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Characterization of the adenosine receptors mediating hypothermia in the conscious mouse

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1 The effects of a range of adenosine receptor-selective ligands on body temperature were investigated following intracerebroventricular (i.c.v.) and intraperitoneal (i.p.) injection in conscious mice. The compounds tested were the non-selective adenosine receptor agonist 5'-N-ethyl-carboxamidoadenosine (NECA), the adenosine A_1 receptor-selective agonists cyclopentyl-adenosine (CPA), N^6-(9**R**-phenyl-isopropyl)-adenosine (**R**-PIA) and N-(1S,*trans*)-[2-hydroxycyclopentyl]-adenosine (GR79236), the A_{2a} receptor selective agonist 2-[*p*-(2-carboxyethyl)phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS-21680), the A_{2b} receptor agonist N-[(2-methylphenyl)methyl]adenosine (metrifudil) and the A_3 receptor agonist N^6-(4-aminophenylethyl)adenosine (APNEA).

2 NECA (0.01–1 μg, i.c.v.), all of the A_1-selective agonists (0.01–1 μg, i.c.v.) and APNEA (0.1–3 μg i.c.v.) produced profound and dose-related hypothermia and sedation. However, CGS-21680 (0.1–10 μg i.c.v.) and metrifudil (0.01–1 μg i.c.v.), produced only mild hypothermia at the highest doses tested.

3 The hypothermic response to the A_1 receptor-selective agonists, GR79236 and **R**-PIA was dose-dependently antagonized by peripheral administration of either the non-selective adenosine receptor antagonist, 8-phenyltheophylline (8-PT, approximately 40 and 30 fold rightward shifts of the dose-response curves respectively at 10 mg kg^{-1}, i.p.), or the adenosine A_1 receptor-selective antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, approximately 20 fold shift of the GR79236 dose-response curve at 1 mg kg^{-1}, i.p.). The hypothermic response to APNEA was similarly dose-dependently antagonized by the A_1 receptor-selective antagonist, DPCPX (5 fold shift at 0.1 mg kg^{-1}, i.p.).

4 8(*p*-Sulphophenyl)theophylline (8-SPT, 10 and 30 mg kg^{-1}, i.p.), a non-selective adenosine receptor antagonist that penetrates the blood brain barrier poorly, produced only modest antagonism (approximately 2 fold shift at 30 mg kg^{-1}, i.p.) of the hypothermic response to GR79236.

5 These data suggest that hypothermia induced by adenosine analogues in the conscious mouse is mediated via adenosine A_1 receptors, which are probably located in the CNS.

Keywords: Hypothermia; adenosine receptors; mouse; N-(1S,*trans*)-[2-hydroxycyclopentyl]adenosine (GR79236), N^6-(4-aminophenylethyl)adenosine (APNEA)

Introduction

Adenosine is an endogenous purine that plays an important role in regulating neuronal excitability. It is released from nerves under conditions of prolonged activity or hypoxia to act at specific cell membrane receptors to modify neuronal activity (Fredholm & Hedqvist, 1980). Adenosine inhibits neurotransmitter release from many neurones including cholinergic, adrenergic and dopaminergic cells through a combination of effects on intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation, calcium and potassium ion fluxes (Fredholm & Dunwiddie, 1988). The actions of adenosine are mediated via distinct extracellular receptors. These receptors have been classified into two major categories, A_1 and A_2, based on their opposing actions on adenylate cyclase, tissue distribution and structure-activity relationships (Hamprecht & Van Calker, 1985). Generally, activation of A_1 receptors inhibits adenylate cyclase via inhibitory G-proteins, and receptors are located in high densities in the cerebral cortex, hippocampus and striatum (Fastbom *et al.*, 1987) and in lower densities in the periphery. In contrast, activation of A_2 receptors stimulates adenylate cyclase activity, and receptors are highly localized in the striatal region, nucleus accumbens and olfactory tubercle (Jarvis & Williams, 1989). The development of the agonist CGS-21680 (2-[*p*-(2-carboxyethyl)phenethylamino]-5'-*N*-ethylcarboxamido adenosine) has facilitated adenosine A_2 receptor sub-classification into two subtypes, A_{2a} receptors with high affinity for CGS-21680 and A_{2b} receptors with low affinity for this compound (Jarvis & Williams, 1989; Hutchison *et al.*, 1990; Gurden *et al.*, 1993). More recently, techniques in molecular biology have identified an adenosine A_3 receptor subtype (Zhou *et al.*, 1992) at which N^6-(4-aminophenylethyl) adenosine (APNEA) acts as an agonist.

Administration of adenosine analogues in mice produces a cluster of physiological effects which include reduced locomotor activity (Barraco *et al.*, 1983), sedation and hypothermia (Yarbrough & McGuffin-Clineschmidt, 1981; Zarrindast & Heidari, 1993). The hypothermic effects of peripherally administered adenosine and 2-chloroadenosine were blocked by the non-selective adenosine antagonists, caffeine and theophylline (Mehta & Kulkarni, 1983). To our knowledge, the specific adenosine receptors mediating this response have not been identified. Accordingly, in the present study the effects of a range of adenosine receptor-selective ligands on body temperature have been investigated in the conscious mouse in order to characterize further the adenosine receptors involved in this response.

Methods

All experiments were performed on conscious male, 8–12 g, weanling CRH mice bred by Glaxo Research and Development Ltd. Agonists or vehicle were administered by intracerebroventricular (i.c.v.) injection at the area fontanelle by means of Hamilton Gas Tight Syringes (Lot No 1704) with

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Luer-Lock fitting hypodermic needles (1/8"). The dose volume was 3 μl. Once dosed, the animals were housed individually in observation beakers and monitored for 30 min. Antagonist compounds were administered as intraperitoneal injections in 0.1 ml dose volume, 10 min prior to agonist dosing. Body (core) temperature was measured 30 min post dose with an oesophageal probe and thermocouple (Digiton Instruments Ltd.) 8-Phenyltheophylline (8-PT), 8(*p*-sulphophenyl)theophylline (8-SPT) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) had no direct effect on body temperature at the doses used in this study.

Statistical analysis

Data points are the mean ± s.e.mean of at least six animals at each dose level. The statistical significance of agonist-induced variations from the control temperatures of vehicle-treated animals at 30 min post-dose was determined by one way analysis of variance followed by Dunnett's test. For antagonist studies, data points obtained in the presence of antagonist were compared with the agonist control curve. Sedation was quantified on an observer rating scale as either mild (reduced exploratory behaviour compared to control mice), moderate (only occasional movement around the observation beaker, lowered body posture) or severe (completely flattened body posture, low muscle tone, no movement around the beaker).

Drugs

The following drugs were used: 5'N-ethyl-carboxamidoadenosine (NECA), N-(1S,*trans*)-[2-hydroxycyclopentyl]-adenosine (GR79236, Glaxo Research and Development Ltd.), N^6-(9R-phenyl-isopropyl)-adenosine (R-PIA, Research Biochemicals Inc., batch PW-688B), cyclopentyladenosine (CPA, Glaxo Research and Development Ltd.), 2-[*p*-(2-carboxyethyl)phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS-21680, Research Biochemicals Inc., batch TB-791D), N-[(2-methylphenyl)methyl]adenosine (Metrifudil, Glaxo Research and Development Ltd), 8-phenyltheophylline (8-PT, Research Biochemicals Inc., batch PW-191C), N^6-(4-aminophenylethyl)adenosine (APNEA, Glaxo Research and Development Ltd.) 8(*p*-sulphophenyl)theophylline, (8-SPT, Research Biochemicals Inc., batch PW-190C), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, Cookson Chemicals Ltd., batch 1442). Agonist compounds were initially dissolved in a small volume of 1 M HCl and made up to volume with 0.9% (w/v) sodium chloride solution (saline). All further dilutions were made in saline. The final concentration of HCl in the injection fluid was 8 mM in experiments with metrifudil, and less than 1 mM for all other compounds. Control animals were injected with the same volume of the highest concentration of HCl in saline. The non-selective adenosine receptor antagonists, 8-PT and 8-SPT, were dissolved in 2 M sodium hydroxide, partially neutralized with 1 M HCl and made to volume with 0.9% sodium chloride solution to form stock solution concentrations of 1 and 3 mg ml^{-1} respectively. The adenosine A_1 receptor-selective antagonist DPCPX was dissolved in dimethylsulphoxide to form a stock solution concentration of 0.1 mg ml^{-1}.

Results

Time-course of hypothermia

Figure 1 shows the time-course of the hypothermic effects of the adenosine A_1 receptor-selective agonist GR79236 (0.3 μg, i.c.v.). GR79236 produced a rapid fall in body temperature in the first 10 min following injection which reached a minimum by 30 min post-dose and was maintained for at least 1 h. The sedative effect appeared to follow the same time-course. In view of this result, body temperature was routinely measured 30 min post-dose in subsequent studies. In contrast, the body temperature of mice injected with the most acidic vehicle used in this study did not vary significantly from the pre-dose value (Figure 1).

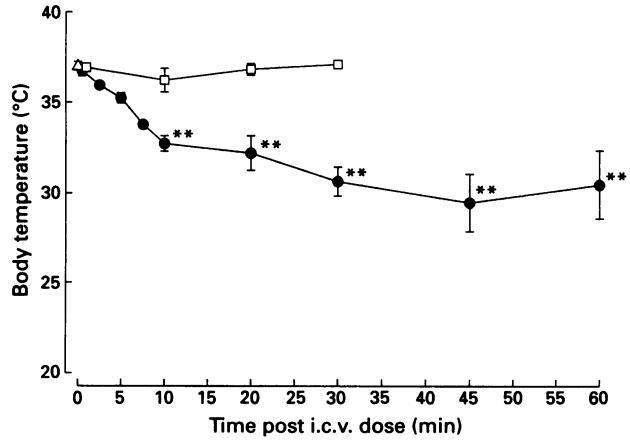

Figure 1 Time course of the hypothermic effects of GR79236 (●, 0.3 μg) and vehicle (□, 8 mM HCl) compared with pre-dose temperature (Δ) following i.c.v. injection in the conscious mouse. Data points are expressed as mean ± s.e.mean of 6 mice per dose group. Statistically significant (**$P<0.01$) reductions in body temperature were determined by one-way analysis of variance followed by Dunnett's test.

Agonist studies

In order to investigate the thermoregulatory effect of adenosine in mice, a range of adenosine analogues were tested over a dose range of 0.01–3 μg, i.c.v. (Figure 2). The non-selective adenosine receptor agonist, NECA, produced dose-dependent and profound hypothermia (Figure 2), and sedation which was rated as mild after 0.03 μg NECA, i.c.v., moderate after 0.1 μg and severe after 0.3 μg. The effects of NECA were mimicked by three adenosine A_1 receptor-selective agonists, CPA, GR79236 and R-PIA, and by the A_3 receptor agonist, APNEA. All five agonists produced a fall in body temperature of at least 6.5°C over a similar (100 fold) dose-range with an apparent molar rank order of agonist activity of NECA > GR79236 > CPA > R-PIA > APNEA. Each agonist also caused sedation in parallel with the degree of hypothermia.

In contrast, the adenosine A_{2a} receptor-selective agonist, CGS-21680 (0.1–10 μg) and the adenosine A_{2b} receptor agonist, metrifudil (0.01–1 μg) produced only mild sedation and hypothermia at the highest doses tested (Figure 2). For example CGS-21680, tested at 10 μg, produced a mean fall of 3.4°C (± 0.7°C; $n = 6$), whereas NECA, tested at 1 μg in the same experiment, produced a mean fall of 11.3°C (± 0.3°C; $n = 6$).

In a further study, GR79236 was administered peripherally as an i.p. injection over a dose-range of 0.1–3 mg kg^{-1}. GR79236 produced falls in body temperature of 0.1 ± 0.4, 3.3 ± 2.0, 12.8 ± 0.1 and 12.0 ± 0.4°C at 0.1, 0.3, 1.0 and 3.0 mg kg^{-1} i.p. respectively ($n = 6$ per dose group).

Antagonist studies

In order to investigate the specific adenosine receptors involved in the hypothermic action of adenosine, the effects of a range of adenosine receptor antagonists on agonist-induced hypothermia were investigated.

The sedative and hypothermic effects of the adenosine A_1 receptor-selective agonists GR79236 (Gurden *et al.*, 1993) and R-PIA were dose-dependently antagonized by peripheral administration of the non-selective adenosine receptor

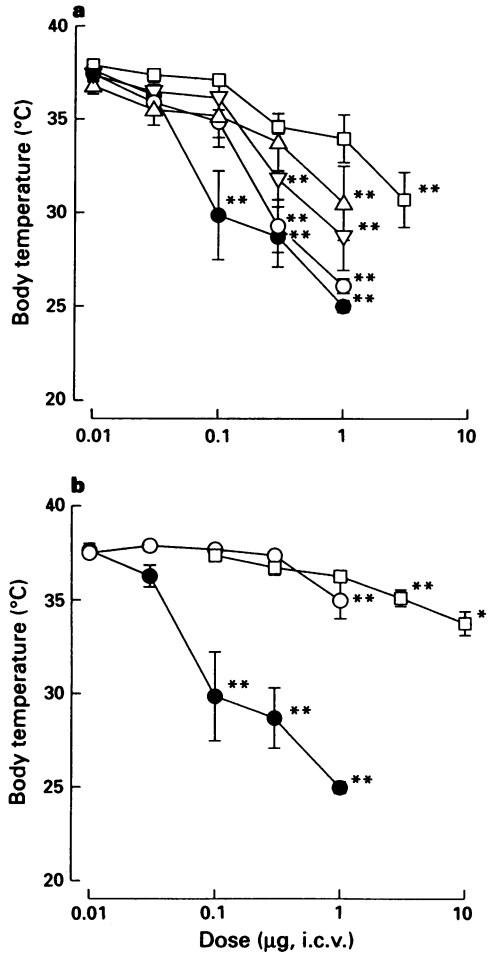

Figure 2 (a) Effects on body temperature of NECA (●), GR79236 (○), CPA (▽), R-PIA (△) and APNEA (□) 30 min following i.c.v. injection in the conscious mouse. Data points are expressed as mean ± s.e.mean of 6 mice at each dose level. Statistically significant (**$P<0.01$) reductions in body temperature were determined by one-way analysis of variance followed by Dunnett's test. (b) Effects on body temperature of NECA (●) metrifudil (○) and CGS-21680 (□) 30 min following i.c.v. injection in the conscious mouse. Data points are expressed as mean ± s.e.mean of 6 mice at each dose level. Statistically significant (**$P<0.01$) reductions in body temperature were determined by one-way analysis of variance followed by Dunnett's test. For abbreviations, see text.

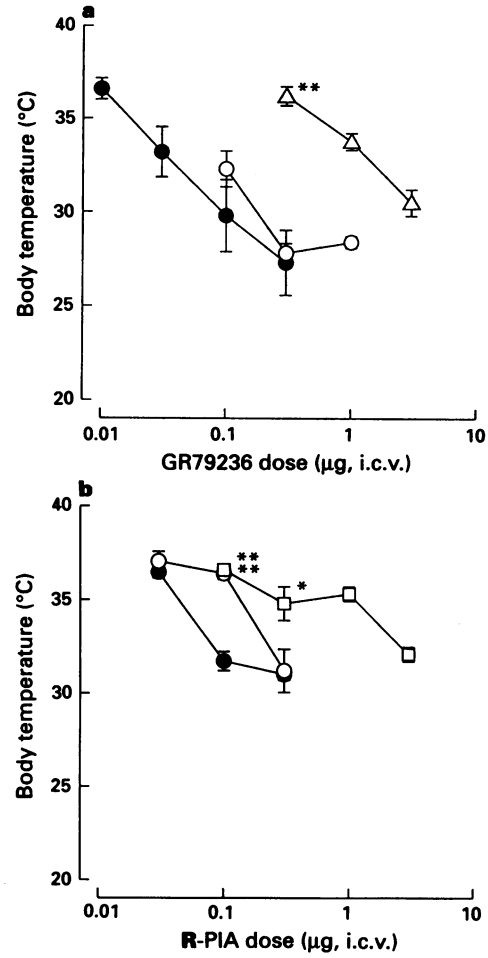

Figure 3 (a) Antagonism of the hypothermic effects of GR79236 i.c.v. (●) by 8-PT at 1.0 (○) and 10 (△) mg kg^{-1} i.p. Data points are expressed as the mean ± s.e.mean of 6 mice at each dose level. Statistically significant (*$P<0.01$) antagonism was determined by one-way analysis of variance followed by Dunnett's test. Vehicle control temperatures were 37.3 ± 0.11°C ($n = 6$). (b) Antagonism of the hypothermic effects of R-PIA i.c.v. (●) by 8-PT at 1 (○) and 10 (□) mg kg^{-1}, i.p. Data points are expressed as the mean of 6 mice at each dose level. Statistically significant (*$P<0.05$, **$P<0.01$) antagonism was determined by one-way analysis of variance followed by Dunnett's test. Vehicle control temperatures were 37 ± 0.15°C ($n = 6$). For abbreviations, see text.

antagonist 8-PT (1–10 mg kg^{-1}, i.p.). For example, there was a 40 fold rightward shift of the GR79236 dose-response curve at 10 mg kg^{-1} (Figure 3). All antagonist shifts were determined at IC_{50} levels. The effects of GR79236 were also antagonized by the potent and selective adenosine A_1 receptor-selective antagonist DPCPX (0.1–1 mg kg^{-1}, i.p.). For example, there was a 16 fold rightward shift at 1 mg kg^{-1} (Figure 4). In addition, the sedative and hypothermic response to APNEA was dose-dependently antagonized by peripheral administration of DPCPX (0.1–1 mg kg^{-1} i.p., Figure 4).

In contrast, the peripherally-acting non-selective antagonist 8-SPT (Daly *et al.*, 1985; 10–30 mg kg^{-1}, i.p.) produced only modest antagonism of the hypothermic response to GR79236 (Figure 5).

Discussion

The aim of the present study was to investigate and characterize the receptors that mediate the hypothermic effects of adenosine in the conscious mouse.

Central administration of the non-selective adenosine receptor agonist, NECA, produced profound, dose-dependent hypothermia and sedation which was mimicked by the adenosine A_1 receptor-selective agonists, CPA, GR79236 and R-PIA and the A_3 receptor agonist, APNEA (Zhou *et al.*, 1992; Gurden *et al.*, 1993). The hypothermic and sedative effects appeared at similar doses for each agonist, although the apparent molar rank order of agonist activity was NECA > GR79236 > CPA > R-PIA > APNEA. This order of potency is somewhat different from that obtained at A_1 receptors *in vitro* (Gurden *et al.*, 1993). However, variations in the lipophilicity of the drugs may partly underlie the variation in activity by modifying access to or removal from the site of action, since we have assumed that all drugs other than GR79236 had achieved equally-effective concentrations 30 min after dosing. The adenosine A_{2a} receptor-selective agonist, CGS-21680, and the modestly selective adenosine A_{2b} receptor agonist, metrifudil, were unable to evoke the same hypothermic effects over a similar dose-range. Indeed these compounds produced only mild responses at much higher doses at which their receptor selectivity may have been compromised. Adenosine A_{2a} receptors present in the nucleus

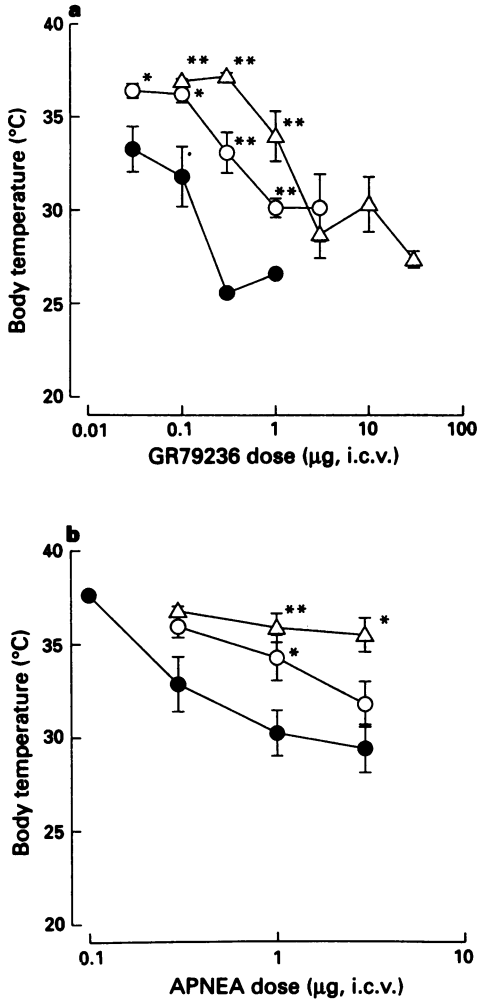

Figure 4 (a) Antagonism of the hypothermic effects of GR79236 i.c.v. (●) by DPCPX at 0.1 (○) and 1.0 (△) mg kg^{-1}, i.p. Data points are expressed as the mean ± s.e.mean of 6 mice at each dose level. Statistically significant (*$P<0.05$, **$P<0.01$) antagonism was determined at each dose level by one-way analysis of variance followed by Dunnett's test. Vehicle control temperatures were (37.4 ± 0.24 $n=6$). (b) Antagonism of the hypothermic effects of APNEA i.c.v. (●) by DPCPX at 0.1 (○) and 1.0 (△) mg kg^{-1}, i.p. Data points are expressed as the mean ± s.e.mean of ($n=6$) mice at each dose level. Statistically significant (*$P<0.05$, **$P<0.01$) antagonism determined by one-way analysis of variance followed by Dunnett's test. Vehicle control temperatures were 36.8 ± 0.28°C ($n=6$). For abbreviations, see text.

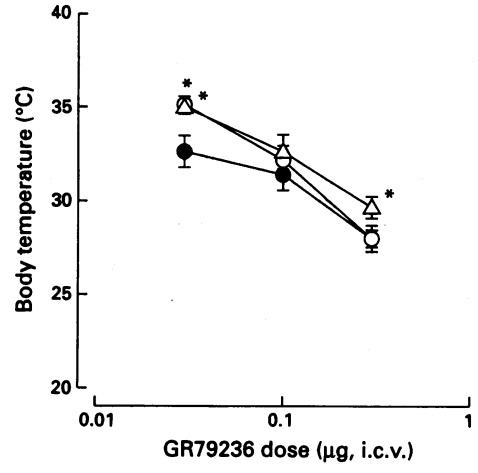

Figure 5 Antagonism of the hypothermic effects of GR79236 i.c.v. (●) by 8-SPT at 10 (○) and 30 (△) mg kg^{-1}, i.p. Data points are expressed as the mean ± s.e.mean of 6 mice at each dose level. Statistically significant (*$P<0.05$) antagonism was determined by one-way analysis of variance followed by Dunnett's test. Vehicle control temperatures were 37.1 ± 0.37°C ($n=6$).

accumbens have been reported to mediate locomotor depression (Barraco *et al.*, 1993) and catalepsy (Ferré *et al.*, 1991) in mice and rats respectively. Presumably, CGS-21680 did not diffuse into the nucleus accumbens at high enough concentrations to evoke these effects in the present study. These results suggest that hypothermia is unlikely to be mediated via activation of either A_{2a} or A_{2b} receptors. Rather they suggest that activation of adenosine A_1 and/or A_3 receptors is likely to be the mechanism involved.

The hypothermic and sedative effects of GR79236 were antagonized by the non-selective adenosine receptor antagonist 8-PT and also by low doses of the adenosine A_1 receptor-selective antagonist, DPCPX. The adenosine receptor agonist, APNEA, has been used to activate the recently-discovered adenosine A_3 receptor although it also has high affinity for A_1 receptors (Zhou *et al.*, 1992). APNEA caused hypothermia and sedation at doses of 0.3–3 μg. However, these effects were antagonized by DPCPX which has been reported to lack affinity for the A_3 receptor (Zhou *et al.*, 1992). There is thus no evidence from our study that the A_3 receptor can mediate either response. These results therefore suggest that adenosine A_1 receptors mediate the hypothermic response. A previous study has described the indirect involvement of adenosine A_1 receptors in neurotensin-induced hypothermia (Jolicoeur & Menard, 1992).

In a recent study (Zarrindast & Heidari, 1993) it was reported that intraperitoneal administration of NECA, CHA and R-PIA reduced core body temperature in mice. Furthermore, the hypothermic effects of R-PIA were potentiated by 8-PT. The latter results are clearly in conflict with our own and further work may be required to clarify the discrepancy.

The mechanism of hypothermia has not been established but may be at a peripheral site (e.g. inhibition of sympathetic nerves or reduction in heart rate), or a central site, or both. In order to investigate whether central or peripheral adenosine receptors were responsible for the hypothermic response, a further antagonist study was undertaken. In contrast to 8-PT, the sulphated analogue, 8-SPT, is a non-selective adenosine receptor antagonist that poorly penetrates the blood brain barrier (as little as 5% penetration occurs as measured by *ex vivo* binding; Baumgold *et al.*, 1992), so preferentially blocking peripheral adenosine receptors. After systemic administration, 8-SPT produced only modest antagonism of the hypothermic effects of GR79236, suggesting that the A_1 receptors responsible for mediating hypothermia are located in the CNS. In addition, the doses of GR79236 which were active on i.c.v. administration were much lower than those which were active peripherally. In the unlikely event of the whole of a maximally effective central dose of GR79236 (1 μg) leaching into the periphery, this would be equivalent to a dose of 0.1 mg kg^{-1}, i.p., which in the present study clearly had no effects on body temperature. Furthermore, Phillis *et al.* (1986) have shown that central (hypoactivity) and peripheral (hypotensive) effects of adenosine analogues could be dissociated by using an intracerebroventricular route of administration. Taken together, these data suggest that central adenosine A_1 receptors mediate the hypothermic effects of adenosine in the mouse.

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