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Characterization of the K^+-channel-coupled adenosine receptor in guinea pig atria

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Summary. In the present work we studied the pharmacological profile of adenosine receptors in guinea pig atria by investigating the effect of different adenosine analogues on $^{86}Rb^+$-efflux from isolated left atria and on binding of the antagonist radioligand 8-cyclopentyl-1,3-[^{3}H]dipropylxanthine ([^{3}H]DPCPX) to atrial membrane preparations. The rate of $^{86}Rb^+$-efflux was increased twofold by the maximally effective concentrations of adenosine receptor agonists. The EC_{50}-values for 2-chloro-N^6-cyclopentyladenosine (CCPA), R-N^6-phenylisopropyladenosine (R-PIA), 5'-N-ethylcarboxamidoadenosine (NECA), and S-N^6-phenylisopropyladenosine (S-PIA) were 0.10, 0.14, 0.24 and 12.9 μM, respectively. DPCPX shifted the R-PIA concentration-response curve to the right in a concentration-dependent manner with a K_B-value of 8.1 nM, indicating competitive antagonism. [^{3}H]DPCPX showed a saturable binding to atrial membranes with a B_{max}-value of 227 fmol/mg protein and a K_D-value of 1.3 nM. Competition experiments showed a similar potency for the three agonists CCPA, R-PIA and NECA. S-PIA is 200 times less potent than R-PIA. Our results suggest that the K^+ channel-coupled adenosine receptor in guinea pig atria is of an A_1 subtype.

Key words: A_1 Adenosine receptors – K^+-channels – Atria – Radioligand binding – $^{86}Rb^+$-efflux

Introduction

Adenosine exerts negative inotropic, chronotropic and dromotropic effects in the heart (Drury and Szent-Györgyi 1929; James 1965; Endoh et al. 1983; Evans et al. 1982). In atrial trabeculae it increases the resting membrane potential and decreases the action potential duration (Johnson and Mc Kinnon 1956; Hollander and Webb 1957; De Gubareff and Sleator 1965). Electrophysiological experiments on atrial myocytes and measurements of $^{42}K^+$-efflux in atrial preparations refered this effect to a receptor-mediated stimulation of the potassium conductance in atrial membranes (Belardinelli and Isenberg 1983; Jochem and Nawrath 1983; West and Belardinelli 1985). These authors suggested that adenosine stimulates the acetylcholine sensitive potassium channel. This adenosine receptor-mediated effect was postulated to be the mechanism of the negative inotropic action of adenosine in guinea pig atria without altering cAMP or cGMP levels (Endoh et al. 1983; Brückner et al. 1985). Several studies showed that a G-protein is involved in the coupling between adenosine receptor and potassium channel in atrial cardiac preparations (Böhm et al. 1986; Kurachi et al. 1986) and in mammalian central neurons (Trussell and Jackson 1987). It is still not clear which adenosine receptor subtype mediates the stimulation of the potassium conductance. In the present study we characterized atrial adenosine receptors by investigating the effect of various adenosine derivatives on the $^{86}Rb^+$-efflux in isolated left guinea pig atria and performing binding experiments on atrial membranes with the A_1 selective antagonist radioligand [^{3}H]8-cylopentyl-1,3-dipropylxanthine [^{3}H]DPCPX.

Abbreviations: CCPA, 2-chloro-N^6-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; NECA, 5'-N-ethylcarboxamidoadenosine; PIA, N^6-phenylisopropyladenosine

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Materials and methods

Materials. [^{3}H]DPCPX and $^{86}Rb^+$ were purchased from Amersham Buchler (Braunschweig, FRG). R-PIA, S-PIA and NECA were obtained from Boehringer Mannheim (Mannheim, FRG). CCPA was synthesized according to Lohse et al. (1988).

Efflux experiments. Guinea pigs were killed by a blow on the neck. The hearts were rapidly excised and the left atria were carefully prepared. They were mounted on stainless steel holders and equilibrated for 15 min at 37° C in incubation buffer bubbled with 95% O_2/5% CO_2. The incubation buffer, pH 7.2, had the following composition in mM: NaCl 128, $NaHCO_3$ 14.4, KCl 4.7, NaH_2PO_4 1.2, MgCl 1.2, Na-Ca-EDTA 0.1, glucose 10, $CaCl_2$ 1.5. The $^{86}Rb^+$-efflux studies were carried out according to the method of Gerstheimer et al. (1987) with slight modifications. The equilibrated quiescent preparations were incubated for 90 min in the buffer solution containing $^{86}Rb^+$ (74 kBq/ml) at 37° C. The holders with the atria were then placed on rotating shafts of a small motor. The atrial preparations were sequentially dipped in tubes containing 5 ml of the mentioned buffer for 3 min in each tube. After 24 min the rate of $^{86}Rb^+$-efflux reached a steady state, then increasing concentrations of agonist were added to the buffer solution. In experiments with antagonist the respective concentrations of the antagonist were added to all tubes of an experiment. At the end of the efflux period the tissue wet weight and the residual radioactivity in the tissue was determined and the radioactivity in the effluents was measured. The rate of $^{86}Rb^+$-efflux was calculated as described (Gerstheimer et al. 1987). The data are means of at least 6 experiments.

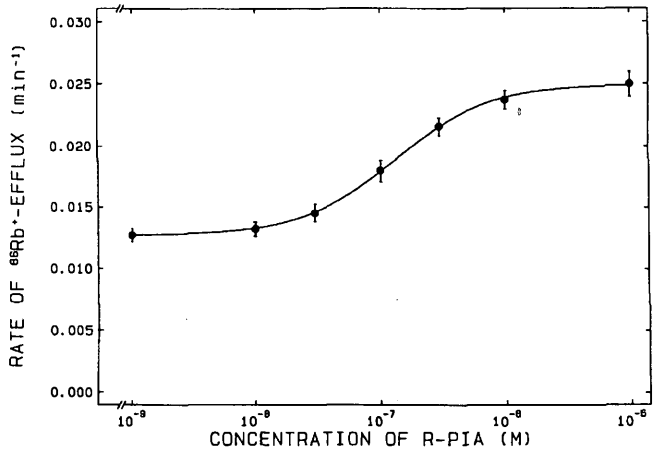

Fig. 1. Effect of R-PIA on the rate of $^{86}Rb^+$-efflux in isolated left guinea pig atria. The concentration of R-PIA was cumulatively increased in the incubation buffer and the effect was studied as described in Methods. The symbols represent the mean value of 6 experiments, the bars are the standard error of the mean

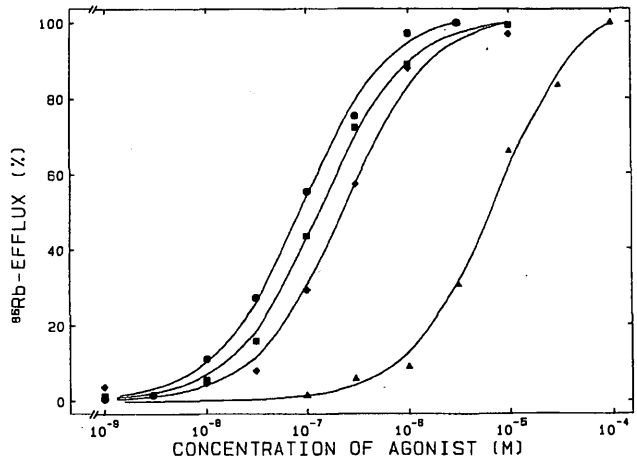

Fig. 2. Effect of different adenosine analogues on the rate of $^{86}Rb^+$ efflux in isolated left guinea pig atria. Data (means of 6 experiments) are expressed as percent of the maximal effect induced by CCPA (●), R-PIA (■), NECA (◆), S-PIA (▲)

Preparation of atrial membranes. The guinea pig atrial membranes were prepared according to Lohse et al. (1985) with some modifications. The atria were suspended in ice cold 10 mM imidazole/5 mM $MgSO_4$/0.3 M sucrose buffer (pH 7) and homogenized with a polytron for 20 s followed by 2 strokes of a glas-teflon potter. The sucrose concentration was then elevated to 0.6 M. The homogenate was centrifuged at 21000 × *g* for 30 min at 4° C. The supernatant was diluted with 1.5 volumes 10 mM imidazole/5 mM $MgSO_4$/160 mM KCl (pH 7) and centrifuged at 30000 × *g* for 45 min at 4° C. The resulting pellet was resuspended in 50 mM Tris-HCl, pH 7.4, and stored at −80° C. Protein concentrations were measured according to Peterson (1977). The yield of membrane protein was about 0.5 mg/10 atria.

Radioligand binding. The binding of [^{3}H]DPCPX to atrial membranes was carried out at a final protein concentration of 14 µg/tube in a total volume of 200 µl according to Lohse et al. (1987). In typical experiments (0.2 nM [^{3}H]DPCPX) total binding was approximately 200 cpm compared to 40 cpm nonspecific binding. The nonspecific binding was defined by the presence of 10 µM R-PIA. In saturation experiments 50 µg protein/tube in a total volume of 500 µl were used. The incubation was carried out at 12° C for 2 h. The reaction was stopped by filtration over Whatman GF/B filters. The radioactivity was determined by liquid scintillation counting for 10 min.

Results

Measurement of $^{86}Rb^+$-efflux

First we tested the effect of R-PIA on K^+ conductance by measuring $^{86}Rb^+$-efflux from atrial tissue in presence of increasing concentrations of R-PIA. Figure 1 shows the concentration-response curve of the R-PIA effect on the rate of $^{86}Rb^+$-efflux from guinea pig left atria. R-PIA caused a concentration-dependent increase in the efflux rate. At maximal concentrations the rate of efflux was twice the basal value. The EC_{50} of R-PIA was 137 nM. The effect of various

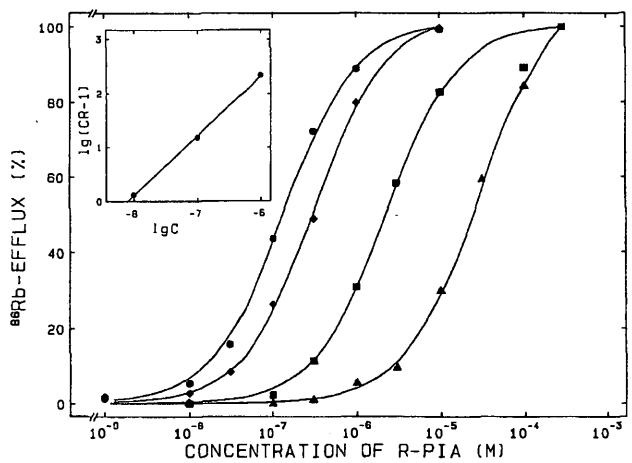

Fig. 3. Effect of the adenosine antagonist DPCPX on R-PIA-stimulated $^{86}Rb^+$-efflux in guinea pig atria. The effect of R-PIA was measured in the absence (●) and the presence of 10 nM (◆), 100 nM (■) and 1000 nM (▲) DPCPX. Data are expressed as percent of the maximal change in $^{86}Rb^+$-efflux caused by R-PIA. *Inset:* Schild plot of the data. C = molar concentration of DPCPX, CR = ratio of the EC_{50} values of R-PIA in the presence and absence of DPCPX, $n = 6$

adenosine derivatives on the $^{86}Rb^+$-efflux is shown in Fig. 2 and the EC_{50} values are 103 nM for CCPA, followed by R-PIA (137 nM), NECA (217 nM) and S-PIA (12905 nM). The $^{86}Rb^+$-efflux was stimulated by the PIA-enantiomers in a highly stereoselective manner with R-PIA being about 100-fold more potent than S-PIA.

The adenosine receptor mediated $^{86}Rb^+$-efflux was then antagonized with DPCPX, which is highly selective for the A_1 subtype. Figure 3 shows concentration-response curves of R-PIA in the absence and presence of different concentrations of DPCPX. DPCPX caused a concentration-dependent shift of the concentration-response curve to the right. A Schild plot of these data gave a line with a slope of 1.1 and a K_B-value of 8.2 nM for DPCPX.

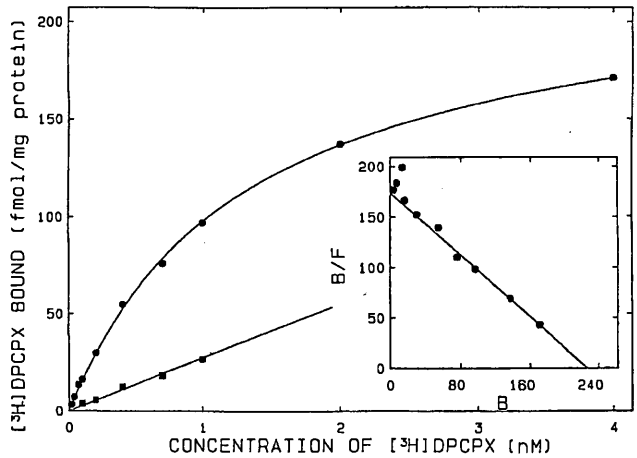

Fig. 4. Saturation of [^{3}H]DPCPX binding to membranes of guinea pig atria. Specific binding (●), nonspecific binding (■). *Inset:* Scatchard plot of the data; B = [^{3}H]DPCPX bound (fmol/mg protein), F = concentration of [^{3}H]DPCPX (nM). Computerized curve fitting gave a B_{max} of 227 fmol/mg protein and a K_D of 1.3 nM

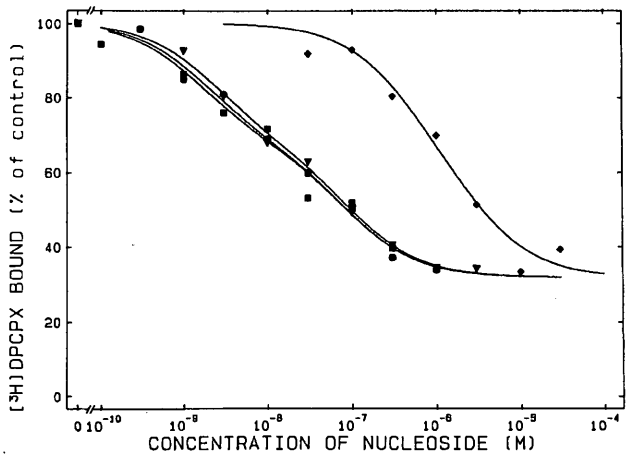

Fig. 5. Competition for [^{3}H]DPCPX binding to guinea pig atrial membranes by adenosine receptor agonists. Membranes were incubated with 0.2 nM [^{3}H]DPCPX in the presence of increasing concentrations of CCPA (●), R-PIA (■), NECA (◆) and S-PIA (▲). Competition curves were simultaneously fitted with the program SCTFIT. The data were best fitted assuming a two side model, and the proportions of receptors in the high and the low affinity state were 48% and 52%, respectively

Radioligand binding studies

To further characterize the atrial adenosine receptors we performed radioligand binding experiments on membrane preparations from guinea pig atria. Figure 4 shows a saturation experiment with [^{3}H]DPCPX. By non-linear curve-fitting of the data a K_D-value of 1.5 nM and a binding capacity of 200 fmol/mg protein was calculated.

Furthermore, we investigated the pharmacological profile of the receptors by competition with different agonists for [^{3}H]DPCPX binding on atrial membranes (Fig. 5). The biphasic character of the competition curves indicates the presence of two affinity states for the agonists, with one half of the binding sites being in the high affinity state and the other half in the low affinity state. R-PIA is about 200-fold more potent than S-PIA. The competition curves of the three agonists CCPA, R-PIA and NECA are almost superimposable. Table 1 summarizes the functional and the binding data. It is obvious that the EC_{50} values from the efflux experiments are in the same concentration range as the K_D-values for the low affinity state.

Table 1. Effect of different adenosine analogues on the binding of [^{3}H]DPCPX to guinea pig atrial membranes and on $^{86}Rb^+$-efflux rate in isolated left guinea pig atria. K_H and K_L are the K_D values of the high and low affinity states for agonist, respectively. The EC_{50}-values for $^{86}Rb^+$-efflux are calculated from 5–6 experiments and confidence limits are given in brackets. The EC_{50}-values of CCPA, R-PIA and NECA are not significantly different

	Radioligand binding		$^{86}Rb^+$-Efflux
	K_H (nM)	K_L (nM)	EC_{50} (nM)
CCPA	1.2	62	103 (37-290)
R-PIA	1.4	61	137 (85-222)
NECA	1.8	68	217 (157-301)
S-PIA	331	1786	12905 (6336-26286)

Discussion

Adenosine exhibits a negative inotropic effect on the heart (Hollander and Webb 1957; De Gubareff and Sleator 1965). The negative inotropic effect on the ventricle could be measured only after prestimulation with isoprenaline, while in the atria it showed a direct inhibition of the basal force of contraction (Schrader et al. 1977; Dobson 1978; Belardinelli et al. 1982; Belardinelli and Isenberg 1983a; Böhm et al. 1984). In the ventricle the inhibitory effect of adenosine on isoprenaline-stimulated force of contraction has been attributed to an inhibition of cardiac adenylate cyclase (Schrader et al. 1977; Dobson 1983; Hosey et al. 1984). In guinea pig ventricular myocytes R-PIA caused an inhibition of forskolin-mediated cAMP accumulation (West et al. 1986). Similarly, studies on rat ventricular myocytes showed an inhibition of the isoprenaline-induced elevation of cAMP levels by adenosine receptor agonists (Henrich et al. 1987; Martens et al. 1987). Radioligand binding experiments using membranes prepared from rat ventricular myocytes showed the existence of A_1 adenosine receptor in ventricular tissue which mediates inhibition of adenylate cyclase (Martens et al. 1987). In atrial tissue, however, the direct negative inotropic effect of adenosine was not accompanied by an alteration in either cAMP or cGMP level (Endoh et al. 1983; Böhm et al. 1984). These authors suggested the existence of an adenosine receptor not coupled to the adenylate cyclase. This suggestion is consistent with previous electrophysiological studies in which it was reported that adenosine caused shortening of the atrial action potential (Johnson and Mc Kinnon 1956; Belardinelli and Isenberg 1983b; West and Belardinelli 1985), an effect attributed to an increased potassium conductance. Jochem and Nawrath (1983) showed that adenosine caused an increase in the rate constant of $^{42}K^+$ efflux in resting guinea pig left atria. Our aim was to clarify via which receptor subtype the atrial action of adenosine is mediated.

In the present study adenosine analogues caused a stimulation of the $^{86}Rb^+$-efflux rate in isolated left guinea pig atria. The rank order of potency for the tested adenosine derivatives was CCPA ≥ R-PIA ≥ NECA ≫ S-PIA which is consistent with an A_1 receptor subtype. The differences between the potencies of the three agonists CCPA, R-PIA and NECA are only small in the functional studies and virtually absent in the binding studies. This is somewhat different from classical A_1 receptor profiles in rat brain membranes (Bruns et al. 1980; Lohse et al. 1984; Lohse et al. 1988), in bovine myocardium (Lohse et al. 1985) and rat ventricular myocytes (Martens et al. 1988), which show a more distinct difference between these agonists. On the other hand, the pronounced stereoselectivity for the PIA enantiomers is characteristic for an A_1 receptor subtype. Our findings also demonstrate that the adenosine receptor-mediated stimulation of $^{86}Rb^+$ efflux is competitively antagonized by the A_1 selective antagonist DPCPX.

Radioligand binding studies on membrane preparation of guinea pig atria with [^{3}H]DPCPX showed a saturable binding which further supports the conclusion that the atrial adenosine receptors are of an A_1 subtype. This finding is in keeping with a recent report of A_1 receptor determined by binding studies on porcine atrial membranes (Leid et al. 1988). These authors, however, reported a higher affinity of S-PIA than NECA which is different from our results obtained on guinea pig atria. It can not be excluded that this A_1 receptor, in addition to modulating a K^+-channel, is also coupled to adenylate cyclase. Endoh et al. (1983) observed that the antiadrenergic effect of adenosine on the force of contraction of rat atria was accompanied by a decrease in the cAMP level.

The EC_{50}-values in the $^{86}Rb^+$ efflux experiments were in the same concentration range as the K_D-values for the low affinity binding sites for agonists. This finding is in agreement with the results of Martens et al. (1988), who reported that the IC_{50}-values of adenosine analogues for the inhibition of the isoprenaline-stimulated cAMP accumulation in ventricular myocytes are in the same concentration range as the K_D-values for the low affinity state. We conclude that the cardiac effect of adenosine receptor agonists in the atria, as in the ventricle, is mediated via the low affinity state of the receptors.

In summary, our results support the suggestion that there are K^+-channel-coupled adenosine receptors in guinea pig atria. They exhibit small differences to the classical adenylate cyclase-coupled A_1 receptor concerning the agonist affinity profile of the receptor. However, we conclude that this receptor is of the A_1 receptor family, since it possesses the main characteristics of this receptor type: (1) The A_1-selective antagonist DPCPX competitively inhibits the adenosine receptor-mediated stimulation of the $^{86}Rb^+$-efflux, (2) radioligand binding showed a saturable high affinity binding of [^{3}H]DPCPX, (3) the pronounced stereoselectivity for the two PIA enantiomeres demonstrated in functional and binding experiments.

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