British Journal of Pharmacology (1998) 125, 437–440
http://www.stockton-press.co.uk/bjp

The role of A_3 adenosine receptors in central regulation of arterial blood pressure

[1,2]Luigi Stella, [1]Vito de Novellis, [1]Ida Marabese, [1]Liberato Berrino, [1]Sabatino Maione, [1]Amelia Filippelli & [1]Francesco Rossi

[1]Institute of Pharmacology and Toxicology, Faculty of Medicine and Surgery, 2nd University of Naples, Via Costantinopoli, 16 - 80138, Naples Italy

1 Pharmacological studies have suggested that A_3 receptors are present on central neurons. Recently this adenosine receptor subtype has been identified in the rat and its presence in the central nervous system has been confirmed.

2 In this study we investigated the effects of acute intracerebroventricular (i.c.v.) injections of N^6-2-(4-aminophenyl)-ethyladenosine (APNEA), a non-selective A_3 adenosine receptor agonist, on arterial blood pressure (ABP) and heart rate (HR), after treatment with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective antagonist of A_1 adenosine receptors.

3 Anaesthetized rats, after DPCPX (12 μg^{-1} kg i.c.v.), were treated with APNEA (0.4–4 $\mu g\ kg^{-1}$ i.c.v.) resulting in a transitory and dose-dependent decrease in arterial blood pressure without a change in heart rate. APNEA also induced hypotensive responses after i.c.v. pretreatment with aminophylline, at a dose of 20 $\mu g\ kg^{-1}$. In contrast, pretreatment 48 h before, with 4 $\mu g\ kg^{-1}$ i.c.v. of pertussis toxin reduced the hypotensive effect induced by APNEA. Administration of APNEA at a higher dose (20 $\mu g\ kg^{-1}$ i.c.v.), after DPCPX, induced a decrease in ABP of -66 ± 5.4 mmHg and after 3 min a decrease in heart rate of -62 ± 6.0 beats min^{-1}. Transection of the spinal cord abolished this significant fall in ABP, but not the decrease of HR.

4 These results suggest that a population of A_3-receptors is present in the CNS, whose activation induces a decrease in blood pressure with no change of heart rate.

Keywords: N^6-2-(4-aminophenyl)-ethyladenosine (APNEA); A_3-receptors; third ventricle; arterial blood pressure; heart rate; anaesthetized rats

Introduction

Recently, the A_3 adenosine receptor subtype has been identified in the rat (Meyerhof *et al*., 1991; Zhou *et al*., 1992) and its presence in the cerebral cortex, cerebellum, striatum, hippocampus and hypothalamus of sheep has been reported (Jacobson *et al*., 1993; Linden *et al*., 1993). The A_3 receptor has been shown to couple with G-proteins (Linden, 1994). Some findings suggested a role for this receptor in mediating hypotension (Carruthers & Fozard, 1993; Fozard & Carruthers, 1993). Although both a fall in systemic vascular resistance and a decrease in cardiac output contribute to the hypotension induced in the rat by activation of adenosine A_3 receptors (Hannon *et al*., 1995), the physiological role(s) of A_3 receptors in cardiovascular function remains to be determined. We reported an important participation of adenosine in the central control of arterial blood pressure *via* the involvement of adenosine A_2 rather than A_1 receptors (Stella *et al*., 1993). In view of this, we have evaluated the effect of acute intracerebroventricular (third ventricle) injection of the non-selective A_1/A_3 agonist, N^6-2-(4-aminophenyl) ethyladenosine (APNEA) (Carruthers & Fozard, 1993; Fozard & Carruthers, 1993; Collis & Hourani, 1993) on arterial blood pressure (ABP) and heart rate (HR).

[2] Author for correspondence.

Methods

Animals

Male Sprague-Dawley rats (weighing 250–270 g, Morini SpA, Reggio Emilia, Italy) were housed at constant temperature ($22 \pm 1°C$) and relative humidity (60%), under regular light/dark schedule (light 0700 to 1900) with free access to food and water. Animal care was in compliance with Italian laws on the protection of animals used for experimental and other scientific purposes (D.M. 116/92), as well as with E.C. regulations (O.I. of E.C. 1358/118/12/1986).

Procedure

Forty-eight hours before experiments, under general anaesthesia (ketamine 120 mg kg^{-1} *ip*), the animals were prepared for direct intracerebroventricular (i.c.v. third ventricle) administration and immediately after insertion of the i.c.v. cannulas some groups received 4 $\mu g\ kg^{-1}$ i.c.v. of pertussis toxin (PTX).

On the day of the experiment the rats, anaesthetized with urethane (1.2 g kg^{-1} intraperitoneally; narcosis maintained with 300 mg kg^{-1} intravenously whenever necessary), were prepared for measurement of arterial blood pressure using previously described methods (Stella *et al*., 1995). Rats were randomized and received one of four doses (from 0.4–20 $\mu g\ kg^{-1}$) of the non-selective A_1/A_3 agonist, N^6-2-(4-aminophenyl)ethyladenosine (APNEA). In the experimental groups rats were pretreated with the adenosine A_1 and A_2-receptor antagonist, aminophylline (APH; 20 $\mu g\ kg^{-1}$ i.c.v.), the A_1 antagonist, 8-cyclopentyl-1,3-dipropylxanthine

(DPCPX; 12 μg kg^{-1} i.c.v.) or had been pretreated with pertussis toxin (PTX), as above and the response to the four doses of APNEA was evaluated. Finally, in another group we tested the cardiovascular effects of an agonist of A_1 adenosine receptors, N^6 cyclohexyladenosine (CHA), alone and after DPCPX.

Complete transection of the spinal cord was performed at the T5-T7 vertebral level.

Drugs and solution/statistical analysis of results

DPCPX and APNEA were initially dissolved in dimethylsulphoxide (DMSO) and diluted with phosphate buffer; the concentration of DMSO was less than 2%. Control animals treated intracerebroventricularly with 0.2 M phosphate buffer or phosphate buffer and DMSO did not show any significant change in blood pressure and heart rate basal values. The volume of injection of drug solution or solvent was 100 nl. The following drugs were used: APNEA, CHA, APH, DPCPX (Research Biochemicals Incorporated MA, U.S.A.); ketamine HCl (Parke-Davis SpA, Lainate-Milan Italy), urethane and pertussis toxin (Sigma Chemical Co. St. Louis MO, U.S.A.).

The changes in the arterial blood pressure (ABP) were calculated as the peak decrease of ABP (mmHg). The statistical differences between group means were performed using ANOVA variance analysis, followed by Newman Keuls test for paired groups. Differences were considered to be significant at $P<0.05$. All results are expressed as means $\pm$ standard error (s.e.m.).

Results

APNEA (0.4–4 μg/kg; $n=8$) injected into the third ventricle of anaesthetized normotensive rats, after DPCPX (12 μg^{-1} kg i.c.v.) injection in the same ventricle, produced a transitory and dose-related decrease in arterial blood pressure (ABP) (Figure 1) without any significant alteration in heart rate (Figure 2). The time course of this effect showed that the response began 7–12 s after i.c.v. administration, peaked between 50–80 s and recovery to basal values occurred between 11–30 min.

In the same way APNEA induced hypotensive responses after pretreatment i.c.v. 5 min) with the A_1 and A_2 antagonist, aminophylline (APH), at the dose of 20 μg kg^{-1} ($n=8$) (Figure 1). In contrast, the APNEA-induced decrease in ABP was significantly inhibited in rats treated, 48 h earlier with pertussis toxin (4 μg kg^{-1} i.c.v., $n=8$) (Figure 1).

Table 1 Arterial blood pressure (ABP; mmHg $\pm$ s.e.m.) and heart rate (HR; beats/min^{-1} $\pm$ s.e.m.) in rats treated with N^6-cyclohexyladenosine (CHA) at doses from 1–5 μg kg^{-1} i.v.) and pretreated with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 12 μg kg^{-1} i.c.v.). Each value is the mean $\pm$ s.e.m. of six experiments

Treatment	*ABP*	*HR*
Controls	121 $\pm$ 6.2	383 $\pm$ 17
CHA 1 μg/kg i.v.	107 $\pm$ 6.1	361 $\pm$ 18
CHA 2.5 μg/kg i.v.	96 $\pm$ 6.4*	344 $\pm$ 16*
CHA 5 μg/kg i.v.	75 $\pm$ 6.0**	314 $\pm$ 19**
CHA 1 μg/kg i.v. + DCPX 12 μg/kg i.c.v.	120 $\pm$ 5.8	380 $\pm$ 18
CHA 2.5 μg/kg i.v. + DCPX 12 μg/kg i.c.v.	118 $\pm$ 5.9	377 $\pm$ 20
CHA 5 μg/kg i.v. + DCPX 12 μg/kg i.c.v.	116 $\pm$ 6.1	373 $\pm$ 21

*$P<0.05$; **$P<0.01$ respect to controls.

Administration of APNEA at dose of 20 μg kg^{-1} i.c.v. ($n=8$) after DPCPX injection induced a -66 ± 5.4 mmHg fall in arterial blood pressure (Figure 1) and after 3 min a -62 ± 6.0 beats/min^{-1} significant ($P<0.05$) decrease in heart rate (Figure 2). The time course of this effect showed that the response began 2.5 min after i.c.v. administration with the maximal decrease at 3 min. Recovery to basal values occurred between 25–30 min.

Transection of the spinal cord abolished the fall in ABP, but did not significantly affect HR decrease (data not shown) induced by APNEA (20 μg kg^{-1} i.c.v. $n=5$) after i.c.v. injection of DPCPX.

The intravenous (i.v.) administration of CHA, a selective agonist at A_1 adenosine receptors, at doses from 1–5 μg kg^{-1}

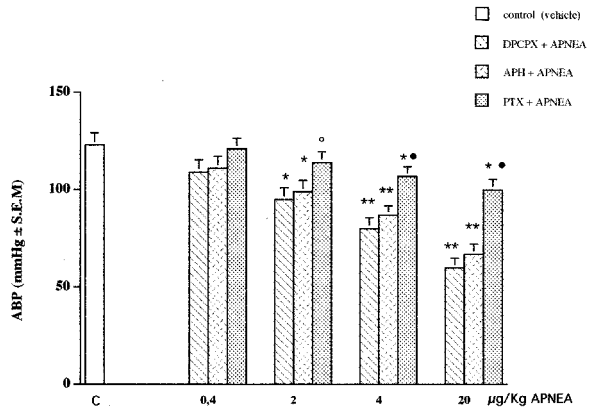

Figure 1 Arterial blood pressure ABP (mmHg $\pm$ s.e.m.) in rats treated i.c.v. with N^6-2-(4-aminophenyl)-ethyladenosine (APNEA) at doses of 0.4–20 μg kg^{-1} i.c.v. and pretreated with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 12 μg kg^{-1} i.c.v.), aminophilline (APH; 20 μg kg^{-1} i.c.v.) and pertussis toxin (PTX; 4 μg kg^{-1} i.c.v.). Each value is the mean $\pm$ s.e.m. of eight experiments. *$P<0.05$, **$P<0.01$ respect to control. $^{\circ}P<0.05$ respect to APNEA + DPCPX and APNEA + APH. $^{\bullet}P<0.01$ respect to APNEA + DPCPX and APNEA + APH.

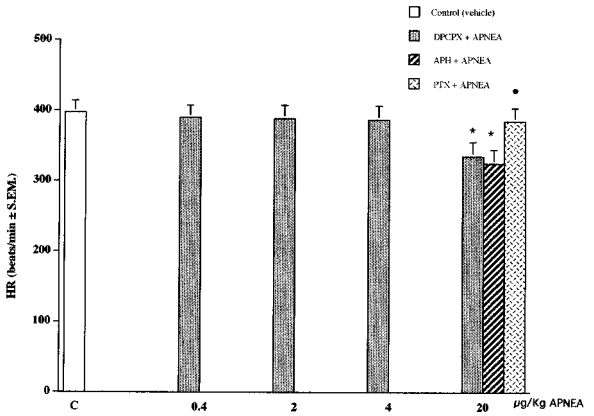

Figure 2 Heart rate (HR; beats/min^{-1} $\pm$ s.e.m.) in rats treated i.c.v. with N^6-2-(4-aminophenyl)-ethyladenosine (APNEA), at doses of 0.4–20 μg kg^{-1} i.c.v., and pretreated with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 12 μg kg^{-1} i.c.v.) aminophilline (APH; 20 μg kg^{-1} i.c.v.) and pertussis toxin (PTX; 4 μg kg^{-1} i.c.v.). The HR decrease at dose of 20 μg kg^{-1} began 2.5 min with maximal decrease at 3 min. Each value is the mean $\pm$ s.e.m. of eight experiments. *$P<0.05$, respect to control. $^{\bullet}P<0.05$, respect to APNEA + DPCPX and APNEA + APH.

($n=5$) induced a significant reduction of blood pressure and heart rate (Table 1) and this effect was completely blocked by pretreatment, 5 min before, with DPCPX (12 μg kg^{-1} i.c.v.).

Administration of APH and DPCPX alone did not cause a significant variation in ABP and HR (APH: -2.3 ± 0.6 mmHg and 8 ± 3.4 beats/min^{-1}); (DPCPX: -1.8 ± 0.4 mmHg and 7 ± 3.2 beats/min^{-1}).

Discussion

It has been reported that the intravenous administration of a A_3 receptor agonist produced a fall in blood pressure of anaesthetized rats (Carruthers & Fozard, 1993) and that the hypotensive response to the adenosine A_3 receptor activation in the rat is largely, if not exclusively, a consequence of mediator release from mast cells (Hannon *et al*., 1995; Fozard *et al*., 1996). Our results show that i.c.v. injection (3rd ventricle) of the A_3 receptor agonist, APNEA, induced a decrease of arterial blood pressure without effects on heart rate at doses from 0.4–4 μg kg^{-1}.

To determine the role of A_3 receptors in the central cardiovascular response to APNEA we investigated the effect of aminophylline, a non-selective A_1 and A_2 antagonist, DPCPX, a selective A_1 antagonist, and pertussis toxin on hypotensive responses to APNEA. After pretreatment with aminophylline, at a dose in excess of those required to block A_1 and A_2 receptors (Stella *et al*., 1993), APNEA induced hypotension. In agreement with other studies, this confirms that in the rat, A_3-receptors are insensitive (Zhou *et al*., 1992) or resistant (Ramkumar *et al*., 1993) to xanthine antagonists. Even after DPCPX pretreatment, APNEA induced a decrease in blood pressure. In contrast, after pretreatment with pertussis toxin (that does not discriminate between A_1 and A_3 receptors), APNEA induced a fall in ABP significantly smaller than that observed after treatment with DPCPX–APNEA or APH+APNEA, demonstrating an involvement of G_i/G_o-proteins. It has been reported, in RBL-2H3 cells, that A_3 receptor activation stimulates inositol 1,4,5-triphosphate production and accumulation of intracellular Ca^{2+} (Ramkumar *et al*., 1993). This response is also sensitive to pertussis toxin, suggesting that is not a $G\alpha_q$-mediated response but may be mediated by the $\beta\gamma$-subunits of G-proteins (Boyer *et al*., 1994). Taken together our results show that the hypotensive effect induced by APNEA was mainly due to the stimulation of A_3 adenosine receptors.

The conclusion that APNEA-induced hypotension was due to the stimulation of central A_3 receptors is based on the observations that: (a) i.c.v. injection of APNEA induced a more intense reduction of arterial blood pressure, than that induced by higher doses administrated i.v. (Carruthers & Fozard, 1993), (b) the latency of this hypotensive effect is very short and (c) the spinal cord transection completely prevented it. Moreover it is interesting to note that, as it is widely reported (Phillis & Wu, 1981; Katims *et al*., 1983; Barraco *et al*., 1986, 1987, 1990, 1991; Dunwiddie, 1985; Marangos & Boulenger, 1985; Biaggioni, 1992, Stella *et al*., 1993, 1995), the central administration of A_1 adenosine receptor agonists always produces bradycardia and decreases arterial blood pressure. In our experiments i.c.v. APNEA was able to induce only a decrease of blood pressure in the presence of an A_1 receptor antagonist. This further confirms that A_3 receptors, which are activated by APNEA together A_1 receptors, are exclusively involved in blood pressure control.

As regard to the relationship between A_3 central receptors and the regulation of heart rate, basing on our experimental data, we exclude the involvement of A_3 central receptors in heart rate regulation. In fact, APNEA, after DPCPX pretreatment, induced bradycardia only with the highest dose (20 μg kg^{-1}), whereas without DPCPX pretreatment even very low doses (0.4 and 2 μg kg^{-1}) of APNEA were able to significantly reduce heart rate, and a dose of 4 μg kg^{-1} was lethal for rats (data not shown). Therefore i.c.v. APNEA injection causes bradycardic effects *via* central A_1 receptor stimulation and these effects are prevented by DPCPX pretreatment. This indirectly confirms that in our experimental conditions central A_3 receptors are not involved in the regulation of heart rate. On the other hand the bradycardic effect induced by 20 μg kg^{-1} of APNEA after DPCPX pretreatment, was surely due to peripheral A_3 receptor stimulation because it appeared 3 min after injection and spinal cord transection was unable to prevent it. It is sure that the dose of DPCPX used is able to block A_1 adenosine receptors because the same i.c.v. dose of DPCPX, which did not prevent APNEA-induced cardiovascular effect, completely prevented the hypotension and bradycardia elicited by intravenous CHA administration, a selective agonist at adenosine A_1 receptors.

In conclusion our data suggest that in the CNS there is a population of A_3-receptors which when activated by an A_3 receptor agonist induces only a decrease in blood pressure without influencing heart rate. Moreover, only the activation of peripheral A_3 adenosine receptors may modulate heart rate.

The authors gratefully acknowledge the support of Italian MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica, 40% and 60%), CNR (Consiglio Nazionale delle Ricerche).

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(Received April 16, 1998
Revised July 20, 1998
Accepted July 21, 1998)