Atrioventricular Nodal Accommodation in Isolated Guinea Pig Hearts: Physiological Significance and Role of Adenosine

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The progressive prolongation of atrioventricular node (AVN) conduction time to a new steady-state value caused by sudden and maintained increases in atrial rate is the most common form of AV nodal accommodation. This study was undertaken to 1) characterize AV nodal accommodation in isolated perfused guinea pig hearts, 2) investigate the influence of potential modulators of this phenomenon such as acetylcholine and adenosine, and 3) determine the physiological significance of AV nodal accommodation on cardiac function. Beat-by-beat changes in AVN conduction time caused by single- or multiple-step increases in atrial pacing rate were measured during control conditions and in the presence of atropine (1 μM), propranolol (1 μM), and the adenosine antagonist BW-A1433 (1 μM). BW-A1433 was the only intervention that significantly reduced the cumulative and frequency-dependent prolongation of AVN conduction time but this was only observed at atrial cycle lengths ≤ 170 msec. In addition, BW-A1433 shortened the Wenckebach cycle length from 163 ± 2 to 153 ± 2 during normoxia and from 172 ± 3 to 164 ± 4 during mild hypoxia. In contrast, dipyridamole (1 μM), an adenosine uptake blocker, markedly accentuated the AVN conduction time prolongation, accentuated the AV block associated with fast atrial rates, and significantly increased the Wenckebach cycle length. These effects of dipyridamole were prevented and antagonized by BW-A1433 and adenosine deaminase. When O₂ supply was limited and at the same time demand increased secondary to fast atrial pacing, the rate of adenosine release increased from a control of 125 ± 27 to 580 ± 54 pmol/min/g. This was accompanied by a significant prolongation in AVN conduction time that invariably progressed to AV block. Once AV block occurred, O₂ consumption decreased, O₂ supply-to-demand ratio improved and the rate of adenosine release dropped to 310 ± 61 pmol/min/g. Reversal of the AV block with adenosine antagonists resulted in a decrease in O₂ supply-to-demand ratio and a severalfold increase in the rate of adenosine release. In this feedback system, adenosine signals the imbalance between O₂ supply and demand, causes AV block and, thus, reduces demand to compensate for the limited O₂ supply. On the other hand, adenosine deaminase and antagonists act as “error signals” by attenuating the effect of adenosine, whereas dipyridamole enhances the “gain” of the system by potentiating the effects of adenosine. We concluded that 1) AV nodal accommodation is an intrinsic property of the AVN that can be influenced by adenosine, and 2) under conditions of low O₂ supply-to-demand ratio, adenosine is produced as a negative feedback signal that protects the ventricles from excessive work by causing AV block. (Circulation Research 1988;63:97-116)
of AV nodal cells are thought to account for the most common form of the phenomenon of AV nodal accommodation, also referred to as "crescendo-like" pattern,\(^1\)\(^-\)\(^3\) that is, the progressive lengthening of AV conduction time to a new steady state in response to sudden and sustained increases in atrial rate. Lehman et al\(^3\) have described in humans two other potential patterns of AV nodal accommodation in response to a sudden increase in atrial rate.

AV nodal accommodation has been studied in the past by many investigators in both laboratory animals and humans.\(^1\)\(^-\)\(^6\) However, neither its mechanism and modulators nor its significance have been systematically investigated. The sympathetic and parasympathetic nervous systems have potent influences on AV node transmission but only limited data are available on their potential modulatory role in AV nodal accommodation. Studies on either chloralose-anesthetized dogs devoid of autonomic innervation of the heart or unanesthetized dogs before and after cardiac transplantation showed that AV nodal accommodation, although still subject to humoral mediation, can occur independently of the sympathetic and parasympathetic nervous system and, hence, is an intrinsic property of the AV node.\(^6\)\(^,\)\(^7\)

Similar to the parasympathetic nervous system, the nucleoside adenosine has inhibitory effects on AV node conduction.\(^8\) In addition, since the formation of this nucleoside is tightly coupled to the oxygen supply-demand ratio of the heart,\(^9\) it could accumulate during fast heart rates and, hence, play a role in the cumulative frequency-dependent AV conduction delay and block.

The purpose of the present study was threefold: 1) to characterize AV nodal accommodation in the isolated perfused guinea pig heart; 2) to investigate the role of potential modulators of this phenomenon, that is, neural transmitters (acetylcholine) and metabolic factors (adenosine); and 3) to determine the physiological significance of AV nodal accommodation on overall cardiac function.

**Materials and Methods**

**General**

The experiments were carried out in isolated perfused guinea pig hearts according to previously described techniques.\(^10\) In brief, adult guinea pigs (Hartley, Charles River, Scottsdale, Pennsylvania) of either sex weighing 250–350 g were killed by cervical dislocation. The hearts were quickly excised to facilitate electrical pacing of the hearts and to expose the AV node for placement of the recording electrodes in the region of the bundle of His. The hearts were paced via bipolar electrodes placed on the left atrium or interatrial septum. A World Precision Instruments (WPI; New Haven, Connecticut) 1830 interval generator and a WPI 1880 stimulus isolation unit were used to deliver square-wave stimulus pulses with a duration of 3 msec and an intensity that was three to four times threshold. His bundle electrograms were recorded with unipolar extracellular electrodes placed in the region of the AV node.\(^10\) His bundle electrogram signals were filtered and amplified using a Tektronix 5A22N (Fullerton, California) differential amplifier and were displayed on a Tektronix 5113 analog dual beam storage oscilloscope. Electrical pacing of the heart and stimulus-to-His bundle (S-H) interval measurements were controlled by a modified Northstar Horizon microcomputer as described previously.\(^11\) This computer system utilized an analog-to-digital converter to sample the His bundle electrogram in real time and, hence, detect the His bundle potential (i.e., H spike). The S-H intervals for each beat were stored for later analysis. Pacing of the hearts was also computer programmed: stimulation involved sending a triggering pulse from a digital-to-analog converter to the WPI stimulator. The structures of the stimulation protocols were programmed in assembly language and Basic and allowed the investigator to choose the desired parameters such as rate and duration of pacing.

After instrumentation, all hearts were allowed to equilibrate for 20–30 minutes before control measurements were taken. Experimental interventions were preceded and followed by control measurements of the S-H interval and if preintervention and postintervention times varied by more than 15% the data were discarded. The data were also discarded whenever the stimulus-to-atrial response increased by more than 3 msec during fast rates of pacing. In the event that an intervention caused second-degree AV block, a maximal S-H interval was assigned, which was the maximal stable S-H conduction time occurring prior to the onset of heart block.

Atropine, DL-propranolol HCL, and adenosine deaminase Type I were purchased from Sigma Chemical, St. Louis, Missouri; 8-(p-sulfophenyl) theophylline (8-PST) was obtained from Research Biochemicals Inc., Natick, Massachusetts; erythro-9-(2-hydroxy-3-nonyl) adenine HCL (EHNA) and the adenosine antagonist E-4-(1,2,3,6-tetrahydro-2,6-dioxo-1,3-dipropyl-9H-puriny-8-yl) cinnamic acid (BW-A1433) were gifts from Burroughs Wellcome, Research Triangle Park, North Carolina; and dipryridamole (DIP) was a gift from Boehringer Ingelheim, Ingelheim, FRG. Stock solutions of the above drugs and enzyme were dissolved in perfusion medium and infused to achieve the desired perfusate concentration. Adenosine deaminase was
dialyzed overnight at 4°C with a spectropor membrane (m.w. cut-off, 6,000–8,000) against 3-[N-morpholino]propanesulfonic acid buffer (50 mM, pH 7.5).

Characterization of the Phenomenon of AV Nodal Accommodation

Two parameters that have been reported\(^1,3,4,12\) to influence AV nodal accommodation are rate and the duration of the period of rapid stimulation. Thus, to characterize this phenomenon, stimulation protocols were developed to allow selection of varying rates and durations of stimulation. Two different stimulation protocols were employed:

1) Single-step protocol. This stimulation protocol consisted of a single-step increase in rate of pacing followed by a single-step decrease to the original rate of stimulation. Several variations of this protocol were performed where the control rate was 2.0 Hz, the duration of rapid pacing was 30 or 60 seconds, and the rapid rate of pacing was varied from 4.0 Hz to 6.25 Hz. The duration of rapid stimulation was chosen based on the determination that a steady-state AV node conduction time was achieved within 60 seconds after the beginning of rapid pacing regardless of the rate of stimulation. Any given stimulation protocol was always preceded by a 20-second initialization period (i.e., basal rate of pacing) and a 120-second recovery period at the control rate of stimulation. AV node conduction data were recorded and stored by the microcomputer system throughout the duration of each protocol. In every heart, a minimum of four protocols were performed, and the order of these stimulation protocols was varied among hearts.

2) Multiple-step protocol. This stimulation protocol consisted of several continuous 30-second intervals of progressively decreasing cycle lengths. One example of the cycle lengths (in msec) of stimulation chosen for this protocol was as follows: 500, 400, 300, 250, 200, 180, 175, 170, 165, 160, and 155.

Effect of Atropine, Propranolol, and BW-A1433

To test whether acetylcholine, catecholamines, and/or adenosine, which could potentially be released during rapid pacing, influenced the time course or degree of AV nodal accommodation, experiments were conducted in the presence of their respective antagonists: atropine (1 μM), propranolol (1 μM), and BW-A1433 (1 μM). Both single- and multiple-step stimulation protocols were employed. The design of this series of experiments was similar for all three interventions. That is, after obtaining the control (no intervention) measurements of AV nodal accommodation, the drugs were infused for at least 5 minutes before evaluation of their effects on AV nodal accommodation. The effects of atropine and propranolol were investigated on the same hearts, whereas those of BW-A1433 were evaluated in a separate group of hearts. Atropine was tested first and then propranolol followed by a 30-minute washout of atropine. Postintervention measurements of AV nodal accommodation were obtained after a 30-minute washout of the drugs.

Effect of Dipyridamole, BW-A1433, and Adenosine Deaminase

Dipyridamole (1 μM), an adenosine transport blocker, was used to evaluate the role of endogenously released adenosine on rapid pacing-induced AV node accommodation. In this series of experiments only the multiple-step protocol was used. This stimulation protocol was carried out during a) control conditions (no intervention), b) in the presence of dipyridamole (seven hearts), and c) in the presence of both dipyridamole and 1 μM BW-A1433 (four hearts) or 5 units/ml of adenosine deaminase (three hearts). Each stimulation protocol was followed by at least a 5-minute recovery period during which the hearts were paced at 2 Hz. Dipyridamole infusion was begun 5 minutes before the second period of rapid pacing. Following the stimulation protocol in the presence of dipyridamole, BW-A1433, or adenosine deaminase infusion was begun. BW-A1433 and adenosine deaminase infusions started 7 and 5 minutes, respectively, before the stimulation protocol was initiated.

Dipyridamole-Induced Second-Degree AV Block and Reversal With BW-A1433

Two different protocols were used to demonstrate the induction of second-degree AV block by rapid atrial pacing in the presence of dipyridamole and its reversal by BW-A1433. In the first of these protocols, the hearts were initially paced for several minutes at a cycle length of 400 msec, after which the atrial cycle length was suddenly shortened to 180 msec until a new steady-state S-H interval was achieved. Then, while the hearts were still being paced at the fast rate (i.e., 180 msec), dipyridamole (1 μM) infusion was begun. When second-degree AV block developed, which at this fast rate of pacing occurred on average in less than 5 minutes, BW-A1433 (1 μM) infusion was started in the presence of continued dipyridamole infusion. Once 1:1 AV conduction was restored (i.e., reversal of AV block), the infusion of the drugs was stopped, and the hearts were allowed to recover for 25 minutes. During the recovery period, the hearts were paced at an atrial cycle length of 400 msec. The ability of BW-A1433 to prevent the occurrence of second-degree AV block was demonstrated by simultaneous infusion of dipyridamole (1 μM) and BW-A1433 (1 μM) while pacing the hearts at an atrial cycle length of 180 msec. The infusion of both dipyridamole and BW-A1433 began at least 5 minutes prior to increasing the atrial cycle length to 180 msec and was maintained throughout the duration of the fast pacing (i.e., 5 minutes).

The second protocol for induction of second-degree AV block with dipyridamole utilized the
effluent samples were obtained during control stimulation period. After a 5-minute washout of BW-A1433 and reoxygenation, the postintervention control protocol was repeated.

Release of Adenosine During Rapid Pacing

In a separate series of experiments (nine hearts), effluent levels of adenosine were measured during different rates of rapid pacing. Effluent samples were collected for 10-second periods during the multiple-step stimulation protocol at cycle lengths of 500 (baseline rate), 210, and 170 msec. Samples were obtained during control conditions (i.e., absence of intervention) and in the presence of 1 μM dipyridamole. The samples were immediately frozen at —70°C for later determination of adenosine content as described below.

Effect of Reduced Perfusate Po2

In six hearts the single-step stimulation protocol was performed during a) normoxic conditions (Po2 500–600 mm Hg), b) mild hypoxia (Po2 225–368 mm Hg) and c) mild hypoxia plus 1 μM BW-A1433. Mild hypoxia was produced by equilibrating the perfusate with either 38% O2 (Po2 225 mm Hg) or 54% O2 (Po2 368 mm Hg) depending on the sensitivity of each individual heart to the low Po2 at the control rate of stimulation (2.5 Hz). If the S-H interval lengthened by more than 3 msec during control stimulation with 38% O2, the heart was immediately reperfused with normoxic solution. After a 10-minute recovery period, the normoxic perfusate was switched to perfusate equilibrated with 54% O2. During perfusion at low Po2, the S-H interval had to be stable during control stimulation for 4–5 minutes prior to starting the rapid pacing (5 Hz). The periods of perfusion at low Po2 were begun 4 minutes before initiating the pacing protocol and were terminated immediately following its completion. After a 10-minute reoxygenation period, infusion of 1 μM BW-A1433 was initiated and 5 minutes later the hearts were once again subjected to the perfusion at low Po2. The BW-A1433 infusion was maintained throughout the rapid stimulation protocol until completion of the protocol. After 20–30 minutes of washout of BW-A1433 and reoxygenation, the postintervention control (normoxic conditions) stimulation protocol was performed.

Rapid Pacing-Induced Release of Adenosine During Normoxia and Low Perfusate Po2

In a separate series of experiments in six hearts, effluent samples were obtained during control stimulation (2.5 Hz) and during 60 seconds of rapid pacing at 5.75 Hz. During the rapid stimulation period, samples were obtained at 25 seconds and 50 seconds. All samples were collected over a 10-second period. The effluent samples were collected during normoxia (95% O2) and reduced perfusate Po2 (38% and 54% O2).

Effect of Reduced Perfusate Po2 (Partial Ischemia)

In these series of experiments, "partial ischemia" was produced by reducing the perfusate flow by 50% to 75% and subsequently increasing the stimulation rate. The single-step stimulation protocol was used to increase the rate of stimulation. After determining the changes in S-H interval caused by the rapid pacing during control conditions (normal perfusion flow), the hearts were allowed to recover for 5 minutes. The rate of perfusion (8 ml/min) was then lowered but not to a level that would prolong the S-H interval when the hearts were stimulated at the control rate (2.5 Hz). After 4 minutes of reduced perfusion flow, the single-step protocol was begun and upon its completion the perfusion rate was returned to the control level. After a 10-minute recovery period, the perfusion flow was again lowered to the same level as before and 4 minutes later, BW-A1433 infusion was begun at a rate to yield a perfusate concentration of 1 μM. After 5 minutes of exposure to BW-A1433 and in its continued presence, the stimulation protocol was repeated. The normal perfusion flow was then restored and the BW-A1433 was washed out of the hearts for at least 20 minutes before a post intervention control protocol was performed.

Myocardial Oxygen Consumption Measurement

Perfusate oxygen pressure was measured using a Clark-type polarographic electrode (model 125/05 INSTECH, Horsham, Pennsylvania). Prior to each experiment the electrode was cleaned, coated with chloride, and tested for linearity of response. The electrode was attached to a temperature-controlled, mechanically stable perfusate flow chamber (INSTECH model SYS600B). Its output was amplified and displayed digitally on a single-channel oxygen electrode amplifier (INSTECH model 102B) and on a Gould 2400 S chart strip recorder (Cleveland, Ohio). A two point calibration was performed before and after each experiment using fluid of known Po2. Since under our experimental conditions arterial (i.e., perfusate) oxygen tension (Pao2) was constant, the myocardial oxygen consumption (MVO2) and O2 supply-to-demand ratio were calculated according to the following equations: 

\[ \text{MVO}_2 = CA \times \frac{(Pao_2 - Pvo_2)}{(c/760) \times 100} \]

where CA is coronary flow (ml/min/g) and C = Bunsen solubility coefficient of O2 dissolved in perfusate at 37°C (mlO2/atm/ml fluid) and O2 supply-to-demand ratio = Pao2/(Pao2 – Pvo2).
Venous Oxygen Tension (PvO₂) and Adenosine Release During Rapid Pacing at High and Low Perfusate O₂

In this series of experiments, the PvO₂ of the perfusate (Pao₂) of both the normoxic (95% O₂) and mild hypoxic (38% or 54% O₂) solution was measured prior to instrumentation of the hearts. In these hearts, the pulmonary artery was cannulated and the venous outflow diverted to the inflow port of the oxygen electrode flow chamber for measurement of venous oxygen tension (PvO₂). The PvO₂ as well as S-H interval were continuously measured and recorded throughout the experiment. To characterize the changes in PvO₂ during increased pacing rates, several single-step stimulation protocols were performed at 95% O₂ by varying the rapid rate of stimulation from 3 Hz to 6.5 Hz. Each protocol was followed by a 3-minute recovery period or until the PvO₂ returned to control. The hearts were then perfused with Krebs-Henseleit solution equilibrated with 38% or 54% O₂ until a new steady-state PvO₂ was reached at a pacing rate of 2 Hz. At this new steady-state PvO₂, the single step protocols were repeated. In a separate group of hearts, in addition to the above measurements of PvO₂ and S-H interval, effluent levels of adenosine were determined. Effluent samples were taken for the last 15 seconds of each rapid stimulation period. Samples were obtained during normoxic (95% O₂) and mild hypoxic (38% or 54% O₂) perfusion and were immediately frozen at -70°C for later determination of adenosine content.

Adenosine Assay

Effluent samples were assayed for adenosine by reverse phase high-performance liquid chromatography (HPLC) according to the method employed by Hartwick et al. with the modification of an isocratic mode instead of a gradient mode. The HPLC system included a Waters 710B injector (Milford, Pennsylvania), an LKB 2150 pump (Bromma, Sweden), and a Beckman 160 absorbance detector (Palo Alto, California). Samples were passed through an Altex Ultrasphere ODS (5 μm) column (Palo Alto, California) containing 50 mM KH₂PO₄ (pH 5.5) with 10% methanol (vol/vol). The adenosine peaks were detected at 254 nm, recorded on a Kipp and Zonen BD 40 strip chart recorder (Bohemia, New York), and integrated with a Hewlett-Packard 3392A integrator (Palo Alto, California).

Data Analysis

All values are reported as mean ± SEM. The Student’s t test was used for paired data analysis. Differences between mean data were considered significant at p < 0.05.

Results

AV Nodal Accommodation

Figure 1 illustrates an example of crescendo-like pattern of AV nodal accommodation in an isolated perfused guinea pig heart. Note that when the pacing rate is increased from 2.5 Hz to 5.0 Hz (Figure 1B), there is an initial large increase in the S-H interval on the first beat at the new rate, that is, 5.0 Hz (cf. H spike "1 to 2"; Figure 1B). On subsequent beats there was an additional and progressive prolongation of the S-H interval until a new steady state was achieved (cf. H spikes "2 to 82"; Figure 1B). The initial increase in AV node conduction time during the first beat at a higher pacing rate and the subsequent increase in S-H interval are hereafter referred to as the "fast and slow component" of AV nodal accommodation, respectively. A similar phenomenon was observed, but in the reverse order, when the pacing rate was decreased (Figure 1C).

Experiments performed using the single-step stimulation protocol revealed two major relations with regard to the AV nodal accommodation phenomenon in the isolated perfused guinea pig heart (Figure 2): 1) The maximum prolongation of the S-H interval (steady-state value) during AV nodal accommodation is dependent on the magnitude of the change in rate of stimulation (Δ rate). Moreover, both the fast and the slow components increase with increasing the delta rate in a parallel manner; that is, the slopes of the curves in Figure 2A are nearly equal. 2) The slow component of AV nodal accommodation is dependent on the duration of pacing at the higher rate, though only to a certain extent. As shown in Figure 2B, the slow component becomes fully developed (i.e., reaches steady state) within 20 to 35 seconds following the initiation of rapid pacing. The mean time required for the S-H interval to reach 90% (t₀.₉) of the maximum prolongation was 17 ± 7 seconds (n = 9). Variations in Δ rate do not significantly affect the time required to achieve steady state. Upon return to the control heart rate, the S-H interval rapidly shortens toward the baseline value. The time required for the S-H interval to return to baseline value varied between 20 and 30 seconds (t₀.₉ = 21 ± 3 seconds, n = 5).

Figure 3 depicts an example of the multiple-step stimulation protocol, showing the prolongation of AV node conduction time in response to stepwise increases in stimulation rate. The differences between each point (S-H interval) on the curve and the baseline S-H interval (i.e., that at the control atrial cycle length: 500 msec) represents the total AV nodal accommodation at each atrial cycle length. The relations found in the single-step stimulation protocol (Figure 2) also apply to the multiple-step stimulation protocol (Figure 3).

Effect of Atropine, Propranolol, and BW-A1433

To test whether acetylcholine, catecholamines, and/or adenosine, which could potentially be released during rapid pacing, influenced the time course or magnitude of AV nodal accommodation, experiments were conducted in the presence of their respective antagonists: atropine (1 μM), propranolol (1 μM), and BW-A1433 (1 μM).
In this series of experiments, the single-step stimulation protocol was applied using rate steps from 2.5 Hz to 5.0 and 5.5 Hz in three hearts (atropine and propranolol group) and from 2.0 Hz to 5.0 and 5.5 Hz in an additional group of five hearts (BW-A1433 group). As can be seen in Table 1, none of the interventions (atropine, propranolol, and BW-A1433) significantly affected either the magnitude of AV nodal accommodation (fast or slow component) or the time required for the S-H interval to achieve a steady-state value. The role of these three drugs on the AV nodal accommodation was further investigated with the multiple-step stimulation protocol. Although not shown, results similar to those in Table 1 were obtained in four hearts using the multiple-step stimulation protocol; atropine (1 μM) or propranolol (1 μM) did not produce any shift in the relation between atrial cycle length and S-H interval. In contrast, in a separate group of seven hearts, 1 μM of the adenosine antagonist (BW-A1433) caused a noticeable shift (Figure 4) in the atrial cycle length versus S-H interval relation during the multiple-step stimulation protocol. As Figure 4 illustrates, there was no significant difference between the control S-H values and those with the adenosine antagonist at atrial cycle lengths between 500 and 175 msec. However, as the atrial cycle length was gradually shortened (i.e., 170, 165, and 160 msec), the difference between “control” and “BW-A1433” became progressively greater and significant at 165 and 160 msec. The inset on Figure 4 depicts the difference (Δ) between “control” and “BW-A1433.” For example, the mean difference (Δ [control-BW-A1433]) was 2.5±1.5 msec at 170 msec, 4.0±2.0 msec at 165 msec, and 8.0±3.5 msec at 160 msec. As shown in Table 2, the Wenckebach cycle length was also significantly shortened by BW-A1433.

**Effect of Dipyridamole**

The role of adenosine in AV nodal accommodation was also studied in hearts pretreated with the nucleoside uptake blocker, dipyridamole. The rationale for this series of experiments was that dipyridamole, by decreasing adenosine uptake by endothelial, vascular smooth muscle and cardiac cells, would lead to a greater accumulation of adenosine in the interstitial space and, hence, longer S-H intervals. Figure 5 shows that 1 μM of dipyridamole causes an increase in AV node accommo-
Dipyridamole-induced second-degree AV block

Since AV node conduction time is prolonged in hearts pretreated with dipyridamole, it is anticipated that dipyridamole alone could precipitate the occurrence of AV block especially at rapid atrial rates. Thus, two stimulation protocols were designed to demonstrate induction of second degree AV block by rapid atrial pacing in the presence of dipyridamole, and its reversal by BW-A1433. An example of the first protocol is depicted in Figure 7. Under control conditions (no drug present), the S-H intervals were 44 msec and 67 msec at atrial cycle lengths of 400 msec and 170 msec, respectively (Figure 7A). In the presence of 1 μM dipyridamole the S-H interval remained 44 msec at an atrial cycle length of 400 msec but was progressively prolonged at the atrial cycle length of 170 msec until AV node block developed (Figure 7B). As shown in Panel C, the addition of 1 μM BW-A1433 to the perfusate not only reversed the AV block but also shortened the S-H interval back to control (i.e., 68 msec).

The second protocol utilized the computer-driven single-step stimulation protocol. The example shown in Figure 8 is an experiment in which a step in rate from 2.5 Hz to 6.0 Hz for 60 seconds was performed during control and in the presence of dipyridamole alone or dipyridamole combined with BW-A1433. The stimulation protocol and time course of the accompanied changes in S-H interval with no intervention (control) are shown in Panel A, whereas Panel B only shows the changes in S-H interval that occurred during the period of rapid pacing. With no intervention, a steady-state S-H interval was reached after 35 seconds of rapid pacing. However, in the presence of 1 μM dipyridamole, a steady state was never achieved; in fact, AV block occurred in less than 20 seconds after the beginning of rapid pacing. BW-A1433 (1 μM) was infused for 5 minutes in the presence of dipyridamole and the same stimulation
protocol was repeated. As can be seen, the dipyridamole-induced S-H prolongation and AV block were completely abolished by the adenosine antagonist. In this series of experiments (n = 6), the maximum S-H prolongation achieved during control conditions was 8.0 ± 0.5 msec whereas when dipyridamole was present the S-H interval (measured prior to AV block) prolonged to 36 ± 2 msec. In these experiments, 1 μM BW-A1433 completely prevented the dipyridamole-induced AV block and the prolongation of S-H interval, that is, during BW-A1433 infusion in the presence of dipyridamole, the S-H interval was prolonged by only 7.0 ± 0.5 msec.

Release of Adenosine During Rapid Pacing

In this series of experiments (n = 9), the rate of adenosine release was measured in the absence and presence of 1 μM dipyridamole at various rates of pacing. Figure 9 summarizes the results of these experiments. As can be seen, at every rate of pacing the effluent adenosine levels were higher in the presence of dipyridamole than in its absence, and adenosine levels increased significantly (p<0.05) during pacing at the cycle length of 170 msec. Note that in the absence of dipyridamole the rate of adenosine release remained unchanged.

Effect of Reduced Perfusate PO2 on AV Node Conduction, Adenosine Release and Myocardial Oxygen Consumption

Experiments were designed to investigate the relationships among S-H interval, effluent adenosine concentrations, MVo2, and the O2 supply-to-demand ratio under normoxic conditions (95% O2) and with reduced perfusate PO2 (38% to 54% O2) during low and fast rates of pacing. The effect of reduced perfusate PO2 on the fast and slow components of AV nodal accommodation are summarized in Figure 10B, and an example of the temporal development of the slow component of AV nodal accommodation during these experiments is shown by guest on May 31, 2017 http://circres.ahajournals.org/ Downloaded from

### Table 1. Lack of Effect of Atropine, Propranolol, and BW-A1433 on AV Nodal Accommodation During Single-Step Stimulation Protocol

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<td>Control</td>
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<td>Atropine (1 μM)</td>
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Values are mean ± SEM of the fast and slow components of AV nodal accommodation (i.e., S-H interval in msec) as defined in the methods section. Group A and B composed of three and five hearts paced at baseline rate of 2.5 and 2.0 Hz, respectively. Differences between control and treatment were not significant (p>0.05). Not shown, the time required for the S-H prolongation to reach steady-state was not altered by any of the interventions.
in Figure 10A. In addition, Figure 10 also shows the effect of BW-A1433 on the S-H interval during conditions of reduced perfusate PO₂. The slow component of AV nodal accommodation during low PO₂ is markedly increased and does not reach steady state, but it is largely attenuated by BW-A1433. As shown in Figure 10B, the fast component of AV nodal accommodation was not significantly affected by the low perfusate PO₂.

The role of adenosine in the prolongation of the S-H interval seen with reduced perfusate PO₂ was further investigated by determining the rate of adenosine release during low and high pacing rates under normoxic conditions (95% O₂) and with reduced perfusate PO₂ (38% to 54% O₂). In this group of experiments (n = 6), the S-H interval and adenosine release were measured simultaneously. As shown in Figure 11, there was no difference in either the rate of adenosine release or S-H interval prolongation between 95% and 38% O₂ at low rates of pacing (2.5 Hz). At low pacing rates the values for adenosine release were 111 ± 12 and 102 ± 12 pmol/min/g with 95% and 38% O₂, respectively; and the S-H interval values were 41.5 ± 1.0 msec and 41.5 ± 0.5 msec with 95% and 38% O₂, respectively. After 1 minute of rapid pacing (5.7 Hz), the S-H interval (Figure 11B) at 38% O₂ was significantly greater than at the low rate and also significantly greater than the S-H interval after 1 minute of rapid pacing at 95% O₂. The S-H interval values after 1 minute of rapid pacing were 71.0 ± 5.0 msec with 95% O₂ and 99.0 ± 14.0 msec with 38% O₂. In addition, the adenosine release after 1 minute of rapid pacing at 38% O₂ was significantly greater than with 95% O₂.

The amount of adenosine released after 1 minute of rapid pacing was 121 ± 12 pmol/min/g with 95% O₂ and 251 ± 60 pmol/min/g with 38% O₂. After 30 seconds of fast pacing, there was no significant difference between either the rate of adenosine release or the S-H interval prolongation at 95% O₂ and those at 38% O₂. In addition, at 30 seconds into the period of rapid pacing, there was no significant difference in the rate of adenosine release at 95% or 38% O₂ when compared with low rates of pacing. Further support for the role of adenosine in the fast pacing-induced S-H prolongation during reduced perfusate PO₂ can be derived from the data shown in Table 2 and Figure 12. As shown in Table 2, the Wenckebach cycle length was significantly longer during reduced perfusate PO₂; it increased from a control (95% O₂) of 163 ± 2 msec to 172 ± 3 msec (45–54% O₂). The adenosine antagonist BW-A1433 (1 μM) significantly shortened the Wenckebach cycle length to 164 ± 4 msec, a value that was not different from control (normoxia), whereas dipyrismole markedly prolonged it to 196 ± 14 msec. BW-A1433 (1 μM) completely antagonized the

**Figure 4. Effect of the adenosine antagonist (BW-A1433) on rate-dependent changes in AV node conduction in isolated guinea pig hearts.** Hearts were subjected to a stimulation protocol consisting of a series of 30-second steps of progressively shorter atrial cycle lengths from 500 to 160 msec. This stimulation protocol was first performed under control conditions (no intervention, n = 7) and then in the presence of 1 μM BW-A1433 (n = 6). Inset depicts more clearly the rate-dependent effect of the adenosine antagonist on AV node conduction. At lower rates there is essentially no difference between results obtained with BW-A1433 and those of control, but as the rate increases, the difference increases. *Significantly (p < 0.05) different from control. Data are mean ± SEM.
dipyridamole-induced prolongation of the Wenckebach cycle length. The recovery time of the S-H interval following 60 seconds of atrial pacing at 5 Hz was significantly longer when using perfusate equilibrated with 38% than with 95% O₂ (Figure 12). The time required for the S-H interval to reach 90% (t0.9) of full recovery (the baseline value) was 21 ± 3 seconds during normoxia (95% O₂) and 112.5 ± 17.0 seconds during 38% O₂. As shown in Figure 12, BW-A1433 (1 μM) significantly reduced the t0.9 to 58 ± 20 seconds.

Figure 13 illustrates the effect of the rate of stimulation on MV₀₂ and the O₂ supply-to-demand ratio. As expected, MV₀₂ (panel A) increases and O₂ supply-to-demand ratio (panel B) decreases as the rate of stimulation increases. The O₂ supply-to-demand ratio at the pacing rate of 2–3 Hz averaged 3.12 ± 0.4 (n = 7) and significantly decreased to 1.87 ± 0.1 (n = 7) when the pacing rate was increased from 5.5 to 6.0 Hz. At fast rates of pacing, when second-degree AV block ensues, MV₀₂ decreases and the ratio of O₂ supply-to-demand improves.

To test the hypothesis that endogenously released adenosine is caused by an imbalance in the O₂ supply-to-demand ratio, a series of experiments (n = 4) were performed in which the rate of adenosine release was measured during fast pacing and reduced perfusate Po₂. As shown in Figure 14, the rate of adenosine release during 1:1 AV conduction at fast pacing was 580 ± 54 pmol/min/g, which was significantly greater than the adenosine released (125 ± 27 pmol/min/g) during slow pacing rates (2 Hz). However, during fast pacing after second degree AV block occurred, the rate of adenosine

**Figure 5.** Effect of dipyridamole (DIP) alone and in combination with the adenosine antagonist BW-A1433 on rate-dependent changes in AV node conduction in isolated perfused guinea pig hearts. Hearts (n=4) were subjected to a stimulation protocol consisting of a series of 30-second steps of progressively shorter atrial cycle lengths from 500 to 170 msec. This protocol was performed under the following conditions: a) control (no intervention, n=4), b) in the presence of 1 μM DIP (n=4), and c) in the presence of both 1 μM DIP and 1 μM BW-A1433 (n=4). Inset illustrates effect of 1 μM DIP on rate-dependent changes in AV node conduction. Note that there is a small effect of DIP at low rates and a greater effect at higher rates. * Significantly (p<0.05) from control and DIP plus BW-A1433 all at cycle length ≤300 msec. Data are mean±SEM.

**Figure 6.** Effect of adenosine deaminase on dipyridamole (DIP)-induced increases in AV nodal accommodation in isolated perfused guinea pig hearts. Hearts were subjected to a stimulation protocol consisting of a series of 30-second steps of progressively shorter atrial cycle lengths from 500 to 170 msec. Only the steady-state values from two cycle lengths (CL) are shown: 300 and 185 msec. The stimulation protocol was performed during a control period, in the presence of 1 μM DIP and in the presence of both 1 μM DIP and 5 units/ml adenosine deaminase (ADA). Note that adenosine deaminase nearly reverses the effect of DIP on AV node conduction at both the low rate (CL=300 msec) and the fast rate of pacing (CL=185 msec). * and † indicate values significantly different (p<0.05) from control and DIP alone, respectively. Values presented are mean±SEM.
FIGURE 7. Example illustrating the occurrence of second degree atrioventricular (AV) node block in the presence of dipyridamole (DIP) and its complete reversal by the adenosine antagonist BW-A1433 in an isolated perfused guinea pig heart. Records in panels A, B, and C were obtained sequentially. Panel A: His bundle electrogram (HBE) recordings with stable S-H intervals at cycle lengths of 400 and 170 msec. Panel B: In the presence of 1 μM dipyridamole (DIP). Note that at a cycle length (CL) of 400 msec, there is no difference in the S-H interval from control, but at a CL of 170 msec, the S-H interval in the presence of DIP prolongs to over 90 msec before second degree AV block occurs (denoted as “block”). In the third tracing of Panel B, additional blocked beats are shown. With DIP still present, addition of 1 μM BW-A1433 reversed second degree AV block into 1:1 AV conduction. When steady state was reached (recording in Panel C), note that BW-A1433 completely reversed the effect of DIP (compare Panel C with second tracing of Panel A). S, stimulus artifact; A, atrial depolarization; H, His bundle depolarization; and V, ventricular depolarization.

FIGURE 8. Example of the effect of dipyridamole on AV node conduction during rapid atrial stimulation in the isolated perfused guinea pig heart. Panel A: Time course of the stimulation protocol and the resulting S-H data during control. Panel B: Progressive prolongation of S-H interval (slow component of accommodation only, ∆S-H interval) as a function of time during the rapid stimulation period of the protocol. In the absence of pharmacological interventions (“control”) the ∆S-H interval reaches a steady-state value after approximately 35 seconds. In contrast, in the presence of dipyridamole, the delta S-H interval becomes very large very soon after the beginning of rapid stimulation, progressing to second degree AV block after approximately 15 seconds. This effect of dipyridamole was completely antagonized by the adenosine antagonist BW-A1433.

Rate of adenosine release were overcome by either direct pacing of the ventricles (Panel A) or restoration of 1:1 AV conduction by the adenosine antagonist 8-PST (Panel B). In the first example (Figure 15A) the atrial cycle length was decreased from 500 to 170 msec resulting in a decrease in PVO₂ while 1:1 AV conduction was maintained. During this period, the effluent adenosine levels increased from 230 pmol/min/g to 982 pmol/min/g shortly before AV block ensued. Once second-degree AV block occurred, the PVO₂ increased gradually until a new steady state was reached and the rate of the adenosine release dropped to 740 pmol/min/g. Direct ventricular pacing at a cycle length of 170 msec resulted in a marked increase in PVO₂ which was accompanied by a large increase in the rate of adenosine release (1,939 pmol/min/g). Upon cessation of direct ventricular pacing, second-degree AV block resumed...
and both the P\textsubscript{vO\textsubscript{2}} and the rate of adenosine release returned to values similar to those before direct ventricular pacing.

Figure 15B depicts an example in which the AV block induced during fast pacing was reversed to 1:1 AV conduction by the adenosine antagonist 8-PST (30 \textmu M). The experimental protocol was similar to the one described in Panel A. In brief, the atrial cycle length was decreased from 500 to 310 msec. This increase in atrial rate resulted in a gradual decrease in P\textsubscript{vO\textsubscript{2}} followed by AV block. Concomitantly, the rate of adenosine release increased from 171 pmol/min/g to 437 pmol/min/g just prior to AV block. During AV block, the P\textsubscript{vO\textsubscript{2}} increased and the rate of adenosine release dropped to 210 pmol/min/g, but resumption of 1:1 AV conduction caused by 8-PST led to a gradual and marked decrease in P\textsubscript{vO\textsubscript{2}} and to a 2.5-fold increase in the effluent concentration of adenosine (518 pmol/min/g). Similar results were observed in five additional experiments, two with direct ventricular pacing and three with 8-PST.

**Effect of Reduced Perfusion Flow (Partial Ischemia)**

To document the applicability of the above findings to more clinically relevant conditions, isolated hearts (n = 7) were subjected to a reduced rate of perfusion. The perfusion flow selected was such that no change in S-H interval occurred at the baseline rate of pacing (270 msec). The experiments were similar to those described above with reduced perfusate P\textsubscript{O\textsubscript{2}}. One example of this protocol is

**FIGURE 9.** Effect of rapid atrial pacing on rate of adenosine release in the absence and presence of dipyridamole (DIP) in isolated perfused guinea pig hearts. The multiple-step stimulation protocol utilized for these experiments consisted of 12 steps (each step of 30-second duration) of decreasing atrial cycle lengths from 500 to 170 msec. Adenosine samples were taken at cycle lengths of 500 msec, 210 msec, and 170 msec. In the presence of dipyridamole, the rate of adenosine release was significantly greater (p<0.05) than during control at every atrial cycle length. Moreover, in the presence of dipyridamole, effluent levels of adenosine increased significantly during fast rates of pacing (170 msec), denoted by the asterisk. Data are expressed as mean±SEM.

**FIGURE 10.** Potentiation of AV nodal accommodation by mild hypoxia in isolated perfused guinea pig hearts and reversal of this effect by the adenosine antagonist BW-A1433. Panel A: Example of progressive prolongation of S-H interval (slow component of accommodation, Δ S-H interval) as a function of time. Before time (t)=0, the heart was paced at 2.0 Hz and had a stable S-H interval of 44 msec. At t=0 seconds, the rate was increased from a control rate of 2.0 to 5.0 Hz for 60 seconds. This procedure was performed under the following conditions: a) 95% O\textsubscript{2}, b) 38% O\textsubscript{2}, and c) 38% O\textsubscript{2} in the presence of 1 \textmu M BW-A1433. At the end of the 60-second rapid stimulation period, the rate of stimulation was returned to control. S-H interval recovered to control values within 1–3 minutes. Note that during the fast stimulation period S-H interval reached steady state after 15–20 seconds when the perfusate was equilibrated with 95% O\textsubscript{2}, whereas it continued to prolong with 38% O\textsubscript{2}. BW-A1433 partially reversed the effect of reduced perfusate P\textsubscript{O\textsubscript{2}}. Panel B summarizes results of six experiments similar to that shown in Panel A. Fast and slow components of AV nodal accommodation were recorded following a single step up in rate from 2.0 to 5.0 Hz during: a) normoxia (95% O\textsubscript{2}, P\textsubscript{O\textsubscript{2}}=591 mm Hg), b) reduced perfusate P\textsubscript{O\textsubscript{2}} (225 or 368 mm Hg), and c) reduced perfusate P\textsubscript{O\textsubscript{2}} in the presence of 1 \textmu M BW-A1433. Note that the fast component of accommodation remained unchanged, whereas the slow component increased during mild hypoxia but was significantly reduced when BW-A1433 was present. * and † indicate values significantly different (p<0.05) from control (95% O\textsubscript{2}) and experimental (38–34% O\textsubscript{2}), respectively. Data are mean±SEM.
A. 350 300 200 150 100
B. 38% O2 38% O2
n=6

FIGURE 11. Effect of low perfusate Po2 (225 mm Hg) and rapid rate of stimulation on adenosine release (Panel A) and S-H interval (Panel B) in isolated perfused guinea pig hearts. During a single step stimulation protocol, effluent adenosine levels were measured at a low rate (2.5 Hz) and a fast rate (5.75 Hz) of stimulation. Effluent samples for measurement of adenosine levels were obtained 25 and 50 seconds after the beginning of the fast rate of stimulation. Note that at low rates of pacing, there was no difference in either adenosine release or S-H interval prolongation between 95% and 38% O2. However, 55 seconds into the rapid period of pacing the S-H interval at 38% O2 was significantly greater than a) at the low rate of pacing (*) and b) after 55 seconds of rapid pacing at 95% O2 (†). The rate of adenosine release after 50 seconds of fast pacing at 38% O2 was also significantly greater than that at 95% O2 (†). * and † indicate that the corresponding value is significantly different (p<0.05) from low rate and 95% O2, respectively. Data are expressed as mean±SEM.

shown in Figure 16. Under normal perfusion conditions (control) the S-H intervals were 35 and 61 msec at atrial cycle lengths of 370 and 158 msec, respectively. After reducing the perfusion flow rate (low flow), the S-H intervals were 35 and 80 msec at atrial cycle lengths of 370 and 158 msec, respectively. Thus, there was no change in the S-H interval at the low rate of pacing, but the S-H interval was prolonged by 19 msec during fast pacing. During continued pacing at the fast rate and the reduced flow rate, 1 μM BW-A1433 reduced the prolongation of the S-H interval to a new steady state of 63 msec, which is only 2 msec longer than the control value. Similar results were obtained in an additional four experiments. Although not shown, in two separate preparations the adenosine antagonist BW-A1433 reversed second-degree AV block to 1:1 AV conduction during conditions of reduced perfusion flow and fast rates of pacing.

FIGURE 12. Effect of mild hypoxia (38% O2) and of the adenosine antagonist BW-A1433 (1 μM) on S-H interval shortening after returning to control pacing rate following 60 seconds of fast atrial pacing. Panel A: Time course of the progressive shortening of the S-H interval (slow component of accommodation, delta S-H). Before time=0, the heart was paced at 5.0 Hz for 60 seconds. At time=0 seconds, the rate of atrial pacing was decreased from 5.0 Hz to 2.5 Hz. When the perfusate was equilibrated with 38% O2, both the maximum S-H prolongation and time for S-H recovery were greater and longer, respectively, than with 95% O2. BW-A1433 partially reversed this effect of 38% O2. Panel B: Summary of recovery time of the S-H interval, i.e., the time required for the S-H interval to reach 90% of full recovery [t0.9] following 60 seconds of fast atrial pacing at 5.0 Hz. * and † indicate values significantly different (p<0.05) from control and those at 38% O2, respectively. Values are expressed as the mean±SEM of five hearts.

Discussion

In addition to confirming that AV nodal accommodation is an intrinsic property of the AV node, our study provides the evidence for the following: 1) that adenosine significantly modulates AV nodal accommodation during imbalances between O2 supply and demand and 2) that AV block due to imbalances in the O2 supply-to-demand ratio is mediated by adenosine which acts as a signal in a
negative feedback system apparently designed to protect the ventricles from excessive increase in O₂ consumption. Furthermore, the data reported here may form the basis for more rational approaches to terminate reentrant tachycardias involving the AV node.

The results of the present study confirm earlier observations in both laboratory animals and humans that AV conduction delay is rate dependent and cumulative. These electrophysiological characteristics are thought to be responsible for the progressive prolongation of AV conduction time to new steady-state values caused by sudden and sustained increases in atrial rate, the so-called "crescendo-like" pattern of AV nodal accommodation phenomenon. Although the studies of Meijler et al. and Heethaar et al. provide detailed analyses and mathematical models of AV node conduction in rat, dog, and human heart during adaptation to stepwise changes in rate of pacing, neither provide a clear understanding of the physiological significance of AV nodal accommodation on cardiac function. Furthermore, a systematic investigation of potential modulators of the AV nodal accommodation phenomenon has not previously been performed.

Characteristics of AV Nodal Accommodation

In the present study, it was possible to establish that the overall increase in AV conduction time during sustained fast atrial pacing has two well-defined components with distinct characteristics. The first component is an initial large prolongation in S-H interval that occurs with the first beat at the new rate and the second component is the subsequent progressive lengthening of the S-H interval to a new steady-state value. These are referred to as the fast and slow components of AV nodal accommodation, respectively. It was found that the time required for the AV node to adapt to the increases in atrial rate varied between 20 and 35 seconds with a t₀.9 of 17 ± 7 msec. This time (20-35 seconds) required for adjustment of the AV nodal conduction to changes in atrial rate is significantly greater than those reported by Meijler et al. for the rat (0.5-1.0 second), dog (1.0-1.5 seconds) and human (1.5-2.0 seconds) heart. These differences could be due to a number of variables, such as a) species, b) the method of stimulation and data acquisition, c) the temperature of the perfusate, in the case of the isolated hearts, and d) the duration of fast pacing. In the present study the fast atrial pacing rate was...
Figure 15. Changes in venous oxygen tension (PvO₂) in isolated perfused guinea pig hearts during 1:1 AV conduction and 2:1 AV block. In record A the AV block was overcome by direct ventricular pacing (V-pacing) and in B by administration of the adenosine antagonist 8-PST. Record A: The rate of atrial pacing was suddenly increased (A-pacing) from 500 msec ("control") to 170 msec ("fast pacing"). The rate of adenosine release (in pmol/min/g) was measured at various times (denoted by arrows) during the procedure and the values are indicated by the numbers in parentheses. Note that after approximately 2.5 minutes of fast pacing, second degree AV block developed and a new steady-state PvO₂ was reached. The AV block was overridden by directly pacing the ventricles at a cycle length of 170 msec (V-pacing). During the V-pacing, the PvO₂ markedly declined and concomitantly the rate of adenosine release increased. The direct ventricular pacing was stopped, atrial pacing (A-pacing) was resumed and hence 2:1 AV block recurred. Record B: The rate of atrial pacing was increased from 500 msec ("control") to 310 msec (FAST PACING). During fast pacing, second degree AV block developed, and after it stabilized, 8-PST (30 μM) was infused. Shortly thereafter, 1:1 conduction was resumed and the PvO₂ began to decrease and the rate of adenosine release increased.

maintained for 60 seconds (single-step protocol) or 30 seconds (multiple-step protocol) at each new cycle length, whereas in other studies the fast pacing rate was maintained either from only 220 msec up to several beats, or from 800 msec to a couple of seconds. In a more recent study in chronically instrumented, unsedated dogs after cardiac transplantation (denervated hearts), it was found that the mean time required to achieve a new steady-state A-H interval after a single step increase in rate for 60 seconds was 41 ± 4 seconds, a value much closer to the one observed in our experiments. Although using a different stimulation protocol than in the present study, Merideth et al reported that the prolongation of AV conduction time in rabbit hearts paced at a cycle length of 156 msec reached a new steady-state nearly 40 seconds after the beginning of fast atrial pacing; a value that, according to the author, was significantly longer than the in situ dog heart. In another study in humans, the length of the A-H interval during right atrial stimulation at 100 and 120 beats/min for periods of 2–5 minutes reached a plateau by 1 minute in most of the patients studied. Unlike laboratory animals, several patterns of response of AV conduction time due to sudden changes in atrial pacing rate have been reported in humans. However, the crescendo-like pattern of AV conduction response, which is consistently observed in hearts from laboratory animals, has also been reported in unsedated humans in virtually 100% of the cases.

Upon return to control conditions (i.e., basal atrial pacing rate) the S-H interval shortens rapidly, attaining baseline values in approximately 30 seconds (t0.9 = 21 ± 3 seconds), which is similar to the time it takes the S-H interval to lengthen when the rate of atrial pacing is increased. This symmetry in the time to reach steady state during both prolongation and shortening of the S-H interval concurs with the results reported by Tuna et al in transplanted (denervated) in situ dog hearts. However, in control dogs (i.e., innervated hearts) there was an asymmetry in the times for the A-H interval to reach steady state during increases and decreases in pacing rate of equal magnitude. Based on these findings, it was postulated that this difference was probably due to asymmetrical neural influences on AV node function.

Modulators of AV Nodal Accommodation

Similar to the present study, some of the previous studies, both in animals and in humans, have found that neither atropine nor propranolol affect the maximum prolongation in A-H interval or the time course of the development of AV nodal accommodation. These observations are somewhat surprising since the AV node is richly innervated and influenced by both divisions of the autonomic nervous system. In fact, in a recent study it was
FIGURE 16. Typical His bundle electrogram (HBE) recordings demonstrating the effects of low perfusion flow and the adenosine antagonist BW-A1433 on AV node conduction in an isolated perfused guinea pig heart. The experimental protocol consisted of a single step up in rate under three different conditions: Panels A-A': Normal flow (8 ml/min); Panels B-B': Low flow (4 ml/min); and Panels C-C': low flow in the presence of 1 μM BW-A1433. When cycle length (CL)=370 msec, there was no difference in the S-H interval between the three conditions (Panels A, B, and C). At CL=158 msec, the S-H interval during low flow alone (Panel B') increased over control by 19 msec whereas in the presence of BW-A1433 (Panel C), the increase in S-H interval was only 2 msec. S, stimulus artifact; A, atrial depolarization; H, His bundle depolarization; and V, ventricular depolarization. Calibration bar in the lower right corner of Panel C applies to all panels.

demonstrated that under basal conditions, AV conduction is predominantly influenced by the parasympathetic activity, which is also the major mediator of the respiratory-related fluctuations in AV conduction time. However, under muscarinic blockade the sympathetic activity was found to modulate AV conduction. Thus, both divisions of the autonomic nervous system can modulate intrinsic AV node function.

The fact that none of the parameters of the AV nodal accommodation phenomenon are altered by blockers of either division of the autonomic nervous system can be interpreted to indicate the AV nodal accommodation is an intrinsic feature of the AV node. However, the above findings do not imply that in the in situ heart neither the sympathetic nor the parasympathetic activity can exert a significant modulatory effect on the AV nodal adaptation to sudden changes in atrial rate.

Like atropine and propranolol, the adenosine antagonist BW-A1433 did not significantly affect any of the parameters of the AV nodal accommodation. Since it is well established that in the guinea pig heart adenosine has marked negative dromotropic effects at concentrations above 1 μM, the results shown in Table I suggest that under those experimental conditions either adenosine is not released in sufficient amounts to influence AV conduction, or unknown factors or conditions impede the nucleoside from exerting its depressant effect on the AV node. However, most of the results of the present study strongly support the view that if adenosine is released in sufficient amounts during fast atrial pacing it can significantly modulate the slow component of AV nodal accommodation.

Role of Adenosine

The negative dromotropic effect of adenosine has been well characterized especially in the guinea pig heart (for review see reference 22). Similar to other actions of adenosine, its depressant effect on the AV node can be competitively and selectively antagonized by alkylxanthines (e.g., BW-A1433) but potentiated by nucleoside transport blockers such as dipyridamole. In addition, strong evidence exists that adenosine plays an important role as mediator of AV conduction disturbances associated with severe hypoxia (P0₂≤50 mm Hg) and ischemia. However, little is known about a) the role of adenosine as modulator of AV nodal conduction under normal physiological conditions, and b) when its rate of formation is regulated by altering the balance between O₂ supply and demand in a controlled fashion, such as reported here.

The results of Table 1 and Figure 4 indicate that endogenously released adenosine may not attain sufficient levels to affect AV conduction time during normoxic conditions (95% O₂) when hearts are paced at atrial cycle lengths between 300 msec and 175 msec. However, based on the results with the
adrenosine antagonist BW-A1433 (Table 2 and Figure 4), at faster atrial pacing rates, that is, near Wenckebach rate (cycle lengths ≤165 msec), endogenously released adenosine may in part determine the conduction delay through the AV node. The results with dipyridamole further substantiate this conclusion. In the presence of dipyridamole there was an overall increase in the concentration of adenosine in the effluent at all rates of atrial pacing, but, more importantly, the increase in effluent levels of adenosine at the atrial cycle length of 170 msec was significantly greater than at lower atrial rates. In addition, dipyridamole significantly increased the Wenckebach cycle length (Table 2). These findings, coupled with observations that BW-A1433 antagonized the dipyridamole-induced rightward shift of the atrial cycle length versus S-H interval relationship (Figure 4), that adenosine deaminase significantly attenuated the effects of dipyridamole on S-H interval at all rates of pacing (Figure 6), and that second degree AV block induced by dipyridamole at fast rates of atrial pacing was reversed (Table 2 and Figure 7) or prevented (Figure 8) by adenosine antagonists, suggests that the increase in AV conduction delay and block caused by dipyridamole is caused by adenosine. Furthermore, these findings indicate that adenosine released during fast atrial pacing (except near Wenckebach rates) does not attain sufficient levels to affect AV conduction because it is rapidly taken up (i.e., removed from its site of action) and that this uptake process (i.e., removal) is prevented by dipyridamole. In keeping with the above findings is the observation that in isolated perfused guinea pig hearts dipyridamole alone causes a dose-dependent increase in coronary venous adenosine release, which is associated with a parallel increase in coronary flow.24 In the same preparation, dipyridamole caused AV block at concentrations ≥1 μM, and both the increase in coronary flow and AV block were prevented by adenosine deaminase and theophylline.24

It is also worth noting that in the absence of dipyridamole (Figure 9), even at the highest rate of atrial pacing, there was no detectable increase in the rate of adenosine released into the venous effluent. Whether this is due to our inability (due to rapid uptake or low assay sensitivity) to measure small changes in adenosine levels is not known. However, the attenuation of the S-H prolongation at fast rates of pacing by BW-A1433, which is a highly selective adenosine receptor blocker,23 raises the possibility that the concentration of adenosine at the site of action was elevated during the fast atrial pacing. On the other hand, consistent with the findings of the present study, Manfredi and Sparks23 found that in anesthetized open-chest dogs, electrical pacing caused an increase in blood flow but not a steady-state increase in adenosine release. Although the results with dipyridamole indicate that AV block ensues when adenosine levels are elevated, they do not imply that adenosine levels in the absence of dipyridamole are sufficient to explain the observed changes in S-H interval during fast rates of atrial pacing.

**Imbalance Between O2 Supply and Demand**

It has now been established that the O2 supply-to-demand ratio is a major determinant of adenosine formation by the heart.9,26 Thus, as originally proposed by Berne,27 the stimulus for production of myocardial adenosine is a decrease in tissue P02 due to an imbalance between O2 supply and demand. Our study strongly supports this concept but it is the first to present compelling evidence that adenosine acts as a feedback signal designed to protect the ventricular myocardium from overwork. Evidence, although indirect, obtained in support of this concept is illustrated in Figures 10–12. That is, in comparing the response to fast atrial pacing, during perfusion with 95% O2 and with 38–54% O2, there was no difference in either the magnitude of the S-H prolongation or in the rate of adenosine release during the initial 20 seconds of the rapid pacing. However, at the end of 1 minute of fast pacing there was a marked difference in both the S-H interval and the rate of adenosine release during normoxia (95% O2) versus mild hypoxia (38–54% O2). The fact that BW-A1433 significantly reduced the S-H prolongation at 38–54% O2 indicates that adenosine was at least in part responsible for the S-H prolongation. Also consistent with the above findings were the observations that the Wenckebach cycle length and the time required for the S-H interval to shorten to baseline upon return to the control rate of pacing were significantly longer at 38–45% than at 95% O2, and that was shortened by BW-A1433 (Table 2 and Figure 12). Furthermore, the fast component of AV nodal accommodation, which was insensitive to adenosine antagonists and dipyridamole, remained unchanged whether the perfusate was equilibrated with 95% O2 or 38–54% O2.

Although the isolated perfused heart model used in the present study is not an isolated working heart preparation, it did respond to changes in rate of pacing with the expected increase in MVO2 and decrease in O2 supply-to-demand ratio. As also expected, when AV block occurred during atrial pacing, MVO2 decreased (Figure 13A) and the O2 supply-to-demand ratio improved (Figure 13B).

If the O2 supply-to-demand ratio is indeed the stimulus for formation of adenosine, one would expect that when AV block occurs, MVO2 should decrease, O2 supply-to-demand ratio should improve, and the rate of adenosine release should decrease. As illustrated in Figure 14, this occurred. That is, during sustained fast atrial pacing just prior to AV block, adenosine levels in the effluent increased by severalfold, but once AV block ensued, ventricular rate decreased and as a consequence MVO2 decreased, O2 supply-to-demand improved, and adenosine levels fell. Under this set of conditions...
AV block during fast atrial rates can be viewed as a protective mechanism from excessive demand on the ventricles when O_2 demand is not matched by O_2 supply. Therefore, we hypothesize that endogenously produced adenosine is a “signal” that is released by the heart to restore the balance between O_2 supply and demand. Moreover, adenosine receptor blockers and adenosine deaminase, by attenuating the effects of adenosine and thereby preventing AV block from occurring, are expected to introduce an “error” to this feedback system. This “error signal” leads to three consequences: 1) MVQO_2 increases; 2) the O_2 supply-to-demand ratio decreases further, and 3) the rate of adenosine (“the signal”) release increases. The results depicted in Figures 14 and 15 confirm the hypothesis that adenosine is a metabolic signal that improves the imbalance between O_2 supply and demand by reducing demand via its depressant action on the AV node.

In contrast to adenosine-receptor blockers and adenosine deaminase, nucleoside transport inhibitors (e.g., dipyridamole) would have the effect of increasing the “gain” of this feedback system by potentiating the action of adenosine. This latter prediction is supported by the observation that in the presence of dipyridamole, fast atrial pacing leads to AV block, which in turn can be prevented and/or reversed by adenosine receptor blockers or adenosine deaminase.

The observation that neither adenosine receptor blockers nor adenosine deaminase shortened the S-H interval at control heart rates indicates that interstitial fluid adenosine levels in the unstressed heart are in the subthreshold region of the concentration-response curve for its negative dromotropic effect. The results with dipyridamole (prolongation of S-H interval and accentuation of AV nodal accommodation) can be explained by the fact that the dose-response curve for the dromotropic effect of adenosine is very steep and, hence, small increases in the interstitial fluid adenosine concentration in the vicinity of AV node cells can result in significant prolongation of S-H interval. In addition, because AV conduction delay is frequency-dependent and the adenosine dose-response curve becomes steeper as the atrial rate increases, the rise in interstitial adenosine concentration required to achieve levels sufficient to cause AV block may be smaller than anticipated. In fact, the amount of adenosine required to produce AV block decreases as heart rate increases. This is consistent with the clinical observation that less adenosine is required to terminate supraventricular tachycardia than to cause AV block during atrial pacing at a rate just above sinus rate. These features make adenosine an effective signal to adjust ventricular oxygen demand in the face of limited supply of oxygen. Whether adenosine plays a more important and broader role in the regulation of cardiac function when the O_2 supply-to-demand ratio is upset cannot be answered by the results of this study. However, based on the fact that the cardiac effects of adenosine lead to either a reduction in O_2 demand or an increase in O_2 supply, it is reasonable to speculate that adenosine can potentially restore the balance of O_2 supply and demand in a unique fashion by increasing supply (coronary vasodilation) and decreasing demand by slowing heart rate, producing AV block, and antagonizing the stimulatory (inotropic and arrhythmogenic) effects of catecholamines.

The majority of actions of adenosine are mediated by activation of two distinct types of cell surface receptors, the A_1 and A_2 subtypes. Activation of A_1 receptors invariably leads to a decrease in oxygen demand whereas activation of A_2 causes an increase in blood flow and, hence, O_2 supply. For example, activation of A_1 receptors results in slowing of heart rate, AV block, decrease in locomotor activity, inhibition of neuronal firing, and inhibition of lipolysis. On the other hand, activation of A_2 receptors causes inhibition of platelet aggregation and vasodilation, the exception being renal arterioles. Based on the above and on the fact that adenosine formation is stimulated in response to the energy state of the cell, Newby et al proposed that adenosine functions in many organs (including the heart) as a “retaliatory metabolite” that protects the cells from excessive demand from external stimulation. The present study is a direct test to this hypothesis, and the results overwhelmingly support the role of adenosine as a “protective” metabolite. Furthermore, our findings demonstrate that adenosine functions as a signal in a negative feedback system that can be modified in a predictable manner by known modulators of its actions. However, it should be pointed out that because the effects of adenosine during normoxia were only evident at fast rates of stimulation, it remains to be determined whether this “protective” action of adenosine from overwork would be elicited during ischemia at atrial rates below the flutter and/or fibrillation range.

Clinical Implications

The results of this study indicate that irrespective of whether O_2 supply is limited by lowering the perfusate PO_2 or, as illustrated in Figure 15, by reducing perfusion flow (ischemia), adenosine plays a major role in the A-H interval prolongation and AV block induced by fast atrial pacing. The example shown in Figure 15 demonstrates that as long as O_2 supply is sufficient to meet the demand, the interstitial concentration of adenosine remains below its effective concentration but when an imbalance between O_2 supply and demand is created by increasing the O_2 demand (i.e., by fast atrial pacing), adenosine is released in sufficient amounts to depress AV node conduction. This condition is analogous to clinical situations in which a critical coronary stenosis causes no symptoms or cardiac dysfunction as long as resting conditions prevail but would appear when O_2 demand increases (e.g., during exercise).
In fact, administration of atropine to patients with type I AV block (e.g., in the case of inferior myocardial infarction) can increase the degree of AV conduction delay and block as a result of the faster atrial rate, which is consistent with what has been observed in isolated perfused guinea pig hearts during hypoxia. Based on the results of the present study, restoration of 1:1 AV conduction may not always be beneficial because the AV block can be a "protective" mechanism for the ventricles from excessive demand when O2 supply is limited. In a recent case report by Wesley et al., it was noted that in the setting of inferior myocardial infarction, reversal of 2:1 AV block by aminophylline (an adenosine antagonist) to 1:1 AV conduction was accompanied by an increase of approximately 0.5 mm in ST segment elevation (Figure 1 in reference 39). Although speculative, it is conceivable that similar to the experiments of the present study, administration of aminophylline and restoration of 1:1 AV conduction in the patient of the abovementioned case report had the effect of introducing an "error signal" that led to an increased O2 demand by increasing ventricular rate and, hence, causing elevation of the ST segment.

Another potential implication of the results reported here is derived from the experiments with dipyridamole, namely those illustrated in Figures 7 and 8. Adenosine is known to be highly effective in terminating supraventricular tachycardias in which the AV node is part of the reentrant circuit. As illustrated in Figure 7, in the presence of dipyridamole, fast atrial pacing led to AV block, which was promptly reversed by the adenosine antagonist BW-A1433. If, however, this was a case of AV node reentrant tachycardia (instead of fast atrial pacing), dipyridamole would have terminated the tachycardia by causing AV block. It is interesting to note that at baseline atrial rate (Figure 7, first strip in Panel B) dipyridamole did not cause a significant prolongation in S-H interval. Furthermore, from the results of Figure 14, it is expected that when AV block occurs and the tachycardia is terminated, adenosine concentration will fall and, thus, normal or quasi-normal AV conduction delay will be restored. On the other hand, when the tachycardia starts, adenosine levels are below the concentration necessary to affect AV conduction; but in the presence of dipyridamole, within a few seconds of the onset of the tachycardia (depending on the rate), the adenosine concentration would increase and, hence, cause AV block, and terminate the tachycardia. The above hypothetical situation is quite similar to the experiment illustrated in Figure 8. Finally, one could also question whether spontaneous termination of AV node reentrant tachycardia under certain conditions could be due to an interstitial concentration of adenosine in the AV nodal area.

Direct extrapolation of the experimental results of the present study to clinical situations cannot be made. In our laboratory model, we can only study the intrinsic rate-dependent properties of the AV node, which are independent of neural influences but still subject to metabolic factors. In addition, one might expect that in blood-perfused intact preparations and in the clinical setting dipyridamole would cause a drop in systemic blood pressure and, hence, trigger neural reflexes such as an increase in sympathetic activity and/or vagal withdrawal. Thus, rather than terminating the AV reentrant tachycardia, dipyridamole might be expected to either speed up the tachycardia or have no effect. However, the fact that adenosine, xanthine derivatives, and dipyridamole have been shown to have the same pharmacological effects in the human AV node as the ones described in the present study give credence and relevancy to the above implications.

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