

## A Trace of Monoamines in Rat Adrenal Gland

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Although norepinephrine and epinephrine are the predominant monoamines in peripheral tissues, peripheral dopamine (DA) and serotonin (5-HT) systems are also physiologically significant. These monoamines are present both in the sympathetic ganglia and in the adrenal gland. We attempted to determine the possible presence of in individual adrenal cells. We found increments in adrenal 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) levels produced by probenecid, an inhibitor of anionic secretory mechanisms. Small amounts of DA, 5-HT, DOPAC and 5-HIAA were detected in the rat adrenal gland by using the high-performance liquid chromatographic electrochemical detection method. Two hr after probenecid treatment (200 mg/kg, i.p.) the concentration of striatal homovanillic acid and 5-HIAA approximately doubled compared with those of control rats and the levels of striatal DOPAC remained much the same. Conversely, the adrenal DOPAC and 5-HIAA were markedly increased with no change in the levels of catecholamines and 5-HT. The existence of DOPAC and 5-HIAA and the increase by probenecid strongly indicate that the adrenal DA and 5-HT may be independently granulated.

### INTRODUCTION

The adrenal gland is one of the most important storage tissue of biogenic amines in mammals. It has been recently recognized that small amounts of dopamine (DA) and serotonin (5-HT) are present in the adrenal gland, although the predominant monoamines are epinephrine (EN) and norepinephrine (NE) (1, 2). The mode of existence of adrenal DA and 5-HT in the adrenal gland has not been understood fully and little is known about the physiologically significance of these traces of monoamines in the

adrenal gland.

Puppi et al (3) separated various granules by different centrifugation from dog adrenal medulla and found that EN accumulated in the 1000A° granule, NE in the 2000-3000A° granule and DA concentrated in rather large granules. The 5-HT also granulated in the rat adrenal gland (2).

Functionally, Ohmiya et al (4) investigated the DA secretion from adrenal medulla during hypotension induced by hemorrhage in the dog. The rates of secretion of EN, NE and DA vary with hemorrhagic alterations, and the pattern of increase in level of DA differs from the patterns in the case of NE and EN in hemorrhagic hypotension.

In the present study, an attempt was made to determine whether or not the adrenal DA and 5-HT are granulated independently. The highly sensitive high performance liquid chromatographic techniques led to investigations of dopaminergic and serotonergic mechanism in the adrenal gland.

We measured a trace of DA and 5-HT in rat adrenal gland, in addition, accumulation of DA and 5-HT metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindolacetic acid (5-HIAA) after the treatment of probenecid, an inhibitor of acid transport process.

## MATERIALS AND METHODS

1) **Animals**: Male Wistar rats weighing 250-300 g were fed a standard diet (Funabashi Farm Co.) and water *ad libitum* and housed at 24°C. These rats were given 10 % sucrose solution orally 24 hr before decapitation since the cereal chow contained tyrosine, DOPA and tryptophan which modify the level of endogenous metabolites of biogenic amines (5).

Probenecid (200 mg/kg, i. p.) in experientmal group and the control group were given the vehicle alone, respectively.

The rats were decapitated 2 hr after administration of probenecid. The brain and adrenal glands were rapidly removed and the striatum was dissected on ice, as described by Glowinski and Iversen (6).

2) **Determination of amines and their metabolites**: Amines and metabolites were assayed using high-performance liquid chromatography (HPLC) with an electrochemical detector (ECD) and a modification of previously reported methods (7, 8, 9).

The adrenal gland and brain regions contain many other components, therefore the author chose the method of purification of DOPAC and homovanillic acid (HVA) described as follows.

Tissue samples were homogenized in 4.0 ml of 0.4 M HClO<sub>4</sub> containing 0.23 mM ascorbic acid as an antioxidant and 3, 4-dihydroxybenzylamine (DHB) as an internal standard for catecholamines (CAs) and N-methyl serotonin (N-Me-5-HT) for 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). The homogenates were centrifuged at 15,000 r. p. m. for 15 min at 2°C.

DOPAC and HVA were extracted from a 3.0ml aliquot of the supernatant by the method of Karasawa et al (10). Briefly, the supernatant was passed through a Sephadex G-10 column. The column was washed with 0.01 M HCl and then DOPAC and HVA were extracted with 0.01 M HCl. The eluate was transferred to the QAE-Sephadex A-25 column. After washing with 0.02 M CH<sub>3</sub>COOH and of 0.2 M NaCl, DOPAC and HVA were eluted with 3.0 ml of 0.2 M NaCl and then assayed simultaneously by HPLC-ECD. The chromatograph (6000A pump, U6K universe injector, Waters Assoc., MA, U.S.A.) was equipped with a glassy carbon amperometric detector (+0.70 V), LC-4A, (Bioanalytical Systems, IN, U.S.A.) and a 0.39 cm x 30 cm reverse phase C<sub>18</sub> ( $\mu$ -Bondapak C<sub>18</sub>, Waters Assoc.) column. The mobile phase, 0.15 M monochloro acetate buffer, pH 3.0, containing 2 mM Na<sub>2</sub>EDTA and 9% methanol was pumped at a rate of 1.5 ml/min.

The other 0.5 ml aliquot of the supernatant containing the CAs was adjusted to pH 8.6 by addition of 3 M Tris-HCl buffer, pH 8.6 and transferred to vial containing acid washed alumina. The absorbed CAs were eluted by 0.5 ml of 0.1 M HClO<sub>4</sub>. The mobile phase of HPLC was 0.15 M monochloroacetate buffer, pH 3.0, containing 5 mM 1-octane sulfonic acid as a paired ion and 2 mM Na<sub>2</sub>EDTA. The flow rate was 1.5 ml/min. The ECD current voltage was set up in + 0.7 volt versus a Ag/AgCl. The NE, EN, DHB and DA eluted from the column with retention times of 4.5, 6.1, 7.5 and 10.5 min, respectively. For the 5-HT and 5-HIAA detection, we applied the supernatant directly to HPLC-ECD (9). The HPLC-ECD conditions were as follows: The mobile phase, 0.15 M monochloro acetate buffer, pH 3.0 containing Na<sub>2</sub>EDTA and 10 % methanol was pumped at a rate of 1.5 ml/min. The ECD current voltage was set up in +0.5 volt.

3) **Drugs**: Probenecid was dissolved in a minimal volume of 1 M NaOH, some saline added and pH adjusted with 2 M HCl to 8.6. Probenecid was a gift from Nippon Merck Banyu Co. DHB HBr (Aldrich Chemical Co., WI, U.S.A.), NE bitartrate, EN bitartrate, DA HCl, DOPAC, HVA, 5-HT creatinine sulfate (Sigma Chemical Co. MO., U.S.A.) 5-hydroxy-N-methyltryptamine oxalate. (Aldrich Chemical Co., WI, U.S.A.)

4) **Others**: Protein concentration of the tissue pellets was determined by the method of Lowry et al (11). Values are expressed as the average  $\pm$  S. E. The statistical significance of differences between mean values was analyzed by using Student's t-test.

## RESULTS:

In the rat adrenal gland, we could detect adrenal DA and 5-HT. And in this study, we used probenecid, an inhibitor of acid transport processes which may clarify the mode of existence of adrenal DA and 5-HT. Additionally, we measured small amounts of metabolites, for example, DOPAC, HVA and 5-HIAA.

The amount of DA is 1.4 % and 5-HT only 0.2 % of all adrenal monoamines. NE was 26.3 % and EN was 72.1 %. Although the percentages of DA and 5-HT minute, the value expressed as ng/mg protein was similar to that in the striatum which is richly innervated with dopaminergic and serotonergic nerve endings. No changes were observed in monoamines (NE, EN, DA and 5-HT) 2 hr after probe necid administration (Table

**Table 1.** Change of Monoamine in Adrenal Gland and Striatum 2 hr After Probenecid Administration

	Adrenal Gland		Striatum	
	Probenecid		Probenecid	
	(-)	(+)	(-)	(+)
5-HT (ng/mg prot.)	8.3 ± 0.5	8.4 ± 0.3	11.1 ± 0.7	12.2 ± 0.2
DA (ng/mg prot.)	63 ± 7	73 ± 4	74 ± 3	86 ± 5
NE (ng/mg prot.)	1171 ± 87	1403 ± 171	4.5 ± 0.7	5.9 ± 1.0
EN (ng/mg prot.)	3206 ± 226	2624 ± 162	n. d.	n. d.

Rats were given probenecid (200 mg/kg, i.p.). 5-HT; serotonin. DA; dopamine. NE; norepinephrine. EN; epinephrine. n.d.; non detectable. Each value is the average ± S.E. of 5 determinations.

1).

Fig. 1 shows typical HPLC profiles of 5-HT and its metabolite, 5-HIAA. The 5-HT and 5-HIAA eluted from HPLC column with retention time of 5.6 and 9.6 min, respectively.

Fig. 2 shows typical HPLC profiles of DA metabolites extracted from the adrenal gland. The DOPAC and HVA eluted from HPLC column with retention times of 8.4 and 21.6 min, respectively.

Fig. 1 and Fig. 2 show a phenomenal increase of adrenal DOPAC and 5-HIAA induced by the probenecid treatment. However, in the striatum DOPAC was not so changed and HVA markedly increased (Table 2).

Interestingly, the adrenal DOPAC/DA and 5-HIAA/5-HT ratio were smaller than the striatal ones. The adrenal DOPAC/DA and 5-HIAA/5-HT ratio increased as same as the striatal HVA/DA ratio (Fig. 3, Fig. 4).

**Table 2.** Accumulation of Metabolites of Serotonin and Dopamine 2 hr After Probenecid Administration

	Adrenal Gland		Striatum	
	Probenecid		Probenecid	
	(-)	(+)	(-)	(+)
5-HIAA (ng/mg prot.)	0.1 ± 0.2	0.3 ± 0.0**	6.7 ± 0.4	11.2 ± 0.6**
DOPAC (ng/mg prot.)	2.7 ± 0.4	7.0 ± 0.5**	9.7 ± 0.6	13.1 ± 1.0*
HVA (ng/mg prot.)	n. d.	n. d.	6.2 ± 0.5	15.9 ± 1.4**

Rats were given probenecid (200 mg/kg, i.p.). 5-HIAA; 5-hydroxyindoleacetic acid. DOPAC; 3,4-dihydroxyphenylacetic acid. HVA; homovanillic acid. n.d.; non detectable. Each value is the average ± S.E. of 5 determinations. \*p<0.05, \*\*p<0.001 with respect to the control value.



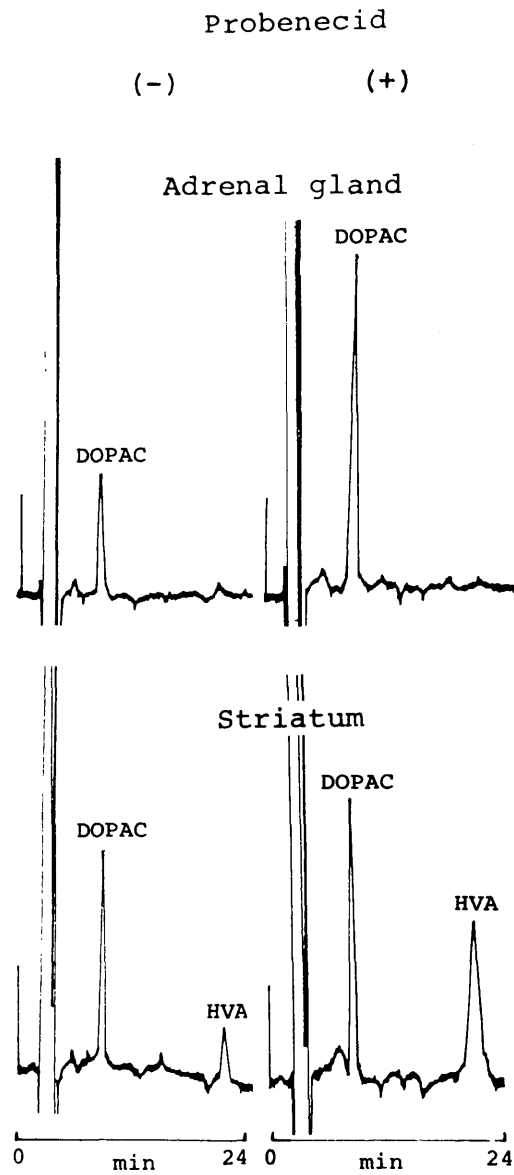


Fig. 2. Typical chromatograms of tissue DOPAC and HVA extracted as described in "Materials and Methods" from a rat adrenal gland (in the upper half) and rat striatum (in the lower half). The left and the right sides are the chromatogram from a control rat and a probenecid (200 mg/kg, i.p., 2 hr after) treated rat, respectively.

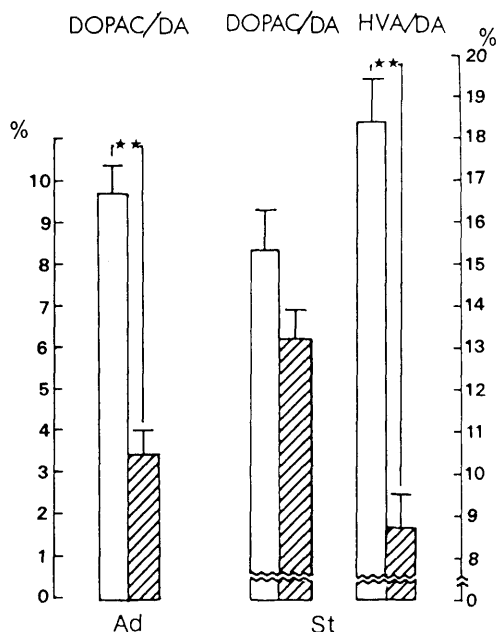


Fig. 3. Effect of probenecid (200 mg/kg, i.p., 2 hr after) treatment on the DOPAC/DA and HVA/DA ratio in rat adrenal gland (Ad) and rat striatum (St). The opened columns denote the data from probenecid treated rats and the shaded columns the data from control rats. Values are the average  $\pm$  S. E. of 5 determinations. \*\* $p < 0.001$  with respect to the control value.

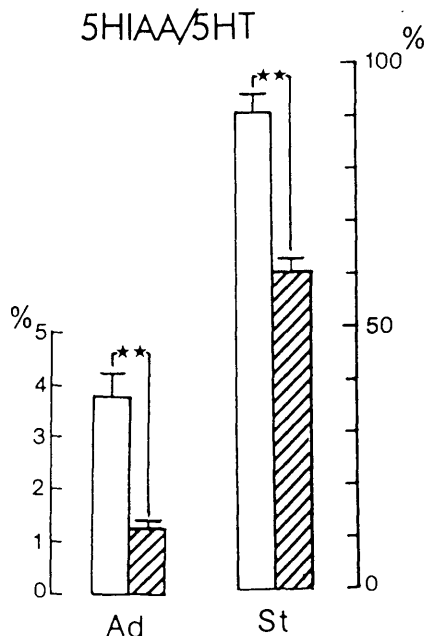


Fig. 4. Effect of probenecid (200 mg/kg, i.p., 2 hr after) treatment on the 5-HIAA/5-HT ratio in rat adrenal gland (Ad) and rat striatum (St). The opened columns denote the data from probenecid treated rats and the shaded columns the data from control rats. Values are the average  $\pm$  S. E. of 5 determinations. \*\* $p < 0.001$  with respect to the control value.

## DISCUSSION :

This study was attempted to measure a small amount of biogenic amines in the adrenal gland, the objective being to determine whether or not adrenal DA and 5-HT could be functional in the sympathoadrenomedullary system, since sensitive and quantitative method of biogenic amines made feasible estimations of minute amounts of adrenal DA and 5-HT (1, 12).

The present study was designed to determine the mode of existence of adrenal DA and 5-HT, as compared with levels in the striatum which is innervated with dopaminergic and serotonergic nerve endings (13). For this purpose, we used probenecid, an inhibitor of acid transport mechanism (14, 15).

In this study we could detect a trace of adrenal DA and 5-HT by the highly sensitive HPLC-ECD method. Previously, CAs were detected by their own pharmacological actions, bioassay procedures. However, this procedure it is not suited to the biochemical approach, and many more physiologically active components are being discovered which

may interfere with these simple biological tests. Thereafter, the fluorescence of oxidation products of EN in alkaline solution was observed by Von Euler (16). With a fluorescence spectrometer of the instrument should be operated at an emission wavelength of 395 nm and an excitation wavelength of 505 nm for NE in the pH 6.5. The second reaction which has been widely used in the fluorometric assay of DA is the condensation with ethylenediamine. DA can be measured fluorometrically by this method, but since the fluorophores of DA and EN have almost the same fluorescence characteristics, complete separation of DA from other catechol compounds is necessary before the assay. These fluorospectrometric methods were cumbersome and the detection limits were relative low.

Recently, an assay method for the determination of NE, EN and DA in biological specimens by application of gas chromatography (5) and of HPLC-ECD has been developed. These studies have showed the advantage of estimation of a small amount of biogenic amines. Especially, the 5-HT and 5-HIAA detection method was more simple and sensitive than other reported methods.

By purification procedures of DOPAC and HVA, we extracted these components simultaneously from the same sample. The adrenal gland and brain regions contain many other components which interfere the peak of DOPAC and HVA. The combined Sephadex G-10 and QAE-Sephadex A-25 column method was appropriate for this purpose.

Probenecid produced a marked elevation of DOPAC and 5-HIAA in the adrenal gland, while the levels of DOPAC in the striatum were little affected. The HVA in the striatum was markedly increased. The HVA in the adrenal gland could not be detected our method and the ratio of DOPAC to DA of the adrenal gland was much smaller than findings in case of striatum.

The reason why probenecid increases HVA but not DOPAC can be explained by probenecid not blocking the transport mechanism or that DOPAC is transported out of the brain by a probenecid insensitive mechanism (15). The acid metabolites produced in the adrenal gland may be extruded by different mechanism in the case of the brain. Van Wijk et al (17) found that probenecid acted not only as an inhibitor of acid transport mechanism in the choroid plexus but also stimulated 5-HT synthesis in serotonergic neurons of the rat brain. Although there is no documented evidence that probenecid stimulates the CAs system, this action of probenecid may contribute to the elevation of adrenal DOPAC.

The existence of adrenal DOPAC and 5-HIAA strongly supports the idea that DA and 5-HT are granulated independently and released to the outside of cells. This is partly supported by findings of other works. (2, 18)

In summary, DA and 5-HT were detected in the adrenal gland. The probenecid increased adrenal DOPAC and 5-HIAA. These biochemical studies indicate that the independent DA and 5-HT system exist in the rat adrenal medulla.



## ACKNOWLEDGEMENTS

This study was supported by Professor M. Ozaki, Assistant Professor M. Niwa, K. Kunisada and Professor M. Tomita. The author thanks them for their valuable advices, technical help and their consideration.

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