Transient increase in release of adenosine during rapid cardiac pacing; transient effects on overdrive suppression of ventricular automaticity

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SUMMARY A study was designed to test the hypothesis that endogenous adenosine concentration may increase during episodes of rapid ventricular pacing and, by virtue of its negative chronotropic effects, contribute to the transient suppression of automaticity that follows the period of overdrive. Isolated, perfused, rat ventricular preparations were subjected to periods of 6.0 Hz overdrive stimulation while adenosine release, oxygen consumption, and subsequent suppression of automaticity were measured. At the end of a 1 min episode of overdrive oxygen consumption and adenosine release were significantly increased, and the initial beating rate after 1 min overdrive was suppressed. At the end of 10 min overdrive oxygen consumption was still increased but adenosine release had returned to control values. Suppression of automaticity after 10 min overdrive was similar to that after 1 min overdrive. The relative magnitude of suppression after 1 min overdrive was decreased by theophylline (10^-4 mol·litre^-1), and increased by the adenosine deaminase inhibitor, EHNA (10^-5 mol·litre^-1). Neither theophylline nor EHNA had any discernible influence on suppression after 10 min overdrive. It is therefore concluded that endogenous adenosine may contribute to the suppression of ventricular automaticity that follows a 1 min episode of overdrive, but because of the transient nature of the increase in adenosine during overdrive endogenous adenosine does not contribute to the suppression that follows prolonged overdrive.

Brief episodes of tachycardia often produce transient decreases in the subsequent heart rate. This phenomenon of overdrive suppression has been shown to occur with atrial as well as with ventricular pacemakers both in vivo and in vitro.1,2 Mechanisms considered responsible for overdrive suppression of the various cardiac pacemakers include transient imbalances in the autonomic neural input as well as local factors, such as changes in extracellular potassium ion concentration, alterations in the electrogenic sodium-potassium pump activity, and changes in calcium ion distribution.3,7 Reports of the depressive effect of exogenous adenosine on atrial and ventricular automaticity8-12 lead to the speculation that endogenous adenosine produced during the overdrive might also affect the subsequent regulation of pacemaker activity. Because ventricular pacemakers have been shown to be more sensitive to adenosine than atrial pacemakers12 and because ventricular adenosine concentrations may be greater than atrial concentrations owing to differences in workload13,14 it is possible that altered adenosine concentrations could be especially important in the overdrive suppression of ventricular pacemakers. Recent work by Wesley and Belardinelli supports this suggestion.15

To test this hypothesis isolated Langendorff perfused rat ventricular preparations were used to obtain information about adenosine's possible...
overdrive stimulation. The direct negative chronotropic effect of exogenous adenosine on this preparation has been previously described.1,2 Two specific questions were addressed in the present study. (a) Does endogenous adenosine release into the venous effluent increase during an episode of rapid overdrive and, if so, is the time course of the recovery to control values after the overdrive similar to the disappearance of the overdrive suppression of pacemaker activity? (b) Do agents that have the potential to either augment or depress the endogenous adenosine concentration (or its negative chronotropic effectiveness) produce a concomitant potentiation or reduction, respectively, in the magnitude of the overdrive suppression?

Material and methods

ISOLATED VENTRICULAR PREPARATION

Adult male Sprague-Dawley rats (250-350 g) anaesthetised with pentobarbital sodium (50 mg·kg⁻¹ ip) and pretreated with heparin (2.5 mg ip) were used for these experiments (n=89). Some of the details of the isolated ventricular preparations have been reported previously.10 Hearts were rapidly removed, pretreated with heparin (2.5 mg ip) and pretreated with heparin (2.5 mg ip) and placed in ice-cold Langendorff solution containing (mmol·litre⁻¹): NaCl 118, KCl 4.7, NaHCO₃ 25.0, CaCl₂ 3.0, MgSO₄ 1.2, KH₂PO₄ 1.2, glucose 10.0, and pH 7.2. Sodium-EDTA (0.5 mmol·litre⁻¹), added to chelate trace amounts of heavy metals, reduced the effective free calcium concentration to 2.5 mmol·litre⁻¹. The solution also contained insulin (10 U·litre⁻¹) and sodium heparin (100 U·litre⁻¹).

The perfusate flow rate was set at approximately 12 ml·min⁻¹·g⁻¹ of total heart weight and was maintained at constant flow (Gilson Volumetric Minipuls 2 pump) for the remainder of the experiment. Perfusion at higher rates did not increase the maximum ventricular systolic pressure production. The perfusion pressure was continually measured from a sidearm located at heart level in the perfusion line. For most of the studies atrial tissue was trimmed from the heart, and the upper 1-2 mm of the interventricular septum was crushed. This procedure removed or destroyed the tissue known to contain atrioventricular nodal cells.10 The spontaneous beating rate was significantly reduced, but the coronary perfusion pressure was not altered. A temperature probe was placed in the right ventricle via the pulmonary outflow tract was used for obtaining periodic anaerobic samples of coronary effluent for oxygen tension determination.

A fluid filled balloon was inserted through the mitral valve into the left ventricle and used to obtain a continuous record of left ventricular pressure. A small piece of polyethylene tubing was placed through a puncture wound in the apex of the left ventricle to allow drainage of any fluid that might accumulate in the left ventricle outside the balloon. The volume of the balloon (and therefore left ventricular volume) was adjusted so as to achieve the end diastolic pressure that produced maximum systolic pressure. Details of this monitoring system have been previously described.17 Except for preparations in which coronary effluent was being collected for adenosine analysis hearts were submerged in a 10 ml bath. Timed collections of overflow were used to check the perfusate flow rate. Leads were attached to the stainless steel aortic cannula and a fixed stainless steel plate electrode was located in the bottom of the bath about 2 mm from the apex of the heart. These electrodes were used for obtaining electrocardiograms of the spontaneously beating heart. Bipolar stimulating electrodes, placed on the surface of the right ventricle, were used to pace the hearts (Grass S88 stimulator). Continuous recordings of left ventricular pressure, aortic perfusion pressure, electrocardiogram, and ventricular pressure were made on a Brush 440 recorder.

MEASUREMENT OF ADENOSINE CONCENTRATION

In 30 of the preparations total coronary effluent samples were collected for intervals of 15 or 20 s before, during, and after the period of overdrive stimulation. Samples were frozen and sent in dry ice to East Lansing, Michigan. Adenosine content was determined using methods previously reported.18 After storage at −90°C for no longer than five days samples were evaporated and redissolved in 400 µl distilled water. The undissolved salts were separated by centrifugation and the supernatant assayed for adenosine by high performance liquid chromatography. Two pumps were programmed for gradient elution of 200 µl samples injected directly on to a reverse phase, 5 µm C-18 column (Beckman). A linear gradient at a flow of 1.1 ml·min⁻¹ over 25 min was used, with initial conditions of 90% 4 mmol·litre⁻¹ KH₂PO₄ and 10% 70/30 methanol/water (v/v). Final conditions were 70% 4 mmol·litre⁻¹ KH₂PO₄ and 30% 70/30 methanol/water. Absorbance of the column eluate was continuously monitored at 254 nm and recorded. Peaks were identified by comparison with retention times of external standards. Adenosine was quantitated by determining the area under the optical density peak and comparing it with the standard. This

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procedure resulted in >90% recovery of adenosine when known quantities were added to samples of coronary effluent in Duluth. Adenosine release (pmol min⁻¹ g⁻¹ wet weight) is defined as the product of the coronary flow and the coronary effluent adenosine concentration.

**PROTOCOL**

After a 45 min equilibration period control values were obtained and an overdrive stimulation of 6.0 Hz applied for either 1 or 10 min. These stimulation conditions were chosen after initial experiments indicated that (a) higher frequencies produced fused contractions and (b) the 10 min train produced a subsequent suppression of automaticity that was similar in magnitude to that produced by the 1 min train. In preparations in which the episodes of overdrive stimulation were repeated periods of at least 15 min were interposed between episodes to allow re-equilibration to occur.

The immediate postoverdrive beating rate was obtained by determining the mean cycle length of the first five beats after the cessation of the stimulation. This method of analysis was chosen to lessen the influence of errors caused by occasional extra beats triggered by the overdrive or irregular rhythms due to ectopic pacemaker firing or re-entry phenomena. Such arrhythmias occurred in 14 of the 89 (about 16%) preparations. This method tended to underestimate the magnitude of the initial pacemaker suppression but did allow us to include preparations that had extra beats or arrhythmic patterns in our data analysis. This calculated immediate post-overdrive rate is henceforth referred to as the postoverdrive rate.

To assess the possible role of endogenous adenosine in overdrive suppression of automaticity experiments were conducted in the presence of 10⁻⁴ mol·litre⁻¹ theophylline, which blocks adenosine receptors,¹⁹ or 10⁻⁵ mol·litre⁻¹ erythro-9-(2 hydroxy-3-nonyl) adenosine hydrochloride (EHNA), which is an adenosine deaminase inhibitor.²⁰ This compound was kindly supplied by Burroughs Wellcome Company. In each case the control response to overdrive stimulation was initially determined and then either theophylline or EHNA added to the perfusate. After a 15 min re-equilibration period the response to overdrive stimulation was again determined.

**STATISTICAL ANALYSIS**

Data are reported as mean(SEM). One way analysis of variance (ANOVA) followed by Scheffé’s test for unequal groups were used for statistical comparisons of heart rate, oxygen consumption, and adenosine release rate before, during, and after periods of overdrive. Paired t tests were used to determine whether the pre-overdrive rate, and the post-overdrive rate, or the relative magnitude of the overdrive suppression obtained under control conditions was altered by the addition of theophylline or EHNA. Significant differences were declared for p values <0.05.

**Results**

**RESPONSES TO 1 MIN OVERDRIVE STIMULATION**

The left ventricular pressure and electrocardiographic records shown in fig 1 were obtained from a preparation subjected to a 1 min period of overdrive.
There were transient alterations in isovolumetric pressure development that accompanied overdrive but, within 30 s, a steady state that was lower than the control amplitude was reached. There were also transient changes in pressure development after the cessation of overdrive. The systolic pressure responses to overdrive will not be reported further in this paper.

At the end of overdrive there was an immediate suppression of pacemaker activity and the initial cycle length (fig 1) was considerably longer than the control (pre-overdrive) cycle length. Subsequent cycle lengths were progressively shorter than the initial length and within 10-20 s the beating rate had returned to control values. The post-overdrive beating rate in this study was determined from the mean of the first five cycle lengths (1-5, fig 1). In this example the pre-overdrive rate was 143 beats·min⁻¹ and the post-overdrive rate 83 beats·min⁻¹.

Figure 2a shows the ventricular beating rate, oxygen consumption, and adenosine release in isolated perfused rat ventricular preparations during and after (a) 1 min and (b) 10 min pacing at 6.0 Hz. Values are mean(SEM). (a) Sample number indicated in parentheses, (b) n=10.*p<0.05, **p<0.01 compared with control values obtained before overdrive (ANOVA with Scheffé's test for multiple comparisons).
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consumption, and adenosine release before, during, and after a 1 minute, 6.0 Hz period of overdrive stimulation. The ventricular rate data and adenosine release data were obtained simultaneously from the same preparations. The pre-overdrive and immediate post-overdrive ventricular rates as well as the pre-overdrive and end-overdrive adenosine release were determined in 20 preparations. Measurements during the subsequent 10 min recovery period were made in only a few of these preparations. (The actual number is indicated at each data point.) In the others samples were not collected, and ventricular rates were undeterminable from the chart records. The oxygen consumption data were obtained from five separate preparations subjected to identical treatment. The nearly 300% increase in ventricular beating rate induced by pacing resulted in a significant increase in oxygen consumption and more than a fourfold increase in adenosine release. At the end of the overdrive period the ventricular rate was initially suppressed to a mean of 62% of the pre-overdrive rate and completely recovered to control values in 30 s. Oxygen consumption also returned very rapidly to control values.

Adenosine release during the first 20 s after overdrive was not different from that during the last 20 s of overdrive. However, during the next 20 s adenosine release decreased to a value lower than that during overdrive but still higher than that before overdrive. After 1 minute release decreased to a value lower than that during overdrive but still higher than that before overdrive. One minute after overdrive adenosine release was not significantly different from pre-overdrive values.

RESPONSES TO 10 MIN OVERDRIVE STIMULATION

The results of 10 experiments in which preparations were subjected to a 10 min period of 6.0 Hz overdrive stimulation are shown in fig 2b. During the 10 min stimulation oxygen consumption increased and remained raised at a nearly constant value above control values throughout the period of stimulation. This was correlated with a nearly constant systolic pressure development after the initial transients, shown in fig 1. Adenosine release initially increased but was not maintained at that high value. After 5 min of overdrive stimulation adenosine release was not significantly different from the pre-overdrive control value.

At the end of the 10 min overdrive stimulation the spontaneous ventricular rate was transiently but significantly suppressed to a value similar to that after a 1 min overdrive stimulation. The adenosine release, however, at the end of the 10 min stimulation was not different from control values. Five minutes after prolonged overdrive stimulation beating rate and oxygen consumption had returned to pre-overdrive values. Adenosine release was now significantly lower than control.

INTACT HEARTS

We also conducted experiments to determine whether the adenosine released during pacing arose from tissue damaged during removal of the atria and crushing of the upper portion of the interventricular septum. For these studies three isolated hearts were perfused with their atria intact and without the balloon in the left ventricle. All other conditions were as previously described. The mean spontaneous heart rate of these preparations was 1.7 times that of the ventricular preparations. Effluent was collected before and during a 10 min episode of ventricular pacing at 6.0 Hz and adenosine release determined. Despite the higher spontaneous beating rates of the intact hearts the control adenosine release (254(44) mol·min⁻¹·g⁻¹) was not significantly different from that of the ventricular preparations (figs 2a and 2b). The adenosine release from the intact hearts increased during pacing to a maximum at 1 min of 1196(328) mol·min⁻¹·g⁻¹, which was not significantly different from that achieved by the ventricular preparations. Furthermore, the adenosine release from the intact hearts during pacing returned to control values with a time course similar to that of the ventricular preparations (fig 2b). These findings indicate that the adenosine release under control conditions and during pacing comes predominantly from normal myocardium and not from damaged tissue.

EFFECTS OF THEOPHYLLINE AND EHNA

The table shows the effects of theophylline and EHNA on the pre-overdrive and post-overdrive ventricular rates and on the relative magnitude of the overdrive suppression, which is defined in this instance as: [(pre-overdrive minus post-overdrive rate)/pre-overdrive rate]100. Responses to overdrive stimulation in the presence of theophylline and EHNA were compared statistically with control responses obtained in the same preparations (paired t test). No significant differences between the control rates of the groups could be detected (ANOVA).

1 min overdrive — The adenosine receptor blocker, theophylline, significantly increased the ventricular rates both before and after 1 min overdrive to above the control rates and decreased the relative magnitude of the overdrive suppression (table). The adenosine deaminase inhibitor, EHNA, decreased the ventricular rates and increased the relative magnitude of the overdrive suppression.

10 min overdrive — The theophylline induced increase in pre-overdrive rate was similar to that found in the preparations subjected to 1 min overdrive. The
TABLE Ventricular beating rates before and after overdrive stimulation and relative magnitude of overdrive suppression

<table>
<thead>
<tr>
<th></th>
<th>Before (a)</th>
<th>After (b)</th>
<th>Relative magnitude [(a-b)/a]100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>124(13)</td>
<td>84(18)</td>
<td>32(8)†</td>
</tr>
<tr>
<td>Plus theophylline</td>
<td>168(15)**</td>
<td>104(14)</td>
<td>37(6)**</td>
</tr>
<tr>
<td>Plus EHNA</td>
<td>136(11)</td>
<td>87(13)</td>
<td>36(7)</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>128(13)</td>
<td>97(14)</td>
<td>23(8)†</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 compared with individual control values (paired t test).
††p<0.01 compared with magnitude of overdrive suppression in the presence of theophylline or EHNA after 1 min stimulation.

rate after 10 min overdrive, however, was not significantly influenced by the presence of theophylline, and the relative magnitude of the overdrive suppression in the presence of theophylline was not different from the control value. Unlike the 1 min overdrive experiments, the addition of EHNA to the perfusate of preparations subjected to 10 min overdrive did not significantly alter the pre-overdrive rate. The post-overdrive rate was also not significantly influenced by EHNA, and the relative magnitude of the overdrive suppression in the presence of EHNA was not different from the control value.

These experiments indicate that both theophylline and EHNA influenced the relative magnitude of the overdrive suppression after a 1 min episode of overdrive stimulation but not after a 10 min episode.

**Discussion**

The results of this study indicate that alterations in endogenous adenosine may contribute to the transient suppression of ventricular pacemakers after a brief (1 min) period of overdrive stimulation but do not contribute to the overdrive suppression that follows a prolonged (10 min) period of overdrive stimulation. These conclusions are based on the observations that (a) adenosine release into the coronary effluent was significantly increased above control values at the end of the 1 min overdrive but had returned to control values at the end of the 10 min overdrive, and (b) theophylline, which blocks the effect of adenogenous adenosine, evoked a decrease and an increase respectively in the relative magnitude of overdrive suppression after 1 min overdrive, whereas these agents had no discernible influence on the relative magnitude of the overdrive suppression which followed 10 min overdrive stimulation.

We have made the assumption that increases and decreases in adenosine release into the venous effluent reflect changes in interstitial adenosine concentrations. However, the relation between adenosine release into the coronary effluent and the actual interstitial adenosine concentration is not well understood. We do know that endogenous adenosine may be compartmentalised within the myocardium and that coronary endothelial cells take up adenosine.22 The capillary wall may represent a significant diffusional and metabolic barrier separating interstitial fluid adenosine from effluent adenosine.24 Therefore, at present it is not possible to use the release of adenosine to calculate the instantaneous concentration of adenosine around pacemaker cells. It seems likely, however, that alterations in adenosine release into the coronary effluent probably occur in parallel with alterations in interstitial adenosine.

We attempted to modulate endogenous adenosine by using two different pharmacological approaches. Each of these interventions individually may have additional effects that could complicate interpretation of the data. For example, theophylline is known to inhibit phosphodiesterase activity and increase cyclic adenosine monophosphate.19 This action has been proposed to account for theophylline’s ability to mimic the effects of catecholamines and increase pacemaker activity of isolated cardiac Purkinje fibres.26 However, phosphodiesterase inhibition by theophylline is generally thought to occur at significantly higher concentrations (100-1000 μmol-litre⁻¹) than adenosine receptor antagonism (10-100 μmol-litre⁻¹).19 The concentration of theophylline used in the present study (100 μmol-litre⁻¹) was such that both effects of theophylline could have been expressed. Thus conclusions about adenosine’s role, based on theophylline’s effects alone, must be
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Tentative. To be certain that adenosine played a role in the overdrive suppression of these preparations both of the interventions used in this study should have produced changes in overdrive suppression that were consistent with their predicted effects on endogenous adenosine. The observations that theophylline decreased, whereas EHNA enhanced, the relative magnitude of overdrive suppression after 1 min overdrive support the conclusion that adenosine is a factor contributing to this phenomenon.

One of the findings of this study was that during 10 min overdrive stimulation the myocardial oxygen consumption increased and was maintained at a raised value for the duration of overdrive, whereas the adenosine release initially increased and then returned to control values, after about 5 min of overdrive. These data indicate that, under the conditions described, adenosine release is not tightly coupled to oxygen consumption. This finding is in agreement with that of DeWitt and colleagues, who reported a phasic increase in adenosine release from isolated guinea pig hearts with sustained increased oxygen consumption induced by noradrenaline infusion. It is also consistent with a report by Manfredi and Sparks that adenosine release from dog myocardium after 10 min of rapid pacing and increased oxygen consumption was not different from control. The mechanisms responsible for the transient nature of the increase in adenosine release in these situations are not clear.

It should be noted that at the end of 1 min overdrive the augmented adenosine release decreased rapidly to control values with a time course that was slightly slower than the recovery of ventricular automaticity. This suggests that either the decline in adenosine release into coronary effluent does not accurately reflect the magnitude or time course, or both, of alterations in interstitial adenosine concentrations in the vicinity of the pacemaker cells or that adenosine plays a relatively minor role in regulating automaticity at the end of overdrive. It is indeed likely that adenosine is only one of several factors that can contribute to the transient decrease in automaticity after overdrive. Since the magnitude of suppression after 10 min overdrive was not different from that after 1 min overdrive it seems likely that the various mechanisms that contribute to this phenomenon operate with different time courses — namely, as the initial adenosine contribution wanes some other factor(s) increase to influence the automaticity.

Some of the results of these experiments also suggest that endogenous adenosine may have been influencing the ventricular pacemaker activity during the control (or pre-overdrive) period. This suggestion is based on the observations that theophylline increased the pre-overdrive beating rate and, in the preparations that were subjected to 1.0 min periods of overdrive stimulation, EHNA decreased the pre-overdrive beating rate. We cannot, however, eliminate the possibility that other effects of these agents might also have contributed to the observed alterations in the pre-overdrive beating rate. The lack of effect of EHNA on the pre-overdrive rates in preparations subjected to 10 min overdrive stimulation is not clear but may somehow be related to the depression in adenosine release that followed the control 10 min overdrive stimulation. Full re-establishment of baseline conditions may not have been achieved in the 15 min re-equilibration period.

In all of these experiments overdrive stimulation was fixed at the constant rate of 6.0 Hz. Thus for preparations with slower spontaneous rates (namely, those tested in the presence of EHNA) the fixed overdrive was relatively more intense than it was for preparations with faster spontaneous rates (namely, those tested with theophylline). Differences in the relative intensity of the overdrive stimulation might therefore account for the changes in the relative magnitude of the overdrive suppression. However, the observation that theophylline increased the pre-overdrive beating rate but did not affect the relative magnitude of overdrive suppression after the 10 min overdrive indicates that the magnitude of suppression compared with the spontaneous rate is not the only determinant of the relative magnitude of overdrive suppression in this study.

There are several mechanisms by which endogenous adenosine might contribute to the transient suppression of automaticity after 1 min overdrive stimulation. Exogenous adenosine is reported to have a variety of electrophysiological effects on cardiac tissue. These effects include (a) hyperpolarisation due to increased potassium conductance, (b) depression of slow calcium mediated action potentials due to a calcium channel blocking effect, and (c) decreases in the slope of the phase IV diastolic depolarisation of spontaneously active ventricular Purkinje fibres, the cause of which has not been elucidated. The results of the present study imply that, by virtue of one or all of these electrophysiological effects, increases in endogenous adenosine may be contributing to the changes in automaticity that occur after brief overdrive. However, because of the transient nature of its increase during sustained rapid pacing endogenous adenosine does not contribute to the changes in automaticity that follow a prolonged overdrive.

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References