British Journal of Pharmacology (1996) 117, 305-308

Functional characterization of adenosine receptors in the nucleus tractus solitarius mediating hypotensive responses in the rat

[1]Paul J. White, Roselyn B. Rose'Meyer & Wendy Hope

Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University. 381 Royal Pde, Parkville, Victoria 3052, Australia

1 The aim of this study was to characterize adenosine receptors located in the nucleus tractus solitarius (NTS) that mediate decreases in blood pressure in the anaesthetized rat. To determine the adenosine receptor subtype involved, a range of selective agonists and antagonists were studied and their relative potencies evaluated.

2 The rank order of agonist potency in inducing decreases in diastolic blood pressure was N^6-cyclopentyladenosine (CPA) > N^6-cyclohexyladenosine (CHA) > N-ethyl-carboxamidoadenosine (NECA) ≥ 2-phenylaminoadenosine (CV1808) > 2-*p*-(carboxyethyl)phenethylamino-5′ N-ethylcarboxamidoadenosine (CGS 21680) > N^6-(2-(4-aminophenyl)ethyl)-adenosine (APNEA).

3 The hypotensive action of CPA following microinjection into the NTS was antagonized by i.v. infusions (50 $\mu g\ kg^{-1}\ min^{-1}$) of adenosine receptor antagonists, 8-cyclopentyl-1,3 dipropylxanthine (DPCPX), 8-phenyltheophylline (8-PT), 8-(*p*-sulphophenyl)theophylline (8-SPT), and 1,3-dipropyl-8-N-(2-(diethylamino)ethyl)-*N* methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo) benzenesulphonamidexanthine (PD 115199). The antagonist potency order was DPCPX > PD115199 ≥ 8-PT. Intravenous infusion of 8-SPT had no effect on blood pressure responses to microinjection of CPA into the NTS.

4 The results suggest that adenosine A_1 receptors in the NTS mediate hypotensive responses in the anaesthetized rat preparation.

Keywords: Adenosine receptors; blood pressure; nucleus tractus solitarius; N^6-cyclopentyl adenosine

Introduction

The nucleoside, adenosine, acts as a neuromodulator in a number of autonomic functions, in addition to being a ubiquitous modulator of cellular function. In the brain, adenosine has been shown to act predominantly by inhibition of neurotransmitter release to produce sedation, decreased locomotor activity and anticonvulsant and anxiolytic effects (see review by Jarvis & Williams, 1990).

In a recent study by Mosqueda-Garcia *et al.* (1991), however, adenosine has been shown to have excitatory cardiovascular effects, in the nucleus tractus solitarius (NTS). Microinjection of adenosine into the NTS was shown to produce decreases in blood pressure and heart rate. The proposed mechanism of the excitatory action of adenosine in the NTS involves the release of glutamate by adenosine, as perfusion of adenosine into the NTS results in increased interstitial concentrations of glutamate. These results suggest that adenosine may play a role in autonomic control of blood pressure, as this brain stem region contains the first synapse for afferent impulses from the carotid baroreceptors (Mosqueda-Garcia *et al.*, 1991). Classification of the adenosine receptor involved in mediating the hypotensive effect of adenosine at the nucleus tractus solitarius may therefore provide further information on the mechanisms of baroreceptor reflex mechanisms.

Significant changes in adenosine receptor classification have occurred since adenosine A_1 and A_2 receptors were first distinguished from each other on the basis of inhibition and stimulation of adenosine 3′5′-cyclic monophosphate (cyclic AMP) production (van Caulker *et al.*, 1979). Adenosine A_2 receptors have been divided into A_{2a}, and A_{2b} receptors on the basis of high and low affinity binding sites, respectively, in rat brain (Daly *et al.*, 1983; Bruns *et al.*, 1986). A novel, xanthine-insensitive adenosine A_3 receptor has been cloned from rat striatum by Zhou *et al.* (1992). A recent review by Collis & Hourani (1993) put forward a framework for the classification of adenosine A_1, A_{2a}, A_{2b} and A_3 receptors on the basis of adenosine receptor agonist potency order and antagonist affinity order.

The present study was designed to characterize the adenosine receptor located in the area of the NTS that is involved in mediating the hypotensive response to adenosine, by use of a range of adenosine analogues. As chronic adenosine receptor inhibition has been linked to trophic changes in blood vessels associated with primary hypertension (Matias *et al.*, 1991), classification of the adenosine receptor involved may allow for treatment aimed at preventing these changes.

Methods

Male Hooded Wistar rats (280-320 g) were used in these studies. The rats were anaesthetized with pentobarbitone sodium (60 $mg\ kg^{-1}$, i.p. initially, then 30 $mg\ kg^{-1}$, i.v. subsequently if required). To maintain a patent airway, the trachea was isolated and cannulated with polyethylene (PE250) tubing. The left jugular vein and right carotid artery were cannulated with polyethylene (PE50) tubing. The jugular and femoral vein cannulae were rinsed with physiological saline, while the carotid cannula was flushed with a heparinized saline solution (100 iu heparin ml^{-1} saline). Diastolic blood pressure was recorded from the carotid artery with a Gould Statham Physiological pressure transducer connected to a Grass 79D polygraph recorder.

The stereotaxic procedure followed was based on the method of Rabkin (1990). Briefly, the animals were placed in a stereotaxic instrument (Kopf), the skull was exposed by a midline incision to the scalp. A burr hole was drilled and a stainless steel needle (o.d. 0.200 mm) was inserted at a point with the co-ordinates AP −11.3, L +2.2, V +8.5. (Paxinos & Watson, 1986).

After surgery, the preparation was allowed 60 min to sta-

[1] Author for correspondence.

bilize before commencement of the protocol. Animals received two intra NTS doses of an adensoine analogue 15 min apart, after which either an adenosine receptor antagonist or its vehicle was infused via the jugular vein at 50 $\mu g\ kg^{-1}\ min^{-1}$ for 15 min, and the agonist administration was repeated in the presence of the antagonist. Volumes for injection were between 0.1 and 10 μl and vehicle controls were performed with no change to diastolic blood pressure.

The site of injection was marked by application of India ink into the burr hole. The brain was removed, frozen, and sectioned to confirm the site of injection. Only in experiments in which little or no spread outside the region of the NTS occurred were the data used.

Drugs

8-Cyclopentyl-1,3 dipropylxanthine (DPCPX), 8-phenyltheophylline (8-PT), 8-(*p*-sulphophenyl)theophylline (8-SPT), N^6-cyclopentyladenosine (CPA), N^6-(2-(4-aminophenyl)ethyl)-adenosine (APNEA), N^6-cyclohexyladenosine (CHA), 2-phenylaminoadenosine (CV1808), 2-*p*-(carboxyethyl) phenethylamino-5′ N-ethylcarboxamidoadenosine (CGS 21680) and N-ethyl-carboxamidoadenosine (NECA) were obtained from Research Biochemicals Inc., Natick U.S.A. Pentobarbitone sodium was obtained from Boehringer Ingelheim. 1,3-dipropyl-8-*N*-(2-(diethylamino)ethyl-*N* methyl-4-C2,3,6,7 - tetrahydro-2,6-dioxo) benzenesulphonamidexanthine (PD115199) was a gift from Parke Davis, Australia. All drugs were dissolved in dimethylsuphoxide (DMSO) and 0.75% v/v 1 M NaOH and diluted to required concentration in saline, with a resulting solution of less than 10% DMSO.

Data analysis

The potency order of the adenosine receptor agonists was determined by comparing the respective ED_{50} values with a computer-generated multiple comparison test (MULTCOMP). This programme performs an analysis of variance which is then followed by a Dunnett's test for significant differences. The effect of each adenosine receptor antagonist was determined by comparison of the ED_{50} values for the hypotensive effect of CPA in the absence and presence of each antagonist. The ED_{50} value was taken as the dose of agonist required to produce 50% of the maximal response for that agonist. The dose which produced the maximal response was taken to be the first dose at which a higher dose produced the same response. A *P*-value of less than 0.05 was considered to indicate statistical significance. ED_{50} values with their associated confidence limits were calculated with a computer programme (Tallarida & Murray, 1987).

Results

Intra NTS administration of adenosine analogues, CPA, CHA, NECA, CV1808 and CGS 21680 (0.3–30 μg) produced dose-dependent hypotension (see Figure 1). APNEA produced no response in the NTS in doses of up to 30 μg. Maximal responses to CPA and CHA were observed within 60 s of microinjection, whilst maximal responses to CGS 21680 and NECA did not occur until at least 5 min after microinjection. CPA, the most potent adenosine receptor agonist in these experiments, produced a maximal decrease in diastolic blood pressure of 63.5±6.6% at a dose of 3 μg, whilst a 30 μg dose of CGS 21680 failed to produce a response of this magnitude (41.4±4.7%). Doses of greater than 30 μg were not used, as these would require volumes in excess of 10 μl, since microinjection of saline (0.1–10 μl) or agonist vehicle (0.1–10 μl) had no effect on blood pressure or on responses to the adenosine analogues. A 30 μl microinjection of saline (0.9%) into the region of the NTS produced a small (5.5±4.9%) decrease in diastolic blood pressure. Table 1 shows the ED_{50} values for the effect of the adenosine receptor agonists on blood pressure. The order of potency of the adenosine analogues in these experiments was CPA > CHA > NECA ≥ CV1808 > CGS 21680.

The effects of the adenosine receptor antagonists were determined by testing responses to the most potent adenosine receptor agonist, CPA, before and after infusion of the antagonist. Intravenous infusion of DPCPX, PD115199 and 8-PT (50 $\mu g\ kg^{-1}\ min^{-1}$) attenuated CPA induced hypotensive responses, whilst 8-SPT was ineffective (see Figure 2). The antagonist infusion itself had no effect on blood pressure. The antagonist affinity order was DPCPX > PD115199 ≥ 8-PT ($n=5$, $P<0.05$).

Discussion

The results from this study suggest that adenosine A_1 receptors are responsible for decreases in diastolic blood pressure produced by adenosine in the NTS. Both the adenosine receptor agonist and antagonist potency orders from these experiments closely follow the framework presented by Collis & Hourani (1993). The agonist potency order from these experiments: CPA > CHA > NECA ≥ CV 1808 > CGS 21680 > APNEA complies with the adenosine A_1 receptor order of CPA > **R**-PIA > CHA > NECA > CADO > **S**-PIA > CV 1808 > CGS 21680. The involvement of adenosine A_2 receptors in the hypotensive response was discounted on the basis of agonist order (e.g. NECA and CGS 21680, the most potent agonists at these two receptor subtypes, were at least 10 fold less potent than CPA in these experiments). The location at which the response is produced also suggests that the responses observed

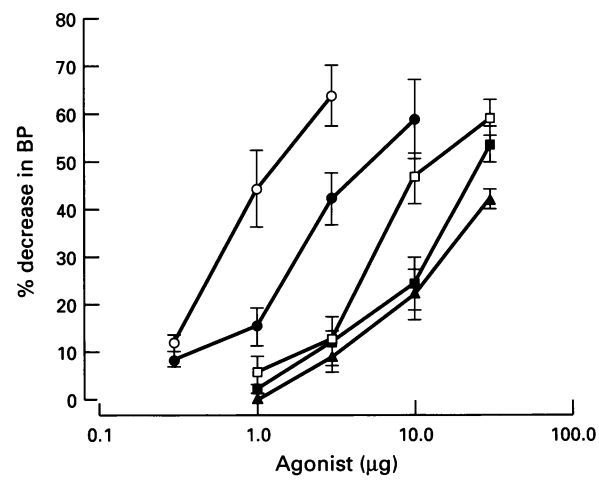

Figure 1 Hypotensive responses to microinjection into NTS of adenosine receptor agonists CPA (○), CHA (●), NECA (□), CV 1808 (■) and CGS 21680 (▲). Values are mean±s.e.mean, $n=5$ for each group. For abbreviations, see text.

Table 1 ED_{50} values and their associated confidence intervals for the hypotensive responses to adenosine receptor agonists

	ED_{50} ($\mu g\ kg^{-1}$)	*95% CI*
CPA	0.93	0.18
CHA	3.59	0.80
NECA	10.97	2.49
CV 1808	16.52	2.01
CGS 21680	21.26	2.79

$n=5$ for each value. For abbreviations, see text.

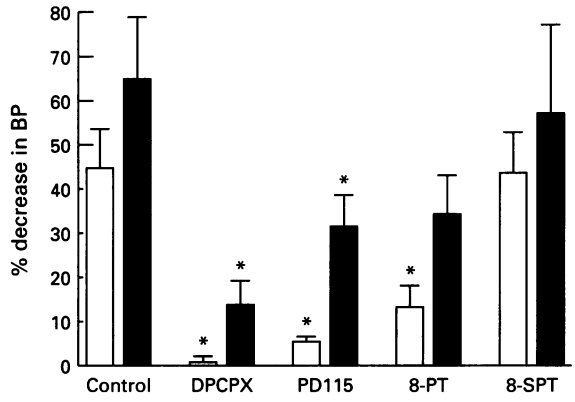

Figure 2 Effect of adenosine receptor antagonists ($50\,\mu g\,kg^{-1}\,min^{-1}$) on hypotensive responses to CPA microinjection into the NTS. CPA, $1.0\,\mu g$ (open columns), CPA, $3\,\mu g$ (solid columns). Values are means ± s.e.mean, $n=5$ for each group. *Indicates a significant difference from the control value for that dose ($P<0.05$, ANOVA, Dunnett's t test). For abbreviations, see text.

were not mediated by adenosine A_2 receptors, as these receptors are predominantly associated with dopaminergic neurones (Ferre *et al.*, 1992), and this region has not been reported to contain dopaminergic pathways.

The antagonist potency order of DPCPX > PD115199 ≥ 8-PT found in these experiments is similar to that from the review by Collis & Hourani (1993) for adenosine A_1 receptors. In order to characterize fully the adenosine receptor involved in the hypotension produced by adenosine in the NTS it is necessary to determine antagonist affinities with a range of antagonist doses. This was not possible in these experiments due to the limits of volume that may be injected into the brain (a 30 μl vehicle injection produced a small hypotensive response). Smaller doses of the antagonists were found to be ineffective, and larger antagonist doses required volumes of agonists to be used which were in excess of 30 μl. A $50\,\mu g\,kg^{-1}\,min^{-1}$ infusion of DPCPX has been shown in previous experiments in our laboratory to produce a selective antagonism of adenosine A_1 receptors (White *et al.*, 1993), and is similar to doses used to achieve a selective blockade of adenosine A_1 receptors by other groups (Munger & Kackson, 1994; St Lambert *et al.*, 1994). Therefore all of the adenosine receptor antagonists were compared at this dose. The results from studies using both agonist relative potency and antagonist potency suggest that adenosine A_1 receptors are responsible for mediating the hypotensive response to adenosine in the NTS.

These results are at variance with those of Barraco & Phillis (1991), who found that adenosine A_2 receptors were responsible for this response, as CGS 21680 produced transient decreases in blood pressure whilst CPA produced more delayed increases in blood pressure with a mean time to maximal response of around 30 min. In the experiments described here, none of the adenosine receptor agonists produced a hypertensive response and resting blood pressure was not elevated over 1 h after the maximal dose of CPA. It is likely that the responses to CHA observed in these experiments occurred by an action at a different region of the CNS from those observed by Barraco & Phillis (1991), as the responses were markedly different in time course and opposite in effect. The co-ordinates used in both studies correspond to the caudal region of the NTS. Tao & Abdel (1993) found that adenosine produced opposite cardiovascular effects in different areas of the NTS. Rostral injection of adenosine produced pressor responses, whilst caudal injection of adenosine produced depressor responses and it is these results that support the premise that the hypotensive effect of adenosine analogues in the current experiments occur in the caudal region of the NTS. The delayed onset of pressor responses to CPA observed by Barraco & Phillis (1991) raises the possibility that these effects occurred at the rostral region of the NTS.

The possibility that adenosine A_3 receptors were responsible for the hypotensive effect of the adenosine analogues in these experiments was discounted since three of the xanthine antagonists used (DPCPX, 8-PT and PD 115199) attenuated responses to CPA, and A_3 receptors have been reported to be insensitive to xanthine antagonists at doses many times greater than those used in these experiments (Zhou *et al.*, 1992). The adenosine A_3 receptor agonist APNEA had no effect on blood pressure when injected into the NTS, which may be considered to be surprising since APNEA has been shown to have some affinity at adenosine A_1 receptors (Fozard & Hannon, 1994). However, a similar result was reported by von Kugelgen *et al.* (1994), who found that high concentrations of APNEA had no effect on adenosine A_1 receptors in the rat brain cortex.

It is unlikely that the hypotensive actions of these adenosine analogues are due to 'leaking' of the drug across the blood-brain barrier and subsequent peripheral action. The amounts of the adenosine analogues injected were insufficient to produce peripheral effects of the magnitudes shown. CPA has been shown to produce a hypotensive effect upon intravenous administration, with a 200 $\mu g\,kg^{-1}$ dose producing a 60% fall in blood pressure (Mathot *et al.*, 1994), compared to 3 μg in this study producing around 60% decrease in blood pressure. Also, the nonselective adenosine receptor antagonist, 8-SPT (which does not cross the blood-brain barrier; Evonuik *et al.*, 1986) was ineffective in attenuating hypotensive responses to adenosine analogues in these experiments. 8-SPT has a far lower potency than 8-PT at adenosine A_1 receptors (Williams, 1989), and hence a comparison of potencies must take this into account. However, since 8-SPT was ineffective in these experiments and the doses of agonists used were insufficient to produce peripheral responses of this magnitude, it is unlikely that the effects of the adenosine agonists are occurring peripherally. It is difficult to determine whether the other antagonists used penetrated the brain to exactly the same degree. DPCPX has been shown to penetrate largely into the brain (Bisserbe *et al.*, 1992), and the other adenosine receptor antagonists which were effective in this study (8-PT and PD 115199) were assumed to have similar abilities to cross the blood-brain barrier.

Changes in the effects of various endogenous agents have been observed at the NTS in hypertensive animals. Altered responses to L-glutamate and acetylcholine at the NTS have been demonstrated in the spontaneously hypertensive rat (SHR, Talman & Lewis, 1991). Since adenosine has been shown to increase release of glutamate at the NTS (Mosqueda-Garcia *et al.*, 1991), and functional defects in purinergic neurotransmission have been reported in spontaneously hypertensive rats (Kamikawa *et al.*, 1980), it is possible that adenosine receptors in the NTS may be targeted for the treatment of the hypertensive state. Further study is required to define fully the role of adenosine in this brain region before any possible link between adenosine and hypertension is defined.

References

BARRACO, R.A. & PHILLIS, J.W. (1991). Subtypes of adenosine receptors in the brainstem mediate opposite blood pressure responses. *Neuropharmacol.*, **30**, 403–407.

BRUNS, R.F., LU, G.H. & PUGSLEY, T.A. (1986). Characterisation of the A2 adenosine receptor labelled by (3H)-NECA in striatal membranes. *Mol. Pharmacol.*, **29**, 331–346.

BISSERBE, J.C., PASCAL, O., DECKERT, J. PUGSLEY, T.A. (1986). Characterisation of the A2 adenosine receptor labelled by (3H)-NECA in striatal membranes. *Mol. Pharmacol.*, **29,** 331–346.

COLLIS, M.G. & HOURANI, S.M.O. (1993). Adenosine receptor subtypes. *Trends Pharmacol. Sci. Rev.*, **14,** 360–366.

DALY, J.W., BUTTS-LAMB, P. & PADGETT, W. (1983). Subclasses of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines. *Cell. Mol. Neurobiol.*, **3,** 69–80.

EVONUIK, G., VON BORSTEL, R.W. & WURTMAN, R.J. (1986). Antagonism of the cardiovascular effects of adenosine by caffeine or 8-(p-sulfophenyl)-theophylline. *J. Pharmacol. Exp. Ther.*, **240,** 428–442.

FERRE, S., VON EULER, G., JOHANSONN, B. & FREDHOLM, B.B. (1992). Adenosine-dopamine interactions in the brain. *Neuroscience*, **51,** 501–512.

FOZARD, J.R. & HANNON, J.P. (1994). BW-A522 blocks adenosine A3 receptor-mediated hypotensive responses in the rat. *Eur. J. Pharmacol.*, **252,** R5–6.

JARVIS, M.F. & WILLIAMS, M. (1990). In *Adenosine and Adenosine Receptors* ed. Williams, M. p. 289. New Jersey: Humana.

KAMIKAWA, Y., CLIN, W.H. & SU, C. (1980). Diminished purinergic modulation of the vascular adrenergic neurotransmission in spontaneously hypertensive rats. *Eur. J. Pharmacol.*, **66,** 347–352.

MATHOT, R.A.A., VAN SCHAICK, E.A., LANGEMEIJER, M.W.E., SOUDIJN, W., BREIMER, D.D., IJZERMAN, A.P. & DANHOF, M. (1994). Pharmacokinetic-pharmacodynamic relationship of the cardiovascular effects of adenosine A_1 receptor agonist N^6-cyclopentyladenosine in the rat. *J. Pharmacol. Exp. Ther.*, **268,** 616–624.

MATIAS, A., ALBINO-TEIXEIRA, A., POLONIA, J. & AZEVEDO, I. (1991). Long term administration of 1,3-dipropyl-8-sulfophenyl-xanthine causes arterial hypotension. *Eur. J. Pharmacol.*, **193,** 101–108.

MOSQUEDA-GARCIA, R., TESTING, C.J., APPALSAMY, C.B. & ROBERTSON, D. (1991). Cardiovascular excitatory effects of adenosine in the nucleus of the solitary tract. *Hypertension*, **18,** 494–502.

MUNGER, K.A. & JACKSON, E.K. (1994). Effects of selective a1 receptor blockade on glomerular haemodynamics: involvement of renin-angiotensin system. *Am. J. Physiol.*, **267,** 783–790.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. Figure 63. Sydney: Academic Press.

RABKIN, S.W. (1990). D-ala-2-Me-Phe-4-Met-(O)-ol-enkephalin in the nucleus tractus solitarius of the rat produces cardiorespiratory depression. *Gen. Pharmacol.*, **22,** 479–484.

ST LAMBERT, J.H., DAWID-MILNER, M.S., SILVA-CARVALO, L. & SPYER, K.M. (1994). Action of adenosine receptor antagonists in the cardiovascular response to defence area stimulation in the rat. *Br. J. Pharmacol.*, **113,** 159–164.

TALLARIDA, R.J. & MURRAY, R.B. (1987). *Manual of Pharmacological Calculations with Computer Progams*. Berlin: Springer Verlag.

TALMAN, W.T. & LEWIS, S.J. (1991). Altered cardiovascular responses to glutamate and acetylcholine microinjected into the nucleus tractus solitarii of the SHR. *Clin. Exp. Hypertens. A.*, **13,** 661–668.

TAO, S. & ABDEL-RAHMAN, A.A. (1993). Neuronal and cardiovascular responses to adenosine microinjected into the nucleus tractus solitarius. *Brain Res. Bull.*, **32,** 407–417.

VAN CALKER, D., MULLER, M. & HAMPRECHT, B. (1979). Adenosine regulates two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J. Neurochem.*, **33,** 999–1005.

VON KUGELGEN, I., SPATH, L. & STARKE, K. (1994). Evidence for P2-purinoceptor-mediated inhibition of noradrenaline release in the rat brain cortex. *Br. J. Pharmacol.*, **113,** 815–822.

WHITE, P.J., ROSE'MEYER, R.B. & HOPE, W. (1993). The role of adenosine in the cardiovascular actions of morphine. *Clin. Exp. Pharmacol. Physiol.*, suppl., 1, 77.

WILLIAMS, M. (1989). Adenosine antagonists. *Med. Res. Rev.*, **9,** 219–243.

ZHOU, Q-Y, LI, C., OLAH, M.E., JOHNSON, R.A., STILES, G.L. & CIVELLI, O. (1992). Molecular cloning and characterization of an adenosine receptor: the adenosine A3 receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 7432.

(Received June 9, 1995
Revised August 30, 1995
Accepted September 26, 1995)