Br. J. Pharmacol. (1991), **104**, 399–405

Adenosine receptors and nucleoside transport sites in cardiac cells

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1 Potential mechanisms responsible for the prominent depression of atrioventricular conduction by adenosine have been investigated in guinea-pig heart.

2 Adenosine A_1 receptors and nucleoside transport (NT) sites were identified and enumerated in cardiac myocytes, atrioventricular conduction cells and coronary endothelial cells in 10 μm sections by autoradiographical analysis of the binding of the A_1 selective antagonist 8-cyclopentyl-1,3-[^{3}H]-dipropylxanthine ([^{3}H]-DPCPX) and the NT ligand [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBMPR), respectively.

3 Atrioventricular conduction cells were identified by acetylcholinesterase histochemistry and endothelial cells by von Willebrand factor immunohistochemistry.

4 Site-specific binding of [^{3}H]-DPCPX, when expressed as grains per cell nucleus was significantly higher (30 fold) in conduction cells than in surrounding myocytes. [^{3}H]-DPCPX site density on endothelial cells in adjacent coronary vessels was not significantly different from myocytes.

5 In contrast, autoradiography of [^{3}H]-NBMPR sites in these areas indicated that, relative to myocytes, conduction cells and endothelial cells were significantly enriched (2 fold and 4.5 fold, respectively) in NT sites.

6 The pronounced dromotropic effect of adenosine in guinea-pig heart is correlated with a higher density of adenosine A_1 receptors in atrioventricular conduction cells than in myocytes. The NT capacity of these cells, as estimated by [^{3}H]-NBMPR binding site density, is not increased in proportion to A_1 receptors.

Keywords: Adenosine receptors; cardiac myocytes; conduction cells; endothelial cells; nucleoside transport sites; acetylcholinesterase (AChE); cyclopentyldipropylxanthine (DPCPX); nitrobenzylthioinosine (NBMPR)

Introduction

As early as 1929, it was observed that adenosine has negative chronotropic and dromotropic effects in heart; of the species studied, the effect on the sinoatrial node was predominant in dog, cat and rabbit while the atrioventricular nodal depression was predominant in guinea-pig (Drury & Szent-Gyorgi, 1929). In man, both sinus rate and atrioventricular conduction are depressed by the intravenous administration of adenosine and this is of therapeutic benefit in some forms of paroxysmal supraventricular tachycardia (DiMarco *et al.*, 1983). Additionally, it has been proposed that conduction delays, seen in cases of ischaemia and hypoxia, may be due to adenosine released from cardiac cells under hypoxic stress (Belardinelli *et al.*, 1980; West & Belardinelli, 1985; Clemo & Belardinelli, 1986a,b; Wesley & Belardinelli, 1989).

Adenosine is able to eliminate action potentials in sinoatrial and atrioventricular nodal cells (West & Belardinelli, 1985; Clemo & Belardinelli, 1986a). In contrast, adenosine shortens the action potential duration in atrial myocytes (Belardinelli *et al.*, 1983b), and, in the presence of compounds which increase intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) such as isoprenaline, in ventricular myocytes (Belardinelli *et al.*, 1984). These effects of adenosine are mediated by cell surface receptors of the A_1 subtype (Clemo & Belardinelli, 1986a); one resultant of stimulation of these receptors in atrial cells is an increase in potassium conductance that produces hyperpolarization (Sperelakis, 1987). Nucleoside transport inhibitors, such as dipyridamole and nitrobenzylthioinosine (NBMPR), potentiate, whereas the adenosine receptor antagonist, aminophylline, inhibits the cardiovascular actions of adenosine (Belardinelli *et al.*, 1980; 1982).

Adenosine A_1 receptors are present in low density in cardiac tissue. Estimates of the maximum site density are about 15–30 fmol mg^{-1} protein in rat (Linden *et al.*, 1985; Martens *et al.*, 1987), guinea-pig (Wu *et al.*, 1989; Froldi & Belardinelli, 1990), bovine (Lohse *et al.*, 1985), pig (Leid *et al.*, 1988) and diseased human (Böhm *et al.*, 1989) cardiac preparations. Since the density of A_1 receptors has not been determined in cells of the impulse conduction system, it is possible that increased receptor density on these cells may account for the predominant chronotropic and dromotropic effects of adenosine in heart.

Another possibility is that altered adenosine metabolism, for example decreased transport of adenosine into conduction cells, may allow increased adenosine-mediated effects in these areas. In support of this idea, we have shown in previous studies that nucleoside transport sites identified with [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBMPR) have a greater site density in coronary endothelial cells than in ventricular cardiac myocytes (Parkinson & Clanachan, 1989a,b). Other cell types within heart may also exhibit differences in transporter density.

Other possible factors that could contribute to the enhanced sensitivity of conduction cells to adenosine include increased receptor affinity for adenosine, increased coupling efficiency between receptors and transduction mechanisms, or the dependence of conduction cells upon slow Na^+-Ca^{2+} channels for depolarization.

In this study, we examined whether guinea-pig atrioventricular conduction cells had altered receptor or nucleoside transporter densities relative to other cardiac myocytes. At present, it is not possible to purify conduction cells, in amounts sufficient for binding analysis, separate from the other cellular components of heart. Therefore these cell types were identified histologically and autoradiography was performed with the A_1 receptor antagonist, 8-cyclopentyl-1,3-[^{3}H]-dipropylxanthine ([^{3}H]-DPCPX) and the nucleoside transport inhibitor [^{3}H]-NBMPR. In this way, site densities of receptors and

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transport inhibitory sites in conduction cells and endothelial cells relative to other cardiac myocytes were determined.

Methods

Guinea-pig hearts were rapidly removed, and from each heart the area of right atrium containing coronary sinus and inferior vena cava, plus the interatrial septum and the upper part of the interventricular septum was isolated as this preparation contains atrioventricular conduction cells (Clemo & Belardinelli, 1986a). The tissues were embedded in sectioning media (20 parts OCT Compound: 10 parts distilled water: 7 parts tragacanth gum), frozen in isopentane cooled with liquid nitrogen and stored at −70°C for up to two weeks. Sections (10 μm) were cut (−22°C) parallel to the long axis of the heart with a cryostat (International Equipment Company model CT1), mounted onto gelatin-coated slides, desiccated overnight at 4°C and then used immediately or stored at −70°C for up to two weeks.

Acetylcholinesterase histochemistry

Cardiac conduction cells can be reliably identified by positive staining for acetylcholinesterase (AChE) (Bojsen-Møller & Tranum-Jensen, 1971; Anderson, 1972). Vagal innervation of cardiac tissue is concentrated on impulse conducting cells within the atria, and both the nerves and the innervated cells are characterized by having large quantities of AChE. The procedure used for detecting AChE in these cells was that described by El-Badawi & Schenk (1969).

Von Willebrand factor immunohistochemistry

Von Willebrand factor is a component of clotting factor VIII and is a reliable marker for endothelial cells (Jaffe, 1984). Sections were first preincubated for 5 min in ice-cold physiological salt solution (PSS) of the following composition (mM): NaCl 137, Na_2HPO_4 6, KCl 2.7, KH_2PO_4 1.5, $CaCl_2$ 0.9 and $MgCl_2$ 0.5; pH 7.4. This was followed by 10 min in 0.4% formaldehyde in ice-cold PSS and two subsequent 5 min washes in ice-cold PSS. Sections were then incubated in presoak buffer (5% bovine serum albumin (BSA), 0.05% NaN_3, 0.3% Triton X 100 in PSS) for 1 h at 20°C. Washes, at this and later steps, were performed with 1% BSA and 0.05% NaN_3 in PSS, 2 × 5 min at 20°C. Sections were then incubated with the primary antibody, rabbit polyclonal antibody to human von Willebrand factor (1:1600 dilution in 1% BSA, 0.05% NaN_3, 0.3% Triton X 100 in PSS), for 1 h at 20°C. Sections were washed then incubated with the secondary antibody, biotinylated goat anti-rabbit IgG (1 : 400 dilution in the same buffer used for primary antibody), for 1 h at 20°C. Sections were washed again then incubated with horseradish peroxidase avidin D (1:100 dilution in the same buffer used for washes), for 1 h at 20°C. Sections were washed again then incubated with 0.05% diaminobenzidine and 0.001% H_2O_2 in PSS, for 5 min at 20°C in the dark. Sections were washed again, then dehydrated and mounted.

[^{3}H]-nitrobenzylthioinosine binding

Sections were preincubated in ice-cold PSS for 5 min, then incubated in 10 nM [^{3}H]-NBMPR for 30 min at 20°C. Fixation of the tissue sections (10 min) resulted from the addition of neutral buffered formaldehyde (final conc. 0.4%) to the incubation medium for 10 min. Sections were washed twice for 5 min with ice-cold PSS and rinsed three times with ice-cold distilled water. Sections were blown dry with cold air and desiccated overnight. The amount of [^{3}H]-NBMPR that bound in the presence of 30 μM dipyridamole was termed the non-specific binding component.

8-Cyclopentyl-1,3-[^{3}H]-dipropylxanthine binding

Sections were preincubated in PSS, 4°C for 5 min, then incubated in 5 nM [^{3}H]-DPCPX for 2 h at 20°C. The results of preliminary experiments indicated that [^{3}H]-DPCPX bound to guinea-pig cardiac membranes with a K_D value of 1.5 nM (0.6–3.9). Tissue sections were then processed as described for [^{3}H]-NBMPR binding assays. Total [^{3}H]-DPCPX binding was defined as that which occurred in the presence of 5 nM [^{3}H]-DPCPX, 0.01% CHAPS and 5.5 units/assay adenosine deaminase. Non-specific binding was defined as the amount of [^{3}H]-DPCPX that remained bound in the presence of 2-chloroadenosine (100 μM).

Autoradiography

The autoradiographical technique used was modified from that described by Young & Kuhar (1979). Coverslips were prepared by dipping into Kodak NTB2 emulsion (diluted 1:1 with distilled water; 43°C) and stored over desiccant, protected from light, for 12–48 h. The emulsion-coated coverslips were glued at one end to slides containing tissue sections previously exposed to [^{3}H]-NBMPR or [^{3}H]-DPCPX as described above. The slides and coverslips were held tightly together with squares of teflon and binder clips and kept at 4°C for 2–3 ([^{3}H]-NBMPR) or 5–6 ([^{3}H]-DPCPX) weeks. This hinged coverslip method avoided the immersion of the sections in emulsion and the associated risk of diffusion of the ligand from the section prior to the generation of the autoradiogram.

To develop the coverslips the binder clips and teflon squares were removed, and the coverslips were gently propped up from the tissue sections. The emulsions were developed in Dektol Developer (diluted 1:1 with water) for 2 min, then rinsed in water for 10 s, fixed in Kodak Fixer for 5 min and washed in water for 5 min. The sections were then stained with hematoxylin and eosin and the coverslips were permanently mounted. With this technique correct alignment of the emulsion with the tissue sections was maintained.

Quantitative autoradiography

Site densities for [^{3}H]-NBMPR and [^{3}H]-DPCPX were quantitated by counting silver grains in small areas (700 μm^2) on each cardiac section. Sections were randomized and counted by an independent observer who was blind to the experimental treatment of the section (NBMPR or DPCPX; total or nonspecific binding condition). For comparison of conduction cells with other cardiac myocytes, silver grains were counted in three areas of each cell type and three areas of slide background (non-tissue). Background grains were subtracted from tissue values; specific binding was then calculated as the difference between total binding and nonspecific binding in adjacent sections. In order to account for potential cell size differences between areas, cell nuclei in each chosen field were also counted; specific binding site densities were then calculated as grains per cell nucleus. The small difference between grains per nucleus and grains per cell due to the small percentage of bi-nucleated, and larger, cells was not considered significant. Similar methods were used to compare binding site densities on myocytes and coronary endothelial cells. The significance of the difference in grain densities between cell types was determined by two-tailed Student's paired t test and judged to be statistically significant when $P < 0.05$.

Materials

Compounds and radioligands for these studies were obtained from the following sources. [^{3}H]-nitrobenzylthioinosine (Sp. Act. 37 Ci mmol^{-1}), from Moravek Biochemicals, CA, U.S.A. and repurified before use by high performance liquid chroma-

tography (h.p.l.c.); 8-cyclopentyl-1,3-[^{3}H]-dipropylxanthine (Sp. Act. 120 Ci mmol^{-1}), from New England Nuclear, Boston, MA, U.S.A.; rabbit polyclonal anti-human von Willebrand factor, biotinylated anti-rabbit IgG and horseradish peroxidase avidin D, from Dimension Laboratories Inc., Mississauga, Ont., Canada; dipyridamole, diaminobenzidine, CHAPS(3 - [(3 - cholamidopropyl) dimethyl - ammonio] - 1 - propane-sulphonate), and adenosine deaminase type VII, from Sigma Chemical Co., St. Louis, MO, U.S.A.; 2-chloroadenosine, from Research Biochemicals Inc., Natick, MA, U.S.A.; glue (Super Bonder 495), from Loctite Corporation, Newington, CT, U.S.A.; Kodak NTB2 nuclear track emulsion, Dektol Developer and Kodak Fixer, from Calgary Photo, Edmonton, Alta., Canada; coverslips (25 × 60 mm, Corning No. 1), OCT compound and tragacanth gum from Fisher Scientific, Edmonton, Alta., Canada.

Results

Acetylcholinesterase histochemistry

Conduction cells, cholinergic nerves and cholinergic ganglion cells were clearly identified by positive staining for AChE (Figure 1a, 2a). Also, conduction cells were not as tightly packed as myocytes. Conduction cells, including atrioventricular node, His bundle and left and right bundle branches,

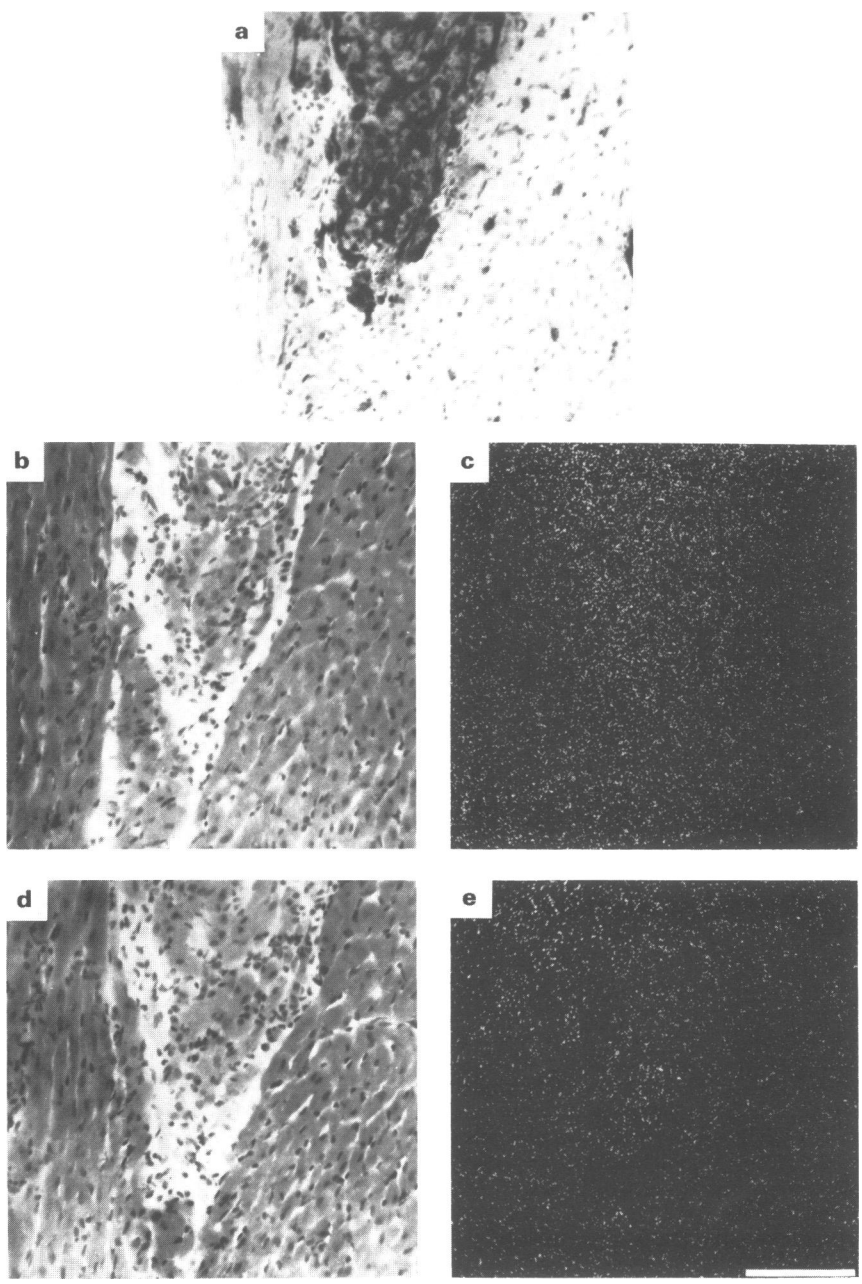

Figure 1 Autoradiographical localization of [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBMPR) binding to atrioventricular conduction cells from guinea-pig. (a) Photomicrograph of conduction cells, identified by dark staining for acetylcholinesterase (AChE). An adjacent section stained for hematoxylin and eosin is shown in (b). A dark field photomicrograph of the same section, (c), shows the distribution of total [^{3}H]-NBMPR binding sites. The density of silver grains over conduction cells and ventricular myocytes appears similar. However, quantitative autoradiography indicated a 2 fold higher density over conduction cells compared to myocytes. An adjacent section, stained for hematoxylin and eosin is shown in (d). This section was used to determine the distribution of silver grains in the non-specific [^{3}H]-NBMPR binding condition and the dark field photomicrograph is shown in (e). A uniform distribution of silver grains is evident over the two cell types. Bar represents 100 μm.

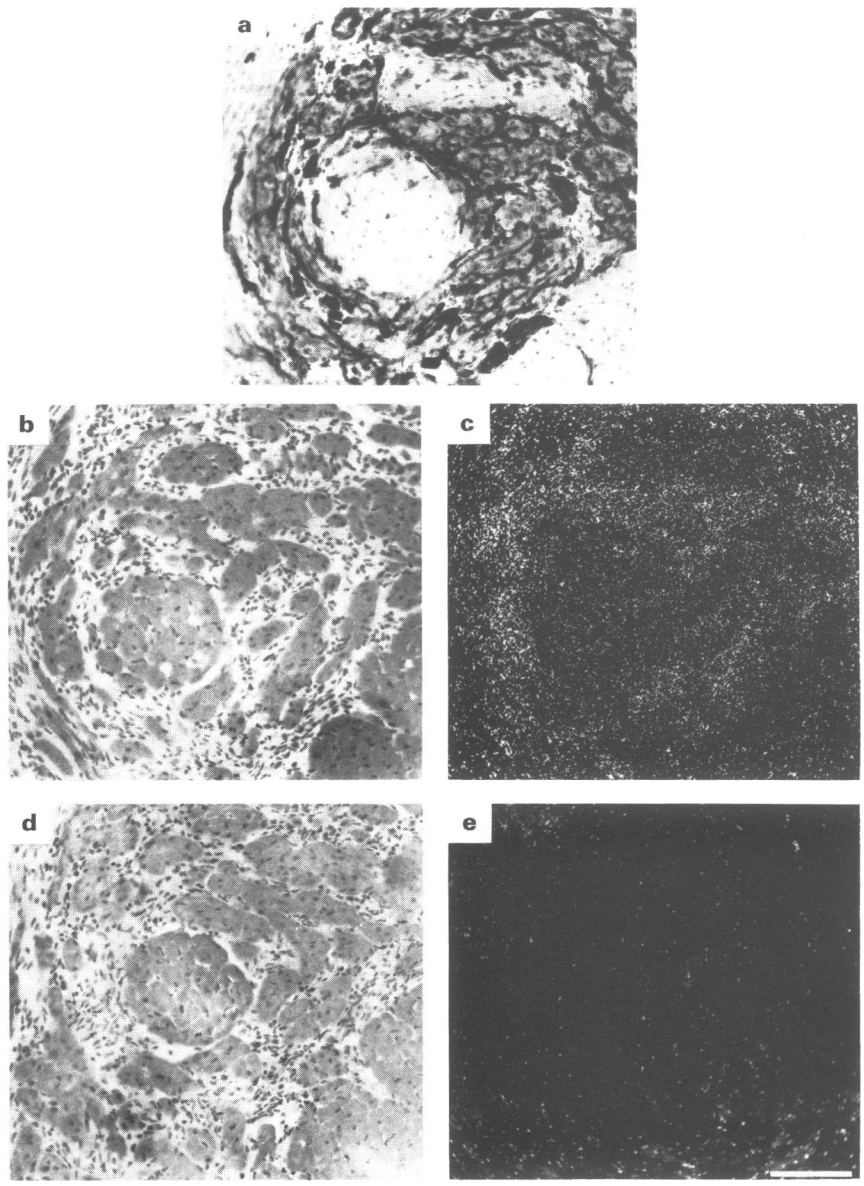

Figure 2 Autoradiographical localization of 8-cyclopentyl-1,3-[^{3}H]-dipropylxanthine ([^{3}H]-DPCPX) binding sites in atrioventricular conduction cells from guinea-pig heart. (a) Photomicrograph of a section stained for acetylcholinesterase; darkly stained cells are conduction cells. An adjacent section, stained with hematoxylin and eosin, is shown in (b). This section was used to determine the distribution of the total [^{3}H]-DPCPX binding sites and the dark field photomicrograph of this section is shown in (c). The grain density is greater over the conduction cells than over ventricular myocytes. An adjacent section, used for the non-specific [^{3}H]-DPCPX binding condition, is shown stained with hematoxylin and eosin in (d). The dark field photomicrograph of this section is shown in (e). The grain density is uniform over conduction cells and ventricular myocytes. Bar represents 100 μm.

were present in approximately 100 sections. The atrioventricular node was often not clearly evident due to the difficulty in obtaining the optimal plane of sectioning. The His bundle was clearly identified in 70% or more of positively staining sections depending on the plane of sectioning.

Von Willebrand factor immunohistochemistry

Immunoperoxidase staining for von Willebrand factor was positive for endothelial cells of blood vessels and the endocardial lining of heart (Figure 3c,f,i).

[^{3}H]-nitrobenzylthioinosine binding

Specific [^{3}H]-NBMPR binding, represented by the difference in density (grains/cell nucleus) of silver grains between total (Figure 1c) and nonspecific (Figure 1e) binding autoradio-

Table 1 Comparison of the site-specific binding of [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBMPR) and 8-cyclopentyl-1,3-[^{3}H]-dipropylxanthine ([^{3}H]-DPCPX) to cardiac myoctyes (Myo) and atroventricular conduction cells (CC) in 10 μm sections of guinea-pig heart

	n	*Myocytes*	*Conduction cells*	*CC/Myo ratio*	P
[^{3}H]-NBMPR	10	11.9 ± 5.1	24.5 ± 5.5	2	0.024
[^{3}H]-DPCPX	19	0.7 ± 3.0	20.3 ± 7	30	0.0002

Site densities are expressed as the number of silver grains divided by the number of cell nuclei. Data are means ± s.e.mean of grain densities in triplicate areas from *n* sections from 5 hearts. *P* values for the significance of the differences between cell types were calculated by Student's paired *t* test.

grams, was slightly higher (2 fold; $P = 0.024$, Table 1) over atrioventricular conduction cells, identified in adjacent sections by heavy staining for AChE (Figure 1a), than over cardiac myocytes. Endothelial cells, identified by immunoperoxidase staining for von Willebrand factor (Figure 3c,f,i), have a much greater grain density, and hence [^{3}H]-NBMPR binding site density (Figure 3b, e, h), than cardiac myocytes (4.5 fold; $P = 0.003$) (Table 2). The high density of [^{3}H]-NBMPR binding sites appeared to be associated with endothelial cells of the endocardium, venules, arterioles, and the aorta, but was not associated with aortic smooth muscle cells, cholinergic nerves, or adipose.

8-Cyclopentyl-1,3-[^{3}H]-dipropylxanthine binding

The distribution of silver grains in the total binding autoradiograms (Figure 2c) was non-uniform in contrast to their

Table 2 Comparison of the densities of site-specific binding of [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBMPR) and 8-cyclopentyl-1,3-[^{3}H]-dipropylxanthine ([^{3}H]-DPCPX) to cardiac myocytes (Myo) and coronary endothelial cells (Endo) in 10 μm sections of guinea-pig heart

	n	*Myocytes*	*Endothelial cells*	*Endo/Myo ratio*	P
[^{3}H]-NBMPR	8	25.1 ± 5.4	114.8 ± 18.2	4.6	0.003
[^{3}H]-DPCPX	6	6.7 ± 2.1	14.1 ± 7.6	2.1	0.270

Site densities are expressed as the number of silver grains divided by the number of cell nuclei. Data are means ± s.e.mean of grain densities in triplicate areas from *n* sections from 4 hearts. *P* values for the significance of the differences between cell types were calculated by Student's paired *t* test.

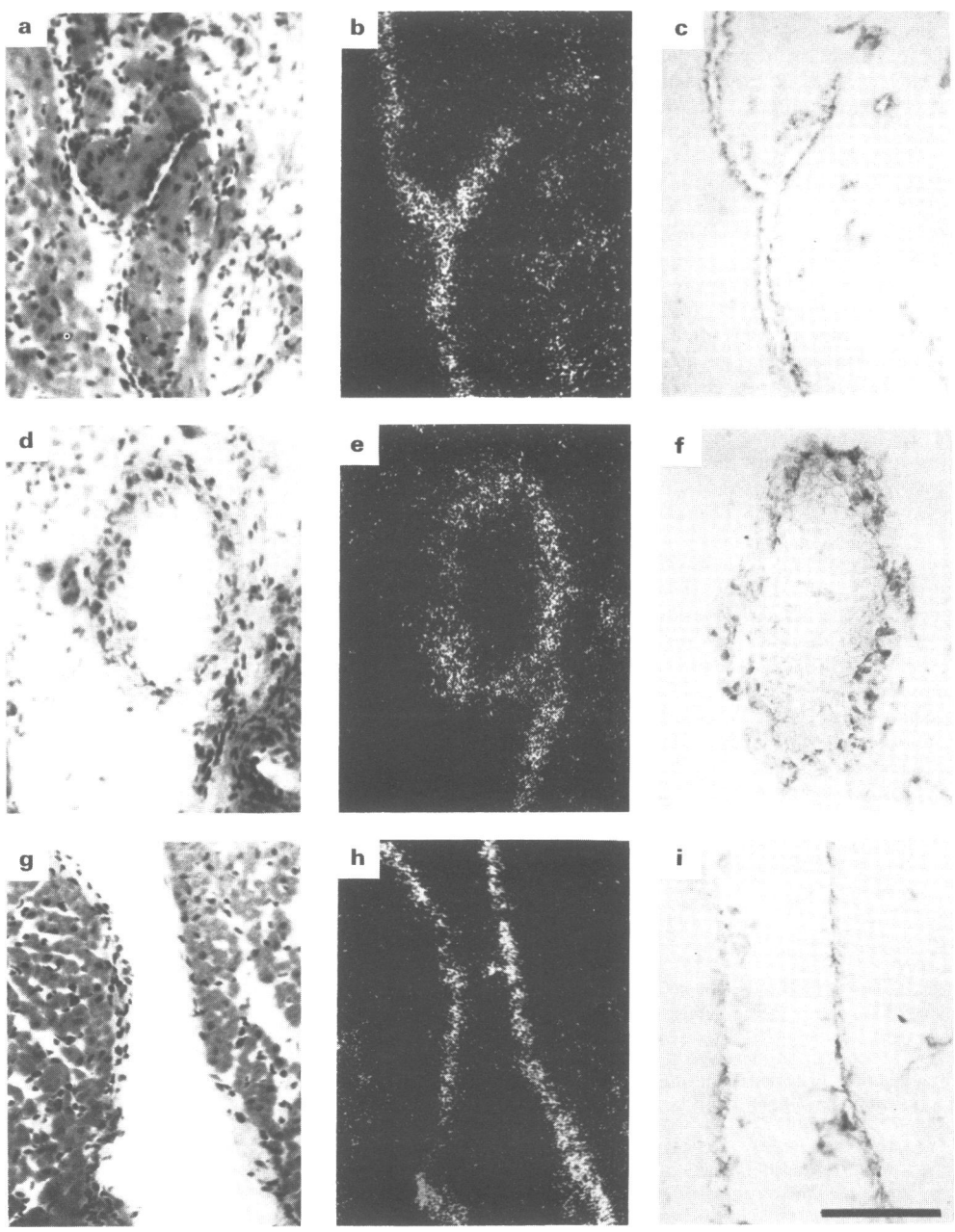

Figure 3 Autoradiographical localization of [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBMPR) binding to cardiac endothelial cells. Bright field photomicrographs of sections stained with hematoxylin and eosin show a blood vessel cut in longitudinal section (a), a blood vessel cut in cross section (d), or endocardial lining (g). Dark field photomicrographs of the same sections show the distributions of total [^{3}H]-NBMPR binding sites associated with these structures (b, e, h). Adjacent sections were used to identify endothelial cells by use of immunoperoxidase staining for von Willebrand factor (c, f, i). Endothelial cells associated with these structures have high densities of [^{3}H]-NBMPR binding sites. Sections used to determine non-specific binding showed uniform grain distributions. Bar represents 100 μm.

distribution in the nonspecific binding condition (Figure 2e). The density of [^{3}H]-DPCPX binding sites was significantly greater in conduction cells (30 fold increase in grain density; $P = 0.0002$, Table 1) identified by dark staining regions for AChE in adjacent sections (Figure 2a), than in cardiac myocytes. The highest density of binding sites for the adenosine A_1 receptor antagonist was associated with conduction cells. No other areas of high site-specific binding of [^{3}H]-DPCPX were observed; the binding to endothelial cells was not significantly different from cardiac myocytes.

Discussion

The main finding of this study is that a higher density of adenosine A_1 receptors exists on atrioventricular conduction cells, indicated by a 30 fold increase in autoradiographic silver grain density, than on cardiac myocytes. Additionally, there is a significantly higher density of NT sites in atrioventricular conduction cells (2 fold increase in grain density) and endothelial cells (4.5 fold increase in grain density) relative to myocytes. The latter finding agrees with our previous low resolution Ultrofilm ^{3}H studies (Parkinson & Clanachan, 1989a,b).

For the localization of adenosine receptors in this study, the radioligand [^{3}H]-DPCPX was used as it is considered relatively selective for adenosine A_1 receptors. It has approximately 500 fold greater affinity for A_1 receptors than for A_2 receptors (Lee & Reddington, 1986; Bruns *et al.*, 1987; Lohse *et al.*, 1987). At the concentration of [^{3}H]-DPCPX used (5 nM), only A_1 receptor binding was detectable and these receptors should have been almost saturated. Therefore our finding of very low silver grain density associated with cardiac myocytes, considering the longer exposure time and the higher specific activity of the ligand relative to [^{3}H]-NBMPR, is in agreement with the low A_1 receptor density found in other cardiac preparations (Linden *et al.*, 1985; Lohse *et al.*, 1985; Martens *et al.*, 1987; Leid *et al.*, 1988; Wu *et al.*, 1989; Froldi & Belardinelli, 1990).

Early work by Belardinelli and co-workers (Belardinelli *et al.*, 1980) showed that the conduction delay produced by adenosine was specific to the atria-His bundle interval, while the His bundle-ventricles interval was unchanged. Subsequently, Clemo & Belardinelli (1986a) found that the effects of adenosine on impulse conduction were predominantly associated with a particular subset of cells (N cells) of the atrioventricular node. Our results, primarily with His bundle and branching bundle cells, show these cells to have increased numbers of A_1 receptors in comparison to atrial and ventricular myocytes and it is possible that N cells have an even greater A_1 receptor density.

In the last few years, several nucleoside transport (NT) systems have been identified (Paterson *et al.*, 1987). [^{3}H]-NBMPR is a useful ligand for the facilitated diffusion system that has high sensitivity to NBMPR. Transport studies with guinea-pig isolated ventricular myocytes have demonstrated that this system is a major component of nucleoside entry in these cells (Heaton & Clanachan, 1987). However, it is possible that other cell types in heart may have additional transport systems, not demonstrable by low nanomolar concentrations of NBMPR.

We have shown that putative NBMPR-sensitive transporters are found in the greatest density in endothelial cells associated with coronary vessels and the endocardial lining. Guinea-pig coronary endothelial cells possess a barrier function to the movement of adenosine between interstitial and intravascular spaces (Nees *et al.*, 1985); this appears due to the high density of nucleoside transporters in these cells in addition to their high catabolic activity for adenosine. Thus, they may be important both in protecting cardiac myocytes from the depressant effects of circulating adenosine and in scavenging adenosine that is released from cardiac myocytes during hypoxic stress.

Previous studies have shown that nucleoside transport inhibitors, such as dipyridamole and NBMPR, potentiate the conduction delay produced by adenosine (Belardinelli & Isenberg, 1983a). From the present autoradiographical analysis of NBMPR binding sites it would appear that this is, in part, due to inhibition of adenosine transport into conduction cells. However, given the higher density of binding sites on endothelial cells, it is likely that these cells also have a significant role. Inhibition of adenosine transport into nearby endothelial cells may contribute to the potentiation of the effects of exogenous adenosine on conduction cells that is seen with these compounds.

In conclusion, the results of this study, showing greatly increased A_1 receptor density and a small increase in nucleoside transporter density, are consistent with increased effects of adenosine in conduction cells versus other cardiac myocytes. However, it is important to recognize that action potentials of nodal cells are different from those of other cardiac myocytes. Therefore, the increased adenosine-mediated effects in conduction cells may be due to the different ionic basis of the action potentials in conjunction with increased receptor density.

We thank Mrs V. Hughes, Ms B. Zeilnik and Mr G. Morrison for technical assistance and to Dr A.R.P. Paterson and his staff for the purification of NBMPR by h.p.l.c. F.E.P. was a recipient of an A.H.F.M.R. Studentship. Supported by the Alberta Heart and Stroke Foundation.

References

ANDERSON, R.H. (1972). The disposition, morphology and innervation of cardiac specialized tissue in the guinea pig. *J. Anat.*, **111**, 453–468.

BELARDINELLI, L., BELLONI, F.L., RUBIO, R. & BERNE, R.M. (1980). Atrioventricular conduction disturbances during hypoxia: possible role of adenosine in rabbit and guinea pig heart. *Circ. Res.*, **47**, 684–691.

BELARDINELLI, L., FENTON, R.A., WEST, G.A., LINDEN, J., ALTHUS, J.S. & BERNE, R.M. (1982). Extracellular action of adenosine and the antagonism by aminophylline on the atrioventricular conduction of isolated perfused guinea pig and rat hearts. *Circ. Res.*, **51**, 569–579.

BELARDINELLI, L., SHRYOCK, J., WEST, G.A., CLEMO, H.F., DI MARCO, J.P. & BERNE, R.M. (1984). Effects of adenosine and adenine nucleotides on the atrioventricular node of isolated guinea pig hearts. *Circulation*, **70**, 1083–1091.

BELARDINELLI, L. & ISENBERG, G. (1983a). Actions of adenosine and isoproterenol on isolated mammalian ventricular myocytes. *Circ. Res.*, **53**, 287–297.

BELARDINELLI, L. & ISENBERG, G. (1983b). Isolated atrial myocytes: adenosine and acetylcholine increase potassium conductance. *Am. J. Physiol.*, **244**, (*Heart Circ. Physiol.*, **13**), H734–H737.

BÖHM, M., PIESKE, B., UNGERER, M. & ERDMAN, E. (1989). Characterization of A_1 adenosine receptors in atrial and ventricular myocardium from diseased human hearts. *Circ. Res.*, **65**, 1201–1211.

BOJSEN-MØLLER, F. & TRANUM-JENSEN, J. (1971). Whole-mount demonstration of cholinesterase-containing nerves in the right atrial wall, nodal tissue, and atrioventricular bundle of the pig heart. *J. Anat.*, **108**, 375–386.

BRUNS, R.F., FERGUS, J.H., BADGER, E.W., BRISTOL, J.A., SANTAY, L.A., HARTMAN, J.D., HAYS, S.J. & HUANG, C.C. (1987). Binding of the A_1-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **335**, 59–63.

CLEMO, H.F. & BELARDINELLI, L. (1986a). Effect of adenosine on atrioventricular conduction. I: Site and characterization of adenosine action in the guinea pig atrioventricular node. *Circ. Res.*, **59**, 427–436.

CLEMO, H.F. & BELARDINELLI, L. (1986b). Effect of adenosine on atrioventricular conduction. II: Modulation of atrioventricular node transmission by adenosine in hypoxic isolated guinea pig hearts. *Circ. Res.*, **59**, 437–446.

DIMARCO, J.P., SELLERS, T.D., BERNE, R.M., WEST, G.A. & BELARDINELLI, L. (1983). Adenosine: electrophysiologic effects and therapeutic use for terminating paroxysmal supraventricular tachycardia. *Circulation*, **68**, 1254–1263.

DRURY, A.N. & SZENT-GYORGYI, A. (1929). The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J. Physiol.*, **68**, 213–237.

EL-BADAWI, A. & SCHENK, E.A. (1969). Histochemical methods for separate, consecutive and simultaneous demonstration of acetylcholinesterase and norepinephrine in cryostat sections. *J. Histochem. Cytochem.*, **15**, 580–588.

FROLDI, G. & BELARDINELLI, L. (1990). Species-dependent effects of adenosine on heart rate and atrioventricular nodal conduction. Mechanism and physiological implications. *Circ. Res.*, **67**, 960–78IS.

HEATON, T.P. & CLANACHAN, A.S. (1987). Nucleoside transport in guinea-pig myocytes: comparison of the affinities and transport velocities for adenosine and 2-chloroadenosine. *Biochem. Pharmacol.*, **36**, 1275–1280.

JAFFE, E.A. (1984). Synthesis of factor VIII by endothelial cells. In *Biology of Endothelial Cells.* ed. Jaffe, E.A. pp. 209–214. The Hague: Martinus Nijhoff.

LEE, K.S. & REDDINGTON, M. (1986). 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX) inhibition of [^{3}H]N-ethylcarboxamidoadenosine (NECA) binding allows the visualization of putative non-A_1 adenosine receptors. *Brain Res.*, **368**, 394–398.

LEID, M., FRANKLIN, P.H. & MURRAY, T.F. (1988). Labelling 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.

LINDEN, J., PATEL, A. & SADEK, S. (1985). [^{125}I]Aminobenxyladenosine, a new radioligand with improved specific binding to adenosine receptors in heart. *Circ. Res.*, **56**, 279–284.

LOHSE, M.J., KLOTZ, K-N., LINDENBORN-FOTINOS, J., REDDINGTON, M., SCHWABE, U. & OLSSON, R.A. (1987). 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX)—a selective high affinity antagonist radioligand for A_1 adenosine receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **336**, 204–210.

LOHSE, M.J., UKENA, D. & SCHWABE, U. (1985). Demonstration of R_i-type adenosine receptors in bovine myocardium by radioligand binding. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 310–316.

MARTENS, D., LOHSE, M.J., RAUCH, B. & SCHWABE, U. (1987). Pharmacological characterization of A_1 adenosine receptors in isolated rat ventricular myocytes. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **336**, 342–348.

NEES, S., HERZOG, V., BECKER, B.F., BOCK, M., DESROSIERS, C. & GERLACH, E. (1985). The coronary endothelium: a highly active metabolic barrier for adenosine. *Basic Res. Cardiol.*, **80**, 515–529.

PARKINSON, F.E. & CLANACHAN, A.S. (1989a). Heterogeneity of nucleoside transport inhibitory sites in heart: a quantitative autoradiographical analysis. *Br. J. Pharmacol.*, **97**, 361–370.

PARKINSON, F.E. & CLANACHAN, A.S. (1989b). Subtypes of nucleoside transport inhibitory sites in heart: a quantitative autoradiographical analysis. *Eur. J. Pharmacol.*, **163**, 69–75.

PATERSON, A.R.P., JAKOBS, E.S., NG, C.Y.C., ODEGARD, R.D. & ADJEI, A.A. (1987). Nucleoside transport inhibition *in vivo* and *in vitro*. In *Topics and Perspectives in Adenosine Research.* ed. Gerlach, E. & Becker, B.F. pp. 89–101. Heidelberg: Springer-Verlag.

SPERELAKIS, N. (1987). Regulation of calcium slow channels of cardiac and smooth muscles by adenine nucleotides. *Prog. Clin. Biol. Res.*, **230**, 135–193.

WESLEY, R.C. Jr. & BELARDINELLI, L. (1989). Role of endogenous adenosine in postdefibrillation bradyarrhythmia and hemodynamic depression. *Circulation*, **80**, 128–137.

WEST, G.A. & BELARDINELLI, L. (1985). Sinus slowing and pacemaker shift caused by adenosine in rabbit SA node. *Pflügers Arch.*, **403**, 66–74.

WU, S.N., LINDEN, J., VISENTIN, S., BOYKIN, M. & BELARDINELLI, L. (1989). Enhanced sensitivity of heart cells to adenosine and up-regulation of receptor number after treatment of guinea-pigs with theophylline. *Circ. Res.*, **65**, 1066–1077.

YOUNG III, W.S. & KUHAR, M.J. (1979). A new method for receptor autoradiography: [^{3}H]opioid receptors in rat brain. *Brain Res.*, **179**, 255–270.

(Received February 15, 1991
Revised May 20, 1991
Accepted June 4, 1991)