PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 7-12 (1976)

A Vasopressor Potentiator for Norepinephrine in Hypertensive Rats (39470)

L. E. SELF, H. D. BATTARBEE, K. A. GAAR, JR., AND G. R. MENEELY

Department of Physiology and Biophysics, Louisiana State University Medical Center, School of Medicine in Shreveport, P. O. Box 3932, Shreveport, Louisiana 71130

Recently, attention has been given to a hyperresponsiveness to vasopressor agents in various forms of hypertension. Hypertrophy and/or "water-logging" of blood vessels associated with prolonged hypertension has been found to narrow vessel lumina and lead to such hyperresponsiveness (1). In addition, subnormal monoamine oxidase activity accompanied by increased tissue norepinephrine concentration has been reported in genetically hypertensive turkeys, which also exhibited a greater vasopressor response to exogenously administered norepinephrine (2).

Lately there has been a renewed interest in a "sensitizing factor" to catecholamines in hypertension, but because of the diverse nature of some studies and the frequent utilization of inadequate controls, reports have been conflicting. Several investigators have found evidence for a vasopressor agent potentiating factor among hypertensive subjects (3-6), and a transmittable humoral factor has been suggested by a study that utilized the parabiotic union of a strain of rats that was genetically susceptible to the development of hypertension (7). In addition, a blood-borne factor in hypertensive humans that sensitized normal assay animals to the vasopressor effects of norepinephrine and angiotensin II has been reported (8). The present study was conducted to determine if a factor exists in the serum of salt-induced hypertensive rats that would enhance the vasopressor activity of exogenously administered norepinephrine in normotensive bioassay animals.

Materials and methods. Development of experimental hypertension. Sixty-eight male rats, initially weighing 166 ± 29 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 divided into three groups of 20-24 animals and placed on diets consisting of Purina Lab Chow with NaCl added to make up 1.3, 5.6, and 8.4% of the ration by weight.[1] Deionized water and feed were provided *ad libitum*. The animals' weights and systolic blood pressures were recorded weekly with a programmed electrosphygmomanometer[2] and a cuff over the caudal artery. At the end of 225 days on the diets, the animals were sacrificed by decapitation, and their trunk blood was collected and allowed to clot at room temperature. Cells were separated from the serum by centrifugation, and the serum was stored at −28° until used. Serum samples were selected from the three dietary groups in such a manner that animals with a wide range of blood pressures were represented in the data.

Bioassay for norepinephrine response. A separate group of male rats weighing 300-400 g, which had previously been screened for blood pressure abnormalities, was used for the bioassay. After bilateral nephrectomy and a 24 hr recovery period, these animals were given dial-urethan[3] as an anesthetic agent (1.8 ml/kg, ip), and pentolinium tartrate[4] was given as an adrenergic blocking agent (5-8 doses of 0.25 mg/25 μl each given iv at 5 min intervals). During the assay, blood pressures were measured via a polyethylene cannula (PE-50)[5] placed in the internal carotid artery connected to a P-1000A pressure transducer and recorded using a Physiograph DMP-4A[2] recorder. A Narco SW-4 Servo-

[1] Nutritional Biochemicals Corp., Cleveland, Ohio.

[2] Narco Biosystems, Houston, Texas.

[3] Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

[4] Wyeth Laboratories, Philadelphia, Pennsylvania.

[5] Intramedic tubing, Beckton-Dickinson, Rutherford, New Jersey.

Writer[2] accessory was used to expand the mean blood pressure recording scale for greater convenience in reading pressure changes. Intravenous injections were made through PE-20 cannulas[5] inserted into both internal jugular veins. The cannula used for injecting norepinephrine was maneuvered into the superior vena cava. Systolic and diastolic pressures, electrocardiogram, and respiration were observed periodically throughout the procedure.

After the administration of the adrenergic blocking agent, a norepinephrine dose response curve was recorded for each animal and used to determine the quantity of norepinephrine required to produce a 10 mm Hg increase in mean blood pressure. The amount thus determined for each animal was subsequently used as a standard dose to be injected iv at 6 min intervals for the duration of the assay. Very small norepinephrine injection volumes were used to avoid the expansion of the extracellular fluid volume that would otherwise accompany multiple injections in the same animal. The mean volume for each injection of norepinephrine was 0.77 ± 0.07 μl (SE) with a total mean volume injected into assay animals of 13.05 ± 1.45 (SE) μl. Responses to the standard dose of norepinephrine were considered acceptable if six consecutive responses varied no more than ± 1 mm Hg mean blood pressure, recorded from a steady blood pressure baseline, varying no more than ± 1.5 mm Hg. After the standard response was established, 15–25 μl of the serum sample was injected through a jugular vein cannula, and the standard dose of norepinephrine was repeated until it became apparent that no further increase in vasopressor response would occur. The three highest consecutive responses to the standard norepinephrine dose, recorded from a mean blood pressure baseline varying no more than ± 3 mm Hg, were selected and averaged, and their mean was used as the test response value.

Statistical analysis of data. Significance of the results obtained was determined by analysis of variance, Fisher's "*F*" test (9).

Results. Effect of diet upon blood pressure. Blood pressure ranges of the animals after 225 days on the 1.3, 5.6, and 8.4% NaCl diets are shown in Fig. 1. The mean

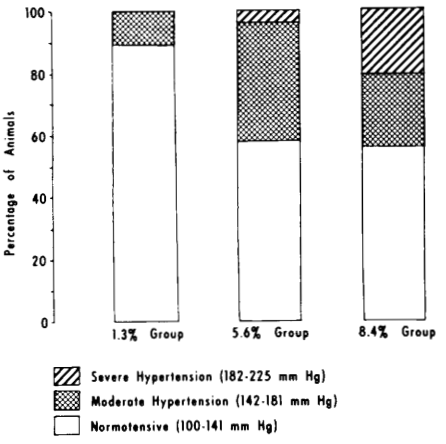

FIG. 1. Systolic blood pressure ranges of rats on 1.3, 5.6 and 8.4% NaCl diets. The proportion of animals on the 5.6 and 8.4% diets that developed hypertension was similar. The severity of hypertension increased as dietary NaCl increased.

systolic blood pressures for the three groups were 129 ± 5 mm Hg (SE), 146 ± 2 mm Hg, and 154 ± 2 mm Hg, respectively. Eighty-nine percent of the animals on the 1.3% NaCl diet remained normotensive, while only 46–48% of the animals on the high salt diets remained normotensive. Greater dietary salt content had little effect upon the percentage of animals developing hypertension (systolic BP > 142 mm Hg), but several of the animals developed very severe hypertension (systolic BP > 180 mm Hg) on the 8.4% NaCl diet.

Effect of serum injection upon mean blood pressure. Ganglionic blockade reduced basal vascular tone with a resultant reduction in mean blood pressure to near 50 mm Hg. An hour or more was required for equilibration following the blocking agent, after which the blood pressure baseline remained stable for several hours. The injection of hypertensive or normotensive serum did not affect basal mean blood pressure, as may be seen in Table I.

Effect of serum injection upon norepinephrine response. The injection of sera from hypertensive rats resulted in an augmented response to standard doses of norepinephrine. After the administration of hypertensive sera, the vasopressor response to norepinephrine ranged from a 7% decrease to a 78% increase in response with a mean

TABLE I. BLOOD PRESSURE CHANGES OCCURRING DURING BIOASSAY PROCEDURE INDUCED BY GANGLIONIC BLOCKING AGENT, TEST SERUM, AND NOREPINEPHRINE. NOREPINEPHRINE VASOPRESSOR RESPONSE TO HYPERTENSIVE SERUM WAS HIGHLY SIGNIFICANT ($P < 0.001$).

		Bioassay Rat						
				Mean blood pressure		BP response to norepinephrine in mm Hg		
Animal	Serum donor systolic BP in mm Hg	Body Weight in g	Systolic BP in mm Hg	Shortly after blocking agent in mm Hg	After serum in mm Hg	Control	Serum test	Norepinephrine response change (%)
4/17	120	370	–	68	44	11.0	11.0	0
4/24	117	350	–	78	50	17.2	16.8	−2
5/23	140	345	90	43	40	16.2	17.7	9
5/23	120	345	90	43	40	17.2	16.5	−4
6/7	117	350	115	53	53	15.8	14.8	−6
6/11	125	440	120	40	41	9.0	11.8	31
7/24	127	370	100	48	48	12.8	14.7	15
7/24	127	370	100	48	48	13.3	20.0	50
7/31	120	335	120	40	44	14.3	14.6	2
7/31	132	335	120	40	44	14.8	14.7	−1
Group Mean ± SD	125 ± 7	361 ± 31	107 ± 13	50 ± 13	45 ± 4	14.2 ± 2.7	15.26 ± 2.66	9.4 ± 18%
2/15	225	300	110	53	53	10.0	13.3	33
2/11	225	360	86	57	58	8.8	12.2	39
3/20	190	410	125	43	43	8.3	13.2	59
4/2	190	385	–	58	47	8.7	15.5	78
4/17	170	370	–	68	44	11.0	13.2	20
4/24	170	350	–	78	50	16.8	22.5	34
5/10	160	560	–	80	44	8.2	10.2	24
6/7	170	350	115	53	53	15.3	22.2	45
6/11	225	440	120	40	41	9.2	13.0	42
6/14	218	500	118	53	51	9.7	11.3	17
6/20	218	430	110	67	67	15.3	18.8	23
7/3	170	385	100	44	42	8.3	8.3	0
7/3	170	385	100	44	42	8.2	8.8	8
7/23	142	360	100	60	42	10.3	13.7	32
7/3	170	310	105	50	62	12.2	19.0	56
7/31	170	335	120	40	44	9.6	9.0	−7
8/6	160	340	125	67	60	11.7	13.2	13
9/12	217	340	85	50	60	13.2	15.5	13
9/19	170	380	92	60	61	14.5	18.7	29
Group Mean ± SD	186 ± 27	384 ± 64	107 ± 13	56 ± 12	51 ± 8	11.0 ± 2.8	14.3 ± 4.3	29.4 ± 21%

increase of 29.4 ± 5% (SE). When compared to the preserum injection response, this difference was found to be highly significant ($P < 0.001$) (see Tables I and II). A typical response to norepinephrine both before and after hypertensive sera administration is shown in Fig. 2. The injection of sera from hypertensive animals usually resulted in rapid augmentation of response that continued for 90–180 min and subsequently declined to the preserum value during the ensuing 90–180 min.

Much smaller and less significant changes in vasopressor response were observed after injection of normotensive serum (see Tables I and II). An example of the norepinephrine response after the injection of normotensive serum is shown in Fig. 3. Responses for this group ranged from a 5% decrease to a 50% increase with a mean increase of 9.4 ± 6% (SE), but the difference was not found to be significant ($P > 0.1$). Half of the normotensive group had a slightly negative or no increase in reactivity, as shown in Fig. 4, while only 11% of the hypertensive group had no augmentation.

Correlation of systolic blood pressure with norepinephrine response. Regression analysis of systolic blood pressures of serum donor animals and the subsequent vasopressor responses to the standard dose of norepinephrine found in bioassay animals revealed no significant correlation between the two ($P > 0.1$).

Discussion. Observations of a hyperresponsiveness to vasopressor agents occurring concomitantly with hypertension

TABLE II. STATISTICAL *F*-TEST SUMMARY OF THE VASOPRESSOR RESPONSE TO NORMOTENSIVE AND HYPERTENSIVE SERA. THE CHANGE IN VASOPRESSOR RESPONSE ATTRIBUTED TO NORMOTENSIVE SERUM WAS NOT STATISTICALLY SIGNIFICANT WHILE THE RESPONSE ATTRIBUTED TO HYPERTENSIVE SERUM WAS HIGHLY SIGNIFICANT.

Normotensive serum	Degrees of freedom	Sum of squares	Mean squares	*F*	*P*
Between treatment sum of squares	1	27	27	$\frac{27}{8.7}= 3.1$	>0.1
Between animals sum of squares	7	292	41.7	$\frac{41.7}{8.7}= 4.8$	<0.05
Interaction sum of squares	7	61	8.7	$\frac{8.7}{.31}= 28$	<0.001
Between responses sum of squares	32	10	.31		
Total	47				
Hypertensive serum					
Between treatment sum of squares	1	310	310	$\frac{310}{6.2}= 50$	<0.001
Between animals sum of squares	18	1296	72	$\frac{72}{6.2}= 11.6$	<0.001
Interaction sum of squares	18	113	6.2	$\frac{6.2}{.39}= 15.9$	<0.001
Between responses sum of squares	36	14	.39		
Total	73				

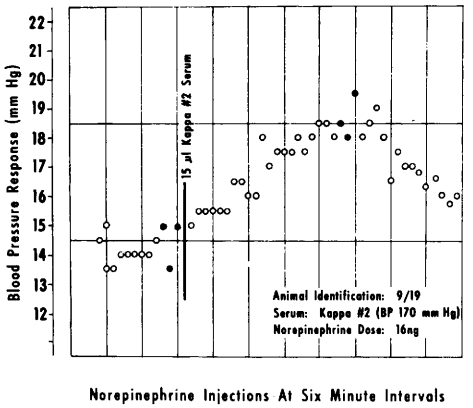

FIG. 2. Norepinephrine response after injection of hypertensive serum. Each circle represents a 16 ng dose of norepinephrine injected at 6 min intervals. The mean of the last three responses before injection of serum (solid circles) was used as control value while the mean of the three highest consecutive responses after serum (solid circles) was used as the test value. In this animal there was a 29% increase in response.

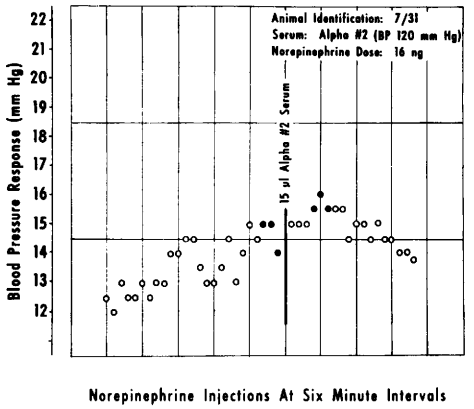

FIG. 3. Norepinephrine response after injection of normotensive serum. Each circle represents a 16 ng dose norepinephrine injected at 6 min intervals. The mean of the last three responses before injection of serum (solid circles) was used as control value while the mean of the three highest consecutive responses after serum (solid circles) was used as the test value. This animal had a 7% increase in response after serum.

have been made in several studies involving both animals (10–13) and man (14–17). Other studies have yielded largely negative results (18–21). Much of the confusion surrounding this important facet of the hypertensive process stems from the nature of the experimental protocols used. Investigations of vascular responsiveness using hypertensive animals themselves to assay for vasopressor response fail to distinguish between changes in apparent response, which can be largely due to geometric changes in resistance vessels and smooth muscle hypertrophy, and real

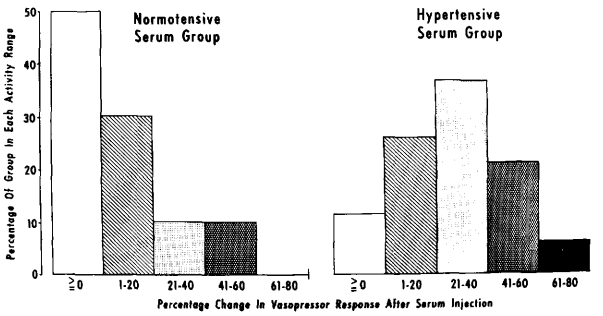

FIG. 4. A comparison of the vasopressor response to norepinephrine in bioassay animals after the injection of normotensive and hypertensive serum. Bars represent the proportion of animals in activity ranges within each serum group. Norepinephrine response after hypertensive serum was much higher than after normotensive serum. The median change in response after normotensive serum was 2 mm Hg, and was 29 mm Hg after hypertensive serum.

changes in response that are due to increased sensitivity to vasopressor agents. Other studies have utilized *in vitro* preparations such as helical strips of femoral artery and aorta (19–22) or portal veins (22), the investigators assuming that large vessels respond quantitatively to vasopressor agents in the same manner as the much smaller resistance vessels.

The use of normotensive animals for the bioassay of humoral vasopressor potentiating agents offers a number of advantages not found in *in vitro* techniques and methods that utilize hypertensive bioassay animals. Such a procedure avoids differences in apparent reactivity that might be due to geometric or hypertrophic changes in the vascular bed brought about by the hypertensive process itself, and since the preparation is an *in vivo* system, it allows for the expression of both direct and indirect effects of injected sera. In addition, there are fewer assumptions as how physiological the assay system is. The effects observed are reflections of changes in the reactivity of the same determinants of total peripheral resistance and blood pressure that exist physiologically.

Recently, a study of Mizukoshi and Michelakis (8) reported a significant increase in vasopressor sensitivity to norepinephrine and angiotensin after the administration of small quantities of serum from hypertensive humans to an assay animal. This material was thought to be a heat-stable, nondialyzable substance that was produced and released by the ischemic kidneys of patients with renovascular hypertension. It was also found in the plasma of patients with essential and malignant hypertension. In addition, sodium restriction in these hypertensive subjects unequivocally reduced the plasma's activity of this substance. It was thought that this substance might play some role in the development of hypertension.

In this study, the sera taken from rats made hypertensive by salt loading were investigated for the presence of a humoral factor that increases reactivity to the vasopressor effects of norepinephrine. Very small aliquots of serum from hypertensive animals were injected into normotensive, nephrectomized, ganglionically blocked rats, and the pressor responses to exogenous norepinephrine were compared to preinjection responses.

The injection of hypertensive sera into the bioassay animals led to a significant increase in vasopressor response to norepinephrine, an observation not made when normotensive sera was used. In contrast to the study of Mizukoshi and Michelakis (8), no change in basal mean blood pressure was observed. No overt attempt was made to characterize the nature or chemical properties of the humoral substance or substances involved, however, the material seems to be relatively heat stable, and its activity persists after repeated freezing and thawing. Studies to elucidate its chemical properties are now in progress.

In summary, the injection of very small aliquots of serum from hypertensive ani-

mals into bioassay animals increased the reactivity of these animals to the vasopressor effects of norepinephrine. These findings suggest the presence of a humoral factor or factors in the serum of hypertensive animals that augments their response to vasopressor agents and that may play some role in the hypertensive process.

The authors gratefully acknowledge the technical assistance of Mr. Glenn E. Farrar and Mrs. Bobbie Gibson in this study, and Mrs. Mary Martin and Mrs. Suzy Moore for aid in the preparation of this manuscript. This work was supported in part by the Mrs. W. C. Feazel Research Award—Louisiana Heart Association.

1. Tobian, L., Fed. Proc. **33,** 138 (1974).
2. El-Halawani, M. E., Waibel, P. E., Appel, J. R., and Good, A. L., Trans. N.Y. Acad. Sci. **1973,** 463 (1973).
3. Page, I. H., Taylor, R. D. and Prince, R., Amer. J. Physiol. **159,** 440 (1949).
4. Doyle, A. E., and Fraser, J. E., Circ. Res. **9,** 755 (1961).
5. Gordon, D. B., Drury, D. R., and Schapiro, S., Amer. J. Physiol. **175,** 123 (1953).
6. Lupu, A. N., Maxwell, M. H., and White, F. N., Proc. Soc. Exp. Biol. Med. **134,** 617 (1970).
7. Dahl, L. K., Kundson, K. D., Heine, M., and Leitl, G., J. Exptl. Med. **126,** 687 (1967).
8. Mizukoshi, H. and Michelakis, A. M., J. Clin. Endocrinol. & Metabol. **34,** 1016 (1972).
9. Batson, H. C. "An Introduction to Statistics in the Medical Sciences." Burgess, Minneapolis, Minn. (1956).
10. Redleaf, P. D. and Tobian, L., Circ. Res. **6,** 185 (1958).
11. McQueen, E. G., Clin. Sci. **21,** 133 (1961).
12. Hinke, J. A. M., Cir. Res. **17,** 359 (1965).
13. McGregor, D. D., and Smirk, F. H., Amer. J. Physiol. **214,** 1429 (1968).
14. Gordon, D. B., and Nogueira, A., Cir. Res. **10,** 269 (1962).
15. Daly, J. J., and Duff, R. S., Clin. Sci. **19,** 457 (1960).
16. Doyle, A. E., and Fraser, J. R. E., Cir. Res. **9,** 755 (1961).
17. Mendlowitz, M., Amer. Heart J. **73,** 121 (1967).
18. Kaplan, N. M., and Shilah, J. G., J. Clin. Invest. **43,** 659 (1964).
19. Redleaf, P. D., and Tobian, L., Circ. Res. **6,** 185 (1958).
20. Bohr, D. F., Fed. Proc. **33,** 127 (1974).
21. Bandick, N. R., and Sparks, H. V., Amer. J. Physiol. **219,** 340 (1970).
22. Hallback, M., Lundgren, Y., and Weiss, L., Acta Physiol. Scand. **81,** 176 (1971).

Received December 1, 1975. P.S.E.B.M. 1976, Vol. 153.