Augmentation of Centrally Induced Alpha-Adrenergic Vasodepression in Spontaneously Hypertensive Rats

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SUMMARY Central α-adrenergic mechanisms for cardiovascular regulation were studied by injecting phenylephrine into a recipient rat whose head was isolated from its body by cross perfusion with a donor rat. Blood pressure increases produced in the donor were accompanied by concurrent reduction of blood pressure and sympathetic nerve activity in the recipient rat's body. These effects were abolished when α-adrenergic receptors in the perfused head were blocked with phentolamine. By contrast, intracarotid injections of angiotensin increased blood pressure not only in donor but also in recipient rats. The magnitude of phenylephrine-induced vasodepression was significantly greater in spontaneously hypertensive rats (SHR) than in normotensive or DOCA-salt hypertensive ones. Distribution of radioactive microspheres indicated that carotid arterial blood went mainly to the cerebrum, midbrain, and hypothalamus, with almost negligible amounts going to the lower brainstem. Collectively, our results suggest that centrally administered phenylephrine reduces sympathetic vasomotor tone and blood pressure by acting on α-adrenergic receptors located in supramedullary brain areas (possibly in the hypothalamus). In SHR, augmented vasodepressor responsiveness may be due to reduced brain levels of endogenous norepinephrine that could increase the α-adrenergic receptors available. (Hypertension 2: 198-206, 1980)

KEY WORDS • α-adrenergic receptors • blood pressure regulation • cross circulation • hypothalamus • phenylephrine • radioactive microspheres • spontaneous hypertension • sympathetic nervous system

THE hypothesis that central adrenergic mechanisms contribute importantly to cardiovascular homeostasis is often tested by recording responses to centrally administered drugs in rats. Hypotension and bradycardia occur when norepinephrine is injected into the cerebral ventricles, anterior hypothalamus, or nucleus tractus solitarius and because both effects are abolished by phentolamine, they have been attributed to α-adrenergic receptors. Larger doses of norepinephrine injected either intracisternally or into cerebral ventricles elicit pressor effects (probably due to peripheral leakage) that are stronger in spontaneously hypertensive rats (SHR) than in normotensive rats. Epinephrine injected into cerebral ventricles or into the anterior hypothalamus in SHR also lowers blood pressure and heart rate, but because these effects are antagonized by propranolol or metoprolol, they have been attributed to β-adrenergic receptors. In general, the information now available implies that unlike peripheral α- and β-adrenergic receptors that produce opposite vascular effects, those in the brain produce similar effects; however, this supposition cannot be confirmed by using naturally occurring catecholamines like norepinephrine or epinephrine because they stimulate both types of receptors.

In an attempt to clarify underlying mechanisms, we tested intrahypothalamic injections of phenylephrine, a drug that stimulates α-adrenergic receptors almost exclusively. Assessment of data thus obtained was difficult because ensuing changes in blood pressure were small, prolonged, and irregular; accordingly, additional experiments were done to develop an isolated head preparation using a donor to perfuse the head of a recipient rat. Brain areas affected and leakage between the recipient rat's head and body were estimated from distribution of radioactivity following injection of tracer microspheres or 125I-labeled albumin. In some experiments, spike potentials were...
recorded from abdominal sympathetic nerves; and to
determine if the central adrenergic mechanisms thus
revealed would be altered during hypertension, rats
with spontaneous or deoxycorticosterone acetate
(DOCA)-salt hypertension were also studied.

Methods

Four groups of 12-week-old Wistar rats were
studied: normotensive, DOCA-salt hypertensive, and
Kyoto-Wistar normotensive (KNR) and SHR. We
purchased KNR and SHR, derived from the strain
originally described by Okamoto and Aoki,12 from
Taconic Farms, Inc. (Germantown, NY); the others
were outbred Wistar rats purchased from Charles
River Breeding Laboratories (Wilmington, MA).
Intrahypothalamic infusions were done on female rats
weighing about 250 g, but since cross perfusion was
best accomplished with large donor rats weighing 350
g or more, male rats were used routinely in all other
experiments. To induce DOCA-salt hypertension, 3-
week-old outbred Wistar rats were anesthetized with
sodium pentobarbital (8 mg/100 g i.p.) and their left
kidneys were removed; silicon rubber molds con-
taining DOCA (20 mg/100 g body weight) were im-
planted subcutaneously13 and 0.9% sodium chloride
(isotonic saline) solution was substituted for drinking
water. Eight weeks later, systolic pressures measured
in these rats with the tail-cuff method16 averaged
183 ± 6 mm Hg

Intracarotid Injections in Isolated Head Preparations

Three rats, anesthetized with urethane, were used
for each preparation: one supplied blood for priming
extravascular connections while the others served as
the donor and recipient respectively. Blood pressure
and heart rate were routinely recorded from femoral
catheters in all donor and recipient rats. Isolation of
the recipient’s head from its body was accomplished
by connecting both jugular veins and common carotid
arteries through a peristaltic pump (Rainin Instru-
ment Co., Brighton, MA) to corresponding vessels of
the donor rat (fig. 1). As soon as one cannula had been
inserted into a carotid artery in the recipient, it was
temporarily connected to the femoral artery to allow
continued supply of arterial blood to the head while
the remaining carotid was being cannulated; hypoxia
resulting from interruption of cerebral blood flow was
thereby kept at a minimum. Perfusion pressure was
recorded from a sidearm of the carotid circuit between
the pump and the perfused head. Pump speed (2.5 to
5.2 ml/min) was always adjusted to keep carotid
pressure in the recipient rat at the same level as
femoral pressure. All catheters were made of silastic
tubing with teflon tips. Total dead space (venous and
arterial connections including the catheters) of 2 ml
was filled with heparinized blood (500 U/100 g) from
a third rat. In preliminary experiments in which a
pump was not used, skin, skeletal muscles, and other
soft tissues were either cut or ligated so that the
recipient’s head was connected to its body only by the
vertebral column; however, many rats became
dyspneic, hypotensive, and died; and since blood
leakage into the body was substantially reduced when

Direct Hypothalamic Injections

Rats were anesthetized with urethane (100 mg/100
g i.p.) while a catheter was inserted into a femoral
artery for recording blood pressure and heart rate.16
A guide cannula (ga 23 stainless steel tubing) was im-
planted at stereotaxic coordinates: 6.6 antero-
posterior, 1.2 lateral, and —2.0 dorsoventral for the
anterior hypothalamus; and 4.8 anteroposterior, 0.8
lateral, and —2.6 dorsoventral for the posterior
hypothalamus. Injections were given by inserting an
injection cannula (ga 30 stainless steel tubing), con-
ected to a 10 microliter syringe mounted on an infu-
sion pump (Sage Instruments, Cambridge, MA), into
the guide cannula.17 The whole system was filled with
the solution to be injected and each injection had a
volume of 1 μl given at a pump rate of 0.84 μl/min.
After each experiment, areas around the cannula tip
were stained by localized injection of potassium
ferrocyanide;18 and brain sections were then compared
with those in a rat brain atlas.19 At the anterior posi-
tion, the cannula tip was located in the anterior
hypothalamic area adjacent to the ventromedial
hypothalamic nucleus, lateral hypothalamic area, me-
dian forebrain bundle, fornix, and paraventricular
hypothalamic nucleus; at the posterior position, it was
in the posterior hypothalamic nucleus immediately
medial to the mamillothalamic tract and adjacent to
the mammilotegmental tract, lateral hypothalamic
area, dorsal premamillary nucleus, and fornix.

FIGURE 1. Diagram showing arrangements for perfused
head experiments.
a pump was used, cervical soft tissues were left intact in all subsequent experiments.

To allow recording of sympathetic nerve activity in some experiments, the abdominal plexus in the recipient's body was exposed and a bipolar stainless steel electrode ( uninsulated tips 1 mm apart) was placed on the major nerve bundle accompanying the superior mesenteric artery immediately below the coeliac ganglion (hereafter referred to as the abdominal sympathetic nerve). Nerves and electrode tips were immersed in mineral oil to reduce tissue drying. Spike potentials for sympathetic nerve activity were recorded together with femoral arterial pressure on magnetic tape and later played back and analyzed as described by Takeda and Buflag. Numbers of individual pulses per second were counted with a rate analyzer (Frederick Haer and Co., Brunswick, ME) and the output recorded as a histogram (see figs. 2 and 3), digitized through a computer interface, and printed by a programmed calculator (Monroe 1860, Litton Industries, Morristown, NJ).

Determination of Brain Areas Affected in the Perfused Head and Amount of Blood Leakage into the Body

Polystyrene tracer microspheres labeled with either strontium-85 or cerium-141 (3M Company, St. Paul, MN) were routinely injected into the perfused head after completion of most experiments to identify brain areas supplied by the carotid arteries. Microspheres 15 ± 3 μ in size were suspended in 10% dextran and 0.05% Tween 80 by 10-minute use of a magnetic stirrer immediately before injection, and a standard dose of approximately 225,000 microspheres in 0.1 ml (containing 3 μCi) was injected. Bovine serum albumin labeled with iodine-125 3 μCi/5 μl (New England Nuclear, Boston, MA) was similarly injected to estimate leakage into the recipient rat's body. Blood for measuring radioactivity was collected for about 1 minute, after which the head was severed and the brain removed.Brains were cut into five sections representing the cerebrum, midbrain, hypothalamus, cerebellum, and lower brain stem; these sections were weighed and their radioactivity measured by a gamma scintillation counter (Searle Analytic Inc., Des Plaines, IL). Fractional distribution of carotid blood flow was calculated by dividing the regional counts per minute of 124I in the liquefied (body) mixture by the amount of 124I injected, and then multiplying by 100.

Statistics and Drugs Injected

Data, expressed as averages ± SEM, from the four groups of rats were compared by an analysis of variance; for F ratios significant at 5% or less, differences between pairs of means were examined by Duncan's multiple range test. Results from only two groups were analyzed using t tests for comparing means of dependent or independent samples; differences at a 5% level (p < 0.05) or less were considered significant.

Drugs used were: phenylephrine hydrochloride (Neoephedrine), 0.1 to 1000 μg (salt)/rat, angiotensin amide (Hypertensin-CIBA), 0.5-2.0 μg/rat, and phenolamine mesylate (Regitine), 50 μg/rat. For perfused head experiments, drugs were diluted in isotonic saline solution and injected via the carotid circuit in a volume of 10 μl.

Results

Cardiovascular Effects of Intrahypothalamic Microinjections of Phenylephrine

Injection of phenylephrine (10 μg) into the anterior or posterior hypothalamus in Kyoto-Wistar rats caused slight but long-lasting falls in mean femoral pressure whose magnitude tended to be larger in KNR than in SHR (table 1). Heart rate changes were irregular and did not differ between groups. Doses 10 times larger (100 μg) had essentially the same effects, but larger doses of 0.5-1.0 mg produced prominent systemic pressor responses resembling those elicited by intravenous injection of 10 μg (table 1). Effects of intrahypothalamic injections were difficult to interpret, not only because they were small, long-lasting, and unrelated to doses injected, but also because centrally induced vasodepression was masked by the increase in systemic blood pressure.

Phenylephrine-Induced Vasodepression in Cross-Perfused Head Preparations

Conditions optimal for recording cardiovascular responses to centrally administered drugs were established using seven pairs of outbred normotensive rats. Blood pressures in each rat pair were almost identical at the onset, but that in donor rats changed (decreased in four and increased in three) soon after vascular connections to the perfused head were opened; thus, the average baseline for mean femoral pressure (mm Hg ± SEM) of 10 ± 4 in donor rats was slightly lower (p > 0.1 < 0.5) than that of 97 ± 5 in recipient rats. Despite blood pressure fluctuations in donor rats, intracarotid injections of phenylephrine (0.1-10 μg) given at 15-minute intervals consistently elevated femoral blood pressure in the donors while simultaneously lowering it in the recipients, with both effects being dose-dependent (table 2). Larger doses (50 μg) elicited larger pressor responses in all donors, but corresponding vasodepression increased in only two of six recipients, and was either reduced or actually reversed to a pressor response in the others.
Table 1. Cardiovascular Responses to Intrahypothalamic Injections of Phenylephrine (10 μg) in Kyoto-Wistar Normotensive (KNR) and Spontaneously Hypertensive (SHR) Rats

<table>
<thead>
<tr>
<th>Injection site</th>
<th>Postinjection time (min)</th>
<th>Mean pressure (mm Hg)</th>
<th>Heart rate (/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KNR</td>
<td>SHR</td>
</tr>
<tr>
<td>Anterior hypothalamus</td>
<td>5</td>
<td>3 ± 4</td>
<td>7 ± 3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>-1 ± 5</td>
<td>2 ± 2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-10 ± 5</td>
<td>-4 ± 1</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>-14 ± 3</td>
<td>-9 ± 1</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-11 ± 5</td>
<td>-6 ± 1</td>
</tr>
<tr>
<td>Posterior hypothalamus</td>
<td>5</td>
<td>1 ± 4</td>
<td>6 ± 3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>-3 ± 5</td>
<td>3 ± 5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-8 ± 7</td>
<td>-1 ± 4</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>-12 ± 6</td>
<td>-5 ± 4</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-14 ± 6</td>
<td>-6 ± 4</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>2</td>
<td>38 ± 8</td>
<td>44 ± 1</td>
</tr>
</tbody>
</table>

*Average ± SEM changes from five rats in each group with baselines of 80 ± 3 in Kyoto-Wistar normotensive rats (KNR) and 104 ± 2 in spontaneously hypertensive rats (SHR) (p < 0.001) for mean femoral pressure, and 310 ± 12 in KNR and 380 ± 9 in SHR (p < 0.001) for heart rate; none of the differences between groups are significant.

Table 2. Average Changes in Mean Femoral Arterial Pressure Occurring in Normotensive Donor and Recipient Rats (n = 7) after Intracarotid Injections of Phenylephrine Given at 15-Minute Intervals

<table>
<thead>
<tr>
<th>Phenylephrine dose (μg)</th>
<th>Mean pressure (mm Hg)</th>
<th>Donor</th>
<th>Recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>8 ± 2</td>
<td>-5 ± 1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>14 ± 2</td>
<td>-11 ± 2</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>24 ± 3</td>
<td>-14 ± 2</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>33 ± 4</td>
<td>-9 ± 6</td>
</tr>
</tbody>
</table>

*Average ± SEM changes from five rats in each group with baselines of 80 ± 4 in donor and 97 ± 5 in recipient rat; all differences between groups significant at 1%.

Spontaneous changes in perfusion pressure were subsequently reduced by interposing a peristaltic pump between circuits connecting donors and recipients.

Pump speed was set to deliver arterial inflow into the perfused head at a pressure approximating that in the femoral artery of the recipient rat. In seven rat pairs, blood pressure in donor rats fell as soon as the pump was turned on and then stabilized 10 to 15 minutes later at a level about 25 mm Hg lower than before; experiments began only after equilibration had occurred. Phenylephrine (5 μg) had the same effects as when a pump was not used: femoral blood pressure rose in the donors and fell in the recipients. Immediately preceding femoral vasodepression in recipient rats, carotid perfusion pressure increased while rate of sympathetic neural firing was reduced (fig. 2B).

To examine the possibility that responses to phenylephrine were caused by increased perfusion pressure, changes produced by accelerating pump speed were compared with those produced by phenylephrine. Average elevations in perfusion pressure produced by both procedures were almost equal: 39 ± 3 for accelerated pumping and 43 ± 4 for phenylephrine. And yet, femoral arterial pressure in recipient rats was unaffected by the increase in pump speed (−2 ± 2) but markedly lowered by phenylephrine (−18 ± 3; p < 0.005). Similarly, sympathetic neural firing was not appreciably affected by increases in pump speed (fig. 2A) but was reduced during phenylephrine-induced vasodepression (fig. 2B).

Specificity of phenylephrine-induced vasodepression was determined by comparison with responses to intracarotid injections of angiotensin (0.5–2.0 μg) in three preparations; angiotensin elevated carotid perfusion pressure consistently, but it also increased femoral pressures in both recipient and donor rats. Thus, although carotid perfusion pressure could be elevated by either accelerated pumping or injected angiotensin, neither procedure lowered femoral blood pressure in recipient rats.

Responses to phenylephrine were next recorded before and after α-adrenergic receptors were blocked by intracarotid injection of phentolamine, 50 μg. In four preparations, baselines for blood pressure were lowered while those for sympathetic neural firing were unchanged. Vasodepressor responses to phenylephrine were completely abolished for about 30 minutes (see fig. 3 for similar results in SHR); then they gradually reappeared and recovered fully an hour later. These results, therefore, suggest that centrally administered phenylephrine reduces blood pressure and sympathetic nerve activity by acting on α-adrenergic receptors in brain areas perfused by the carotid vascular bed.

Blood Flow Distribution in the Perfused Head

Brain areas perfused by the carotid arteries were identified by measuring regional distribution of radioactivity after injection of tracer microspheres. A stan-
Responses of a normotensive recipient rat to: A: mechanically induced increase in perfusion pressure and B: intracarotid injection of phenylephrine (5 μg). Start of stimulation in each panel is indicated by arrows. Tracings from top to bottom indicate: carotid perfusion pressure (mm Hg), phasic femoral pressure (mm Hg), rate of sympathetic neural firing (spikes/sec), and original analog signal of sympathetic nerve activity.

Comparison of Phenylephrine-Induced Vasodepression in Normotensive and Hypertensive Rats

To determine whether central α-adrenergic mechanisms become altered in hypertensive rats, responses to intracarotid injection of phenylephrine (5 μg) were recorded in the following rat pairs: 8 KNR, 7 SHR, and 5 DOCA-salt hypertensive. The DOCA-salt hypertensive recipients were paired with either outbred normotensive or DOCA-salt hypertensive donors. The baseline for mean femoral pressure in SHR recipients (table 4) was higher than that for KNR (t value of 4.22 from Duncan's test significant at 1%) or outbred normotensives (t value of 2.21 not significant) and lower than that for DOCA-salt
FIGURE 3. Responses to intracarotid injection (arrows) of phenylephrine, 5 μg, in a spontaneously hypertensive, recipient rat. From top to bottom: carotid perfusion pressure (mm Hg), phasic femoral pressure (mm Hg), and rate of sympathetic neural firing (spikes/sec). Panel A was recorded during an initial injection and Panel B after intracarotid injection of phentolamine, 50 μg; note abolition of responses to second injection of phenylephrine.

TABLE 3. Relative Distribution of Radioactivity in Brain Areas after Intracarotid or Left Ventricular Injection of Tracer Microspheres

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Percent cerebral flow</th>
<th>Intracarotid</th>
<th>Left ventricular</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midbrain</td>
<td>104.45 ± 13</td>
<td>127.71 ± 7</td>
<td>not significant</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>197.10 ± 26</td>
<td>85.87 ± 9</td>
<td>&lt; 0.005</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>37.00 ± 16</td>
<td>112.00 ± 9</td>
<td>&lt; 0.005</td>
<td></td>
</tr>
<tr>
<td>Brain stem</td>
<td>8.95 ± 5</td>
<td>119.00 ± 25</td>
<td>&lt; 0.005</td>
<td></td>
</tr>
</tbody>
</table>

*Averages ± SEM expressed as percentage of cerebral blood flow.

hypertensives (t value of 4.17 significant at 5%); corresponding baselines for heart rate (/min) were 350 ± 15 in KNR, 421 ± 13 in SHR, 458 ± 10 in outbred normotensives, and 390 ± 9 in DOCA-salt hypertensives (F ratio of 12.98 significant at 1%). Increases in blood pressure produced by phenylephrine among donor rats were larger in SHR and outbred normotensives than in KNR and DOCA-salt hypertensives (using normotensive donors). A slight bradycardia ranging in magnitude from −11 to −19/min occurred in most recipient rats regardless of grouping. Clearly the most significant finding here was that magnitude of phenylephrine-induced vasodepression in SHR recipients was more pronounced than that in
TABLE 4. Average Femoral Pressures (mm Hg) and Responses to Intracarotid Injection of Phenylephrine in Donor and Recipient Rat Pairs*

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Donor baseline</th>
<th>Donor response</th>
<th>Recipient baseline</th>
<th>Recipient response</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNR</td>
<td>67 ± 3</td>
<td>80 ± 6</td>
<td>123 ± 9</td>
<td>194 ± 6</td>
</tr>
<tr>
<td>SHR</td>
<td>95 ± 9</td>
<td>19 ± 3</td>
<td>104 ± 6</td>
<td>170 ± 13</td>
</tr>
<tr>
<td>Outbred NT</td>
<td>80 ± 6</td>
<td>11 ± 2</td>
<td>104 ± 6</td>
<td>170 ± 13</td>
</tr>
<tr>
<td>Doca-salt HT</td>
<td>74 ± 6</td>
<td>11 ± 2</td>
<td>104 ± 6</td>
<td>170 ± 13</td>
</tr>
</tbody>
</table>

*Average ± SEM from eight Kyoto-Wistar normotensive rats (KNR), seven spontaneously hypertensive rats (SHR), six outbred normotensives (NT), and five DOCA-salt hypertensives (HT). For $f_1 = 3$ and $f_2 = 25$, $F$ ratios of 4.86 or more are required for significance at 1% and of 2.99 or more for significance at 5%.

Discussion

Central localization by drug injection into a recipient animal, whose head is connected to its body solely through the spinal cord, was first described in dogs by Heymans and Bouckaert²³ and later perfected by Taylor and Page.²⁴ The method has apparently not other groups (fig. 4 and table 4); $t$ values of 4.58 comparing SHR with KNR are significant at 1%, and of 3.35 and 3.31 for similar comparisons with outbred normotensives and DOCA-salt hypertensives are significant at 5%. Vasodepression in SHR recipients was also accompanied by reduced sympathetic neural firing, and both responses were abolished by intracarotid injection of phentolamine (fig. 3).

Carotid blood flow was uniformly distributed in all brain areas except the hypothalamus, which had disproportionately higher flows in SHR than in other rats. Actual values (flow distribution/g tissue) (fig. 5) for the hypothalamus ($F$ ratio of 7.88 significant at 1%) of 169.62 ± 17 in SHR were higher than those of 114.53 ± 11 for KNR ($t$ value of 3.21 significant at 5%), 120 ± 10 for outbred normotensives ($t$ value of 2.68 not significant), or 78.05 ± 11 for DOCA-salt hypertensives ($t$ value of 4.72 significant at 1%).

Because vascular separation between head and body of recipient rats was incomplete (i.e., vascular connections through cervical soft tissues and vertebral column were patent), magnitude of leakage was estimated from the percentage of $^{18}$I found in the body after injection into the perfused head. Leakage was generally higher in KNR (18.7 ± 1%) and SHR (15.42 ± 2%) than in either outbred normotensives (11.89 ± 2%) or DOCA-salt hypertensives (9.46 ± 1%). The $F$ ratio of 5.78 is significant at 1%, but of the different comparisons between pairs of means, only the $t$ value for KNR versus DOCA-salt hypertensives (3.80) is significant at 5%. In general, therefore, leakage from head to body was less than 20% so that if distribution of phenylephrine parallels that of the microspheres, for every 5 ng of phenylephrine injected, less than 1 ng would reach vascular beds in the recipient's body.
been used in rats until now, but the underlying principle is still that neurally mediated effects can be separated from others exerted on the cardiovascular system. Despite several modifications (i.e., a peristaltic pump was used to maintain blood flow to and from the head constant; cervical tissues and buffer nerves were left intact; and vertebral arteries were not occluded), leakage from the head into the body was less than 20%, and our donor-recipient pairs usually remained viable. Vascular isolation of the head was not exactly the same; yet our results agree with those described by Taylor and Page in that centrally injected adrenergic agonists, which normally produce pressor effects, consistently produced vasodepression instead.

How phenylephrine injected into the head caused blood pressure in the body of a recipient rat to fall, or why vasodepression thus induced lasted twice as long as the pressor response to intravenous injection, is uncertain. Because most vessels connecting the head and body had been cut, it seems reasonable to assume that systemic effects were mediated neurally through the spinal cord and sympathetic nerves. Although the blood-brain barrier is generally believed to prevent entry of most amines from the blood into the brain, the induction of systemic vasodepression here implies that phenylephrine did cross the barrier at some point. Presumably this happens at brain regions like the median eminence or area postrema where capillaries with fenestrated endothelia provide specific sites for transferring solutes regardless of size or lipid solubility. Our results suggest that after crossing the blood-brain barrier, phenylephrine acted on $\alpha$-adrenergic receptors in the hypothalamus to reduce efferent sympathetic vasomotor tone and thereby lower blood pressure. The brain receptors involved were probably $\alpha$-adrenergic because they were stimulated by phenylephrine and because resulting effects were abolished by phentolamine. Furthermore, regional distribution of carotid blood flow indicated that exposure to phenylephrine was highest in supramedullary brain areas, including the hypothalamus; and mapping done with microinjections has shown that the anterior preoptic region located close to the median eminence is among the areas most responsive to norepinephrine.

Intrahypothalamic infusion appears unreliable for studying drugs acting on the brain because effective stimulation depends on delivery of highly concentrated solutions in volumes that must not exceed 1 $\mu$L. Aside from the fact that direct injections may cause neural damage, accurate infusion of such minute amounts is technically difficult, and physical characteristics of the chemical (i.e., rate of dissolution, metabolic degradation, and extent of tissue dispersion) injected determine the duration of ensuing effects. Although our standard 10 $\mu$g dose (about 4 nmol) for phenylephrine was smaller than that used by Struyker Boudier et al. (70 nmol), it sufficed to elicit pressor effects upon intravenous injection; and with infusion of larger doses into the hypothalamus, we obtained pressor responses just as they did. Thus, any

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**Figure 5.** Distribution of radioactivity after intracarotid injection of $^{89}$Sr-labeled microspheres in four different rat groups. Wistar = Wistar normotensive rat; DOCA = Wistar DOCA-salt hypertensive rat; KNR = Kyoto-Wistar normotensive rat; SHR = Kyoto-Wistar spontaneously hypertensive rat.
centrally induced vasodepression would have been obscured by systemic pressor effects produced by phenylephrine absorbed from the site of infusion.

Decreases in sympathetic activity and blood pressure resembling those shown here are also produced by clonidine, which presumably acts on an inhibitory α-adrenergic system in the nucleus tractus solitarius to activate baroreceptor reflexes. 37 If it is assumed that phenylephrine acts mainly on the posterior hypothalamus, then our results could mean that the aforementioned inhibitory system extends above the medulla. By contrast, the existence of an excitatory α-adrenergic system in the hypothalamus has been proposed based on drug-induced changes in pressor responsiveness to electrical stimulation of the posterior hypothalamus (i.e., inhibition by α-adrenergic blockade with tolazoline or piperoxan, and enhancement upon reduction of norepinephrine reuptake with desipramine). 8 But although these studies seem linked to ours by common involvement of central α-adrenergic mechanisms, further comparisons cannot be made because of obvious differences not only in methods of stimulation employed, but also in brain areas affected.

Why responsiveness to phenylephrine-induced vasodepression was augmented selectively in SHR is conjectural. Although blood-brain barrier dysfunction has been reported after abrupt pressure elevations in renal hypertensive rats, 26 or when carotid arterial pressure is increased beyond 200 mm Hg, 23 this would not account for our results, because the blood pressure elevation in SHR was not very high and was in fact lower than that in DOCA-salt hypertensive rats (table 4). A possible explanation is that with increased flow to the hypothalamus (see fig. 5), amounts of phenylephrine delivered to α-adrenergic receptors there were higher in SHR than in other rats. Alternatively, a derangement of catecholamine metabolism in the hypothalamus could be involved. Based on the diminished hypothalamic levels of norepinephrine first reported by Yamori et al. 39 and recently confirmed by Saavedra et al., 50 it can be argued that reduced levels of endogenous norepinephrine would leave more α-adrenergic receptors available for phenylephrine, such that ensuing vasodepression would therefore become more pronounced.

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H Takahashi and R D Buñag

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