Peptides in Urine of DCA-Treated and Figure-of-8 Rats.* (28395)

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It has previously been reported(1) that accompanying DCA hypertension in rats there is increased uterotonic activity of the extract prepared from their urine. The material responsible for the increased activity was thought to be associated with anephrotensin, a polypeptide obtained from blood (2), because the increase in activity of the urine appeared to be related, chronologically, to an increase in anephrotensin substrate in the serum.

The present study concerns the nature of the biologically active substance, and the relationship between its appearance in the urine and the elevation of the blood pressure in desoxycorticosterone- and renal-hypertensive rats.

Methods. 250-350 g Sprague-Dawley male rats were treated in 3 groups as follows:

Group I: 36 animals each received a daily subcutaneous injection of 10 mg DCA per kg; 12 animals served as controls. All were given 1% NaCl as drinking solution.

Group II: 12 animals each received 2.5 mg DCA per kg daily; 12 animals served as controls. All were given 1% NaCl as drinking solution.

Group III: 24 rats with figure-of-8 ligature on one kidney, the other kidney removed (operations 1 week apart); 24 uninephrectomized animals without figure-of-8 ligature served as controls. All were given tap water *ad libitum.*

Blood pressure was measured at weekly intervals under ether anesthesia, using the tail microphone technique described by Friedman and Freed(3).

Sixteen-hour samples of urine were collected at weekly intervals, during 6 weeks for animals of Group I, 9 weeks for Group II, and 12 weeks for Group III. The samples were collected under toluene and glacial acetic acid (0.1 mg/rat). Peptides were extracted from the urine samples by the method proposed by Nobel, Rinderkneckt and Williams(4) for neurohypophyseal hormone extraction; it consists of precipitation with $ZnSO_4$ and $K_3Fe(CN)_6$ at pH 5 and density 1010, followed by alcoholic extraction.

Pressor activity of the pooled extract obtained was assayed by its effect on the blood pressure of a rat nephrectomized 16-24 hours previously, anesthetized with Na pentobarbital (35 mg/kg, i.p.) and blocked with pentolinium bitartrate (5 mg/kg). Oxytocic activity of the extract was assayed by its effect on the contraction of isolated rat uterine tissue suspended in a modified Tyrode's solution.‡ Synthetic angiotensin and oxytocin, respectively, were used as standards for the above mentioned tests.

Results. Appearance of increased amounts of biologically active material in urine of treated rats. Hypertension became evident in Group I in the 3rd week of treatment ($p = <0.01$, Table I), and in the 4th week in rats in Group II ($p = <0.01$, Table II). The volume of urine excreted by DCA-treated rats was, in general, greater than that by controls. Urine flow was not the same in the two control groups. Since the pressor and uterotonic activity of the urine extract from controls of the two groups was approximately the same, we conclude that there is no relation between urine flow and output of vasoactive peptides. We have expressed extract activity in terms of weight of the animal, and urine volume has not entered into our calculations.

Pressor and uterotonic activity of the peptide extract from urine of rats receiving 10 mg DCA per day was increased from the 2nd week. The average activity, per kg rat in

* This work was supported in large part by grant USPHS H-2578 C4 of the National Institutes of Health.

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‡ The composition of the Tyrode's solution in mM/l is: NaCl, 137.0 KCl, 4.05; MgCl, 1.0; $CaCl_2$, 1.0; $NaHCO_3$, 12.0; NaH_2PO_4, 0.36; and dextrose, 5.6.

TABLE I. Blood Pressure, Urine Excretion and Urine Peptide Activity of Rats Treated with 10 mg DCA Daily, and Controls (Group I).

	Week	0	1	2	3	4	5	6
Blood pressure in mm Hg	DCA	101 ± 8	110 ± 12	122 ± 14	140 ± 14	158 ± 17	179 ± 16	203 ± 24
	Control	104 ± 10	112 ± 5	111 ± 9	113 ± 11	112 ± 10	115 ± 11	123 ± 11
Urine excretion in ml/day/kg	DCA	—	88.1	135.8	217.2	197.8	171.8	197.0
	Control	51.6	62.2	49.2	54.5	57.1	37.9	49.9
Pressor activity of urine peptides in μg angio/day/kg	DCA	—	.03	2.28	1.70	3.61	7.74	38.95
	Control	.01	.03	1.22	.29	.62	2.72	.30
Uterotonic activity of urine peptides in mU oxytocin/day/kg	DCA	—	.70	47.2	120.0	624.0	1440.0	17280
	Control	—	2.04	15.98	1.02	2.04	14.4	15.99

Blood pressure values are given with standard deviation.

TABLE II. Blood Pressure, Urine Excretion and Urine Peptide Activity of Rats Treated with 2.5 mg DCA Daily, and Controls (Group II).

	Week	0	1	2	3	4	5	6	7	8	9
Blood pressure in mm Hg	DCA	115 ± 16	129 ± 7	131 ± 10	140 ± 18	145 ± 14	158 ± 18	171 ± 10	186 ± 14	187 ± 24	191 ± 22
	Control	111 ± 20	117 ± 6	119 ± 10	131 ± 8	120 ± 14	117 ± 15	124 ± 11	111 ± 15	125 ± 21	142 ± 9
Urine excretion in ml/day/kg	DCA	—	—	194	356	—	214	280	243	—	266
	Control	—	—	137	243	—	283	173	152	—	233
Pressor activity of urine peptides in μg angio/day/kg	DCA	—	—	.23	.53	—	.59	.21	.61	—	23.13
	Control	—	—	.49	.35	—	.07	.12	.22	—	.66
Uterotonic activity of urine peptides in mU oxytocin/day/kg	DCA	—	—	3.3	6.0	—	38.4	24.8	96.0	—	1152
	Control	—	—	19.2	14.4	—	12.8	6.6	.0?	—	19.2

Blood pressure values are given with standard deviation.

24 hours, of the extracts from the urine of the control group throughout the 6-week experimental period was equivalent to 0.86 μg of angiotensin in its effect on blood pressure, and to 8.4 mU of oxytocin in its effect on the isolated uterus. Activity of the extract from the urine of these DCA-treated rats at the 6th week was equivalent to 39 μg of angiotensin in its effect on blood pressure and to 17,280 mU of oxytocin in its uterotonic effect. Maximum change in both pressor and uterotonic activity occurred between the 5th and 6th (final week) (Table I). The increase in biological activity was greater as measured by the uterotonic effect of the extract than as measured by its pressor effect; it would seem, therefore, that the increase must be due to more than one substance.

Pressor activity of the urine extract from rats receiving 2.5 mg DCA per day was increased in the 9th week and uterotonic activity was increased from the 5th week, though blood pressure elevation was apparent from the 4th week on (Table II).

Figure-of-8 rats were separated, after 3 weeks, into 2 groups, those that had become hypertensive and those that remained normotensive. Pressor activity of the urine peptides of both hypertensives and normotensives was the same as that of the controls from the 3rd to the 12th (final) week of the experiment. Uterotonic activity was not measured.

Evidence for the peptide nature of the pressor component of the urine. In addition to the fact that the separation procedure was devised for the extraction of peptides in the urine, there is evidence that the active substance is peptide in nature in that it has certain properties common to polypeptides: it is heat stable (in boiling water 30 minutes, pH 5-9), butanol soluble, and fully dialyzable. Moreover, the activity is destroyed by the proteolytic enzyme, chymotrypsin.

Evidence excluding angiotensin as the pressor substance. Intact and nephrectomized animals, previously injected with pentolinium, are equally responsive to the pressor effect of angiotensin(5). When urine extract

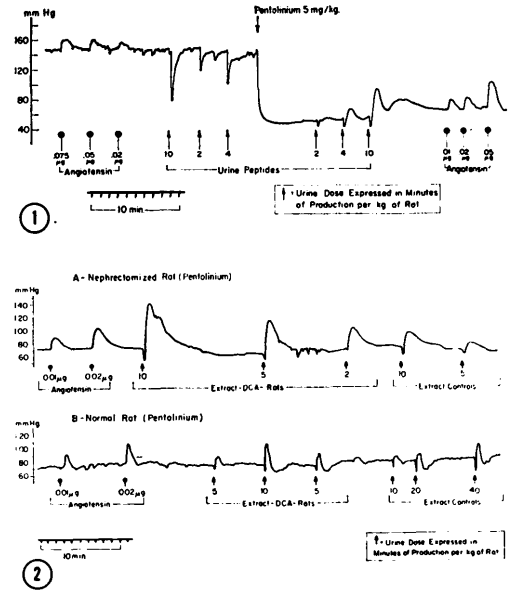

FIG. 1. Effect of urine extract on blood pressure of non-nephrectomized rat before and after pentolinium blockade. Effect of angiotensin serves as reference.

FIG. 2. Comparison of blood pressure responses of nephrectomized and non-nephrectomized rat, both blocked with pentolinium, to urine extract from control and DCA-treated rats. Response to angiotensin serves as reference.

is injected into a normal, anesthetized rat, the blood pressure falls momentarily but returns rapidly to normal levels (Fig. 1). When it is injected into a 16-24 hour nephrectomized, anesthetized rat, there is usually an immediate decrease in blood pressure followed by a temporary increase (5-10 minutes) slightly above pre-injection level, after which the blood pressure gradually returns to normal levels. If in either type of assay rat the sympathetic system is blocked with pentolinium bitartrate (.5 mg/100 g rat), the urine extract elicits a marked increase in blood pressure (Fig. 1 and 2), but the response of the nephrectomized-blocked rat to the urine extract is greater than is that of the intact-blocked rat (Fig. 2). Often a slight, rapid hypotensive reaction precedes the hypertensive response to the axtract. Urine extract is, therefore, unlike angiotensin in its effect on arterial pressure in normal rats and in the difference in effect in normal and nephrectomized blocked rats. This evidence does not rule out the possibility that

some angiotensin is present, since its effect could be obscured by other vasoactive substances, but it does show that, if any is present, it is not the effective peptide of the urine extract.

Evidence excluding vasopressin as the pressor substance. When vasopressin is incubated with Na thioglycollate (400 mU to 2 mg Na thioglycollate) at 38°C, pH 7.7, for 2 hours, it loses approximately 95% of its pressor activity. The pressor activity of the urine extract, on the other hand, remains unchanged by incubation with Na thioglycollate under the same conditions.

In hyperhydrated rats, 0.25 mU of vasopressin has a strong antidiuretic effect(6). When urine extracts from DCA-treated and control rats, in pressor doses equivalent to 7 and 20 mU of vasopressin, were tested under the same assay conditions, no antidiuretic activity was found.

Additional evidence that the pressor substance of the extract is not angiotensin or vasopressin is to be found in the fact that the pressor activity of the extract is destroyed by chymotrypsin and not by trypsin; both angiotensin and vasopressin are destroyed by trypsin.

Comparison with bradykinin. Bradykinin lowers blood pressure in unblocked rats and increases blood pressure in blocked rats(7). It can be seen from Fig. 1 that the urine peptides are like bradykinin in producing these effects.

Bradykinin (30 μg) and urine extract (3 ml) were each incubated with trypsin (40 mg) at 38°C, pH 7, for 30 minutes. Both bradykinin and urine extract maintained activity following the period of incubation.

Bradykinin injected intraarterially into a rat hind limb perfused at constant rate with autogenous blood, has a vasodilator action, and this effect is not changed by pentolinium blockade of the injected animal. The urine extract acts similarly under these conditions.

Discussion. The chronological relationship between the increase in blood pressure and in urine peptides in rats receiving 10 mg of DCA daily, caused us to think that the two might be related. The experiments with 2.5 mg DCA-treated and figure-of-8 hypertensive rats, however, showed no chronological relation between increase in blood pressure and pressor action of the urine extract. Increase in uterotonic activity, however, did parallel increase in blood pressure.

It is of interest that the urine extract has a hypotensive effect in rats whose sympathetic system is not blocked, and a hypertensive effect in rats blocked with pentolinium bitartrate. Blocked renoprival rats are more sensitive to the urine peptides than blocked intact rats. These findings suggest that different substances are being tested in the different animal conditions, or that the mechanism of action of the urine peptides is changed by the condition of the animal.

The greater activity of extract from urine of animals treated with DCA must be due to an increase of more than one substance, since when biological activity increases, the uterotonic effect increases more than the pressor action. The presence of multiple substances should not be surprising, since the method used extracts any small peptides from the urine.

Our experiments permit the conclusion that neither angiotensin nor vasopressin can account for the activity. They allow the possibility that anephrotensin may be among the active components, since the extract shows the characteristics of rat anephrotensin in its action on blood pressure and uterine muscle, and in its greater effect on nephrectomized than on intact rats(8). This possibility receives further support from the fact that anephrotensinogen in the plasma is increased late in DCA treatment(1). We have not ruled out the possibility that the substance(s) is bradykinin(9) and/or kallidin(10). Each of these substances has pharmacological activity similar to that of urine extract(7,9,11). Other peptides described as present in the urine, *i.e.*, substance Z(12) and substance U(13), with pharmacological properties similar to those of bradykinin, also may be present.

These results are in agreement with those published by Gomes(14,15), who, from experiments using the same extraction method with human urine, concluded that the peptide responsible for the pharmacological ac-

tivity found in normal human urine is not vasopressin, but, probably, a kinin.

The increase in urine peptides after DCA treatment may be the result of: 1) An increase in amount of polypeptides circulating and cleared by the kidney. 2) An increase in peptides split off from the plasma protein by the kidney cells, and subsequently excreted in the urine. There is evidence that the kidney may play some role in protein metabolism(16-18). 3) An increase in active polypeptides liberated in the urine itself, as kallidin is liberated by kallikrein (19). 4) Slower degradation of the urine peptides.

Whatever the mechanism for the change, the striking increase in these pharmacologically active agents must be reckoned with in considering the chronic effects of DCA treatment in the rat.

Summary. A peptide material has been extracted from rat urine which decreases blood pressure of normal rats, raises blood pressure of rats with sympathetic system blocked, and causes contraction of uterine muscle. The activity of the extract is greatly increased in rats treated several weeks with DCA, but there is no clear relationship between the increase in the active substance and the onset of the hypertension. In renal hypertension pressor activity of the urine extract was not increased, even after hypertension had developed. It is highly probable that 2 or more peptides are involved. Anephrotensin, bradykinin and/or kallidin may be the active agents, or the activity may be due to some as yet undescribed substance or substances. The possibility that angiotensin or vasopressin is the active component has been ruled out.

We are grateful to Dr. Ruth McVaugh for editing this manuscript, to Mr. Michael Gross for the perfused hind limb experiments, and to Miss Eleanor Overll for technical assistance.

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Received March 18, 1963. P.S.E.B.M., 1963, v113.