Vasodepressor Reaction Induced by Inferior Vena Cava Occlusion and Isoproterenol in the Rat
Role of \( \beta_1 \) - and \( \beta_2 \)-Adrenergic Receptors

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**Background** Testing for the susceptibility of vasodepressor reaction in humans involves the combination of restriction of venous return by passive upright tilting and the administration of isoproterenol. We developed an experimental rat model in which vasodepressor reactions are induced when the inferior vena cava is occluded during an infusion of isoproterenol. The reactions are characterized by the development of paradoxical bradycardia during the period of inferior vena cava occlusion.

**Methods and Results** Inferior vena cava occlusion was performed for 60 seconds, and the maximal changes in RR interval were measured during seven states as follows: (1) when inferior vena cava occlusion was performed under control conditions in 40 rats, the rate accelerated in all 40 rats (ARR, \(-15.6 \pm 1.9 \) milliseconds in 25 rats, \( P < .001 \); \( \Delta RR, -13.3 \pm 1.7 \) milliseconds in 10 rats, \( P < .001 \)); (2) when inferior vena cava occlusion was performed in 25 rats during an infusion of isoproterenol, a vasodepressor reaction was observed in all rats as the heart rate slowed (ARR, \(+92.7 \pm 8.3 \) milliseconds, \( P < .001 \)); (3) when inferior vena cava occlusion was performed in 10 rats during an infusion of dobutamine, a selective \( \beta_1 \)-agonist, a vasodepressor reaction was observed in all rats as the heart rate slowed (ARR, \(+63.3 \pm 10.6 \) milliseconds, \( P < .001 \)); (4) when inferior vena cava occlusion was performed in 5 rats during an infusion of salbutamol, a selective \( \beta_2 \)-agonist, vasodepressor reaction was not observed as the heart rate accelerated in all rats (ARR, \(-11.4 \pm 2.8 \) milliseconds, \( P < .002 \)); (5) the vasodepressor reaction induced by either dobutamine or isoproterenol was inhibited by atenolol, a selective \( \beta_1 \)-adrenergic receptor antagonist; (6) the vasodepressor reaction induced by isoproterenol was inhibited by propranolol (lipophilic) and sotalol (nonlipophilic) \( \beta \)-blockers and there was a dose-dependent attenuation by propranolol of the maximal RR interval slowing during inferior vena cava occlusion; and (7) butoxamine, a selective \( \beta_2 \)-adrenergic receptor antagonist, attenuated but did not block the vasodepressor reaction observed during an infusion of isoproterenol.

**Conclusions** Reduced cardiac volume combined with \( \beta_1 \)-adrenergic stimulation can stimulate a vasodepressor reaction in rats. \( \beta_2 \)-Adrenergic receptors play little or no role in the reaction. The vasodepressor reaction can be blocked by selective or nonselective \( \beta_1 \)-adrenergic antagonists independent of the drug's ability to penetrate the central nervous system. The application of these findings to humans remains to be elucidated. (Circulation. 1994;89:2401-2411.)

**Key Words** • vasovagal nerve • \( \beta \)-adrenergic receptors • cardiac volume

We recently developed a laboratory test for the induction of vasodepressor reactions in humans. The test consists of the administration of isoproterenol during passive upright tilting. In 70% of patients who suffer from spontaneous syncopal episodes manifested as vasodepressor reactions, upright tilting combined with isoproterenol administration reproduces the signs and symptoms of a vasodepressor reaction. The striking features seen are the development of bradycardia and hypotension and symptoms of presyncope or syncope. By contrast, passive upright tilting alone in the same patients for as long as 15 minutes did not provoke vasodepressor reactions. These observations have been confirmed by others.

To advance our knowledge of the physiology of this new clinical test, we developed a model of the vasodepressor reaction in the rats. In these experiments, vasodepressor reactions are induced by inferior vena cava occlusion during an infusion of isoproterenol. The reactions are characterized by paradoxical bradycardia during superior vena cava occlusion. The paradoxical bradycardia depends on an afferent signal originating in the heart that is carried centrally by the vagus nerves but is independent of efferent vagal tone, because muscarinic receptor blockade with atropine did not affect the bradycardic response. The slowing is secondary to a reflex withdrawal sympathetic tone, because the bradycardia is inhibited when efferent sympathetic tone is interrupted by surgical or chemical sympathectomy.

Because prolonged upright tilting alone or tilting in combination with isoproterenol can induce vasodepressor reaction in humans and because these interventions activate adrenergic receptors, \( \beta \)-adrenergic antagonists have been used to inhibit spontaneous vasodepressor reaction in humans. Because adrenergic tone plays an important role in human vasodepressor reactions as well as in our rat model, studies were undertaken with two primary objectives: (1) we investigated the \( \beta_1 \) - and \( \beta_2 \)-adrenergic receptor components of the vasodepressor reaction in this model by using selective agonists and antagonists, and...
(2) because isoproterenol might act in the central nervous system, we assessed whether \(\beta\)-adrenergic receptor blockers that penetrate the central nervous system freely and that penetrate the central nervous system poorly are able to inhibit this reaction.16,17

In the present study, we used isoproterenol as a nonselective \(\beta\)-adrenergic agonist,18,19 dobutamine as a selective \(\beta_1\)-adrenergic agonist,19,20 and salbutamol as a selective \(\beta_2\)-adrenergic agonist.21 Atenolol was used as a selective \(\beta_1\)-adrenergic antagonist17,22; propranolol and sotalol were used as nonselective \(\beta\)-adrenergic antagonists, the former penetrating the central nervous system readily and the latter penetrating the central nervous system poorly16,17; and butoxamine was used as a selective \(\beta_2\)-adrenergic antagonist.23-25

Methods

Adult male Wistar rats (368.4±28 g) were used in these studies. The methods used have been extensively described.26 Two animals were anesthetized with intraperitoneal inactin, paralyzed with intravenous pancuronium, and ventilated at a rate of 70 min\(^{-1}\) through a tracheostomy with a constant-volume respirator (model 680, Harvard Apparatus). The expiratory tube was submerged under water to provide a constant end-expiratory resistance of 2 to 5 cm. The respirator's tidal volume was adjusted using a standard nomogram (Harvard Apparatus). The animal was placed on a warming plate, and the core temperature was maintained at 37±0.5°C. A Silastic cannula was inserted into the carotid artery and attached to a transducer (P23db, Statham) for blood pressure measurement. Another Silastic cannula was inserted into the jugular vein for fluid and drug administration. The thorax was opened through a low sternotomy, and thin umbilical tape was passed around the inferior vena cava just as it enters the chest for periodic occlusion. The following signals were recorded on a polygraph (model 7D, Grass) and stored on magnetic tape (model 101, Honeywell): surface ECG lead, blood pressure, beat-to-beat RR interval, and event marker. In each animal, the inferior vena cava was occluded for 60 seconds, and this was done twice. The sequential occlusions were separated by a rest period of 5 minutes.

A total of 40 rats were used in these experiments, and they underwent inferior vena cava occlusion as follows: (1) 40 rats under control conditions; (2) 25 rats during an infusion of isoproterenol; of these 25 rats, 10 were also studied after receiving propranolol, 5 were studied after receiving atenolol, 5 were studied after receiving sotalol, and 5 were studied after receiving butoxamine; (3) 10 rats were studied during an infusion of dobutamine; of these 10 rats, 5 animals were also studied after receiving atenolol; and (4) 5 rats were studied during an infusion of salbutamol.

Protocol

Thirty minutes after the surgical preparation was completed, the following experiments were performed.

Induction of Vasodepressor Reaction by Dobutamine and Its Comparison to Induction of Vasodepressor Reaction by Isoproterenol

Dobutamine. The inferior vena cava was occluded for 60 seconds during control conditions twice in each animal (n=10 rats). After control measurements, dobutamine was infused. In five experiments, the infusion was started at a rate of 1 \(\mu\)g \cdot min\(^{-1}\), and the dosage was increased to 2.5, 5.0, or a maximum of 10 \(\mu\)g \cdot min\(^{-1}\). The end point sought was RR interval prolongation (≥20 milliseconds) during inferior vena cava occlusion. In five other experiments, the dobutamine infusion was started at 5 \(\mu\)g \cdot min\(^{-1}\), and if the RR interval did not prolong (≥20 milliseconds) during inferior vena cava occlusion, the dose infused was doubled. Dobutamine was infused for 10 minutes before inferior vena cava occlusion was performed.

Isoproterenol. Twenty-five additional rats were studied before and during an infusion of isoproterenol. The inferior vena cava was occluded for 60 seconds during control conditions twice in each animal (n=25 rats). After control measurements, isoproterenol was infused starting at a rate of 0.25 \(\mu\)g \cdot min\(^{-1}\), and this was doubled until the RR interval prolonged (≥20 milliseconds) during inferior vena cava occlusion (0.8±0.13 \(\mu\)g \cdot min\(^{-1}\); range, 0.25 to 1.0 \(\mu\)g \cdot min\(^{-1}\)).

Induction of Vasodepressor Reaction by Salbutamol

The inferior vena cava was occluded for 60 seconds during control conditions twice in each animal (n=5). After these measurements, salbutamol was infused at a rate of 2 to 4 \(\mu\)g \cdot min\(^{-1}\) (2.4±0.4 \(\mu\)g \cdot min\(^{-1}\)). The dose selected reduced the mean arterial blood pressure from 128.6±5.4 to 100.2±8.4 mm Hg. The reduction in mean arterial blood pressure was the same as that seen during an infusion of isoproterenol. The inferior vena cava was occluded for 60 seconds twice during the infusion of salbutamol.

Inhibition of Vasodepressor Reaction by Atenolol

Isoproterenol. The inferior vena cava was occluded during an infusion of isoproterenol in 5 rats, as outlined above, until the RR interval prolonged (≥20 milliseconds) during inferior vena cava occlusion (0.7±0.12 \(\mu\)g \cdot min\(^{-1}\); range, 0.25 to 1.0 \(\mu\)g \cdot min\(^{-1}\)). After this, the animals received atenolol as a bolus of 1 \(\mu\)g IV, and this was sequentially doubled to 2, 4, 8, and 16 \(\mu\)g. The end point was inhibition of the RR interval prolongation (≥20 milliseconds) during inferior vena cava occlusion. Inferior vena cava occlusion was repeated twice during the isoproterenol infusion.

Dobutamine. The inferior vena cava was occluded twice during an infusion of dobutamine in 10 rats as outlined above. After this, 5 animals received atenolol starting at 2 \(\mu\)g, and this was sequentially doubled up to a maximum of 16 \(\mu\)g or until the RR interval did not prolong during inferior vena cava occlusion. The inferior vena cava occlusion was repeated twice after each dose of atenolol while the dobutamine infusion continued.

Inhibition of Vasodepressor Reaction by Butoxamine

The inferior vena cava was occluded during an infusion of isoproterenol (0.8±0.12 \(\mu\)g \cdot min\(^{-1}\); range, 0.5 to 1.0 \(\mu\)g \cdot min\(^{-1}\)) in 5 rats. At this dose, the RR interval prolonged (≥20 milliseconds) during inferior vena cava occlusion. After basal observations, \(\beta_1\)-adrenergic receptor blockade was achieved by the intravenous administration of butoxamine (0.4±0.2 mg; range, 0.25 to 0.5 mg). After this, the inferior vena cava was occluded while the isoproterenol was continued. After these observations, another dose of butoxamine was given to make a total dose of 1.0 mg in each rat, and inferior vena cava occlusion was repeated. Once these observations were completed, 8 \(\mu\)g atenolol was administered, and inferior vena cava occlusion was repeated.

Inhibition of Vasodepressor Reaction by Propranolol and Sotalol

Propranolol. The inferior vena cava was occluded during an infusion of isoproterenol (range, 0.5 to 1.0 \(\mu\)g \cdot min\(^{-1}\)) in 10 rats. After this, the animals received propranolol (a lipophilic \(\beta\)-adrenergic receptor antagonist that readily penetrates the central nervous system), and the inferior vena cava occlusion was repeated while the isoproterenol infusion was ongoing. The initial dose of propranolol was 2.5 \(\mu\)g, and this was doubled sequentially until the paradoxical bradycardia during inferior vena cava occlusion was blocked. From this point on,
the RR interval shortened during inferior vena cava occlusion. Consequently, 1.0 mg atropine sulfate IP was administered to produce muscarinic receptor blockade, so that subsequent rate acceleration during inferior vena cava occlusion could be attributed to alterations in sympathetic tone rather than reflex alterations in vagal tone. After inhibition of the paradoxical bradycardia during inferior vena cava occlusion by propranolol, additional doses of propranolol were administered until there was no further increase in heart rate during inferior vena cava occlusion.

Sotalol. The inferior vena cava was occluded during an infusion of isoproterenol (0.8±0.12 μg · min⁻¹; range, 0.5 to 1.0 μg · min⁻¹) in 5 rats. After this, the animals received sotalol (16.0±2.4 μg; range, 10 to 20 μg · min⁻¹), a nonlipophilic β-adrenergic receptor antagonist that penetrates the central nervous system to a very limited degree, and the inferior vena cava occlusion was repeated while the isoproterenol was ongoing.

Data Analysis

The RR interval was measured on-line by a microcomputer system as previously described. The output of the ECG amplifier triggered another amplifier, which generated positive square-wave pulses. These were fed into an interval-measuring circuit that produced a DC voltage proportional to the RR interval. The measuring accuracy of this system is ±0.5 millisecond. The DC voltage was entered into analog-digital converters (GW Instruments) and processed by a microcomputer (model SE, Macintosh). The DC voltage is sampled every 20 milliseconds, and after 50 samples, the computer generates and stores 1-second averages. The analog signal from the amplifier receiving the blood pressure signal from the transducer was similarly digitized, and the voltage is sampled every 20 milliseconds. After 50 samples, the computer produces an average blood pressure for each second. When the data are analyzed, the 1-second samples of RR interval and blood pressure are averaged into 5-second segments to cover the control period and the period of inferior vena cava occlusion. The data measured during the 10 seconds before inferior vena cava occlusion are averaged, and a single value is calculated for the resting RR interval and blood pressure. Data were measured during the 60 seconds of inferior vena cava occlusion and for an additional 20 seconds. The maximal change (increase or decrease) during the 60-second period of inferior vena cava occlusion was measured. An analog-marking pulse defined the beginning and end of the control period as well as the period of inferior vena cava occlusion. This signal also provided the timing needed for the computer analysis.

Statistical Analysis

The Student's t test for paired and unpaired data compared resting RR intervals within each group of experiments. During inferior vena cava occlusion, the maximal change in RR interval from control was measured in each group of experiments. The Student's t test for paired data compared the RR interval during resting conditions with the maximal change during inferior vena cava occlusion in each group of experiments. Dunnett's test (treatment against isoproterenol alone) compared the change in RR interval during inferior vena cava occlusion in the different groups. A linear regression analysis calculated the relation between the dose of administered propranolol and the resting RR interval. A similar regression analysis calculated the relation between the subthreshold to the threshold dose of propranolol (threshold defined as the dose that blocked the paradoxical bradycardia during inferior vena cava occlusion) and the maximal changes in RR interval during inferior vena cava occlusion. A one-way ANOVA compared the changes in RR interval in response to inferior vena cava occlusion after subsequent doses of propranolol (doses above threshold). A one-way ANOVA compared the maximal blood pressure decrease during inferior vena cava occlusion in the different states. A value of P<.05 was considered significant. The methods used in these experiments conformed to the 1984 guidelines on animal use by the American Heart Association. The protocols were reviewed and approved by the Animal Care Committee of the University of Toronto.

Results

Effects of Different Drugs on Resting Blood Pressure and Effects of Inferior Vena Cava Occlusion on Blood Pressure

During the control state and under resting conditions, the mean blood pressure was 132.7±3.8 mm Hg (n=40 rats). During an infusion of isoproterenol, the mean blood pressure decreased to 94.9±2.8 mm Hg (n=25 rats, P<.001 compared with control state, unpaired Student's t test). During an ongoing infusion of isoproterenol, the addition of propranolol (n=10 rats), sotalol (n=5 rats), atenolol (n=5 rats), and butoxamine (n=5 rats) resulted in a mean blood pressure that ranged from 94.9±2.8 to 105.3±5.1 mm Hg (P=NS, ANOVA). During an infusion of dobutamine, the resting mean blood pressure was 119.8±4.5 mm Hg (n=10 rats, P<.01 compared with control state, unpaired Student's t test). The decrease in blood pressure during dobutamine was less than the decrease during an infusion of isoproterenol (P<.01, unpaired Student's t test).

Inferior Vena Cava Occlusion During Control Conditions

In the entire group of 40 rats, the RR interval at rest was 144.3±3.7 milliseconds. During inferior vena cava occlusion, the RR interval shortened by a maximum of 11.4±1.9 milliseconds (paired Student's t test, P<.001).

Inferior Vena Cava Occlusion During Infusion of Dobutamine and Its Comparison to Inferior Vena Cava Occlusion During Infusion of Isoproterenol Before and After Atenolol

During the infusion of dobutamine (7.5±0.8 μg · min⁻¹), the resting RR interval shortened (144.3±3.7 to 126.4±4.2 milliseconds, n=10 rats, P<.001; see Fig 1 and Table 1, A). An infusion of isoproterenol (0.70±0.12 μg · min⁻¹) shortened the resting RR interval (147.6±1.9 to 124.0±1.8 milliseconds, P<.001; see Fig 1 and Table 2). As in the case of isoproterenol, inferior vena cava occlusion during dobutamine caused the RR interval to lengthen gradually initially, and then it prolonged abruptly. When the inferior vena cava occlusion was released, the RR interval returned to its original value. The time course of the RR interval prolongation during inferior vena cava occlusion was very similar for dobuta-
In five other paired experiments, in which the rats received dobutamine, treatment with atenolol lengthened the resting RR interval by a small but significant amount (121.7±1.7 to 136.0±5.4 milliseconds, P<.05), and blood pressure was unchanged (P=NS). After pretreatment with atenolol (4.6±2.2 µg), inferior vena cava occlusion caused no significant change in RR interval (+3.2±1.5 milliseconds, P=NS compared with resting value) but a highly significant difference compared with isoproterenol alone (P<.001; see Table 2).

In five other paired experiments, in which the rats received dobutamine, treatment with atenolol lengthened the resting RR interval (128.7±2.7 to 147.9±7.6 milliseconds, P<.05), and blood pressure was unchanged. As in the case of isoproterenol, atenolol completely eliminated the RR interval prolongation seen during the combination of dobutamine and inferior vena cava occlusion (−0.3±1.7 milliseconds, P=NS; see Table 1, A).

**Inferior Vena Cava Occlusion During Infusion of Salbutamol and After Butoxamine**

In five experiments, salbutamol did not change the resting RR interval (158.5±6.1 versus 147.6±3.6, P=NS; see Table 1, B). However, mean arterial blood pressure was significantly reduced (128.0±5.4 versus 100.2±8.4 mm Hg, P<.001), and this reduction in pressure was similar to that observed during an infusion of isoproterenol (P=NS, ANOVA). During inferior vena cava occlusion, the RR interval shortened by a maximum of 17.3±3.1 (P<.001) in control, and this is slightly increased compared with the maximal RR interval shortening after salbutamol (−11.4±2.8 milliseconds, P<.05; see Table 1, B).

In five paired experiments in which the rats received an infusion of isoproterenol, treatment with 0.4 mg butoxamine (dose A) caused a small but significant prolongation of the basal RR interval (123.3±0.5 to 132.5±2.2 milliseconds, P<.001), but blood pressure did not change (99.2±2.8 versus 94.9±2.8 mm Hg, P=NS). After butoxamine, the maximal RR interval prolonged significantly during inferior vena cava occlusion (63.1±13.2 milliseconds, P<.001), and although this was less, it was not significantly different from the RR interval prolongation during inferior vena cava occlusion.

**Table 1.** Effects of Dobutamine, Dobutamine and Atenolol, and Salbutamol on Heart Rate Response During Inferior Vena Cava Occlusion

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=10)</td>
<td>Dobutamine (n=10)</td>
</tr>
<tr>
<td>Resting RR interval, ms</td>
<td>144.3±3.7</td>
<td>128.4±4.2</td>
</tr>
<tr>
<td>Maximal change in RR interval during IVC occlusion, ms</td>
<td>−13.4±1.7</td>
<td>+63.3±10.6</td>
</tr>
<tr>
<td>*P value for rest versus IVC occlusion (paired Student's t test)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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</tbody>
</table>

* Dobutamine vs control A, and salbutamol vs control B (paired Student's t test).
† Dobutamine+atenolol vs dobutamine alone using five paired experiments (paired Student's t test).

Section A describes the resting RR interval and the maximal change in RR interval during inferior vena cava (IVC) occlusion for 60 seconds during control, during the administration of dobutamine, and during the combination of dobutamine and atenolol. A paired Student's t test compared the resting RR interval with the maximal change in RR interval during IVC occlusion in each state. The 10 dobutamine experiments were compared with the 10 control experiments, using a paired Student's t test. The five experiments that used dobutamine and atenolol were compared with their five paired experiments that used dobutamine alone. Section B describes the resting RR interval as well as the maximal change in RR interval during IVC occlusion during control and after salbutamol.
TABLE 2. Effects of Isoproterenol Alone and Isoproterenol Combined With Nonselective β₁, Selective β₁, and Selective β₂-Adrenergic Receptor Antagonists on Heart Rate Response During Inferior Vena Cava Occlusion

<table>
<thead>
<tr>
<th></th>
<th>1: Control</th>
<th>2: Isoproterenol</th>
<th>3: Isoproterenol + Propranolol</th>
<th>4: Isoproterenol + Sotalol</th>
<th>5: Isoproterenol + Atenolol</th>
<th>6: Isoproterenol + Butoxamine (A)</th>
<th>7: Isoproterenol + Butoxamine (B)</th>
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<tr>
<td></td>
<td>(n=25)</td>
<td>(n=25)</td>
<td>(n=10)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Resting RR interval, ms</td>
<td>147.6±1.9</td>
<td>124.0±1.8</td>
<td>149.9±5.2</td>
<td>128.2±7.8</td>
<td>136.0±5.4</td>
<td>132.5±2.2</td>
<td>133.6±4.6</td>
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<tr>
<td>Maximal change</td>
<td>149.9±5.2</td>
<td>128.2±7.8</td>
<td>136.0±5.4</td>
<td>132.5±2.2</td>
<td>133.6±4.6</td>
<td>132.5±2.2</td>
<td>133.6±4.6</td>
</tr>
<tr>
<td>RR interval during IVC occlusion, ms</td>
<td>-15.6±1.6</td>
<td>-92.7±8.3</td>
<td>-15.7±3.9</td>
<td>-2.8±1.9</td>
<td>-3.2±1.5</td>
<td>-3.2±1.5</td>
<td>-3.2±1.5</td>
</tr>
<tr>
<td>P value for rest RR versus IVC occlusion (paired Student's t test)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>NS</td>
<td>NS</td>
<td>&lt;.001</td>
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</tr>
</tbody>
</table>

Butoxamine (A), dose 0.4 mg; butoxamine (B), dose 1.0 mg.
*Isoproterenol alone vs control, paired Student's t test.
†Columns 3 through 7 compared with isoproterenol alone (column 2) using paired experiments, paired Student's t test.
‡Compared with isoproterenol alone (column 2), Dunnett's test.

shown when the inferior vena cava occlusion was performed during isoproterenol alone (see Table 2). After a much larger dose of butoxamine (1.0 mg; dose B), the resting RR interval was unaltered. During inferior vena cava occlusion, the RR interval still prolonged significantly (36.7±4.8 milliseconds), but this was significantly reduced compared with isoproterenol alone (P<.02, see Table 2). After this, atenolol was administered, and occlusion of the inferior vena cava no longer caused RR interval prolongation.

Inferior Vena Cava Occlusion During Infusion of Isoproterenol After Propranolol or Sotalol

In 10 paired experiments in which the rats received an infusion of isoproterenol, the additional administration of 10 μg propranolol prolonged the resting RR interval (124.6±1.4 to 149.7±5.2 milliseconds, P<.001). However, blood pressure did not change. Under these conditions, inferior vena cava occlusion did not cause RR interval prolongation; instead, the RR interval shortened significantly (−15.7±3.9 milliseconds, P<.001; see Table 2).

In five paired experiments in which the rats received an infusion of isoproterenol, the additional administration of sotalol prolonged the resting RR interval (123.8±7.1 to 128.0±7.8 milliseconds, P<.05). However, blood pressure did not change. Inferior vena cava occlusion caused a minor decrease in RR interval (−2.8±1.9 milliseconds, P=NS), but this was, of course, highly significant compared with isoproterenol alone (P<.001; see Table 2).

With linear regression analysis, there was a significant dose-dependent increase in the resting RR interval in response to propranolol doses as follows: [resting RR interval = 0.282 (propranolol dose) + 143.4] (r=.757, P<.001; see Table 3). Again, with linear regression analysis, during different doses of propranolol there was a significant dose-dependent attenuation of the RR interval prolongation observed during the combination of inferior vena cava occlusion and isoproterenol as follows: [change in RR = −11.5 (dose of propranolol) + 91.0] (r=.831, P<.001; see Table 3). After 10 μg propranolol, inferior vena cava occlusion no longer caused RR interval prolongation but instead caused RR interval shortening that was identical to the RR interval shortening seen during inferior vena cava occlusion before the administration of any drugs (see Fig 2). After the 10-μg dose of propranolol, 1.0 mg atropine was given to block any RR interval changes that could be due to alterations in vagal tone.20 Incremental doses of propranolol progressively raised the resting RR interval, and during subsequent occlusions of the inferior vena cava, the RR interval shortened, but the extent of shortening was not different until a dose of 320 μg propranolol, at which point the RR interval shortening became minimal during inferior vena cava occlusion (see Table 3). Thus, it is obvious that although a dose of 10 μg propranolol was sufficient to block the paradoxical bradycardia during inferior vena cava occlusion, this dose and subsequent doses up to and including 160 μg did not fully block adrenergic receptors in the heart, because heart rate accelerated in response to inferior vena cava occlusion after 10 μg propranolol. It is also notable that after a large dose of propranolol (≥160 μg), the resting RR interval prolonged to levels that are
TABLE 3. Effects of Incremental Doses of Propranolol on Effects of Inferior Vena Cava Occlusion During Infusion of Isoproterenol

<table>
<thead>
<tr>
<th></th>
<th>1: Control (n=10)</th>
<th>2: Isoproterenol Alone (n=10)</th>
<th>3: Isoproterenol+ Propranolol 2.5 µg (n=10)</th>
<th>4: Isoproterenol+ Propranolol 5.0 µg (n=10)</th>
<th>5: Isoproterenol+ Propranolol 10 µg (n=10)</th>
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<tbody>
<tr>
<td>Resting RR interval, ms</td>
<td>153.6±3.3</td>
<td>124.6±1.4 <strong>P&lt;.001</strong></td>
<td>132.3±4.9</td>
<td>138.2±5.2</td>
<td>149.7±5.2</td>
</tr>
<tr>
<td>Maximal change in RR interval during IVC occlusion, ms</td>
<td>-17.6±1.3</td>
<td>+96.0±8.9 <strong>§</strong></td>
<td>+45.2±13.4</td>
<td>+26.4±11.2</td>
<td>-15.7±3.9</td>
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<tr>
<td>P value for rest versus IVC occlusion (paired Student's t test)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.05</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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</tbody>
</table>

*Isoproterenol alone vs control, paired Student's t test.
†ANOVA, P=NS.
§ANOVA, P<.05.
¶Regression analysis propranolol dose vs resting RR interval (columns 2 through 10); r=.757, P<.001 (see text).
‖Regression analysis propranolol dose vs maximal change in RR interval during inferior vena cava occlusion (columns 2 through 5); r=.831, P<.001 (see text).

Shown are the resting RR interval and the maximal change in RR interval during inferior vena cava (IVC) occlusion for 60 seconds, during control conditions, with isoproterenol alone, and after the combination of isoproterenol and increasing doses of propranolol, starting at 2.5 µg and sequentially doubled up to a maximum of 320 µg. A paired Student's t test compared the resting RR interval with the maximal RR interval change during IVC occlusion in each state. A regression analysis compared the dose of propranolol with the resting RR interval for columns 2 through 10. Another regression analysis compared the dose of propranolol with the change in RR interval for columns 2 through 5. An ANOVA compared the extent of change of RR interval between columns 6 and 10.

Fig 2. Plot of the mean RR interval (R-R) every 5 seconds before, during, and after inferior vena cava (IVC) occlusion. Graph shows the data during the following conditions: control (no drug), during an infusion of isoproterenol (0.75±0.15 µg·min⁻¹), and during the combination of isoproterenol (same dose) and incremental doses of propranolol (2.5 to 320 µg). For purposes of clarity, this figure contains the responses of a representative number of propranolol doses (2.5, 10, 160, and 320 µg). Not shown are the responses to 5.0, 20, 40, and 80 µg. Also, the SEM values have been omitted in the interest of visual clarity. It should be noted that after the response to 10 µg propranolol, the rats received 1 mg atropine to effect full muscarinic blockade.

Discussion

Results of the present study reveal that temporary occlusion of the inferior vena cava in rats during an infusion of isoproterenol or dobutamine causes paradoxical heart rate slowing. By contrast, under control conditions, the RR interval shortens during inferior vena cava occlusion. Heart rate acceleration is the expected physiological response to baroreceptor deactivation during hypotension, whereas rate slowing in the face of hypotension is the hallmark of a vasodepressor reaction clinically and experimentally. Previous
TABLE 3. Continued

<table>
<thead>
<tr>
<th>Column</th>
<th>Isoproterenol+ Propranolol 20 μg (n=10)</th>
<th>Isoproterenol+ Propranolol 40 μg (n=10)</th>
<th>Isoproterenol+ Propranolol 80 μg (n=10)</th>
<th>Isoproterenol+ Propranolol 160 μg (n=6)</th>
<th>Isoproterenol+ Propranolol 320 μg (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:</td>
<td>160.4±6.9</td>
<td>165.5±8.1</td>
<td>176.5±10.5</td>
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<td>223.2±7.6</td>
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<tr>
<td>7:</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
</tr>
<tr>
<td>8:</td>
<td>-20.9±4.1</td>
<td>-24.6±2.6</td>
<td>-23.9±3.9</td>
<td>-19.7±6.1</td>
<td>-5.2±2.1</td>
</tr>
<tr>
<td>9:</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

or no role. The findings favoring this conclusion are as follows. (1) Selective β₁-adrenergic receptor stimulation by dobutamine induces the vasodepressor reaction. (2) Selective β₂-adrenergic receptor stimulation by salbutamol does not induce the reaction. (3) Selective β₁-adrenergic receptor blockade by atenolol inhibits the isoproterenol- or dobutamine-induced vasodepressor reaction. (4) Selective β₂-adrenergic receptor blockade by butoxamine does not inhibit the isoproterenol-induced vasodepressor reaction. Assuming that isoproterenol induces a vasodepressor reaction in the rat by acting on the heart, the lack of a significant role for the β₂-adrenergic receptor may reflect the small number of these receptors compared with β₁-adrenergic receptors in the rat heart.²⁴

However, one cannot completely dismiss a role for β₂-adrenergic receptors in the isoproterenol-induced paradoxical bradycardia for the following reasons. First, the maximal RR interval prolongation with dobutamine was less than that with isoproterenol. Second, a higher dose of butoxamine attenuated the RR interval prolongation during inferior vena cava occlusion. These findings could reflect the existence of β₂-receptors in the rat.

![Fig 3](image3.png)

**Fig 3.** Bar graph of summary of the maximal RR interval (R-R) change in response to inferior vena cava occlusion during control conditions, during isoproterenol (0.75±0.15 μg·min⁻¹), and during the combination of isoproterenol (same dose) and incremental doses of propranolol (2.5 to 320 μg). The significance of these changes is shown in Table 3.

![Fig 4](image4.png)

**Fig 4.** Bar graph of summary of the maximal RR interval (R-R) changes in response to inferior vena cava occlusion during various states. Left, Responses to inferior vena cava occlusion during (1) control state, (2) isoproterenol, (3) isoproterenol and propranolol, (4) isoproterenol and sotalol, (5) isoproterenol and atenolol, (6) isoproterenol and butoxamine dose A, and (7) isoproterenol and butoxamine dose B. Middle, Responses to inferior vena cava occlusion during (8) control conditions, (9) dobutamine, and (10) dobutamine and atenolol. Right, Response during (11) control and (12) salbutamol. The significance of these changes is shown in Tables 1 through 3.
heart that subserve specialized functions associated with the activation of cardiac vagal afferents. Alternatively, high doses of butoxamine may block β1-adrenergic receptors and therefore reduce the effect of isoproterenol. Nevertheless, the difference in the ratio of β1 to β2-adrenergic receptors in rat hearts compared with human hearts limits our ability to transfer the present results to humans.

The site of action of isoproterenol in the experimental vasodepressor reaction or in the human vasodepressor reaction is not known. It is widely assumed that isoproterenol elicits a vasodepressor reaction through its effects on the heart, where it contributes to the activation of cardiac vagal afferents. However, other possible sites of action have not been considered. In this regard, it is notable that vasodepressor reactions can develop in humans who have undergone cardiac transplantation. Because transplant patients are believed to be denervated, the development of vasodepressor reaction in these patients suggests that an extra cardiac site may trigger the reaction. Thus, the central nervous system could be proposed as the site of action by isoproterenol in causing the paradoxical bradycardia during inferior vena cava occlusion. In this regard, the withdrawal of sympathetic tone during hemorrhage-induced vasodepressor reactions in rabbits is mediated by centrally released opiates.

We tested for a possible central site of action of isoproterenol indirectly by using β-adrenergic receptor antagonists with different abilities to penetrate the central nervous system. The blood-brain barrier acts like a lipid membrane, and the ability of β-adrenergic antagonists to penetrate the brain correlates very well with the drug’s lipid solubility in vitro. Propranolol, a nonselective β-adrenergic antagonist, is highly lipid soluble and thus readily penetrates the central nervous system, whereas sotalol, a nonspecific β-adrenergic receptor blocker, has low fat solubility, and therefore its penetration into the central nervous system is minimal. Atenolol, a selective β1-adrenergic receptor antagonist, is not fat soluble and therefore penetrates the brain minimally.

Despite the differences in central nervous system penetration between propranolol and sotalol on the one hand and atenolol on the other hand, all these agents completely inhibited the vasodepressor reaction. In addition, atenolol, a β1-selective drug, and propranolol or sotalol, nonselective β-adrenergic blockers, were equally effective at inhibiting the paradoxical bradycardia during inferior vena cava occlusion. These findings support the conclusion that isoproterenol is not acting in the brain. This agrees with other evidence that isoproterenol crosses the blood-brain barrier minimally. Isoproterenol and epinephrine increase the cyclic AMP concentration in the brain of 7-day-old rats but not in mature rats. Thus, isoproterenol does not penetrate the mature rat brain. The administration of isoproterenol into a carotid artery in humans also reveals minimal uptake of isoproterenol by the brain.

With the same model, previous research showed that inferior vena cava occlusion during an infusion of isoproterenol causes marked paradoxical bradycardia. The bradycardia was unaffected by pretreatment with atropine but was blocked by bilateral vagotomy or intrapericardial lidocaine. This suggested that the bradycardia was due to a cardiac signal carried centrally by the vagus nerves, which then leads to a reflex withdrawal of sympathetic tone. In support of this, the paradoxical bradycardia was prevented by bilateral stellate ganglion removal, an intervention that slowed the resting rate to values very similar to those achieved by inferior vena cava occlusion. The role of the sympathetic nervous system was examined further as follows. (1) Chemical sympathectomy by 6-hydroxydopamine also blocked the paradoxical bradycardia. (2) Right stellate ganglion removal slowed the background heart rate, and because the sinus node is primarily innervated by the right stellate ganglion, there was no further rate slowing during inferior vena cava occlusion. (3) Left stellate ganglion removal did not change the resting heart rate, but it also blocked the paradoxical bradycardia. These findings were interpreted to be consistent with the concept that enhanced left ventricular contraction during inferior vena cava occlusion is potentiated by a reflex rise in sympathetic tone via the left stellate ganglion was an essential prerequisite for triggering the vasodepressor reaction.

The role of β-adrenergic blockers in preventing spontaneous human vasodepressor reaction has not been fully addressed. Some early reports indicate they are beneficial whereas other reports show limited or no benefit. Moreover, the mechanism of inhibition of the vasodepressor reaction by β-adrenergic blockers is unknown. Two mechanisms can be proposed. First, propranolol reduces the firing of cardiac vagal afferents, the fibers believed to be important in triggering vasodepressor reactions. Second, β-adrenergic blockade attenuates the reduction in cardiac volume during passive upright tilting in humans, a state believed to be a prerequisite for vasodepressor reaction during upright tilting in humans. β-Adrenergic blockers might complement the withdrawal of sympathetic tone that occurs during experimental or clinical vasodepressor reactions. It has been postulated that the vasodepressor reaction results from an excessively vigorous cardiac contraction around an empty left ventricular cavity. This could easily be the case in our experiments because the inferior vena cava is obstructed and the animal is receiving isoproterenol. Therefore, a reduction in sympathetic tone in this circumstance could reduce cardiac contractility, facilitate normalization of intraventricular pressures, abort the reaction, and promote cardiac filling.

One of the intriguing findings of this study is that very low doses of propranolol, atenolol, or sotalol prevented the reaction. These doses of β-adrenergic receptor-blocking drugs are clearly too small to antagonize all the actions of isoproterenol on the heart. This suggests that the receptors responsible for the vasodepressor reaction may have a much different sensitivity than the receptors governing contractility or heart rate. When sequential doses of propranolol were administered, not only was the paradoxical bradycardia during inferior vena cava occlusion blocked but also reflex tachycardia was observed during subsequent inferior vena cava occlusion. The reflex tachycardia at this stage occurred despite pretreatment with atropine, implying that it was secondary to increased sympathetic tone during the hypotension.
potensive stimulus provided by inferior vena cava occlusion.28 The occurrence of reflex tachycardia after propranolol pretreatment indicates that adrenergic receptor blockade was incomplete. This is not surprising, as the dose of propranolol that inhibited the paradoxical bradycardia during inferior vena cava occlusion was very small (10 μg total dose).

The ability of β-adrenergic antagonists to inhibit the vasodepressor reaction may be seen to be occurring at two levels—efferent and afferent. In response to high doses of propranolol, the resting heart rate slows to levels similar to those of the paradoxical bradycardia observed during inferior vena cava occlusion with isoproterenol alone (see Fig 2). At these high doses of propranolol, the effects of alterations in efferent sympathetic tone are nullified because the β-adrenergic receptors are blocked. Thus, high doses of propranolol act on the efferent side of the reflex. Low doses of propranolol affected the resting rate minimally and obviously did not block the β-adrenergic receptors strongly, yet this was sufficient to inhibit the vasodepressor reaction. At this dose level, we postulate that the afferent side of the vasodepressor reaction is blocked. The effects of low and high doses of propranolol can be compared with the separate effects of right and left stellotomy on the vasodepressor reaction in our previous experiment with this model.28

In contrast to vasodepressor reactions in other animal species30,31 and humans3 in which the bradycardia is due to increased efferent vagal tone, the bradycardia seen in our rat model is due to reflex withdrawal of sympathetic tone.2,7 In related experiments, pretreatment of rats with propranolol significantly reduced the paradoxical bradycardia during hemorrhage-induced vasodepressor reactions.7,8 The withdrawal of sympathetic tone as the cause of the bradycardia in our experiment is analogous to the reduction of sympathetic tone as the major cause of hypotension during experimental32,33,34 or human35 vasodepressor reactions.

In keeping with the present experiments, the sympathetic nervous system plays an essential role in the vasodepressor reaction. The classic causes of vasodepressor reaction in humans, such as pain, fear,39 orthostasis,9 or experimental hemorrhage58 increase sympathetic tone.51 Activation of the sympathetic nervous system maintains blood pressure in the face of hypovolemia due to hemorrhage in experimental animals or in simulated volume depletion in humans by tilting or lower body negative pressure. When volume depletion reaches a critical stage, there is a reversal of effect, and sympathetic tone is withdrawn.58 The reduction in sympathetic tone may, however, be quite specific to certain organ systems, because hemorrhage-induced vasodepressor reactions in rats is accompanied by a paradoxical decrease in renal sympathetic nerve activity and an increase in adrenal sympathetic nerve activity.50

As previously stated, it is postulated that the vasodepressor reaction during orthostasis or hemorrhage is initiated by ventricular vagal nerve endings called C-fibers.30,51 The empty hypercontractile left ventricle activates C-fibers, which, like the carotid sinus baroreceptors,28 respond to pressure changes, send afferent vagal impulses to the medulla, and initiate a reflex response characterized by reduced sympathetic tone and increased vagal tone.30,56 In addition to intramyocardial pressure, C-fibers are activated by catecholamines and sympathetic nerve stimulation,61-65 but their firing is reduced by β-adrenergic receptor antagonists.48,49 Numerous observations have shown that adrenergic stimulation combined with reduced cardiac volume excites cardiac C-fibers and initiates a vasodepressor reaction as follows. (1) Isoproterenol facilitates the development of vasodepressor reactions in humans during passive upright tilting.1 (2) Inferior vena cava occlusion in rats during an infusion of isoproterenol leads to dramatic paradoxical bradycardia mediated by cardiac vagal afferents.7,8 (3) In conscious dogs, the tachycardia response to temporary occlusion of the inferior vena cava is significantly reduced after intracoronary epinephrine secondary to activation of cardiac C-fibers by epinephrine.33 (4) Ultrasound studies during upright tilt-testing in humans support the view that syncope results from reduced cardiac volume interacting with vigorous cardiac contractility.56

Study Limitations

The present study was carried out in anesthetized rats, and therefore the reflex responses may have been altered significantly. It must be appreciated that the ratio of β1- to β2-adrenergic receptors is much different in the rat than in other mammals, including humans,34 and therefore one cannot extrapolate our findings to humans. Similar work should be repeated in larger mammals and humans. Simulation of hypovolemia by inferior vena cava occlusion is technically attractive, but the sudden obstruction of venous return differs from the more gradual decrease in venous return seen with hemorrhage or orthostasis. In addition, occlusion of the inferior vena cava may elicit certain reflexes from the viscera drained by the inferior vena cava. It would be useful to have a mechanism of grading the inferior vena cava occlusion. The β1 receptor is paramount in this model, in which isoproterenol or dobutamine provokes paradoxical bradycardia during inferior vena cava occlusion. Presumably, the β1-adrenergic receptor stimulation activates cardiac C-fibers. Recordings of cardiac C-fibers cardiac are needed to confirm their role in the vasodepressor reaction. Future experiments should also assess the role of the β1-adrenergic receptor in cases where C-fibers are activated by reflex or direct cardiac sympathetic stimulation.

Conclusions

A reduced cardiac volume combined with β1-adrenergic stimulation can stimulate a vasodepressor reaction in the rat. The β1-adrenergic receptors play little or no role in the reaction, which is characterized by paradoxical bradycardia in the face of marked hypotension. The vasodepressor reaction can be blocked by selective or nonselective β-adrenergic antagonists independent of the drug's ability to penetrate the central nervous system.

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