β-Adrenoceptors in vascular capacitance responses to unloading of carotid baroreceptors in anesthetized dogs

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Karim, F., and S. M. Poucher. β-Adrenoceptors in vascular capacitance responses to unloading of carotid baroreceptors in anesthetized dogs. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1713–H1718, 1997.—The role of β- and α-adrenoceptors in the total vascular capacitance responses to changing pressure in vascularly isolated carotid sinuses of anesthetized and atropinized dogs was investigated. A change in vascular capacitance was determined by measuring the shift of blood in and out of a reservoir that was connected to the aorta and maintained at a constant pressure. Changes in carotid sinus pressure from 135 to 57 mmHg and back to 137 mmHg resulted in a rapid vascular capacitance response of ~30 ml in the absence of adrenoceptor antagonists. Administration of a β2-adrenoceptor antagonist (ICI-118551) caused a significant enhancement of the capacitance responses to similar decreases and increases in carotid sinus pressure (~130%). Administration of a β1-adrenoceptor antagonist (CGP-20712A) did not cause any further enhancement of the responses. However, an α-blocker (phenolamine) reduced the responses by 75%. The results suggest that in the presence of a β2-adrenoceptor antagonist vascular capacitance responses to loading and unloading of baroreceptors are greatly enhanced and that patients suffering from orthostatic syncope may benefit from this kind of drug.

METHODS

Beagle dogs (male, 13–16 kg, n = 7; obtained from Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, UK) were anesthetized by thiopental sodium (Intraval sodium, 500 mg; May and Baker), followed by chloralose (0.1 g/kg; British Drug Houses) through a catheter placed via the right lateral saphenous vein under local anesthesia (2% xylocaine, Astra Pharmaceuticals) so that its tip lay in the inferior vena cava, as described previously (20). After the insertion of a cuffed tracheal tube, positive-pressure ventilation, with 40% oxygen in air, was started at a rate of 18 strokes/min and a stroke volume of ~17 ml/kg. During experiments, arterial blood gases and pH were measured (Corning 178 pH/blood gas analyzer, Corning Scientific Instruments, Medfield, MA) frequently and maintained within their normal limits by infusion of sodium bicarbonate and/or by altering the setting of the respiration pump and the flow of oxygen in inspired air.

Surgical procedure, cannulation, and hemodynamic measurements. Aortic pressure was measured through a cannula that was passed through the cardiac end of the left femoral artery. Right atrial pressure was measured through a cannula inserted via the left femoral vein. Pressures were recorded with Statham strain gauges (model P23 ID) connected to appropriate cannulas. Both carotid sinuses were vascularly isolated and perfused with arterial blood from the proximal end of the left common carotid artery at a constant flow as described previously (20). Carotid sinus pressure was regulated by altering the outflow resistance of the carotid sinus perfusion circuit (Fig. 1). Whole body capacitance responses were measured by connecting the animal to a graduated blood reservoir via the right common carotid (input into the reservoir) and femoral arteries (output from the reservoir; Fig. 1). To prevent stagnation of blood in the reservoir, blood was pumped to the top of the reservoir at a constant rate (MRHE pump, Watson Marlow) from the right common carotid artery. The mean pressure in the abdominal aorta was controlled by connecting it, via the right femoral artery, to the graduated blood reservoir maintained at a constant pressure by means of a Starling resistance and compressed air (see Fig. 1 and Ref. 15 for details). An electromagnetic flow probe (Gould Statham cannulating type,
ID 4 mm) was placed in the circuit between the reservoir and the femoral artery. A decrease in whole body vascular capacitance was measured as both an increase in the volume of blood in the reservoir (3) and a change in the signal from the flow probe. The flowmeter (model SP2202, Gould Statham) was calibrated at the end of the experiment using the dog's own blood.

Before connecting the perfusion circuits to the animals, we gave heparin (185 U/kg iv) followed by a continuous infusion into the carotid circuit at $1 \text{ U} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Perfusion circuits were primed with 40–50 ml of 50% dextran-saline, and returned to animal via heat exchanger (HE$_{1}$) and cannula connected to right femoral artery. Desired pressure was initially set in system by pressure bottle (PB) connected to mercury manometer (MM). When aortic pressure exceeded set level, flow of air through Starling resistance and outflow tube (OT), which was placed in water, was enhanced and thus pressure in control system returned to initial level. Blood flowed at constant pressure from arterial reservoir into animal via cannulating electromagnetic flow probe and cannula in femoral artery. CB, carotid body.

Experimental protocol. Three series of experiments were performed. All animals were given atropine sulfate (0.4 mg/kg iv) to prevent chronotropic effects of altered baroreceptor output and to minimize the contribution from cardiopulmonary stretch receptors. In the first series ($n = 7$), effects on vascular capacitance of lowering the pressure in the vascularly isolated carotid sinuses from $-140$ to $-50$ mmHg were determined before the carotid sinus pressure was raised to 140 mmHg. In the second series, this protocol was repeated in five of these animals in the presence of the $\beta_2$-adrenoceptor blocker (ICI-118551, 0.2 mg/kg iv over 5 min), followed by the $\beta_1$-adrenoceptor blocker (CGP-20712A, 0.2 mg/kg iv over 5 min), followed by the $\alpha$-adrenoceptor blocker phentolamine (Rothine, 1–2 mg/kg iv over 5 min). In the third series, in two animals, the carotid sinus pressure was altered before and after administration of CGP-20712A alone. In three of the animals, the capacitance responses after inhibition of nitric oxide synthase using $N^\text{G}$-nitro-L-arginine methyl ester (L-NAME; 50 mg/kg iv) and administration of adenosine (50–150 mg/kg iv), vasopressin (30 IU iv), and sodium nitroprusside (100 µg/kg iv) were also investigated. In all cases, carotid sinus pressure was maintained for 6 min.

Statistical analysis. All results are expressed as means ± SE. Statistical analysis was performed by two-factor analysis of variance. When a significant F value was found ($P < 0.05$), Student’s t-test for paired data was used to locate differences;
statistical significance was deemed if \( P < 0.05 \). Corrections for multiple comparisons such as Bonferroni, Tukey, and Scheffé were not used in the current study because these methods of multicomparison analysis were designed to take into account between-group variation when each treatment group is from separate animals. The current study used the same animal for all drug treatments, thereby reducing variation due to use of different animals.

**RESULTS**

Efficacy and selectivity of \( \beta \)-adrenergic antagonists. In pilot studies, the efficacy and selectivity of ICI-118551 (2 mg/kg) and CGP-20712A (2 mg/kg) in anesthetized dogs was determined by the relative inhibition of isoproterenol (1 \( \mu \)g/kg iv)-induced tachycardia (\( \beta_1 \)) and hindlimb vasodilatation (\( \beta_2 \); Ref. 10). Isoproterenol produced an increase in heart rate of 108 \( \pm \) 9 min\(^{-1} \) and a decrease in perfusion pressure of 57 \( \pm \) 3 mmHg in the constant flow-perfused hindlimb before \( \beta \)-adrenergic antagonist treatment. In animals treated with ICI-118551, the responses to isoproterenol were 108 \( \pm \) 8 min\(^{-1} \) and 27 \( \pm \) 3 mmHg for heart rate and hindlimb perfusion pressure, respectively (\( n = 2 \)). In animals treated with CGP-20712A, the responses to isoproterenol were 8 \( \pm \) 4 beats/min and 58 \( \pm \) 4 mmHg for heart rate and hindlimb perfusion pressure, respectively (\( n = 2 \)).

Arterial \( pH \) and blood gases. Throughout the experiment arterial \( pH \), \( PCO_2 \), and \( PO_2 \) were maintained at 7.38 \( \pm \) 0.01, 40.6 \( \pm \) 1.4 mmHg and 106 \( \pm \) 1.4 mmHg, respectively. The hematocrit and esophageal temperature were 38 \( \pm \) 1.1% and 36.9 \( \pm \) 0.2°C, respectively.

Responses to changes in carotid sinus pressure. Alteration of carotid sinus pressure resulted in a rapid change in whole body total vascular capacitance in the absence of changes in mean arterial blood pressure, right atrial blood pressure, and heart rate in the animals treated with atropine alone (Fig. 2). Lowering of carotid sinus pressure from 135 \( \pm \) 4 to 57 \( \pm \) 3 mmHg followed by a return to 137 \( \pm \) 6 mmHg resulted in whole body vascular capacitance responses of 28.5 \( \pm \) 5.3 and 29.9 \( \pm \) 4.1 ml, respectively (\( n = 5 \)), in the absence of hemodynamic changes (Figs. 3 and 4).

**Effect of adrenoceptor antagonist on capacitance responses.** Treatment of the animals with ICI-118551 did not affect mean arterial blood pressure but caused a small, although statistically significant, reduction in heart rate (7 \( \pm \) 1%, \( P < 0.01 \); Fig. 3). Subsequent lowering of carotid sinus pressure from 147 \( \pm \) 4 to 54 \( \pm \) 5 mmHg followed by a return to 154 \( \pm \) 5 mmHg resulted in whole body vascular capacitance responses of 68.1 \( \pm \) 14.8 and 75.2 \( \pm \) 16.2 ml, respectively (\( n = 5 \)), in the absence of hemodynamic changes (Figs. 3 and 4). The mean increase in capacitance response to both changes in carotid sinus pressure after ICI-118551 was 42.4 ml (95% confidence interval: 11.4–73.4 ml). This represents a potentiation of the response by \( \beta_2 \)-adrenoceptors (\( P < 0.05 \)).

Although administration of CGP-20712A resulted in significant reductions in mean arterial blood pressure and heart rate and an increase in mean right atrial pressure, these were maintained constant during the remaining protocol (Fig. 3). However, changing carotid sinus pressure had no further effect on whole body capacitance responses (Fig. 4). The mean difference in capacitance response to changes in carotid sinus pressure between administration of ICI-118551 and CGP-20712A was 10.8 ml (95% confidence interval: 1.5 to +36.9 ml). In addition, CGP-20712A was administered before ICI-118551 in two dogs. In these animals, the

**Fig. 2.** Response to large step decrease in CSP in dog weighing 14 kg. Blood was collected from left common carotid artery using roller pump into arterial reservoir held at constant pressure and returned (Inflow) into animal from reservoir via right femoral artery. Decreasing CSP caused expulsion of 38 ml of blood from animal into reservoir before blockade of \( \beta_2 \)-adrenergic receptors (left) and 49 ml after injection of ICI-118551 (\( \beta_2 \)-adrenergic receptor blocker, right). Large change in systemic arterial pressure was prevented by pressure-control system (see Fig. 1). Note that unloading of carotid baroreceptors caused expulsion (reduction of vascular capacitance) of greater amount of blood after blockade of \( \beta_2 \)-adrenergic receptors.
initial capacitance responses were 30.0 and 60.0 ml before antagonists. CGP-20712A resulted in either no increase or a small increase (1.5 ml) in the vascular capacitance response. After ICI-118551, the vascular capacitance response was increased by 55% in both animals.

Phentolamine produced a further reduction in both mean arterial blood pressure and heart rate (14 ± 6.6 and 9 ± 2%, respectively, P < 0.05; Fig. 3). The magnitude of the whole body capacitance response to carotid sinus pressure alteration was reduced to 8 ml, which represents 28% of the response observed before administration of β-adrenoceptor antagonist (Figs. 2 and 4). The mean difference in capacitance response to changes in carotid sinus pressure between administration of phentolamine and control was 21.6 ml (95% confidence interval: 9.8–33.5 ml).

To determine whether the lack of a capacitance response after phentolamine was due to general deterioration of the preparation, the capacitance responses to adenosine, vasopressin and L-NAME were investigated. After administration of adrenergic blockers, the

Table 1. Whole body vascular capacitance responses to vasoactive agents

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Compound</th>
<th>MABP, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Capacitance Response, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Control</td>
<td>89</td>
<td>120</td>
<td>–38</td>
</tr>
<tr>
<td></td>
<td>Vasopressin (30 IU)</td>
<td>103</td>
<td>120</td>
<td>–38</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenosine (50 mg/kg)</td>
<td>91</td>
<td>109</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>93</td>
<td>120</td>
<td>–87</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>54</td>
<td>132</td>
<td>–95</td>
</tr>
<tr>
<td></td>
<td>L-NAME (50 mg/kg)</td>
<td>55</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium nitroprusside (100 µg/kg)</td>
<td>30</td>
<td>132</td>
<td>96</td>
</tr>
</tbody>
</table>

MABP, mean arterial blood pressure; L-NAME, N^G^-nitro-L-arginine methyl ester.
above vasoactive agents were able to both increase and decrease the whole body vascular capacity (Table 1).

**DISCUSSION**

The present investigation has clearly demonstrated that the responses of the total vascular capacitance to loading and unloading of carotid baroreceptors can be greatly enhanced by administration of a $\beta_2$-adrenoceptor antagonist and greatly reduced by administration of an $\alpha$-adrenoceptor blocker (Figs. 2 and 4). Additionally, antagonism of the $\beta_1$-adrenoceptor with CGP-20712A either alone or in combination with ICI-118551 did not further enhance the capacitance response.

Lowering intracarotid sinus pressure always resulted in a shift of part of the blood volume of the animal into a graduated extracorporeal reservoir that was held at a constant pressure. Conversely, raising the carotid sinus pressure resulted in an equivalent shift of blood back to the animal (see Figs. 2 and 4). Blood was circulated through the reservoir at a constant flow and at a constant pressure to keep the systemic arterial pressure constant, and the right atrial pressure did not change. Under this condition, a shift of blood in and out of the reservoir must have been caused by changes in the total blood volume from different vascular beds including the spleen. The present study was not designed to determine the specific organ involvement. Because a large proportion of this volume shift occurred within 1 min of changes in carotid sinus pressure, a considerable part of this volume shift was most likely caused by a change in the total vascular capacitance from reflexly induced active vasoconstriction or vasodilation (see Fig. 2). Passive elastic recoil or distension of the venous wall and fluid absorption or filtration at the capillary bed (4, 23) were unlikely to make any significant contribution. The hematocrit level did not change significantly, indicating that fluid shift at the capillaries was not significant during the determination of the capacitance response (see Arterial pH and blood gases).

The shift of blood volume into the rigid extracorporeal reservoir in response to changes in carotid sinus pressure in the present series of experiments was similar in direction, but somewhat smaller in magnitude, to that reported in the dog previously (15, 29, 30). This difference was due to the larger step changes in intrasinus pressure in earlier experiments than in the present series. Although the experiments were not designed to identify the sources of the volume shift, it was likely that much of this blood was shifted from the abdominal capacitance vessels via the central circulation to the arterial side and then to the reservoir (6, 29). However, whether the abdominal vascular bed is the main source of blood cannot be determined without subjecting the animal to extensive traumatic surgery as used previously (6).

Atropine was given to prevent the chronotropic effect and to minimize the potential contribution of cardiopulmonary and aortic receptors. It is possible that vagal afferent fibers exert an inhibitory effect on sympathetic efferent nerve activity that, when intact, could reduce the capacitance response to unloading the carotid baro...

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**REFERENCES**


