Atrioventricular Conduction Disturbances during Hypoxia

Possible Role of Adenosine in Rabbit and Guinea Pig Heart

LUIZ BELARDINELLI, FRANCIS L. BELLONI, RAFAEL RUBIO, AND ROBERT M. BERNE

SUMMARY Adenosine and related compounds can produce atrioventricular (A-V) conduction block. Similar conduction disturbances are observed in myocardial hypoxia. To investigate the possibility that adenosine might be causally involved in hypoxic conduction disturbances, we measured A-V conduction times, subdivided into atrial-to-His bundle (A-H) and His bundle-to-ventricular (H-V) intervals, with extracellular electrodes in isolated rabbit and guinea pig hearts perfused with modified Krebs-Henseleit solution. Adenosine produced dose-dependent prolongation of A-V conduction time in both species, although guinea pig hearts responded to lower doses (10⁻⁷ M) and showed a steeper dose-response relationship than rabbit hearts. Higher adenosine doses produced second-degree heart block in both species. Conduction delay was confined to the A-H interval, implicating action on A-V node cells. Further investigation of guinea pig hearts revealed a specific antagonism towards adenosine's effects by 10⁻⁶ M aminophylline. Conduction disturbances produced by acetylcholine or MnCl₂ were unaffected by aminophylline as were adenosine's effects by atropine. Perfusion with hypoxic perfusate caused A-V conduction delays and second-degree block in guinea pig hearts. This effect was dramatically attenuated by aminophylline. We conclude that endogenously released adenosine may cause at least some of the A-V conduction disturbances associated with acute myocardial hypoxia. Furthermore, methylxanthines may prove to be of therapeutic value in combatting such disturbances in a clinical setting. Circ Res 47: 684-691, 1980

AS EARLY as 1929 it was observed that adenosine and related compounds can cause heart block (Drury and Szent-Gyorgyi, 1929). Stafford (1966) reported that adenosine and adenine nucleotides produce dose-dependent atrioventricular conduction block (A-V block) in guinea pig hearts. Adenosine is also known to depress Ca²⁺-mediated action potentials in mammalian atria (Schrader et al., 1975; Belardinelli et al., 1979), and recent observations by Rubio et al. (1979) suggest that the A-V block caused by adenosine is accompanied by an inhibition or blockade of the "slow," Ca²⁺-Na⁺-mediated action potentials in the A-V node cells (Paes de Carvalho et al., 1969; Zipes and Mendez, 1973; Akiyama and Fozzard, 1979). This inhibitory effect of adenosine on the action potential of nodal cells could explain the A-V block previously reported.

During ischemia, hypoxia, and asphyxia, A-V conduction is impaired (Zumino et al., 1970; Bagdonas et al., 1961; Senges et al., 1979; Alanis et al., 1959). Furthermore, it has been shown that AV node action potentials are depressed by hypoxia and, concomitantly, the atria-to-His bundle conduction time is markedly increased (Senges et al., 1979). With inadequate oxygenation of the myocardium, the adenosine levels in atrial (Thomas et al., 1975) and ventricular tissue have been found to be elevated significantly (Rubio and Berne, 1969; Rubio et al., 1974). Hence, it is possible that adenosine mediates some of the A-V node conduction disturbances observed during hypoxia, and investigation of this hypothesis constitutes the goal of the present study.

Methods

General

Experiments were carried out using isolated, perfused rabbit and guinea pig hearts. Adult rabbits of either sex (New Zealand White), weighing 6–8 kg, were anesthetized with sodium pentobarbital (25 mg/kg, iv), whereas guinea pigs of either sex (Harley), weighing 450–700 g, were stunned by a blow to the head. The hearts were removed rapidly and rinsed with ice cold Ringer's solution. Retrograde aortic perfusion at a constant flow (Gilson pump, Minipuls-2) of 3–5 ml/min per g was initiated immediately. In all experiments the non-recirculating perfusion fluid was modified Krebs-Henseleit solution (pH 7.4) with the following composition
tations were gassed with 95% O₂ + 5% CO₂ and the physiological stimulator (Grass model S-4) provided filled with Krebs-Henseleit solution and positioned in such a way that it was completely immersed. The heart was placed in a bath region was excised. The heart was placed in a bath and positioned at 34° ± 1°C.

KH₂PO₄, 1.18; NaHCO₃, 25; glucose, 11. The solution for the His bundle electrogram (HBE) consisted of cristal terminalis and left ventricle. The electrode coated, stainless steel wires (o.d., 0.0045") on the position of the HBE electrode until a discernible stimuli through a stimulus isolation unit as illustrated in Figs. 4 and 5). The bipolar electrograms were obtained by connecting these two electrodes to a differential amplifier (Tektronix model 2A61). The signals from the right atrial electrogram (RAE) and HBE were amplified and displayed on a dual-beam oscilloscope (Tektronix model 302) and recorded on a strip-chart recorder (Gould-Brush model 220). Oscilloscope signals were displayed at sweep speeds of 10 to 50 msec/cm and photographed with a Kymographic camera (Grass). Stray chart recordings were obtained at a paper speed of 125 mm/sec.

Measurements

On the right atrial and His bundle electrograms the stimulus artifact, the onsets of atrial (A) and ventricular (V) depolarization and the His spike (H) were identified. From these features, we measured (1) cycle length, defined as the interval (S-N) between the stimulus artifact, the onsets of atrial (A) and ventricular (V) depolarization and the His spike (H); (2) A-H interval, which represents the conduction time from the atrial tissue to the bundle of His; (3) H-V interval, which represents the conduction time from the bundle of His to the ventricular tissue; and (4) A-V interval, which represents the atrial to ventricular conduction time (A-VCT). These measurements are expressed in milliseconds.

Protocols

After securing the electrodes, we waited 30 minutes before beginning control measurements. Control measurements preceded and followed all experimental interventions. When total A-V conduction time in the pre- and post-control differed by more than 10%, the intervening experimental data were discarded. Since A-V node conduction is very dependent upon cycle length, control and experimental measurements were made at similar cycle lengths. Furthermore, the effects of each experimental maneuver were observed at more than one cycle length.

Effects of Adenosine

Adenosine prolonged A-V conduction time in isolated perfused rabbit hearts. In these experiments, displayed in Figures 1 and 2, the isolated heart preparation was paced at several different rates during a control (no infusion) period. Adeno-
Adenosine was then infused to achieve a perfusion fluid concentration of $10^{-7}$, $10^{-6}$, $10^{-5}$, or $10^{-4}$ M and the paced rates were repeated. Finally, a post-control series of measurements was made. As seen in Figure 2, adenosine prolonged the A-H interval without changing the H-V interval. Figure 1 summarizes the data from 20 rabbit heart preparations. No effect of adenosine was seen at $10^{-7}$ M, but the higher doses clearly prolonged the A-V conduction time. Statistical analysis was hampered somewhat by small numbers of replicate points at certain cycle lengths. The trend of the data was clear, however, as 150 of 155 trials at the three highest doses showed prolonged A-V conduction time with adenosine. Another statistical problem was caused by the tendency of $10^{-5}$ and $10^{-4}$ M adenosine to cause second-degree heart block at the higher heart rates in some preparations (11 trials indicated by crosses in Fig. 2). Although this made quantification difficult, it clearly strengthens the conclusion that adenosine prolongs A-V conduction in isolated rabbit hearts.

Figure 1 also demonstrates the tendency for A-V conduction time to increase with heart rate, i.e., as cycle length decreases. This is a well-known effect (Merideth et al., 1968). We subdivided the A-V conduction time into its A-H and H-V intervals in 14 of the 20 rabbit hearts in this series (i.e., those in which we obtained a His electrogram). The H-V interval averaged $25 \pm 1$ msec for these hearts and showed no dependence on cycle length. Therefore, all the changes in A-V conduction time as a function of cycle length are accounted for by changes in the A-H interval. In addition to showing that the adenosine-induced changes in A-V conduction time were due solely to changes in the A-H interval, Figure 2 also indicates a significant concentration-dependence of this adenosine effect. The percentage increments in A-V conduction time showed only very weak dependence on cycle length for any adenosine dose. Linear regression analysis of these percent increment values against cycle length yielded low $r^2$ values (0.05 – 0.46) and shallow slopes (1–3% per 100 msec cycle length) for the groups of data for the three highest adenosine concentrations. We, therefore, pooled these values for each dose. Cases in which second-degree heart block was induced by adenosine were not included in these calculations, leading to some underestimation of the effects of the high adenosine concentrations. The lowest adenosine concentration ($10^{-7}$ M) caused a $1 \pm 1\%$ (mean $\pm$ SEM, $n = 23$ paired comparisons) increase in A-V conduction time. This was not significantly different from no effect. But, adenosine at $10^{-6}$ M caused an $8 \pm 1\%$ ($n = 52$) increase in A-V conduction time; adenosine at $10^{-5}$ M caused a $21 \pm 2\%$ ($n = 59$) increase; adenosine at $10^{-4}$ M caused a $31 \pm 2\%$ ($n = 44$) increase. These values were all significantly different from zero and from each other [analysis of variance for one-way classification by adenosine dose (Snedecor and Cochran, 1967)].

Adenosine also prolonged A-V conduction time.
and A-H interval in isolated, perfused guinea pig hearts (Fig. 3). A protocol similar to that used for rabbit hearts was followed. A-H and H-V intervals, as well as A-V conduction time, were measured for each preparation. The threshold for effects in the guinea pig was lower, with 1.2 X 10^-7 M adenosine causing a 4 ± 1% increase in A-V conduction time in this species (n = 20 paired comparisons from five hearts, all cycle lengths, different from zero at P < 0.001 level) compared to the statistically insignificant change of 1 ± 1% seen with 1.3 X 10^-7 M adenosine in the rabbit hearts. Furthermore, the dose-response relationship appeared to be much steeper in guinea pig hearts. Adenosine at 1.2 X 10^-6 M caused a 31 ± 4% (n = 9 paired comparisons from four hearts, all cycle lengths) increase in A-V conduction time in this species as compared to only an 8 ± 1% increase at a similar concentration in rabbit hearts. Furthermore, adenosine at 5 X 10^-6 M produced second-degree heart block in 17 of 17 trials with guinea pig hearts (cycle lengths = 260-330 msec), and 1.2 X 10^-5 M adenosine produced complete A-V block (A-V dissociation) in each of eight guinea pig hearts (not shown). Comparable concentrations in rabbit hearts produced only relatively mild A-V conduction delay or, at most, occasional second-degree block at high heart rates (Fig. 1).

In the guinea pig heart, as in the rabbit heart (Fig. 2), both the effects of adenosine and the conduction changes with cycle length were confined to the A-H interval (Fig. 3), whereas the H-V interval remained unaltered at 14 ± 1 msec (n = the 7 hearts represented in Figure 3). Our conclusions about the relative threshold and dose-response steepness for the two species are equally as valid for A-H interval as for A-V conduction time although, of course, the percentages calculated are slightly higher for A-H intervals.

**Effect of Aminophylline on Adenosine-Induced Changes in Conduction**

Figure 4 shows an electrogram from an experiment in which 10^-6 M adenosine induced second-degree heart block (4:3, panel B) in a guinea pig heart. Two minutes after the initiation of an aminophylline (10^-5 M) infusion (panel C), in the continued presence of adenosine, the heart block was eliminated and the A-H interval was only 16% greater than the control value (panel C vs. panel A).

This pattern was seen in eight hearts at cycle lengths between 176 and 332 msec. In other experiments, for example the one summarized in Table 1, adenosine did not cause second-degree heart block at certain cycle lengths, but only prolonged the A-H and A-V intervals. In three experiments of this type, involving several different pacing rates, 10^-5 M aminophylline reduced the 10^-6 M adenosine-induced increment in A-H interval by 65-100%. Aminophylline by itself had no significant effect on A-V conduction (Figs. 5 and 6).

Aminophylline’s antagonistic action appears to be relatively specific for adenosine. Acetylcholine and manganese chloride were administered as infusions to several guinea pig heart preparations. The concentrations of these agents were adjusted to achieve A-H interval prolongation similar in magnitude to that caused by adenosine at comparable pace rates (Table 1, one experiment shown). Aminophylline (2 X 10^-5 M) blocked or greatly attenuated the adenosine responses, as already discussed, but was without effect on the responses to acetylcholine or manganese chloride. Atropine (10^-6 M), however, eliminated the effects of acetylcholine while exerting no apparent antagonism against adenosine. Qualitatively similar effects of atropine and aminophylline on responses induced by adenosine, acetylcholine, and manganese chloride were obtained from a total of three preparations.

**Effects of Hypoxia**

Hypoxia was induced in guinea pig heart preparations by switching from an oxygenated to an hypoxic perfusion fluid. As shown in the top panels of Figure 5, the A-H interval was prolonged and second-degree heart block eventually ensued. There was no measurable significant change in the H-V...
FIGURE 4 Typical His bundle electrograms showing aminophylline antagonism of adenosine-induced conduction disturbances in a guinea pig heart. Panel A is a control trace. Panel B is a trace obtained in the presence of $10^{-5}$ M adenosine. Panel C is a trace obtained 2 minutes after the introduction of $10^{-5}$ M aminophylline in the continued presence of $10^{-5}$ M adenosine. The cycle length was 308 msec for all three traces. St = stimulus artifact. A, H, and V denote atrial, His bundle, and ventricular depolarization, respectively. The atrial depolarization is partially hidden by the stimulus artifact. The numbers represent the A-H interval in msec. Note that adenosine caused a second-degree (4:3) heart block (panel B) and that aminophylline reversed this (panel C).

interval. Aminophylline, at a concentration ($2.5 \times 10^{-5}$ M) which sharply attenuated the A-V nodal conduction effects of adenosine (Fig. 4; Table 1), also decreased the prolongation of conduction induced by hypoxia (Fig. 5, bottom panels). These experiments are summarized in Figure 6. Two control runs, consisting of 5 minutes of hypoxic perfusion, were sandwiched around a hypoxic perfusion in the presence of $2.5 \times 10^{-5}$ M aminophylline. Thirty minutes of normoxic perfusion intervened between hypoxic periods. Note in Figure 6, summarizing those experiments performed at a pace rate of $\sim 3$ Hz, that recovery was complete after each hypoxic period and that the initial A-H intervals were approximately equal in all three runs. The first effects of hypoxia on the A-H interval were noticed about 2.5–3 minutes after perfusates were changed. The minimal transit time of our system, as measured by appearance of trypan blue at the aortic cannula tip after switching from a colorless to a trypan blue-containing perfusion fluid, was $\sim 1.5$ minutes. The trypan blue concentration at the cannula tip reached a plateau at $\sim 3.2$ minutes after the change of perfusion fluids. Therefore, much of the delay in onset of the effects of hypoxia can be attributed to the delay in onset of the hypoxic

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fills symbols signify that one or more preparations had fused from 3 minutes before the second hypoxic period; hearts is plotted. X signifies that all hearts had developed second-degree heart block by that time, in which case the average A-H interval of the remaining hearts is plotted. X signifies that all hearts had developed second-degree block. Measurements were made every 30 seconds. n = number of preparations. CL = cycle length.

**Figure 6** Average time course of A-H interval changes during hypoxic perfusion and during recovery. Control, aminophylline, and post-control runs were performed in order, with no intervening time. Aminophylline was infused from 3 minutes before the second hypoxic period until 5 minutes of the subsequent recovery period. Half-filled symbols signify that one or more preparations had developed second-degree heart block by that time, in which case the average A-H interval of the remaining hearts is plotted. X signifies that all hearts had developed second-degree block. Measurements were made every 30 seconds. n = number of preparations. CL = cycle length.

The appearance of second-degree heart block was more common and occurred sooner with the higher rate. In some cases, the post-control run displayed some deterioration of the preparation, as noted by an incomplete recovery. Nonetheless, the same pattern of amelioration by aminophylline was observed.

**Discussion**

The results presented here confirm the previous observations (Drury and Szent-Györgyi 1929; Stafford, 1966; Schrader et al., 1977a) that adenosine induces A-V node conduction delay and block. An additional finding is that the effects of adenosine on A-V node conduction are due to increased conduction delay between the atria and His bundle, i.e., an increased A-H interval. In contrast, the H-V interval is not affected by adenosine even at high concentrations (10^-4 M). These new findings are consistent with the idea that adenosine depresses slow channel-mediated action potentials (atria and/or A-V node) whereas fast channel-mediated action potentials are not affected (Schrader et al., 1975; Belardinelli et al., 1979). The selectivity of adenosine's action on the A-V node is similar to that of other slow-channel blockers, such as MnCl2 and verapamil (Benitez et al., 1973; Zipes and Mendez, 1973). In comparison to the effects of MnCl2 and verapamil (Kohlhardt et al., 1973), however, the effects of adenosine have a rapid onset (<1 min) and can be reversed rapidly on washout.

Guinea pig hearts were more sensitive to the effects of adenosine than rabbit hearts, i.e., the threshold dose was lower and A-V block was seen more frequently in guinea pig hearts. Species differences with respect to sensitivity to the effects of adenosine on A-V node conduction have been reported previously (Drury and Szent-Györgyi, 1929). The basis for these differences is not understood.

Temperature can modify the action of adenosine on A-V conduction. Drury and Szent-Györgyi (1929) noted a decreased sensitivity of the guinea pig heart (induction of A-V block) to adenosine at higher temperatures. Our pilot experiments at 37°C also showed a right-shifted adenosine dose-response curve compared to our data, presented here, obtained at 34°C. We chose the lower temperature because of the superior preparation stability it provided. Even at 37°C, however, we obtained complete A-V block in the guinea pig heart with 10^-5 M adenosine (three experiments). As noted below, hypoxic myocardial adenosine levels can approach this concentration. Therefore, our basic conclusion is not altered because of our use of a low temperature.

In our study the A-H interval prolongation induced by adenosine was blocked by 10^-5 M aminophylline, and this antagonism was specific, inasmuch as similar acetylcholine- and MnCl2-induced effects were not blocked by aminophylline. These
findings are consistent with the notion that adenosine is competitively inhibited by theophylline (Bünger et al., 1975), and probably affects the A-V node conducting system by acting on the plasma membrane, as has been demonstrated for other tissues using adenosine that had been complexed to high molecular weight substances (Olsson et al., 1976; Schrader et al., 1977b; Hartzell, 1979).

Our working hypothesis is that there are at least three mechanisms whereby the A-V node action potential can be depressed. Manganese appears to have a direct effect on the slow channel (Vitek and Trautwein, 1971; Kohlhardt et al., 1973), whereas adenosine and acetylcholine may act by either inhibiting the slow channel (Schrader et al., 1975; Belardinelli et al., 1979; Ten Eick et al., 1976) or by increasing the outward potassium current (De Gabareff and Sleator, 1965; Paes de Carvalho et al., 1969; Ten Eick et al., 1976; Hartzell, 1979).

Methylxanthines are known to inhibit phosphodiesterase activity and consequently increase cellular cyclic AMP (cAMP) (Chasin and Harris, 1976; Watanabe and Besch, 1974). There is also evidence that increased cAMP is associated with enhancement of the inward calcium current and also the slow action potential (Watanabe and Besch, 1974; Scholz and Reuter, 1976). Thus, if aminophylline were to induce the formation of slow channels or increase the conductivity of the existing slow channels (Schneider and Sperelakis, 1975) by its inhibitory action on phosphodiesterase activity, the effects of adenosine, acetylcholine, and Mn⁺⁺ on the A-V node action potential each should have been counteracted. Since aminophylline antagonized only the effects of adenosine, however, its action cannot be explained on the basis of such a final common mechanism. Rather, selective interference with the actions of adenosine, probably at the level of a membrane receptor, seems the most likely interpretation.

The concentration of methylxanthines which are effective in inhibiting phosphodiesterase are on the order of 10⁻⁴ to 10⁻³ M (Butcher and Sutherland, 1962). These are the concentrations used to mimic the cardiac electrophysiological effects of epinephrine (Tsien et al., 1972; Tsien, 1974), which are thought to be mediated via changes in intracellular cAMP concentration (Tsien, 1973; Tsien, 1974, Yamasaki et al., 1974). Furthermore, the inhibition of phosphodiesterase takes several minutes to develop, presumably to allow penetration of the methylxanthine into the cell. In our study, we used concentrations of aminophylline (1 to 5 × 10⁻⁵ M) below those effective for phosphodiesterase inhibition. We saw no electrophysiological effects of aminophylline itself at this concentration (Fig. 6). Finally, aminophylline's adenosine antagonism developed very quickly and faded rapidly upon removal of the aminophylline. All these facts lead us to conclude that aminophylline's antagonism toward adenosine in this study is not mediated via phosphodiesterase inhibition.

It is well established that myocardial tissue is capable of producing adenosine under ischemic or hypoxic conditions (Rubio et al., 1974; Thomas et al., 1975). Ischemia and hypoxia are also known to produce A-V node conduction disturbances. Senges et al. (1979) recently reported that A-V node action potentials are depressed by hypoxia concomitantly with a marked increase in atrial-to-His bundle conduction time. Thus, a causal relationship between the inhibition of the nodal action potential and impairment of the A-V node conduction may exist. Adenosine depresses the A-V node action potential and increases the atrial-to-His bundle conduction time in a manner similar to that observed with hypoxia. Considering the similarities between the effects of hypoxia and those of adenosine upon the A-V node, it is reasonable to postulate a causal role for adenosine in the A-V node conduction disturbances seen during hypoxia. Aminophylline appears to be a potent and specific antagonist of adenosine's actions. Thus, if the effects of hypoxia on the A-V node are mediated by endogenous adenosine, aminophylline should attenuate them. The results presented in Figures 5 and 6 clearly illustrate that aminophylline does attenuate the A-V node conduction delay and second-degree heart block which arise during hypoxic perfusion. This finding supports a causal role for adenosine in the A-V node conduction disturbances seen during hypoxia.

We would also predict a role for adenosine in causing conduction defects in slow channel-dependent tissues during myocardial ischemia. Ischemia also causes conduction defects in fast channel-dependent tissues (Cranefield, 1975), but these are probably not due to adenosine which does not appear to affect fast channels.

Any substance proposed to cause hypoxic A-V node conduction disturbances must meet certain criteria: (1) the substance should be produced during hypoxia and attain a concentration sufficient to account for its effects, (2) the substance should increase in concentration at the site of action with a sufficiently fast time course to account for the effects of hypoxia, (3) the substance should mimic the effects of hypoxia, and (4) agents known to attenuate or potentiate the action of the substance should, respectively, reduce or enhance the effect of hypoxia. We have presented evidence showing that adenosine appears to fulfill at least the last two of these criteria. Analysis of the first two criteria is more speculative. Literature values for hypoxic adenosine levels vary with species, severity of hypoxia, and site of sampling (Thomas et al., 1975; Rubio et al., 1974). From the values reported, we would anticipate a myocardial adenosine level, after 5 minutes of hypoxic perfusion, to be high enough to yield an interstitial concentration of about 10⁻⁵ to 10⁻⁴ M. In our guinea pig hearts, after 5 minutes of hypoxia, marked increases in A-V node conduction delay or block were observed. Adenosine at 10⁻⁵ M could account for these changes in guinea pig heart. However, it is not known whether the A-
V node cells produce adenosine in response to hypoxia or whether the adenosine released by atrial or ventricular cells could be responsible for the observed effects. Further investigations concerning the adenosine concentrations in the A-V node tissue are required.

If the prolongation of A-V conduction and the production of heart block by myocardial hypoxia is indeed mediated, at least in part, by adenosine, then methylxanthines like aminophylline or theophylline may be of therapeutic value in combating the A-V conduction disturbances observed in clinical cases of myocardial hypoxia or ischemia.

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