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Effects of adenosine agonists on consumptive behaviour and body temperature

Ian M. Coupar and Binh L. T. Tran

Abstract

This study was designed to determine the effects of the A_1-receptor selective agonist N^6-cyclopentyladenosine (CPA), and the A_2-selective agonist, 2-*p*-(2-carboxyethyl)-phenethyl-amino-5′-*N*-ethylcarboxamidoadenosine-hydrochloride (CGS-21680) on consumptive behaviour and body temperature in rats in relation to the non-selective A_1/A_2 adenosine agonist, *N*-ethylcarboxamidoadenosine (NECA), and to morphine. It was shown that two subcutaneous injections of 0.1 and 0.3 mg kg^{-1} CPA caused a similar decrease in food consumption to NECA (2×0.03 mg kg^{-1}) and morphine (2×10 mg kg^{-1}). However, two doses of 0.03 mg kg^{-1} CPA and 0.1 and 0.3 mg kg^{-1} CGS-21680 enhanced feeding. These effects were not directly correlated to faecal output at all doses of the selective agonists, as NECA and morphine induced constipation. The doses of CPA and 0.1 and 0.3 mg kg^{-1} of CGS-21680 enhanced water consumption, as did NECA, but not morphine. The stimulation of drinking by CPA was not absolutely associated with diuresis. Instead, urine output was reduced by 0.03 and 0.1 mg kg^{-1} and increased by 0.3 mg kg^{-1}. CGS-21680 at 0.1 and 0.3 mg kg^{-1} and NECA also induced diuresis, which was opposite to the effect of morphine. CPA and CGS-21680 both caused significant dose-dependent decreases in body temperature after the two-injection treatment, but their effects were significantly less after 36 h when four doses had been administered. The study indicates that highly selective A_1 and A_{2A} adenosine agonists might have the ability to interfere with consumptive behaviour, induce constipation, affect renal function and to lower body temperature.

Introduction

Adenosine produces an array of effects in the body by acting at A_1, A_{2A}, A_{2B} and A_3 adenosine receptors. A number of metabolically stable adenosine analogues are now available and some, such as the non-selective agonist NECA (*N*-ethyl-carboxamidoadenosine), have actions remarkably similar to opiates. They also have unrelated actions such as lowering blood pressure. Consequently, the development of subclass receptor-selective agonists is seen to hold the promise of developing a variety of new treatments for clinical conditions. Some suggested applications are pain control and infectious diarrhoea and patents have been lodged for adenosine compounds claimed to have antipsychotic, antihypertensive and diuretic applications (See Prous 1996). Several studies have shown that adenosine and its analogues induce hypothermia in experimental animals (Mehta & Kulkarni 1983; Ticho & Radulovacki 1991; Anderson et al 1994), but the mechanism of this action is not yet clear. In other systems it is known that tolerance occurs to adenosine and its stable analogues when studied in-vivo and in-vitro (Abbracchio et al 1992; Casati et al 1994; Aley et al 1995).

Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia

Ian M. Coupar, Binh L. T. Tran

Correspondence: I. M. Coupar, Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, 381, Royal Parade, Parkville, Victoria 3052, Australia. E-mail: ian.coupar@vcp.monash.edu.au

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The purpose of this study was to investigate the effects of the A_1-receptor selective agonist, N^6-cyclopentyladenosine (CPA), and the A_2-selective agonist, 2-*p*-(2-carboxyethyl)-phenethylamino-5′-*N*-ethyl carboxamidoadenosine-hydrochloride (CGS-21680) (Bruns et al 1986; Jarvis et al 1989) on consumptive behaviour in rats in relation to the non-selective A_1/A_2 adenosine agonist, NECA, and to morphine. Additionally, experiments were designed to determine whether tolerance occurs to the hypothermic action of CPA and CGS-21680 (Bruns et al 1986; Jarvis et al 1989).

Materials and Methods

Drugs

The drugs used were N^6-cyclopentyladenosine (CPA), 2-*p*-(2-carboxyethyl)-phenethylamino-5′-*N*-ethylcarboxamidoadenosine-hydrochloride (CGS-21680), 5′-*N*-ethylcarboxamidoadenosine (NECA) (Research Biochemicals Inc., Natick USA) and morphine hydrochloride (GlaxoWellcome, Melbourne, Australia). CPA, NECA and morphine were dissolved in normal saline. CGS-21680 was dissolved in dimethyl sulfoxide (DMSO) and diluted to required concentrations in saline, with a resulting solution containing less than 2% v/v DMSO.

The dose of morphine was selected on the basis of numerous previous studies showing it to induce dependence within 48 h. The treatment period for the adenosine agonists was controlled to 48 h also, because of the evidence that adenosine receptors are involved in mediating opiate effects. The doses of the adenosine agonists were chosen again on the basis of literature reports that the doses were shown to be pharmacologically effective in our pilot experiments.

Animals

All experiments were carried out on equal populations of male and female Hooded Wistar rats (optimal weight ranges were 230–400 g for males and 200–300 g for females). Each rat was placed individually in a separate metabolism cage (200 mm diameter, 175 mm height, standing on 25-mm length leg stands) with attached Urimax funnels designed to collect urine and faeces into separate containers. Rats were kept in the laboratory under a 12-h light–dark cycle in a temperature-controlled environment (21 ± 2°C).

Food and water consumption, faecal and urine excretion

At 0900 h (designated as time = 0 h), cage-familiarised rats were placed into their individual original metabolism cages, which were set up with pre-weighed amounts of food, water, urine and faecal containers. Twenty-four hours later (time = 24 h), the amount of food and water remaining, as well as urinary and faecal excretion of each rat was measured. Each rat was then administered a treatment, by subcutaneous (s.c.) injection. A second dose of the test drug was given 12 h later (time = 36 h). The dose referred to in the text is the amount of each of the two single doses. Twelve hours after the last dose (time = 48 h), the amounts of food and water remaining, as well as the rats' urinary and faecal excretions, were measured.

Body temperature

Male and female Hooded Wistar rats were allocated equally into groups with free access to food and water. Four doses of the test drugs (0.03, 0.1 or 0.3 mg kg^{-1} CPA or CGS-21680, 0.03 mg kg^{-1} NECA or 10 mg kg^{-1} morphine) or appropriate vehicle controls were administered. The doses were given subcutaneously, separated by 12 h from the previous dose, over a 48-h period. The laboratory environment was a 12-h day–night cycle with temperature at 20 ± 2°C.

Core body temperature was measured 30 min after the second (time = 12 h) and fourth dose (time = 36 h). This was performed using a Model 2100 Tele-Thermometer rectal probe thermistor lubricated with white soft paraffin and inserted 6–7 cm into the rectum.

Statistics

Student's paired *t*-test was used to compare the effects of the treatment means on their respective pre-treatment control values and unpaired *t*-tests to compare the difference between two individual means. One-way analysis of variance followed by Dunnett's *t*-test was used for multi-comparisons of means to a common control and two-way analysis of variance was used to assess the significance of differences between dose–response curves. Means and curves were considered statistically different when $P < 0.05$. All graphics and statistical analyses were performed using the computer program GraphPad Prism (GraphPad Software Inc., San Diego, CA).

Results

Food consumption and faecal elimination

Morphine and NECA significantly reduced food intake at doses of 0.03 and 10 mg kg^{-1}, respectively (Figure 1A). Similar effects were observed in rats treated with moderate and high doses of the adenosine A_1 receptor agonist, CPA (0.1 and 0.3 mg kg^{-1}, s.c.). However, the lowest dose of CPA tested (0.03 mg kg^{-1}) significantly enhanced feeding behaviour, an effect also induced by the moderate and high doses of the adenosine A_{2A} receptor agonist, CGS-21680 (0.1 and 0.3 mg kg^{-1}, s.c.).

Morphine and NECA caused a significant reduction in faecal output following acute administration (Figure 1B). Significant constipating effects were also induced by CPA and CGS-21680, but this was inversely dose-related.

Water consumption and urinary excretion

NECA significantly increased the amount of water consumed by the rats (Figure 2A). In contrast, morphine did not significantly affect the drinking rate at the dose tested (10 mg kg^{-1}, s.c.). Rats treated with CPA and CGS-21680 also drank more water, with the effect being more pronounced at the highest doses (0.3 mg kg^{-1}, s.c.). However, the lowest dose of CGS-21680 (0.03 mg kg^{-1}) caused a small inhibition in water consumption.

A corresponding diuresis was also observed in NECA-treated rats (Figure 2B). In contrast, morphine, which

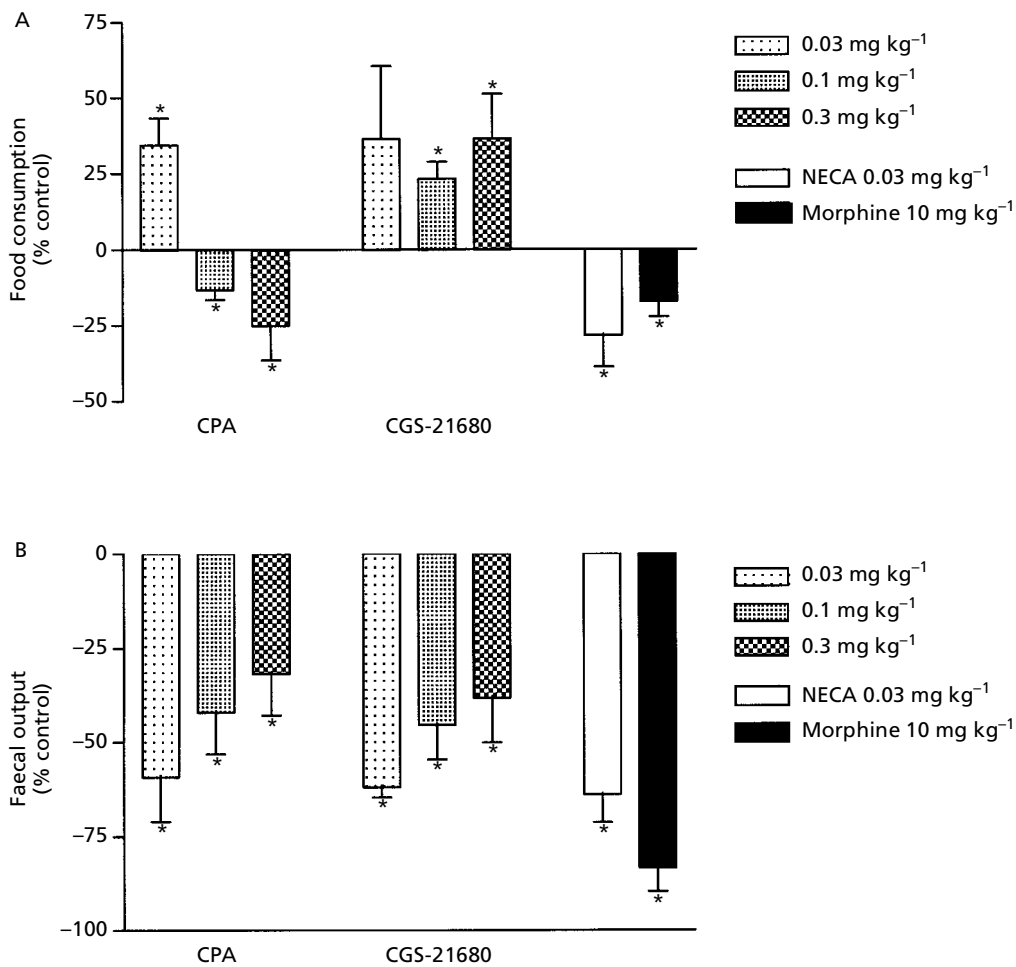

Figure 1 Effect of CPA and CGS-21680 (2×0.03, 0.1, 0.3 mg kg^{-1}, s.c.), NECA (2×0.03 mg kg^{-1}, s.c.) and morphine (2×10 mg kg^{-1}, s.c.) on food consumption (A) and faecal output (B) of rats. Each column represents the mean overall change in the amount of food consumed or faecal output (±s.d.m., n = 5–6) at the end of 24 h of treatment (two injections, 12 h apart). *$P < 0.05$, n = 5–6 (Student's paired *t*-test), compared with data obtained during the 24 h of non-treatment.

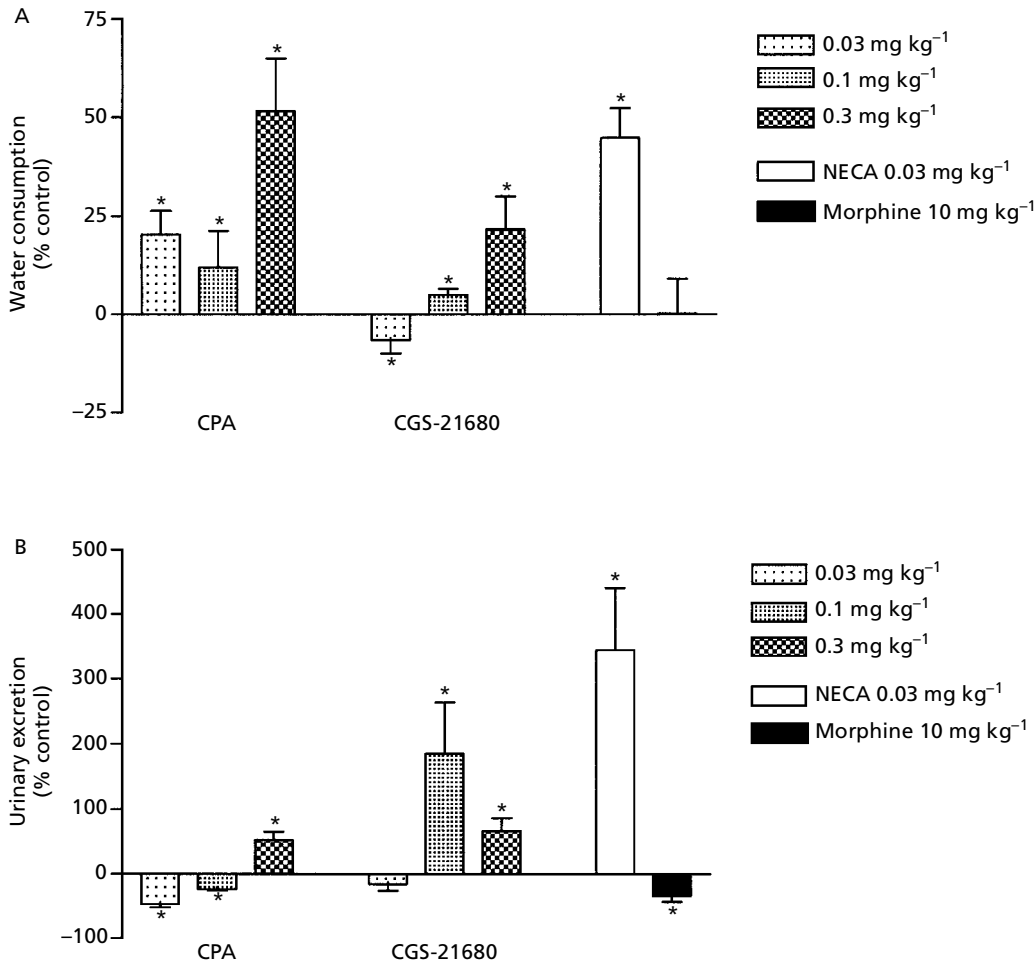

Figure 2 Effect of CPA and CGS-21680 (2×0.03, 0.1, 0.3 mg kg^{-1}, s.c.), NECA (2×0.03 mg kg^{-1}, s.c.) and morphine (2×10 mg kg^{-1}, s.c.) on water consumption (A) and urinary excretion (B) of rats. Each column represents the mean overall change in the amount of water consumed or urine excreted (±s.d.m., n = 5–6) at the end of 24 h of treatment (two injections, 12 h apart). *$P < 0.05$, n = 5–6 (Student's paired *t*-test), compared with data obtained during the 24 h of non-treatment.

had no significant effect on water consumption (Figure 2A), caused a small reduction in urinary volume (Figure 2B). This inhibitory effect was also induced by the low and medium doses of CPA (0.03 and 0.1 mg kg^{-1}, s.c.). However, the high dose (0.3 mg kg^{-1}) caused a significant increase in urination, an effect also induced by CGS-21680 (0.1 and 0.3 mg kg^{-1}, s.c.).

Body temperature

NECA (0.03 mg kg^{-1}, s.c.) and morphine (10 mg kg^{-1}, s.c.) both significantly reduced the body temperature (t = 12 h, two doses completed) compared with vehicle treated rats ($P < 0.05$, n = 6–16). The response to NECA after 36 h of treatment (four doses completed) was not significantly different from the results obtained after a 12-h treatment period ($P > 0.05$, n = 6). In contrast, tolerance developed to the hypothermic effect of morphine, whereby the effect on body temperature after 36 h of treatment was significantly less than the response obtained after a 12-h treatment period ($P < 0.01$, n = 14–16).

CPA and CGS-21680 both caused a significant dose-dependent decrease in body temperature after 12 h of treatment ($P < 0.01$, n = 7–16; Figure 3). The effects of both drugs were significantly less after 36 h of treatment (four doses of 0.03, 0.1 or 0.3 mg kg^{-1} completed), ($P < 0.0001$, n = 7–16, two-way analysis of variance; Figure 3).

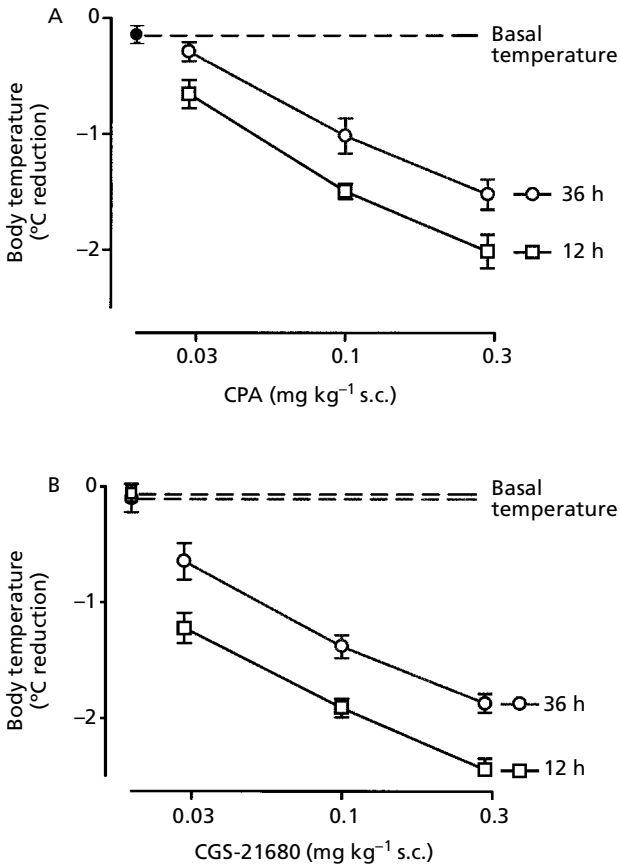

Figure 3 Effect of CPA (0.03, 0.1 and 0.3 mg kg^{-1}, s.c.) (A) and CGS-21680 (0.03, 0.1 and 0.3 mg kg^{-1}, s.c.) (B) on body temperature of rats. Each dose was administered four times over a period of 48 h, on a twice daily basis, 12 h apart; at t = 12 h (i.e. 12 h of treatment), two injections had been completed and at t = 36 h, four injections had been completed. Each data point or column represents the mean decrease in body temperature, measured 30 min after administration of test drugs, ± s.e.m., n = 6–16. *$P < 0.05$, **$P < 0.01$, n = 6–16 (one-way analysis of variance, Dunnett's multi-comparison for CPA and CGS-21680, unpaired t-test for NECA and morphine) compared with vehicle-treated rats.

Discussion

The results show that CPA and CGS-21680 are both considerably more potent at inducing constipation compared with morphine. This effect can be explained on the basis of earlier studies that demonstrated that NECA inhibited both intestinal secretion and peristalsis in rats (Coupar & Hancock 1994). These processes have been shown to involve peripheral adenosine A_{2B} (Hancock & Coupar 1995a) and A_1 (Hancock & Coupar 1995b) receptors, respectively.

It is interesting to note, however, that even though CGS-21680 inhibited faecal output, it increased food consumption at the medium and high doses used. In contrast, CPA dose-dependently decreased food intake at 0.1 and 0.3 mg kg^{-1}, causing a similar response to NECA (0.03 mg kg^{-1}, s.c.) and morphine (10 mg kg^{-1}, s.c.), but increased food intake at 0.03 mg kg^{-1}. The reduction in food intake at the higher doses of CPA is not surprising given its potent sedative action (Dunwiddie & Worth 1982). The inhibitory effect of morphine on food consumption is in agreement with a previous study utilising metabolism cages (Koyuncuoglu et al 1984).

The sedative actions of adenosine A_1 receptor agonists (Dunwiddie & Worth 1982) and the locomotor depressant effects of A_2 receptor agonists (Durcan & Morgan 1989) do not appear to affect the ability of CPA- or CGS-21680-treated animals to drink water. In fact, both CPA and CGS-21680 induced a dose-related stimulation of drinking. This contrasts with the inhibitory effect of CPA on food intake noted in this study.

Previous studies of the effect of adenosine agonists on renal function have reported variable results. This in-vivo study shows that one factor accounting for the variation is the dose. Hence, in our study, acute administration of the low and medium doses of CPA (0.03 and 0.1 mg kg^{-1}, s.c.) reduced the amount of urine output, whereas the high dose induced diuresis. The anti-diuretic response may be explained by an adenosine-A_1-receptor-mediated renal vasoconstriction and decreased glomerular filtration rate (Murray & Churchill 1984; Lopex-Novoa et al 1987), whereas at a high dose, diuresis may be due to an A_1-receptor-mediated inhibition of renin secretion (Churchill & Churchill 1985), inhibition of vasopressin-stimulated cAMP production (Hayslett et al 1995) or a direct stimulation of tubule sodium and water excretion (Yagil 1993).

In contrast, the adenosine A_{2A} receptor agonist, CGS-21680, caused a dose-related diuresis. This may be explained by the stimulation of A_{2A} receptors leading to activation of adenylate cyclase and subsequent relaxation of renal arteries and increased glomerular filtration rate (Murray & Churchill 1984; Levens et al 1991).

This study also investigated the hypothermia induced by adenosine agonists and showed for the first time that the study drugs produce tolerance to this effect. In this respect, the adenosine agonists are similar to morphine, which also reduces body temperature. Tolerance to morphine-induced hypothermia has been reported previously in other studies (Gunne 1960; Rauhala et al 1995). The results of this study further demonstrate that both the A_1-selective agonist, CPA, and the A_2-selective agonist, CGS-21680, induce hypothermia and that tolerance develops to both. The result that tolerance failed

to show with the non-selective adenosine agonist, NECA, was probably due to the dose being too high to reveal tolerance under the experimental conditions used.

Since tolerance to both CPA- and CGS-21680-induced hypothermia was demonstrated in this study, it is possible that desensitisation of both the adenosine A_1 and A_{2A} receptors are involved in temperature regulation. Other in-vivo and in-vitro studies have shown that tolerance develops to the antihypertensive effect of the adenosine A_1 receptor agonist, CCPA, following repeated administration (Casati et al 1994) and that prolonged exposure of the rat brain to selective adenosine receptor agonists desensitises both A_1 (Abbracchio et al 1992) and A_2 receptors (Porter et al 1988).

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