used to precipitate withdrawal. Signs have been classified into dominant, which are observed when high total doses of opiate are used to induce dependence, and recessive, which are expressed more strongly on withdrawal from low total doses (6). The method used in the experiments described here has the advantage that both dominant (jumping, teeth-chattering) and recessive signs (diarrhoea, body-shakes, paw-shakes) are observed following naloxone administration. This indicates that the dependence induced in our animals was moderate.

The two adenosine agonists with activity at adenosine A_1 receptors were remarkably effective at reducing the incidence of opiate withdrawal behaviours. The A_1-selective agonist R-PIA significantly reduced the incidence of both paw-shakes and teeth-chattering while the nonselective agonist NECA was even more potent in this respect. Both withdrawal signs are influenced by a cholinergic pathway, paw-shakes being inhibited and teeth-chattering being exacerbated by muscarinic agonists (18). Body-shakes, a sign mediated by a CNS tryptaminergic pathway that involves 5-hydroxytryptamine-2 (5-HT_2) receptors (51) was abolished by both the adenosine agonists and again NECA was more effective than R-PIA. However, R-PIA and NECA were equiactive at inhibiting jumping.

On the basis of these results, it seems reasonable to conclude that adenosine A_1 receptors mediate the antiwithdrawal behaviours. The difficulty with this assumption lies in the relative potencies of R-PIA and NECA. Classification of adenosine receptors is based on the rank order of agonist potency (12,41), with NECA > R-PIA, as occurred for the behaviours, generally indicating the presence of an adenosine A_2 receptor population (38). However, the physicochemical properties of these agonists are quite different, R-PIA being lipophilic and thus likely to accumulate in cells and tissues while NECA is hydrophilic and so more likely to be confined to the receptor environment of the extracellular fluid. Consequently, Phillis et al. (36) stress that caution should be exercised when comparing the potencies of the two drugs in vivo. Indeed, the negative results obtained with the selective adenosine A_2 agonist CV-1808 indicate that A_2 receptor stimulation is not associated with reduction of withdrawal behaviours. The one inhibitory effect of CV-1808 on teeth-chattering is probably biologically insignificant since it occurred at the low dose only. As with behavioural signs of opiate withdrawal, the adenosine analogues with A_1 agonist activity were also effective antidiarrhoeals, but it is interesting to note that R-PIA was as potent as NECA. CV-1808 did cause a small decrease in total faecal matter but its biological significance is uncertain because the effect was not dose dependent. Again, it is reasonable to assume that the antidiarrhoeal action of the adenosine agonists is exerted via adenosine A_1 receptors.

The diarrhoea associated with the morphine withdrawal syndrome is a consequence of both increased intestinal motility (8) and decreased ability of the intestinal mucosa to absorb fluid (4,13). Although some authors suggest that the effect is largely peripheral (5), there is in fact good evidence that changes in mucosal function during morphine withdrawal are partly initiated in the CNS (13,49).

It is well documented that clonidine inhibits morphine withdrawal diarrhoea in rats (10,34,42,47) and in opiate addicts (26). It is used clinically either alone or in combination with narcotic antagonists to suppress withdrawal symptoms in addicts undergoing opiate detoxification [see (28) for review] with some authors reporting striking success rates (7). However, in our experiments we were unable to demonstrate effective inhibition of either total diarrhoea or behavioural signs of withdrawal with the exception of body-shakes. Clonidine (100 and 300 μg/kg) reduced the amount of dry faecal matter, which suggests that it reduces motility but not intestinal fluid secretion. This limited effect is surprising since α_2-adrenoceptors in the gut mediate inhibition of both motility and intestinal secretion (22).

The inability of clonidine to reduce teeth-chattering has been previously reported (10). In addition, the present results show that clonidine does not alter the incidence of paw-shakes. We did observe a decreased incidence of body-shakes, which is in agreement with the finding of other workers (23,44,48). Clonidine actually augmented jumping, which can

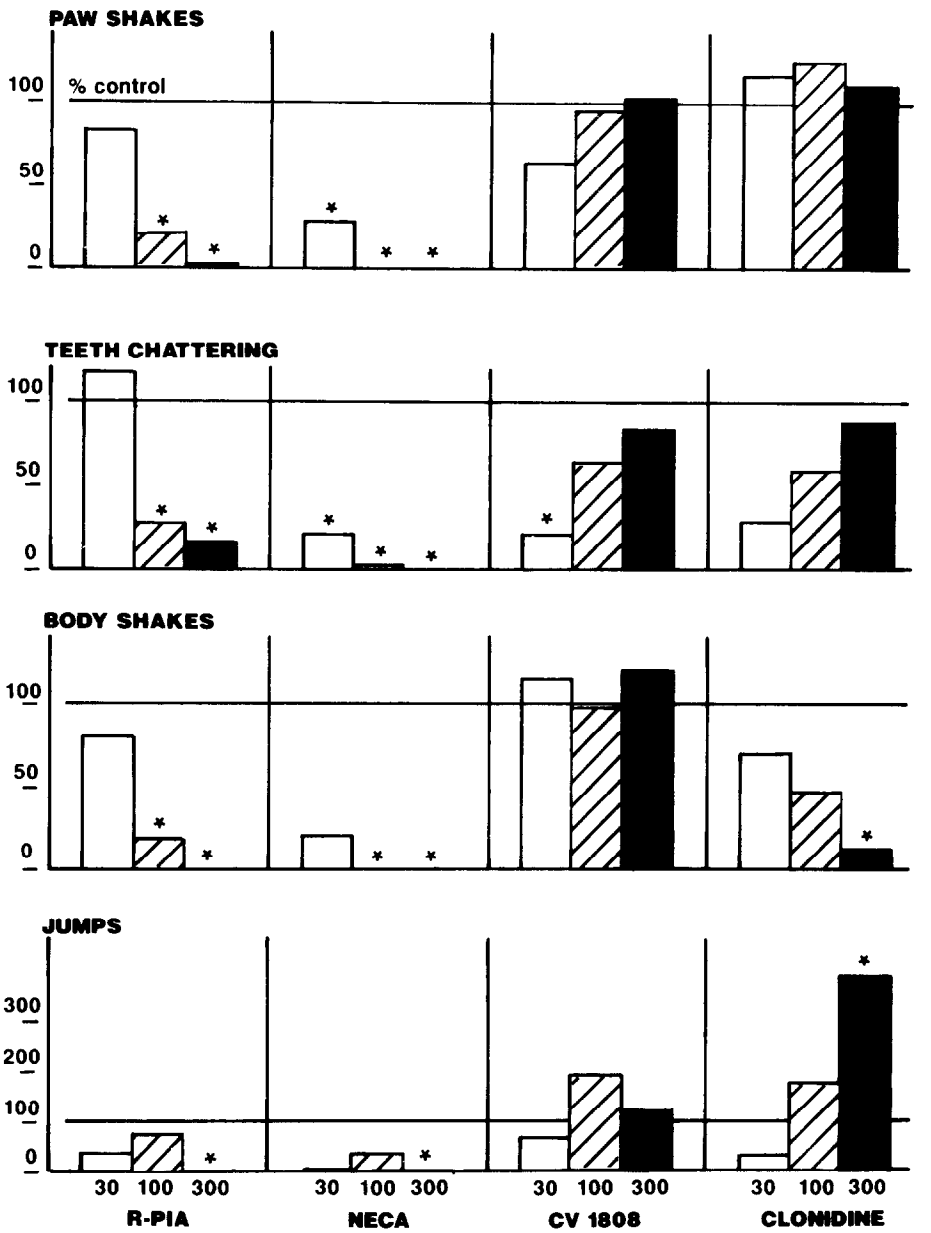

FIG. 2. Effect of adenosine agonists and clonidine on naloxone-precipitated morphine withdrawal behaviours. Rats were treated as in Fig. 1. The columns show the effect of drugs on the incidence of behaviours adjusted to percentage of the incidence of behaviors occurring in the vehicle-treated group. Actual control incidence of behaviours per 20 min were paw-shakes = 16.6, teeth-chattering = 19.7, body-shakes = 7.9, and jumps = 3.7 (n = 7). The control values for CV-1808 (vehicle 1:1 saline:ethanol) were paw-shakes = 10.7, teeth-chattering = 49.7, body-shakes = 4, and jumps = 1.6 (n = 7). Asterisks indicate that the mean is significantly different from its appropriate control value ($p < 0.05$, n = 6–7 all test groups, control group, n = 7–12, Mann-Whitney U-test).

be considered escape behaviour, and again this effect has been previously described. The same effect is seen with a number of selective α_2-adrenoceptor agonists so it has been suggested that clonidine potentiates jumping by stimulating α_2-adrenoceptors (47).

Interactions between morphine and adenosine have been demonstrated in a number of different systems (1,4,32,33,35) and it has been previously reported that adenosine analogues inhibit opiate withdrawal symptoms in morphine-dependent mice (45). Collier's hypothesis of convergent dependence (20) states that adenylate cyclase activity is enhanced during chronic exposure to opiates and that drugs that act via adenylate cyclase should alter opiate withdrawal. The results described here provide support for the involvement of the adenylate cyclase/cyclic 3′,5′AMP system in the signs of opiate withdrawal.

Adenosine A_1 analogues are negatively coupled to adenylate cyclase in many different organ systems including brain (46) and gut (29), and it can be argued that R-PIA and NECA produced their behavioural effects by lowering levels of cyclic 3′,5′AMP that are reported to be elevated during opiate withdrawal (16). Since adenosine A_2 receptors are positively coupled to adenylate cyclase and thus stimulate cyclic 3′,5′AMP accumulation (46), it was expected that the A_2 selective adenosine analogue CV-1808 (9) would exacerbate opiate withdrawal. The lack of effect of CV-1808 in our experiments may, however, be explained in terms of receptor distribution. Opiate receptors are widely distributed throughout the brain (27), whereas A_2 adenosine receptors have a discrete location, being confined to the striatum and nucleus accumbens (37). It is also possible that during morphine withdrawal levels of cyclic 3′,5′AMP are already maximally elevated and further stimulation by A_2 agonists is thus ineffective.

Although it has been suggested that α_2-adrenoceptors are negatively coupled to adenylate cyclase in adipocytes (11), the lack of effect of clonidine on paw-shakes and teeth-chattering and the increased incidence of jumping seen in the experiments described here provide a quite different spectrum of activity than that of the adenosine analogues. It is unlikely therefore that there is any involvement of cAMP in the effects of clonidine on withdrawal behaviour except perhaps body-shakes.

Both NECA and R-PIA are potent depressants of locomotor activity and it could be argued that these drugs alleviate opiate withdrawal behaviour by nonselective sedation rather than specific activity at adenosine A_1 receptors. This is unlikely, however, as both clonidine and CV-1808, which were much less effective at inhibiting withdrawal signs, also caused marked depression of locomotor activity.

In conclusion, although it is unlikely that the entire spectrum of the opiate withdrawal syndrome can be explained in terms of a supersensitive adenylate cyclase some of the signs do appear to be associated with this system. Consequently, there is a possible clinical application of adenosine A_1 receptor analogues in the treatment of opiate withdrawal in addicts undergoing detoxification.

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