Role of the Sympathetic Nervous System in Blood Pressure Maintenance and in the Antihypertensive Effects of Calcium Antagonists in Spontaneously Hypertensive Rats

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SUMMARY In conscious spontaneously hypertensive rats (SHR), 2, 3, 6, 9, 12, and 16 months of age, the blockade of autonomic ganglia (with chlorisondamine) or postjunctional α1-adrenergic receptors (with prazosin) or the depletion of peripheral norepinephrine stores (with syrosingopine), in contrast to the blockade of α2-adrenergic receptors (with yohimbine, rauwolscine), produced a sustained decrease in the directly measured mean tail artery blood pressure. In 3- to 9-month-old SHR, the fall in blood pressure after prazosin pretreatment was significantly smaller than that after chlorisondamine or syrosingopine pretreatment. In ganglion-blocked SHR, prazosin decreased blood pressure only when this parameter had been elevated by an intra-arterial infusion of epinephrine or norepinephrine. In contrast, under the same experimental conditions, yohimbine or rauwolscine administration failed to modify the pressor effects of either phenylephrine or epinephrine but partially reduced those of norepinephrine and, unlike prazosin, strongly antagonized those of B-HT 920. In either intact or ganglion-blocked SHR, a 30-minute intra-arterial infusion of diltiazem at 100.0, but not 25.0, µg/kg/min significantly decreased baseline mean tail artery blood pressure. In ganglion-blocked SHR, the smaller dose of diltiazem antagonized by 40 and 80% the pressor effects of norepinephrine and B-HT 920, respectively, but failed to change the vasoconstrictor responses of phenylephrine, epinephrine, or vasopressin, which were, however, reduced by the higher dose of diltiazem. These results indicate that, in conscious adult SHR, norepinephrine released by peripheral sympathetic nervous terminals and humoral ly borne epinephrine stimulate almost exclusively postjunctional α1-adrenergic receptors. The latter findings may account for the lack of blood pressure-lowering effects of the studied calcium antagonists at doses that effectively antagonize α2-adrenergic receptor-mediated vasoconstriction in conscious SHR. (Hypertension 11: 360-370, 1988)

KEY WORDS • spontaneously hypertensive rats • vascular postjunctional α-adrenergic receptors • B-HT 920 • phenylephrine • calcium antagonists • prazosin • yohimbine • enalapril • SKF 100273 • V1 vasopressin receptor antagonist

CALCIUM ions play a pivotal role in several of the multiple cellular processes linking the activation of plasmalemma receptors to vascular smooth muscle tension development. In particular, receptor stimulation may evoke changes in cytosolic Ca2+ concentration essential to the initiation of the contractile processes, either by releasing it from intra-cellularly located stores (e.g., inner membrane sites, sarcoplasmic reticulum) or by promoting its cellular entry from the extracellular space through plasmalemma (voltage- or receptor-operated) calcium channels, or by both actions.1-3 The relative contribution of the extracellular or intracellular calcium pool to the extrinsic regulation of myogenic tone appears to depend on several variables, such as the receptor subtype within the studied section (conductance, resistance, or capacitance) of a vascular region, the animal species, and complex, as yet not clearly understood, intracellular regulatory mechanisms.1-3
In pithed rats, full agonists of \(\alpha_1\)-adrenergic or \(\alpha_2\)-adrenergic receptors increase aortic blood pressure.\(^6\)\(^{-10}\) Calcium entry blockers, such as diltiazem, nifedipine, or verapamil, reduce preferentially and in an apparently noncompetitive manner the pressor responses mediated by \(\alpha_2\)-adrenergic receptors.\(^{10\text{-}12}\) On the basis of this finding in anesthetized animal preparations, it was suggested that the antihypertensive and vasodilator action of calcium antagonists may partly be the functional consequence of the blockade of postjunctional \(\alpha_2\)-adrenergic receptors.\(^{11\text{-}15}\) Furthermore, calcium entry blockers were found to impair responses evoked either by partial agonists of \(\alpha_1\)-adrenergic receptors in control pithed rats\(^6\) or by full agonists in animals that were pretreated with the irreversible blocker of \(\alpha_1\)-adrenergic receptors, phenoxybenzamine.\(^{10\text{-}11}\) This finding indicates that, under certain experimental conditions, this class of pharmacological agents can interfere with either subtype of postjunctional \(\alpha_2\)-adrenergic receptors.

The substantial contribution of the sympathetic nervous system to the maintenance of elevated blood pressure of spontaneously hypertensive rats (SHR) appears to be well established.\(^8\)\(^{-12}\) The goals of this investigation were to determine the role of the autonomic nervous system and, in particular, of each subtype of postjunctional \(\alpha_2\)-adrenergic receptors in the maintenance of aortic blood pressure in conscious SHR. As a corollary to this problem, we have assessed whether the antihypertensive activity of calcium entry blockers in SHR could be explained solely on the basis of their functional blockade of postjunctional \(\alpha_2\)-adrenergic receptor–mediated vasoconstriction.

**Materials and Methods**

SHR (Okamoto strain, Charles River, Saint Aubin-lès-Elbeuf, France; 2–17 months of age) were implant ed with a tail artery catheter during a brief period of ether anesthesia. Each animal was placed in a custom-made box where it could move freely while tail artery blood pressure was measured through a catheter connected with a swivel to a transducer (Statham P23Ge, Stag Instruments, Oxon, England) fitted with a dome\(^28\) that allowed intra-arterial (i.a.) infusions through small catheters without affecting the quality of pressure signals. Pulsatile and mean blood pressure and heart rate (measured with a cardiotachometer triggered by the pulse pressure signals) were recorded on a Grass polygraph (Model 7D; Stag Instruments). The mean tail artery blood pressure is 10 to 20 mm Hg higher than the mean blood pressure measured at the level of the upper abdominal aorta.

Each experimental procedure was begun 2 to 4 hours after the rats had recovered from anesthesia, at which time cardiovascular parameters had been in a steady state for at least 30 minutes. Most of the studies were performed in 6- to 9-month-old rats, with the exception of Series 1 and part of Series 3 (effects of prazosin and yohimbine on epinephrine response) in which SHR aged 2, 3, 6, 9, and 12 months were used.

**Series 1**
The blood pressure effects of i.a. administration of saline (0.2 ml/kg), prazosin (0.03 mg/kg), rauwolscine (0.5 mg/kg), yohimbine (0.5 mg/kg), and enalapril (1.0 mg/kg) plus the \(\mathrm{V}_{\text{1}}\) vasopressor receptor antagonist [1-(\(\beta\)-mercapto-\(\beta\)-cyclopentamethylene-propionic acid), 8-D-argininyl]vasopressin (SKF 100273; 1.0 mg/kg), infused over 5 minutes, were studied for a 60-minute period in intact rats. Furthermore, chlorisondamine (0.6 mg/kg i.a.) followed 45 minutes later by 0.6 mg/kg s.c.) was given to block autonomic ganglia transmission. This treatment produced a long-lasting fall in mean arterial pressure and heart rate. The achievement of an effective blockade of ganglionic transmission was indicated in preliminary studies by the failure of prazosin (0.03 mg/kg i.a.) or yohimbine (0.5 mg/kg i.a.) and that of propranolol (0.75 mg/kg i.a.) to decrease further blood pressure and heart rate, respectively. Furthermore, in pithed 7- to 8-month-old SHR, the peak pressor response (102 ± 3 mm Hg; \(n = 13\)) to the electrical stimulation (1.0 Hz, 0.5 msec, 60 V for 20 seconds) of the spinal cord was blocked entirely by chlorisondamine. Finally, one group of SHR was given syrosingopine (5.0 mg/kg s.c.) 16 hours before undertaking the study of their blood pressure.

**Series 2**
In independent groups of SHR pretreated with chlorisondamine, mean tail artery blood pressure was restored to levels similar to those measured before ganglionic blockade by a 90-minute i.a. infusion of either B-HT 920 (alefexole; 4.0–7.5 \(\mu g/kg/min\), nor epinephrine (2.0–3.75 \(\mu g/kg/min\), epinephrine (2.0–4.0 \(\mu g/kg/min\), phenylephrine (4.0–7.5 \(\mu g/kg/min\), or vasopressin (3.5–4.0 \(\mu l/U/kg/min\). To prevent possible vasodilator effects mediated by vascular \(\alpha_2\)-adrenergic receptor stimulation, the animals in which norepinephrine, epinephrine, or phenylephrine was studied received propranolol (0.75 mg/kg i.a.).\(^9\)\(^{-10}\)

**Series 3**
Saline (0.2 ml/kg), prazosin (0.03 mg/kg), rauwolscine (0.5 mg/kg), or yohimbine (0.5 mg/kg) was infused intra-arterially over a 5-minute period in ganglion-blocked SHR in which the low blood pressure was either left unchanged or elevated by an i.a. infusion of B-HT 920, epinephrine, norepinephrine, or phenylephrine, as described in Series 1.

**Series 4**
Diltiazem (12.5, 25.0, 50.0, and 100.0 \(\mu g/kg/min\)), verapamil (6.25, 25.0, 50.0, and 100.0 \(\mu g/kg/min\), or nifedipine (1.0, 3.0, 6.25, 12.5, and 25.0 \(\mu g/kg/min\), was infused intra-arterially in intact (not ganglion-blocked) SHR for 30 minutes, and their blood pressure effects were followed for an additional 60 minutes.

**Series 5**
Diltiazem (25.0 and 100.0 \(\mu g/kg/min\)), verapamil (6.25 and 100.0 \(\mu g/kg/min\), or nifedipine (1.0 and 25.0 \(\mu g/kg/min\) was infused for 30 minutes in ganglion-blocked SHR. Diltiazem was also studied in SHR.
pretreated with prazosin (0.1 mg/kg i.a.) plus yohimbine (0.5 mg/kg i.a.).

Series 6
The effects of diltiazem (25.0 and 100.0 μg/kg/min i.a. over a 30-minute period) were studied in ganglion-blocked SHR in which the low level of blood pressure was increased, as described in Series 1, by i.a. infusions of B-HT 920, epinephrine, norepinephrine, phenylephrine, or vasopressin, which were given throughout the experimental procedure (60–90 minutes). Furthermore, the effects of verapamil (100.0 μg/kg/min i.a.) and nifedipine (25.0 μg/kg/min i.a.) were studied in ganglion-blocked SHR in which the blood pressure was elevated by an infusion of B-HT 920 or phenylephrine.

Series 7
The blood pressure effects of enalapril (1.0 mg/kg i.a. infused over 5 minutes) were studied in ganglion-blocked SHR. The blockade of angiotensin I converting enzyme by this dose of enalapril was assessed by determining its antagonist effects on angiotensin I (0.35–0.45 μg/kg/min i.a. over 10 minutes)–evoked increases in mean tail artery blood pressure. Furthermore, the blood pressure effects of SKF 100273 (1.0 μg/kg i.a. infused over 5 minutes), a vasopressin antagonist, were assessed in ganglion-blocked SHR. The V1 vasopressin receptor blocking activity of this dose of SKF 100273 was determined against the vasopressin (3.75 mIU/kg/min i.a.)–evoked pressor response.

Analysis of Results
All results are given as means ± SEM. The statistical evaluation was done by using a two-way analysis of variance or Student’s t test. Significance was accepted at a p level below 0.05.

Drugs
Drugs used were B-HT 920 HCl (Karl Thomae), chlorisondamine HCl (CIBA-Geigy), diltiazem HCl (Tanabe Seiyaku), enalapril (Merck Sharp & Dohme), l-epinephrine bitartrate (Sigma), nifedipine (synthesized by Laboratoires d’Etudes et de Recherches Synthélabo, Paris, France), l-norepinephrine bitartrate (Sigma), l-phenylephrine HCl (Sigma), prazosin (synthesized by Laboratoires d’Etudes et de Recherches Synthélabo), rauwolscine HCl (Serlabo), SKF 100273 (Smith Kline & French), syrosingopine (CIBA-Geigy), vasopressin (Parke-Davis), verapamil HCl (synthesized by Laboratoires d’Etudes et de Recherches Synthélabo), and yohimbine HCl (Boyer). All doses reported in the text refer to the bases of the compounds, which were generally dissolved in saline (9.0 g of NaCl per liter of distilled water).

Results
Role of the Autonomic Nervous System in the Maintenance of Blood Pressure in Conscious SHR
In conscious SHR of various ages, the administration of saline (control) did not significantly change baseline tail artery blood pressure during a 90-minute observation period. The blood pressure level exhibited by young (2–3 months) SHR was significantly lower than that of the older (6–17 months) animals with established hypertension (Table 1).

Effects of Chlorisondamine, Prazosin, Rauwolscine, Syrosingopine, and Yohimbine on Mean Tail Artery Blood Pressure and Heart Rate of SHR
In SHR of any studied age (2–17 months), either blockade of postfunctional α1-adrenergic receptors with prazosin or impairment of ganglionic transmission with chlorisondamine produced a pronounced, long-lasting reduction in mean tail artery blood pressure, in contrast to treatment with rauwolscine or yohimbine, which failed to modify this parameter significantly (Figure 1). SHR pretreated with syrosingopine exhibited a level of blood pressure that was as low as that measured after ganglionic blockade. In contrast, after prazosin treatment, the blood pressure values of 2- to 3-, 6-, and 8- to 9-month-old SHR were significantly higher than those of animals in which the central autonomic drive was blocked with either chlorisondamine or syrosingopine (see Table 1). This difference was not due to an insufficient dose (0.03 mg/kg i.a.) of prazosin, since by increasing the latter, the level of blood pressure did not fall further.

In chlorisondamine-pretreated or syrosingopine-pretreated rats aged 3 to 16 months, administration of prazosin, rauwolscine, or yohimbine did not change the level of resting blood pressure.

The administration of enalapril failed to produce a significant fall in blood pressure in all studied ages except the 12- to 17-month-old rats. The combination of enalapril plus SKF 100273 (V1 vasopressin receptor antagonist) produced the same effects as administration of enalapril alone (see Table 1).

Prazosin, rauwolscine, or yohimbine did not significantly alter heart rate as compared with saline administration, in contrast to chlorisondamine, which reduced markedly this parameter (from 406 ± 4 to 304 ± 8 beats/min; n = 30). This effect probably was not responsible for the greater reduction in blood pressure produced by chlorisondamine with respect to prazosin. In fact, in 16- to 17-month-old SHR, the combination of enalapril plus SKF 100273 in prazosin-pretreated rats decreased blood pressure to the same levels as those observed after chlorisondamine (see Table 1), but it did not modify heart rate. Furthermore, in 9-month-old SHR, propranolol decreased heart rate (from 428 ± 10 to 347 ± 12 beats/min; n = 8), but not blood pressure (from 189 ± 2 to 191 ± 3 mm Hg). In these animals, prazosin reduced blood pressure to 140 ± 6 mm Hg, a value not different from that found in SHR not given propranolol (see Table 1).

Effects of i.a. Infusion of B-HT 920, Epinephrine, Norepinephrine, Phenylephrine, or Vasopressin in Ganglion-blocked SHR
The i.a. infusions of appropriate doses of epinephrine, phenylephrine (α1-adrenergic receptor agonist), B-HT 920 (α2-adrenergic receptor agonist), norepi-
Table 1. Effects of Various Treatments on the Mean Tail Artery Blood Pressure of SHR of Various Ages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>2 mo</th>
<th>3 mo</th>
<th>6 mo</th>
<th>8–9 mo</th>
<th>12 mo</th>
<th>16–17 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5–41)</td>
<td>—</td>
<td>169 ± 2</td>
<td>175 ± 2</td>
<td>200 ± 2</td>
<td>195 ± 2</td>
<td>199 ± 3</td>
<td>204 ± 2</td>
</tr>
<tr>
<td>YO (n = 5–9)</td>
<td>0.5</td>
<td>169 ± 2</td>
<td>174 ± 2</td>
<td>199 ± 4</td>
<td>194 ± 3</td>
<td>199 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>RA (n = 5–7)</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>200 ± 2</td>
<td>192 ± 3</td>
<td>—</td>
<td>200 ± 2</td>
</tr>
<tr>
<td>EN (n = 7–8)</td>
<td>1.0</td>
<td>—</td>
<td>171 ± 2</td>
<td>192 ± 4</td>
<td>184 ± 4</td>
<td>176 ± 5*</td>
<td>174 ± 6*</td>
</tr>
<tr>
<td>EN + SKF (n = 7–8)</td>
<td>1.0 + 0.001</td>
<td>—</td>
<td>170 ± 3</td>
<td>192 ± 4</td>
<td>185 ± 3</td>
<td>177 ± 5*</td>
<td>173 ± 6*</td>
</tr>
<tr>
<td>PR (n = 5–10)</td>
<td>0.03</td>
<td>146 ± 4*</td>
<td>148 ± 3*</td>
<td>157 ± 6*</td>
<td>138 ± 7*</td>
<td>149 ± 7*</td>
<td>—</td>
</tr>
<tr>
<td>SY (n = 5–8)</td>
<td>0.1</td>
<td>—</td>
<td>131 ± 4*</td>
<td>161 ± 4*</td>
<td>137 ± 4*</td>
<td>146 ± 5*</td>
<td>142 ± 8*</td>
</tr>
<tr>
<td>CH (n = 5–7)</td>
<td>0.2</td>
<td>—</td>
<td>143 ± 6*</td>
<td>166 ± 3*</td>
<td>143 ± 4*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>YO + PR (n = 3–6)</td>
<td>0.5 + 0.1</td>
<td>—</td>
<td>133 ± 7*</td>
<td>156 ± 7*</td>
<td>145 ± 8*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EN + SKF + PR (n = 4–6)</td>
<td>1.0 + 0.001</td>
<td>—</td>
<td>132 ± 3*</td>
<td>143 ± 8*</td>
<td>135 ± 4*</td>
<td>123 ± 7*†</td>
<td>101 ± 2*†</td>
</tr>
<tr>
<td>SY (n = 7–10)</td>
<td>5.0</td>
<td>—</td>
<td>105 ± 2*†</td>
<td>116 ± 4*†</td>
<td>114 ± 1*†</td>
<td>117 ± 2*†</td>
<td>110 ± 4*†</td>
</tr>
<tr>
<td>CH (n = 5–41)</td>
<td>0.6</td>
<td>106 ± 7*†</td>
<td>105 ± 2*†</td>
<td>116 ± 2*†</td>
<td>117 ± 2*†</td>
<td>118 ± 2*†</td>
<td>113 ± 2*†</td>
</tr>
<tr>
<td>SY + PR (n = 4)</td>
<td>5.0 ± 0.1</td>
<td>—</td>
<td>105 ± 2*†</td>
<td>116 ± 4*†</td>
<td>110 ± 3*†</td>
<td>110 ± 4*†</td>
<td>108 ± 6*†</td>
</tr>
<tr>
<td>CH + PR (n = 4–9)</td>
<td>0.6 ± 0.1</td>
<td>106 ± 7*†</td>
<td>107 ± 2*†</td>
<td>109 ± 2*†</td>
<td>119 ± 2*†</td>
<td>117 ± 3*†</td>
<td>111 ± 5*†</td>
</tr>
<tr>
<td>CH + YO (n = 4–7)</td>
<td>0.6 ± 0.5</td>
<td>—</td>
<td>108 ± 2*†</td>
<td>120 ± 5*†</td>
<td>120 ± 8*†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EN + SKF + CH (n = 3–5)</td>
<td>1.0 + 0.001</td>
<td>108 ± 10*†</td>
<td>116 ± 8*†</td>
<td>117 ± 8*†</td>
<td>117 ± 7*†</td>
<td>97 ± 6*†</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means ± SEM. All drugs were given i.a., except for syrosingopine, which was given i.p., 16 hours before the experimental procedure. The reported values are those measured at an apparently steady state, which was generally achieved 15 to 30 minutes after each treatment.

MAP = mean tail artery blood pressure; YO = yohimbine; RA = rauwolscine; EN = enalapril; SKF = SKF 100273; PR = prazosin; SY = syrosingopine; CH = chlorisondamine.

*p < 0.05, compared with control (saline) values (by t test).
†p < 0.05, compared with all the values without a dagger in the same column (by t test).

Nephrine, or vasopressin restored the low blood pressure of ganglion-blocked SHR to levels close to those existing before the blockade. These effects persisted throughout the duration of the infusion of the agonists (Figure 2 illustrates the results obtained with B-HT 920 and phenylephrine) and were not modified by the administration of saline, which, in the studies described subsequently, was replaced by various antagonists.

**Figure 1.** Left panel. Changes in mean tail artery blood pressure (ΔMAP) produced by i.a. administration of prazosin (PR; 0.03 mg/kg; n = 7), yohimbine (YO; 0.5 mg/kg; n = 9), rauwolscine (RA; 0.5 mg/kg; n = 7), or chlorisondamine (CH; 0.6 mg/kg; n = 9) in conscious 8- to 9-month-old SHR. The initial blood pressure in the four groups was similar and their combined mean value was 195 ± 2 mm Hg (n = 32). Right panel. Maximal changes in mean blood pressure (ΔMAP max) produced by YO, PR, and CH in 3-, 6-, and 9-month-old SHR. ΔMAP max produced by PR and CH were significantly (p < 0.05, by paired t test) greater than those after PR.
Effects of Prazosin, Rauwolscine, and Yohimbine on the Pressor Responses Evoked by B-HT 920, Norepinephrine, Epinephrine, and Phenylephrine in Ganglion-Blocked SHR

Prazosin but not rauwolscine (results not shown) or yohimbine antagonized the pressor responses evoked by phenylephrine and epinephrine. However, the two α2-adrenergic receptor antagonists, in contrast to prazosin, strongly inhibited the pressor effects of B-HT 920. Prazosin antagonized the norepinephrine-induced increase in mean tail artery blood pressure significantly more than did yohimbine. However, this inhibition was less pronounced than that produced by prazosin and yohimbine against phenylephrine and B-HT 920, respectively (Figure 3). Interestingly, prazosin inhibited (75–90%) epinephrine pressor response in SHR at each age studied (3, 6, 8–9, 12, and 16–17 months; Figure 3 reports results for the 8- to 9-month-old rats).

Mechanism of the Antihypertensive Effects of Calcium Entry Blockers in 8- to 9-Month-Old SHR

Effects of Diltiazem, Nifedipine, or Verapamil on the Mean Tail Artery Blood Pressure and Heart Rate of Intact SHR

A 30-minute i.a. infusion of diltiazem, nifedipine, or verapamil produced dose-related reductions in mean tail artery blood pressure in intact SHR that attained a maximum within the first few minutes after the end of the infusion. The fall in blood pressure was still significant 60 minutes later, although the response had waned by approximately 50% (Figure 4). On the basis of the results of the dose-response curves, diltiazem has an antihypertensive potency similar to that of verapamil and these two compounds are approximately four times less potent than nifedipine (see Figure 4). However, diltiazem, nifedipine, and verapamil, at all doses studied, failed to change heart rate significantly as compared with saline treatment.

Effects of Diltiazem, Nifedipine, or Verapamil on Mean Tail Artery Blood Pressure of Ganglion-Blocked SHR

In this preparation, the highest i.a. dose of diltiazem, nifedipine, or verapamil used in this study pro-
produced a significant decrease in blood pressure. When this effect was expressed as a percentage of the baseline blood pressure value exhibited by ganglion-blocked SHR, its magnitude was found to be comparable to that induced by these agents in SHR with an intact autonomic drive (Figure 5).

Effects of Diltiazem in SHR Pretreated with Prazosin and Yohimbine in Combination

The mean tail artery blood pressure of SHR (195 ± 3 mm Hg; n = 5) was not changed by yohimbine (193 ± 4 mm Hg), 0.5 mg/kg i.a., but was significantly decreased (130 ± 3 mm Hg) by prazosin, 0.1 mg/kg i.a. In this preparation, at the end of the 30-minute infusion of diltiazem (0.1 mg/kg/min i.a.), blood pressure was 87 ± 1 mm Hg. Thus, the change was −43 ± 2 mm Hg, which corresponds to a 35 ± 3% decrease from the blood pressure level measured after administration of yohimbine plus prazosin.

Effects of a Low Dose of Diltiazem on Pressor Responses Evoked by B-HT 920, Norepinephrine, Epinephrine, Phenylephrine, or Vasopressin in Ganglion-Blocked SHR

Diltiazem, at a dose (25.0 μg/kg/min i.a. for 30 minutes) that did not significantly decrease blood pressure in intact SHR, inhibited the pressor response induced by B-HT 920 and norepinephrine by 82 and 40%, respectively. In contrast, it failed to modify the vasoconstrictor effects of phenylephrine, epinephrine, and vasopressin (Figure 6).

Effects of a Low Dose of Nifedipine or Verapamil on Pressor Responses Evoked by B-HT 920 and Phenylephrine

Verapamil (6.25 μg/kg/min i.a. for 30 minutes) and nifedipine (1.0 μg/kg/min i.a. for 30 minutes) behaved similarly to diltiazem inasmuch as, at the end of their administration, they significantly decreased (55 ± 9 and 56 ± 7%, respectively; n = 5/group) the blood pressure response of B-HT 920 without significantly affecting the vasoconstrictor effects of phenylephrine.

Effects of a Dose of Diltiazem Exerting an Antihypertensive Action in Intact SHR on the Pressor Response Evoked by Phenylephrine, Epinephrine, or Vasopressin in Ganglion-Blocked SHR

Diltiazem (100.0 μg/kg/min i.a. for 30 minutes), at a dose that substantially decreased mean tail artery blood pressure in both intact and ganglion-blocked rats (see Figures 4 and 5), inhibited the pressor response evoked in the latter preparation by an infusion of phenylephrine, epinephrine, or vasopressin (see Figure 6). As described in a previous section, a smaller dose of diltiazem (25.0 μg/kg/min i.a. for 30 minutes), which antagonized by 82% the pressor response to B-HT 920, lacked antihypertensive activity and failed to reduce the pressor response to phenylephrine, epinephrine, or vasopressin. In contrast to diltiazem, prazosin (0.03 mg/kg i.a.) in a dose blocking more than 90% of the increase in blood pressure evoked by phenylephrine, did not modify the pressor effects of vasopressin (see Figure 3).
FIGURE 5. Effects (expressed as percentages of baseline mean blood pressure; ΔMAP) on the mean tail artery blood pressure produced by diltiazem (DIL-100; 100.0 μg/kg/min i.a.; n = 7–9), nifedipine (NIF; 25.0 μg/kg/min i.a.; n = 5–7) in intact or ganglion-blocked (with chlorisondamine; CH) conscious SHR. The baseline values of blood pressure in these preparations were 196 ± 4 (n = 22) and 123 ± 3 (n = 16) mm Hg, respectively. The left panel shows the time-course effects of diltiazem, whereas the right panel gives the maximal effect that occurred 5 minutes after the end of the 30-minute infusion of each studied calcium entry blocker. The effects of the three compounds were significant (p < 0.05, by t test on responses measured at the end of infusion) and similar in the two preparations.

Effects of Enalapril on Mean Tail Artery Blood Pressure in Conscious Ganglion-Blocked SHR

Enalapril (1.0 mg/kg i.a.), an angiotensin I converting enzyme inhibitor, blocked the sustained pressor response (70 ± 10 mm Hg; n = 3) evoked by an infusion of angiotensin I (0.35–0.45 μg/kg/min i.a. over 10 minutes) in ganglion-blocked SHR. However, this dose of enalapril failed to reduce significantly the blood pressure in the latter preparation (baseline mean tail artery blood pressure after ganglion blockade, 125 ± 0 mm Hg, n = 3; change, −5 ± 3 and −7 ± 4 mm Hg, respectively, 15 and 30 minutes after enalapril), in contrast to diltiazem (100.0 μg/kg/min i.a. over 30 minutes; n = 7) which produced a −26 ± 2 mm Hg change in blood pressure (see Figure 5).

Effects of SKF 100273 on Mean Tail Artery Blood Pressure in Conscious Ganglion-Blocked SHR

SKF 100273 (1.0 μg/kg i.a. over 5 minutes) inhibited (−79 ± 8%; n = 5) the sustained pressor effect (86 ± 10 mm Hg) evoked by a continuous i.a. infusion of vasopressin. This dose of SKF 100273 did not change baseline blood pressure in either intact or ganglion-blocked SHR. In contrast, a dose of diltiazem (100.0 μg/kg/min i.a. for 30 minutes), exerting the same inhibitory effects as SKF 100273 against the vasopressin-induced pressor response (see Figure 6), lowered blood pressure in these two preparations (see Figures 4 and 5).

Discussion

A clear-cut, albeit expected, result of this investigation is that the sympathetic nervous system plays a cardinal role in the maintenance of aortic blood pressure in young, adult, and old SHR, as indicated by the large fall in blood pressure following the blockade of ganglionic transmission with chlorisondamine or the depletion of peripheral stores of norepinephrine with syrosingopine. In contrast, the renin-angiotensin system (as studied by inhibiting the angiotensin I converting enzyme with enalapril) does not appear to intervene in the blood pressure regulation of 3- to 9-month-old SHR, although it seemed to play a minor but significant role in the oldest (12–17 months) animals used in this study (see Table 1). However, we failed to obtain evidence that V1 vasopressin receptors participate in the maintenance of blood pressure in SHR of all studied ages.

In SHR of various ages, the level of blood pressure attained after treatment with either chlorisondamine or syrosingopine was virtually the same and did not depend on the initial values of blood pressure, which were significantly lower in young than in adult or old SHR. This finding suggests that the vascular tone remaining after the removal of the sympathetic nervous system contribution is relatively constant from the 2nd to the 17th month of age. However, for all studied ages, chlorisondamine and syrosingopine produced a similar fall in blood pressure that was significantly greater than that evoked by prazosin. In pithed 7- to 8-month-old SHR, the peak pressor response (102 ± 3 mm Hg; n = 13) to electrical stimulation (1.0 Hz, 0.5 msec, 60 V for 20 seconds) of the spinal cord was blocked by chlorisondamine, but it was reduced only by 72% after prazosin (0.2 mg/kg i.v.; remaining response, 28 ± 3 mm Hg; n = 5) and 75% after prazosin plus yohimbine (0.5 mg/kg i.v.; remaining response, 26 ± 4 mm Hg; n = 8). In 2- to 9-month-old rats, the residual tone after blockade of α1-adrenergic receptors was due neither to α2-adrenergic receptors nor to the renin-angiotensin or vasopressin system; thus, its possible sympathetic (norepinephrine stimulating adrenergic receptors other than α1-adrenergic or α2-adrenergic receptors) or nonadrenergic (e.g., neuropep-
Figure 6. Effects (expressed as percentages of steady state control pressor responses) produced by diltiazem (DIL-25; 25.0 μg/kg/min; DIL-100, 100.0 μg/kg/min i.a., during 30 minutes; n = 6-7) in conscious ganglion-blocked SHR in which i.a. infusion of either B-HT 920 (BHT), norepinephrine (NE), epinephrine (EPI), phenylephrine (PHE), or vasopressin (VAS) was performed to evoke a sustained pressor effect (60-80 mm Hg). The left panel reports the time-course effects of diltiazem (DIL-25) on the pressor response evoked by a continuous i.a. infusion of B-HT 920 or phenylephrine. The right panel reports the effects produced by diltiazem 5 minutes after the end of its infusion on the pressor responses to various agonists. Asterisk indicates that the lower dose of diltiazem significantly inhibited (p<0.05, by paired t test) the pressor effects of NE and BHT but not those of PHE, EPI, and VAS, while the higher dose of diltiazem significantly inhibited the responses to PHE, EPI, and VAS.

ditions) inasmuch as the residual vascular tone was abolished by syrosingopine, it is likely to be dependent on nerve endings of sympathetic origin. Furthermore, in rats of 12 and 17 months of age, the renin-angiotensin system contributed at least partly to the residual tone (see Table 1).

A primary goal of this investigation was to determine the role played by each of the two subtypes of vascular postjunctional α-adrenergic receptors in the maintenance of aortic blood pressure in conscious SHR. On the basis that rauwolscine or yohimbine failed to exert an antihypertensive effect, postjunctional vascular α1-adrenergic receptors would not appear to play a major physiological role in the peripheral regulation of blood pressure. However, yohimbine and rauwolscine can also block α2-adrenergic receptors located prejunctionally or activate central sympathetic outflow. These mechanisms are known to be accompanied by an enhancement in the neuronal release of norepinephrine from peripheral sympathetic nerve terminals, which functionally can manifest itself as an increase in blood pressure and heart rate in anesthetized preparations. Therefore, these prejunctional or central effects could counteract the decrease in total peripheral vascular resistance induced by yohimbine or rauwolscine through the blockade of postjunctional α2-adrenergic receptors. On the other hand, if these antagonists enhanced the efferent sympathetic drive by either a central or a peripheral prejunctional mechanism in SHR, they would have increased heart rate (effect mediated by postjunctional β-adrenergic receptors, which are not blocked by yohimbine or rauwolscine). However, in the present experiments in conscious SHR, yohimbine or rauwolscine did not produce a significant degree of tachycardia. The possibility exists that SHR already have a relatively high level of cardiac sympathetic tone, and thus, yohimbine is unable to enhance it further. Additionally, published works indicate that prejunctional α2-adrenergic receptors in SHR do not respond to agonists and antagonists in the same manner as do normotensive rats. 

In fact, these receptors on sympathetic nerve terminals required higher doses of phentolamine for blockade than did those in the normotensive WKY. It may be argued that yohimbine or rauwolscine are not sufficiently selective α2-adrenergic receptor antagonists and thus other compounds possessing this mechanism of action, such as idazoxan, should be investigated before concluding that α2-adrenergic receptors are not involved in the blood pressure regulation of the conscious SHR. This justified concern, however, is not supported by the experimental results of this investigation, in which the dose of yohimbine or rauwolscine chosen produced a selective inhibition of α2-adrenergic receptor-mediated responses. Furthermore, idazoxan (unpublished observation), like yohimbine or rauwolscine, also failed to lower blood pressure in conscious SHR at doses blocking vascular α2-adrenergic receptors.

A rather more convincing argument against the substantial participation of postjunctional vascular α1-adrenergic receptors in the maintenance of blood pressure in SHR is provided by the effects of the highly selective α1-adrenergic receptor antagonist prazosin, which, in a small dose (0.03 mg/kg i.a.), decreased...
blood pressure to levels that were 20% higher than those observed after the blockade of ganglionic transmission with chlorisondamine. Furthermore, neither yohimbine nor rauwolscine reduced blood pressure in either prazosin-pretreated or ganglion-blocked SHR (of each studied age), suggesting that α₂-adrenergic receptors do not actively contribute to the total peripheral vascular resistance of the SHR. This conclusion is apparently not supported by a study performed in anesthetized SHR, in which rauwolscine reduced diastolic blood pressure even after prazosin pretreatment. This α₂-adrenergic receptor contribution, however, was shown to be of adrenal medulla origin. The discrepancy between our conscious animal investigation and the latter study (pentobarbital plus ether anesthesia) may be due to the substantially different experimental conditions.

A further argument against a functional role of α₂-adrenergic receptors in the blood pressure regulation of SHR is provided by the experimental results obtained with diltiazem, nifedipine, and verapamil. These compounds, in a dose devoid of antihypertensive activity in untreated SHR, markedly increased the increase in blood pressure evoked by an i.a. infusion of the relatively selective α₂-adrenergic receptor agonist B-HT 920, but not of phenylephrine, in ganglion-blocked SHR. Interestingly, the same low dose of diltiazem reduced by 40% the vasoconstrictor effects of exogenously administered norepinephrine. Thus, this catecholamine, when delivered humorally to resistance vessels, can stimulate postjunctional α₁-adrenergic receptors mediating vasoconstriction. However, when released into the synaptic cleft, norepinephrine appears to stimulate only postjunctional adrenergic receptors mediating vasoconstriction. However, when released into the synaptic cleft, norepinephrine appears to stimulate only postjunctional adrenergic receptors of the α₁ subtype inasmuch as, in intact SHR, yohimbine did not further lower blood pressure after prazosin administration. This conclusion is further strengthened by results obtained in the pithed SHR preparation, in which the pressor responses to the electrical stimulation of the sympathetic outflow were selectively inhibited by α₁-adrenergic receptor antagonists. In addition, it is also supported by the report of Sawyer et al. which showed that α₂-adrenergic receptors are targets of circulating catecholamines.

To our surprise, epinephrine appeared to be a selective α₁-adrenergic receptor agonist in conscious SHR, since its pressor effects, in propranolol-pretreated ganglion-blocked SHR, were almost entirely antagonized by prazosin and not affected by yohimbine, rauwolscine, or a dose of diltiazem that selectively blocked the vasoconstrictor response evoked by B-HT 920. However, it was inhibited by a dose of diltiazem that depressed the effects of phenylephrine. Thus, epinephrine behaves differently from norepinephrine, which, as discussed, produced a pressor effect that was partly antagonized by a dose of calcium entry blockers that did not modify the vasoconstrictor response to phenylephrine. The finding that yohimbine as well as prazosin partially inhibited the pressor effects of norepinephrine confirms the generally held view that this catecholamine is an agonist of α₁-adrenergic and α₂-adrenergic receptors. The selective inhibition of epinephrine by prazosin, but not yohimbine, was not limited to the adult SHR, it was also demonstrated in young (2–3 months) and old (16–17 months) animals.

Our results in conscious SHR replicate those obtained in pithed rats, in which it was found that there are doses of diltiazem, nifedipine, and verapamil that markedly inhibit the pressor responses mediated by α₂-adrenergic receptor stimulation but modify only slightly those evoked by full α₁-adrenergic receptor agonists. These findings were used to propose an original hypothesis that the mechanism of the antihypertensive and vasodilator activity of calcium entry blockers could be in part the consequence of a functional blockade of α₁-adrenergic receptor-mediated vasoconstriction. This contention, however, cannot be held for the conscious SHR, an animal model that, for the last 2 decades, has been widely used to study the pathophysiology of human hypertension and the mechanisms of action of antihypertensive drugs.

In the past few years, numerous reports have dealt with the modalities of coupling used by vascular α₁-adrenergic and α₂-adrenergic receptors in the rat. Results from functional studies like the present one cannot establish whether vascular α₁-adrenergic receptors on resistance vessels are less dependent than α₂-adrenergic receptors on the availability of extracellular calcium to couple the signal initiated by their stimulation to the intracellular milieu. What appears clearly from results obtained in conscious (present investigation) and anesthetized pithed rats is that there are doses of calcium entry blockers that inhibit the pressor responses to α₁-adrenergic receptor agonists, without affecting those to full α₂-adrenergic receptor agonists. Possible mechanisms of this observation were discussed by us elsewhere.

An important question that requires some consideration concerns the mechanism of the antihypertensive effects of calcium antagonists in conscious SHR. The sympathetic system appears an improbable primary target of action, inasmuch as the dose of diltiazem, nifedipine, or verapamil decreasing blood pressure in intact SHR was hypotensive in ganglion-blocked SHR and in prazosin plus yohimbine–pretreated rats. The magnitude of this effect was similar in both preparations when the results were expressed as a percentage of initial blood pressure value, which was much lower (30–40%) in SHR deprived of autonomic regulation of the cardiovascular system by blockade of ganglionic transmission. Furthermore, the fall in blood pressure, in absolute values, was not too different in intact (47 ± 4 mm Hg) or ganglion-blocked SHR with the baseline blood pressure elevated with an infusion of vasopressin (54 ± 6 mm Hg), phenylephrine (50 ± 5 mm Hg), or epinephrine (67 ± 8 mm Hg), at least for diltiazem, which was studied in all these preparations (n = 6–9/group). Thus, the calcium antagonists investigated in this study, at doses that reduced blood pressure in conscious intact SHR, not...
only can produce vascular smooth muscle relaxation in the absence of an adrenergic vasconstrictor tone, but they can also inhibit pressor responses evoked by various vasconstrictor agents with distinct mechanisms of action (α₁ and α₂ agonists, vasopressin, or angiotensin II).

In pithed SHR, diltiazem antagonized the increase in blood pressure evoked by angiotensin II. This observation prompted us to investigate whether, in conscious SHR, circulating vasopressor hormones such as angiotensin II or vasopressin played a major role in the maintenance of vascular resistance. This possibility was discarded for rats aged 3 to 9 months since enalapril or SKF 100273, at doses blocking the angiotensin I converting enzyme activity or vasopressin vasconstriction, respectively, failed to lower blood pressure. However, enalapril slightly but significantly decreased blood pressure in 12- to 17-month-old rats, implying a minor contribution of the renin-angiotensin system to the maintenance of blood pressure of adult and old SHR. The hypotensive effects of calcium antagonists in the conscious ganglion-blocked SHR are unlikely to result from the antagonism of a vasconstrictor tone contributed by blood-borne angiotensin II or vasopressin, inasmuch as enalapril or SKF 100273 failed to lower blood pressure in this preparation.

The calcium antagonists studied in this report may lower blood pressure in intact or ganglion-blocked SHR by reducing cardiac output. However, this hemodynamic mechanism is not supported by published results indicating that, in the conscious intact SHR, verapamil, nifedipine, and diltiazem lower total peripheral resistance and slightly increase cardiac output. As a final consideration, our results do not entirely preclude the suggestion of several investigators that calcium entry blockers may be of particular therapeutic benefit in the treatment of hypertensive patients with elevated vascular resistance, which may partly be due to vasoconstriction mediated by stimulation of vascular postjunctional α₁-adrenergic receptors. Although, we are not aware of experimental results supporting a major contribution of postsynaptic α₁-adrenergic receptors to the etiology of human hypertension, some available results indicate that α₁-adrenergic receptors may participate in the maintenance of blood pressure in rats with deoxycorticosterone acetate–salt hypertension (personal observation, 1987).

Acknowledgments

The authors are grateful to Jacqueline Lechaire and Angélique Vidal for the excellent performance of the experimental work and to Nicole Sieller for the careful preparation of the manuscript. They extend their thanks to Dr. Alan G. Roach for his advice during the preparation of this manuscript and to Dr. Barry Berkowitz for the gift of SKF 100273.

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Hypertension. 1988;11:360-370
doi: 10.1161/01.HYP.11.4.360

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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