Determination of Urinary 5-S-Cysteinyldopamine by High-Performance Liquid Chromatography with Electrochemical Detection

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Key Words
Column liquid chromatography
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Summary
5-S-Cysteinyldopamine, a new metabolite of dopamine, was determined in urine by high-performance liquid chromatography with electrochemical detection. The catechol was detected in 14 of 21 melanoma patients and 7 of 21 normal subjects; the highest values were 657 μg/day for melanoma patients and 44 μg/day for normal subjects. These results suggest that the cysteine conjugate may arise from autoxidation of dopamine but tyrosinase may also participate in the oxidation.

Introduction
We have recently demonstrated that on incubation of rat tissue homogenates with dopamine, the new metabolites 5-S- and 2-S-cysteinyldopamine are formed in a ratio of 7:1 [1]. 5-S-Glutathionyldopamine was also detected in the incubation mixture. It thus appears that the cysteine conjugates are formed by a reaction of dopamine o-quinone with glutathione followed by enzymic hydrolysis. The oxidation of dopamine to dopamine o-quinone may be brought about by a variety of biological oxidation systems such as tyrosinase-O₂, peroxidase-H₂O₂, superoxide radical, hydroxyl radical, and iron-chelates (cf. ref. [1]).

Urinary 5-S-cysteinyldopamine has been widely used as a biochemical marker of melanoma metastasis [2]. It is also known that the level of urinary dopamine may be elevated in certain melanoma patients [3]. Therefore, we have determined the levels of 5-S-cysteinyldopamine in the urine of melanoma patients and healthy subjects.

Experimental
24-h urine samples were collected in bottles containing 50 ml of acetic acid and 1 g of Na₂S₂O₅. The urine samples from melanoma patients were obtained from Dr. K. Jimbow (Sapporo Medical College, Sapporo, Japan). In a microcentrifuge tube (1.5-ml volume) were placed 50 mg of acid-washed alumina and 1 g of 2 % Na₂S₂O₅. The tube were added 100 μl of a urine sample or 10 μl of a standard solution containing 1 μg each of 5-S- and 2-S-cysteinyldopamine and dopamine in 1 ml of 0.1 M HCl and 10 μl of 1 μg/ml 3,4-dihydroxybenzylamine (DHBA) in 0.1 M HCl as an internal standard. To this was added 0.8 ml of 1.5 M Tris-HCl buffer containing 2 % Na₂EDTA, pH 8.6, and the mixture was immediately shaken for 5 min. The alumina was washed twice with water and catechols were then eluted with 150 μl of 0.4 M HClO₄.

The catechol extracts, usually 50 μl, were analyzed by HPLC with electrochemical detection as follows [1]. A Yanaco Model L-5000 liquid chromatograph (Yanagimoto, Kyoto, Japan) was used with a Yanaco Model VMD-101A electrochemical detector. The detector was set at +600 mV vs. an Ag/AgCl reference electrode and sensitivity was 20 nA f.s.

Results
We analyzed 21 urine samples each from melanoma patients and normal subjects. Among them, two melanoma samples contained very high levels of a compound appearing at the position of 5-S-cysteinyldopamine (Fig. 1). The identity was confirmed by (a) the coincidence of retention time
with the standard under HPLC conditions for 5-S-cysteinyldopa determination [4], (b) the close parallelism of voltammograms between the sample and standard in comparison with those of dopamine (Fig. 2), and (c) the identical k' values between the sample and standard in response to a temperature change (35–60 °C; data not shown).

In the chromatograms of the samples containing high levels of 5-S-cysteinyldopamine, the 2-S isomer was also detected at much lower levels, the ratios of 5-S isomer to 2-S isomer being 5.3 : 1 and 8.7 : 1 in the samples in Fig. 1.

Acid hydrolysis of the above two samples in 0.1 M HCl for 20 min did not increase the 5-S-cysteinyldopamine values, indicating that the catechol is present mostly in the free form.

Table I shows the urinary excretion of 5-S-cysteinyldopamine in melanoma patients and normal subjects, in comparison with those of 5-S-cysteinyldopa and dopamine. 5-S-cysteinyldopamine was detected in 14 of 21 melanoma patients but only 7 of 21 healthy subjects. Four values for melanoma patients exceeded the highest value for normal subjects. There were no clear correlations among 5-S-cysteinyldopamine, 5-S-cysteinyldopa, and dopamine levels both in melanoma patients and normal subjects except for one exception: in normal subjects 5-S-cysteinyldopamine level (Y) was correlated with 5-S-cysteinyldopa level (X): Y = 0.144X − 15.6, r = 0.756, p < 0.01.

**Discussion**

The present study demonstrates for the first time the occurrence of 5-S-cysteinyldopamine in the urine from normal subjects as well as from melanoma patients. The occurrence of the new dopamine metabolite in human brain was recently reported; the 5-S-cysteinyldopamine level was about 5 % of the dopamine level [5]. More recently, the same group reported the occurrence of 5-S-cysteinyl derivatives of dopamine, dopa, and 3,4-dihydroxyphenylacetic acid in the brains of eight mammalian species [6]. They have suggested that the 5-S-cysteinyldopamine metabolites are formed by autoxidation of catechols to o-quinones and subsequent coupling to glutathione and the conjugates thus formed are split to the cysteine conjugates by the action of peptidases.

The present finding of 5-S-cysteinyldopamine in urine further supports the presence of such an oxidative process *in vivo*. The detection rate and the level were much higher in melanoma patients than in normal subjects. This seems to suggest that tyrosinase may also participate in the oxidation of dopamine in melanoma patients. The fact that 5-S-cysteinyldopamine was not detected in one-third of melanoma patients and two-thirds of normal subjects may be ascribed to the presence of sufficient amounts of o-quinone scavenger(s) such as ascorbic acid in the tissues [7].

Fig. 1
Chromatograms of urine samples from melanoma patients. (A) subject 2 in Table I. (B) subject 1 in Table I. Peaks: 1 = norepinephrine (5.6 min), 2 = 2-S-cysteinyldopamine (6.7 min), 3 = DHBA (8.8 min), 4 = 5-S-Cysteinyldopamine (11.0 min), 5 = dopamine (12.8 min).

Fig. 2
Voltammograms of 5-S-cysteinyldopamine and dopamine in sample (Subject 1 in Table I) and standard. □, 5-S-cysteinyldopamine in sample; ○, 5-S-cysteinyldopamine in standard; △, dopamine in sample; ▲, dopamine in standard. 50 μl of the urine sample after alumina extraction or 10 μl of a standard solution containing 1 μg each of the catechols per ml was injected into the HPLC.