Arterial Tone Factor in Extract of Rat Arterial Wall[1] (35484)

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Recent investigations from this laboratory demonstrated that distilled water extracts of normal rat aorta wall (NAE) contain a pressor factor not identified with known vasoactive substances such as catecholamine or renin (1). This factor elevates blood pressure only in rats made hypotensive through administration of protein synthesis inhibitors such as actinomycin D or acetoxycycloheximide (2). NAE is ineffective in normtensive rats or those made hypotensive by potassium depletion. It was suggested that antimetabolites suppress synthesis of this factor, normally present in arterial tissue, resulting in depression of blood pressure. Administration of NAE to these animals restores blood pressure through alleviating the deficiency. This hypothesis is supported by showing that extracts of aortas from rats treated with these antimetabolites have little or no pressor activity in test rats.

A number of mechanisms may be involved in NAE pressor response. The one considered most likely was through increasing peripheral vascular resistance. Studies were therefore made on effectiveness of NAE on contraction of arterial muscle *in vitro*. The conditions of these experiments duplicated those in which pressor responses were obtained in the intact rat to determine a possible correlation between *in vitro* and systemic NAE responses.

Methods and Results. Arterial muscle tension was measured on helically-cut aorta strips as described by Bohr and associates (3, 4). Thoracic aortas from male Long-Evans rats (250–300 g) were cut helically, resulting in a strip measuring approximately 1.5 × 0.2 cm. The strip was suspended in a bath containing 1.5 ml of PSS (physiologic salt and sugar solution) designed by these investigators with composition of these ingredients (mmoles/liter): NaCl, 119.0; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$, 1.17; $NaHCO_3$, 14.9; dextrose, 5.5; sucrose, 50.0; $CaCl_2$, 1.6; calcium disodium versenate, 0.026. The bath was maintained at 37.5° and was aerated with a mixture of 95% O_2 and 5% CO_2. The free end of the strip was attached to an isometric force transducer (Bionix 1705) and connected to a Bausch and Lomb chart recorder, the chart moving 2.5 in./hr. As suggested by Bohr and associates, the strip tension was maintained by manual regulation at about 100 mg for 90 min before testing responses to various substances. The viability of the strip was evaluated before each experiment by determining tension increase after 3 ng of *l*-norepinephrine was added to the bath. This dosage was chosen after numerous trials with varying amounts had shown that 3 ng induced an average tension of 190 ± 12 mg, about 70% maximum. Strips which failed to show a tension increase of 150 mg were discarded, arbitrarily.

NAE was prepared by adding 3 ml of distilled water to 1 g of chilled minced aortas from male rats weighing 250–300 g and the mixture allowed to stand overnight at 4°. NAE was separated from the residue and preserved with merthiolate. Control extracts of other muscle tissues and aortas from other experimental rats were similarly prepared. These extracts as well as drugs were added to the muscle bath dissolved in PSS to make a total volume of 0.1 ml.

A. Tension response to l-norepinephrine, angiotension, and NAE. Three procedures were followed in studying the tension responses of the strip to the above preparations. In the first, the preparations were left in the

[1] This work was aided by Grant HE-01006 from the National Institutes of Health, National Heart Institute.

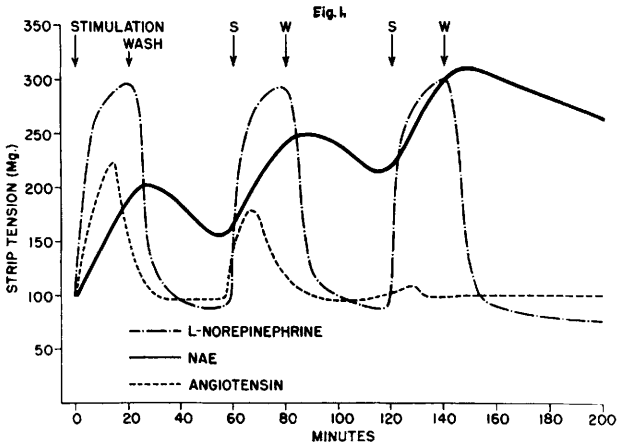

FIG. 1. Superimposed charts of arterial strip tension responses after exposure to three repeated doses of 3 ng of *l*-norepinephrine, 0.05 ml of aorta wall extract (NAE), and 3 ng of angiotensin (retouched for graphic purposes).

muscle bath for 20 min, drained and replaced with fresh PSS. Tension response resulting from adding 3 ng of *l*-norepinephrine was prompt and reached a plateau after 5 min at an average of 190 ± 12 mg (14 trials) above base level. After washing, tension declined to original level in about 10 min. After adding 3 ng of angiotensin to muscle bath, tension increased promptly to 160 mg (6 trials) but started to decline immediately and returned to its base in 2 to 3 min, even before washing. The response to NAE differed considerably from these vasoactive agents. It was slower in onset and reached a plateau in about 10 min averaging 140 ± 11 mg (12 trials) above base. After washing, the tension declined slowly and 1 hr after washing still registered an average of 70 ± 7 mg above starting level.

The second procedure consisted of adding two additional doses of each substance 30 min after each washing. The response to each of three doses of 3 ng of *l*-norepinephrine was equally high at onset and prompt in returning to original levels. Angiotensin response to the second dose was approximately one-half that of the first and practically none with the third. Tachyphylaxis to this substance was previously noted by Bohr and Johannson (4). The response to NAE was quite distinctive. After each dose, the tension rose higher than with the proceeding one so that with the third dose the tension averaged 220 mg (6 trials) above the base line. After washing, it slowly declined to 160 mg level 60 min later (Fig. 1).

With the third procedure, the arterial strip was allowed contact with *l*-norepinephrine and NAE for 60 min before washing out the bath. Angiotensin was not used since tension declined rapidly in spite of its presence in the bath. Strips bathed with 3 ng of *l*-norepinephrine displayed an increased tension averaging 200 mg (6 trials) at the peak, which lasted 30 to 40 min and then slowly declined to original level during the next 30 to 40 min. The response to NAE was considerably slower, tension increased to an average of 100 mg in 20 min and continued to increase to an average of 220 mg (6 trials) even after washing. It then declined slowly to about 100 mg above base level at the end of 120 min (Fig. 2).

B. Effect of NAE on tension after alpha-adrenergic blockade. It was demonstrated that NAE pressor response in rats was not impaired significantly by previously administering the alpha-adrenergic blocking drug, phenoxybenzamine. NAE was tested on arterial strips in a bath containing 0.05 μg of phenoxybenzamine, a dose shown capable of blocking 3 ng of *l*-norepinephrine. The initial response of the strip to exposure for 20 min of 0.05 ml of NAE was considerably slower than with a normal strip requiring 30 min or more to reach the peak which averaged 100

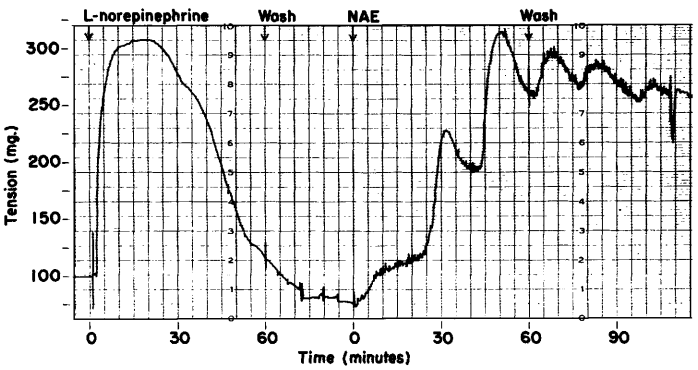

FIG. 2. Tracing of chart of arterial strip responses after 60-min exposure to 3 ng of *l*-norepinephrine and to 0.05 ml of aorta wall extract (NAE).

± 8 mg (12 trials) above the base line. After washing, the tension declined slowly but remained elevated approximately 70 mg above the base line for 30 min or more. The difference in responses to NAE between the blocked and unblocked strips is apparently due to catecholamine content sufficient to induce a tension increase of about 40 mg.

C. Effect on tension of extracts of other muscle tissue. For control purposes, extracts were made from heart, stomach, and skeletal muscle. Arterial strips were exposed for 20 min to 0.05-ml extracts. Tension increases averaged 120 mg for skeletal muscle, 160 mg for stomach, and 240 for heart (6 trials each). After phenoxybenzamine blockade none of these extracts induced significant changes in tension. Apparently the extracts, especially myocardial, have considerable catecholamine, but practically none of the factor present in NAE capable of inducing nonneurogenic muscle shortening.

D. Tension induced by extracts of aortas from rats treated with antimetabolites. As previously demonstrated, extracts prepared from aortas of rats treated with actinomycin D or acetoxycycloheximide had little effect on blood pressure of test rats. Such extracts were prepared from animals receiving daily injections of 40 μg of actinomycin D and 50 μg of acetoxycycloheximide for 4 days. Extracts were tested by adding 0.05 ml to the muscle bath for 20 min. The strips responded by increases of tension averaging about 60 mg for either extract (6 trials). However, after alpha-adrenergic blocking, the tension increases averaged 20 mg for either extract, compared to 100 mg for normal aorta extract. Apparently nonneurogenic vasoactivity of arterial tissue was significantly suppressed by antimetabolites.

In addition, an extract was made from aortas of rats injected with 15 mg of puromycin HCl daily for 3 days. This extract induced about 40-mg tension response on the arterial strip but had no effect after phenoxybenzamine block (5 trials). Thus, the puromycin treatment completely suppressed the arterial tissue factor responsible for nonneurogenic muscle shortening.

It was noted incidentally that the aortas of rats treated with actinomycin D showed normal responses to 3 ng of *l*-norepinephrine and reacted to 0.05 ml of NAE with an average tension increase of 160 mg (6 trials) after phenoxybenzamine blocking thus indicating no serious impairment in muscle shortening.

E. NAE heat stability of vasoactive factor. Heating NAE to 100° for 10 min does not reduce appreciably pressor activity in the test rat. After such treatment, 0.05 ml of NAE induced strip tension increase of an average of 94 mg (5 trials) compared to 140 mg with unheated NAE. Since heating destroys catecholamine, the difference in tension response was possibly due to loss of this agent.

F. Effect of trypsin digestion on NAE vasoactivity. It was also demonstrated that pressor activity of NAE was destroyed by trypsin digestion. Present studies indicate that such treatment also obliterates tension effect *in vitro*. Thus, 0.25 ml of trypsin solution (Gibco 0.25%) was added to 1 ml of NAE. This mixture was heated to 100° for 3

min to destroy the enzyme and immediately tested on phenoxybenzamine blocked strips. Tension response to 20-min exposure to 0.05 ml of mixture was about 100 mg, normal for heated NAE. Another portion was incubated for 45 min at 37.5° and then heated similarly. Little or no effect on isometric tension was observed, indicating that trypsin destroyed the factor responsible for nonneurogenic muscle shortening.

Discussion. The present studies clearly show that distilled water extract of normal rat aortic tissue (NAE) contains a vasoactive factor capable of shortening helically-cut arterial strips *in vitro*. This response is apparently unrelated to neurogenic, catecholamine, or renin activity. The tension pattern induced by NAE can be distinguished from that by *l*-norepinephrine or angiotensin, the most obvious difference being the more prolonged tension response. This is especially graphic in strips subjected to three consecutive doses of each substance. *l*-Norepinephrine induces prompt and equal rises and declines after all three doses while each dose of angiotensin has a diminished response. On the other hand, tension from NAE increases with each dose and it is sustained for relatively long periods.

Furthermore, NAE effect is only moderately reduced (about 25%) by heating to 100° for 10 min or by alpha-adrenergic blocking of strip with phenoxybenzamine. This decrease in response is more than likely due to destruction of the catecholamine content in NAE.

The nonneurogenic vasoactivity of arterial tissue extract is apparently specific as indicated by failure to create tension in blocked strips with extracts of other muscle tissues such as skeletal, myocardial, and stomach.

It would appear that increased tension of arterial strips induced by NAE *in vitro* may be related to the pressor response to intravenous NAE in hypotensive test rats. Thus, strip tension may be equated with shortening the circular smooth muscle of arteries, the lumen thus is narrowed, peripheral vascular resistance is increased, and pressure is elevated. The conditions under which muscle shortening is obtained are similar to those where blood pressure is increased, *i.e.*, after alpha-adrenergic blocking or after heating extracts. On the other hand, muscle shortening is absent under conditions where blood pressure levels were not elevated, *i.e.*, after trypsin digestion of NAE and after administration of arterial extracts obtained from animals treated with actinomycin D or acetoxycycloheximide.

One exception in correlating NAE response in muscle shortening with blood pressure elevation was noted. Puromycin injections fail to reduce blood pressure in rats (2), yet such treatment suppresses the activity of extracts of arterial tissue on muscle shortening. This discrepancy may be related to the fundamentally different manner of protein synthesis inhibition between puromycin and the other antimetabolites (2). Thus, actinomycin D and acetoxycycloheximide suppress polypeptide synthesis while puromycin releases polypeptides prematurely.

The present and earlier studies showed that extract of arterial wall induced prolonged shortening of arterial muscle *in vitro* and sustained elevation of diastolic pressure in intact test rats, through mechanisms unrelated to neurogenic, renal, or catecholamine influences. It would appear, therefore, that an increased basal myogenic arterial tone rather than phasic muscle contraction is involved in increasing tension *in vitro* or pressor response in intact rats. This hypothesis may be questioned on the basis that arterial muscle of large vessels such as the aorta is considered to have relatively little myogenic tone (5). In the absence of intrinsic tone, arterial smooth muscle may, however, respond tonally to humoral vasoactive factors (3). Thus, Uchida and Bohr (5) stated that an unidentified vasoactive factor in plasma "may be responsible for nonneurogenic vascular tone of vessels whose smooth muscle does not have intrinsic myogenic tone and may also enhance intrinsic myogenic tone of those vessels that do have it." It remains to be determined whether the hypothetical arterial tonal factor of arterial extracts enters the blood stream as the unidentified muscle shortening plasma factor (4) and thus exerts pressor effect or whether it acts *in situ* on the smooth muscle.

It was suggested earlier that the hypotheti-

cal tone factor may be a polypeptide. *In vitro* studies above are compatible with such a suggestion, namely (i) effectiveness of extraction of arterial tissue with distilled water, (ii) heat stability, and (iii) loss of potency after digestion by trypsin. Further studies of this problem await discovery of more abundant sources of material than is now available.

Summary. Aqueous extracts of rat aorta (NAE) induces increased tension in helically-cut arterial strips *in vitro*. Muscle shortening is not seriously impaired in strips treated with an alpha-adrenergic blocking drug. This effect is not obtained with similar extracts of stomach, myocardial, or skeletal muscle. Also, no significant strip tension is observed with extracts of aortas from rats treated with protein synthesis inhibitors, actinomycin D, puromycin, or acetoxycycloheximide. Vasoactivity of NAE persists after heating to 100° but is destroyed by trypsin digestion.

The author thanks the John L. Smith Memorial for Cancer Research, Chas. Pfizer & Co., Inc., Maywood, N.J., for the supplies of acetoxycycloheximide under support of NIH contract PH 43-68-45 with the National Cancer Institute through the courtesy of Dr. M. T. J. McBride.

1. Freed, S. C., Proc. Soc. Exp. Biol. Med. **133**, 1035 (1970).

2. Freed, S. C., Proc. Soc. Exp. Biol. Med. **124**, 225 (1967).

3. Zuberbuhler, R. C., and Bohr, D. F., Circ. Res. **16**, 431 (1965).

4. Bohr, D. F., and Johannson, B., Circ. Res. **19**, 593 (1966).

5. Uchida, E., and Bohr, D. F., Circ. Res. **25**, 549 (1969).

Received Sept. 3, 1970. P.S.E.B.M., 1971, Vol. 136.