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CONTENTS OF DOPAMINE SULFOCONJUGATE ISOMERS AND THEIR DESULFATION IN DOG ARTERIES

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Abstract—In humans and dogs, the plasma contains little free dopamine, and dopamine is almost all present in two isomeric forms of conjugates with sulfate esters, dopamine-3-O-sulfate and dopamine 4-O-sulfate. These two isomers differ in metabolic stability and biological activity. The physiological role of dopamine sulfates is controversial. In the present study on dogs, noradrenaline, dopamine and the two dopamine sulfate isomers in the peripheral arteries were measured by high-performance liquid chromatography, and the possibility that dopamine sulfate isomers are deconjugated in the arteries as a source of free dopamine was examined. The arteries were found to contain free dopamine and dopamine 3-O-sulfate at concentrations of 0.09–0.54 and 0.008–0.015 pmol/mg wet tissue, respectively. No dopamine 4-O-sulfate was found in the arteries or the plasma. Arylsulfatase activity was assayed by incubating a crude extract of arterial homogenate with dopamine 3-O-sulfate or dopamine 4-O-sulfate and measuring the dopamine produced. Exogenous dopamine 4-O-sulfate was desulfated by the extract, but dopamine 3-O-sulfate, which is the predominant isomer in the artery, was not desulfated by the extract. These results suggest that dopamine sulfate is not converted to dopamine in physiological conditions in the dog.

Dopamine exerts vasodilatory activity through its specific peripheral receptors in vascular smooth muscle in the cerebral, coronary, mesenteric and renal arteries [1–3]. Dopaminergic neurons are also thought to exist in peripheral nerves [4]. But in the dog and monkey and man, dopamine is present in the circulation almost entirely in conjugated forms, as sulfate esters [5, 6], and the plasma level of free dopamine is too low to exert any intrinsic activity in physiological conditions.

The physiological role of the high levels of dopamine conjugates is unknown, and the problem of whether they are metabolites of dopamine is controversial; for example, there are reports suggesting that conjugated dopamine itself has physiological activity [7, 8] or is converted to noradrenaline [9] or adrenaline [10] directly or has no activity [11]. Dopamine conjugates are also considered to be related to the pathophysiology of certain diseases such as essential hypertension [12].

There are two isomers of dopamine sulfate, dopamine 3-O-sulfate and dopamine 4-O-sulfate. As the biological and physiological characteristics of these isomers are suggested to be different [8, 13], in studies on the roles of dopamine sulfates, these two isomers must be examined separately.

In this work, we studied the possible contributions of dopamine sulfates to vasodilation by dopamine in Beagle dogs by measuring dopamine and dopamine sulfate isomers in the arteries and investigating the desulfation of each isomer by crude extracts of the arteries.

METHODS

Beagle dogs (body weight 13.7 ± 1.6 kg) were killed by an intravenous injection of an overdose of sodium pentobarbital. The carotid, coronary, pulmonary, mesenteric, renal and femoral arteries and thoracic and abdominal aortae were promptly removed, washed with cold saline and freed of connective tissues. Half of the samples were used for determination of amine contents and the other half for assay of arylsulfatase activity.

Determination of the contents of noradrenaline, dopamine and dopamine sulfate isomers. The arteries were homogenized in 5 volumes of 3% (w/v) perchloric acid containing 0.2% (w/v) disodium EDTA and 1 mM sodium metabisulfate, and the homogenates were centrifuged at 10,000 *g* for 30 min at 4°. Unconjugated dopamine in the deproteinized supernatant was analyzed together with noradrenaline in a fully automated HPLC-fluorometric system (Model HLC-8030 Catecholamine Analyzer, TOSOH, Tokyo) using the post-column diphenylethylenediamine condensation method [14]. Isomers of dopamine sulfate in partially purified supernatant were determined by an HPLC fluorometric method as follows. A sample of 1 ml of supernatant was adjusted to pH 6.0 by addition of several drops of a mixture of 2M KOH and 1M K_2HPO_4. The sample was then centrifuged to remove potassium perchlorate and applied to a disposable ODS column (Baker-10 SPE, No. 7020, J. T. Baker, NJ). Material was eluted with methanol, dried under a stream of nitrogen, dissolved in 100 μl of 0.5 M acetic acid, and injected into an HPLC column (4.6 mm i.d. × 250 mm) packed with an anion exchanger (DEAE-2SW, particle size 5 μm, Tosoh, Tokyo). The two

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Fig. 1

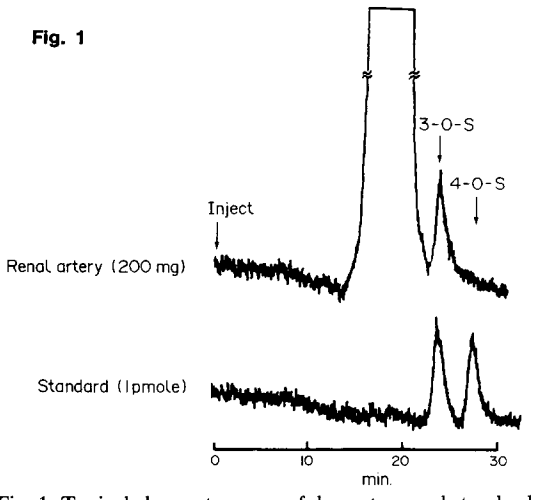

Fig. 1. Typical chromatograms of dog artery and standard. (Top) Elution pattern of deproteinized dog renal artery. The sample was concentrated 10-fold by the procedure for partial purification. The concentration of dopamine 3-O-sulfate was 0.0047 pmol/mg wet tissue. Dopamine 4-O-sulfate was not detectable. (Bottom) Elution pattern of a standard mixture of 1 pmol each of dopamine 3- and 4-O-sulfate. Abbreviations: 3-O-S, dopamine-3-O-sulfate; 4-O-S, dopamine-4-O-sulfate.

isomers of dopamine sulfate were resolved on the column and determined fluorometrically by the *para*-aminobenzoic acid condensation method described previously [15].

Assay of arylsulfatase activity. Arylsulfatase activity in the crude extract of dog arteries was measured as formation of dopamine determined by HPLC. Preparation of the sample and incubation were carried out by the method of O'Fagain *et al.* [16]. Fresh vascular tissues were homogenized in 10 volumes of 10 mM Tris–acetate buffer pH 7.2, containing 2 mM EDTA, and centrifuged at 43,000 *g* for 60 min. The resulting supernatant was dialysed against 10 mM Tris–acetate buffer pH 7.2, and used for assay of arylsulfatase activity. The assay was conducted at 37° in a final volume of 0.5 ml containing 0.33 M sodium acetate buffer pH 5.6, and 40 μM of one of the dopamine sulfate isomers, which were synthesized and purified as described previously [15]. The reaction was started by addition of 50 μl of crude extract and terminated by addition of 25 μl of 60% perchloric acid. Then the dopamine produced was measured by a HPLC (TSK-IEX 510)-PABA method [17].

RESULTS

Concentrations of catecholamines and dopamine sulfate isomers in dog arteries

Figure 1 shows typical chromatograms of dopamine sulfate isomers in the extract of dog arteries and standard solution. The detection limit, assuming a signal-to-noise ratio of two, was 1 pmol/ml (= 0.5 fmol/mg wet tissue).

The arteries were found to contain 3.9–12.7 pmol/mg of noradrenaline and 0.09–0.54 pmol/mg of dopamine (Table 1). The highest concentrations of noradrenaline and dopamine were found in the mesenteric artery, and the lowest in the femoral artery. The noradrenaline and dopamine contents in the femoral artery were significantly lower than those in the pulmonary artery, renal artery and thoracic aorta, but the contents in the mesenteric artery were not significantly different from those in other arteries because of the limited number of data. The dopamine/noradrenaline ratio was highest in the pulmonary artery, and lowest in the femoral artery (range 2.4–6.0%). Low levels (0.008–0.015 pmol/mg) of dopamine 3-O-sulfate were detected in all the arteries examined (Table 1) but these levels were much lower than those of total dopamine (2.4–5.3%). The levels of 3-O-sulfate in different arteries did not differ significantly. The general pattern of distributions of dopamine and dopamine 3-O-sulfate did not differ in different arteries. Dopamine-4-O-sulfate was not detectable in any artery (Fig. 1).

Desulfation of dopamine sulfate isomers by dog artery arylsulfatase

As shown in Fig. 2, a crude extract of dog arteries desulfated dopamine 4-O-sulfate, but not dopamine-3-O-sulfate. The results were obtained with a crude extract of thoracic aorta, but similar results were obtained with extracts of other arteries. With dopamine-4-O-sulfate as substrate, the highest specific activities of desulfation were observed in the pulmonary artery and abdominal aorta, and the lowest in the femoral artery. The differences between the activity in the femoral artery and those in the pulmonary artery and abdominal aorta were significant ($P < 0.05$). There were no significant differences in the specific activities of arylsulfatase in the various other arteries examined.

DISCUSSION

The level of dopamine in the circulation is very low, and several hundred times the plasma concentration is required to affect the blood pressure [18]. But the plasma contains high levels of dopamine sulfates. In the present study, we examined whether arylsulfatase in the arteries deconjugated plasma dopamine sulfate.

First, we compared the contents of dopamine and dopamine sulfate isomers with those of noradrenaline in vascular tissues. In various arteries of dogs, the range of levels of noradrenaline was 3.9–12.7 pmol/mg wet tissue whereas those of dopamine were 0.09–0.54 pmol/mg wet tissue (Table 1). This difference is similar to that found by Bell *et al.* [19] in mesenteric artery, who showed that, in dogs, the range of values is related to the relative proportions of noradrenergic axons [19]. The lowest concentrations of noradrenaline and dopamine were found in the femoral artery. Toda examined the relaxant effects of dopamine in different arteries of dogs, finding that dopamine caused relaxation in the mesenteric, renal, small femoral, coronary and cerebral arteries, but contraction in large femoral arteries [1]. This difference in responses to dopamine might be related to the affinity and number of dopamine receptors, which reflect the contents of dopamine in the arteries. The dopamine/noradrenaline

Table 1. Levels of noradrenaline, dopamine and dopamine sulfate isomers in normal dogs (pmol/mg wet tissues)

Artery	N	Noradrenaline	Dopamine	DA-3-O-sulfate
Carotid	3	6.9 ± 0.6	0.24 ± 0.04	0.010 ± 0.002
Coronary	2	5.6 ± 0.6	0.30 ± 0.06	0.010 ± 0.001
Pulmonary	4	8.7 ± 1.1	0.52 ± 0.09	0.015 ± 0.005
Mesenteric	2	12.7 ± 7.1	0.54 ± 0.35	0.010 ± 0.001
Renal	3	12.2 ± 2.7	0.49 ± 0.11	0.008 ± 0.001
Femoral	4	3.9 ± 0.8	0.09 ± 0.03	0.012 ± 0.002
Thoracic aorta	4	10.4 ± 1.8	0.38 ± 0.05	0.012 ± 0.005
Abdominal aorta	3	6.9 ± 0.3	0.23 ± 0.01	0.013 ± 0.002

Dopamine-4-O-sulfate was not detectable (<0.0005 pmol/mg wet tissue). The noradrenaline and dopamine contents in the femoral artery were significantly lower than those in the pulmonary artery, renal artery and thoracic aorta ($P < 0.01$). The highest concentrations of noradrenaline and dopamine were found in the mesenteric artery, but their levels were not significantly different from those in other arteries. Values are means ± SE. Abbreviations used: DA, dopamine; 3-O-S, dopamine-3-O-sulfate.

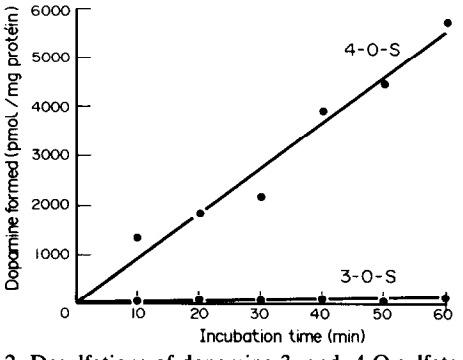

Fig. 2. Desulfations of dopamine-3- and -4-O-sulfate by a crude extract of dog artery. Arylsulfatase activity in the crude extract of dog artery was assayed as formation of dopamine, determined by HPLC. Preparation of the sample and incubation were carried out as described by O'Fagain [16]. Points are means for three determinations. Dopamine 4-O-sulfate was desulfated linearly with time but dopamine 3-O-sulfate was not desulfated. The results were obtained with a crude extract of thoracic aorta, but similar results were obtained with extracts of other arteries.

Abbreviations are as for Fig. 1.

ratio was highest in the pulmonary artery. There is experimental evidence, such as that specific dopamine receptors are located on the postsynaptic muscle membrane of the rabbit pulmonary artery [20], that dopamine may play an important role in regulation of the pulmonary circulation [21, 22], as suggested by our finding of a high content of dopamine in the pulmonary artery as in the renal artery.

The only conjugated dopamine isomer found in dog arteries was 3-O-sulfate, and the ratios of 3-O-sulfate to dopamine were low, compared with those in the plasma. Thus sulfo-conjugation in the vasculature seems to be a minor pathway in dopamine metabolism.

No 4-O-sulfate was found in the dog arteries. Previously, we observed that the ratio of 4-O-sulfate to 3-O-sulfate is different in different species: in humans 3-O-sulfate is the predominant isomer and the level of 4-O-sulfate is about 20% of the total level of dopamine sulfates but little 4-O-sulfate is present in dog plasma [23]. Phenolsulfotransferase (PST, EC 2.8.2.1), the enzyme responsible for the formation of dopamine sulfates, has two isozymes, PST-A and PST-B [24]. Using partially purified preparations from dog liver, Yamamoto found that PST-A produces only 3-O-sulfate whereas PST-B produces mainly 3-O-sulfate with a little 4-O-sulfate which depends on the pH of the reaction [23]. After oral admininstration of L-dopa, the plasma levels of dopamine sulfates increased significantly and in humans not only 3-O-sulfate but also 4-O-sulfate increased [18], but in dogs, L-dopa administration caused increase of 3-O-sulfate but not 4-O-sulfate (unpublished data). This finding suggests that in dogs only 3-O-sulfation is present *in vivo*.

Using a crude extract of dog artery, we found that 4-O-sulfate but not 3-O-sulfate was desulfated to produce free dopamine. We also obtained the same result using dog liver arylsulfatase [23]. Jenner and Rose also reported that 4-O-sulfate was desulfated more readily than the 3-isomer by rat liver arylsulfatase [13]. In a previous study, we infused the two isomers at a rate of 0.1 μmol/kg/min, and found that neither isomer influenced the blood pressure, heart rate or urine output, but we observed that a small portion of the administered dopamine-4-O-sulfate was desulfated to form dopamine [23], so we suspected that 4-O-sulfate was desulfated more readily than the 3-isomer *in vivo*. Arylsulfatase is a rather non-specific glycosulfatase, and can hydrolyse all monosaccharide sulfates [25], but even the most active enzyme, arylsulfatase A, has very low affinity (K_m 360 mM) for the 4-O-sulfate [13]. Thus, further studies are required on the characteristics of this sulfatase.

In the dog, the only form of dopamine sulfate in the circulation and tissues is 3-O-sulfate, which is stable against hydrolysis. The physiological role of dopamine sulfate is controversial. Some reports suggest that dopamine sulfate itself has physiological activity [7, 8] while there are other reports that dopamine sulfate has no biological activity on the renal

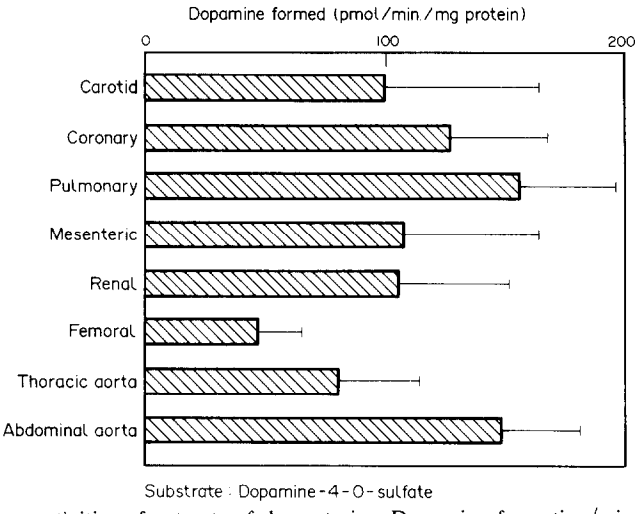

Fig. 3. Arysulfatase activities of extracts of dog arteries. Dopamine formation/min was significantly lower in the extract of the femoral artery than in those of the pulmonary artery and abdominal aorta ($P < 0.05$).

vasculature [26, 27]. The present results suggest that dopamine sulfates are not desulfated in the vasculature and do not contribute to vasodilation in dogs. However, in humans activation of sulfatase in various conditions where sympathetic activation is known to occur [28] may regulate catecholamine metabolism, and dopamine sulfates may have a buffer action.

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