Antiadrenergic Effects of Adenosine on His-Purkinje Automaticity

Evidence for Accentuated Antagonism

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Abstract

The effects of adenosine on the human His-Purkinje system (HPS) were studied in nine patients with complete atrioventricular (AV) block. Adenosine had minimal effect on the control HPS cycle length, but in the presence of isoproterenol increased it from 906±183 to 1,449±350 ms, $P < 0.001$. Aminophylline, a competitive adenosine antagonist, completely abolished this antiadrenergic effect of adenosine.

In isolated guinea pig hearts with surgically induced AV block, isoproterenol decreased the HPS rate by 36%, whereas in the presence of 1,3-dipropyl-8-phenyl-xanthine, a potent adenosine antagonist, the HPS rate decreased by 48% and was associated with an increased release of adenosine. Therefore, by blocking the effects of adenosine at the receptor level, the physiologic negative feedback mechanism by which adenosine antagonizes the effects of catecholamines was uncoupled.

The results of this study indicate that adenosine's effects on the human HPS are primarily antiadrenergic and are thus consistent with the concept of accentuated antagonism. These effects of adenosine may serve as a counterregulatory metabolic response that improves the O_2 supply-demand ratio perturbed by enhanced sympathetic tone. Some catecholamine-mediated ventricular arrhythmias that occur during ischemia or enhanced adrenergic stress may be due to an imbalance in this negative feedback system.

Introduction

It is well recognized that many myocardial functions are regulated by the autonomic nervous system. A nonlinear antagonism between sympathetic stimulatory effects and inhibitory parasympathetic responses exists (1, 2). Parasympathetic stimulation is thought to have a protective myocardial influence under hypoxic conditions, whereas sympathetic effects may create an unfavorable O_2 supply-demand ratio (3–6). As part of this function, vagal stimulation has a property thought to be unique to the autonomic nervous system, designated by Levy as accentuated antagonism (1, 2). This term refers to the enhanced or augmented inhibitory myocardial response to vagal stimulation in the presence of background sympathetic tone, and is particularly evident in ventricular myocardium (7–14).

Although not as extensively studied, the intermediate metabolite adenosine, an endogenous nucleoside whose production is increased in response to ischemia or hypoxia (15), has cardiac actions that in many respects resemble acetylcholine (ACh)-mediated[1] vagal effects. Experimentally, adenosine improves O_2 supply through coronary vasodilation (16) and reduces O_2 demand through its negative inotropic effects on atrial and ventricular myocardium (17, 18). In addition, the electrophysiologic effects of adenosine on supraventricular tissues also reduce O_2 demand (19) through its negative chronotropic (20) and dromotropic (21) effects in sinoatrial and atrioventricular (AV) nodal tissues, respectively. Like ACh, adenosine increases potassium conductance and hyperpolarizes supraventricular tissues toward the K^+-equilibrium potential, E_k (22, 23). Similar to ACh, these effects of adenosine are cAMP independent (i.e., direct effects) and are mediated through cell surface receptors that are coupled to a guanine nucleotide-binding regulatory protein, most likely G_i[-3] (24–26).

The effects and role of adenosine in ventricular myocardium and the His-Purkinje system (HPS) are not as well understood as those in supraventricular tissues. Experimentally, adenosine slows HPS automaticity both in isolated preparations and in vivo (27–31), suggesting a direct effect in the absence of β-adrenergic cAMP stimulation in these tissues. Other studies in single isolated ventricular myocytes, however, indicate that adenosine has no direct effect but rather antagonizes the electrophysiologic effects of catecholamines that are mediated through stimulation of the adenylate cyclase-cAMP system (32, 33), i.e., lengthening of action potential duration, positive displacement of the plateau phase, and delayed after depolarizations.

The purposes of this study were to (*a*) characterize the direct (cAMP-independent) and indirect (cAMP-dependent) electrophysiologic effects of adenosine in the human HPS and (*b*) determine the physiologic significance of adenosine's effects in these tissues. We hypothesized that, as in other myocardial tissues, adenosine functions in the HPS to reduce O_2 demand. It was anticipated that adenosine would show minimal direct effects under basal conditions, but that during catecholamine stimulation and increased O_2 demand adenosine would demonstrate accentuated antagonism to restore a favorable O_2 supply-demand ratio.

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1. *Abbreviations used in this paper:* ACh, acetylcholine; AV, atrioventricular; HPS, His-Purkinje system; QRS, ventricular complex; VT, ventricular tachycardia.

Methods

Clinical protocol

All studies were performed with patients in the unsedated, postabsorptive state after informed consent was obtained. All patients had a temporary ventricular pacing catheter inserted before the study. Intracardiac recordings were filtered at 30–500 Hz and simultaneously displayed with three electrocardiographic (ECG) leads on a multichannel oscilloscope (model VR-16; Electronics for Medicine, Pleasantville, NY). Data were stored on magnetic tape (model 101; Honeywell Information Systems, Inc., Waltham, MA). Real-time recordings were made with an ink-jet recorder (Elma mingograph; Siemens Corp., Iselin, NJ) and a three-channel ECG recorder. Systemic arterial pressure was continuously monitored (Dinamap; Critikon, Inc., Tampa, FL).

All patients had fixed third degree AV block and were referred for implantation of permanent pacemakers. Each patient was hemodynamically stable and asymptomatic in the supine position. The mean age was 72±15 yr with a range from 35 to 82 yr. Seven patients had idiopathic heart block, one patient had heart block secondary to an atrial septal defect repair, and the remaining patient had undergone His bundle ablation for medically refractory supraventricular tachycardia. There were four men and five women.

The level of block was infra-Hisian as documented by His-bundle recordings in five patients. In the remaining four patients infranodal (AV) block was inferred by the following findings: escape cycle length ≥ 1,250 ms, ventricular complex (QRS) duration ≥ 120 ms, and complete heart block despite vagolytic or sympathomimetic agents such as atropine and isoproterenol, respectively.

Patients with congestive heart failure, angina (> New York Heart Association Class II), chronic obstructive pulmonary disease, recent use of either adenosine uptake inhibitors (i.e., dipyridamole, diazepam, or phenobarbital), or competitive adenosine antagonists such as xanthine derivatives were excluded from the study. The procedures described in this study were performed in accordance with a protocol approved by the Human Investigations Committee of the University of Virginia.

Protocol

Direct effects of adenosine. Recordings of a stable HPS rhythm were documented for 5 min before any intervention. Direct effects of adenosine on the HPS escape rhythm were assessed by rapidly injecting a bolus of adenosine into a central line and flushing it with 10 ml saline. Crystalline adenosine (Sigma Chemical Co., St. Louis, MO) was dissolved in normal saline at a concentration of 5 mg/ml. The concentration of adenosine in the stock solution was confirmed by HPLC. Adenosine was infused at doses between 150 and 300 μg/kg. The half-life adenosine in the central compartment is < 5 s (34).

Modulation of the direct effects of adenosine by β-adrenergic blockade was examined by pretreating two patients with an intravenous infusion of 0.1 mg/kg propranolol and then reevaluating the effects of adenosine.

Autonomic effects. The effects of autonomic modulation on the HPS rhythm were evaluated with atropine and isoproterenol. Atropine, 0.04 mg/kg, was administered and effects were observed for 15 min. Isoproterenol was then infused starting at a rate of 2 μg/min and increasing by 2 μg/min every minute until an infusion rate of 12 μg/min was achieved or side effects occurred.

The antiadrenergic (indirect), cAMP-dependent effects of adenosine were assessed by injecting adenosine during the maximal shortening of cycle length induced by isoproterenol. The adenosine dose was identical to that used earlier to evaluate the direct effects of adenosine.

Effects of adenosine inhibitors and potentiators. The ability of aminophylline (5.6 mg/kg), a competitive adenosine antagonist, to attenuate the antiadrenergic effects of adenosine was evaluated in five patients during simultaneous infusion of isoproterenol.

The antiadrenergic effects of dipyridamole, an adenosine transport blocker, on the HPS cycle length were demonstrated by infusing 0.56 mg/kg over 4 min during simultaneous isoproterenol infusion. Dipyridamole, which has antiplatelet effects, could only be infused in two patients since the other patients underwent implantation of a permanent pacemaker immediately after the studies described.

Isolated perfused hearts

To examine the proposed inhibitory feedback loop between adenosine and catecholamines on the HPS, experiments were carried out in nine adult guinea pigs weighing 250–300 g with a protocol approved by the Institutional Animal Care and Use Committee. The guinea pigs were stunned by cervical dislocation and the hearts were quickly excised. The hearts were retrogradely perfused via the aorta at a constant flow rate of 4–5 ml/min per g with an oxygenated Krebs-Henseleit solution with the following composition in mM: NaCl, 130; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.18; $NaHCO_3$, 25.0; dextrose, 11.0; pyruvate, 2.5. The pH and PO_2 of Krebs-Henseleit solution when equilibrated with 95% O_2 −5% CO_2 were 7.4 and 523±11 mm Hg (mean±SEM), respectively. The perfusate temperature was maintained at 35.5±1°C.

The following procedure was used to produce complete AV block (30). After an incision along the lateral margin of the right atrial appendage, an 8-0 silk suture was placed in the tip of the appendage for temporary retraction and access to the AV node area. An incision was then made anterior to the ostium of the coronary sinus above the insertion of the septal leaflet of the tricuspid valve.

Extracellular electrograms were recorded from the left atrium and His bundle as previously described (35). Electrograms were amplified and displayed on a dual-beam oscilloscope (model 5110; Tektronix, Inc., Beaverton, OR) and recorded on a strip-chart recorder (model 220; Gould, Inc., Cleveland, OH). The rate of the escape rhythm (His bundle) was continuously monitored by a tachometer (Biotech, Gould Inc., Cleveland, OH) triggered by the ventricular deflection on the His bundle electrogram.

Isoproterenol (Sigma Chemical Co.) was infused for 1 min via a polyethylene catheter positioned in the aortic root above the coronary ostia at a concentration of 6×10^{-8} M. The His bundle rate was continuously monitored and multiple venous effluent samples (each sample was collected for 15 s) for measuring adenosine were obtained during and after the infusion.

To block the effects of adenosine, a novel alkylxanthine (1,3-dipropyl-8-phenylxanthine; BW A1433) that binds to the adenosine A_1 receptor (36) was infused into the perfusion line at a rate to yield a final concentration of 5 μM (before isoproterenol infusion). Adenosine measurements were then repeated at the intervals described above. BW A1433 was kindly provided by Dr. S. Daluge from Burroughs Wellcome, Research Triangle Park, NC.

Analytical procedures

Effluent adenosine. The adenosine concentration in the effluent of the isolated perfused guinea pig hearts was measured as follows: 4-ml samples of effluent were collected in chilled tubes, filtered through 0.22-μm millipore filters, and assayed for adenosine using reversed phase HPLC in the isocratic mode according to the method of Hartwick et al. (37).

Statistical analysis. Effects of autonomic and pharmacologic interventions on the HPS cycle length (longest R-R interval) were analyzed by *t* test. Differences were considered significant for $P < 0.05$. All data are expressed as mean±SD unless otherwise indicated.

Results

Effects of adenosine and atropine

All escape rhythms were regular, stable, and hemodynamically well tolerated. The cycle length of the escape rhythms was 1,518±152 ms, with a range between 1,280 and 1,750 ms (Table I). Adenosine had a small but consistent effect on the

Table I. Antiadrenergic Effects of Adenosine on His-Purkinje System Cycle Length

Patient	Age/sex	ADO dose	Control	ADO	Control	Atropine	Control	ISO	ISO + ADO	ISO + Aminophylline	ISO + Aminophylline + ADO
		μg/kg	*ms*	*ms*	*ms*	*ms*	*ms*	*ms*	*ms*	*ms*	*ms*
1	77 F	150 (12 mg)	1,600	1,640	ND	ND	1,600	800	1,600	ND	ND
2	70 M	150 (11 mg)	1,410	1,580	1,480	1,400	1,380	910	1,140	1,110	1,160
3	68 F	187.5 (12 mg)	1,380	1,500	1,420	1,460	1,380	690	920	930	970
4	79 M	187.5 (16 mg)	1,500	1,550	1,500	1,480	1,500	900	1,840	990	1,055
5	82 M	225 (15 mg)	1,700	1,780	1,680	1,520	1,520	1,080	1,440	ND	ND
6	79 F	225 (13 mg)	1,480	1,720	1,480	1,340	1,480	880	1,620	1,130	1,130
7	79 F	225 (16 mg)	1,750	1,840	1,760	1,760	1,750	1,250	1,880	ND	ND
8	82 F	225 (13 mg)	1,560	1,660	1,560	1,520	1,560	740	1,150	940	920
9	35 M	300 (25 mg)	1,280	1,360	1,410	1,400	ND	ND	ND	ND	ND

Abbreviations: ADO, adenosine; ISO, isoproterenol; ND, not done.

escape rhythm, slowing the cycle length to 1,626±148 ms (7.3±4.4%), $P < 0.001$ (Figs. 1 and 2). Maximum effects were observed 7–20 s after injection. To determine whether the observed results were actually due to direct effects of adenosine or were related to antagonism of elevated levels of endogenous catecholamines, the effects of adenosine were studied after β-blockade with propranolol in two patients. In patient 5 the HPS cycle length slowed from 1,700 to 1,780 ms in response to adenosine. After propranolol, the escape rhythm cycle length increased by 120 ms. Adenosine injection (300 mg) at this time had no further effect on the cycle length. Similar results were observed in patient 9 (Fig. 2). Thus, the apparent "direct"

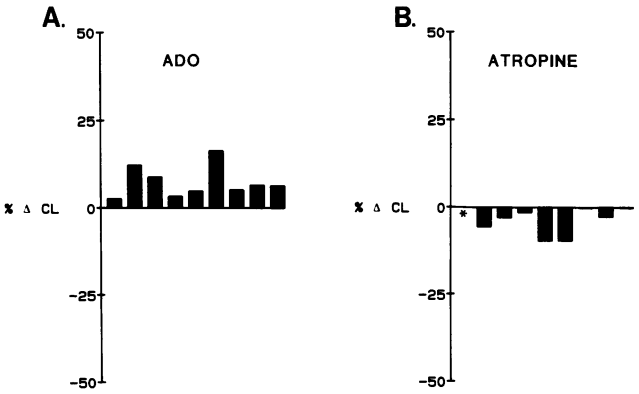

Figure 1. Effects of adenosine and atropine on the HPS escape rhythm. The bars represent the percent change in cycle length (*CL*) in response to the intervention with respect to control (0%) for each patient. The horizontal zero line represents the control or immediate cycle length before the intervention. Thus, a positive change indicates an increase in cycle length or slowing of the heart rate. Panels in Figs. 3 and 6 are arranged in a similar manner. (*A*) Effects of adenosine (*ADO*) under basal conditions in patients 1–9. (*B*) Direct effects of atropine. There is only a minimal direct effect of atropine on the HPS cycle length. Note that atropine was given to patients 2–9. *, atropine was not given to patient 1.

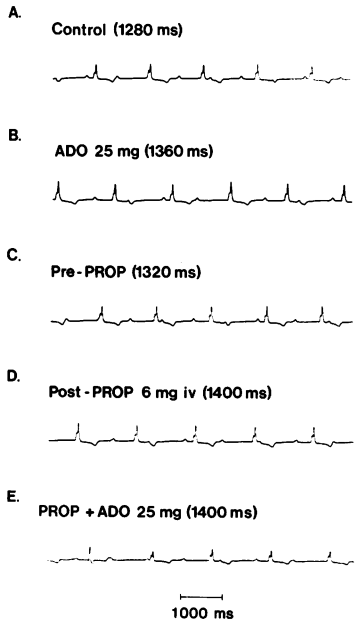

Figure 2. Effects of adenosine on HPS escape rhythm. (*A*) Control HPS rhythm in patient 9. (*B*) Adenosine has an apparent "direct" though small effect on the infranodal conduction system since it increases the escape rhythm cycle length by 80 ms, from 1,280 to 1,360 ms. (*C*) After washout of adenosine the escape rhythm cycle length is 1,320 ms. (*D*) After an infusion of 6 mg of propranolol (*PROP*) the escape rhythm cycle length shows to 1,400 ms. (*E*) Adenosine in the presence of propanolol has no effect on the escape rhythm cycle length. This suggests that the initial "direct" effects of adenosine were due to its indirect effects in response to endogenous β-adrenergic stimulation. All tracings are from ECG lead I and are recorded at chart speed of 25 mm/s.

effect of adenosine was completely abolished by pretreatment with propranolol.

Parasympathetic blockade with atropine had minimal effect on the HPS rhythm. It decreased the cycle length from 1,536±124 to 1,485±128 ms (4.0±3.8%), $P = 0.08$ (Fig. 1). QRS morphology and duration were unchanged by atropine. The effects of increased vagal tone on the escape rhythm were assessed in three patients. Valsalva maneuvers and right and left carotid sinus massage had no effect on the escape cycle length.

Antiadrenergic effects of adenosine

Adrenergic stimulation of the HPS with isoproterenol markedly decreased the escape rhythm cycle length in eight patients from 1,521±121 to 906±183 ms, $P = 0.0001$. The mean decrease was 41±9.6% (Fig. 3). In two patients the QRS duration was prolonged during isoproterenol stimulation suggesting either increased intraventricular conduction delay or preferential acceleration of an escape focus distal to the control site (Fig. 4). Atrioventricular conduction was not restored in any patient during isoproterenol infusion.

Adenosine showed marked antiadrenergic effects on the HPS escape rhythm. In the presence of isoproterenol, doses of adenosine between 150 and 300 μg/kg decreased the escape cycle length by 61±31% from 906±183 to 1,449±350 ms, $P < 0.001$. In two patients the escape rhythm shifted to a more proximal but still infranodal site (same site as the control escape rhythm) in response to adenosine (Fig. 4).

Antiadrenergic effects of dipyridamole

The antiadrenergic effects of dipyridamole were evaluated in two patients. In the first patient (patient 8), during isoproterenol infusion (12 μg/ml) dipyridamole (0.56 mg/kg) increased the escape rhythm cycle length from 640 to 1,000 ms within 3 min (Fig. 5), whereas adenosine (in the presence of isoproterenol) slowed the ventricular escape cycle length from 740 to 1,150 ms. In another patient, a 38-yr-old man who underwent a His bundle ablation, direct effects of adenosine could not be evaluated because of a very slow idioventricular escape rhythm. This patient required continuous ventricular pacing;

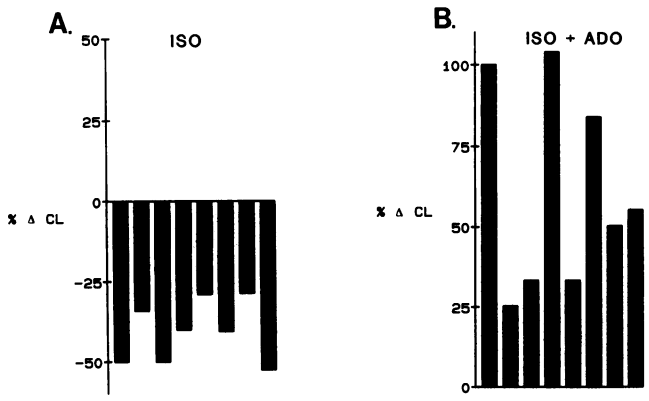

Figure 3. Antiadrenergic effects of adenosine. (*A*) Isoprotenerol (*ISO*) markedly decreases the HPS cycle length in patients 1–8. (*B*) Antiadrenergic effects of adenosine. The horizontal zero line corresponds to the control cycle length during ISO infusion just before the bolus administration of adenosine.

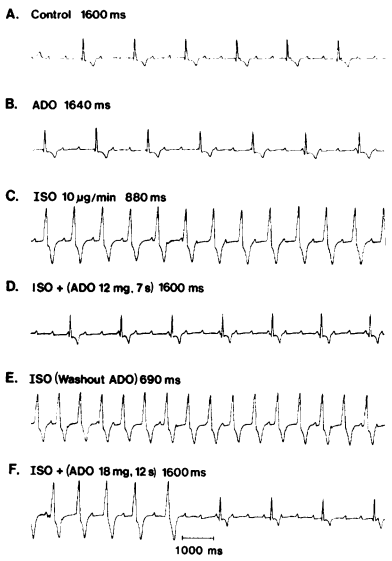

Figure 4. Antiadrenergic effects of adenosine on the HPS (Patient 1). (*A*) Complete heart block with an infra-Hisian escape rhythm cycle length of 1,600 ms. (*B*) Adenosine has minimal direct effect on the escape rhythm cycle length. (*C*) Isoproterenol increases the sensitivity of a more distal conduction site resulting in prolongation of the QRS complex and a marked decrease in the escape rhythm cycle length. (*D*) Adenosine completely antagonized the effects of isoproterenol and resulted in a proximal shift in pacemaker site identical to that observed during control (*A*). (*E*) After adenosine washout and during continued isoproterenol infusion (10 μg/min) the escape rhythm focus again shifted to a more distal site and the cycle length decreased to 690 ms. (*F*) The reproducible antiadrenergic effects of adenosine are shown. Abbreviations as previously designated. All recordings are from surface ECG lead 2.

therefore, his data are not included in Table I. However, during isoproterenol infusion dipyridamole increased the His bundle cycle length from 900 to 1,040 ms. Similarly, during isoproterenol infusion adenosine increased the escape cycle length from 780 to 1,080 ms. There was no appreciable effect on BP.

Effects of aminophylline

Adenosine either had no effect or minimal antiadrenergic effects on the HPS cycle length in the presence of both isoproterenol and aminophylline in five patients (Fig. 6), 1,020±94 (control) vs. 1,047±102 ms (adenosine), P = NS.

Isolated guinea pig hearts

The His bundle rhythm showed a time-dependent response to isoproterenol (Fig. 7 *A*). The maximum rate response was ob-

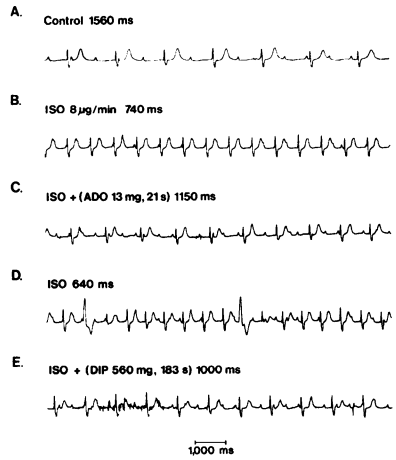

Figure 5. Effects of dipyridamole on the HPS. (*A*) Escape HPS cycle length of 1,560 ms during control in patient 8. (*B*) Response of HPS to isoproterenol. (*C*) Antiadrenergic effects of adenosine. (*D*) Antiadrenergic effects of dipyridamole (*DIP*) in the presence of isoproterenol (8 μg/min). All tracings are from ECG lead I. See text for discussion.

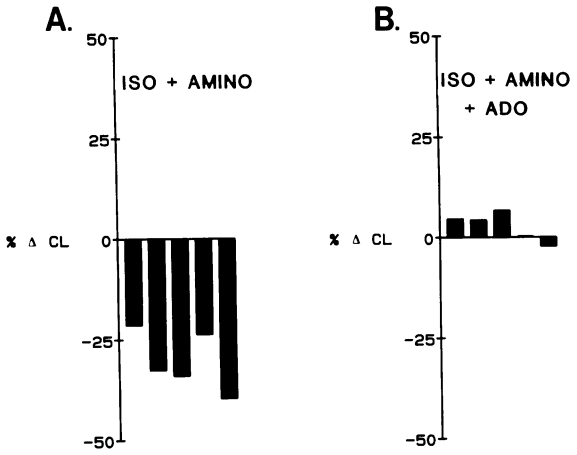

Figure 6. Effects of aminophylline (*AMINO*) on the HPS escape rhythm. (*A*) The HPS cycle length decreases in response to both isoproterenol and aminophylline in patients 2–4, 6, and 8. (*B*) Addition of aminophylline results in inhibition of the antiadrenergic effects of adenosine. The control cycle length refers to the HPS cycle length in response to isoproterenol and aminophylline.

served 40 s after the beginning of the infusion, 99±3 beats/min (0 s) vs. 156±5 beats/min (40 s), $P < 0.05$ (mean±SE). The His bundle rate returned to control 180 s after completion of the 1-min infusion.

The adenosine antagonist BW A1433 had no effect on the His bundle rate in the absence of isoproterenol, 101±3 beats/min vs. 99±3 beats/min (control), P = NS (mean±SE); however, between 40 and 80 s after initiation of isoproterenol infusion the increase in His bundle rate was significantly greater in the presence of BW A1433 than during control (Fig. 7 *A*). Likewise, in comparison with control the isoproterenol-induced increase in His bundle rate (in the presence of BW A1433) was associated with a greater release of adenosine in the venous effluent (Fig. 7 *B*).

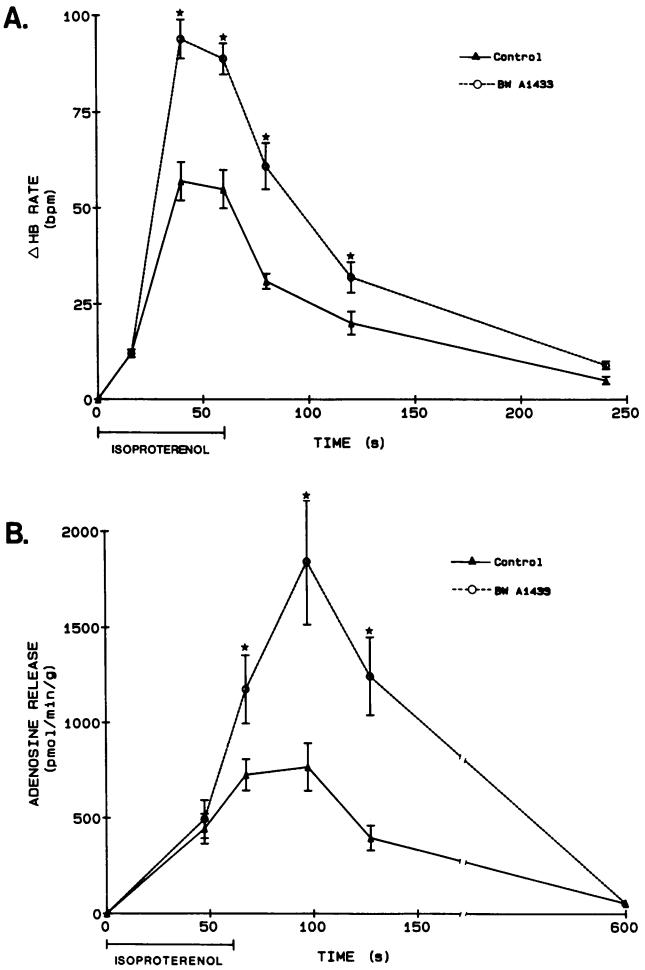

Figure 7. Effect of isoproterenol and of the adenosine A_1 receptor antagonist BW A1433 on His bundle (*HB*) rate and adenosine release in isolated guinea pig hearts with complete AV block. (*A*) Isoproterenol (6×10^{-8} M) markedly increases the HB rate. After the addition of BW A1433, the HB rate during isoproterenol infusion is further accelerated. (*B*) Isoproterenol infusion (control) results in a time-dependent release of adenosine in the venous effluent, which is significantly increased in the presence of BW A1433. These results suggest that adenosine released in response to isoproterenol functions as a feedback inhibitor of isoproterenol-induced increase in HB automaticity. Uncoupling of this feedback loop with BW A1433 further augments HB automaticity and the amount of adenosine released. See text for discussion. *, $P < 0.05$.

Discussion

A major finding of this study is that adenosine is an important antagonist of catecholamine-induced electrophysiologic effects in the human HPS. In contrast, adenosine has minimal direct effect on HPS automaticity. The primary effects of adenosine in the HPS are indirect or antiadrenergic. These findings are consistent with the phenomenon of accentuated antagonism, a term originally used to describe the enhanced inhibitory response of the myocardium to parasympathetic stimulation in the presence of sympathetic tone (1, 2).

Evidence supporting accentuated antagonism

The effects of adenosine on HPS automaticity under basal conditions were abolished when two patients were pretreated with propranolol. These findings suggest that the apparent "direct" electrophysiologic actions of adenosine were in fact due to antagonism of endogenous catecholamine effects (Fig. 2). Although adenosine has been reported to have significant direct effects in Purkinje tissue (27–31), other studies in Purkinje fibers pretreated with propranolol (38) or in isolated ventricular myocytes (32, 33) suggest that adenosine has minimal or no direct effect.

The observed antiadrenergic effects of adenosine were consistent and reproducible in all patients. These antiadrenergic effects on HPS automaticity were antagonized by the xanthine derivative, aminophylline. Alkylxanthines are well-known competitive adenosine antagonists (36) and have been shown to antagonize the negative dromotropic effects of adenosine at concentrations considerably lower than those required to either inhibit phosphodiesterase activity or to release catecholamines from nerve terminals (39).

A unique finding in this study, although examined in only two patients, was that dipyridamole induced His-Purkinje slowing during isoproterenol infusion (Fig. 5). Dipyridamole, a

nucleoside transport blocker that potentiates the actions of adenosine (39), has been shown to elevate plasma levels of adenosine in humans (40). In the absence of exogenous adenosine it is likely that dipyridamole potentiated the effects of endogenous adenosine released by isoproterenol (41), although direct negative chronotropic effects of dipyridamole independent of adenosine cannot be excluded (42).

Previous clinical studies have shown that adenosine has significant negative chronotropic (43) and dromotropic effects in the sinoatrial and AV nodes (43, 44). Adenosine is thought to have a direct action (effect in the absence of β-adrenergic-cAMP stimulation) in these tissues by increasing steady state outward K^+ current (22, 24). Similar to atrial muscarinic cholinergic receptors, the adenosine A_1 receptor is coupled to K^+ channels via an inhibitory guanine nucleotide binding regulatory protein that mediates its effects independent of cAMP stimulation (24–26). In contrast, the electrophysiologic actions of adenosine in the human HPS are primarily antiadrenergic and are consistent with the concept of accentuated antagonism. This phenomenon was originally observed for sympathetic-parasympathetic interactions in both in vitro and in vivo studies in ventricular muscle and Purkinje fibers (1, 2, 7–14) and appears to serve as a protective mechanism for the ventricular myocardium from excessive catecholamine stimulation.

The likely cellular mechanism by which adenosine mediates accentuated antagonism involves the guanine nucleotide-binding regulatory protein (G_i) that couples the A_1 receptor with the catalytic subunit of adenylate cyclase to reduce cAMP levels (45). Acetylcholine mediates its electrophysiologic effects on ventricular myocardium (accentuated antagonism) via an identical transduction process after binding to muscarinic cholinergic receptor (24, 46).

Adenosine has previously been described as having antiadrenergic activity with respect to its inotropic effects in ventricular myocardium (18, 41). These effects are independent of muscarinic cholinergic stimulation (47). Similar to its electrophysiologic action in the ventricle, adenosine has minimal effects on ventricular contractility in the absence of catecholamine stimulation and does not antagonize increased inotropy due to elevated extracellular [Ca^{2+}] (48) or α-adrenergic stimulation (49). Attenuation of the contractile response to catecholamines by an increase in endogenous adenosine production is paralleled by a decrease in cellular cAMP levels (41), suggesting that the antiadrenergic effects of adenosine are cAMP-dependent. Since catecholamines increase the rate of formation of adenosine in both hypoxic and normoxic hearts, it has been proposed by Schrader et al. (41) and Dobson et al. (17, 18), that adenosine functions as a negative feedback inhibitor of catecholamine stimulation of myocardial contractility to attenuate adverse metabolic consequences.

To determine the physiologic role of adenosine's antiadrenergic activity in the HPS, a series of experiments was performed in normoxic guinea pig hearts. These studies were designed to demonstrate a negative feedback mechanism between adenosine and catecholamine-cAMP–mediated electrophysiologic effects on the HPS. Although catecholamine stimulation was shown to increase the chronotropic response of the HPS, this effect was amplified in the presence of the specific and potent adenosine antagonist, BW A1433 (Fig. 7 *A*). Of significance is the finding that catecholamine-induced release of adenosine is also enhanced by the addition of BW A1433 to the perfusate (Fig. 7 *B*). Thus, by blocking the effects of adenosine at the receptor level with a resultant increase in adenosine release, the inhibitory effect of adenosine on catecholamine stimulation of HPS rate was uncoupled (therefore HPS rate increased). Since under these conditions release of the control signal (adenosine) was enhanced but its effector response blunted, a physiologic negative feedback control relationship between adenosine and catecholamines appears operative. Similarly, a positive feedback relationship between catecholamines and adenosines is suggested by the data demonstrating catecholamine-induced release of the nucleoside. These findings identify an important electrophysiologic role for adenosine in the HPS, that of a counterregulatory signal that protects the HPS from excessive catecholamine stimulation.

Autonomic effects on the HPS

As expected from in vivo canine studies, catecholamine infusion produced a dose-dependent increase in HPS automaticity (50, 51). Vagal maneuvers such as Valsalva and carotid sinus massage had little effect on the HPS rate. Consistent with the results from experimental studies, muscarinic cholinergic blockade with atropine had no effect on the HPS rate (52–54).

There is extensive evidence confirming parasympathetic innervation of the mammalian ventricle as demonstrated by choline acetyltransferase activity, the presence of ACh, and muscarinic cholinergic receptors (55–59). However, there are conflicting data regarding the importance of direct vagal effects in the ventricular myocardium and the HPS. Several studies have shown that vagal stimulation and ACh infusion exert a negative chronotropic effect on His-Purkinje tissues in vitro and in vivo (52, 60–63). In addition, ventricular refractory periods are shortened in response to atropine in patients pretreated with propranolol, suggesting a direct cholinergic effect in this tissue (64). Despite these findings, however, the weight of evidence indicates that in ventricular myocardium vagal effects are primarily antiadrenergic (accentuated antagonism) (1, 2, 7–14). Vagal antagonism of sympathetic stimulation occurs at both pre- and postsynaptic levels (1, 2, 46).

Significant parallels appear to exist between the electrophysiologic effects of vagal stimulation (ACh) and adenosine in the ventricle. For example, (*a*) both mediators demonstrate evidence of accentuated antagonism, (*b*) have relatively minor direct electrophysiologic effects, (*c*) share a similar transduction process which involves the guanine nucleotide binding regulatory protein G_i, and (*d*) manifest sympathetic antagonism at pre- and postsynaptic levels (1, 2, 24, 32, 33, 46, 65). In addition, both systems attenuate the effects of enhanced sympathetic tone precipitated by acute ischemia. In general, sympathetic activity during acute ischemia in normal hearts (66) or in previously infarcted hearts is arrhythmogenic (67, 68). In contrast, increased vagal tone under these circumstances has a protective effect, decreasing the vulnerability for ventricular fibrillation (10, 67). Adenosine is released from cardiac myocytes into the interstitium in response to ischemia, catecholamines, and ACh stimulation (69). It therefore appears that during ischemia the parasympathetic nervous system and the intermediate metabolite adenosine are linked at several levels to function in parallel to antagonize the potentially adverse electrophysiologic (and inotropic) effects of catecholamine

stimulation. The existence of these essentially redundant systems may be viewed as a protective physiologic response to perturbation of the homeostatic O_2 supply-demand ratio.

Significance

The results of this study suggest that the primary electrophysiologic effect of adenosine in the human ventricular myocardium is to antagonize the effects of catecholamine stimulation of adenylate cyclase. It has been previously shown that exogenous adenosine effectively terminates ventricular tachycardia (VT) thought to be due to cAMP-mediated triggered activity (32, 33, 70). The present study shows that automatic rhythms originating from the HPS that are mediated by catecholamine stimulation of cAMP are also sensitive to adenosine. The response of automatic or cAMP-mediated, triggered ventricular rhythms to adenosine may be relatively specific since adenosine has no effect in catecholamine-facilitated reentrant VT (70) in patients with remote myocardial infarction, suggesting that the mechanism of catecholamine facilitation in these patients is independent of cAMP stimulation (71). The effects of adenosine in catecholamine-induced reentrant VT due to acute ischemia has yet to be systematically evaluated.

The physiologic significance of adenosine-mediated accentuated antagonism is to restore a favorable O_2 supply-demand ratio and, hence, to protect the myocardium from excessive catecholamine stimulation. The relationship between sympathetic stimulation and adenosine can be understood in terms of a feedback schema with a positive and negative limb. In the positive feedback limb, adenosine (signal) is released from myocytes in response to catecholamines and ischemia. Adenosine, in turn, attenuates (negative feedback limb) catecholamine release and end-organ responses at pre- and postsynaptic levels. Based on the results of this study and previous findings (67, 70) it appears that some catecholamine-mediated ventricular arrhythmias that occur under either normoxic or hypoxic conditions may result from inadequate feedback inhibition of sympathetic stimulation by adenosine and the parasympathetic nervous system.

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