Plasma Levels of Free and Sulfoconjugated Catecholamines in Patients with Atherosclerosis

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We have studied the relationship between free and sulfoconjugated catecholamines (CAs) in the plasma of patients with various cardiovascular diseases and have described the physiological significance of sulfoconjugated CAs in plasma. In the present study, we measured free and sulfoconjugated dopamine (DA) and noradrenaline (NA) in the plasma of patients with atherosclerosis (AS). Results showed that the plasma levels of free DA and NA in patients with atherosclerosis were higher than those in control subjects. Moreover, it was also observed that the plasma levels of conjugated DA and NA in patients had a tendency to be higher than the levels in control subjects. These results suggest the involvement of free CAs in atherosclerosis and that elevated free CAs may be converted to sulfoconjugated forms in patients with atherosclerosis.

Key words sulfoconjugation; dopamine; noradrenaline; arteriosclerosis

It is known that catecholamines (CAs) are inactivated both catabolically, by their deamination or O-methylation, and by conjugation.[1,2] Dopamine (DA) is the main CA undergoing conjugation because there is a high concentration of sulfoconjugated dopamine in human plasma.[3,4] These sulfoconjugated CAs have attracted much attention because of the possibility of their conversion to active free CAs, especially DA.[5] Previously, we reported that sulfoconjugated CAs are converted to active free CAs by tissue arylsulfatase,[6] suggesting that sulfoconjugation might act as a reservoir of potential free CAs.

It is known that CAs promote lipid metabolism and are also involved in atherogenesis[7] although the relationship between atherosclerosis (AS) and the role of plasma CAs is still unclear. Previously, we have reported that the plasma levels of free and sulfoconjugated CAs are elevated in patients with heart disease[8] or hypertension.[9] In the present study, to investigate the relationship between atherosclerosis and plasma CAs, we measured the plasma levels of free and sulfoconjugated DA and noradrenaline (NA) in patients with arteriosclerotic diseases.

MATERIALS AND METHODS

Subjects The study group consisted of 28 patients, 22 men and 6 women, with a mean age of 60.6±13.6 years (Table 1). The patients were divided into two groups, with or without atherosclerosis, as follows: 16 patients with atherosclerosis obliterans or aortic aneurysm or old myocardial infarction, the AS group, and 12 patients without atherosclerosis as cotrols, the NC group. All subjects gave informed consent to participate in the study and the study protocol was approved by the ethics committee of the University of Tokushima. Blood (5 ml) was collected during angiographic examination and plasma total cholesterol (T-cho) and triglycerides (TG) were measured using a Hitachi 7350 (Hitachi Ltd., Tokyo, Japan) instrument. Samples were placed in a cooled test-tube containing 40 mg EDTA·2Na and 8 mg $Na_2S_2O_5$ as stabilizers and immediately centrifuged at 3000 rpm for 5 min at 4 °C. The separated plasma samples were stored at −70 °C until analysis.

Measurements of Free and Sulfoconjugated DA and NA in Plasma DA, NA and their sulfoconjugated forms were extracted from plasma as described below, and their concentrations were measured by HPLC as described previously.[10] For the measurement of free DA and NA in plasma, 100 ng deoxyepinephrine hydrochloride were added to 1 ml plasma as an internal standard (IS). Three milliliters 0.4 N perchrolic acid was also added to the plasma, which was then vigorously shaken. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4 °C. The resultant supernatant was then subjected to alumina adsorption. One hundred milligrams activated alumina and 3 ml 2 M Tris–HCl buffer (pH 8.6) were added to the supernatant and mixed for 10 min using a rotary mixer. The mixture was allowed to settle and the supernatant was removed by aspiration. The residual alumina was washed twice with 1.5 ml methanol and twice with 1.5 ml deionized water. CAs absorbed on the alumina were eluted with 200 μl 2 N acetic acid. The eluate was stored at −20 °C until the HPLC determination. For the measurement of total (free and conjugated) DA and NA, 500 ng deoxyepinephrine hydrochloride as IS and 1 ml 0.4 N perchloric acid were added to 0.5 ml plasma and the mixture was vigorously shaken. After centrifugation at 3000 rpm for 5 min at 4 °C, 0.24 ml concentrated HCl was added to the supernatant, and the mixture was then heated for 30 min at 100 °C. After being cooled, 0.5 mg EGTA·2Na and 0.5 mg $Na_2S_2O_5$ were added to the reaction mixture which was treated with 192 ml 28% NH_4OH to make it mildly acidic. Then, the solutions were prepared for HPLC analyses by the alumina adsorption method as described above. The conjugated form was determined from the difference between the concentration of free DA and NA, and total (free and conjugated) DA and NA. For HPLC measurement, a Shimadzu LC-10AD high performance liquid chromatograph (Shimadzu Co., Ltd., Kyoto, Japan) was used with an coulochemical detector, model Coulochem II (ESA Inc., Bedford, MA, U.S.A.). The analytical column employed was a MCM column C18 DF-5-120A (MC Medical, Inc., Tokyo, Japan, 150×4.6 mm i.d.). The chromatographic conditions were as follows: solvent, 0.1 M

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NaH_2PO_4 (pH 3.0) containing EGTA·2Na (0.1 mM)–MeOH (95 : 5) which contained sodium 1-hexane-sulfonate (3 mM); flow rate, 1.0 ml/min; temperature, ambient; detection, coulometric at +0.300 V *versus* Ag/AgCl. We were unable to separate adrenaline from impurities completely, when we subjected plasma extracts to HPLC. So we did not report data on adrenaline in this study.

Chemicals Dopamine hydrochloride and noradrenaline hydrogen tartrate were obtained from Nakalai Tesque Co. (Kyoto, Japan). Deoxyepinephrine hydrochloride was purchased from Serva Feinbiochemica Co. (Heidelberg, Germany). Activated alumina for DA and NA absorption was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were commercial products of reagent grade.

Statistical Analysis Values are expressed as the mean±S.E. in the experiments throughout the study. A comparison of values was performed by the analysis of variance with post-hoc tests. A value of $p<0.01$ versus the control basal value was considered to be statistically significant.

RESULTS

In the AS group, the concentrations of T-cho and TG were 214.5±6.6 and 192.7±19.9 mg/dl, respectively (Table1). The concentration of free DA in plasma of patients with AS was 80.3±7.6 pg/ml which was higher than that of the NC group at 41.5±8 pg/ml. The sulfoconjugated DA level in plasma of patients with AS was 3780.6±610.8 pg/ml which was not statistically different from that of the NC group at 3370.6±495.9 pg/ml (Fig. 1).

On the other hand, the free NA level in plasma of patients with AS was 125.0±18.3 pg/ml which was higher than that in the NC group (without atherosclerosis) at 96.3±24.7 pg/ml. The concentration of sulfoconjugated NA level in plasma of patients with AS was 1976.7±696.6 pg/ml which was generally higher than that in the NC group at 1645.3±368.3 pg/ml. However, there was no statistically significant difference in these values among the groups (Fig. 2).

DISCUSSION

In the present study, we have found that the free CAs in plasma increased significantly and the conjugated CAs in plasma tended to increase in patients with atherosclerosis (Figs. 1, 2). Since it has been reported that CAs promote the metabolism of lipids,[7] increased free CAs in plasma may facilitate the metabolism of TG to free fatty acids (FFA). On the other hand, it has also been reported that there is a reverse pathway from FFA to TG, by which increased levels of FFA are converted to TG, resulting in atherogenesis.[7] In patients with atherosclerosis, increased free CAs may be converted to their conjugated forms which may prevent atherogenesis by free CAs. Since it has also been reported that the plasma levels of free and sulfoconjugated CAs increase in patients with hypertension,[9,11] the results of the present study may also apply in such cases. Hauss *et al.* proposed the hypothesis that CAs, adrenaline (A) and/or NA, could act as chemical mediators in the pathogenesis of arteriosclerosis. They have shown that culutured endothelial and smooth muscle cells exposed to A or NA exhibit increased proliferation.

Table 1. Patient Characteristics

Patient	Sex	Age	Diagnosis	T-cho (mg/dl)	TG (mg/dl)
1	M	63	AP/OMI	203	180
2	M	62	AAA/BA	239	220
3	M	71	AAA	201	201
4	M	63	ASO/RA/DM	192	163
5	M	63	ASO/CI/DM	196	151
6	M	62	ASO/HT/DM	178	255
7	M	61	Lt. CIA	230	119
8	M	63	AP/OMI	203	180
9	M	69	ASO	220	300
10	M	25	TAA	240	375
11	M	72	ASO	214	171
12	M	63	ASO/DM/HT	214	192
13	M	67	ASO	191	80
14	M	69	AAA/HT	229	115
15	M	55	OMI/AP/DM	193	190
16	F	59	ASO	277	249

T-cho, total cholesterol; TG, triglycerides; AP, angina pectoris; OMI, old myocardial infarction; AAA, abdominal aortic aneurysm; BA, bronchial asthma; ASO, arteriosclerosis obliterans; RA, rheumatoid arthritis; DM, diabetes mellitus; CI, cerebral infarction; HT, hypertension; Lt. CIA, left common iliac aneurysm; TAA, thoracic aortic aneurysm.

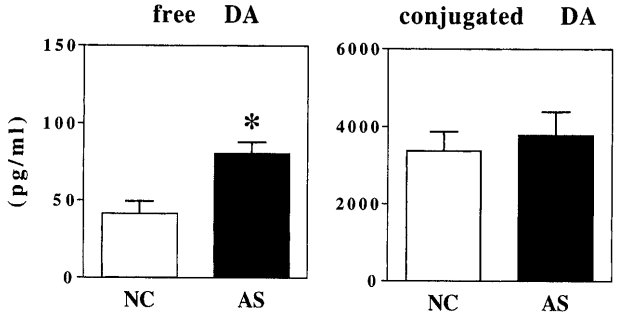

Fig. 1. Plasma Levels of Free and Sulfoconjugated Dopamine (DA) in AS and NC

Values are means±S.E.M. *: $p<0.01$ *versus* the value for the NC group.

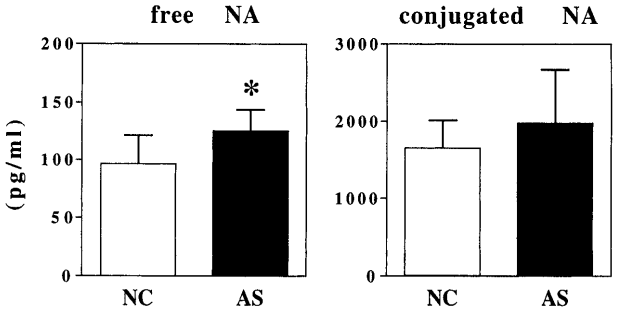

Fig. 2. Plasma Levels of Free and Sulfoconjugated Noradrenaline (NA) in AS and NC

Values are means±S.E.M. *: $p<0.01$ *versus* the value for the NC group.

Moreover, plasma concentrations of A and NA also correlated with certain stages of arteriosclerosis in patients[12] and in this study, we found that plasma free CAs increased in patients with arteriosclerosis. Hence, CAs may play an important role in the pathogenesis of arteriosclerosis.

However, as far as the changes in DA and NA are considered, it has been reported that DA administration to patients results in an increase in FFA,[13] whereas NA infusion increase plasma TG.[7] From these findings, it is conceivable

that the plasma DA and NA levels may affect the reversible conversion of FFA and TG. From our present findings, the increased plasma free CAs may affect the balance between TG and FFA in the body. We need further studies to clarify the role of each CA in the pathogenesis of arteriosclerosis.

In conclusion, CAs may promote lipid metabolism, while also causing atherogenesis. Although the physiological role of free and conjugated CAs in atherosclerosis is still unclear, our present study demonstrated that free DA and NA increased in the plasma of patients with atherosclerosis. These findings suggest that free plasma CAs are involved in atherosclerotic diseases.

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