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The Cardiac Effects of a Novel A_1-Adenosine Receptor Agonist in Guinea Pig Isolated Heart

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ABSTRACT

Adenosine increases atrioventricular (AV) nodal conduction time and is used for termination of AV nodal re-entrant tachycardias, but it is rapidly metabolized. The purposes of the present study were to characterize the cardiac actions and effects of an orally active and stable adenosine analog, N^6-cyclohexyl-2-O-methyladenosine (SDZ WAG-994) and to evaluate its potential as an antiarrhythmic agent. Guinea pig hearts were isolated and perfused with oxygenated Krebs-Henseleit solution. SDZ WAG-994 slowed the atrial rate and prolonged the AV nodal conduction time of spontaneously beating hearts in a concentration-dependent manner. The EC_{50} values for the negative chronotropic and dromotropic effects of SDZ WAG-994 were 0.69 ± 0.04 and 1.49 ± 0.54 μM, respectively. The A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (0.2 μM) significantly antagonized SDZ WAG-994-induced stimulus-to-His bundle (S-H) interval prolongation. The negative dromotropic effect of SDZ WAG-994 showed very strong frequency dependence. In hearts paced at an atrial cycle length of 300 msec (200 beats/min), the EC_{50} value of SDZ WAG-994 to prolong the S-H interval was 3.7-fold lower (0.40 ± 0.02 μM) than in unpaced hearts, and at atrial pacing cycle lengths of 500 and 250 msec, 0.3 μM SDZ WAG-994 prolonged the S-H interval by 8 and 26 msec, respectively. SDZ WAG-994 also decreased coronary perfusion pressure (EC_{50} = 1.50 ± 0.80 μM); this effect of SDZ WAG-994 was attenuated by adenosine deaminase and by 8-cyclopentyltheophylline (2 μM). Radioligand binding assays revealed that SDZ WAG-994 had a 280-fold greater affinity for A_1- than for A_{2a} receptors of the guinea pig brain. The marked frequency dependence of the negative dromotropic effect of SDZ WAG-994 suggests that this A_1 agonist may be highly effective in the termination of AV nodal re-entrant tachycardias.

Adenosine activates both A_1 and A_2 subtypes of cell membrane AdoRs in the heart. A_1-AdoR mediate 1) the antagonism by adenosine of the stimulatory effects of catecholamines (anti-*beta*-adrenergic action), 2) the slowing of the heart rate (negative chronotropy) and impulse propagation through the AV node (negative dromotropy) and 3) the reduction in atrial contractility (negative inotropy; Belardinelli *et al.*, 1989). A_2-AdoRs mediate the coronary dilatation caused by adenosine (Belardinelli *et al.*, 1989). In recent years, considerable interest has been shown in the action of adenosine to slow AV nodal conduction because this action is the basis for adenosine's efficacy in the termination of AV nodal re-entrant supraventricular tachycardia (Belardinelli and Lerman, 1990; Lerman and Belardinelli, 1991; Camm and Garratt, 1991).

The negative dromotropic effect of adenosine has been demonstrated in experiments with isolated AV nodal preparations (Clemo and Belardinelli, 1986a; Belardinelli *et al.*, 1987) and in isolated perfused and *in situ* hearts of laboratory animals (Belardinelli *et al.*, 1982, Clemo and Belardinelli, 1986ab) and humans (DiMarco *et al.*, 1990; Lerman and Belardinelli, 1991). The site of adenosine action appears to the N cell zone of the AV node (Clemo and Belardinelli, 1986a). Inhibitors of adenosine uptake or metabolism (Belardinelli *et al.*, 1981, 1982, 1984) and allosteric enhancers of adenosine binding to the A_1-AdoR (Amoah-Apraku *et al.*, 1993) both potentiate the action of adenosine to slow AV nodal conduction, whereas AdoR antagonists and ADA both attenuate the negative dromotropic effect of adenosine (Belardinelli *et al.*, 1984, 1987, 1989). There is strong evidence to indicate that the slowing of AV nodal conduction observed in the hypoxic and ischemic heart is caused by endogenous adenosine (Belardinelli *et al.*, 1981; Clemo and Belardinelli, 1986b; Froldi and Belardinelli, 1990; Xu *et al.*, 1993).

An important feature of AV nodal transmission is its fre-

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ABBREVIATIONS: SDZ WAG-994, N^6-cyclohexyl-2-O-methyladenosine; AV, atrioventricular; S-H, stimulus-to-His bundle; AdoR, adenosine receptor; CPX, 8-cyclopentyl-1,3-dipropylxanthine; CPT, 8-cyclopentyltheophylline; CPA, N^6-cyclopentyladenosine; R-PIA, N^6-R-phenylisopropyladenosine; N-0861, (±)N^6-endonorborman-2-yl-9-methyladenine; QNB, quinuclidinylbenzilate methyl chloride; NBMPR, nitrobenzylthioinosine; CCPA, 2-chloro-N^6-cyclopentyladenosine; CGS 21,680, 2-*p*-(2-carboxyethyl)-phenethylamino-5-N- ethylcarboxamidoadenosine; ADA, adenosine deaminase.

quency dependence (Merideth *et al.*, 1968). Conduction of the electrical impulse through the AV node becomes progressively slower (conduction time increases) as the atrial pacing rate is increased (*i.e.*, as the pacing cycle length decreases) until conduction fails. Failure of impulse conduction in the AV node can protect the ventricles from the effects of abnormally high rates of electrical activity in the atria.

Drugs the negative dromotropic action of which is frequency dependent are used to reduce ventricular rate during atrial tachycardias (Roth *et al.*, 1986; Ellenbogen, 1992). The frequency-dependent slowing of AV nodal conduction by calcium channel blockers is the basis for the high efficacy of this class of agents to terminate AV nodal re-entrant supraventricular tachycardias and to control ventricular rate during atrial fibrillation (Talajic and Nattel, 1986; Talajic *et al.*, 1989, 1990). The depressant effect of adenosine on AV nodal conduction also appears to depend on the underlying heart rate (Belardinelli and Shryock, 1992; Stark *et al.*, 1993a). However, it is not known whether the actions of stable analogs of adenosine, which are long acting and therefore suitable for chronic therapy of supraventricular tachycardias, are also frequency dependent. Therefore, the objective of this study was to characterize the frequency dependence of the effects of SDZ WAG-994 on AV nodal transmission of guinea pig hearts. SDZ WAG-994 is a new, potent, orally active and selective A_1-AdoR agonist (Wagner *et al.*, 1994; Fozard *et al.*, 1994). To facilitate interpretation of the functional responses of hearts to SDZ WAG-994, we also evaluated the pharmacological properties (*e.g.* potency, receptor selectivity and affinity) of the drug. We hope our findings will lead to an assessment of the frequency dependence of the actions of other adenosine analogs on the AV node, with the goal to develop efficacious drug therapy of supraventricular arrhythmias.

Methods

SDZ WAG-994 was a gift from Dr. John Fozard of Sandoz Pharma, Ltd. (Basle, Switzerland). CPX, CPT, CPA and R-PIA were purchased from Research Biochemicals (Natick, MA). Atropine was purchased from Sigma (St. Louis, MO). N-0861 was a gift from Discovery Theraeutics Inc. (Richmond, VA). Stock solutions of these above drugs were dissolved in perfusion medium and infused to achieve the desired perfusate concentration. [^{3}H]QNB, [^{3}H]NBMPR, [^{3}H]CCPA and [^{3}H]CGS 21,680 were purchased from New England Nuclear (Doraville, GA).

Isolated Perfused Hearts

Guinea pigs of either sex that weighed 250 to 300 g were anesthetized with methoxyflurane and sacrificed by cervical dislocation. The hearts were quickly removed and rinsed in ice-cold Krebs-Henseleit solution. The aorta was cannulated for perfusion of the coronary arteries at a constant flow rate of 8 or 10 ml/min with Krebs-Henseleit solution oxygenated with 95% oxygen and 5% CO_2. The pO_2, temperature and pH of the Krebs-Henseleit solution were maintained at 500 to 600 mm Hg, 35 ± 0.5°C and 7.3 to 7.4, respectively.

To facilitate pacing of the heart and measurement of the His bundle electrogram, the sinoatrial nodal region (which included the vena cava) and part of the right atrium were excised (Clemo and Belardinelli, 1986a; Jenkins and Belardinelli, 1988). The hearts were electrically paced at a cycle length of 300 msec (unless otherwise indicated) by a bipolar electrode placed on the atrium. An interval generator (model 1830, WPI, New Haven, CT) delivered the stimuli through a stimulus isolation unit (model 1880, WPI) as square-wave pulses of 3 msec in duration and at least twice the threshold intensity. AV nodal conduction time was measured from His bundle electrograms during constant atrial pacing. The S-H interval was used as the index of AV nodal conduction time and was measured on-line with an AT & T 6 (LisPe, IL) 300 microcomputer, as previously described (Jenkins and Belardinelli, 1988).

In experiments designed to study the chronotropic and dromotropic effects of SDZ WAG-994 in spontaneously beating guinea pig heart preparations, the entire right atrium (which included part of the superior and inferior venae cavae) was left undissected, *i.e.*, only the extraneous tissue was trimmed away. A unipolar extracellular electrogram was recorded from the surface of the right atrium with a Teflon-coated stainless steel electrode. The atrial rate and AV nodal conduction time were determined from measurements of atrial cycle length and AV intervals, respectively.

After completion of dissection and instrumentation, the hearts were allowed to equilibrate for 30 min before experiments were begun. Experimental interventions were always preceded and followed by measurements of the atrial rate (spontaneously beating preparations) or S-H interval (atrial paced preparations). Whenever precontrol and postcontrol values differed by more than 15%, the intervening data were discarded. When an intervention caused second-degree AV block, the longest stable S-H interval before the onset of AV block was considered the maximal dromotropic effect and that value was used for data analysis.

Membrane Preparation

Cardiac. Dissected atrial and ventricular tissues were minced and homogenized (Polytron, Brinkman Instruments, Westburg, NY) for 10 to 15 sec in 10 volumes of ice-cold buffer (50 mM Tris HCl, pH 7.4). The homogenate was spun in a centrifuge at 48,000 × *g* for 15 min at 4°C to pellet membranes. The pellet was resuspended in 30 ml of buffer and spun again. Then the pellets were washed twice more by resuspension and centrifugation. The final pellet was resuspended in 1 volume of buffer used for assays. Membrane suspensions were stored at −80°C. The protein content was determined by the Bradford protein dye-binding method (Bio-Rad, Cambridge, MA) with bovine serum albumin as the standard.

Brain. Pieces of freshly isolated guinea pig forebrain and striatum were immersed in 10 volumes of ice-cold 50 mM Tris HCl buffer, pH 7.4, and homogenized for 5 sec. The homogenate was spun at 48,000 × *g* for 15 minutes at 4°C. The supernatant was discarded and the pellet was resuspended in 10 volumes of the same buffer and washed three times by centrifugation and resuspension in fresh buffer. The washed pellet was resuspended to yield a protein concentration of 0.4 to 0.6 mg/ml in 50 mM Tris HCl buffer, pH 7.4, and stored at −80°C. The protein content was determined by the Bradford method.

Radioligand Binding Assay

Competition assays to determine the affinities of SDZ WAG-994 for the A_1- and A_2-AdoRs, the muscarinic cholinergic receptor and the nucleoside transporter(s) were performed with 100-μl aliquots of membrane suspension (0.2–0.7 μg) incubated at room temperature in 50 mM Tris HCl buffer (pH 7.4), which contained ADA (5 U/ml) and one of the following radioligands: 6 nM [^{3}H]-CCPA for the A_1-AdoR (Schwabe, 1991), 5 nM [^{3}H]CGS 21,680 for the A_{2a}-AdoR (Schwabe, 1991), 0.1 nM [^{3}H]QNB for the muscarinic cholinergic receptor (Baker and Posner, 1986) and 2 nM [^{3}H]NBMPR for the nucleoside transporter binding site (Williams *et al.*, 1984). Nonspecific binding was determined with 10 μM CPT, 10 μM unlabeled CGS 21,680, 20 μM atropine and 10 μM dipyridamole to displace specific binding of [^{3}H]CCPA, [^{3}H]CGS 21,680, [^{3}H]QNB and [^{3}H]NBMPR, respectively. Binding parameters were determined from competition assays with the computer program LIGAND (Biosoft, Cambridge, UK). Dissociation constants for the binding of [^{3}H]CCPA to guinea pig atrial, human atrial, guinea pig ventricle and forebrain membrane preparations were 2, 2, 4 and 1 nM, respectively. The dissoci-

ation constant for the binding of [^{3}H]CGS 21,680 to guinea pig striatal membranes was 4 nM. The dissociation constants for the binding of [^{3}H]NBMPR to guinea pig atrial and ventricular membranes were 1.1 and 1.7 nM, respectively. The value of K_i for the displacement of each [^{3}H]ligand by SDZ WAG-994 was calculated with the Cheng-Prusoff transformation (Cheng and Prusoff, 1973).

Adenosine Assay

Samples of 0.5 ml of the heart's effluent were collected in chilled tubes, frozen and stored at −80°C for later analysis of adenosine content. A 100-μl aliquot of each sample was assayed for adenosine content with reversed-phase high-performance liquid chromatography (model M6000A pump, model 710B autosampler, model 441 ultraviolet light detector and model 730 data module, Waters, Marlborough, Ma). Adenosine was separated from other compounds by the use of a Beckman Arlington Height, IL Ultrasphere octadecylsilane 4.6 × 250-mm column and elution with 10% methanol in aqueous 50 mM KH_2PO_4, pH 5.6, at a flow rate of 1.5 ml/min. The absorbance of sample peaks at 254 nm was measured and the adenosine content of samples was quantified by comparison with standards of known adenosine content.

Data Analysis

All measurements are reported as the mean ± S.E.M. To determine the EC_{50} values for effects of SDZ WAG-994 on AV nodal conduction time and S-H interval and the coronary conductance and heart rate, SDZ WAG-994 concentration-response relationships were fitted with either a nonlinear (Marquardt-Levenberg) regression algorithm to a parabolic (equation 1) or a dose-response logistic equation (equation 2), respectively (Table Curve program, Jandel Scientific, San Rafael, CA).

In equation 1, $y = a + bx^2$, where y, x, a and b denote either AV nodal conduction time or S-H interval (in milliseconds), the concentration of SDZ WAG-994 (in micromolar amounts) and two curve-fitting parameters, respectively. The dose-response relationships for the effects of SDZ WAG-994 on S-H interval and AV nodal conduction time were parabolic in form.

In equation 2, $y = d + (a - d)/(1 + (x/c)^b$, where y, x, a, b, c and d denote coronary conductance (in milliliters per millimeter of mercury per minute), concentration of SDZ WAG-994 (in micromolar amounts), extrapolated maximal value of coronary conductance (in milliliters per millimeter of mercury per minute), apparent Hill coefficient, concentration of SDZ WAG-994 that causes a half-maximal increase, *i.e.*, EC_{50}, in coronary conductance (in micromolar amounts) and extrapolated minimum value of coronary conductance (in milliliters per millimeter of mercury per minute), respectively. The EC_{50} for SDZ WAG-994 to decrease spontaneous heart rate was calculated similarly.

The time constants τ_{on} and τ_{off} characterize the frequency-dependent responses of the S-H interval to an abrupt increase or decrease, respectively, in atrial pacing rate. The time constants were determined with the Table Curve software program. Estimates of τ_{on} and τ_{off} were determined by fitting the experimental data to equations 3 and 4, respectively.

In equation 3, $SH = SH_o + (SH_{ss} - SH_o)(1 - e^{-T/\tau on})$, where SH, T, SH_o, SH_{ss} and τ_{on} denote S-H interval (in milliseconds), time (in seconds), S-H interval of either the first or second conducted beat after an abrupt increase in atrial pacing rate (in milliseconds), extrapolated steady-state value of the S-H interval and the time constant of change in S-H interval (in seconds), respectively.

In equation 4, $SH = SH_o\, e^{-T/\tau off}$, where SH, T, SH_o and τ_{off} denote the S-H interval (in milliseconds), time (in seconds), S-H interval of either the first or second conducted beat after an abrupt decrease in atrial pacing rate (in milliseconds) and time constant of the change in S-H interval (in seconds), respectively. Note the S-H interval of the first conducted beat was usually included in the data for analysis and used as SH_o (equations 3 and 4). However, occasionally, the S-H interval of the first conducted beat was very different from that of the second conducted beat, presumably because the drug action was just beginning. In this case, the data point for the first interval was deleted from the data set.

The two-tailed Student's t distribution was used to analyze paired data. One-way repeated-measures analysis of variance followed by the Student-Newman-Keuls test was used to analyze multiple comparisons among control and interventions. Differences between or among group means were considered significant at the level of $P < .05$.

Protocols

Chronotropic effect of SDZ WAG-994. Experiments to determine the chronotropic effect of SDZ WAG-994 were carried out in each of six spontaneously beating hearts. In the same hearts, the effect of SDZ WAG-994 on AV nodal conduction time was also determined. After control atrial and ventricular electrograms were recorded, successively higher doses of SDZ WAG-994 were infused to achieve perfusate concentrations ranging from 0.1 to 10 μM. The effects of SDZ WAG-994 on atrial rate and AV nodal conduction time were recorded simultaneously. For each heart, the relationship between slowing of atrial rate and prolongation of AV nodal conduction time at each concentration of SDZ WAG-994 was determined.

Dromotropic effect of SDZ WAG-994. In this series of experiments (n = 8), concentration-response relationships for the negative dromotropic effect of SDZ WAG-994 (*i.e.*, prolongation of S-H interval) were obtained during pacing of hearts at atrial cycle lengths of 500, 300 and 200 msec. After a control His bundle electrogram was recorded, SDZ WAG-994 was administered at successively higher concentrations, starting at 0.1 μM and ending at a concentration that caused second-degree AV block. The effect of a given concentration of SDZ WAG-994 was measured when the response had reached a steady-state (≤ 10 min after onset of infusion). To investigate the negative dromotropic effect and its frequency dependence of SDZ WAG-994 further, determinations were made of 1) Wenckebach cycle length, 2) effective refractory period of the AV node and 3) prolongation of the S-H interval in response to an abrupt and transient increase in atrial pacing rate. Three programmed stimulation protocols were used as follows. The Wenckebach cycle length was determined by decreasing the atrial pacing cycle length in 3-msec steps every 10 stimuli until second-degree AV block occurred. After the control cycle length was determined, the effects of 0.05 and 0.1 μM SDZ WAG-994 alone and in the presence of CPX (0.1 μM) were determined. In the premature stimulus protocol, the right atrium was stimulated at a fixed basic cycle length (S_1S_1 interval) of 300 msec. After a train of 15 stimuli (S_1), a single premature (test) stimulus (S_2) was introduced and the S-H interval was determined. The coupling interval (S_1S_2) between the last S_1 and the test stimulus (S_2) was progressively shortened in 3-msec steps after every train of stimuli. The longest S_1S_2 interval for a stimulus that did not conduct through the AV node and produce a His bundle response was defined as the AV nodal effective refractory period. After the control refractory period was determined, the effects of 0.05 and 0.1 μM of SDZ WAG-994 alone and in the presence of CPX (0.1 μM) were determined. In the single-step protocol (tachycardia experiments), after 30 sec of pacing at a fixed atrial cycle length of 300 msec, pacing at an atrial cycle length of either 200 or 180 msec was begun and maintained for 1 min, followed by a return to the original pacing cycle length. The 1-min duration of rapid pacing was shown in a previous study (Jenkins and Belardinelli, 1988) to be sufficient in most cases for the AV nodal conduction time to achieve steady state, regardless of the base-line atrial pacing cycle length. In each heart of this series (n = 5), a maximum of four pacing protocols was performed, *i.e.*, in the absence (control) and in the presence of 0.05 and 0.1 μM SDZ WAG-994. In two hearts of this series, a further stimulation protocol in the presence of SDZ WAG-994 plus CPX (0.1 μM) was performed.

Specificity of the negative dromotropic effect of SDZ WAG-994. Hearts (n = 5) were perfused with a concentration of SDZ WAG-994 that caused a stable S-H interval prolongation of about 12 to 15 msec. After SDZ WAG-994 had prolonged the S-H interval to the new steady-state value, the muscarinic antagonist atropine (1 μM) and later the A_1-AdoR antagonist CPX (0.2 μM; Bruns *et al.*, 1987) were added to the perfusate.

Coronary vasodilatory effect of SDZ WAG-994. Hearts were perfused at a constant flow of 10 ml/min and ventricles were paced at a cycle length of 300 msec. The concentration-response relationship for the action of SDZ WAG-994 (0.05–20 μM) to increase coronary conductance was obtained by a determination of the effects of progressively higher concentrations of SDZ WAG-994 on coronary perfusion pressure. Once the concentration-response relation was completed, SDZ WAG-994 was washed out and ADA (3 U/ml) was added to the perfusate. After a 10-min equilibration period in the presence of ADA, SDZ WAG-994 was infused at rates to achieve the same perfusate concentrations as were achieved before the administration of ADA. Measurements of coronary perfusion pressure were made when steady-state effects of SDZ WAG-994 and ADA were attained. Coronary vascular conductance was calculated as the ratio of flow rate (10 ml/min) to coronary perfusion pressure. To assess the attenuation of SDZ WAG-994-induced coronary vasodilation by AdoR antagonists, hearts (n = 6) were perfused with SDZ WAG-994 (5 μM). After the effect of SDZ WAG-994 reached steady state, first the A_1-AdoR-selective antagonist N-0861 (5 μM; Shryock *et al.*, 1992) and then CPT (2 μM) were added to the perfusate and the changes in coronary perfusion pressure were recorded. CPT is less A_1 selective than N-0861; it has not been reported to distinguish between A_{2a}- and A_{2b}-AdoR subtypes.

In seven experiments, the coronary perfusion pressure and S-H interval were recorded simultaneously. SDZ WAG-994 was infused to achieve a perfusate concentration of 0.4 μM. After the effects of SDZ WAG-994 on coronary perfusion pressure and S-H interval reached a steady state, N-0861 (5 μM) was added to the perfusate and the responses were recorded. After a 15-min drug-free perfusion to wash out N-0861, the same protocol was repeated with CPT (2 μM).

Effect of SDZ WAG-994 on effluent levels of adenosine. Hearts (n = 5) were perfused with various concentrations of adenosine (0, 3, 6 and 12 μM) in the absence and presence of SDZ WAG-994 (5 μM). The heart ventricles were paced at a cycle length of 300 msec. After collection of control effluent samples, adenosine was infused to achieve perfusate concentrations of 3, 6 and 12 μM. Effluent samples (0.5 ml) for the measurement of adenosine concentration were collected at 5 min after each change in perfusate adenosine concentration. After a 20-min period of perfusion with drug-free medium, perfusion with SDZ WAG-994 (5 μM) was started and maintained and the infusions of adenosine were repeated.

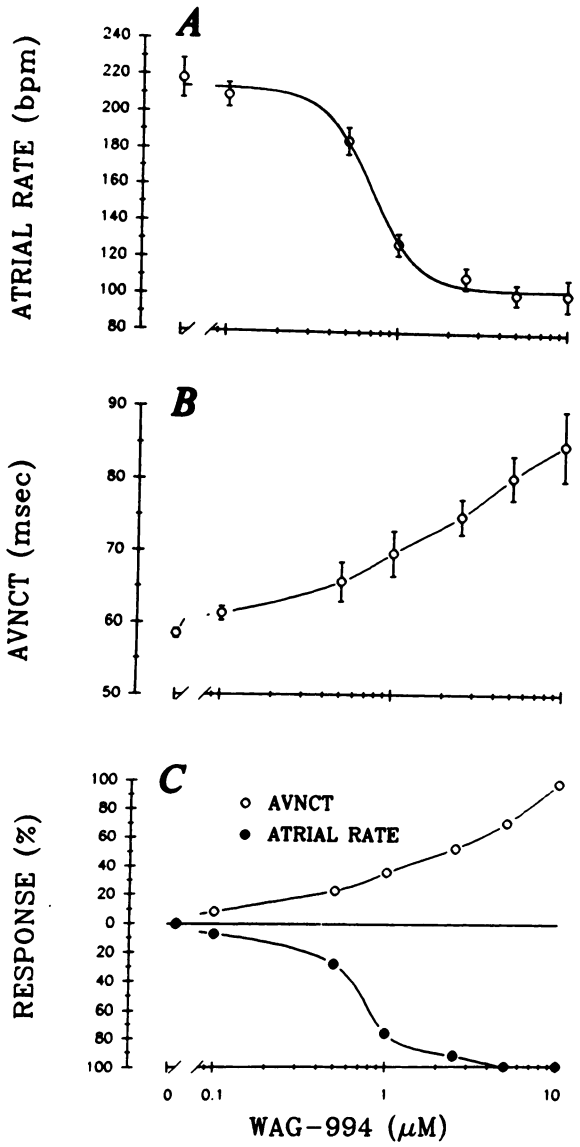

Fig. 1. Concentration-response relationship for the A) negative chronotropic (atrial rate in beats/minute) and B) negative dromotropic effects (AV nodal conduction time, AVNCT) of SDZ WAG-994 in spontaneously beating guinea pig hearts. C) Comparison of the concentration-response relations shown in A and B. Data are plotted as percent of maximal response. Each point is mean ± S.E.M. of responses of eight (atrial rate) and six (AVNCT) guinea pig hearts.

Results

Chronotropic effect of SDZ WAG-994. The rate of spontaneous beating of isolated hearts (n = 8) was 219 ± 10 beats/min. SDZ WAG-994 caused a concentration-dependent slowing of spontaneous atrial rate (fig. 1A). The EC_{50} value for SDZ WAG-994-induced slowing of spontaneous atrial rate was 0.69 ± 0.04 μM (fig. 1A; table 1). This negative chronotropic effect of SDZ WAG-994 could be completely antagonized by either N-0861 (5 μM) or CPX (0.2 μM) but not by atropine (5 μM, not shown).

Dromotropic effect of SDZ WAG-994. SDZ WAG-994 caused a concentration-dependent prolongation of AV nodal conduction time of spontaneously beating hearts (n = 6, fig. 1B). The maximal effect of SDZ WAG-994 (10 μM) was a prolongation of AV nodal conduction time from 58 ± 1 to 85 ± 5 msec; SDZ WAG-994 (10 μM) did not cause second-degree AV block. The EC_{50} value for prolongation of AV nodal conduction time by SDZ WAG-994 was 1.49 ± 0.54 μM (fig. 1B; table 1).

The negative dromotropic action of SDZ WAG-994 was also investigated in hearts paced at atrial pacing cycle lengths of 500, 300 and 200 msec. In atrial-paced hearts, as in spontaneously beating hearts, SDZ WAG-994 prolonged the S-H interval in a concentration-dependent manner (fig. 2). The prolongation of S-H interval caused by SDZ WAG-994 was greater at faster atrial rates. When the atrial cycle length was shortened from 500 to 300 to 200 msec, the concentration-response relationships for SDZ WAG-994 were shifted leftward. The threshold and EC_{50} values for SDZ WAG-994-induced S-H prolongation in hearts paced at a cycle length of

TABLE 1

Concentration of SDZ WAG-994 required to cause 50% of maximal effect on heart rate and AV nodal conduction time in guinea pig isolated hearts

Values (in micromolar quantities) are mean ± S.E.M. Number of experiments indicated in parentheses. Paced hearts were stimulated at an atrial cycle length of 300 msec.

Cardiac Response	Paced	Nonpaced
Chronotropic	—	0.69 ± 0.04 (6)
Dromotropic	0.40 ± 0.02 (8)	1.49 ± 0.54[a] (6)

[a] Indicates an EC_{50} value that overestimates the true potency of SDZ WAG-994 because second-degree AV nodal conduction block was not attained in nonpaced hearts.

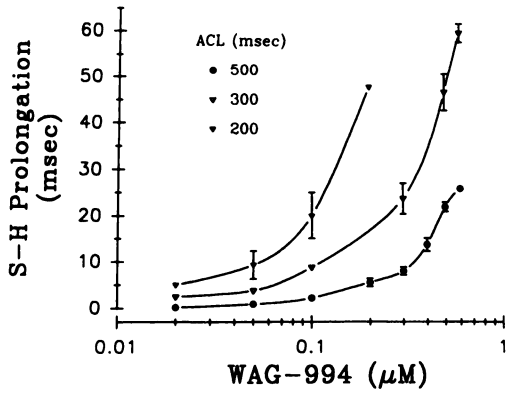

Fig. 2. Concentration-response relationship for the negative dromotropic effect (increase of S-H interval) of SDZ WAG-994 in guinea pig isolated perfused hearts paced at atrial cycle lengths (ACL) of 500 (●), 300 (▽) and 200 (▼) msec. Note that, as the atrial pacing rate increases (*i.e.*, as ACL is reduced from 500 to 200 msec), the S-H prolongation is greater at any given concentration of SDZ WAG-994. Each point is mean ± S.E.M. of data from eight guinea pig hearts.

300 msec were 0.05 μM and 0.40 ± 0.02 μM, respectively (fig. 2; table 1).

The concentration-response relations for the negative dromotropic effects of SDZ WAG-994 on hearts paced at a constant atrial cycle length of 300 msec and on spontaneously beating hearts were directly compared by replotting the data from figures 1B and 2. These are presented in figure 3. The concentration-response curve for the negative dromotropic effect of SDZ WAG-994 in paced hearts was steeper than and shifted to the left of that for unpaced hearts.

To characterize the negative dromotropic properties of SDZ WAG-994 further, the effects of this AdoR agonist on Wenckebach cycle length and AV nodal effective refractory period were determined (fig. 4). The Wenckebach cycle length, *i.e.*, the atrial pacing cycle length at which second-degree AV block occurs, was significantly and concentration-dependently prolonged by SDZ WAG-994 (fig. 4A). In the absence of SDZ WAG-994, the Wenckebach cycle length was 161 ± 3 msec. It increased to 179 ± 5 and 196 ± 11 msec in the presence of 0.05 and 0.10 μM of SDZ WAG-994, respectively. CPX (0.2 μM) significantly reduced the prolongation by SDZ WAG-994 of Wenckebach cycle length. Likewise, the AV nodal effective refractory period was prolonged by SDZ WAG-994 (fig. 4B). The control AV nodal effective refractory period was 130 ± 3 msec and it was increased to 142 ± 2 and 160 ± 6 msec in the presence of 0.05 and 0.10 μM SDZ WAG-994,

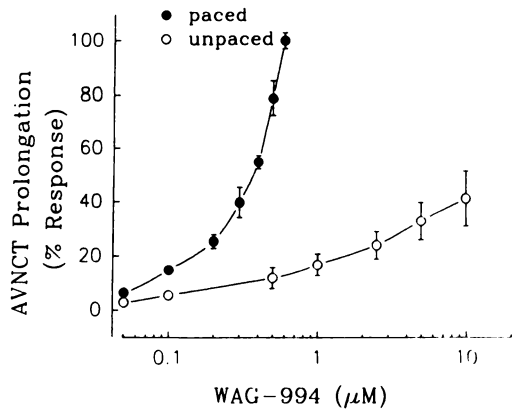

Fig. 3. Comparison of concentration-response relationships for the negative dromotropic effect (increase of AV nodal conduction time, AVNCT) of SDZ WAG-994 in spontaneously beating and in paced (atrial cycle length = 300 msec) isolated guinea pig hearts. Each point is the mean ± S.E.M. of data from five unpaced and eight paced hearts.

respectively. CPX (0.2 μM) significantly reversed prolongation of the effective refractory period by SDZ WAG-994.

Evidence that the negative dromotropic effect of SDZ WAG-994 was mediated by the A_1-AdoR and not by the muscarinic acetylcholine receptor is presented in figure 5. The S-H interval of hearts paced at an atrial cycle length of 300 msec was prolonged approximately 12 msec by SDZ WAG-994 (0.2 μM). The muscarinic receptor antagonist atropine (1 μM) had no effect on the S-H prolongation caused by SDZ WAG-994. In contrast, the selective A_1-AdoR antagonist CPX (0.2 μM) almost completely reversed the negative dromotropic effect of SDZ WAG-994. ADA (3 U/ml) did not inhibit the prolongation of S-H interval caused by SDZ WAG-994 (not shown).

Comparison of the chronotropic and dromotropic responses to SDZ WAG-994. To compare the effects of SDZ WAG-994 on heart rate and AV nodal conduction time, data from figure 1, A and B were recalculated and presented as the percentages of maximal decrease in heart rate and increase in AV nodal conduction time, respectively (fig. 1C). The EC_{50} value for SDZ WAG-994-induced prolongation of AV conduction time in spontaneously beating hearts appeared to be greater than that which caused slowing of the heart rate, *i.e.*, 1.49 *versus* 0.69 μM (table 1). However, when the heart rate was held constant by pacing at a cycle length of 300 msec, the EC_{50} value for SDZ WAG-994-induced S-H interval prolongation was lowered to 0.40 ± 0.02 μM (fig. 2; table 1). Thus, when hearts were paced at a fixed rate to prevent atrial slowing, SDZ WAG-994 appeared to become more potent in prolonging AV nodal conduction time (fig. 3).

Frequency-dependent effect of SDZ WAG-994 on AV nodal conduction. The frequency-dependent negative dromotropic effect of SDZ WAG-994 was investigated by comparison of prolongations of the S-H interval in response to an abrupt and transient increase in atrial rate, in the absence and presence of 0.1 and 0.2 μM SDZ WAG-994. The results are depicted in figures 6 and 7 and presented in table 2. In the example shown in figure 6, the atrial pacing cycle length were decreased in a single step from 300 to 200 msec and held at 200 msec for 60 sec (bottom panel). At an atrial cycle length of 300 msec, 0.1 μM SDZ WAG-994 caused a 6-msec prolongation of the S-H interval (from 42 to 48 msec), whereas the same concentration of SDZ WAG-994 at an

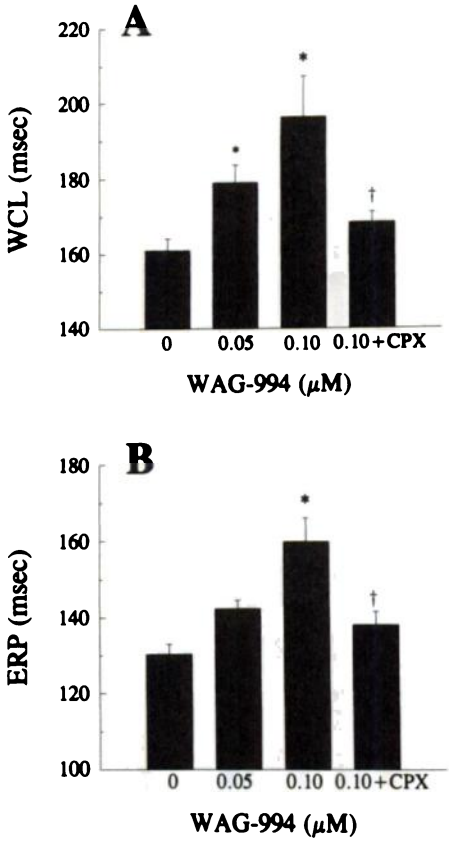

Fig. 4. Effect of SDZ WAG-994, in the absence and presence of CPX, on A) Wenckebach cycle length (WCL) and B) AV nodal effective refractory period (ERP) in guinea pig isolated hearts. SDZ WAG-994 significantly (*P < .05) increased WCL and ERP in a concentration-dependent manner. CPX, a selective A_1-AdoR antagonist, significantly attenuated the effect of 0.1 μM of SDZ WAG-994 on WCL and ERP. Not shown, CPX alone did not significantly reduce the base-line values of WCL and ERP. Bars indicate mean and S.E.M. of single determinations in each of four hearts.

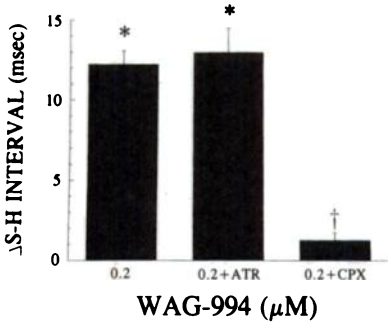

Fig. 5. Selective antagonism of the negative dromotropic effect (▲ S-H interval) of SDZ WAG-994 by CPX. Hearts (n = 5) were paced at an atrial cycle length of 300 msec. CPX (0.2 μM) significantly antagonized the S-H interval prolongation caused by 0.2 μM SDZ WAG-994. Atropine (ATR, 1 μM) had no effect on the S-H prolongation caused by SDZ WAG-994.

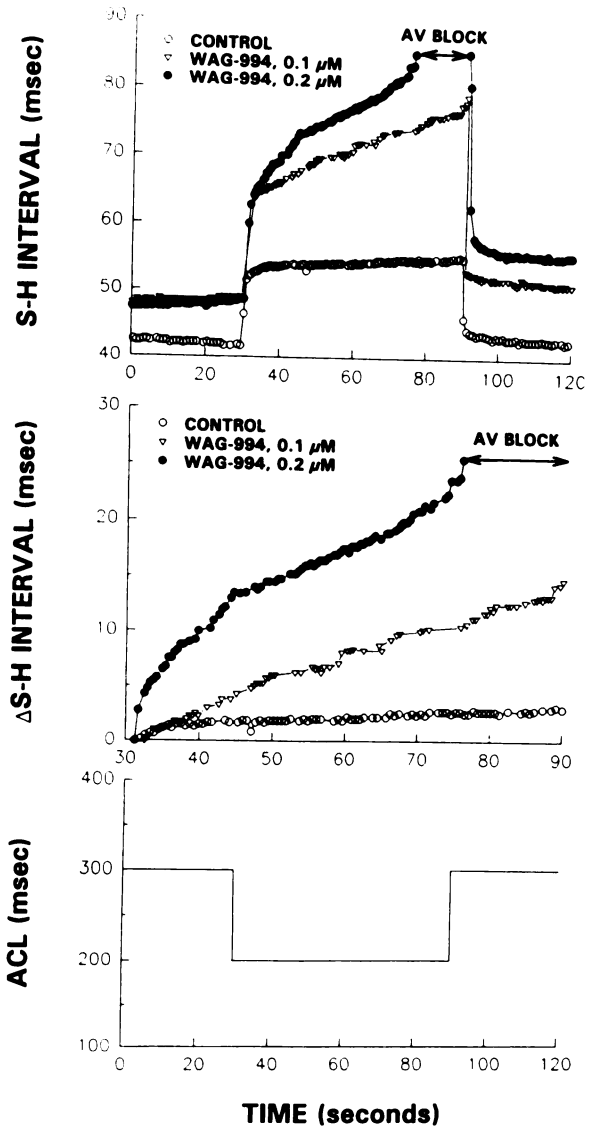

Fig. 6. Effect of SDZ WAG-994 on S-H interval during rapid atrial pacing of a guinea pig isolated heart. Top panel: progressive prolongation of S-H interval in the absence and presence of SDZ WAG-994 as a function of time after an abrupt increase in rate of pacing. Middle panel: Data from the top panel is replotted to show the S-H interval prolongation (Δ S-H) from the first to the last beat during the 60-sec period of fast pacing. Bottom panel: Time course of the pacing protocol; atrial cycle length (ACL) was abruptly shortened from 300 to 200 msec, held at 200 msec for 60 sec and then returned to 300 msec. In comparison with the control, in the presence of SDZ WAG-994, prolongation of the S-H interval (Δ S-H) was greater and did not reach a steady state. In the presence of 0.2 μM SDZ WAG-994, second-degree AV block ensued at approximately 35 sec after initiation of fast atrial pacing. Not shown, the effects of SDZ WAG-994 were antagonized by the adenosine antagonist CPX.

atrial cycle length of 200 msec caused a 25-msec prolongation of the S-H interval from 55 to 80 msec (fig. 6, top panel, also replotted in middle panel). Hence, the ratio between S-H interval prolongation at fast and slow pacing rates (*i.e.*, short and long atrial pacing cycle length; Δ $S\text{-}H_{200}$/Δ $S\text{-}H_{300}$) in the presence of 0.1 μM SDZ WAG-994 was 25/6 or 4.2. A ratio more than 1 indicates a greater effect at a faster than at a slower pacing rate. Table 2 and figure 7 summarize the results that show the frequency-dependent prolongation of the S-H interval by SDZ WAG-994 at two different atrial pacing cycle lengths of 200 and 180 msec. The magnitude and time course of the S-H interval prolongation were greater at an atrial pacing cycle length of 180 than at 200 msec and at the higher concentration (0.1 *versus* 0.05 μM) of SDZ WAG-994 (table 2). Likewise, as illustrated in figure 7, the ratios between S-H interval prolongation at fast and slow pacing

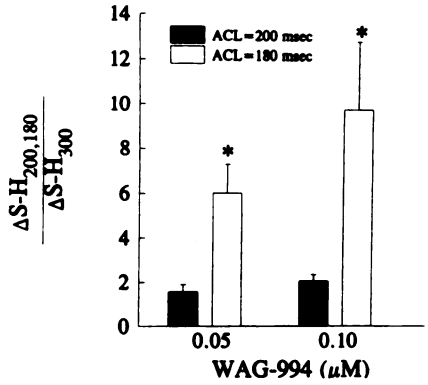

Fig. 7. Frequency-dependent prolongation of S-H interval by SDZ WAG-994. The ratios of S-H prolongations caused by 0.05 and 0.1 μM SDZ WAG-994 at atrial pacing cycle lengths (ACL) of 200 or 180 ($\Delta S\text{-}H_{200,180}$) and 300 ($\Delta S\text{-}H_{300}$) msec, respectively, are shown. Bars represent the mean ± S.E.M. of data from four hearts.

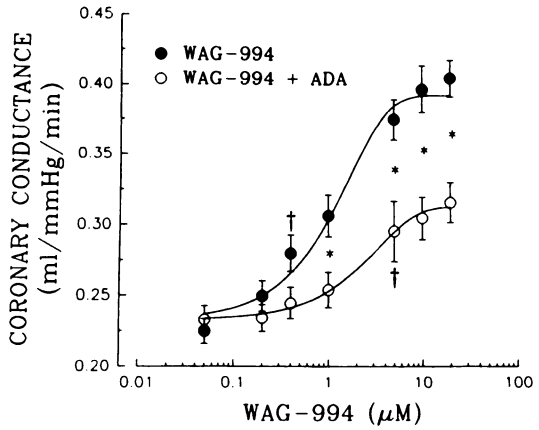

Fig. 8. Concentration-response relationships for the vasodilatory effect of SDZ WAG-994 in the absence and presence of ADA. SDZ WAG-994 caused a concentration-dependent increase in coronary conductance, which was significantly (*P < .05) reduced by 3 U/ml of ADA at SDZ WAG-994 concentrations ≥ 1 μM. The concentration of SDZ WAG-994 that caused a significant increase in coronary conductance above control (absence of drug) is indicated by †. Each point represents the mean ± S.E.M. of data from eight experiments (eight hearts).

rates were greater at the shorter atrial pacing cycle length and at the higher concentration of SDZ WAG-994.

Coronary vasodilatory effect of SDZ WAG-994. SDZ WAG-994 significantly increased coronary conductance in a concentration-dependent manner (fig. 8). The threshold and EC_{50} values for the SDZ WAG-994-induced increase in coronary conductance were 0.4 and 1.50 ± 0.80 μM, respectively. However, the addition of ADA (3 U/ml) to the perfusate significantly attenuated the concentration-dependent increase in coronary conductance caused by SDZ WAG-994. When ADA was present, the lowest concentration of SDZ WAG-994 that caused a significant (P < .05) increase in coronary conductance was 5 μM and 10 or 20 μM SDZ WAG-994 caused no further increase in coronary conductance. ADA did not attenuate the effect of SDZ WAG-994 on S-H interval prolongation (data not shown).

To determine the AdoR subtype (A_1 *versus* A_2) responsible for the coronary vasodilation observed in the presence of SDZ WAG-994, the effects of SDZ WAG-994 (5 μM) on coronary conductance were determined alone and in the presence of N-0861 (5 μM), a highly selective A_1-AdoR antagonist (Shryock *et al.*, 1992), and CPT (2 μM), a less selective A_1- and A_2-AdoR antagonist. As depicted in figure 9, SDZ WAG-994 increased coronary conductance to 0.31 ± 0.02 ml mm Hg^{-1} min^{-1} from a base-line value of 0.19 ± 0.01 ml mm Hg^{-1} min^{-1}. This increase in coronary conductance caused by 5 μM SDZ WAG-994 was not affected by N-0861 but was almost completely reversed by CPT. Neither N-0861 nor CPT alone had any significant effect on coronary conductance (not shown). In a separate group of hearts (n = 7), the negative dromotropic and coronary vasodilatory effects of SDZ WAG-994 were measured simultaneously and compared. N-0861 (5 μM) attenuated the A_1-AdoR-mediated S-H prolongation caused by SDZ WAG-994 (0.4 μM) from 37.0 ± 3.0 to 6.0 ± 1.5 msec but had no effect on the A_2-AdoR-mediated increase in coronary conductance (fig. 10). In comparison, CPT (2 μM) reversed both the S-H prolongation and the increase in coronary conductance caused by SDZ WAG-994.

Binding of SDZ WAG-994 to the A_1- and A_2-AdoRs and nucleoside transporter. Binding of SDZ WAG-994 to the A_1-AdoR, the muscarinic cholinergic receptor (fig. 11) and the nucleoside transporter (fig. 12) of atrial and ventricular membranes was measured with competitive binding assays. [^{3}H]CCPA and [^{3}H]QNB were used for assay of the A_1-AdoR and the muscarinic cholinergic receptor, respectively, and [^{3}H]NBMPR was used for the assay of the nucleoside trans-

TABLE 2

Effect of abrupt change in atrial pacing rate on the magnitude and time course of SDZ WAG-994-induced slowing of AV nodal conduction time

Shown are the mean ± S.E.M. of results of five experiments in which the atrial pacing cycle length was abruptly decreased from 300 to either 200 or 180 msec. ΔS-H = S-H interval prolongation measured from the first or second to the last beat during rapid atrial pacing cycle lengths of 200 and 180 msec; τ_{on} = time constant of ΔS-H after the *increase* in pacing rate; τ_{off} = time constant of ΔS-H after the *decrease* in pacing rate.

Atrial Pacing Cycle Length	Control		SDZ WAG-994 Concentration			
			0.05		0.10	
	200	180	200	180	200	180
msec				*μM*		
ΔS-H	5.5 ± 0.5	10.0 ± 1.5***	8.0 ± 1.1	28.0 ± 7.0**	17.0 ± 3.0*,a	41 ± 3.0**,***,b
τ_{on}	15.5 ± 2.5	23.0 ± 6.0	49.0 ± 14.5	73.5 ± 19.0**	53.5 ± 19.0*	114.0 ± 40.0**
τ_{off}	17.5 ± 4.0	17.0 ± 3.0	18.5 ± 4.0	25.5 ± 4.0	20.0 ± 4.0	35.5 ± 7.0**

[a] AV block occurred in one of five hearts.
[b] AV block occurred in all five hearts.
* P < .05, control *vs.* SDZ WAG-994-treated hearts at ACL = 200.
** P < .05, control *vs.* SDZ WAG-994-treated hearts at ACL = 180.
*** P < .05, ACL = 200 *vs.* ACL = 180.

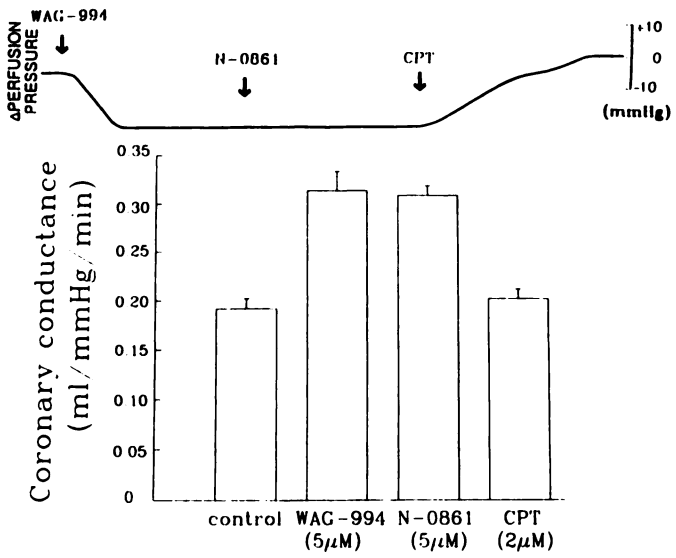

Fig. 9. Effects of N-0861 and CPT on the coronary vasodilation caused by SDZ WAG-994 in guinea pig isolated hearts perfused at a constant flow 10 ml/min. Heart ventricles were paced at a cycle length of 300 msec. Upper panel: Analog record of the decrease in coronary perfusion pressure (CPP) caused by SDZ WAG-994 (5 μM), in the absence of ADA. N-0861 (5 μM), an A_1-AdoR antagonist did not antagonize the decrease in CPP caused by SDZ WAG-994, whereas the less A_1-AdoR-selective antagonist CPT (2 μM) completely reversed the effect of SDZ WAG-994. Lower panel: Summary of data (mean and S.E.M. of results from six hearts) demonstrating that SDZ WAG-994 significantly increased coronary conductance. The increase in coronary conductance caused by SDZ WAG-994 was reversed by CPT but not by N-0861.

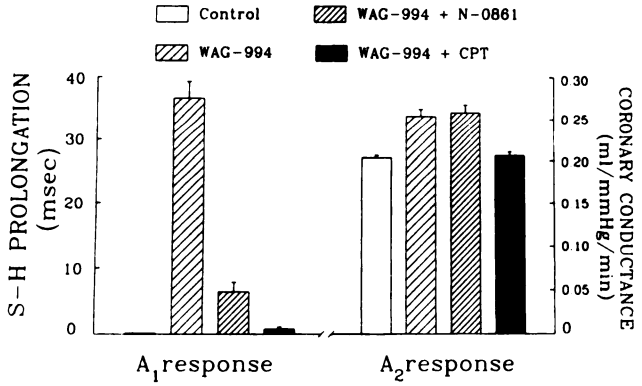

Fig. 10. Antagonism of the negative dromotropic and vasodilatory actions of SDZ WAG-994 by N-0861 and CPT in guinea pig isolated perfused hearts (n = 7). Heart atria were paced at a cycle length of 300 msec and hearts were perfused at a constant flow (10 ml/min) throughout an experiment. The S-H interval and the coronary perfusion pressure were recorded simultaneously. SDZ WAG-994 (0.4 μM) caused a significant prolongation of the S-H interval and increased coronary conductance. N-0861 significantly attenuated the A_1-AdoR-mediated S-H prolongation caused by SDZ WAG-994 but had no effect on A_2-AdoR-mediated increase in coronary conductance. In contrast, CPT (2 μM) reversed both the S-H prolongation and the increase in coronary conductance induced by SDZ WAG-994.

porter. Displacement of [^{3}H]CCPA (4 nM) binding to cardiac atrial and ventricular membrane preparations by SDZ WAG-994 was concentration dependent (fig. 11). The K_i values of SDZ WAG-994 used to displace [^{3}H]CCPA binding to atrial and ventricular membranes of guinea pig hearts were 0.07 ± 0.01 and 0.26 ± 0.01 μM, respectively, and to atrial membranes of human hearts, 0.60 ± 0.09 μM (fig. 11; table 3). The

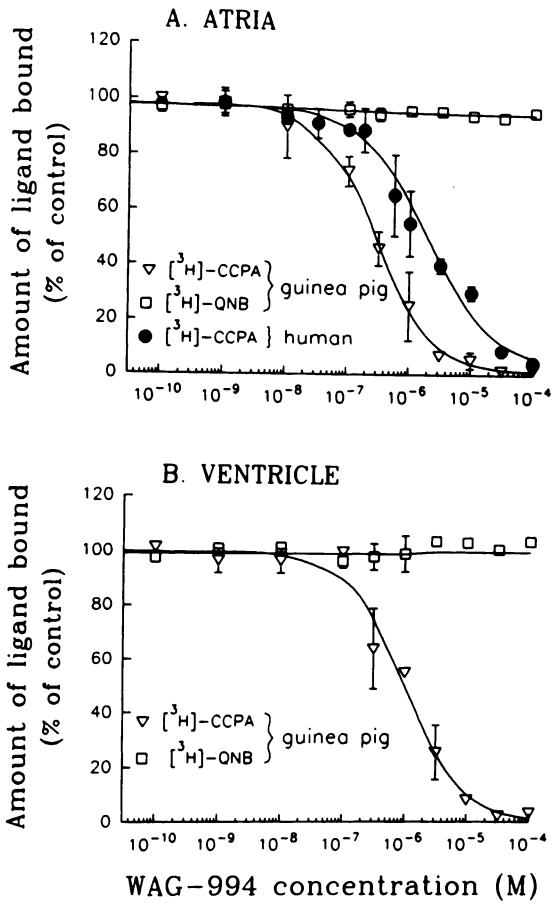

Fig. 11. Effect of SDZ WAG-994 on specific binding of [^{3}H]CCPA (A_1-AdoR agonist) and [^{3}H]QNB (muscarinic acetylcholine receptor antagonist) to atrial and ventricular membranes. Atrial or ventricular membrane preparations, unlabeled SDZ WAG-994 and [^{3}H]CCPA (4 nM) or [^{3}H]QNB (0.1 nM) were incubated together for 2 hr at room temperature. Nonspecific binding to A_1-AdoR and muscarinic cholinergic receptors was defined as binding not displaced by 10 μM unlabeled CCPA and 1 μM atropine, respectively. Each symbol represents the mean of triplicate determinations from two experiments. The K_i values for SDZ WAG-994 displacement of [^{3}H]CCPA binding to guinea pig atrial and ventricular membranes and to human atrial membranes were 0.07, 0.26 and 0.60 μM, respectively (table 3).

displacement of specific [^{3}H]CCPA binding by SDZ WAG-994 (100 μM) was complete in both atrial and ventricular membranes. To determine the A_1- *versus* A_2-AdoR subtype selectivity of SDZ WAG-994, binding of SDZ WAG-994 to the A_1- and A_2-AdoRs of guinea pig forebrain and striatum, respectively, was measured with competition binding assays. [^{3}H]CCPA and [^{3}H]CGS 21,680 were used for the assays of A_1- and A_2-AdoRs, respectively. Displacement of [^{3}H]CCPA (2 nM) and [^{3}H]CGS 21,860 (5 nM) binding to brain membrane preparations by SDZ WAG-994 was concentration dependent (not shown). The K_i values of SDZ WAG-994 to displace [^{3}H]CCPA and [^{3}H]CGS 21,860 binding to guinea pig forebrain (A_1-AdoR) and striatum (A_2-AdoR) were 0.15 ± 0.02 and 42 ± 3 μM, respectively (table 3). Hence, the A_1/A_2 selectivity ratio for SDZ WAG-994 binding was 280. SDZ WAG-994 did not displace [^{3}H]QNB binding to cardiac membranes (fig. 11).

SDZ WAG-994 also inhibited binding of [^{3}H]NBMPR to nucleoside transport sites. SDZ WAG-994 displaced [^{3}H] NBMPR binding to guinea pig atrial and ventricular mem-

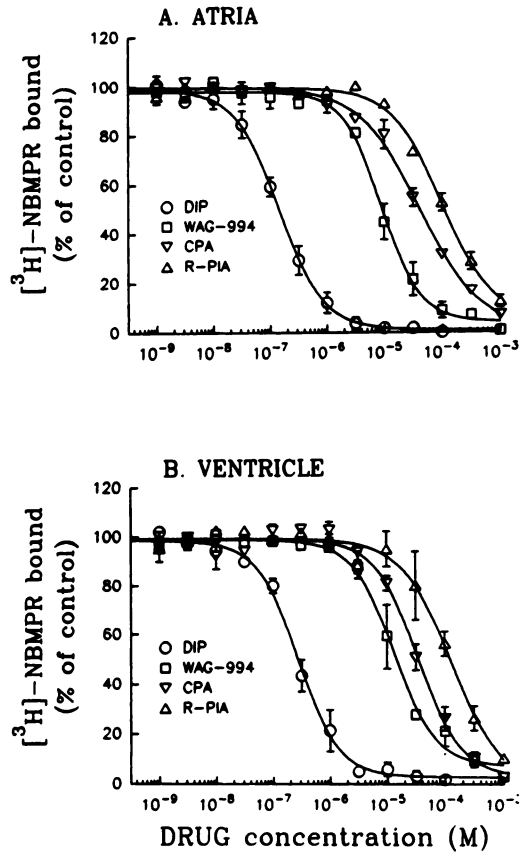

Fig. 12. Displacement by SDZ WAG-994, CPA, R-PIA and dipyridamole (DIP) of specific binding of [^{3}H]NBMPR to guinea pig atrial and ventricular membranes. Crude atrial and ventricular membrane preparations, [^{3}H]NBMPR (4 nM) and either unlabeled SDZ WAG-994, CPA, R-PIA or DIP were incubated together for 2 hr at room temperature. Nonspecific binding to nucleoside transport binding sites was defined as the binding not displaced by 10 μM unlabeled NBMPR. Each symbol represents the mean of triplicate determinations from two experiments. The K_i values for SDZ WAG-994, CPA, R-PIA and DIP displacement of [^{3}H]NBMPR binding to atrial and to ventricular membranes are given in table 4.

TABLE 3

Binding affinities of SDZ WAG-994 for the A$_1$- and A$_2$-AdoR in cardiac and brain membranes

Affinities (K_i values) measured as inhibition of [^{3}H]CCPA (A$_1$-AdoR) and [^{3}H]CGS 21,680 (A$_2$-AdoR) to guinea pig (GP) and human cardiac and brain membranes. Values are the means of triplicate determinations from two to three experiments.

Tissue	Species	Receptor Subtype	K_i
			μM
Atrium	GP	A_1	0.07 ± 0.01
Atrium	Human	A_1	0.60 ± 0.09
Ventricle	GP	A_1	0.26 ± 0.01
Forebrain	GP	A_1	0.15 ± 0.02
Striatum	GP	A_2	42.0 ± 3

branes with K_i values of 1.6 ± 0.5 and 4.2 ± 1.4 μM, respectively (fig. 12; table 4). Complete displacement of [^{3}H]NBMPR binding was achieved at SDZ WAG-994 concentrations ≥ 100 μM. The N^6-substituted adenosine derivatives, R-PIA and CPA, also displaced specific [^{3}H]NBMPR binding but with a lower potency than SDZ WAG-994. The nucleoside uptake blocker dipyridamole was approximately 100-fold more potent than SDZ WAG-994 at displacing [^{3}H]NBMPR binding.

TABLE 4

Binding affinities of AdoR agonists and dipyridamole for the nucleoside transport sites in cardiac membranes

Affinities (K_i values) measured as inhibition of [^{3}H]NBMPR binding to guinea pig atrial and ventricular membranes. Values are the means of triplicate determinations from two experiments.

	K_i	
	Atrium	Ventricle
	μM	
Dipyridamole	0.02 ± 0.005	0.04 ± 0.002
SDZ WAG-994	1.6 ± 0.5	4.2 ± 1.4
CPA	7.0 ± 2.0	5.2 ± 1.0
R-PIA	18.0 ± 0.01	19.0 ± 3.0

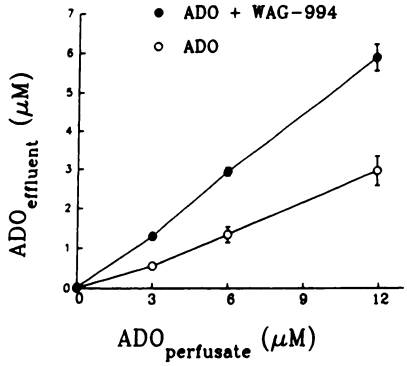

Fig. 13. The effect of SDZ WAG-994 to increase recovery of adenosine in the effluent of guinea pig isolated hearts that were perfused with medium containing adenosine. Heart ventricles were paced at a cycle length of 300 msec. SDZ WAG-994 (5 μM) significantly ($P < .01$) increased the effluent levels of adenosine at each perfusate concentration of the nucleoside. Not shown, the base-line concentration of adenosine in the effluent (0.02 ± 0.01 μM) was not affected by SDZ WAG-994. Each point is the mean ± S.E.M. of data from five hearts.

To investigate the effect of SDZ WAG-994 on adenosine uptake by the heart, the concentration of adenosine in the cardiac effluent was measured in the absence and presence of 5 μM SDZ WAG-994. This drug did not significantly increase the concentration of adenosine in the cardiac effluent of normoxic hearts paced at an atrial cycle length of 300 msec. The concentration of adenosine in the effluent was 0.02 ± 0.01 μM in the absence and 0.03 ± 0.01 μM in the presence of SDZ WAG-994. However, the concentration of adenosine in the cardiac effluent during infusion of adenosine was higher in the presence of SDZ WAG-994 than in its absence (control) (fig. 13). At each perfusate (arterial) concentration of adenosine (3, 6 and 12 μM), the effluent (venous) levels of adenosine were significantly ($P < .01$) higher when SDZ WAG-994 was present in the perfusate. That is, the recovery of intra-arterially infused adenosine (*i.e.*, adenosine added to the perfusate) in the heart's effluent was significantly increased by SDZ WAG-994.

Discussion

This report is the first to describe the frequency dependence of action of an adenosine analog on the AV node. The results of this study show that the slowing of heart rate and AV nodal conduction time caused by SDZ WAG-994 was most likely mediated by the A$_1$ subtype of AdoR. This conclusion is based on the following findings: 1) both the negative chrono-

tropic and dromotropic effects of adenosine and its N^6-substituted derivatives have been shown to be mediated by the A_1-AdoR (Clemo and Belardinelli, 1986a; West *et al.*, 1987); 2) the effects of SDZ WAG-994 were significantly attenuated by the adenosine antagonists, CPX and N-0861, which have 740- and 610-fold selectivity for A_1- *versus* A_2-AdoR, respectively (Bruns *et al.*, 1987; Shryock *et al.*, 1992; May *et al.*, 1991); and 3) SDZ WAG-994 displaced binding of [^{3}H]CCPA to atrial and ventricular membranes in a concentration-dependent manner. The EC_{50} values of 0.69 and 0.40 μM for the negative chronotropic and dromotropic effects of SDZ WAG-994 on guinea pig hearts are similar to the K_i values of SDZ WAG-994 calculated from displacement of [^{3}H]CCPA binding to guinea pig atrial and ventricular A_1-AdoRs (0.07 and 0.26 μM, respectively). This finding is consistent with the interpretation that the sites to which SDZ WAG-994 binds in atrial and ventricular myocytes are representative of those that mediate the slowings of heart rate and AV nodal conduction.

The threshold and EC_{50} values for SDZ WAG-994 to depress AV nodal conduction were dependent on the atrial rate. The EC_{50} value of 0.40 μM for SDZ WAG-994 to prolong AV nodal conduction time in paced hearts was approximately 3.7-fold less than the EC_{50} value of 1.49 μM obtained in spontaneously beating hearts (table 1). In spontaneously beating hearts, the EC_{50} value of SDZ WAG-994 to slow heart rate (negative chronotropic effect) was approximately 2-fold less than that to slow AV nodal conduction (negative dromotropic effect). In contrast, in hearts paced at a constant atrial rate, SDZ WAG-994 was 1.5-fold more potent in slowing AV nodal conduction than in slowing heart rate. This indicates that the effect of SDZ WAG-994 to slow AV nodal conduction of spontaneously beating hearts was significantly diminished by concomitant slowing of the atrial rate.

It is well known that atrial rate modulates AV nodal conduction time (Merideth *et al.*, 1968). AV nodal conduction time increases progressively as atrial rate is increased (Froldi and Belardinelli, 1990; Merideth *et al.*, 1968; Jenkins and Belardinelli, 1988). Because SDZ WAG-994 slows atrial rate, it is not surprising that in unpaced hearts (*i.e.*, spontaneously beating hearts), SDZ WAG-994 caused significantly less prolongation of the S-H interval than in paced hearts (fig. 3) and that a concentration of SDZ WAG-994 as high as 10 μM did not cause second-degree AV block in unpaced hearts (fig. 1B), whereas second-degree AV block of paced hearts occurred at SDZ WAG-994 concentrations lower than 1 μM (fig. 6). Thus, modulation of AV nodal conduction by atrial rate and the finding that the potency of SDZ WAG-994 to slow the heart rate (EC_{50} = 0.69 μM) was similar to that to prolong AV nodal conduction (EC_{50} = 0.40 μM) in paced hearts could fully explain the difference in responses of paced and unpaced hearts to SDZ WAG-994 (fig. 3). It is therefore anticipated that, in spontaneously beating hearts, the predominant effect of SDZ WAG-994 will be to decrease the heart rate (sinus bradycardia).

The failure of atropine to attenuate the negative chronotropic and dromotropic effects of SDZ WAG-994 demonstrates that SDZ WAG-994 is specific for AdoRs and does not activate cardiac muscarinic cholinergic receptors. This interpretation is also consistent with the finding that SDZ WAG-994 did not displace binding of [^{3}H]QNB to either atrial or ventricular membranes.

Vasodilatory effect of SDZ WAG-994. In addition to the A_1-AdoR-mediated slowing of heart rate and increase in AV nodal conduction time, SDZ WAG-994 significantly increased coronary conductance of guinea pig isolated, perfused hearts. This effect of SDZ WAG-994 was concentration dependent and was attenuated by ADA (fig. 8). The finding that the selective A_1-AdoR antagonist N-0861 (May *et al.*, 1991) did not antagonize the increase in coronary conductance caused by SDZ WAG-994, but CPT (5-fold less selective for the A_1-AdoR in the brain than N-0861) (Bruns *et al.*, 1987; Shryock *et al.*, 1992; May *et al.*, 1991) completely reversed this effect of SDZ WAG-994 (fig. 9) suggests that the effect was mediated by an A_2-AdoR. However, these findings do not provide a conclusive answer to the question whether the increase in coronary conductance is the result of a direct activation of A_2-AdoRs by SDZ WAG-994 and/or by endogenous adenosine accumulated as a consequence of inhibition of adenosine uptake by SDZ WAG-994 (fig. 13). Likewise, because CPT cannot be used to distinguish between A_{2a}- *versus* A_{2b}-mediated responses, the subtype of A_2-AdoR activated by SDZ WAG-994 cannot be deduced from our studies. The results of radioligand binding studies indicated that the affinity of SDZ WAG-994 for the brain A_1-AdoR was 280- to 1090-fold greater than that for the brain A_2-AdoR (table 3; Wagner *et al.*, 1994). If this degree of receptor selectivity can be extrapolated to the heart, it is unlikely that the highest concentration of SDZ WAG-994 used in the present study (*i.e.*, 20 μM) was sufficient to activate a substantial fraction of A_{2a}-AdoRs directly and thereby elicit an A_{2a}-AdoR-mediated coronary vasodilation. However, activation of A_{2b}-AdoRs by SDZ WAG-994 cannot be ruled out.

Does SDZ WAG-994 inhibit adenosine uptake? The attenuation by ADA of the coronary vasodilation caused by SDZ WAG-994 suggests that this effect of SDZ WAG-994 is at least in part mediated by endogenous adenosine present in the preparation. We propose that SDZ WAG-994 inhibits the cellular uptake of adenosine. At least four observations lend support to this interpretation: 1) the recovery of intra-arterially infused adenosine in the effluent of the heart was significantly greater in the presence than in the absence of SDZ WAG-994 (fig. 13); 2) SDZ WAG-994 displaced in a concentration-dependent manner the binding of [^{3}H]NBMPR, a radioligand that binds to and inhibits the function of the nucleoside transporter (Williams *et al.*, 1984), to atrial and ventricular membranes (fig. 12); 3) the vasodilatory effect of SDZ WAG-994 was significantly attenuated by ADA; and 4) the differences between the concentration-response relationship for the vasodilatory effect of SDZ WAG-994 in absence and presence of ADA (fig. 8) became statistically significant ($P < .05$) at concentrations of WAG-994 $\geq$ 1 μM, a concentration of SDZ WAG-994 that, according to binding data (table 2; fig. 12), should be sufficient to inhibit NBMPR-sensitive adenosine transport. Although these observations do not prove that the two phenomena, *i.e.*, vasodilatation and inhibition of NBMPR-sensitive adenosine uptake, are related as cause and effect, they are consistent with the interpretation that a component of the vasodilation caused by SDZ WAG-994 is due to endogenous adenosine. It is worth noting that not only SDZ WAG-994 but also R-PIA and CPA, both N^6-substituted adenosine analogs, inhibited specific binding of [^{3}H]NBMPR to atrial and ventricular membranes in a concentration-dependent manner. This finding is consistent

with a previous report that A_1-AdoR agonists, such as R-PIA and N^6-cyclohexyladenosine, which are structurally similar to SDZ WAG-994, are competitive antagonists at the nucleoside transporter in Novikoff rat hepatoma cells (Plagemann and Wohlhueter, 1984).

ADA attenuated the coronary vasodilation but not the S-H interval prolongation caused by SDZ WAG-994. This differential effect of ADA can be explained as follows. First, the concentration of adenosine required to prolong AV nodal conduction time exceeds that needed to cause coronary vasodilation by at least 10-fold (Belardinelli and Shryock, 1992). Thus, a small increase in interstitial adenosine concentration caused by SDZ WAG-994 may be sufficient to cause coronary vasodilation but not S-H interval prolongation. Second, SDZ WAG-994 binds to A_1-AdoRs to cause S-H interval prolongation at concentrations equal to or lower than those at which it inhibits nucleoside transport. This direct effect of SDZ WAG-994 to cause S-H interval prolongation is not attenuated by ADA.

Antiarrhythmic effect and potential therapeutic implications. The negative chronotropic and dromotropic effects of SDZ WAG-994 may confer important antiarrhythmic properties to this A_1-AdoR agonist. In particular, the negative dromotropic effect of SDZ WAG-994 may be the basis for using this drug for the treatment of supraventricular tachycardias in which the AV node is part of a re-entrant circuit (*e.g.*, AV nodal re-entrant tachycardias). The increase in Wenckebach cycle length and AV nodal refractory period caused by SDZ WAG-994 should enhance the "filtering" capacity of the AV node and reduce transmission of atrial impulses to the ventricles.

The negative dromotropic effect of SDZ WAG-994 was greater as the atrial pacing rate was increased (fig. 2). This suggests that the effectiveness of SDZ WAG-994 to cause second-degree AV nodal block will increase as a function of the atrial rate and is consistent with the finding that the ratio between S-H interval prolongation at fast and slow pacing rates is increased as the atrial pacing cycle length is shortened (fig. 7). The observations that the magnitude and time constant of S-H interval prolongation caused by SDZ WAG-994 were greater at a faster than at a slower rate of pacing (table 2) are similar to those reported for calcium channel antagonists (Talajic *et al.*, 1989) and adenosine (Belardinelli and Shryock, 1992; Stark *et al.*, 1993a; Nayebpour *et al.*, 1993). The frequency-dependent effects of calcium channel antagonists on AV nodal conduction have been extensively investigated and are thought to explain the prompt and effective control by these drugs of ventricular rate during supraventricular tachyarrhythmias, such as atrial fibrillation (Roth *et al.*, 1986; Ellenbogen, 1992; Talajic and Nattel, 1986; Talajic *et al.*, 1989, 1990; Hondeghem and Katzung, 1984). Adenosine is a therapeutic alternative to calcium channel blockers for clinical modulation of AV nodal conduction (Lerman and Belardinelli, 1991; Camm and Garratt, 1991; DiMarco *et al.*, 1990). Although no direct comparisons were made among adenosine, SDZ WAG-994 and calcium channel antagonists in the present study, Stark *et al.* (1993a) showed that the frequency-dependent effect of adenosine on AV nodal conduction is greater than that of verapamil. A detailed study of the rate-dependent negative dromotropic action of adenosine in rabbit and guinea pig isolated AV nodal preparations revealed that increased AV nodal fatigue and reduced facilitation are the basis for the rate-dependent AV nodal depression caused by adenosine (Nayebpour *et al.*, 1993).

It is tempting to speculate that SDZ WAG-994 has the potential to be an effective and safe antiarrhythmic agent. At normal heart rates, SDZ WAG-994 would cause minimal slowing of AV nodal conduction, whereas at fast heart rates (*i.e.*, during tachycardia) SDZ WAG-994 should cause a marked prolongation of AV nodal conduction time, an increase in AV nodal refractory period and a termination of re-entrant tachycardias that involve the AV node. Thus, SDZ WAG-994 could prove to be effective for the management of supraventricular tachycardias and for the control of ventricular rate during atrial flutter or fibrillation, at concentrations that cause little or no slowing of AV nodal conduction during normal sinus rhythm. On the other hand, because adenosine shortens the atrial action potential (Belardinelli and Isenberg, 1983a) and refractory period (Stark *et al.*, 1993b), it facilitates the induction of atrial flutter and fibrillation. Thus, SDZ WAG-994 may facilitate the induction and/or maintenance of re-entrant atrial arrhythmias. In contrast, because adenosine does not shorten the ventricular action potential (Belardinelli and Isenberg, 1983b), it is unlikely that SDZ WAG-994 will have a proarrhythmic action in ventricular myocardium. Finally, because in spontaneously beating hearts SDZ WAG-994 was at least 2-fold more potent to slow atrial rate than to prolong AV nodal conduction time, bradycardia instead of first- or second-degree AV block is expected to be the predominant effect of SDZ WAG-994 at resting heart rates. Further investigation of the frequency-dependent negative dromotropic effect of adenosine and an elucidation of structure-activity relationships for A_1-AdoR to exert frequency-dependent actions may be worthy research endeavors with important therapeutic implications.

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