Peripheral Origin of Plasma Dopamine

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To clarify the peripheral origin of plasma dopamine (DA), we studied the changes in plasma levels of free and conjugated catecholamines after nephrectomy, adrenalectomy, chemical sympathectomy and renal denervation. Nephrectomy markedly increased conjugated DA levels, indicating that plasma DA is rapidly excreted through the kidney and originates outside the kidney. Adrenalectomy reduced plasma total epinephrine (E) to undetectable limits, whereas total norepinephrine (NE) and DA levels remained unchanged. In addition, the subsequent immobilization stress significantly increased both total NE and DA, but not E. Chemical sympathectomy with 6-hydroxydopamine decreased both NE and DA by 66% and 72%, respectively. E level, however, was not affected by sympathectomy. Although the following immobilization stress significantly increased all catecholamines levels, the magnitude of increase in concentrations of NE and DA were much less than that of E. These results suggest that plasma DA is mainly derived from the peripheral sympathetic nerve terminals. However, the renal nerve, one of the sympathetic nerves, did not serve as a source of plasma DA because renal denervation had no effect on plasma DA levels in spite of the marked depletion of free DA in the kidney.

DOPAMINE (DA) is an important neurotransmitter in the central nervous system. Several dopaminergic neuron systems have been identified in the brain! But in peripheral tissues DA has been thought to be only a precursor for norepinephrine (NE) and epinephrine (E). However, specific dopaminergic receptors have been identified in many peripheral tissues such as kidneys, carotid bodies, peripheral arteries, autonomic ganalia and sympathetic nerve terminals.2-4 In addition, recent studies on conjugated catecholamines suggest that DA is the most abundant catecholamine in plasma5-7 and may have important peripheral functions as a neurotransmitter.

The origin of plasma DA remains unknown. Studies on the sources of plasma DA have been based on the measurement of plasma free DA. However, plasma free DA is usually close to the limit of detection since more than 99% of plasma DA circulates in conjugated form. Therefore, measurement of plasma free DA is not always useful in the study of peripheral dopaminergic mechanism. To evaluate the role of endogenous DA in the periphery, it may be useful to measure plasma conjugated DA. Our method for enzy-

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mamic hydrolysis of conjugated catecholamines permits us to measure plasma free, sulfated, glucuronidated catecholamines.\(^7\)

Fig. 1. Effects of nephrectomy (Nx) on plasma free, sulfated and glucuronidated catecholamines. Mean ± SEM, \(n = 6\). Significant difference from before Nx: \(*p < 0.01\), ns not significant.

Fig. 2. Effects of adrenalectomy (Adx) and the following immobilization stress on plasma free, sulfated and glucuronidated norepinephrine (NE) and dopamine (DA). Mean ± SEM, \(n = 6\). Significant difference from after Adx: \(*p < 0.01\).

The aim of the present study was to identify the peripheral sources of plasma dopamine in the rat.

MATERIALS AND METHODS

Materials: Chemicals were purchased from the following sources: Sulfatase (from Aerobacter aerogenes, Type VI, 4.64 units/mg protein), β-glucuronidase (from E. coli, Type IX, 570,000 units/g solid), norepinephrine bitartrate, epinephrine bitartrate, 3-methoxytyramine (Sigma Chemical Co., St. Louis, MO); [3H]-S-adenosyl-l-methionine (12.8 Ci/mmol), Econofluor (New England Nuclear, Boston, MA); TLC plates silica gel 60 F254 (E. Merk AG, Darmstadt, Germany). All other chemicals were obtained from Nakarai Chemicals LTD, Kyoto, Japan. Catechol-O-methyltransferase (COMT) was partially purified from rat livers according to the method of Nikodejevic et al.8 as modified by Weise and Kopin.9

Animals and preparations: Male Wistar rats, weighing 250-300 grams, were purchased from Japan Animal Co., Osaka, Japan. The rats were divided into 6 groups: 1) nephrectomized group, 2) adrenalectomized group, 3) chemical sympathectomized group, 4) control group for sympathectomized rats, 5) renal denervated group and 6) sham-operated group for renal denervation. Rats were anesthetized with ether and a polyethylene catheter filled with heparin-saline was inserted into the lower aorta through the femoral artery and the other end exteriorized at the back 24 hours before these surgical procedures (nephrectomy and adrenalectomy). First blood sampling was done just before these operations. Bilateral nephrectomy was carried out 24 hours prior to second blood sampling. Bilateral adrenalectomy was performed 48 hours before the second blood sampling. Peripheral sympathectomy was accomplished by the administration of 6-hydroxydopamine (6-OHDA, 100 mg/kg i.v. on Days 1, 7 and 9) in 0.5% ascorbic acid in saline. Control rats were injected only 0.5% ascorbic acid saline. 6-OHDA destroys only peripheral sympathetic neurons, leaving the adrenal gland and brain intact. Blood sampling was carried out on Day 10. To further ascertain the effect of the adrenalectomy and sympathectomy, rats were immobilized supine on a metallic board for 15 minutes because this stress increases all free and conjugated catecholamines.10 Renal denervation was performed by isolating the renal artery and vein, and stripping the nerves and connective tissues from them; finally the vessels were painted with 10% phenol in absolute ethanolalcohol to destroy any remaining nerve fibers.

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Fig. 3. Plasma concentrations of free, sulfated and glucuronidated catecholamines at rest (R) and during immobilization stress (S) in controls (Cont) and sympathectomized (Symp) rats. Mean ± SEM, n = 8. Significant difference from at rest: * p < 0.01, Significant difference from control: **p < 0.01.
sham-operated rats received the same surgical procedure except for the damaging of the renal nerve. Blood sampling was done 4 days after the operation. All the animals were given penicillin postoperatively. Rats received food and water ad libitum. Regular chow did not affect the plasma concentrations of DA because starvation for 24 hours had no effect on DA (data not shown). Adrenalectomized rats were not given saline because a high salt diet increased plasma conjugated DA (Fukuyama M. et al. unpublished data).

Sample collection: An extension was connected to the end of the catheter and a 0.5 ml of blood sample was collected while rats were at rest in home cage. Blood samples were mixed immediately in ice cold tubes with a solution of glutathione (GSH) and ethylenediamine tetraacetic acid (EGTA), pH 5.5, 20 μl per ml and centri-fuged. Plasma was stored at -80°C until assay.

Analysis of catecholamines: Plasma free catecholamines were measured by a modification of the radioenzymatic assay of Peuler and Johnson. Conjugated catecholamines were determined by enzymatic hydrolysis simultaneously with radioenzymatic assay. To measure conjugated catecholamines each sample was assayed for free and total (free + conjugated) catecholamines. Enzymatic hydrolysis for catecholamine sulfates and glucuronides were performed by adding 10 mU sulfotase or 25 U β-glucuronidase directly to the reaction mixture of free catecholamines assay. Conjugated catecholamines were estimated from the difference between total and free values.

Statistical analysis: The results were expressed as mean ± SEM and statistically analyzed by means of paired or unpaired Student’s t-test.

RESULTS

Effects of nephrectomy on plasma catecholamines (Fig. 1): In rats, the main conjugate of NE was sulfate, whereas E and DA were conjugated mainly with glucuronic acid. Nephrectomy significantly increased the concentrations of plasma free, sulfated and glucuronidated DA. Plasma levels of conjugated (sulfated and glucuronidated) NE and E markedly increased following nephrectomy, whereas those of free

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TABLE I EFFECT OF RENAL DENERVATION ON FREE CATECHOLAMINES IN THE KIDNEY

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (S)</td>
<td>142.4 ± 17.5</td>
<td>7.23 ± 0.21</td>
</tr>
<tr>
<td>Denervated (D)</td>
<td>2.1 ± 0.8*</td>
<td>2.69 ± 0.29*</td>
</tr>
<tr>
<td>D/S</td>
<td>1.5%</td>
<td>37.2%</td>
</tr>
</tbody>
</table>

Mean ± SEM ng/g tissue, n = 6
* = p versus sham = p < 0.001
NE and E remained unchanged.  

Effects of adrenalectomy on plasma catecholamines (Fig. 2): Adrenalectomy reduced the concentrations of plasma free and conjugated E to undetectable or close to the detection limit. The level of plasma E in adrenalectomized rat did not change during stress. Adrenalectomy had no significant effect on the concentrations of free and conjugated NE and DA. Immobilization stress significantly increased plasma free and conjugated NE and DA in adrenalectomized rats.

Effects of sympathectomy on plasma catecholamines (Fig. 3): In resting rats, treatment with 6-OHDA significantly reduced the plasma levels of total NE and DA by 66% and 72%, respectively. On the contrary, the difference in plasma total E concentration was not significant between the two groups of rats at rest. Immobilization stress resulted in the increase of all catecholamines in both controls and 6-OHDA treated rats. But the magnitude of increase in concentrations of total NE and DA in sympathectomized rats was much less than that in control rats. On the other hand, the increase of total E in 6-OHDA treated rats was of higher magnitude than that in controls.

Effects of renal denervation on catecholamines in plasma and kidney (Table 1 and Fig. 4): Although renal denervation decreased the concentrations of free NE and DA in rat kidney by 98.5% and 63% respectively, there were no differences in plasma total NE and DA levels between renal denervated rats and sham operated ones.

DISCUSSION.

To identify the endogenous origin of plasma DA, we measured plasma conjugated DA together with free DA. Because the plasma level of free DA is too low to measure, the measurement of conjugated DA may be used as magnified index of the peripheral dopaminergic activity. In this study, plasma free and conjugated catecholamines were determined after nephrectomy, adrenalectomy, sympathectomy and renal denervation, respectively. Plasma conjugated DA markedly increased after nephrectomy, which indicates that conjugated DA is rapidly excreted through the kidney and that the source of plasma DA exists outside the kidney. Nephrectomy markedly increased the plasma levels of conjugated NE and E too, while those of free NE and E remained unchanged. These findings mean that conjugation plays an important role for inactiva-
ference. With regard to plasma conjugated DA, our previous report in rat plasma was similar to their results. Therefore, methodological difference cannot account for this difference. Ueshima et al, however, found that adrenalectomy did not reduce the plasma level of total DA in rabbits, using acid hydrolysis with heating, which is in agreement with our results in rats. Second, they showed that sympathectomy significantly decreased only sulfoxoconjugated DA, but not glucuronidated DA, which is the main conjugate in rats, resulting in no significant changes in plasma total DA. Contrary to their results, sympathectomy decreased all forms of DA in our study. Supposing that dopaminergic neurons are widely distributed in the periphery, this difference may be explained by the differences of the drugs used for chemical sympathectomy. 6-OHDA destroys both noradrenergic and dopaminergic neurons, while guanethidine, which they used, damages only noradrenergic neurons, leaving dopaminergic neurons intact. Taking it the other way round, this discrepancy may indicate the existence of dopaminergic neurons in the peripheral tissues.

At present there is no convincing evidence that dopaminergic neuronal system distributes widely in the periphery. Dopaminergic neurons have been indentified only in autonomic ganglia, the kidney, and the paw pad of dog and postulated to exist in mesenteric artery, stomach, vas deferens and spinal nerve root. High levels of dopamine have been reported in small intensely fluorescent (SIF) cells in sympathetic ganglia, glomus cells of carotid body, kidney and gastrointestinal tract. In addition, specific DA receptors have been identified in many peripheral tissues. Postsynaptic DA-1 receptors, which mediate the relaxation of vessels, exist in kidney, carotid body, peripheral arteries and parts of the gastrointestinal, genito-urinary and endocrine system. Presynaptic DA-2 receptors on sympathetic nerve terminals inhibit the release of NE. These results indicate that DA is an important neurotransmitter not only in the central nervous system but also in peripheral tissues.

In this study, we demonstrated that plasma DA is mainly derived from the sympathetic nerve terminals. Taking account of the widely distributed DA-1 and DA-2 receptors on sympathetic nerve terminals, this study indicates that measurement of plasma conjugated DA is useful as one of the indices of free DA which acts on these receptors in peripheral organs.

REFERENCES


