0026-895X/90/060916-05$02.00/0

MOLECULAR PHARMACOLOGY, 37:916-920

Pharmacological Characterization of the Adenylate Cyclase-Coupled Adenosine Receptor in Isolated Guinea Pig Atrial Myocytes

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Received November 9, 1989; Accepted March 28, 1990

SUMMARY

Although adenosine is known to activate K^+ conduction in atrial tissue, there is still debate as to the involvement of cAMP-dependent mechanisms. In isolated adult guinea pig atrial myocytes, we demonstrate that the highly A_1-selective adenosine receptor agonist 2-chloro-N^6-cyclopentyladenosine reduced basal cAMP levels by 30–40% in the absence and presence of the nonxanthine phosphodiesterase inhibitor Ro 20-1724. Isoprenaline caused a concentration-dependent increase in cAMP levels, which was more pronounced in the presence of the phosphodiesterase inhibitor. Several adenosine derivatives suppressed the isoprenaline-induced cAMP increase by approximately 80%. The rank order of potency was 2-chloro-N^6-cyclopentyladenosine (IC_{50}, 93 nM) > (*R*)-N^6-phenylisopropyladenosine (IC_{50}, 309 nM) > 5′-*N*-ethylcarboxamidoadenosine (IC_{50}, 813 nM) ≫ (*S*)-N^6-phenylisopropyladenosine (IC_{50}, 26,300 nM). A similar but complete suppression of the isoprenaline-induced cAMP increase was produced by the muscarinic receptor agonist carbachol (IC_{50}, 398 nM), which like adenosine is known to activate atrial K^+ channels. The A_1-adenosine receptor-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine antagonized the effect of 2-chloro-N^6-cyclopentyladenosine concentration-dependently, with a K_B value of 9.6 nM. In atrial myocytes isolated from guinea pigs pretreated with pertussis toxin, the inhibitory effects of adenosine analogs on basal and isoprenaline-stimulated cAMP accumulation were markedly attenuated. It is concluded that the adenosine receptor in guinea pig atrial myocytes, which is known to be linked to K^+ channels, is also coupled to adenylate cyclase via a pertussis toxin-sensitive guanine nucleotide-binding protein and shows the characteristics of the A_1-adenosine receptor subtype.

Adenosine has a number of cardiac effects, which generally are inhibitory. In ventricular tissue, it has little direct effect on contractility or electrical activity, but it antagonizes positive inotropic effects elicited by cAMP-increasing agents (1–3). The ionic basis for these indirect adenosine actions has been proposed to be a decrease in catecholamine-stimulated Ca^{2+} conductance (4, 5). Controversial findings have been made concerning the contribution of changes in adenylate cyclase activity to these effects, i.e., stimulation (6), no effect (3), or inhibition (7). However, it has been shown in ventricular myocytes that the cAMP increase induced by compounds such as isoprenaline and forskolin, which enhance the activity of adenylate cyclase, is reduced by several adenosine derivatives (8–11).

In the atrium, adenosine exerts direct inhibitory effects, which are suggested to be mediated by A_1-adenosine receptors (12–14). Regarding the underlying mechanism, major importance has been ascribed to the role of K^+ channels. In atrial myocytes, adenosine increases a K^+ conductance of the cell membrane (15–17), causing hyperpolarization and, hence, shortening of the action potential duration. This effect is mediated via a GTP-dependent regulatory protein that is inhibited by pertussis toxin (18).

Although A_1 receptors appear to mediate the negative inotropic effects of adenosine in atrial tissue, it is still subject to debate whether changes in adenylate cyclase activity are involved. While Endoh *et al.* (19) reported that in atrial preparations the antagonism by adenosine of β-adrenergic effects was accompanied by decreases in elevated cAMP levels, others failed to observe concomitant reductions of cAMP content (3, 20).

These divergent results might be related to the cellular heterogeneity of cardiac tissue. Therefore, we used a unicellular preparation of guinea pig atria and studied the inhibitory effects

This study was supported by the Deutsche Forschungsgemeinschaft.

ABBREVIATIONS: PIA, N^6-phenylisopropyladenosine; CCPA, 2-chloro-N^6-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; NECA, 5′-*N*-ethylcarboxamidoadenosine; Ro 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; ScAMPTME, 2′-*O*-monosuccinyladenosine-3′,5′-cyclic monophosphate tyrosyl methyl ester; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; G protein, guanine nucleotide-binding protein.

of different adenosine analogs on cAMP levels in isolated adult guinea pig atrial myocytes. Furthermore, we examined the effect of a pretreatment with pertussis toxin on the action of the adenosine derivatives.

Experimental Procedures

Materials. Carrier-free $Na^{125}I$ was purchased from Amersham-Buchler (Braunschweig, FRG). Adenosine deaminase (200 units/mg), (R)-PIA, (*S*)-PIA, and NECA were obtained from Boehringer Mannheim GmbH (Mannheim, FRG). DPCPX and CCPA were synthesized according to the method of Lohse *et al.* (21, 22). Ro 20-1724 was a gift from Dr. W. E. Scott (Hoffmann-La Roche, Nutley, NJ). Isoprenaline, ScAMPTME, bovine serum albumin fraction V, crude collagenase (C2139), deoxyribonuclease I, protease type XIV, and pertussis toxin (islet-activating protein) were from Sigma (Deisenhofen, FRG). Elastase 20931 was purchased from Serva (Heidelberg, FRG). All chemicals were of analytical grade or the best commercial grade available.

Preparation of ^{125}I-ScAMPTME. ^{125}I-ScAMPTME was prepared by the chloramine-T method (23), and the reaction product was separated by gel filtration on Sephadex G-25 superfine and stored at −25°.

Isolation of atrial myocytes. Atrial myocytes were isolated from hearts of adult male guinea pigs (300–350 g). For some experiments, guinea pigs were pretreated with pertussis toxin (180 μg/kg intravenously) 27 hr before the experiments, during brief ether anesthesia. Control animals were treated in the same way with the solvent alone (sodium phosphate, pH 7.5, 0.5 M NaCl; 750 μl/100 g of body weight). Dispersed atrial cells were obtained according to the method of Bechem *et al.* (24), with minor modifications. The animals were injected with 500 units of sodium heparin intraperitoneally and killed 20 min later by a blow on the neck. They were bled from the carotid arteries, and the hearts were rapidly excised and mounted on a cannula of a Langendorff perfusion system. The isolated hearts were perfused for 5–10 min, at a pressure of 50 cm of water, with a calcium-free perfusion solution (solution A) consisting of (mM): NaCl, 140; KCl, 5.4; $MgCl_2$, 1.0; glucose, 5.0; and HEPES-NaOH, 10; pH 7.4, which was continuously gassed with O_2 and maintained at 37°. All following steps were done at 37°. The hearts were subsequently perfused in a recirculating manner with a solution of the same composition containing 0.5 mM EGTA and supplemented with collagenase (1 mg/ml), elastase (10 μl of suspension/ml), deoxyribonuclease (0.3 mg/ml), protease (0.1 mg/ml), and bovine serum albumin (0.5 mg/ml). After 20 min, the hearts were removed from the perfusion apparatus and the atria were transferred into Petri dishes containing 2 ml of solution A with added bovine serum albumin (0.5 mg/ml) and deoxyribonuclease (0.3 mg/ml). The atria were cut into three or four pieces and dissolved by gently moving the pieces of tissue through the solution. The resulting cell suspension was transferred into solution A and passed through 150-μm mesh nylon gauze. Calcium was gradually increased to 1.0 mM. Myocyte yield (about 1.2×10^6 cells/heart) and viability (about 65–80%) were determined by microscopic examination. Noncardiomyocyte cell contributions were negligible. Cells were considered viable when they were spindle-shaped or beginning to round up but showed no blebs or granulation. Isolated atrial myocytes remained viable for at least 60 min in solution A supplemented with bovine serum albumin and deoxyribonuclease.

TABLE 1

Effect of isoprenaline, CCPA, and Ro 20-1724 on cAMP levels of isolated guinea pig atrial myocytes

cAMP was measured after 15-min incubation of myocytes with adenosine deaminase (0.5 units/ml) and the indicated additions. Data are means ± standard errors of six experiments.

Additions	cAMP	
	Control	+ Ro 20-1724 (0.5 mM)
	pmol/10⁶ cells	
None	7.1 ± 1.2	11.8 ± 1.0
CCPA (1 μM)	4.2 ± 0.6*	8.4 ± 0.9*
Isoprenaline (1 μM)	25.5 ± 2.7	62.7 ± 3.7
+ CCPA (1 μM)	8.6 ± 1.4*	22.0 ± 2.6*

*$p < 0.05$ versus preceeding value (*t* test).

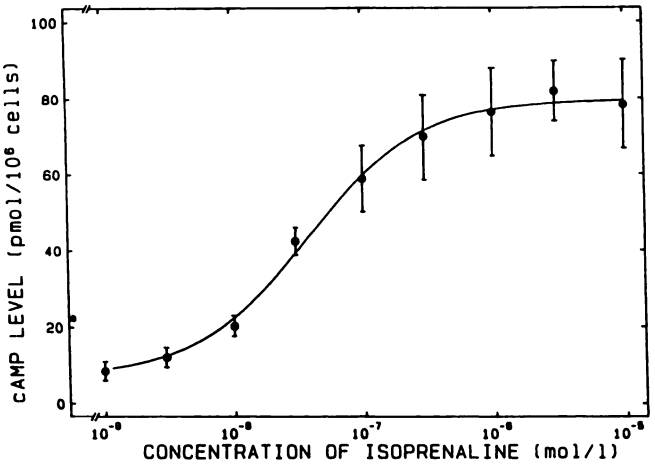

Fig. 1. Effect of isoprenaline on cAMP levels of guinea pig atrial myocytes. The cAMP content was measured 15 min after addition of increasing concentrations of isoprenaline, in the presence of Ro 20-1724 (0.5 mM) and adenosine deaminase (0.5 units/ml), as described in Experimental Procedures. The basal value was 8.4 ± 2.8 pmol of cAMP/10^6 cells. Values are means ± standard errors of three experiments.

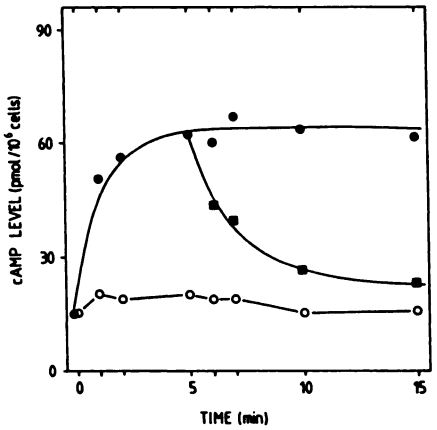

Fig. 2. Time course of the effects of isoprenaline and CCPA on cAMP levels. Myocytes were incubated either in the absence (○) or in the presence (●) of 1 μM isoprenaline. The basal value was 14.0 pmol of cAMP/10^6 cells. After 5 min, CCPA (1 μM) was added to the isoprenaline-stimulated cells (■). Data are from one representative experiment.

Measurement of cAMP. cAMP content of freshly isolated atrial myocytes (7,000–10,000 cells/tube) was measured at 37° in a total volume of 100 μl of solution A (pH 7.4) supplemented with 1.0 mM $CaCl_2$. Substances routinely added were 0.5 units/ml adenosine deaminase to remove endogeneous adenosine, 0.5 mM Ro 20-1724 to inhibit phosphodiesterase activity, and 1 μM isoprenaline to stimulate cAMP formation. The incubation was started by the addition of 70 μl of the cell suspension and was terminated after 15 min by the addition of 900 μl of 0.2 M sodium acetate buffer (95°; pH 6.0). cAMP was determined by a sensitive radioimmunoassay, using ^{125}I-ScAMPTME as labeled tracer (25).

Data analysis. Concentration-response curves were fitted to the Hill equation by a nonweighted nonlinear curve-fitting program, as described earlier (26).

Results

Incubation of isolated adult guinea pig atrial myocytes with 1 μM CCPA resulted in a 40% decrease in basal cAMP levels

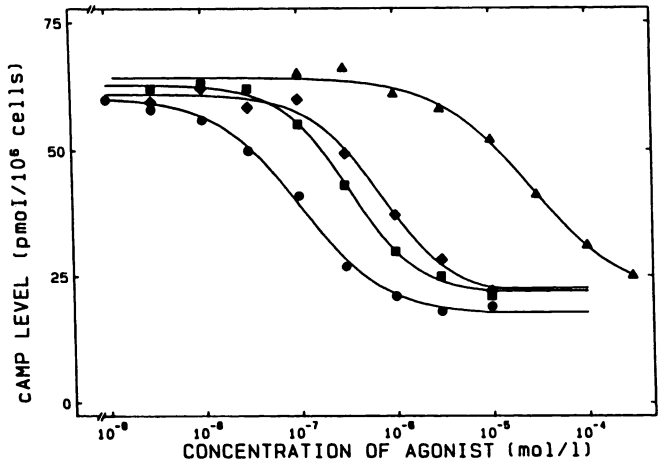

Fig. 3. Effect of adenosine analogs on cAMP levels of guinea pig atrial myocytes. Myocytes were incubated with 1 μM isoprenaline and increasing concentrations of CCPA (●), (*R*)-PIA (■), NECA (♦), or (*S*)-PIA (▲), for 15 min. The basal value (mean ± standard error) was 11.6 ± 3.2 pmol of cAMP/10^6 cells. Data are means of four experiments.

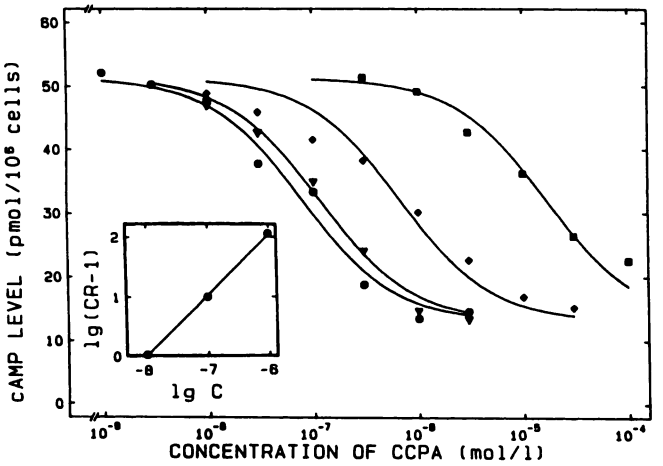

Fig. 4. Effect of the adenosine antagonist DPCPX on the CCPA-induced reduction of cAMP levels in guinea pig atrial myocytes. cAMP levels were raised by 1 μM isoprenaline. The basal value (mean ± standard error) was 9.6 ± 2.5 pmol of cAMP/10^6 cells. Myocytes were incubated for 15 min before the cAMP content was measured as described in Experimental Procedures. The effect of CCPA was examined in the absence (●) and in the presence of 10 nM (▼), 100 nM (♦), and 1000 nM (■) DPCPX. *Inset*, Schild plot of the data. *C*, concentration of DPCPX (in M); *CR*, ratio of the IC_{50} values of CCPA in the presence and absence of DPCPX. Data are means of three experiments.

(Table 1). Isoprenaline (1 μM) caused a 3-fold increase in cAMP content, which was almost completely suppressed by 1 μM CCPA.

In the presence of the nonxanthine phosphodiesterase inhibitor Ro 20-1724 (0.5 mM), basal cAMP levels were elevated by 70%. Under these conditions, 1 μM CCPA produced a 30% reduction in basal cAMP levels, whereas 1 μM isoprenaline induced an almost 6-fold increase in cAMP accumulation (Table 1). CCPA (1 μM) reduced isoprenaline-enhanced cAMP levels by 80%. The isoprenaline-induced cAMP increase was larger in the presence of the phosphodiesterase inhibitor, and the inhibitory effect of CCPA was more easily detectable. Therefore, all other experiments were performed in the presence of 0.5 mM Ro 20-1724.

Fig. 1 shows the concentration-response curve for isoprenaline. The maximally effective concentration of isoprenaline (1 μM) produced a pronounced increase in cAMP levels. The EC_{50} value for this effect was 37 nM.

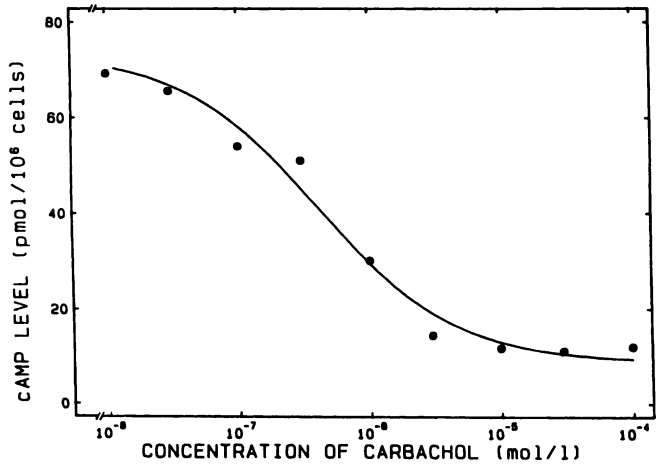

Fig. 5. Effect of carbachol on cAMP levels of guinea pig atrial myocytes. cAMP levels were enhanced by 1 μM isoprenaline. The basal value (mean ± standard error) was 11.8 ± 1.7 pmol of cAMP/10^6 cells. Myocytes were incubated for 15 min before the cAMP content was measured as described in Experimental Procedures. Data are means of three experiments.

A time course of the effects of isoprenaline (1 μM) and CCPA (1 μM) on cAMP formation is illustrated in Fig. 2. The rise in cAMP was rapid, being about 4-fold after 1 min and maximal after 5 min. Addition of the adenosine receptor agonist CCPA resulted in a rapid decrease of isoprenaline-stimulated cAMP levels.

The following set of experiments was done to study the effects of various adenosine derivatives on isoprenaline-stimulated cAMP formation. In Fig. 3, the concentration-response curves for agonists are shown. All agonists reduced cAMP levels in a concentration-dependent manner. CCPA was the most potent agonist, with an IC_{50} value of 93 nM, being about 3-fold more potent than (*R*)-PIA and about 9-fold more potent than NECA (Table 2). (*R*)-PIA was about 85 times more potent than (*S*)-PIA in reducing the isoprenaline-mediated cAMP increase (Table 2), indicating the stereospecific action of the PIA diastereomers.

The influence of pertussis toxin on the effects of adenosine analogs on basal and isoprenaline-stimulated cAMP accumulation is shown in Table 3. The reduction of basal cAMP levels by CCPA was abolished by pretreatment of guinea pigs with pertussis toxin before the isolation of atrial myocytes. The inhibitory effects of several adenosine derivatives on isoprenaline-induced cAMP increases were partially reversed by pretreatment with pertussis toxin.

The A_1 receptor-selective antagonist DPCPX reversed the inhibitory effect of CCPA (Fig. 4). DPCPX shifted the concentration-response curve of CCPA to the right, in a concentration-dependent manner. A Schild plot of the data gave a line with a slope near unity and a K_B value of 9.6 nM. Isoprenaline-stimulated cAMP levels were not substantially altered by DPCPX, confirming that DPCPX has no inhibitory effect on phosphodiesterase activity (21).

Furthermore, the effect of the muscarinic receptor agonist carbachol on the cAMP content of guinea pig atrial myocytes was examined (Fig. 5). The isoprenaline-elevated cAMP levels were reduced in a concentration-dependent manner, with a

TABLE 2

Effect of different adenosine analogs and the muscarinic receptor agonist carbachol on cAMP accumulation in guinea pig atrial myocytes, on the binding of [^{3}H]DPCPX to guinea pig atrial membranes, and on $^{86}Rb^+$ efflux rate in isolated left guinea pig atria

Values presented are geometric means of three to six experiments. The 95% confidence limits are given in parentheses.

Compounds	cAMP in atrial myocytes IC_{50}	Radioligand binding in membranes		$^{86}Rb^+$ efflux in atria, EC_{50}
		K_H	K_L	
		nM		
CCPA	93 (57–154)	1.2[a]	62[a]	103[a] (37–290)
(*R*)-PIA	309[b] (245–389)	1.4[a]	61[a]	137[a] (85–222)
NECA	813[b] (562–1,202)	1.8[a]	68[a]	217[a] (157–301)
(*S*)-PIA	26,300[b] (17,378–41,686)	331.0[a]	1,786[a]	12,905[a] (6,336–26,286)
Carbachol	398 (133–1,183)	ND[c]	ND	ND

[a] Data from Ref. 29.
[b] $p < 0.05$ versus preceeding substance (*t* test).
[c] ND, not determined.

TABLE 3

Influence of pertussis toxin on the effects of isoprenaline and different adenosine analogs on cAMP levels of isolated guinea pig atrial myocytes

Animals were pretreated with pertussis toxin or the solvent alone, as described in Experimental Procedures. cAMP was measured after 15-min incubation of myocytes with adenosine deaminase (0.5 units/ml), Ro 20-1724 (0.5 mM), and the indicated additions. Data are means ± standard errors of three experiments.

Additions	cAMP	
	Control	Pretreatment with pertussis toxin
	pmol/10^6 cells	
None	14.4 ± 3.7	14.9 ± 4.1
CCPA (10 μM)	9.5 ± 2.5	16.3 ± 1.6
Isoprenaline (1 μM)	74.0 ± 2.1	69.6 ± 8.8
+ CCPA (10 μM)	16.8 ± 0.7[a]	32.4 ± 2.9[a]
+ (*R*)-PIA (10 μM)	22.8 ± 0.6[a]	39.3 ± 4.6[a]
+ NECA (10 μM)	22.1 ± 0.9[a]	38.0 ± 3.3[a]
+ (*S*)-PIA (300 μM)	28.3 ± 0.4[a]	48.2 ± 4.7

[a] $p < 0.05$ versus corresponding isoprenaline-stimulated value (*t* test).

half-maximal inhibition at a concentration of 398 nM (Table 2). Maximally effective concentrations of carbachol completely suppressed isoprenaline-stimulated cAMP levels.

Discussion

Similar to earlier studies with ventricular myocytes, the present study shows that, in guinea pig atrial myocytes, different adenosine analogs caused concentration-dependent decreases in isoprenaline-stimulated cAMP levels.

In atrial myocytes, two major findings were obtained suggesting the involvement of an A_1 adenosine receptor in the mediation of these effects. Firstly, the rank order of potency for adenosine analogs was CCPA > (*R*)-PIA > NECA ≫ (*S*)-PIA. Thus, including the marked stereoselectivity between (*R*)- and (*S*)-PIA, it closely resembles the pharmacological profile of cardiac A_1 receptors described in ventricular preparations. This has been demonstrated in functional studies on the inhibition of cAMP accumulation in rat ventricular myocytes (10, 11) and in radioligand binding experiments in bovine myocardium (27) or in rat ventricular myocytes (28). Secondly, the inhibitory effect of the adenosine derivatives on isoprenaline-stimulated cAMP accumulation in atrial myocytes was antagonized by the A_1 receptor-selective antagonist DPCPX.

The agonist profile in this study differs somewhat from our recent report (29), which, by means of $^{86}Rb^+$ efflux measurements, demonstrated a K^+ channel-coupled adenosine receptor of the A_1 receptor family in guinea pig atria. Contrary to the present work, only small differences in the potencies of CCPA, (*R*)-PIA, and NECA were observed (Table 2). In radioligand binding studies, these differences were virtually absent, because almost identical K_i values for the agonists were obtained (Table 2). The reason for this discrepancy remains to be elucidated. One possibility is a heterogeneity of A_1 receptors, although the results obtained in binding experiments do not favor this hypothesis. Another explanation for the divergent results would be that two different G proteins are involved in the atrial effects of adenosine, which are coupled to a homogeneous A_1 receptor population. The G proteins involved in these processes, mediating the activation of K^+ channels and the inhibition of adenylate cyclase activity, have been termed G_K and G_i, respectively. Their identities remain to be established (30). It is even unknown whether G_k and G_i represent distinct G proteins. Recently, it has been reported that G_i subunits had G_k activity in guinea pig atrial cells (31). Previously, it has been demonstrated that pertussis toxin-sensitive G proteins are involved in the activation of K^+ channels in guinea pig atrial myocytes (18). Similarly, the present results indicate the existence of a pertussis toxin-sensitive G protein that mediates the inhibitory effects of the atrial A_1 receptor on adenylate cyclase activity. Yet, it cannot be distinguished at present whether the above-mentioned differences in the pharmacological profiles of atrial A_1-adenosine receptors are due to a heterogeneity of A_1 receptors, G proteins, or the involvement of additional factors.

Inconsistent results have been obtained in previous studies on the effect of adenosine on cAMP levels in isolated atria (3, 19, 20). The interpretation of these results might be complicated by the fact that isolated atria contain a mixture of cell types. An A_1 receptor-mediated decrease of cAMP in myocytes might, therefore, be masked by a concomitant activation of A_2 receptors of endothelial cells (32). By the use of isolated atrial myocytes, the disadvantages of whole organ preparations can be excluded.

Additional support for the assumption that atrial adenosine receptors are of the A_1 subtype has been provided recently. Radioligand binding studies showed the presence of an A_1-adenosine receptor in porcine atrium (33, 34). Furthermore, in cultured embryonic chick atrial myocytes, the existence of A_1-adenosine receptors, which are coupled to adenylate cyclase and to the contractile state in an inhibitory pertussis toxin-sensitive manner, was demonstrated. (35). This study also showed that adenosine analogs were capable of inhibiting both the basal and isoprenaline-stimulated adenylate cyclase activity, although the extent of the inhibitory effect was much less than in the present study. In all of the studies cited, somewhat different agonist profiles were obtained but the involvement of the A_1 receptor subtype was suggested, because the highly A_1-selective antagonist DPCPX showed the expected properties. It should be considered that deviations from the prototypical A_1 profile for agonists might partly be due to species differences.

The extent of inhibition of isoprenaline-induced cAMP accumulation by adenosine was different in atrial and ventricular cellular preparations. In our present study on guinea pig atrial myocytes, adenosine analogs caused a marked reduction (80–90%) of isoprenaline-stimulated cAMP formation, whereas in

rat ventricular myocytes (10, 11) and in guinea pig ventricular myocytes (36) only a 40–50% inhibition of isoprenaline-enhanced cAMP content was observed. This difference in efficacy may be explained by a higher adenosine receptor density in atrial than in ventricular myocardium (14, 37).

It is generally accepted that adenosine and acetylcholine exert similar actions via different receptors but common mechanisms. These include stimulation of K^+ conductance (15, 18, 38) and inhibition of adenylate cyclase (39, 40). Thus, in comparison with adenosine analogs, the muscarinic receptor agonist carbachol showed a similar but even more effective inhibition of isoprenaline-stimulated cAMP accumulation. The greater efficacy of carbachol might be related to the greater density of muscarinic receptors than adenosine receptors in atrial tissue (37).

In summary, our results suggest that the adenylate cyclase-coupled adenosine receptor present in isolated adult guinea pig atrial myocytes has the characteristics of the A_1 subtype. Together with the data from previous studies, atrial adenosine receptors in guinea pig heart appear to be linked to K^+ channels, as well as to adenylate cyclase, in a pertussis toxin-sensitive manner.

References

1. Schrader, J., J. Baumann, and E. Gerlach. Adenosine as inhibitor of myocardial effects of catecholamines. *Pflügers Arch.* **372:**29–35 (1977).
2. Dobson, J. G., Jr. Mechanism of adenosine inhibition of catecholamine-induced responses in heart. *Circ. Res.* **52:**151–160 (1983).
3. Böhm, M., R. Brückner, I. Hackbarth, B. Haubitz, R. Linhart, W. Meyer, B. Schmidt, W. Schmitz, and H. Scholz. Adenosine inhibition of catecholamine-induced increase in force of contraction in guinea-pig atrial and ventricular heart preparations: evidence against a cyclic AMP- and cyclic GMP-dependent effect. *J. Pharmacol. Exp. Ther.* **230:**483–492 (1984).
4. Belardinelli, L., and G. Isenberg. Actions of adenosine and isoproterenol on isolated mammalian ventricular myocytes. *Circ. Res.* **53:**287–297 (1983).
5. Isenberg, G., and L. Belardinelli. Ionic basis for the antagonism between adenosine and isoproterenol on isolated mammalian ventricular myocytes. *Circ. Res.* **55:**309–325 (1984).
6. Anand-Srivastava, M. B., and M. Cantin. Regulation of adenylate cyclase in cultured cardiocytes from neonatal rats by adenosine and other agonists. *Arch. Biochem. Biophys.* **223:**468–476 (1983).
7. Leung, E., C. I. Johnston, and E. A. Woodcock. A comparison between the adenosine receptors mediating adenylate cyclase inhibition and cardiac depression in the guinea-pig heart. *J. Cardiovasc. Pharmacol.* **8:**1003–1008 (1986).
8. Hazeki, O., and M. Ui. Modification by islet-activating protein of receptor-mediated regulation of cyclic AMP accumulation in isolated rat heart cells. *J. Biol. Chem.* **256:**2856–2862 (1981).
9. West, G. A., G. Isenberg, and L. Belardinelli. Antagonism of forskolin effects by adenosine in isolated hearts and ventricular myocytes. *Am. J. Physiol.* **250:**H769–H777 (1986).
10. Henrich, M., H. M. Piper, and J. Schrader. Evidence for adenylate cyclase-coupled A_1-adenosine receptors on ventricular cardiomyocytes from adult rat and dog heart. *Life Sci.* **41:**2381–2388 (1987).
11. Martens, D., M. J. Lohse, B. Rauch, and U. Schwabe. Pharmacological characterization of A_1 adenosine receptors in isolated rat ventricular myocytes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **336:**342–348 (1987).
12. Evans, D. B., J. A. Schenden, and J. A. Bristol. Adenosine receptors mediating cardiac depression. *Life Sci.* **31:**2425–2432 (1982).
13. Collis, M. G. Evidence for an A_1-adenosine receptor in the guinea-pig atrium. *Br. J. Pharmacol.* **78:**207–212 (1983).
14. Böhm, M., B. Pieske, M. Ungerer, and E. Erdmann. Characterization of A_1 adenosine receptors in atrial and ventricular myocardium from diseased human hearts. *Circ. Res.* **65:**1201–1211 (1989).
15. Belardinelli, L., and G. Isenberg. Isolated atrial myocytes: adenosine and acetylcholine increase potassium conductance. *Am. J. Physiol.* **244:**H734–H737 (1983).
16. West, G. A., and L. Belardinelli. Correlation of sinus slowing and hyperpolarization caused by adenosine in sinus node. *Pflügers Arch.* **403:**75–81 (1985).
17. Cerbai, E., U. Klöckner, and G. Isenberg. Ca-antagonistic effects of adenosine in guinea pig atrial cells. *Am. J. Physiol.* **255:**H872–H878 (1988).
18. Kurachi, Y., T. Nakajima, and T. Sugimoto. On the mechanism of activation of muscarinic K^+ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflügers Arch.* **407:**264–274 (1986).
19. Endoh, M., M. Maruyama, and N. Taira. Modification by islet-activating protein of direct and indirect inhibitory actions of adenosine on rat atrial contraction in relation to cyclic nucleotide metabolism. *J. Cardiovasc. Pharmacol.* **5:**131–142 (1983).
20. Brückner, R., A. Fenner, W. Meyer, T.-M. Nobis, W. Schmitz, and H. Scholz. Cardiac effects of adenosine and adenosine analogs in guinea-pig atrial and ventricular preparations: evidence against a role of cyclic AMP and cyclic GMP. *J. Pharmacol. Exp. Ther.* **234:**766–774 (1985).
21. Lohse, M. J., K.-N. Klotz, J. Lindenborn-Fotinos, M. Reddington, U. Schwabe, and R. A. Olsson. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX): a selective high affinity antagonist radioligand for A_1 adenosine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **336:**204–210 (1987).
22. Lohse, M. J., K.-N. Klotz, U. Schwabe, G. Cristalli, S. Vittori, and M. Grifantini. 2-Chloro-N^6-cyclopentyladenosine: a highly selective agonist at A_1 adenosine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **337:**687–689 (1988).
23. Hunter, W. M., and F. C. Greenwood. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature (Lond.)* **194:**495–496 (1962).
24. Bechem, M., L. Pott, and H. Rennebaum. Atrial muscle cells from hearts of adult guinea-pigs in culture: a new preparation for cellular electrophysiology. *Eur. J. Cell. Biol.* **31:**366–369 (1983).
25. Harper, J. F., and G. Brooker. Femtomole sensitive radioimmuoassay for cyclic AMP and cyclic GMP after 2′-*O*-acetylation by acetic anhydride in aqueous solution. *J. Cyclic Nucleotide Res.* **1:**207–218 (1975).
26. Lohse, M. J., K.-N. Klotz, and U. Schwabe. Agonist photoaffinity labeling of A_1 adenosine receptors: persistent activation reveals spare receptors. *Mol. Pharmacol.* **30:**403–409 (1986).
27. Lohse, M. J., D. Ukena, and U. Schwabe. Demonstration of R_i-type adenosine receptors in bovine myocardium by radioligand binding. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **328:**310–316 (1985).
28. Martens, D., M. J. Lohse, and U. Schwabe. [^{3}H]-8-Cyclopentyl-1,3-dipropylxanthine binding to A_1 adenosine receptors of intact rat ventricular myocytes. *Circ. Res.* **63:**613–620 (1988).
29. Tawfik-Schlieper, H., K.-N. Klotz, V. A. W. Kreye, and U. Schwabe. Characterization of the K^+-channel-coupled adenosine receptor in guinea pig atria. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **340:**684–688 (1989).
30. Iyengar, R., and L. Birnbaumer. Signal transduction by G-proteins. *ISI Atlas Sci. Pharmacol.* **1:**213–221 (1987).
31. Yatani, A., R. Matttera, J. Codina, R. Graf, K. Okabe, E. Padrell, R. Iyengar, A. M. Brown, and L. Birnbaumer. The G protein-gated atrial K^+ channel is stimulated by three distinct G_i-subunits. *Nature (Lond.)* **336:**680–682 (1988).
32. Goldman, S. J., E. S. Dickinson, and L. L. Slakey. Effect of adenosine on synthesis and release of cyclic AMP by cultured vascular cells from swine. *J. Cyclic Nucleotide Protein Phosphorylation Res.* **9:**69–78 (1983).
33. Leid, M., P. H. Franklin, and T. F. Murray. Labeling of A_1 adenosine receptors in porcine atria with the antagonist radioligand 8-cyclopentyl-1,3-[^{3}H]dipropylxanthine. *Eur. J. Pharmacol.* **147:**141–144 (1988).
34. Leid, M., M. I. Schimerlik, and T. F. Murray. Characterization of agonist radioligand interactions with porcine atrial A_1 adenosine receptors. *Mol. Pharmacol.* **34:**334–339 (1988).
35. Liang, B. T. Characterization of the adenosine receptor in cultured embryonic chick atrial myocytes: coupling to modulation of contractility and adenylate cyclase activity and identification by direct radioligand binding. *J. Pharmacol. Exp. Ther.* **249:**775–784 (1989).
36. Neumann, J., W. Schmitz, H. Scholz, and B. Stein. Effects of adenosine analogues on contactile response and cAMP content in guinea-pig isolated ventricular myocytes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **340:**689–695 (1989).
37. Linden, J., C. E. Hollen, and A. Patel. The mechanism by which adenosine and cholinergic agents reduce contractility in rat myocardium. *Circ. Res.* **56:**728–735 (1985).
38. Sorota, S., Y. Tsuji, T. Tajima, and A. J. Pappano. Pertussis toxin treatment blocks hyperpolarization by muscarinic agonists in chick atrium. *Circ. Res.* **57:**748–758 (1985).
39. Kurose, H., and M. Ui. Functional uncoupling of muscarinic receptors from adenylate cyclase in rat cardiac membranes by the active component of islet-activating protein, pertussis toxin. *J. Cyclic Nucleotide Protein Phosphorylation Res.* **9:**305–318 (1983).
40. Endoh, M., M. Maruyama, and T. Ijima. Attenuation of muscarinic cholinergic inhibition by islet-activating protein in the heart. *Am. J. Physiol.* **249:**H309–H3230 (1985).

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