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A Humoral Sensitizing Factor for Norepinephrine in the Spontaneously Hypertensive Rat (41146)

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Abstract. The vasopressor effect of exogenous norepinephrine was compared before and after iv injection of sera from Aoki-Okamoto spontaneously hypertensive rats into bioassay animals. Changes in the pressor responses were compared to those produced by the sera of normotensive Sprague–Dawley-derived rats. The systolic blood pressures of serum donor animals were periodically taken via a cuff over the caudal artery. The animals were decapitated and trunk blood was collected. The mean systolic blood pressure of the spontaneously hypertensive group was 184 ± 4 mm Hg just prior to killing. A separate group of normotensive rats was bilaterally nephrectonized and after 24 hr was ganglionically blocked with hexamethonium and used as bioassay animals. Bioassay animals were given standardized doses of norepinephrine iv at 6-min intervals both before and after iv injection of 15–25 μl of either normotensive or spontaneously hypertensive rat serum. Spontaneously hypertensive rat serum increased the average pressor response to norepinephrine 22 ± 7% ($P < 0.02$). Normotensive serum did not increase the pressor response to norepinephrine. These data suggest there is a humoral factor in spontaneously hypertensive rat serum that potentiates the pressor effect of norepinephrine when injected into bioassay animals.

Studies in older and more recent literature suggest the presence of a slowly acting humoral substance in various hypertensive models and forms of human hypertension that renders normotensive bioassay animals hyperresponsive to exogenous pressor agonists. Mizukoshi and Michelakis (1) reported that very small (20 μl) volumes of serum from humans with essential, malignant, or renovascular hypertension would slowly raise the blood pressure of bioassay rats and nonspecifically increase the effects of pressor agonists. The active substance appeared to be a polypeptide and renal in origin. Studies by Michelakis were extended to include various models of experimental hypertension: the one-kidney, one-clip dog (2); the one-kidney perinephritic dog; and the one-kidney, one-clip rat (3). Serum from these animals was found to be active both in bioassay rats and in isolated rat mesenteric arterioles. The active substance was found to have a molecular weight of about 1000 (2, 3) and possibly to be polypeptide in nature (2) but it is not angiotensin (3). Self *et al.* (4), using the ganglionically blocked nephrectomized rat, demonstrated a similar factor in the serum of the dietary-sodium-induced hypertensive rat. Haddy *et al.* (5) recently reviewed the evidence for such an agent(s) in volume expanded hypertension and suggests that a slowly acting ouabain-like humoral agent that acts directly on cardiovascular muscle to increase contractility and on nerve endings to reduce reflex compensation might exist. The present study was conducted to determine if such a "sensitizing factor" is present in the serum of the spontaneously hypertensive rat.

Materials and Methods. *The spontaneously hypertensive rat.* Male SHR/Cox spontaneously hypertensive rats (NIH descendents) obtained from Laboratory Supply Company, Inc., Indianapolis, Indiana, and initially weighing 206 ± 8 g (SD) were quartered in a climate-controlled room (25 ± 2° and 50–60% relative humidity) with 12 hr of light each day (6 AM to 6 PM). Animals were fed a diet of Purina Lab Chow and

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given deionized water *ad libitum*. The animals' weights and systolic blood pressures (SBP) were recorded weekly with a programmed electrosphygmomanometer[1] and a cuff over the caudal artery. On the day of sample collection, the animals were decapitated and their trunk blood was collected and allowed to clot at room temperature. Cells were separated by centrifugation, and the serum was stored at −28° until bioassayed. Control serum samples were collected from normotensive randomly selected and similarly treated Sprague–Dawley-derived rats with weights ranging from 295 to 490 g (see Table I).

Bioassay. The method used for the bioassay for norepinephrine (NE) response has been previously described (4). Briefly, it consisted of bilaterally nephrectomizing a normotensive Sprague–Dawley rat. The animals were anesthetized with DIAL–urethane and, after a 24-hr recovery period, ganglionically blocked with hexamethonium bromide. Standardized doses of NE were injected at 6-min intervals into the rat via the internal jugular vein and the mean blood pressure response was measured by means of a carotid cannula. Once a stable response to NE was obtained, 15–25 μl of a serum sample was injected and the standardized dose of NE repeated until it became apparent that there would be no further increase in the vasopressor response. The three highest consecutive responses to the standardized dose were selected and averaged and their mean used as the test response value.

Statistical analysis of data. Significance of the results obtained was determined by linear regression analysis, one-way analysis of variance (ANOVA), and Student's paired *t* test (6).

Results. *Systolic blood pressure and body weight*. The mean SBP of the spontaneously hypertensive (SHR) group was 184 ± 4 mm Hg (SE) and their body weights were 364 ± 6 g (SE) at the time of serum collections. The SBP of each of the normotensive control animals was <140 mm Hg and their body weights ranged between 350 and 400 g.

Effect of serum injection upon mean blood pressure of assay animal. Ganglionic blockade with hexamethonium reduced the mean BP of bioassay animals to near 50 mm Hg. The injection of hypertensive serum or normotensive serum did not affect assay animal basal mean blood pressure (see Table I).

Effect of serum injection upon norepinephrine response. The injection of sera from SHR rats resulted in an augmented response to the standard dose of NE. After administration of the hypertensive sera, the pressor response ranged from an 11% decrease to a 65% increase with a mean increase of 22 ± 7% (SE). When compared to the preinjection response, this increase was found to be significant at the $P < 0.02$ level (see Table I). The injection of sera from hypertensive animals usually resulted in a prompt augmentation of response that increased over the ensuing 30 min, the time course being dependent on the amount of activity present (see Figs. 1, 2, and 3). The response then often declined to the preinjection value providing adequate time was allowed. Most assays were discontinued once it became obvious that the maximal response to the standardized dose had been reached. Much smaller and insignificant changes in the pressor response were observed after the injection of normotensive sera (see Table I). Responses to this serum group ranged from a 4% decrease to a 23% increase to the standardized dose of NE with a mean increase of 5 ± 3% (SE).

Correlation of systolic blood pressure with norepinephrine response. Regression analysis of the SBP of serum donor animals and the percentage augmentation of response subsequent to serum injection revealed no significant correlation.

Discussion. Increased vascular reactivity has been described in human hypertension (7–9) and most hypertensive animal models. In established hypertension, a great deal of evidence has accumulated revealing that structural redesign is an important contributor to this "apparent" hyperreactivity to pressor agonists. Folkow

[1] Narco Biosystems, Houston, Tex.

TABLE I. BLOOD PRESSURE CHANGES OCCURRING DURING BIOASSAY PROCEDURE INDUCED BY GANGLIONIC BLOCKING AGENT, TEST SERUM, AND NOREPINEPHRINE

Serum donor data		Bioassay animal data						
				BP (mm Hg)		NE response (mm Hg)		
Systolic BP (mm Hg)	Body wt (g)	Body wt (g)	Systolic BP (mm Hg)	After hexamethonium	After serum	Preserum	Postserum	NE response change (%)
Hypertensive								
190	339	385	120	40	46	12.7	18.3	45
184	336	355	115	50	52	12.3	14.0	20
184	365	490	75	40	45	10.2	11.0	8
184	365	490	120	55	45	7.5	6.7	−11
194	370	465	115	52	55	9.8	16.2	65
194	370	560	105	55	50	6.7	8.8	32
190	380	315	95	45	60	15.2	16.7	10
190	380	460	118	50	50	9.8	9.7	−1
164	389	375	128	46	44	9.0	10.5	17
163	347	365	110	50	57	9.3	12.8	38
184 ± 4	364 ± 6	426 ± 25	110 ± 5	48 ± 2	50 ± 2	10.3 ± 0.8	12.5 ± 1.2	22 ± 7% ($P < 0.02$)
Normotensive								
		355	115	50	52	11.7	12.3	5
		490	75	40	45	10.0	10.3	3
<140	350−400	315	95	45	60	13.3	14.0	5
		460	118	50	50	9.8	9.7	−1
		385	90	60	47	8.2	8.8	7
		295	127	60	50	8.0	9.8	23
		370	120	68	44	11.0	11.0	0
		345	90	43	40	16.2	17.7	9
		345	90	43	40	17.2	16.5	−4
		373 ± 21	120 ± 6	51 ± 3	48 ± 2	11.7 ± 1.1	12.2 ± 1.1	5 ± 3%

Note. Norepinephrine response after hypertensive serum was significantly different from control, $P < .02$.

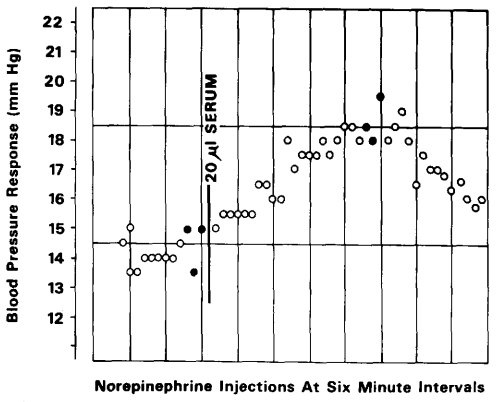

FIG. 1. Norepinephrine response after injection of hypertensive serum. Each circle represents a standardized dose of NE injected at 6-min intervals. The mean of the last three responses before injection of serum (solid circles) was used as the control value, while the mean of the three highest consecutive responses after serum (solid circles) was used as the test value.

and his associates provided the most detailed exposition of this topic and have found in both hypertensive humans and experimental models that changes in peripheral resistance and reactivity appear to follow what would be predicted from a simple increase in wall mass relative to luminal diameter (10–14). Other investigators purport that there may also be true changes in sensitivity to agonists that are additive to changes conferred by vessel wall geometry (15, 16). Particularly suggestive are the studies of Doyle and Fraser (17) who demonstrated that the vascular bed of the hand was hyperresponsive in normotensive children of hypertensive parents.

Previous studies conducted using the SHR rat have revealed a vascular hyperresponsiveness, but it has been difficult to dissociate changes in apparent reactivity due to geometric factors and true changes in sensitivity. It is well documented that changes in sensitivity to NE occur in animals with established hypertension (15), and it is likely that changes in sensitivity have occurred at a time when hypertension is first detectable. Such changes may be the harbinger of the hypertensive process (16, 18) and perhaps undergo some evolution in that hypersensitivity to NE appears to be only one-third as great in long-standing hypertension when compared with reactivity in the 3-week-old SHR rat (15, 16). This hypersensitivity consists of two components—a specific hypersensitivity to NE and a nonspecific component that could derive from altered excitation–contraction coupling and/or from geometric mechanisms already present when pressure differences first began to appear (16). These components appear to operate in an additive fashion and are probably important in sustaining a high peripheral resistance.

Data from the present study indicate a humoral substance in the SHR rat with established hypertension that increases reactivity to NE when injected into normotensive, nephrectonized, ganglionically blocked bioassay rats. Very small amounts of sera from spontaneously hypertensive rats induced a 22% increase in BP response to NE, whereas normotensive sera were without effect. The physiological and chemical characteristics of this material resemble closely those described in a previ-

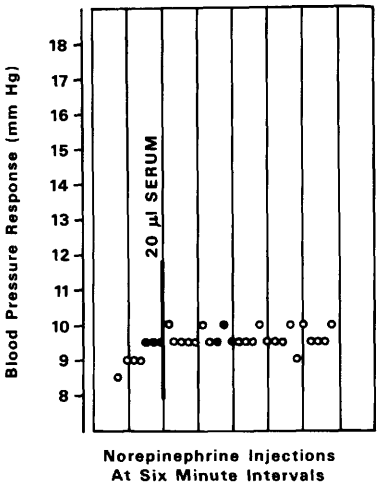

FIG. 2. Norepinephrine response after injection of normotensive serum. Each circle represents a standardized dose of NE injected at 6-min intervals. The mean of the last three responses before injection of serum (solid circles) was used as the control value, while the mean of the three highest consecutive responses after serum (solid circles) was used as the test value.

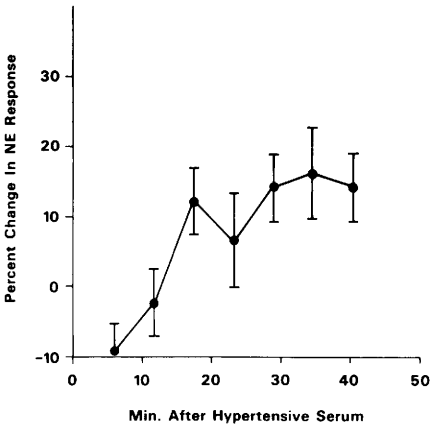

FIG. 3. Effect of SHR serum on the pressor response to norepinephrine with respect to time. Percentage change in response represents percentage difference in pressor response before and after hypertensive serum injections. Vertical lines represent SE.

ous study conducted by the authors using the dietary-sodium-loaded, renin-suppressed hypertensive rat (4). It is a heat-stabile compound (serum retains activity after heating at 56° for 30 min and after standing at room temperature for up to 6 hr) that has a slow time course with respect to onset of activity (maximal potentation of NE occurs approximately 30 min after injection), it resists degradation and loss of activity due to repeated freezing and thawing, and the basal pressure of the bioassay animal preparation is not affected. As in the authors' previous study (4), attempts to correlate serum potentiating activity with previously recorded systolic blood pressures were not successful. This, however, is not surprising when the complexity of the determinants of vascular reactivity, the crudeness of the assay, and the small range of blood pressures in the hypertensive group are considered. Earlier studies in the laboratory of Mizukoski and Michelakis (1) indicated such a substance in the plasma of hypertensive patients that was apparently renal in origin and related to sodium balance and extracellular fluid volume. Using an assay system very similar to that of the present study, Michaelakis' group found that plasma from essential hypertensives with suppressed renin produced the greatest augmentation to pressor agonists followed by the sera from those with malignant hypertension, then renovascular hypertension, and lastly high-renin essential hypertensives. Subsequent studies from the same laboratory using both human hypertensive sera and sera from dogs with renovascular hypertension revealed a substance that was polypeptide in nature or perhaps a small protein (2, 3). This substance was active both *in vivo* and *in vitro* (mesenteric arteriole of rat). The substance is apparently not renin or angiotensin, since it can be extracted away from these two compounds (3, 19) and it is found in greater quantities in renin-suppressed hypertension. The SHR rat with established hypertension is thought to be a low-renin model of hypertension (20). These observations suggest the presence of a humoral factor in the spontaneously hypertensive rat that augments responses to pressor agonists. The role of this factor in the pathogenesis of hypertension in this model is presently unknown.

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