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URINARY DOPAMINE LEVELS AND KIDNEY MONOAMINE OXIDASE ACTIVITY IN HYPERTENSIVE RATS*

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Abstract—The relationship among urinary dopamine levels, arterial blood pressure, and kidney monoamine oxidase (MAO) activity was studied in male rats with experimentally induced renal hypertension.

Urinary dopamine levels rose throughout the experimental period from an average control value of 673 μg/l to a high of 1,022 μg/l. The excretion of dopamine was found to be quite variable.

A regression analysis was conducted by regressing blood pressure on logarithm dopamine concentration. The coefficient of regression was + 0·85. As the weight of the experimental (hypertensive) animals increased, the urinary dopamine levels (μg/kg/24 hr) increased, whereas in the sham-operated control animals, the urinary excretion of dopamine (μg/kg/24 hr) decreased with increasing weight.

Kidney MAO activity decreased after the induction of hypertension, went through what appeared to be a compensatory rise, and then dropped off sharply to the end of the experimental period. A clear and consistent relationship between decreased MAO activity and urinary dopamine levels was not established.

INFORMATION on catecholamine levels in the presence of arterial hypertension has been sparse and conflicting. Reports have shown on the one hand that circulating catecholamine levels are normal in hypertensive patients and, on the other hand, that the urinary excretion of these substances increases.[1, 2]

The injection of a preparation of tyrosinase in suitable doses into rats and dogs exhibiting renal hypertension results in a reduction in blood pressure, whereas the blood pressure of normal animals is less or not at all affected. The most probable explanation for the hypotensive action of tyrosinase is that in the hypertensive state some substrate of the enzyme is altered. As tyrosinase acts only on compounds containing a mono- or dihydroxybenzene structure in the molecule, the catecholamines are implicated in the hypertensive process.[3]

The kidney still receives extensive support as the producer of pressor materials in hypertension. Bing[4] reported that the production of a pressor substance, presumably dopamine, by decarboxylation of dihydroxyphenylalanine (DOPA) occurred in extracts of guinea pig kidneys incubated with DOPA under anaerobic conditions. He noted that a similar reaction took place in the cat's ischemic kidney perfused with blood containing DOPA.

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It has been reported that deamination but not decarboxylation of certain amino acids is incomplete in kidneys deficient in their supply of oxygen. A decrease in MAO activity in the kidneys of rabbits rendered hypertensive by renal ischemia has been demonstrated.[5, 6]

In the case of renal insufficiency, the presence of continuing decarboxylation without deamination could lead to an accumulation of dopamine which could not be metabolized.

The aim of this investigation was to determine whether there is, in fact, an increase in dopamine levels during renal insufficiency and what effect this might have on the arterial blood pressure.

MATERIALS AND METHODS

Male albino rats of the Sprague-Dawley strain, weighing 90 to 100 g, were used for this investigation. Experimental hypertension was effected by renal insufficiency. The rats were subjected to two separate operations; the first involved removal of the right kidney; the second, compression of the renal artery to the remaining organ.[7, 8]

The rats were divided into groups of eight and kept in pairs in cages equipped with stainless steel funnels for urine collections which were made weekly; samples from each group were pooled. A sham-operated control group of 8 animals was run concurrently with the experimental groups. These animals were subjected to a right nephrectomy followed in 2 weeks by manipulation of the left renal artery.

Indirect blood pressure of each animal was obtained weekly by use of the photoelectric tensometer.*

Dopamine was extracted from the urine in the following manner: 1 mg/ of ascorbic acid/ml and 2 volumes of distilled water were added to a maximum of 15 ml of urine. The samples were then adjusted to pH 6·5 with 2·0 N sodium hydroxide.[9] The dopamine was extracted by passing a 20-ml aliquot of the processed urine sample through a strongly acidic cationic exchange resin, Dowex 50 W-X8.† Elution was performed with 8·0 ml of 2·0 N hydrochloric acid.[10] Bertler *et al.*[10] have shown that the H^+ form of the Dowex-50 resin will bind not only norepinephrine, epinephrine, and dopamine but also DOPA. They further showed that DOPA passed freely through the Na^+ form of the resin. It is advantageous not to have DOPA present in the eluate since it possesses fluorescent characteristics similar to those of dopamine and would interfere with the fluorometric determination of this substance. Dowex 50 W-X8 is supplied in the H^+ form and was converted to the Na^+ form before use.

The determination of dopamine was carried out according to the method of Carlsson and Waldeck.[11]

The fluorescence of the samples was determined on an Aminco-Bowman spectrophotofluorometer.‡ The activating and fluorescent wave lengths were 325 and 380 mμ (uncorrected instrumental values), respectively, and were determined by a standard solution of dopamine§ (0·05 μg/ml). A linear relationship was found to exist between

* Obtained from Metro Industries, Inc., Long Island City, N.Y.

† Dowex 50 W-X8, 200–400 mesh, obtained from J. T. Baker Chemical Co., Phillipsburg, N.J. A column with the dimensions 20 mm^2 × 12 mm was used.

‡ American Instrument Co., Inc. Silver Spring, Md. Specifications for the test: xenon lamp, slit arrangement No. 5, photomultiplier tube IP 28; fused quartz cells were used.

§ Dopamine was obtained from the Winthrop Laboratories, Rensselaer, N.Y.

the fluorescent intensity and concentrations of dopamine up to 0·4 μg/ml. A standard dopamine curve was plotted with each group of unknown samples. The values of the unknown dopamine samples were extrapolated from the calculated standard line.

Internal standards were employed at various times throughout the experimental period. This was accomplished by the addition of known amounts of dopamine to urine samples before processing. The average recovery ± standard error ($E = \sqrt{\{\Sigma d^2/[n(n-1)]\}}$) for several samples was 74·52 ± 2·93%.

In order to determined the MAO activity, one animal from each group was sacrificed every 2 weeks during the experimental period. The kidneys were immediately removed and frozen. The kidneys removed during the first operation were used to obtain control values.

MAO levels in kidney were determined according to Cotzias and Dole.[12] A unit of MAO activity (U) is defined as the quantity capable of catalyzing the production of ammonia at the rate of 1·0 μmole/hr under the conditions of the test. Tyramine was used as the substrate in the studies.

RESULTS AND DISCUSSION

The results presented in Table 1 represent pooled data from six groups of male rats.

TABLE 1. RELATIONSHIP OF URINARY DOPAMINE LEVELS, ARTERIAL BLOOD PRESSURE, AND MAO ACTIVITY*

Week after 2nd oper.	Dopamine		Blood pressure		Monoamine oxidase activity ($U \times 10^4$)†
	No. of animals	(μg/l ± SE)	(mmHG ± SE)	No. of animals	U ± SE
Control†	42	673 ± 67·8	122 ± 1·8	6	34 ± 3·8
1	25	542 ± 44·1	140 ± 2·9		
2	25	562 ± 45·7	148 ± 4·2	6	22 ± 1·2
3	23	585 ± 29·8	164 ± 3·4		
4	22	572 ± 73·1	166 ± 3·9	6	25 ± 6·6
5	21	627 ± 102·4	173 ± 4·6		
6	19	785 ± 76·3	185 ± 7·6		
7	16	752 ± 93·9	188 ± 2·2	6	31 ± 2·2
8	17	838 ± 133·9	183 ± 5·7		
9	15	831 ± 137·9	196 ± 2·3		
10	12	950 ± 137·9	193 ± 3·8	6	19 ± 2·3
11	12	757 ± 140·0	183 ± 6·9		
12	9	1022 ± 183·3	184 ± 3·9	6	15 ± 2·3

* All values represent means with standard error of 6 groups of male rats at the time specified in column one. The standard error was determined from the equation shown in the methods section.

† Control values represent pooled data from animals prior to left renal arterial compression.

‡ U = quantity capable of catalyzing the production of ammonia at the rate of 1·0 μmole/hr.

The average control blood pressure (taken prior to the second operation) was 122 mm Hg and rose steadily throughout the experimental period until week 11 when it dropped slightly and leveled off at approximately 183 mm Hg.

Urinary dopamine levels rose consistently throughout the experimental period with an unexplainable drop occurring during week 11. It had previously been reported that

the urinary excretion of dopamine was extremely variable,[1, 13] and this was found to be true in the present investigation.

A simple regression was conducted by regressing the arterial blood pressure on the logarithm dopamine concentration. The regression was run independently of time. The regression coefficient was 0·85. In a test of the null hypothesis H_0:P = 0, taking the percentage points of the t-distribution at the 0·05 level. $|T|$ = 5·094 was greater than $t_{.05}(N - 2) = 2{\cdot}228$. Rejection of this hypothesis indicates that the two variables considered here are, in fact, statistically dependent.

The urinary excretion of dopamine measured in micrograms per kilogram per 24 hr has been reported to decrease in normal rats as their body weight increases.[14] In order to substantiate further that dopamine levels were in fact rising, the dopamine excretion on a μg/kg/24 hr basis between sham-operated control animals and hypertensive animals was compared (Fig. 1).

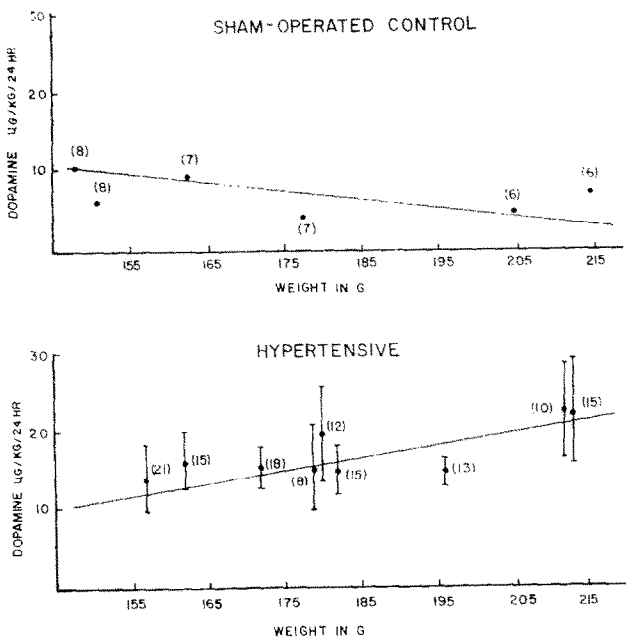

FIG. 1. Comparison of urinary dopamine excretion in μg/kg/24 hr between sham-operated control and hypertensive male rats. With the control group, each point represents the pooled dopamine values from the number of animals shown in the parentheses plotted against the mean weight of each of these groups of animals. With the experimental, the animals were grouped according to the number of the week following the second operation (left renal arterial compression), and the mean dopamine values $\pm$SE of each of these groups is plotted against the mean weight of each group. (SE calculated according to equation shown in footnote in Methods section.) The numbers in the parentheses indicate the total number of animals considered in each case.

Results from the comparison indicated a gradual increase in the dopamine excreted in the experimental (hypertensive) groups, whereas in the sham-operated control group a decrease was noted.

Kidney MAO activity dropped during the first 2 to 3 weeks after the second operation. During the succeeding 5 weeks there appeared to be a compensatory rise and then

a sharp drop reaching a value at the end of the experimental period that was approximately 45% of the original control activity (Table 1).

No positive statement can be made from this investigation of the relationship between dopamine levels and decreased kidney MAO activity. Some correlation was established between urinary dopamine levels and blood pressure during the experimental period. The urinary dopamine excretion increased gradually on a μg/kg/24 hr basis; however, no large increase in total urinary dopamine excretion was observed until about week 12.

REFERENCES

1. H. Weil-Malherbe and A. D. Bone, *J. clin. Path.* **10**, 138 (1957).
2. U. S. von Euler, *Noradrenaline*. Thomas, Springfield, Ill. (1956).
3. H. Schroeder and K. Adams, *J. exp. Med.* **73**, 531 (1941).
4. R. J. Bing, *Amer. J. Physiol.* **132**, 497 (1941).
5. H. Schroeder, *Science* **95**, 306 (1942).
6. C. Giordano, A. H. Samily, J. Bloom, F. W. Haynes and J. R. Merrill, *Fed. Proc.* **100**, 19 (1959).
7. H. Goldblatt, J. Lynch, R. F. Hanzel and W. W. Summerville, *J. exp. Med.* **59**, 334 (1934).
8. D. R. Dury, *J. exp. Med.* **68**, 695 (1938).
9. T. B. B. Crawford and W. Law, *J. Pharm. Pharmacol.* **10**, 179 (1958).
10. A. Bertler, A. Carlsson and E. Rosengren, *Acta physiol. scand.* **44**, 273 (1958).
11. A. Carlsson and B. Waldeck, *Acta physiol. scand.* **44**, 295 (1958).
12. C. G. Cotzias and V. P. Dole, *J. biol. Chem.* **90**, 665 (1951).
13. R. D. Drujan, T. L. Sourkes, D. S. Layne and C. F. Murphy, *Canad. J. Biochem.* **37**, 1153 (1959).
14. J. Leduc, *Acta physiol. scand.* **53**, Suppl. 183 (1961).