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Deconjugating activity for sulfoconjugated dopamine in homogenates of organs from dogs

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Abstract—To clarify the possibility that sulfoconjugated dopamine (DA) may play a physiological role by being converted to active free DA, we examined the deconjugating activity in homogenates of organs from dogs. Each tissue homogenate was incubated with sulfoconjugated DA, and the deconjugating activity of the organs was compared. The kidney and liver exhibited the highest deconjugating activities. In contrast, the intestine and heart showed lower arylsulfatase activities, and almost no activity was found in the brain or skeletal muscle. Moreover, in the heart, the deconjugating activity for sulfoconjugated DA was higher in the atrium than the ventricle. These results indicate that sulfoconjugated DA is converted to active free DA in homogenates of organs from dogs and that the deconjugating activity varies between different parts of an organ. Sulfoconjugated DA must be looked upon as a possible precursor or reservoir for the production of active free DA.

Circulating catecholamines are known to be inactivated by three major metabolic pathways, i.e. deamination, O-methylation and conjugation [1, 2]. In humans, monkeys and dogs, the major conjugation pathway is sulfoconjugation [3], whereas glucuronide conjugation is predominant in rats and rabbits [1]. A dopamine (DA) conjugate has recently attracted much attention because of its relatively high concentration in the plasma. More than 98% of plasma DA is present in a sulfoconjugated form in humans [4–6]. The DA conjugate may have some biological effects itself [7–10], and it may be converted to free DA through a deconjugation pathway by tissue arylsulfatase [11]. In our previous study using isolated perfused rat hearts, we found that sulfoconjugated DA is converted to active free DA by tissue arylsulfatase [12].

In the present study, to clarify whether sulfoconjugated DA may play some physiological role by being converted to active free DA, we examined the deconjugating activity in homogenates of organs from dogs. Each tissue homogenate was incubated with sulfoconjugated DA, and then the deconjugating activity of the organs was compared.

Materials and Methods

Dogs were killed by intravenous injection of an overdose of sodium pentobarbital. The heart, skeletal muscle, brain, intestine, liver and kidney were promptly removed, washed with cold saline and freed of connective tissue. Each organ was sliced with a hand slicer, and about 1.0 g of each slice was homogenized with 4 mL of Tris–HCl buffer (10 mM, pH 7.4), $MgCl_2$ (2 mM) and EGTA (2 mM) in a Teflon homogenizer. A 100-μL aliquot of each homogenate was added to an incubation medium containing 800 μL of Krebs–Henseleit HCO_3^- buffer solution (pH 7.4) and 100 μL of DA 4-sulfate dissolved in the same buffer (final concentration: 1×10^{-3} M). For the blanks, 100 μL of distilled water was added instead of the homogenate

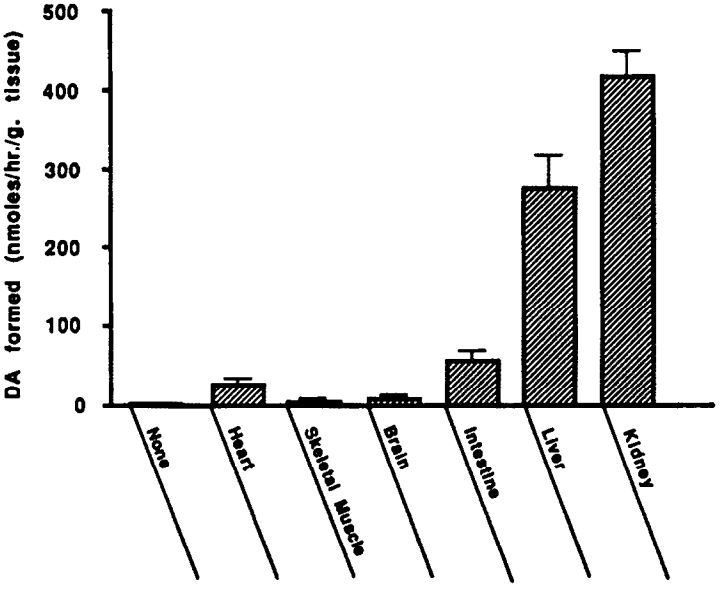

Fig. 1. Deconjugating activities of various organs of the dog. A homogenate of each organ was incubated with sulfoconjugated DA (1×10^{-3} M) in Krebs–Henseleit HCO_3^- buffer solution (pH 7.4) for 1 hr. The results are expressed as the amount of free DA formed (nmol/hr/g wet tissue). The values are the means ± SEM for three to four dogs.

sample. The reaction mixture was incubated for 1 hr at 37°, and the reaction was terminated by addition of 25 μL of 60% perchloric acid. Then the reaction mixture was centrifuged at 8000 *g* for 20 min, and the resultant supernatant was applied to an HPLC column after being passed through a microfilter. The deconjugating activity in the crude extract of each dog organ was measured as the formation of DA, determined by HPLC. The blank value of DA was below 100 pmol/g tissue, which may be considered to be the limit of sensitivity of the present method. Linearity of the results was obtained in the range from 100 pmol/g tissue to 1 μmol/g tissue. When separate aliquots of homogenate were analysed on three repetitive occasions, the intra-assay variability of DA was within 10%. The chromatographic conditions for measurement were as follows: Yanapac ODS-A (particle size: 5 μm) 4.6 mm × 250 mm; solvent, 0.05 M NaH_2PO_4 (pH 3.1); flow rate, 1.0 mL/min; temperature, ambient; detection, amperometric at +0.800 V vs Ag/AgCl. The results were expressed as the amount of DA formed.

Chemicals. Dopamine 4-sulfate (4-sulfoconjugated DA) was synthesized chemically from DA-HCl and concentrated H_2SO_4 according to the method of Jenner and Rose [13]. The purity and identity of DA 4-sulfate were verified by HPLC using an authentic standard of DA 4-sulfate analysed by ^1H NMR spectroscopy. Contamination of the sulfate ester by free DA did not exceed 100 ppm. Tris (hydroxymethyl) aminomethane and EGTA were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). DA-HCl, concentrated H_2SO_4 and $MgCl_2$ were purchased from the Wako Pure Chemical Co. (Tokyo, Japan). All other chemicals used were of commercial reagent grade.

Results and Discussion

Figure 1 shows the deconjugating activity for DA-sulfate in the various dog organ homogenates. The kidney and liver showed the highest deconjugating activities for DA-sulfate: 415 ± 33 and 275 ± 41 nmol of DA formed/hr/g wet tissue, respectively. In contrast, the intestine and heart showed lower arylsulfatase activities (56 ± 13 and 27 ± 8, respectively), and almost no activity was found in the brain or skeletal muscle. These results suggest that the deconjugating activity for DA-sulfate differs among the various organ homogenates.

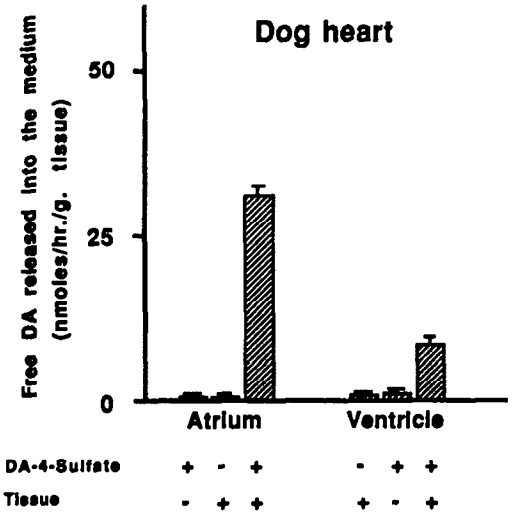

Fig. 2. Comparison of deconjugating activity between the ventricle and atrium of the dog heart. Homogenates of the ventricle and atrium were incubated with sulfoconjugated DA (1×10^{-3} M) in Krebs–Henseleit HCO_3^- buffer solution (pH 7.4) for 1 hr. The results are expressed as the amount of free DA formed (nmol/hr/g wet tissue). The values are the means ± SEM for three to four dogs.

In our previous study using isolated perfused rat hearts, we found that sulfoconjugated DA was converted to active free DA [12]. In addition, it has been reported that administered DA-sulfate showed a positive inotropic effect on the perfused rat heart by conversion to free DA [14]. Therefore, we further analysed the deconjugating activity of the dog heart, which was divided into the atrium and ventricle. As shown in Fig. 2, the atrium showed higher deconjugating activity for DA-sulfate than the ventricle. These results suggest that the deconjugating activity differs not only between organs but even within the same organ. Furthermore, in the brain, deconjugating activity was higher in the brain stem than in the cerebral cortex, and in the kidney, it was higher in the medulla than in the cortex (data not shown).

Based on the above findings, it became apparent that sulfoconjugated DA is converted to active free DA in many organs, and the deconjugating activity varies between different parts of an organ. DA conjugates have long been believed to be only metabolites of free DA. However, it has been proposed recently that DA conjugates may have some biological effects [7–10] because they may be converted to free DA through a deconjugation pathway involving tissue arylsulfatase [11]. In an *in vivo* study, we reported that sulfoconjugated DA was converted to free DA during marathon running [15]. Joyce *et al.* [16] also reported that the plasma levels of free noradrenaline and adrenaline increased during bicycle exercise, while the levels of both sulfoconjugated noradrenaline and adrenaline decreased. These findings indicate the possibility that sulfoconjugated DA in the plasma might serve as a reservoir from which active free DA is converted.

DA production in the central nervous system is well established, while the origin of plasma free DA has not yet been elucidated. Moreover, although DA receptors in the peripheral nerves have been investigated [17], dopaminergic innervation of the peripheral organs has hardly been studied. Therefore, it may be that sulfoconjugated DA, which exists in large amounts in the plasma, serves as a source in plasma of free DA which might work as a peripheral neurotransmitter. With regard to the target organs of sulfoconjugated DA, it is conceivable that the organs might have DA receptors and deconjugating activity for the sulfoconjugated DA. The organs examined in the present study may be among the targets for circulating sulfoconjugated DA.

In conclusion, we have demonstrated the possibility that sulfoconjugated DA is converted to active free DA in homogenates of organs from dogs and that the deconjugating activity varies between different parts of an organ. Sulfoconjugated DA, which has been regarded as a mere metabolite of free DA, now must be looked upon as a possible precursor or reservoir for the production of active free DA in the plasma.

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