Sympathetic Nerves and Adrenal Medulla: Contributions to Cardiovascular-Conditioned Emotional Responses in Spontaneously Hypertensive Rats

AKIRA SAKAGUCHI, M.D., JOSEPH E. LEDOUX, PH.D., AND DONALD J. REIS, M.D.

SUMMARY This report investigates the contributions of the sympathetic nerves and adrenal medulla to resting mean arterial pressure (MAP) and to emotionally conditioned MAP and heart rate (HR) responses in unrestrained spontaneously hypertensive rats (SHR) and Wistar-Kyoto normotensive control rats (WKY). Resting MAP (in mm Hg), which was higher in SHR (WKY = 120 ± 4; SHR = 163 ± 4; p < 0.01), did not differ in the two strains following chemosympathectomy (WKY = 105 ± 2; SHR = 101 ± 2; n.s.). Adrenal medullectomy did not affect resting MAP in WKY (125 ± 6; n.s.) but lowered it in SHR (146 ± 5; p < 0.05), relative to controls (see above). The conditioned pressor response (in mm Hg) in controls consisted of two peaks (I, II) in both strains, but was exaggerated in SHR (I = WKY, 13 ± 1; SHR, 25 ± 2; p < 0.01; II = WKY 10 ± 2; SHR 20 ± 2; p < 0.01). Chemosympathectomy suppressed (relative to controls) the first peak, but not the second, in both strains (WKY: I = 4 ± 1, p < 0.01; II = 12 ± 2, n.s.; SHR: I = 6 ± 1, p < 0.01; II = 15 ± 2, n.s.). Adrenal medullectomy alone had little effect on the pressor response, but when combined with chemosympathectomy both peaks were largely eliminated (WKY: I = 2 ± 1; II = 5 ± 1; SHR: I = 1 ± 0; II = 2 ± 0). These data indicate that: 1) hypertension in conscious, freely behaving SHR is largely sustained by the sympathetic vasomotor nerves but that the adrenal medulla contributes to the magnitude of the elevation; 2) the early component of the exaggerated pressor response during aversive stimulation is mediated by sympathetic vasomotor excitation; and 3) the later component of the exaggerated pressor response reflects coactivation of the sympathetic vasomotor nerves and the adrenal medulla. (Hypertension 5: 728-738, 1983)

KEY WORDS • sympathetic nerves • adrenal medulla • classical conditioning • fear • conditioned emotional response • spontaneously hypertensive rats

THE magnitude of mean arterial pressure (MAP) changes associated with the expression of certain behaviors is exaggerated in spontaneously hypertensive rats (SHR). We have observed that during conditioned emotional (fear) responses and during drinking, but not during feeding, grooming, or exploration, greater increases in MAP occur in SHR than in Wistar-Kyoto (WKY) rats.

In the present study we have sought to determine the peripheral efferent mechanisms through which the exaggerated MAP changes associated with conditioned emotional responses in SHR are expressed. We have examined SHR and WKY subjected to classical fear conditioning and chronically implanted for computer-assisted recording of MAP and heart rate (HR) while awake and unrestrained. The experiments involved surgical and pharmacological manipulations of the peripheral autonomic nervous system and allowed us to determine the relative contributions of the sympathetic nerves and adrenal medulla to the maintenance of hypertension and to the expression of the exaggerated pressor response during emotional arousal in SHR.

Methods

General

Male SHR and WKY, aged 14–16 weeks (275–325 g), obtained from Taconic Farms, Germantown, New York, were individually housed in clear plastic cages with a stainless steel wire top that held a water dispenser and lab chow. The cages were kept in the animal housing area, which was thermally controlled (at 20°C), sealed to sunlight, and maintained on a fluorescent light cycle (on at 0600, off at 0800).
**Surgery and Drug Administration**

**Chronic Catheterization for Cardiovascular Recording**

Procedures for chronic implantation of cannulas have been used in earlier studies. Animals were anesthetized with halothane (2.5%–3% in O₂). For recording arterial pressure and HR, a plastic cannula (Tygon, Westvaco, Cleveland, Ohio) (0.012 inch, i.d., connected to the Tygon tubing of 0.02 inch, i.d.) filled with saline containing heparin (50 units/ml of 0.9% saline), was inserted into the thoracic aorta via the left common carotid artery, and its tip was placed at the level of the diaphragm. At the same time, another plastic cannula was inserted into the superior vena cava via the left external jugular vein. With the catheters fixed to the soft tissues with sutures, the free ends were passed subcutaneously behind the ear to the back of the neck, brought through the skin through a stab wound, and sealed with stainless steel obturators. The neck wound was closed with sutures, and the rat was returned to its home cage.

**Adrenal Medullectomy and Chemosympathectomy**

Some animals were subjected to bilateral adrenal medullectomy while under anesthesia for implantation of catheters. The adrenal glands were exposed through the back. A small incision was made on the surface of the gland, and the medulla was removed with smooth-surfaced forceps. Sham surgery was performed similarly, but without incision. The skin wound was closed by suture and the animals were returned to their cages. All tests of adrenal medullectomized animals were conducted on the day following surgery.

Chemosympathectomy was produced in two ways. Guanethidine sulfate (10 mg/ml/kg, Ciba-Geigy, Summit, New Jersey) dissolved in 0.9% saline was administered (i.v.) 15 minutes prior to tests, or the neurotoxin 6-hydroxydopamine (6-OHDA) (100 mg/ml/kg, Sigma Chemical Company, St. Louis, Missouri) was administered 24 hours before the tests. Guanethidine sympathectomy is achieved by the acute, reversible blockade of transmission in peripheral postganglionic sympathetic nerves through norepinephrine depletion, while 6-OHDA results in the destruction of the noradrenergic terminals of the sympathetic nerves through norepinephrine depletion. Both procedures spare the adrenal medulla. The effects of guanethidine on resting cardiovascular activity and on conditioned cardiovascular responses were assessed, but the effects of 6-OHDA were only examined with respect to resting MAP and HR.

**Drug Treatment**

Phentolamine mesylate (10 mg/ml/kg, Ciba-Geigy), propranolol HCl (1 mg/ml/kg; Ayerst Laboratories, New York, New York), and atropine sulfate (2 mg/ml/kg, Sigma) were administered, each to separate animals. All drugs were dissolved in 0.9% saline and administered (i.v.) 10 minutes prior to tests. These doses were selected from a survey of the literature and through pilot studies.

**Classical Fear Conditioning**

Classical fear conditioning procedures, involving the repeated pairing of an auditory tone with electric footshock, were used to establish cardiovascular responses to the tone, as previously described. In brief, at the time of conditioning, each rat was removed from its home cage and placed in a standard conditioning chamber enclosed by a sound attenuating cubicle. After 5 minutes of acclimation to the chamber, the tone conditioned emotional stimulus (CES) (800 Hz, 80 db, 10 sec) was presented through a speaker mounted in the chamber 40 times at an average intertrial interval of 150 seconds (range = 100–200 sec).

During the first 10 trials, the tone was presented alone, in order to extinguish unconditioned responses. Over the following 30 trials, the termination of the tone was coextensive with a 0.5-second delivery of the electric footshock unconditioned stimulus (US) (1.5 mA) distributed across the grid floor of the chamber. At the end of 40 trials, the animal was returned to its home cage. We have found this procedure to be effective for the establishment of cardiovascular and behavioral responses to the CES in SHR and WKY.

**Assessment of Resting Mean Arterial Pressure and Heart Rate and Conditioned Cardiovascular Responses**

Pulsatile pressure and MAP were recorded from a strain gauge transducer (Statham P23D6, Statham Instruments, Hata Rey, Puerto Rico), which was placed 2 cm above the floor, approximately at the level of the heart, via an input coupler by the polygraph (Beckman R611, Beckman Instruments, Schiller Park, Illinois). The HR was derived from a cardiotachometer. In addition to display on paper by the polygraph, all data were recorded on-line by a microcomputer (Varian V-76, Vortex II Multitask Digital Computer, Irvine, California) which sampled MAP at a rate of 100/sec. Incoming data from each experiment were digitized, processed, and stored on disc for subsequent analysis.

At the time of data acquisition, the distal end of the arterial cannula was connected via extension tubing (Tygon 0.02 inch i.d., and 55 cm long) to the transducer (see above), which allowed the animal to move freely in its home cage. The animals were continuously observed to ensure that the catheters were not disturbed during recordings. The home cage was transferred to an enclosed observation chamber and, after 15 minutes, resting MAP and HR were continuously recorded for 5 minutes and stored on disk. Rest in these experiments is defined as a quiet period during which relatively little ambulation or other movement occurs.

Three extinction trials (tone CES delivered without shock US) were presented 10–15 minutes following drug treatment or immediately following the assessment of resting MAP and HR in animals not receiving drug treatment (adrenal operated). MAP and HR were
continuously recorded throughout the extinction trials, which were presented on the same schedule as the conditioning trials.

Following the extinction test, a table was generated from the computer which listed the average value (mean) and variability (standard deviation) of the MAP and HR during the following time periods: pre-CES, CES, and post-CES periods, and CES. In this way, the absolute value of MAP and HR during the pre-CES, CES, and post-CES periods, and CES were computed. The pre-CES periods, in addition to serving as the baseline for conditioned response measures, were used to determine the effect of drug treatment on resting MAP and HR.

Conduct of the Experiment

SHR and WKY were subjected to classical fear conditioning and at least 2 hours later were implanted with catheters while under anesthesia. The adrenal medulla was also removed in some animals while they were anesthetized. The following day, the animal’s home cage was placed in the observation chamber. After a 15-minute delay, resting MAP and HR were measured. Immediately thereafter, animals received either a drug or vehicle injection through the intravenous catheter, if appropriate. MAP and HR responses elicited by the CES were measured 10-15 minutes later.

Statistical Analysis

The data were analyzed using Dunnett’s test after analysis of variance or Student’s t test for absolute values and Mann-Whitney U test for percent changes. The particular analysis employed is indicated with the data.

Table 1. Effects of Peripheral Autonomic Receptor Blockade on Resting Mean Arterial Pressure and Heart Rate in WKY and SHR

<table>
<thead>
<tr>
<th>Rat group</th>
<th>No. of rats</th>
<th>Before blockade</th>
<th>After blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MAP (mm Hg)</td>
<td>HR (bpm)</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>6</td>
<td>122±4</td>
<td>311±6</td>
</tr>
<tr>
<td>Phentolamine (10 mg/kg)</td>
<td>5</td>
<td>116±2</td>
<td>289±11</td>
</tr>
<tr>
<td>Propranolol (1 mg/kg)</td>
<td>5</td>
<td>126±3</td>
<td>334±11</td>
</tr>
<tr>
<td>Atropine (2 mg/kg)</td>
<td>6</td>
<td>120±5</td>
<td>319±9</td>
</tr>
</tbody>
</table>

| SHR       |             |              |            |              |           |
| Saline    | 6           | 177±4***     | 368±8+++  | 167±7+++   | 350±9+++  |
| Phentolamine (10 mg/kg) | 10 | 171±3++     | 371±11+++ | 76±3**    | 442±131** |
| Propranolol (1 mg/kg) | 10 | 171±3+++   | 384±12++  | 166±7++   | 335±7     |
| Atropine (2 mg/kg) | 10 | 165±4+++  | 371±6+++  | 161±3+++  | 424±4++   |

Values represent means ± SE. Significance determined using Dunnett’s test after analysis of variance (for absolute values) and Mann-Whitney U test (for % change).

Results

Resting Mean Arterial Pressure and Heart Rate

Before treatment, resting MAP and HR (table 1) were both higher in SHR than in WKY. The magnitude of the hypertension in SHR is consistent with that commonly reported for animals of comparable age (14–16 weeks old). The elevated HR is consistent with our earlier finding that resting HR is elevated in conditioned SHR relative to unconditioned SHR, which did not differ from WKY.

Autonomic Receptor Blockade

Administration of the alpha adrenergic antagonist phentolamine (10 mg/ml/kg) resulted in a pronounced reduction in MAP accompanied by tachycardia in both rat strains (table 1). Resting MAP and HR, which were both elevated in saline-treated SHR relative to similarly treated WKY, did not differ in phentolamine-treated SHR and WKY.

Propranolol (1 mg/ml/kg), the beta-adrenergic antagonist, equated the resting HR in SHR with that in WKY, which differed before treatment (table 1). Propranolol had no effect on resting MAP in either strain.

Atropine (2 mg/ml/kg) increased the resting HR without affecting the resting MAP in both strains (table 1). The increase in resting HR was, however, greater in WKY. Thus, resting HR did not differ between strains after treatment.

Chemosympathectomy

Chemosympathectomy was produced in the present study through administration of the postganglionic adrenergic neuron blocking agent, guanethidine sulfate, or through the neurotoxin 6-OHDA. Guanethidine (10 mg/ml/kg), like phentolamine, lowered resting MAP and increased resting HR in both SHR and WKY (table 2). Although the magnitude of the fall in MAP was greater in SHR and the magnitude of the
increase in HR was greater in WKY following guanethidine treatment, MAP and HR did not differ in the two strains. Similar effects on resting MAP and HR were produced in SHR by 6-OHDA (100 mg/ml/kg) (table 2).

Adrenal Demedullation

Removal of the adrenal medulla had no effect on resting MAP in WKY, but produced a modest hypotension in SHR (table 2). Resting HR, following demedullation, was elevated in both SHR and WKY (table 2). However, resting HR was higher in demedullated SHR than in demedullated WKY, as in control SHR and WKY. When combined with sympathectomy, adrenal demedullation produced no additional changes in resting MAP or HR beyond the effects of guanethidine alone.

Emotionally Conditioned Mean Arterial Pressure and Heart Rate Responses

The conditioned MAP response (fig. 1) was a biphasic pressor response in both strains, as we have reported.4 In WKY (fig. 1 A), the first pressor peak (I) occurred at the 3rd second and the second peak (II) at the 9th second following CES onset. The MAP remained elevated above baseline at the 15th and 30th second following the termination of the CES.

The conditioned HR response in WKY (fig. 1 A) consisted of a tachycardic peak (Ia) at the 6th second after CES onset in association with the MAP drop which followed pressor peak I. The tachycardia then declined toward baseline. By the 8th second following termination of the CES, the HR was below baseline, where it remained through 30th post-CES second.

The pressor response in SHR (fig. 1 B) was greater than in WKY throughout the CES. The first peak (I) in SHR occurred at the same time as in WKY (3rd sec), but the second peak (II) occurred somewhat earlier in SHR (7th sec) than in WKY (9th sec). The MAP in SHR remained elevated above baseline, but not above that seen in WKY, throughout the post-CES period.

The HR conditioned response in SHR (fig. 1 B) was biphasic, with the first peak (Ia) occurring during the CES (5th sec) and the second (IIa) occurring shortly after CES termination (4th post sec). Each pressor peak was associated with a relatively low level of HR and each MAP trough was accompanied by a rise in HR. Thus, the peak HR response (Ia) during the CES occurred at the MAP drop following pressor peak I and the post-CES HR peak (IIa) appeared in conjunction with the MAP drop following the second pressor peak (II).

In summary, the conditioned pressor response was biphasic in both strains, with the two peaks occurring during the CES. The MAP response was greater in SHR throughout the CES, but was not different in SHR and WKY during most of the post-CES period. The conditioned HR response in WKY was a monophasic tachycardia that peaked during the CES. In contrast, SHR exhibited a biphasic tachycardia that peaked during the CES and again shortly after CES termination.

Efferent Mechanisms Underlying Emotionally Conditioned Cardiovascular Responses

Intact SHR and WKY

Efferent regulation of CES-elicited changes in MAP and HR in intact SHR and WKY was examined through receptor blockade experiments involving alpha- and beta-adrenergic and cholinergic (muscarinic) receptor antagonism.

Alpha-adrenergic blockade suppressed the two peaks of the MAP response to the CES in both strains (fig. 2 A; table 3). Although MAP remained near baseline after CES termination in SHR, MAP in WKY was below baseline throughout the post-CES period (fig. 2 A). The conditioned tachycardia was prolonged in both strains. While the tachycardia was stable in SHR, the HR continued to rise in WKY following CES termination (fig. 2 A).

Beta-blockade suppressed pressor peak I and converted the tachycardic HR response to a large magni-
Figure 1. Exaggerated conditioned pressor and tachycardic responses in SHR. Presentation of the CES elicited a biphasic pressor response and tachycardia in WKY (A) and in SHR (B). Both the first (I) and second (II) pressor peak were exaggerated in SHR relative to WKY. The MAP remained elevated through the 30th (IV) second following the termination of the CES. The peak conditioned HR response (la) during the CES did not differ in SHR and WKY. SHR exhibited a second HR peak (Ha) immediately following the offset of the CES. While HR returned to baseline in SHR during the post-CES period, in WKY the tachycardia converted to a bradycardia in the early post-CES period and remained below baseline throughout the post-CES period. Data represent means ± se. Statistical differences computed using Student's t test (*p < 0.05; ** p < 0.01).

Sympathectomy

Sympathectomy through post-ganglionic adrenergic neuron blockade suppressed the first peak of the pressor response and produced a greatly enhanced and delayed second peak in both SHR and WKY (fig. 3 A). Moreover, in both strains the tachycardia was converted into a large magnitude decelerative HR response, which was greater in WKY (fig. 3 A). Thus, a delayed and amplified pressor peak associated with a pronounced bradycardia remains in sympathectomized SHR and WKY.

Adrenal Demedullation

Adrenal demedullation suppressed slightly the first pressor peak in WKY but had no effect on the MAP response in SHR (fig. 3 B; table 3). Tachycardia during the CES was not altered by adrenal demedullation in either strain, but the second HR peak, characteristic only of SHR, was completely eliminated by adrenal demedullation (fig. 3 B).

Adrenal Demedullation Plus Sympathectomy

The combined treatment involving adrenal demedullation and sympathectomy suppressed almost completely the MAP response elicited by the CES in both SHR and WKY (fig. 3 C; table 3). The tachycardia elicited by the CES was converted into a slight bradycardia in both strains following the combined treatment.

The delayed prolonged pressor response in sympathectomized animals thus appears to be due to adrenomedullary catecholamine release. This is indicated by the fact that pressor activity is eliminated by sympathectomy in adrenal-demedullated animals or by alpha-adrenergic blockade in intact animals.
FIGURE 2. Effects of autonomic nerve receptor blockade on the conditioned MAP and HR responses. Adrenergic alpha-receptor blockade (A) by administration of phentolamine (10 mg/ml/kg) substantially suppressed the pressor response and prolonged the tachycardia in both strains. Adrenergic beta-receptor blockade (B) with propranolol (1 mg/ml/kg) slightly suppressed the first peak of the pressor response and delayed the second peak in both strains. Although the effect on the MAP response was minimal, propranolol converted the tachycardic response to a bradycardia, which mirrored the pressor response in both strains. Moreover, the magnitude of the bradycardia was greater in WKY than in SHR, in spite of the similar magnitude of the pressor response in the two strains. Cholinergic muscarinic receptor blockade (C) with atropine (2 mg/ml/kg) had little effect on the pressor response in either strain, but produced an intensified monophasic tachycardia that lasted into the post-CES period in both strains. Thus, alpha-adrenergic vasomotor nerve activation is the primary factor contributing to the conditioned pressor response and the interaction of vagal and beta-adrenergic sympathetic neural activities determines the conditioned HR response in both strains. Values represent means ± se. Statistical differences computed using Dunnett’s test after analysis of variance. (*p < 0.05; **p < 0.01; significant differences indicated for the 3rd, 5th, 7th and 9th second of the CES-period and the 5th, 15th and 30th second of the post-CES period).
FIGURE 3. Effects of sympathectomy, adrenal medullectomy, and the combined treatment involving adrenal medullectomy and sympathectomy on the conditioned MAP and HR responses. Sympathectomy (A) achieved by administration of guanethidine (10 mg/ml/kg) suppressed the first peak and elicited a delayed and long lasting pressor response in both strains. Furthermore, it converted the tachycardia into a bradycardia, which is relatively greater in WKY, in spite of the smaller pressor response than in SHR. Although the adrenal medullectomy (B) had little effect on the conditioned MAP response in either strain, it eliminated the second peak of the tachycardia in SHR. The combined treatment (C) largely eliminated both the MAP and HR conditioned responses in both strains. Thus, the conditioned MAP response is determined by the sympathetic vasomotor nerves, although adrenomedullary catecholamines are normally released. In SHR, the second HR peak, which is absent in WKY, is determined solely by the adrenal medulla. Values represent means ± se. Statistical differences computed using Dunnett's test after analysis of variance (*p < 0.05; **p < 0.01, significant differences indicated for the 3rd, 5th, 7th and 9th second of the CES-period and the 5th, 15th and 30th second of the post-CES period).
## Table 3. Conditioned MAP and HR Responses

<table>
<thead>
<tr>
<th>Rat group</th>
<th>No. of rats</th>
<th>MAP peak responses</th>
<th>HR peak responses</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td><strong>WKY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>13 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Phentolamine (10 mg/kg)</td>
<td>5</td>
<td>4 ± 1**</td>
<td>1 ± 1**</td>
</tr>
<tr>
<td>Propranolol (1 mg/kg)</td>
<td>5</td>
<td>8 ± 1</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Atropine (2 mg/kg)</td>
<td>6</td>
<td>9 ± 2</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Adrenal demedullation</td>
<td>6</td>
<td>6 ± 1**</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Guanethidine (10 mg/kg)</td>
<td>8</td>
<td>4 ± 1**</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Adrenal demedullation</td>
<td>7</td>
<td>2 ± 1**</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>

**SHR**

| Control | 19 | 25 ± 2††       | 20 ± 2††       | 13 ± 2           | 13 ± 3           |
| Phentolamine (10 mg/kg) | 10 | 5 ± 1**         | 4 ± 1**        | 6 ± 3           | 12 ± 5           |
| Propranolol (1 mg/kg)    | 10 | 17 ± 3t*        | 13 ± 2*        | -5 ± 2**        | -12 ± 3**        |
| Atropine (2 mg/kg)       | 10 | 28 ± 2††        | 25 ± 2††       | 19 ± 3          | 14 ± 3           |
| Adrenal demedullation    | 10 | 21 ± 3††        | 16 ± 3††       | 11 ± 2          | 0 ± 2**          |
| Guanethidine (10 mg/kg)  | 10 | 6 ± 1**         | 15 ± 2         | -2 ± 1**        | -7 ± 3**         |
| Adrenal demedullation    | 10 | 1 ± 0**         | 2 ± 0**        | -3 ± 1**        | -4 ± 1**         |

I = 3rd second of CES; Ia = 5th (SHR) and 6th (WKY) second of CES; II = 7th (SHR) and 9th (WKY) second of CES; Ila = 4th second of post-CES.

Values represent means ± se. Significance determined using Dunnett's test after analysis of variance.

* p < 0.05; **p < 0.01; relative to control group within strain.

† p < 0.05; †† p < 0.01; strain differences for each treatment.

**Discussion**

We have examined SHR and WKY chronically implanted for continuous computer-assisted recording of MAP and HR while awake and unrestrained. The objective of the study was to determine the relative contribution of the sympathetic vasomotor nerves and the adrenal medulla to the control of resting MAP and HR and to the expression of the exaggerated changes in MAP which accompany conditioned fear reactions in SHR. The animals were subjected to chemosympathectomy, adrenal demedullation, and peripheral blockade of alpha- and beta-adrenergic, as well as cholinergic-muscarinic, receptors. The findings indicate specific roles for the different peripheral autonomic effectors in the maintenance of hypertension in SHR and in the expression of the cardiovascular concomitants of emotional arousal in normotensive animals and in SHR.

### Maintenance of Hypertension by Peripheral Autonomic Effectors

Studies based on direct recordings of sympathetic nerve activity, measurements of plasma catecholamines, and pharmacological blockade of sympathetic outflow have indicated that the elevated blood pressure in SHR is maintained by enhanced sympathetic vasomotor tone. Many of these studies, however, have examined anesthetized animals.

The present findings, involving conscious SHR and WKY, are consistent with the view that the strain difference in basal MAP is due to enhanced sympathetic influences. Thus, elimination of endogenous peripheral catecholamines by the combined treatment involving sympathectomy and adrenal demedullation lowered MAP in both strains such that the strain difference in resting MAP was abolished. Sympathectomy alone lowered MAP in both strains almost to the same level as the combined treatment. Adrenal demedullation alone did not change MAP in WKY but lowered MAP in SHR, which nevertheless remained hypertensive relative to WKY. These results indicate that resting MAP, without the influence of endogenous catecholamines, is the same in SHR and WKY and that, while the sympathetic nerves are crucial to the maintenance of hypertension, adrenal catecholamines also contribute to the magnitude of the elevation in MAP.

It is of interest to note that the hypotension produced by phentolamine was greater than that produced by sympathectomy combined with adrenal demedullation. The difference could reflect stimulation, by circulating catecholamines, of the vasodilating beta-2 receptors, which are spared by alpha-blockade.

Our findings differ somewhat from those of Touw et al., who observed that ganglionic blockade with hexamethonium left MAP elevated in SHR above that in similarly treated WKY. However, Kubo has shown...
MAP in SHR and WKY is higher following hexamethonium treatment than following 6-OHDA sympathectomy. This suggests that hexamethonium, with the dosage used in these experiments, may not completely block peripheral catecholaminergic transmission.

The elevated basal HR in SHR appears to be due to diminished vagal and increased sympathetic influences. Propranolol, which had no effect on MAP in either strain, made the resting HR in SHR equal to that in WKY. Atropine, which also had no effect on resting MAP in either strain, induced a greater increase in resting HR in WKY than in SHR, and also made equal the HR in SHR with that in WKY.

Thus, in summary, the elevated resting blood pressure in unanesthetized and unrestrained SHR is maintained mainly by increased sympathetic neural vaso-motor tone, but adrenal catecholamines also contribute. The higher resting HR in SHR involves diminished vagal and increased sympathetic influences on the heart.

**Emotionally Conditioned Cardiovascular Responses**

Studies of autonomic effector processes in conscious animals have focused on the measurement of levels of plasma catecholamines and on the use of receptor blockade techniques during exposure to various forms of aversive stimulation. The humoral measurements have been used to index sympathetic nerve and adrenomedullary activity, but suffer in several ways. While such findings indicate that more or less of a substance is released, the results, with respect to cardiovascular control, are only correlational. In addition, since blood must be collected over a relatively long period, plasma measurements are not capable of indicating the temporal relationship between the secretions and cardiovascular changes. Moreover, it is not possible to separate the unique contributions of epinephrine and norepinephrine, and thus of the adrenal medulla and sympathetic nerves, to a given cardiovascular response. The receptor blockade approach does allow the direct assessment of the select contribu-
tion of several different peripheral effectors to cardio-
vascular control, but fails to distinguish between the alpha-adrenergic effects of sympathetic vasomotor nerve excitation and release of adrenal catecholamines. Through a combination of receptor blockade studies, selective disruption of sympathetic transmission by chemosympathectomy, and removal of the adrenal medulla, we have examined the peripheral mechanisms through which the cardiovascular responses are expressed in SHR and in normotensive WKY.

**Conditioned Responses in Normotensive Animals**

Presentation of the 10-second CES to WKY while at rest in their home cage resulted in a biphasic pressor response accompanied by tachycardia. The tachycardia, however, converted to a prolonged bradycardia with the offset of the CES. This response pattern is similar to that which we previously described, except that bradycardia was observed during CES in our earlier study. This difference could reflect individual differences in conditioned HR responses, as well as the fact that a higher intensity footshock was employed in the present study.

The biphasic pressor response in WKY appears, for two reasons, to be primarily attributable to sympathetic neurogenic control. First, both peaks of the pressor response were largely eliminated by alpha adrenergic blockade. Second, sympathectomy eliminated the first peak and produced a delayed, prolonged second peak. Although adrenal demedullation alone did not substantially alter the pressor response, both pressor peaks were eliminated by demedullation combined with sympathectomy. Thus, the pressor response is normally mediated by sympathetic neurogenic mechanisms. While adrenal catecholamines are released, their pressor potential is masked when the sympathetic nerves are intact. Similar findings have been observed by Del Bo et al. in studies of cerebellar fastigial nucleus stimulation in sympathectomized and adrenal demedullated rats. The enhanced pressor response in sympathectomized animals could reflect either supersensitivity to circulating catecholamines or enhanced secretion of adrenal catecholamines following chemosympathectomy.

The tachycardia during the CES in WKY appears to be due to excitation of beta-adrenergic receptors activated by sympathetic nerves, since it was converted to a bradycardia by either propranolol or chemosympathectomy, but not by adrenal demedullation. However, coexcitation of the cardiovagal nerves is indicated by several observations: first, the tachycardia appears earlier and was larger in atropine treated animals than in controls, suggesting that vagal influences normally serve to suppress HR acceleration during emotional arousal; second, the small, but consistent, delayed bradycardia in the combined sympathectomized-adrenal demedullated group, which lacked a pressor response, suggests direct vagal excitation in the absence of baroreceptor activation; third, the bradycardia in propranolol-treated and sympathectomized animals, which formed the mirror image of the pressor response, was greater than that in the combined treated animals. This suggests that the baroreflex, which is normally activated, but largely masked, was unmasked by blockade of beta-adrenergic influences.

Shortly after termination of the CES, the HR fell below baseline in WKY. This bradycardia, which was absent in the atropine-treated group, suggests the manifestation of the combined direct and indirect (through baroreflex) vagal influences which were activated during the CES and unmasked as the stimulus terminated.

Past studies of cardiovascular conditioned responses in normotensive animals have focused on the acquisition of the responses and have thus measured the responses during sessions in which the unconditioned stimulus (US), usually electric footshock, is presented in conjunction with the CES. In our experiments, however, we have taken care to separate the establishment of conditioned emotional reactions from the evaluation...
of the responses. In this way, the cardiovascular changes elicited by the CES could be measured without the confounding effects of footshock. Responses in such a situation are a pure reflection of the emotional reaction to the CES rather than to the interaction of the CES and US. Moreover, while most studies which have examined the acquisition of conditioned responses have, in order to facilitate cardiovascular recordings, utilized restrained animals, our experimental conditions allowed the assessment of freely behaving animals. Also, relatively few studies have examined both blood pressure and HR conditioned responses. These differences with past studies thus make our observations on normotensive WKY interesting on their own, in addition to their value as a control for the SHR.

Cardiovascular Hyperreactivity in SHR

The cardiovascular response to the arousal of fear in SHR consisted of a biphasic pressor response and a biphasic tachycardia. Both peaks of the pressor response in SHR, as in WKY, appear to be due to sympathetic vasomotor neural excitation. This is indicated by the failure of adrenal demedullation to alter the response and by the almost complete suppression of the response in phenolamine treated animals and in animals subjected to the combined treatment involving sympathectomy plus adrenal demedullation. That catecholamines are normally released from the adrenal medulla during the CES is demonstrated by the delayed, large magnitude, adrenal-dependent pressor response in sympathectomized animals. This pressor action is, however, normally masked by the dominant control of the sympathetic nerves. While similar if not identical mechanisms underlie the biphasic pressor response in intact WKY and the delayed pressor response in sympathectomized WKY (see above), the larger magnitude responses in SHR suggest that both sympathetic neural and adrenomedullary humoral activity are enhanced in SHR during emotional arousal. That the exaggerated response coupled to emotional behavior is not simply a reflection of structural changes in the vessels of SHR is suggested by our earlier finding that the elevation in blood pressure associated with several other behaviors is comparable in SHR and WKY.

The tachycardia during the CES in SHR closely corresponds to the tachycardia observed in WKY. Moreover, as in WKY, the HR acceleration during the CES is primarily due to neural excitation. That vagal influences tend to suppress the tachycardia in both strains is indicated by the enhanced HR response in atropine treated animals. The HR responses in atropine treated SHR and WKY were in fact similar. However, while propranolol resulted in a bradycardia in both SHR and WKY during the CES, the deceleration was greater in WKY. Since the MAP response to the CES was similar in propranolol treated SHR and WKY, the smaller bradycardia suggests that the baroreceptor reflex is less effectively activated during emotional arousal in SHR. This conclusion is consistent with a variety of studies of anesthetized SHR suggesting abberations in baroreflex control.13,27,28

Although the adrenal medulla contributes little to the initial tachycardia in SHR, it is solely responsible for the tachycardia that follows the offset of the CES. This contrasts with the WKY, where the termination of the CES was followed by a bradycardia. This strain difference perhaps indicates in part why the vagal influence is less effective in SHR: it is unable to suppress cardiac excitation in the presence of excess catecholamines. That epinephrine release is greater during fear arousal in SHR is demonstrated by studies that have measured plasma level of epinephrine during conditioned fear reactions.3 Moreover, our findings in sympathectomized animals indicate either that more catecholamines are released or that their pressor capacity is greater in SHR.

In summary, we have used surgical and pharmacological manipulations of the autonomic nervous system to evaluate the relative contributions of the sympathetic vasomotor nerves and adrenal medulla to the maintenance of hypertension and to the expression of the exaggerated pressor response during emotional arousal in SHR. Our findings indicate that: (1) both the sympathetic vasomotor nerves and adrenal medulla contribute to the hypertension; (2) the enhanced pressor response and tachycardia in SHR are largely determined by sympathetic activation, but adrenomedullary catecholamines are also involved; and (3) baroreceptor reflexes are diminished in SHR.

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