A New Pressor Factor in Extract of Rat Aorta Wall[1] (34621)

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It was recently demonstrated in this laboratory that administration of several inhibitors of protein synthesis, actinomycin D, acetoxycycloheximide, and chloramphenicol, induced prompt and profound reduction in blood pressure of normotensive and hypertensive rats (1, 2). From these investigations it was deduced that such extravascular factors as malnutrition, potassium deficiency, adrenal cortex suppression, or pharmacological toxicity were probably not responsible for blood pressure depression. Additional studies indicated that the depressor effect did not involve impairment of the relatively stable phasic contractile proteins in arterial muscle (3) nor interference with kidney renin production (4).

It seemed possible therefore, that the depressor effect of antimetabolites might be due to suppression of a labile vasoactive substance normally present in arterial tissue. It was also suspected, on a theoretical basis, that such a hypothetical factor might be a polypeptide. This concept was challenged by testing the hemodynamic effects of an extract of arterial tissue designed to remove polypeptides on rats made hypotensive by administration of antimetabolites.

Methods. Extracts were prepared in the following manner: Aortas were removed immediately from freshly killed rats, chilled, and then minced; to 1.0 g of arterial tissue 3.0 ml distilled water was added, and the mixture remained overnight at 4°; the supernatant solution was removed for testing. Hypotensive test rats were prepared by injections of 40 μg actinomycin D intraperitoneally morning and afternoon of day 1 and 20 μg on days 2 and 3. On the fourth day, the test animal was anesthetized with ether, the abdomen opened, and a polyethylene catheter inserted into the abdominal aorta after heparinization. The catheter was attached to a pressure transducer (Statham P23) and connected to an apparatus which photographed pulse rate and blood pressure level (Electronics for Medicine DR 8). A 0.5-ml extract of normal rat aorta (NAE) was injected intravenously into the test animal three times at 3-min intervals and blood pressure responses photographed. Measurements of changes in pressure levels were obtained from the photographs.

TABLE I. Average Blood Pressures after Successive Intravenous Injections of Arterial Extract (0.5 ml) at 3-min Intervals (19 rats).

Extract injection	Blood pressure (mm Hg)	
	Systolic	Diastolic
None	77 ± 1.6	40 ± 1.4
First	84 ± 1.3	45 ± 1.6
Second	90 ± 1.3	50 ± 1.4
Third	98 ± 1.8	55 ± 1.9

Results. Blood pressure responses. Typical hemodynamic changes in the hypotensive test animal injected with NAE consisted of a rapid blood pressure rise of 10–20 mm Hg and a decline of the same magnitude within 30 sec with each injection, but in the interim there was a gradual rise of both systolic and diastolic blood pressure levels of approximately 2 and 1.5 mm Hg per minute respectively, for about 10 to 15 min, after which there was little further change (Fig. 1). Table I shows that at the end of 9 min, systolic pressure has increased an average of 21 ± 1.0 and diastolic 14 ± 1.2 mm Hg in 19 rats. The elevation in blood pressure was sustained for a relatively long period; thus, there was no appreciable decline in six ani-

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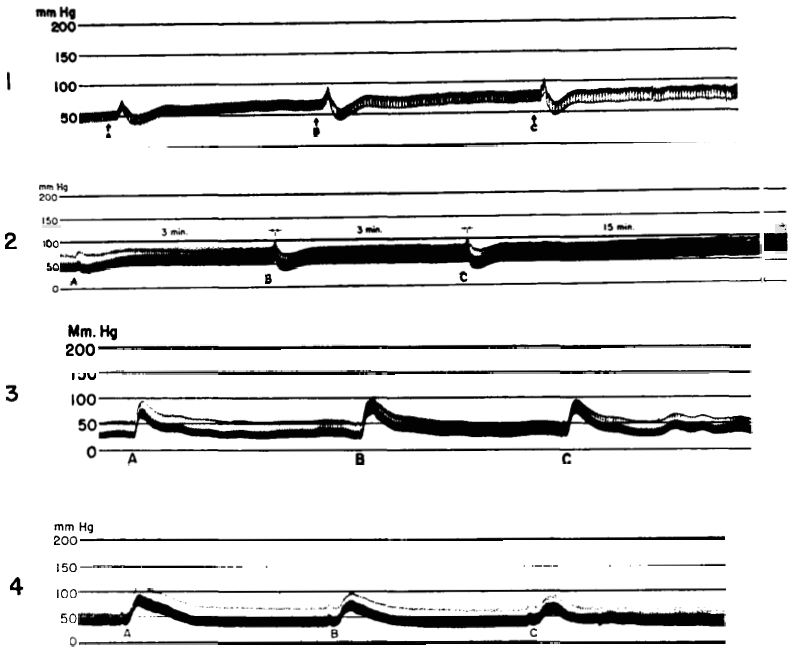

FIG. 1. Blood pressure responses to three intravenous injections at 3-min intervals of 0.5-ml aorta extract (1 and 2, 0.1-μg *l*-norepinephrine (3) and 0.1-μg angiotensin (4).

mals observed for 90 min after the third injection of NAE. Observation for longer periods were not made because of possible complications from excessively prolonged ether anesthesia. Normal rats were also injected with NAE but showed no similar elevation in blood pressure level.

Comparison to L-norepinephrine and angiotensin. A number of investigators have already noted the presence of pressor agents, catecholamines and renin, in extracts of arteries. However, the pattern of blood pressure response to NAE does not resemble that induced by either of these substances. The pattern of pressure changes following three intravenous injections of 0.5 ml NAE, 0.1 μg *l*-norepinephrine and 0.1 μg angiotensin at 3-min intervals are seen in the Fig. 1. After each administration of *l*-norepinephrine or angiotensin blood pressure levels rose rapidly but returned promptly to previous values within 1 min, as distinguished from the step-like and sustained elevations in systolic and diastolic pressure after each injection of NAE.

Furthermore, NAE could be readily differentiated from catecholamine when rats were subjects to alpha-adrenergic blockade. The subcutaneous administration of 5 mg phenoxybenzamine to test rats 15 min previously completely suppressed blood pressure elevation to intravenous injection of 0.1 μg *l*-norepinephrine. NAE injections in seven rats under similar conditions increased the average blood pressure from 72/44 to 93/57 mm Hg.

Other tissue extracts and electrolytes. The possibility was considered that the blood pressure response to NAE might be due to nonspecific vasoactive substances often found in tissue extracts (adenosine compounds, electrolytes, serotonin, or histamine). Extracts were prepared from heart, intestine, stomach, and skeletal muscle and injected similarly into test rats. Ringer's solution was also administered to the hypotensive rats to determine whether increase in blood volume was a factor in elevation of blood pressure as well as the possible influence of electrolytes extracted from arterial tissue. The hemodynamic responses after injecting each of these extracts consisted of a rapid rise of blood pressure (10–20 mm Hg) for 20–30 sec and an equally rapid decline to original levels in

contrast to the sustained cumulative elevation after each injection of arterial wall extract (Fig. 1). Injections of 0.5 ml Ringer's solution had no demonstrable effect on blood pressure levels.

Acetoxycycloheximide hypotension. NAE was also shown to be equally as effective in restoring depressed blood pressure of rats subjected to another antimetabolite, acetoxycycloheximide.[2] Test rats were made hypotensive by administering 50 μg of this compound in the morning and afternoon of day 1 and 40 μg on days 2 and 3. On the fourth day, they received three intravenous injections of 0.5 ml NAE at 3-min intervals. In all seven rats tested, blood pressures were elevated from an average of 76/43 to 95/55 mm Hg at the end of 9 min.

K-Deficiency hypotension. The effect of NAE on blood pressure of rats made hypotensive by a method other than through administering inhibitors of protein synthesis was also studied. Hypotension was induced by potassium depletion as described previously (5). Six rats (60–70 days old) were fed a diet deficient in potassium and injected subcutaneously with 20 mg desoxycorticosterone dimethylacetate once weekly for 3 weeks to accelerate potassium excretion. By the fourth week, systolic blood pressures were reduced to an average of 83 mm Hg. Three intravenous injections of 0.5 ml NAE at 3-min intervals did not elevate blood pressure.

Antimetabolites on extract potency. It would appear, thus far, that normal arterial tissue contains a water-soluble vasopressor substance whose activity is limited to restoring blood pressure of hypotensive test rats through replacement of a deficiencey induced by administration of actinomycin D or acetoxycycloheximide. Evidence of such deficiency was demonstrated as follows: Extracts were made from aortas obtained from rats injected with 40 μg actinomycin D daily for 4 days and administered to hypotensive test animals, as described above; blood pressures of test rats were not increased in six attempts. Similarly, extracts prepared from aortas of rats which had received injections of 50 μg acetoxycycloheximide daily for 4 days had no significant pressor effect in five trials.

Polypeptide properties. The following experiments provide evidence suggesting that the vasoactive factor in NAE might be a polypeptide. Extracts of the tissue with distilled water may be considered presumptive evidence. Heat stability was indicated by observing that heating NAE to 100° for 10 min did not alter its potency. Thus, injections of the heated extract increased blood pressure of test rats an average of 18 and 13 mm Hg systolic and diastolic, respectively, in five tests. Additionally, incubation of NAE with trypsin for 15 min at 37° destroyed the pressor activity of NAE in all four trials. Furthermore, NAE was filtered through an Amicon UM-3 membrane designed to obstruct molecules larger than 2000 m.w. A 0.5 ml filtrate injected three times elevated blood pressures in six test rats from an average of 77/48 to 98/62 mm Hg; the residue on the filter was redissolved and found inactive, indicating that the molecular size of the vasoactive factor is apparently small enough to be a polypeptide.

Discussion. It appears from the present studies that aqueous extract of rat arterial wall (NAE) contains a vasopressor substance capable of sustaining an elevation of systolic and diastolic blood pressure of rats made hypotensive by the administration of protein synthesis inhibitors actinomycin D and acetoxycycloheximide. The specificity of this factor in NAE is indicated by failure to obtain this hemodynamic response after injecting similarly prepared extracts of other muscle tissues, either smooth or striated (stomach, intestine, myocardium, and skeletal). In addition, NAE has no apparent pressor response when injected into normal rats or those made hypotensive by another procedure (potassium depletion). Such results suggest that the pressor response to NAE is due to replacement of a deficiency of a specific vasoac-

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tive factor induced by administering these antimetabolites.

This postulation is supported by demonstrating that extracts prepared from arteries of rats subjected to depressor doses of actinomycin D or acetoxycycloheximide have no pressor activity when injected into test rats. Suppression of synthesis of this hypothetical pressor factor by these inhibitors is offered as a possible explanation for their ability to reduce blood pressure in normotensive and hypertensive rats (1) (2).

The hemodynamic response to NAE is distinctive and differs in a number of ways from that observed after administering known pressor agents. Thus, systolic and diastolic blood pressure levels are elevated in a gradual and cumulative pattern after repeated injections of NAE, and the increase is sustained for relatively long periods (at least 90 min from last treatment), in contrast to the rapid rise and decline of blood pressure to the original levels induced by repeated injections of *l*-norepinephrine or angiotensin. In addition, alpha-adrenergic blockade with phenoxybenzamine effectively nullifies catecholamine activity but does not impair the blood pressure response to NAE.

The physiologic mechanisms involved in restoration of blood pressure in hypotensive test rats have not yet been determined. The possibility was considered that NAE improves myocardial function and restores blood pressure through increased cardiac output similar to the plasma factor "kinekard" which has a pressor effect through improving myocardial contraction (6). However, "kinekard" but not NAE is blocked by phenoxybenzamine. Studies are now in progress on the effectiveness of NAE in shortening arterial muscle *in vitro* to determine whether it influences peripheral vasculature directly. The sustained elevation of diastolic blood pressure in the test rat indicates that the vasoactive factor in NAE may increase arterial tone. Its effectiveness in the presence of alpha-adrenergic blockade suggests that such tonal response involves myogenic rather than adrenergic or catecholamine mechanisms.

The vasoactive factor may be a polypeptide based on (a) theoretical considerations (2), (b) method of extraction, (c) heat stability, (d) destruction by incubation with trypsin, and (e) appropriate molecular size.

Summary. Aqueous extract of rat aorta wall (NAE) induces gradual and sustained elevation of systolic and diastolic blood pressure in rats made hypotensive by inhibitors of protein synthesis, actinomycin D and acetoxycycloheximide. The pressor response is obtained only from extracts of arterial and not other smooth or striated muscle tissue. NAE has no significant effect on normal or other hypotensive rats. It apparently replaces a specific deficiency of a vasoactive factor induced by these antimetabolites. This factor differs physiologically from other pressor agents by acting apparently on arterial tone rather than phasic contractility. Preliminary data suggest it may be a polypeptide.

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