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Evidence Using Human Arterial Tissue for a Circulating Vascular Sensitizing Agent in Essential Hypertension*

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ABSTRACT. We studied the effect of plasma from 12 patients with essential hypertension and 12 normotensive subjects on the contractile response to norepinephrine in human isolated arterial spiral strips. Human mesenteric and uterine arteries were obtained during abdominal surgery; they were cut into spiral strips and set up in isolated organ baths. After the equilibration period, arterial strips were incubated for 20 min in plasma from either normotensive subjects or hypertensive patients, and the contractile responses to norepinephrine (2.96×10^{-7} M) were recorded. Plasma from hypertensive subjects significantly increased the contractile response to norepinephrine by 25.8% ($P < 0.02$). Plasma from normotensive subjects did not increase the contractile response to the pressor agent (−3.2%; P = NS). The mean change in contractile response to norepinephrine in the presence of plasma from hypertensive patients was significantly higher than that after incubation of the human arterial strips in plasma from normotensive subjects ($P < 0.02$). When both groups were considered as a whole, there was a significant correlation between diastolic pressure and the change in the contractile response to norepinephrine ($r = 0.52$; $P < 0.01$). These results suggest the existence of a circulating vascular sensitizing substance in patients with essential hypertension. (*J Clin Endocrinol Metab* **63:** 463, 1986)

AN INCREASE in peripheral arteriolar resistance to flow is a characteristic manifestation of essential hypertension. Both structural and functional changes have been proposed to account for this increase in peripheral resistance (1, 2). These functional changes in arteriolar smooth muscle could be due to neurogenic, myogenic, or humoral mechanisms. Mizukoshi and Michelakis (3) demonstrated that plasma from hypertensive patients increased the pressor response to norepinephrine and angiotensin II when injected in bilaterally nephrectomized rats and suggested the presence of a circulating vascular sensitizing factor in patients with essential hypertension. In view of the difficulties in interpreting whole animal experiments and allowing for species differences in response to possible circulating humoral factors, we directly measured and compared contractile responses to norepinephrine in human isolated arterial muscle before and after incubation with human plasma obtained from either normotensive or hypertensive subjects.

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Subjects and Methods

Plasma donors

Twelve patients with essential hypertension referred to the Blood Pressure Unit by local general practitioners (9 men and 3 women; all white; mean age, 41.6 yr; range, 20–60 yr), whose diastolic pressure after 2 months of observation in the absence of treatment was between 90–130 mm Hg, and 12 normotensive subjects from the Hospital staff (11 men and 1 woman; all white, mean age, 38.8 yr; range, 20–70 yr), whose diastolic pressure was below 90 mm Hg on at least 2 different occasions, were studied. Table 1 shows the characteristics of the study subjects. All subjects gave their informed consent. Patients and controls were excluded if they were taking any drugs or oral

TABLE 1. Characteristics of the study subjects

	Normotensive subjects (n = 12)	Hypertensive patients (n = 12)
Age (yr)	38.8 ± 17.1	41.6 ± 14.1
Systolic blood pressure (mm Hg)	122.5 ± 9.4	165.8 ± 17.8[a]
Diastolic blood pressure (mm Hg)	77.6 ± 6.9	106.2 ± 9.5[a]
PRA (ng/ml·h)	1.57 ± 0.93	1.58 ± 1.96
Plasma aldosterone (ng/dl)	10.81 ± 5.70	12.41 ± 8.15

Results are given as the mean ± SD.
[a] $P < 0.001$.

contraceptives or had evidence of renal failure, heart failure, or cerebrovascular disease. All subjects were studied while consuming their normal diet. Average blood pressure in the hypertensive group was 165.8/106.2 ± 17.8/9.5 mm Hg (mean ± SD), and that in the normotensive group was 122.5/77.6 ± 9.4/6.9 mm Hg.

Blood pressure was measured by nurses, using semiautomatic ultrasound sphygmomanometers (Arteriosonde) (4) with attached recorders, as the mean of five readings taken every 1–2 min while subjects were in the supine position. Venous blood was taken without stasis after the subjects had been sitting upright for 10 min between 1000–1200 h. Twenty-five milliliters of blood were collected in heparin tubes from the antecubital vein of subjects and immediately centrifuged at 4 C. The plasma was either kept on ice and used the same day or frozen immediately at −20 C and assayed within 26 days. Just before testing, the plasma was centrifuged to remove any particulate matter. Plasma electrolytes, urea, and creatinine were measured with standard procedures, as previously described (5). PRA and plasma aldosterone were measured by RIA (6, 7).

Tissue donors

Human mesenteric (n = 6) and uterine arteries (n = 7; internal diameter, ~1–4 mm) were obtained from 13 normotensive patients (4 men and 9 women; mean age, 56.7 yr; range, 34–86 yr) undergoing gastric resection (left and right gastric, left and right gastric-epiploic arteries), colonic resection (left colic, inferior mesenteric, sigmoid, and superior rectal arteries), or total abdominal hysterectomy. The blood vessels were dissected from gastric, colonic, or uterine specimens in the operating theater, then immediately transported to the laboratory in freshly prepared cold Krebs' solution bubbled with 5% CO_2 in oxygen. All experiments were done on the same day that the tissue specimen was obtained.

Laboratory procedure

After collection of the blood vessels from the operating theater, they were dissected free of connective tissue and fat, cut into spiral strips of 2–4 mm width and about 5 cm long, and set up in 5 ml isolated organ baths under a load of 0.5 g. They were bathed in Krebs' solution (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 1.2 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 2.4 mM; $NaHCO_3$, 25 mM; glucose, 11 mM) at 37 C bubbled with 5% CO_2 in oxygen, as previously described (8).

Before testing for contractility, the strips were left to equilibrate for 60–90 min. Contractions were then recorded through isotonic transducers (Bio-Science, Palmer-Washington, Kent, U.K.) connected to pen recorders (Rikadenki, Kogyo Co. Ltd., Tokyo, Japan). One minute was found to be the optimum contact time with the agonist, followed by a 30-sec washout. The interval between successive norepinephrine doses was 10 min.

After defining the dose-response curve (dose range, 1.48×10^{-8} to 2.96×10^{-6} M), a submaximal dose (EC_{80}, 2.96×10^{-7} M) of norepinephrine (Levophed, Winthrop Laboratories, Surrey, U.K.) was added to the bath until several consecutive doses gave the same response. The mean of these last consecutive responses (usually three to six responses) was taken as the initial response to norepinephrine. The human arterial strips were incubated with the same procedure either for 20 min in Krebs' solution or, in a blind fashion, in 5 ml plasma obtained from a normotensive subject or a hypertensive patient. Eight experiments were carried out in pairs using one normotensive and one hypertensive plasma sample on separate arterial strips obtained from the same specimen, with one strip from the same specimen incubated in Krebs' solution to control the stability of the tissue during the time course of the experiment (time-matched control strip). The remaining plasma samples were tested in parallel with a time-matched control strip obtained from the same arterial specimen. Within an experiment, each plasma incubation was, therefore, always performed on separate strips obtained from the same arterial specimen, and a single strip was not used to test more than one plasma sample. After the 20-min incubation, the response to norepinephrine was retested in each strip in the presence of plasma. Responses were measured as millimeters of pen deflection on the chart and converted to percentages of the initial response to norepinephrine; therefore, results were expressed as the percent change from the mean initial response to norepinephrine obtained in the same strip before the incubation. Tracings were read and responses calculated separately by two observers blinded as to the source of plasma, and results were compared by correlation analysis; a correlation coefficient of 0.990 indicated a high degree of correspondence in the calculations. The average time of storage of plasma samples did not differ between hypertensive and normotensive subjects, and within the normotensive group there was no difference in response between unfrozen and frozen plasma.

Human arterial strips were stable in Krebs' solution; after the equilibration period, the mean within-assay coefficient of variation for the contractile response to norepinephrine was 7.0% (range, 0–18%) when calculated on 16 control strips, and the between-assay coefficient of variation was 16.0%. Three plasma samples taken from 3 hypertensive patients (not included in the 24 age-, sex-, and race-matched subjects described) were tested in duplicate on strips from the same artery on the same day, and the responses were reasonably consistent (7.0% and 10.3%, 34.8% and 32.0%, 0% and −3.9%). Two plasma samples were also tested on 2 different days, and results were −1.9% and 3.5%, and −15.3% and −9.9%, respectively.

Statistical analysis

As human mesenteric and uterine arterial strips had comparable dose-response curves to norepinephrine, the results were pooled for analysis. Results are expressed as the mean ± SEM. Because of the possible non-Gaussian distribution of the data, both parametric (paired and unpaired Student's *t* tests and Pearson correlation analysis) and nonparametric (paired and unpaired Wilcoxon's tests and Spearman correlation analysis) tests were performed (9).

Results

Figure 1 shows an original tracing recording the contractile response of an isolated human arterial strip induced by norepinephrine before and after incubation for 20 min in Krebs' solution, representing a time-matched control experiment. After incubation in Krebs'

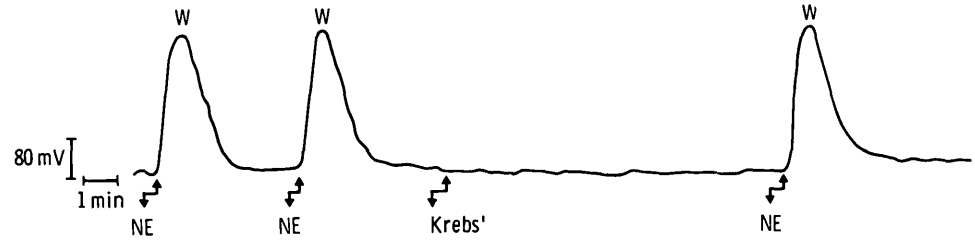

FIG. 1. Original recording of the contractile response of an isolated human arterial strip to norepinephrine (NE; 2.96×10^{-7} M). W, Washout.

solution for 20 min, human arterial strips were stable; the mean contractile response to norepinephrine was 6.5 ± 3.7% (n = 16; P = NS).

Incubation of human arterial strips in plasma obtained from hypertensive patients resulted in increased response to the standard dose of norepinephrine; after 20 min of incubation, the individual contractile responses ranged from a 13.4% decrease to a 100% increase, with a significant mean increase of 25.8 ± 8.8% compared to the initial response of the same strip before incubation with plasma (n = 12; $P < 0.02$, by paired Student's t test; $P < 0.01$, by paired Wilcoxon's test; Fig. 2, *right panel*). No significant change in the contractile response to norepinephrine was found after incubation of the human arterial tissues in plasma obtained from normotensive subjects; the individual contractile responses ranged from a 52.9% decrease to a 30.8% increase, with a mean change of −3.2 ± 6.4% (n = 12; both paired tests, P = NS, Fig. 2, *left panel*).

The change in contractile response to norepinephrine in the presence of hypertensive plasma was significantly higher than that in the presence of plasma from normotensive subjects ($P < 0.02$, by unpaired Student's t test; $P < 0.02$, by unpaired Wilcoxon's test; Fig. 3).

Considering all subjects, there was a significant correlation between the change in the contractile response to norepinephrine during incubation in plasma and diastolic pressure (Fig. 4) but not systolic pressure (Table 2). Age, PRA, potassium, and aldosterone were not significantly different in the two groups, and there was no significant relationship between those variables and the contractile response to norepinephrine (Table 2).

Discussion

Our results demonstrate for the first time, using human vascular tissue, that plasma from patients with essential hypertension increases the contractile response to norepinephrine of human isolated arterial strips.

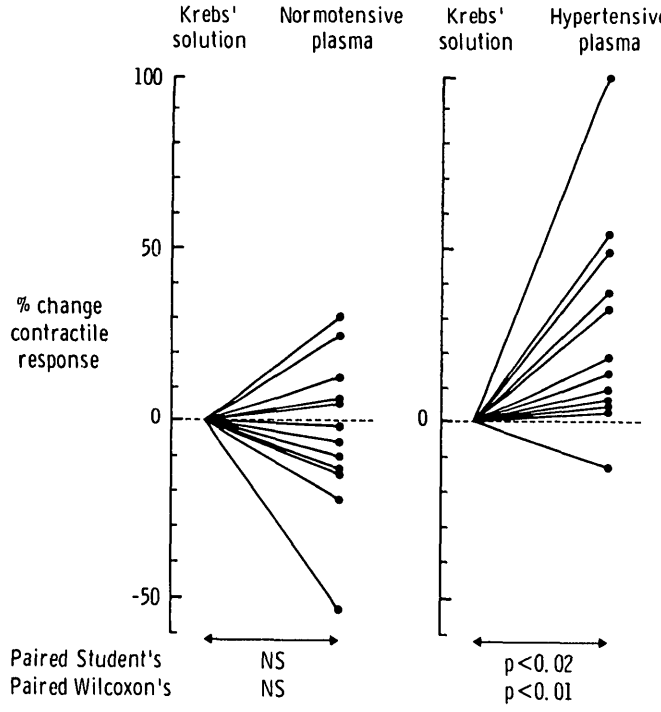

FIG. 2. Effect of plasma from normotensive subjects (*left panel*) and hypertensive patients (*right panel*) on the contractile response to norepinephrine (2.96×10^{-7} M) of human isolated arterial spiral strips. The contractile response to norepinephrine in the presence of hypertensive plasma was significantly different from the initial response in the same strip before incubation with plasma $P < 0.02$, by paired Student's t test; $P < 0.01$, by paired Wilcoxon's test), but not (both paired tests) in the presence of normotensive plasma.

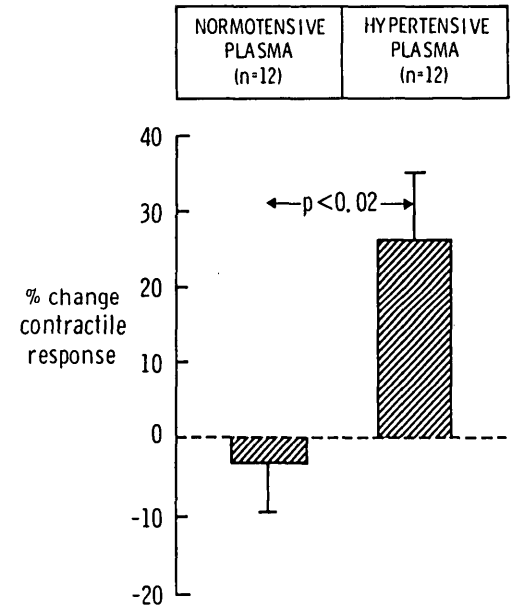

FIG. 3. Comparison between the effect of plasma from normotensive subjects (n = 12) and hypertensive patients (n = 12) on the contractile response to norepinephrine in human isolated arterial spiral strips. The mean change in the contractile response to norepinephrine in the presence of plasma from hypertensive patients was significantly greater than that in the presence of plasma from normotensive subjects ($P < 0.02$, by unpaired Student's t test; $P < 0.02$, by Wilcoxon's test). Values are the means ± SEM.

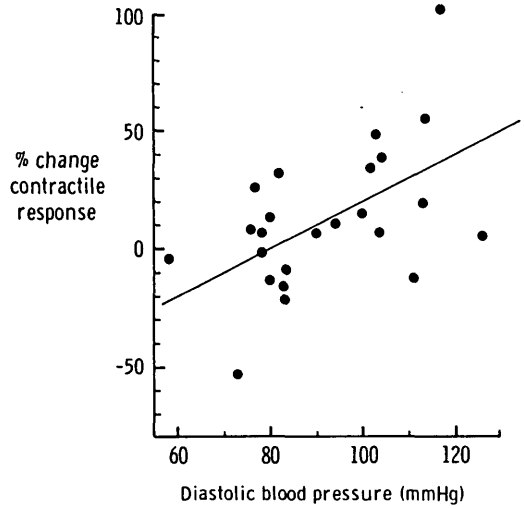

FIG. 4. Relationship between the change in the contractile response to norepinephrine of human isolated arterial spiral strips in the presence of plasma and the diastolic blood pressure of plasma donors (n = 24; Pearson's r = 0.52 and $P < 0.01$; Spearman's R = 0.43 and $P < 0.05$).

TABLE 2. Relationships between the change in the contractile response to norepinephrine after incubation in plasma and other variables (n = 24)

	Pearson's r	Spearman's R
Age	0.06	0.09
PRA	−0.21	−0.33
Plasma aldosterone	0.12	0.03
Plasma potassium	−0.15	0.07
Systolic blood pressure	0.39	0.38
Diastolic blood pressure	0.52[a]	0.43[b]

[a] $P < 0.01$.
[b] $P < 0.05$.

They, thus, support an increasing body of evidence that plasma from hypertensive patients increases the contractile response to norepinephrine. For instance, Mizukoshi and Michelakis (3) found that small amounts of plasma from hypertensive subjects increased the pressor response to norepinephrine and angiotensin II in bilaterally nephrectomized pentolinium-treated rats. Bloom *et al.* (10) perfused the rabbit isolated femoral artery with human plasma using a constant flow pump. The addition of norepinephrine to plasma from hypertensive patients caused a greater rise in perfusion pressure than the addition of norepinephrine to plasma from normotensive subjects. However, Greenberg *et al.* (11) reported that in large rats (525–585 g) not pretreated with pentolinium, plasma from patients with essential hypertension enhanced the pressor response to tyramine, but not to angiotensin II or norepinephrine.

In animal hypertension, Michelakis and co-workers (12, 13) found that plasma obtained from one-kidney, one-clip hypertensive dogs increased the blood pressure response of control rats to norepinephrine and angiotensin II. Longer term pressor hyperresponsiveness to norepinephrine and angiotensin II also was demonstrated, more recently, in normal rats infused twice a day for 3 weeks with serum from chronic one-kidney, one-clip hypertensive rats (14). Self *et al.* (15) and Battarbee *et al.* (16) reported that the serum obtained from hypertensive rats increased the pressor response to norepinephrine in bioassay rats, and Johnson *et al.* (17) also found an increase in pressor responsiveness to norepinephrine in rabbits with experimental hypertension which they attributed to the presence of a circulating vascular sensitizing factor.

The increase in vascular reactivity to vasoactive agents has also been associated with salt sensitivity (18) and acute blood volume expansion (19). For instance, Plunkett *et al.* (20) demonstrated that plasma extracts from saline-loaded dogs increased vascular responsiveness to norepinephrine, arginine vasopressin, and angiotensin II when injected in cremasteric arterioles from normal rats. Finally, Wright (21) examined the effects of plasma from spontaneously hypertensive rats on the contractile properties of aortic strips from normotensive rats; when incubated in plasma from spontaneously hypertensive rats, the vascular tissue exhibited hyperresponsiveness to norepinephrine compared to tissue incubated in plasma obtained from Wistar-Kyoto or Sprague-Dawley rats.

The nature and mechanism of action of the effect of plasma from hypertensive patients in potentiating the contractile response to norepinephrine in human vascular tissue is not known. Possible mechanisms include a direct effect on plasma membrane permeability, ionic fluxes (22), or the agonist-receptor interaction or a direct or indirect effect on intracellular calcium. The concentration of intracellular calcium is under the control of several different mechanisms (23). One hypothesis suggests that it may be influenced by intracellular sodium via a Na^+-Ca^{2+} exchange mechanism (24). An increase in intracellular sodium could be due to the increased levels of a circulating sodium transport inhibitor with ouabain-like properties that has been described in patients with essential hypertension (25, 26). Ouabain and digoxin, by inhibiting sodium transport, at least in the short term, increase intracellular sodium (27) and potentiate the constrictor response to norepinephrine in isolated vascular smooth muscle from animals (28, 29) and man (30). Furthermore, oral digoxin given to normotensive human subjects for 4 days caused pressor hyperresponsiveness to norepinephrine and angiotensin II (31). We cannot exclude the possibility that the enhanced response to norepinephrine that we found with plasma from some patients with essential hypertension might be due to increased levels of a known potentiator of the

contractile response to norepinephrine, for instance 5-hydroxytryptamine (32, 33). It is unlikely to be due to increased levels of angiotensin II, as there was no significant difference in PRA between the two groups. Besides, recent reports have shown no consistent differences in the vascular reactivity to various vasoconstrictor agents of isolated arteries from both normotensive and hypertensive subjects (34–36), thus supporting the view that structural vascular changes are unlikely to play a major role in the increased vascular reactivity in hypertension. Our results are in agreement with many previous results in animal experiments and support an increasing body of evidence that plasma from both hypertensive humans and animals increases the reactivity of both isolated arteries and intact animals to vasoactive substances. Moreover, they provide direct evidence for the presence of a circulating vascular sensitizing agent in human essential hypertension which increases the contractile response of human isolated arterial strips to norepinephrine. Although more evidence is needed to characterize the structure and mechanism of action, our findings strengthen the view of the potential importance of humoral factors in essential hypertension.

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