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EFFECTS OF ADMINISTRATION OF DOPAMINE AND L-DOPA TO DOGS ON THEIR PLASMA LEVEL OF DOPAMINE SULFATE

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Abstract—The effect of intravenous administration of dopamine (DA) or L-3,4-dihydroxyphenylalanine (L-DOPA), its immediate precursor, on the level of DA sulfate in dog plasma was examined, to clarify the source and physiological significance of DA sulfate which is present at high level in the plasma. After DA administration, the plasma level of free DA increased markedly, but the level of DA sulfate did not change. However, after administration of L-DOPA, the levels of both free DA and DA sulfate increased greatly. After a single injection of L-DOPA, increase in the level of free DA was transient, but that of DA sulfate persisted for a long time. These results suggest that some of the DA sulfate in dog plasma is formed from circulating L-DOPA, not from circulating DA, and that formation of DA conjugate may play a role in regulating the plasma level of free DA.

Catecholamines (CA) are known to be inactivated both catabolically by their deamination or *O*-methylation, and by their formation of conjugates [1, 2]. Of the conjugated forms of CA, dopamine (DA) conjugate has recently attracted much attention, because it is present at high levels in the plasma of humans and experimental animals: in humans, monkeys and dogs mostly as a sulfate conjugate and in guinea pigs, rats and rabbits mostly as a glucuronide conjugate [3–10]. Of the levels of free CA (unconjugated CA) in the plasma, that of noradrenaline (NA) is the highest, followed in order by those of adrenaline (Ad) and DA. However, of the levels of free plus conjugated CA, that of DA is the highest. More than 98% of the plasma DA is present in a conjugated form, whereas only 50–70% of the plasma NA and Ad are conjugated [6, 11–14]. The DA conjugate may have some biological effects itself [15–23] and may be converted to free DA through a deconjugation pathway [20].

To clarify the source of this large amount of DA conjugate in the plasma, in this study we examined the effect of intravenous administration of DA or its immediate precursor, L-3, 4-dihydroxyphenylalanine (L-DOPA) to dogs on their plasma level of DA sulfate. Results showed that after DA administration the level of free DA increased markedly, but the level of DA conjugate did not change, whereas after L-DOPA administration the levels of both free DA and DA conjugate increased and that the increased level of plasma DA sulfate persisted for a long time even after the level of free DA had returned to normal.

MATERIALS AND METHODS

Dogs (10–20 kg) was starved overnight and then anesthetized with pentobarbital (30 mg/kg). Polyethylene catheters were then inserted into a cephalic vein for intravenous infusion of DA or L-DOPA and into a femoral vein for blood sampling. In some experiments, L-DOPA was administered to non-anesthetized dogs by intravenous injection.

Blood samples were collected in syringes containing heparin (500 IU/ml) and promptly transferred to cooled tubes on ice. The plasma was separated from the cells by centrifugation at 4°, and deproteinized by addition of 3 N perchloric acid (final concentrations, 0.3 N). The mixtures were stood at 0° for 20 min, and then centrifuged for 2 min in the microcentrifuge at room temperature, and the resulting supernatants were stored at −20° for assay.

Free and conjugated CA were determined by radioenzymatic assay with and without arylsulfatase by the method of Johnson *et al.* [6, 24] with minor modifications. Volumes of 100 μl of each sample were added to incubation medium containing 40 μl of 2 M Tris–HCl buffer (pH 9.6), 20 μl of 100 mM dithiothreitol, 20 μl of 75 mM $MgCl_2$, 20 μl of 50 mM EGTA, 50 μl of catechol-*O*-methyltransferase (COMT) purified from rat liver [25] and 50 μl of ^{3}H-methyl-*S*-adenosylmethionine(^{3}H-SAM) (2.5 μCi), with or without 10 μl (25 m units) of arylsulfatase. As blanks, 100 μl of distilled water was added instead of samples. CA sulfate levels were determined as the difference between the levels of free CA and "total" CA measured in the presence of the sulfatase.

The reaction mixture was incubated for 60 min at 37° in a water bath with shaking. After incubation, 200 μl of borate buffer (1 M, pH 10.5) was added to stop the reaction and metanephrine, normetanephrine and methoxytyramine were added as non-radioactive carriers. Toluene and isoamylalcohol (3:2, 5 ml) were used to extract methoxy CA. The organic phase was extracted with 150 μl of 0.1 N acetic acid with vigorous agitation. Volumes of 100 μl of the extracts were spotted on TLC plates and developed with chloroform–ethanol–ethylamine (70%) (16:3:2) for about 1 hr. The separated

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methoxy CA was located under UV light (286 nm). Appropriate spots were scraped into vials containing 0.5 ml of 0.1 N HCl and scintillator, and radioactivity was counted in an Aloka 703 liquid scintillation counter.

The following compounds and solvents were used: ^{3}H-SAM, specific activity 10.2 Ci/mmol (New England Nuclear Corp.); arylsulfatase (type VI), L-DOPA, DA-HCl, normetanephrine–HCl, metanephrine–HCl, 3-methoxy-tyramine–HCl, and dithiothreitol (Sigma Chemical Co); EGTA, $MgCl_2$, toluene and isoamyl alcohol (Wako Pure Chemical Industry); TLC (Kieselgel 60 F254) (Merk).

RESULTS AND DISCUSSION

Figure 1 shows the effect of intravenous infusion of DA into dogs on the plasma level of DA sulfate. DA was infused successively at rates 5 μg/min for 5 min, 25 μg/min for 5 min and 125 μg/min for 5 min. The plasma level of free DA increased dose-dependently, but that of DA sulfate did not increase significantly even during the last stage of infusion of DA (125 μg/min for 5 min), indicating that DA sulfate in the plasma was not generated from circulating DA that was administered exogenously.

Figure 2 shows the effect of intravenous infusion of L-DOPA, the immediate precursor of DA, on the plasma levels of free DA and DA sulfate. L-DOPA was infused at first 50 μg/min for 15 min, 250 μg/min for 15 min, and then 1250 μg/min for 15 min, and blood samples were collected at 5, 10 and 15 min during each infusion period. The levels of both free

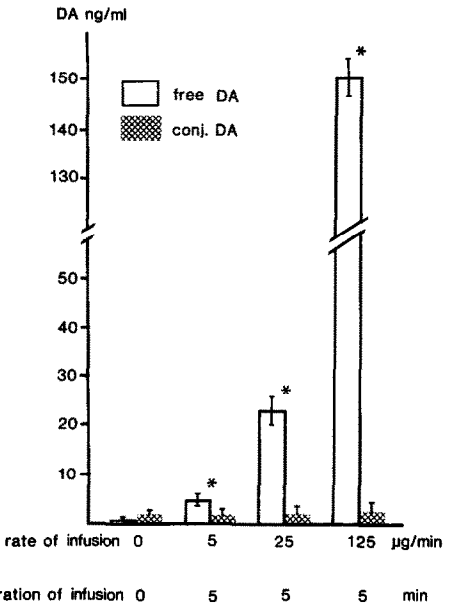

Fig. 1. Effect of DA administration on the plasma levels of free DA and DA sulfate in dogs. DA was infused into anesthetized dog (pentobarbital 30 mg/kg i.v.) through a cephalic vein at a rate of 5 μg/min, 25 μg/min for 5 min and then 125 μg/min for 5 min, and blood samples were collected from a femoral vein at the end of each infusion period. Concentrations of free DA and DA sulfate were measured as described in Materials and Methods. Before DA infusion (at infusion 0) the levels (used as control values) were free DA, 125 ± 20 pg/ml; DA sulfate, 2400 ± 380 pg/ml. Values are means ± SEM for three dogs. *P < 0.005 vs control.

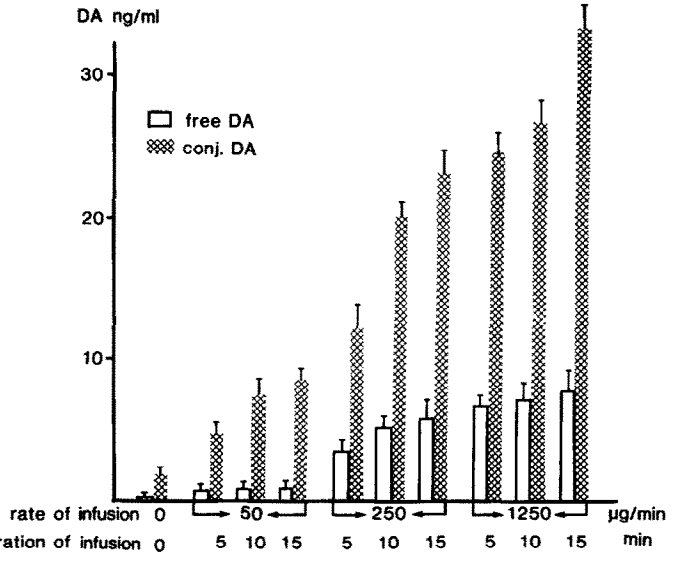

Fig. 2. Effect of L-DOPA administration on the plasma levels of free DA and DA sulfate in dogs. L-DOPA was infused into anesthetized dogs (pentobarbital 30 mg/kg i.v.) through a cephalic vein at a rate of 50 μg/min for 15 min, 250 μg/min for 15 min and then 1250 μg/min for 15 min. Blood samples were collected from a femoral vein at 5, 10, 15 min during each infusion. The levels of free DA after L-DOPA infusion were similar to those after DA infusion (5 μg/min for 5 min or 25 μg/min for 5 min) as shown in Fig. 1. Values are means ± SEM for three dogs. P < 0.005 vs level before L-DOPA infusion.

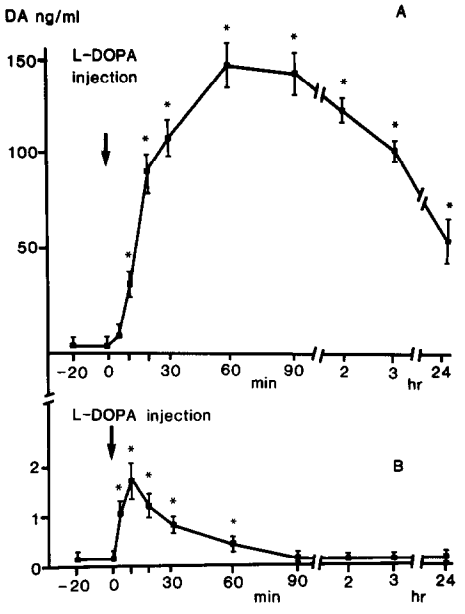

Fig. 3. Time courses of change in the plasma levels of free DA and DA sulfate in dogs after a single i.v. injection of L-DOPA. L-DOPA (2 mg/kg) was injected i.v. into non-anesthetized dogs, and blood samples were collected from a femoral vein at the indicated times after the injection. (A) Change in plasma DA sulfate level. (B) Changes in plasma free DA level. Before L-DOPA injection the level of DA sulfate was 5592 ± 891 pg/ml and that of free DA was 175 ± 21 pg/ml. Values are means ± SEM for three dogs. *$P < 0.005$ vs level before L-DOPA injection.

DA and DA sulfate increased greatly, the increases depending on both the dose of L-DOPA and the duration of infusion. These results suggest that the decarboxylation and sulfoconjugation pathways are very efficient. Under these conditions, the levels of free NA and NA sulfate did not change.

Next, we examined the plasma levels of free DA and DA sulfate at different times after single injection of L-DOPA (2 mg/kg i.v.). As shown in Fig. 3, increase in the free DA level reached a maximum 10 min after L-DOPA injection and decreased to one-third of this maximum level 60 min after the injection. In contrast, the DA sulfate level increased to a maximum 60 to 90 min after L-DOPA injection and remained high for at least 24 hr. It is of interest that increase of free DA was transient, but that of conjugated DA was long lasting.

The effect of L-DOPA on the level of DA sulfate in dog plasma, as a function of time, has not been reported previously, but there are reports that in healthy subjects and patients with Parkinson's disease administration of L-DOPA increases the plasma level of DA conjugate [25–28]. Recently, increase in the plasma level of DA sulfate after L-DOPA administration was also observed in rats, in which DA sulfate is a minor conjugate of DA [29]. These findings and our results suggest that at least some of the DA sulfate present in the plasma originates from circulating L-DOPA. Probably L-DOPA is taken up by the kidney or liver, where DOPA-decarboxylase activity is high, and is converted to DA which is then rapidly conjugated with sulfate and released into the circulation, probably through an acid transport system, although it is possible that L-DOPA itself is first converted to a conjugated form and then decarboxylated to form DA conjugate.

The formation of DA conjugate may have a significant role in regulating the level of free DA when this is synthesized excessively in the tissue, and the DA conjugate released from the tissue into the circulation may be a storage or reserve form of DA. Formation of a DA conjugate is reversible and so free DA could be formed through a deconjugation pathway.

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