Role of Adenosine Receptors in the Paradoxic Bradycardia Response of Rats to Inferior Vena Cava Occlusion During an Infusion of Isoproterenol

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Background—In susceptible humans, vasodepressor reactions are induced by restriction of venous return (upright tilting) and administration of isoproterenol. Because paradoxic bradycardia is a major manifestation of vasodepressor reactions, and allowing for extrapolation between paradoxic bradycardia in rats and vasodepressor reactions, we examined whether adenosine receptors mediate the paradoxic bradycardia reaction.

Methods and Results—Paradoxic bradycardia was induced in rats by inferior vena cava occlusion during an isoproterenol infusion. We studied whether dipyridamole, an adenosine transport inhibitor, and aminophylline (nonselective) or DPCPX (selective) A1 antagonists augmented or inhibited paradoxic bradycardia, respectively, during inferior vena cava occlusion. The maximum changes in R-R during 60 seconds of inferior vena cava occlusion were that (1) in control, the rate accelerated ($\Delta$R-R, $-9.7\pm0.8$ ms, $P<0.001$); (2) during isoproterenol (0.8 $\mu$g $\cdot$ min$^{-1}$), paradoxic bradycardia occurred ($\Delta$R-R, $+92.0\pm32.0$ ms, $P<0.001$); (3) during isoproterenol but after dipyridamole, paradoxic bradycardia occurred at a much lower dose of isoproterenol (0.2 $\mu$g $\cdot$ min$^{-1}$), and the magnitude was increased at all doses (at 0.8 $\mu$g $\cdot$ min$^{-1}$ isoproterenol, $\Delta$R-R, $+195.6\pm27.6$ ms, $P<0.001$ versus isoproterenol alone, $\Delta$R-R, $+92.0\pm32$ ms); (4) during isoproterenol and dipyridamole, atropine did not block paradoxic bradycardia, but cervical vagotomy inhibited paradoxic bradycardia ($\Delta$R-R, $+5.6\pm1.8$ ms, $P<0.001$ compared with isoproterenol and dipyridamole alone); and (5) during isoproterenol alone, aminophylline or DPCPX blocked paradoxic bradycardia ($\Delta$R-R, $-5.4\pm1.0$ ms, and $\Delta$R-R, $-2.6\pm0.5$ ms, respectively, each $P<0.001$ compared with isoproterenol alone).

Conclusions—The adenosine A1 receptor mediates the paradoxic bradycardia reflex during inferior vena cava occlusion in the face of isoproterenol via vagal afferents. (Circulation. 1998;98:1228-1235.)

Key Words: heart rate $\bullet$ adenosine $\bullet$ receptors $\bullet$ reflex

Despite extensive research, our understanding of the mechanism and the therapy of vasodepressor reactions remains limited.1 Vasodepressor reactions occur in response to pain, fright, orthostasis, blood loss, myocardial ischemia, vasodilator drugs, or after extreme exercise.1–3 Reactions also occur in fighter pilots and motor vehicle drivers, and fatal crashes can ensue.4 Thus, vasodepressor reactions are important because they occur in response to a wide variety of stimuli and they can lead to hypotension, cardiac asystole, syncope, injury, or even death. Development of effective treatment requires an understanding of the triggering mechanism.

We developed a clinical test to assess human susceptibility to the vasodepressor reaction and its treatment.5 Isoproterenol administered during restricted venous return ($+60^\circ$ tilt) provokes paradoxic bradycardia and hypotension in persons prone to such reactions. Subsequently, we developed a related model in the rat consisting of inferior vena cava occlusion combined with an infusion of isoproterenol.6–9 Inferior vena cava occlusion causes marked hypotension, and without isoproterenol, it increases heart rate through reflexes. When isoproterenol is administered, however, inferior vena cava occlusion causes paradoxic bradycardia. Bradycardia during hypotension is a major manifestation of clinical1,2 and experimental1,10 vasodepressor reactions.

Although our previous work defined some of the neural pathways involved in reflex bradycardia,6–9 we have not examined whether endogenous chemicals play a role in initiating the reaction. We believe that adenosine may play a central role in the vasodepressor reaction, for the following reasons: (1) adenosine, an endogenous purine, is elaborated during low blood flow11 and high adrenergic drive,12 conditions that are present in our model; (2) ischemia13 and adenosine14 can excite vagal afferents; (3) paradoxic bradycardia reaction in our model depends on cardiac vagal afferents6–9; (4) exogenously administered adenosine during upright tilting can elicit a vasovagal reaction in humans15; and (5) in 2 reports, patients have been successfully treated for vasodepressor reactions with theophylline.16,17

We investigated whether adenosine participates in paradoxic bradycardia in our rat model. We measured the para-
doxic bradycardia response to inferior vena cava occlusion and isoproterenol without and after pretreatment with diprydiamole, an adenosine transport inhibitor, and aminophylline, a nonselective, as well as 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), a selective A1 adenosine receptor antagonist.

**Methods**

**Protocol**
A total of 35 adult male Wistar rats (360.2±5.8 g) were used in these studies; the methods have been extensively described previously.6–9 The animals were anesthetized by intraperitoneal inactin, paralyzed with intravenous pancuronium, and ventilated at 70 breaths per minute through a tracheostomy (Harvard Apparatus, model 680). In preliminary experiments, it was shown that inactin completely inhibited any withdrawal reflex in response to pressure applied to the extremities for the entire experimental period before pancuronium was used. The animal’s core temperature was kept at 37±0.5°C. A Silastic cannula inserted into the carotid artery was attached to a transducer (Statham, P23db) for blood pressure measurement. Another Silastic cannula inserted in the jugular vein was used for fluid and drug administration. Through a low sternotomy, a thin umbilical tape was passed around the inferior vena cava just as it enters the chest for periodic occlusion. Under the conditions of the protocols described below, the inferior vena cava was occluded for 60 seconds twice during any particular intervention or drug dosage.

**Inferior Vena Cava Occlusion During Administration of Isoproterenol**
Six rats were studied before and during isoproterenol infusion. The inferior vena cava was occluded for 60 seconds during control conditions. Isoproterenol was then infused starting at 0.1 μg·min⁻¹ and sequentially doubled until we observed R-R interval prolongation (≥20 ms) during 60 seconds of inferior vena cava occlusion to a concentration of 0.8 μg·min⁻¹. If R-R prolongation was not observed, the dose of isoproterenol was increased to a maximum of 1.0 μg·min⁻¹, at which dose R-R prolongation occurred.

**Inferior Vena Cava Occlusion During Administration of Isoproterenol in Rats Pretreated With Diprydiamole Before and After Treatment With Atropine and Bilateral Cervical Vagotomy**
Nine rats treated with diprydiamole 2.0 mg were studied before and during isoproterenol. The inferior vena cava was occluded for 60 seconds during control conditions. Isoproterenol was infused starting at 0.1 μg·min⁻¹ and sequentially doubled to a maximum concentration of 1.0 μg·min⁻¹ (as above), and at each step the inferior vena cava was occluded. In separate experiments, diprydiamole (2 mg) caused a 4-fold increase in the R-R interval prolongation in response to adenosine (20 to 80 μg) (see Results). Another group of 5 rats were pretreated with 2 mg diprydiamole, and the inferior vena cava was occluded during an infusion of isoproterenol 0.4 μg·min⁻¹. The dose chosen was insufficient to cause paradoxic bradycardia by itself, but when combined with diprydiamole, it resulted in paradoxic bradycardia equal to or greater than that seen in response to 0.8 μg·min⁻¹ isoproterenol without diprydiamole. The experiments were repeated after pretreatment with atropine 1 mg IP and after bilateral cervical vagotomy. The bradycardia response to electrical stimulation of the sectioned efferent cervical vagus nerves was blocked in each rat after pretreatment with 1 mg atropine IP.

**Inferior Vena Cava Occlusion During Administration of Isoproterenol After Treatment With Aminophylline or DPCPX**
Ten rats were studied before and during an infusion of isoproterenol. The inferior vena cava was occluded for 60 seconds during control, after which isoproterenol was infused starting at 0.25 μg·min⁻¹, and this was sequentially doubled to a maximum of 1.0 μg·min⁻¹ or until we observed R-R interval prolongation (≥20 ms) during inferior vena cava occlusion. With infusion of isoproterenol maintained, the rats received aminophylline as a 0.5-mg bolus, and this was sequentially doubled to a maximum of 4.0 mg or until inhibition of R-R interval prolongation during inferior vena cava occlusion was achieved (mean dose, 2.5±0.3; range, 1 to 4 mg). In separate experiments, aminophylline (2 mg) caused a 3.44-fold decrease in the R-R interval prolongation after administered adenosine (20 to 80 μg) (see Results).

Five additional rats were studied after DPCPX treatment. After the dose of isoproterenol that resulted in paradoxic bradycardia during inferior vena cava occlusion had been established, DPCPX was administered as a bolus starting at 25 μg, and this was sequentially doubled up to a maximum of 200 μg or until paradoxic bradycardia was blocked (150±25 μg). In separate experiments, DPCPX (150 μg) caused a 3.8-fold reduction in the R-R interval prolongation in response to adenosine (20 to 80 μg) (see Results).

**Preliminary Experiments to Establish Effects of Aminophylline, Diprydiamole, and DPCPX on Response of Heart Rate to Exogenously Administered Adenosine**
In a separate group of 9 closed-chest anesthetized rats, we administered adenosine 20, 40, and 80 μg IV and measured the R-R interval change in control and after diprydiamole (0.5 to 2 mg), aminophylline (0.5 to 4 mg), and DPCPX (25 to 200 μg).

**Data Analysis and Statistical Analysis**
ECG and blood pressure signals were digitized and sampled continuously every 20 ms and after 50 samples, were stored as 1-second values by a microcomputer system.6–9 The 1-second samples were averaged into 5-second segments covering the control period (20 seconds), the inferior vena cava occlusion (60 seconds), and after release (20 seconds). The data during the 20 seconds of control were averaged into a single value for the resting R-R interval and blood pressure. The maximum change (increase or decrease) during the 60-second period of inferior vena cava occlusion was measured.

All data were expressed as mean±SEM. The blood pressure response to drug administration and the R-R interval during resting conditions in each group of experiments were analyzed with Student’s t test for paired data. A 1-way ANOVA for repeated measurements was used when a comparison involved ≥2 states and was followed by post hoc testing with Dunnett’s test to identify the significant comparisons. Linear regression analyses calculated the relationship between the dose of administered isoproterenol and the change in R-R interval before and after diprydiamole. A 1-way ANOVA compared the maximum blood pressure fall during inferior vena cava occlusion in the different states. A probability value of <0.05 was considered significant.21

The methods used in these experiments conformed to the guidelines on animal use of the American Heart Association (1984). The protocols were reviewed and approved by the Animal Care Committee of the University of Toronto.

**Results**

**Effects of Aminophylline, Diprydiamole, and DPCPX on Response of Heart Rate to Exogenously Administered Adenosine**
There was a dose-dependent prolongation in the ΔR-R interval after the administration of adenosine. The effect of adenosine was enhanced by diprydiamole and blocked by aminophylline and DPCPX. Plots of the log ΔR-R versus log doses of adenosine under control, diprydiamole, aminophylline, and DPCPX were obtained. Dose ratios were then calculated for the doses of diprydiamole, aminophylline, and DPCPX that approximated the doses of these adenosine agonist/antagonists that were closest to the average dose in the experiments that enhanced or blocked the R-R response to...
Effects of Isoproterenol on Resting Blood Pressure and Effects of Inferior Vena Cava Occlusion on Blood Pressure and Pulse Pressure

During an infusion of isoproterenol (0.8 ± 0.13 μg · min⁻¹), the mean blood pressure fell from 122.7 ± 6.2 to 91.3 ± 8.1 mm Hg (n = 30 rats, P < 0.001, paired Student’s t test), and the pulse pressure increased from 29.2 ± 2.2 to 64.6 ± 2.7 mm Hg (P < 0.001, paired Student’s t test). During inferior vena cava occlusion in all the different states, the mean blood pressure fell to a minimum of 17.3 ± 5.3 mm Hg (range, 14 to 27 mm Hg, P = NS, ANOVA). During inferior vena cava occlusion, the pulse pressure fell to 11.4 ± 1.3 and 12.7 ± 0.64 mm Hg during control and isoproterenol, respectively (P = NS, ANOVA).

Figure 1. Plot of maximum ΔR-R response to 60 seconds of inferior vena cava occlusion during graded doses of isoproterenol without and after dipyridamole.

**Table 1.**

<table>
<thead>
<tr>
<th></th>
<th>Isoproterenol 0.1 μg · min⁻¹</th>
<th>Isoproterenol 0.2 μg · min⁻¹</th>
<th>Isoproterenol 0.4 μg · min⁻¹</th>
<th>Isoproterenol 0.8 μg · min⁻¹</th>
<th>Isoproterenol 1.0 μg · min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Resting R-R, ms</td>
<td>152.6 ± 2.2</td>
<td>146.9 ± 4.2</td>
<td>145.6 ± 4.8</td>
<td>140.2 ± 3.6</td>
<td>139.3 ± 5.2</td>
</tr>
<tr>
<td>Maximal ΔR-R during IVC occlusion, ms</td>
<td>−9.7 ± 0.8</td>
<td>−13.9 ± 1.1</td>
<td>−15.7 ± 1.4</td>
<td>−7.2 ± 1.1</td>
<td>+92.0 ± 32.0</td>
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<td></td>
<td>P = NS</td>
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<td>P = NS</td>
<td>P = NS</td>
<td>P &lt; 0.001†</td>
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</table>

*Resting R-R isoproterenol vs R-R control, paired Student’s t test (across row 1 and across row 3).
†ΔR-R isoproterenol vs ΔR-R control, ANOVA followed by Dunnett’s test (across row 2 and across row 4).
‡Resting R-R vs R-R during IVC occlusion, paired Student’s t test (row 1 vs 2 in columns 1–6; row 3 vs 4 in columns 1–6).

Inferior Vena Cava Occlusion During Graded Infusion of Isoproterenol

Without isoproterenol, inferior vena cava occlusion shortened the R-R interval (−9.7 ± 0.8 ms, paired Student’s t test, P < 0.001). During an infusion of a lower amount of isoproterenol (0.1 to 0.4 μg · min⁻¹), inferior vena cava occlusion produced significant shortening of the R-R interval (−13.9 ± 1.1, −15.7 ± 1.4, and −7.2 ± 1.1 ms, respectively, P = NS compared with control). During higher amounts of isoproterenol (0.8 and 1.0 μg · min⁻¹), inferior vena cava occlusion caused paradoxical R-R interval prolongation (+92.0 ± 32.0 and +118.8 ± 40.5 ms, respectively, P < 0.001 compared with control; Table, row 2, columns 1 through 6; ANOVA).

Inferior Vena Cava Occlusion During Administration of Isoproterenol in Rats Pretreated With Dipyridamole

After dipyridamole treatment, inferior vena cava occlusion without isoproterenol prolonged the R-R interval minimally (+5.2 ± 2.6 ms). During isoproterenol alone (without dipyridamole), inferior vena cava occlusion did not cause paradoxical bradycardia at doses of isoproterenol <0.8 μg · min⁻¹. After dipyridamole, however, inferior vena cava occlusion resulted in significant R-R prolongation at isoproterenol doses of 0.2 μg · min⁻¹, and the R-R prolongation during inferior vena cava occlusion after dipyridamole pretreatment was significantly higher than with isoproterenol alone (Figure 1 and Table, row 4, columns 1 through 6).

Linear regression analysis showed a significant increase in the change in R-R in response to isoproterenol (ΔR-R
interval $= 163.3$ (dose of isoproterenol) $- 46.9$; $r = 0.94$, $P < 0.01$. Similarly, linear regression analysis also showed a significant dose-dependent increase in the changes in R-R interval in the group pretreated with dipyridamole $\Delta$R-R $= 184.7$ (dose of isoproterenol) $- 26.7$; $r = 0.75$, $P < 0.01$. There was a significant shift in the slope of the 2 dose-response curves, showing a difference of $+ 75.1$ ms ($P < 0.01$).

**Inferior Vena Cava Occlusion During Administration of Isoproterenol in Rats Pretreated With Dipyridamole After Treatment With Atropine and After Bilateral Cervical Vagotomy**

In 5 separate experiments, rats were pretreated with dipyridamole. An infusion of isoproterenol (0.4 $\mu$g $\cdot$ min$^{-1}$) was administered. In untreated rats, this dose was insufficient to provoke paradoxic bradycardia during inferior vena cava occlusion, but after treatment with dipyridamole (see above), this dose caused paradoxic bradycardia equal to or greater than that seen in response to 0.8 $\mu$g $\cdot$ min$^{-1}$ of isoproterenol. After treatment with atropine, the R-R interval prolongation during inferior vena cava occlusion was unchanged ($+ 126.2 \pm 22.3$ ms, $P = $NS compared with isoproterenol + dipyridamole). Bilateral cervical vagotomy blocked the paradoxic bradycardia response to inferior vena cava occlusion ($\Delta$R-R, $- 5.6 \pm 1.8$ ms, $P < 0.001$ compared with isoproterenol + dipyridamole with intact vagus nerves by ANOVA).

**Inferior Vena Cava Occlusion During Administration of Isoproterenol Before and After Treatment With Aminophylline or DPCPX**

Without isoproterenol, inferior vena cava occlusion shortened the R-R interval ($- 13.2 \pm 2.1$ ms, $P < 0.001$ by paired Student’s $t$ test). During an infusion of isoproterenol (0.8 $\mu$g $\cdot$ min$^{-1}$), inferior vena cava occlusion significantly prolonged the R-R interval ($+ 108.9 \pm 13.0$ ms, $P < 0.001$, ANOVA). Aminophylline (2.5 $\pm$ 0.3 mg; range, 1 to 4 mg) blocked the R-R prolongation during isoproterenol and inferior vena cava occlusion ($- 5.4 \pm 1.0$ ms, $P < 0.001$, ANOVA; see Figure 2).

In 5 additional rats, the paradoxic bradycardia response to isoproterenol (0.8 $\mu$g $\cdot$ min$^{-1}$) was established ($\Delta$R-R, $+ 132.4 \pm 20.4$ ms, $P < 0.001$ compared with control). DPCPX blocked paradoxic bradycardia ($\Delta$R-R, $- 2.6 \pm 0.5$ ms, $P < 0.001$ compared with isoproterenol, $P = $NS compared with control; Figure 3).

**Summary of Responses to Inferior Vena Cava Occlusion**

A graphic summary of the maximum changes in R-R interval during inferior vena cava occlusion in 10 states (columns 1 through 10) is shown in Figure 4. Section 1 is recorded after pretreatment with dipyridamole. The R-R interval shortened during control conditions (column 1); the R-R prolonged during occlusion, but after treatment with dipyridamole (see above), this dose caused paradoxic bradycardia equal to or greater than that seen in response to 0.8 $\mu$g $\cdot$ min$^{-1}$ of isoproterenol. After treatment with atropine, the R-R interval prolongation during inferior vena cava occlusion was unchanged ($+ 126.2 \pm 22.3$ ms, $P = $NS compared with isoproterenol + dipyridamole). Bilateral cervical vagotomy blocked the paradoxic bradycardia response to inferior vena cava occlusion ($\Delta$R-R, $- 5.6 \pm 1.8$ ms, $P < 0.001$ compared with isoproterenol + dipyridamole with intact vagus nerves by ANOVA).

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Paradoxic Bradycardia, Adenosine, Isoproterenol

Discussion

In our model, there is low blood flow because of inferior vena cava occlusion as well as intense adrenergic stimulation caused by the infusion of isoproterenol and reflex activation of sympathetic tone. Because adenosine is elaborated in response to such conditions, we examined the role of adenosine receptors on the paradoxic bradycardia reflex in this model. Two aspects of this study show that adenosine receptors mediate the paradoxic bradycardia response to inferior vena cava occlusion and isoproterenol. First, dipyridamole blocks the uptake of adenosine into cells, thereby raising the extracellular adenosine concentration, and this allowed paradoxic bradycardia to occur at a much lower dose of isoproterenol and enhanced paradoxic bradycardia in response to inferior vena cava occlusion during an infusion of isoproterenol. Second, aminophylline, a nonselective, and DPCPX, a selective A1 adenosine receptor antagonist inhibited the paradoxic bradycardia response to inferior vena cava occlusion during an infusion of isoproterenol. In this regard, 2 recent publications claim success in treating humans for vasodepressor reactions with theophylline. Earlier studies showed that the paradoxic bradycardia in this rat model depends on cardiac vagal afferents: it is blocked by cervical vagotomy or intrapericardial lidocaine but is independent of efferent vagal tone because it is unaffected by muscarinic receptor blockade with atropine. Similarly, the paradoxic bradycardia response to inferior vena cava occlusion during isoproterenol and dipyridamole was not affected by pretreatment with atropine, but it was blocked by cervical vagotomy. Thus, paradoxic bradycardia is independent of muscarinic receptors but depends on vagal afferents. To demonstrate this, we administered a dose of isoproterenol (0.4 μg · min⁻¹) that was insufficient to cause paradoxic bradycardia during inferior vena cava occlusion. However, when rats were pretreated with dipyridamole, this dose of isoproterenol resulted in paradoxic bradycardia equal to or greater than that seen with 0.8 μg · min⁻¹ isoproterenol. Thus, we set up conditions in which both dipyridamole and isoproterenol were essential in producing paradoxic bradycardia.

Because it has been suggested that anomalous firing of carotid sinus baroreceptors might be the afferent source of vasodepressor reactions experimentally and in humans, we considered this possibility in our experiment. In this regard, it is notable that isoproterenol markedly increased the pulse pressure, and this can exaggerate afferent firing from the baroreceptors at any level of mean arterial pressure. However, an augmented pulse pressure is unlikely to be responsible for paradoxic bradycardia, because the pulse pressure during inferior vena cava occlusion with or without isoproterenol fell to the same levels. Furthermore, without inferior vena cava occlusion, isoproterenol augmented the pulse pressure, and yet paradoxic bradycardia was not seen. In addition, in recent experiments we demonstrated that paradoxic bradycardia in this model was unaffected by carotid sinus nerve section.

Previous work showed that the bradycardia is secondary to a reflex withdrawal sympathetic tone, because bilateral stel late ganglionectomy or chemical sympathectomy (6-hydroxydopamine) or the administration of propranolol slows the heart rate to values that occur during inferior vena cava occlusion, and after these interventions, the bradycardia during inferior vena cava occlusion is inhibited. In a related experiment involving inferior vena cava occlusion in the rabbit, bradycardia was due to sympathoinhibition. By contrast, bradycardia in human vasodepressor reactions is usually due to increased efferent vagal tone. Adenosine modulates and regulates cell metabolism through specific surface receptors A1 and A2 and through intracellular binding sites. Apart from coronary vasodilatation, which is regulated by A2 receptors, all other cardiac...
adenosine actions are mediated via $A_1$ receptors.\textsuperscript{19,26} During decreased oxygen supply (ischemia)\textsuperscript{11} or accelerated ATP usage (excessive catecholamine drive),\textsuperscript{12} adenosine production rises. By decreasing metabolism and by causing vasodilation to increase blood flow and oxygen delivery during increased metabolic activity and during ischemia, adenosine protects the heart during ischemia.\textsuperscript{27} Adenosine production correlates with the extent of ischemia.\textsuperscript{11}

The mechanism whereby inferior vena cava occlusion and isoproterenol trigger the reflex has not been established. It is believed that an increase in left ventricular contractility is salient to triggering the vasodepressor reaction clinically and experimentally.\textsuperscript{28} In this regard, we recently showed that pretreatment with verapamil inhibited paradoxic bradycardia,\textsuperscript{29} thereby offering support to the hypothesis that augmented contractility is part of the triggering mechanism.\textsuperscript{28} In further support of the concept that an increase in contractility is needed to trigger the reflex, we found that if we prevent a rise in sympathetic tone during the initial phase of inferior vena cava occlusion by maintaining the carotid sinuses at normal pressure, the paradoxic bradycardia reflex is inhibited.\textsuperscript{28}

Adenosine interacts with adrenergic neurons at prejunctional and postjunctional sites by altering catecholamine release and by directly altering the effects of catecholamines at the receptor level.\textsuperscript{26} Adenosine inhibits norepinephrine release directly via purinergic receptors in open-chest dogs.\textsuperscript{30} Furthermore, adenosine inhibits neurotransmission in sympathetic ganglia, leading to inhibition of norepinephrine release from effenter sympathetic nerves.\textsuperscript{31} The main actions of catecholamines on the myocardium are mediated by cAMP.\textsuperscript{26} $\beta$-Adrenergic stimulation augments adenosine levels, which work through $A_1$ receptors to decrease cAMP and act as a natural feedback inhibitor of catecholamines in the myocardium.\textsuperscript{32} Ultimately, adenosine attenuates the adrenergically stimulated rise in cAMP and the increase in L-type calcium inward current ($I_{Ca,L}$) in atrial and ventricular tissue.\textsuperscript{32} Adenosine working through prejunctional and postjunctional mechanisms could reduce the release of norepinephrine as well as the cellular response to adrenergic tone. Thus, adenosine could act on the efferent side of the reflex, where its actions would be additive with the reflex sympathoinhibition that is responsible for paradoxical bradycardia in our model.\textsuperscript{6,7} This could explain the augmented bradycardia after dipyridamole.

Adenosine-mediated sympathoinhibition could also act on the afferent side of the reflex. Previously, we showed that paradoxical bradycardia required a reflex rise in sympathetic tone to trigger the afferent mechanism of the reaction.\textsuperscript{24} Thus, adenosine, by its ability to reduce sympathetic transmission,\textsuperscript{31} release of norepinephrine,\textsuperscript{30} and the intracellular effects of adrenergic stimulation,\textsuperscript{26,32} could block the reaction. However, such an action would not fit with the observed results, because dipyridamole enhanced rather than blocked the reaction.

A further effect of adenosine on paradoxical bradycardia is possible. In a recent publication in which adenosine elicited vasovagal reactions in humans undergoing upright tilting, it was postulated that adenosine works by causing sympathetic activation.\textsuperscript{15} Because an increase in sympathetic tone is essential for the paradoxical bradycardia reflex in our model,\textsuperscript{24} adenosine may initially act at this level to trigger the reflex. In summary, adenosine may act by sympathetic excitation to initiate the reflex, and once started, adenosine may also augment the sympathoinhibition on the efferent side that is responsible for paradoxical bradycardia.

Previously, we showed that phenylephrine inhibits the paradoxical bradycardia response to inferior vena cava occlusion, whereas prazosin facilitated paradoxical bradycardia.\textsuperscript{9} $\alpha_1$-Adrenergic stimulation and $\alpha_1$-blockade augment and decrease the release of adenosine from ischemic myocardium, respectively.\textsuperscript{33} Furthermore, $\alpha_1$-adrenergic blockade with prazosin decreases adenosine release from ischemic myocardium and decreases coronary blood flow and reduces reactive hyperemia.\textsuperscript{33} The finding that $\alpha$-stimulation raises adenosine levels, which in turn counteracts the effects of ischemia,\textsuperscript{28} and the fact that phenylephrine, an $\alpha_1$-agonist, blocks paradoxical bradycardia in our model, whereas phenolamine and prazosin, $\alpha_1$-receptor antagonists, facilitate the reaction,\textsuperscript{9} lead us to believe that phenylephrine and prazosin are not working through adenosine in our model. Alternatively, the conditions of our experiments may not be applicable to some models of experimental ischemia.

Adenosine and a related purine ATP slow the sinus rate in humans, animals, and isolated tissues by hyperpolarization via a time-independent potassium current ($I_{Kcat}$) and a reduced rate of diastolic depolarization.\textsuperscript{29} As in our rat model, in which the bradycardia is independent of atropine,\textsuperscript{6,7} the bradycardia induced by adenosine given systemically or into the sinoatrial node is unaffected by atropine in dogs.\textsuperscript{34–37} The bradycardia caused by adenosine or ATP and the bradycardia in our experiment are blocked by aminophylline, an $A_1$-receptor antagonist.\textsuperscript{37} However, unlike our rat model, in which vagotomy blocks bradycardia,\textsuperscript{3,9} the bradycardia after exogenous adenosine is not blocked by vagotomy in dogs\textsuperscript{44} or cats.\textsuperscript{38}

ATP is rapidly hydrolyzed to adenosine, and when a nonhydrolyzable ATP is injected into the sinus node artery, there is no bradycardia.\textsuperscript{37} Thus, the action of ATP on the sinus node is probably via ATP conversion to adenosine, and the latter acts on $A_1$ receptors to slow the rate.\textsuperscript{26} In addition to a direct effect on the sinus node, ATP also induces reflex slowing via cardiac vagal afferents in cats and dogs\textsuperscript{34,39} but not in rabbits.\textsuperscript{38,39} Thus, ATP causes bradycardia by direct effects on the sinus node as well as by vagally mediated reflex–induced slowing. Like the bradycardic effects of ATP, which require conversion of ATP to adenosine when it is injected directly into the sinoatrial node, the reflex stimulation of vagal afferents by ATP may also work through conversion to adenosine. According to this reasoning, endogenously formed adenosine may trigger vagal afferents. Thus, not only may ATP induce a vagal afferent bradycardic reflex via adenosine, but it is also possible that when adenosine is released endogenously as a result of ischemia, etc, the ensuing bradycardia may involve a vagal afferent reflex. Thus, released adenosine could work directly on the sinoatrial node to slow the rate, or it may slow the rate by stimulating
vagal afferents in our experiment that cause reflex sympa-thoinhibition and bradycardia.

Experimental and clinical evidence suggests that adenosine release during ischemia might be responsible for cardiac pain.6–8 Experimentally induced myocardial ischemia60 or the intracoronary administration of adenosine62 in dogs increases renal sympathetic nerve activity. The increase in renal sympathetic nerve activity is augmented by dipyridamole in both cases41,42 and attenuated by aminophylline.41 These experiments are offered as proof, albeit indirect, that ischemia acting via adenosine receptors may stimulate sympathetic afferents that transmit impulses to the central nervous system, where they are perceived as pain. However, recent neurographic recordings in cats have failed to show that adenosine excites the same sympathetic afferents as are activated by coronary occlusion.43 Although adenosine excites sympathetic afferents, which are thought to be the primary mediators of cardiac pain, exogenously applied adenosine also excites vagal afferents.14 In a related vein, recent direct neurographic recordings of cardiac vagal afferents has shown that myocardial ischemia activates vagal afferents in rats.44 Although the preponderant evidence at present suggests that ischemia and exogenous adenosine activate sympathetic afferents,40 this question has not been conclusively resolved.

Our present experiments and our previous work suggest that paradoxic bradycardia is mediated by vagal afferents,6,7 but a role for sympathetic afferents has not been completely dismissed, because vagotomy and sympathectomy both block the reaction.5,7 By recording vagal and sympathetic afferents, future experiments will identify conclusively through which afferents adenosine is working.

Limitations of the Study

Attempting to further evaluate the role of the A1 receptor in mediating paradoxic bradycardia, we performed experiments (not included in the present results) with cyclopentyladenosine (CPA),6,7,10,19,20 a selective A1 receptor agonist. Consistent with the hypothesis that A1 receptors mediate paradoxic bradycardia, CPA produced a dose-dependent R-R prolongation that was associated with a proportionately diminished R-R prolongation in response to inferior vena cava occlusion. Against this hypothesis, CPA alone did not induce paradoxic bradycardia during inferior vena cava occlusion, and it did not shift the dose of isoproterenol needed to induce paradoxic bradycardia during inferior vena cava occlusion. At higher doses of CPA and isoproterenol together, the paradoxic bradycardia response to inferior vena cava occlusion was extremely prolonged, often lasting ≥5 minutes after release of the inferior vena cava occlusion. This contrasts with other experiments involving isoproterenol and inferior vena cava occlusion, in which paradoxic bradycardia disappears promptly after the release of the inferior vena cava.5–9,24,29

Despite the failure of CPA by itself to produce paradoxic bradycardia during inferior vena cava occlusion or to shift the dose of isoproterenol needed to cause paradoxic bradycardia during inferior vena cava occlusion, we concluded that A1 receptors were probably the basis for the reflex, because DPCPX, a selective A1 antagonist, blocked the reaction. The different conclusions from the CPA and DPCPX experiments may reside in a difference between exogenous adenosine (CPA) and endogenously released adenosine, as reported by other investigators.27 Further experiments are needed to address the difference between exogenous A1 agonist (CPA) and endogenous adenosine released in our experimental model.

These experiments were carried out during anesthesia, which may alter reflex activity. Future experiments in awake animals are needed to address this issue.

Conclusions

A reduced cardiac volume combined with β1-adrenergic stimulation (isoproterenol) stimulates paradoxic bradycardia in the rat. Augmenting extracellular adenosine by dipyridamole markedly increased the bradycardia-isoproterenol dose response. Blocking the adenosine receptor by aminophylline, a nonselective, or DPCPX, a selective A1 antagonist inhibited the reaction. Therefore, drugs that increase adenosine concentration or block the adenosine receptors may increase or inhibit paradoxic bradycardia, respectively. These findings may have relevance to other animal models of vasodepressor reaction.

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