ACETALDEHYDE-MEDIATED ALCOHOL SENSITIVITY AND ELEVATION OF PLASMA CATECHOLAMINE IN MAN

Junko ADACHI and Yasuhiko MIZOI
Department of Legal Medicine, Kobe University School of Medicine,
Kusunoki-cho 7-chome, Chuo-ku, Kobe 650, Japan
Accepted January 20, 1983

Abstract—According to the presence and absence of aldehyde dehydrogenase (ALDH) I isozyme which had low $K_m$ for acetaldehyde, subjects were divided into two groups: the former, the usual ALDH group and the latter, the unusual ALDH one. Blood alcohol and acetaldehyde levels, plasma norepinephrine and epinephrine levels, and urinary excretion of norepinephrine, epinephrine, dopamine, vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were determined; and the differences in these values and cardiovascular symptoms after alcohol intake between the two groups were investigated. Fifty-six healthy male volunteers were studied after they ingested 0.4 g of alcohol per kg of body weight. There was no difference in blood alcohol level between the two groups. In the unusual ALDH group, facial flushing, increase of pulse rate and decrease in diastolic blood pressure associated with accumulation of blood acetaldehyde were shown. In addition, rises in plasma catecholamine and urinary excretion of catecholamine were also observed. However, in the usual ALDH group, in which blood acetaldehyde level scarcely increased, these changes were not significant. The alteration of catecholamine metabolism, decrease in urinary VMA and increase in urinary MHPG was recognized in both groups.

As biogenic amines have been considered to cause emotional and behavioral changes or mental disorders after acute and chronic alcohol administrations, the relationship between alcohol administration and changes of catecholamine metabolism and distribution has been studied (1). On the other hand, in Japanese, individual differences in the cardiovascular responses which may be sympathomimetic effects such as facial flushing and increase in pulse rate after alcohol intake has been demonstrated (2).

Ethanol is oxidized to acetaldehyde by alcohol dehydrogenase and then metabolized to acetate by aldehyde dehydrogenase (ALDH) in human liver. Acetaldehyde has been reported not only to have more pronounced effects on the cardiovascular system than alcohol, but also has been reported to release catecholamines from tissues (3). From these observations, it would follow that the cardiovascular symptoms may represent catecholamine-mediated effects of acetaldehyde.

Liver ALDH in man was classified into two groups on the basis of the presence or the absence of the low $K_m$ isozyme for acetaldehyde. The subjects that were deficient in low $K_m$ ALDH showed very high levels of blood acetaldehyde (4).

Biogenic amines are oxidized to their aldehydes by monoamine oxidase and then in man, mainly to their carboxylic acids by ALDH. Because after alcohol intake both aldehydes from alcohol and biogenic amines need ALDH for each metabolism, the metabolic pathway for catecholamine might be altered.
The relationship between alcohol sensitivity and change of catecholamine metabolism has not been clarified yet since levels of blood acetaldehyde, plasma catecholamine and urinary metabolites of catecholamine were not determined in the same experiment using various subjects.

In the present experiments, normal subjects divided into two groups by the phenotypes of ALDH were studied after they ingested alcohol. Concentrations of blood alcohol and acetaldehyde, levels of plasma noradrenaline and epinephrine, and urinary excretion of norepinephrine, epinephrine, dopamine, vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in order to clarify the interrelation between these values and cardiovascular symptoms in the two groups. The difference in the catecholamine metabolic pathways of both these groups was also investigated.

Materials and Methods

Experimental procedures: Fifty-six healthy Japanese male volunteers, aged 20–32 yr, were used in the study. These subjects were explained the outline of the research projects, and they freely consented to the tests. Each subject was kept without breakfast on the day of the experiment and was given “sake” (Japanese rice wine with 16 v/v% alcoholic content), 0.4 g of alcohol per kg of body weight, to ingest with a side dish of 30 g okaki (rice cracker) between 10:00 and 10:10 a.m., followed by rest on a sofa. The room temperature was maintained at 20–22°C.

Determination of ALDH phenotypes: The phenotypes of ALDH in 36 out of 56 subjects were determined using 30 hair roots by isoelectric focusing in the range of pH 3.5–10.0 according to the method of Harada et al. (5). The subjects were divided into two groups based on the ALDH phenotypes: one was the usual ALDH group in which subjects possessed the ALDH I isozyme that had a low Km for acetaldehyde, and the other was the unusual group in which subjects were deficient in ALDH I isozyme.

Determination of blood alcohol and acetaldehyde: Blood samples were collected from the median cubital vein of each subject at 30 min and 1, 2, 3 and 4 hr after the drinking. Blood alcohol and acetaldehyde levels were measured using a Perkin-Elmer F45 Head Space Analyzer according to the previous report (4).

Determination of plasma norepinephrine and epinephrine: Blood samples were collected before the experiment and at 45 min and 3.5 hr after alcohol ingestion. Blood was drawn into heparinized tubes placed on ice, and this was centrifuged promptly. Three ml plasma was deproteinized by 0.8 M perchloric acid, and the supernatant was used. The extraction procedure was a modified version of the method of Keller et al. (6) and was as follows: 1) Addition of an internal standard, 3 ng of 3,4-dihydroxybenzyl amine (Aldrich) to the supernatant. 2) Addition of an antioxidant, freshly prepared sodium metabisulfite. 3) Addition of 1 M Tris buffer, pH 8.3 (Sigma). 4) Adjustment to pH 8.4–8.6 by sodium hydroxide. 5) Addition of 20 mg acid-washed alumina and vigorous shaking for 10 min. 6) The alumina was washed three times with water. After the last washing, the supernatant was removed, and the alumina was dried. 7) Desorption of catecholamine with 100 ml of 0.1 N hydrochloric acid. 8) 20 ml of the supernatant were injected into the column packed with octadecylsilane. The eluant was a phosphate buffer (pH 3.1) which was prepared from 890 ml of 0.1 M KH₂PO₄, 110 ml of methanol, 20 mg of EDTA-2Na and 300 mg of I-heptane sulfonic sodium. Levels of norepinephrine, epinephrine and dopamine were determined using a high performance liquid chromatograph.
(Yanaco L-2000L) equipped with an electrochemical detector (VMD-101).

**Determination of urinary norepinephrine, epinephrine and dopamine:** Each subject was requested to void immediately before the drinking. Urine was collected at 1, 2, 3 and 4 hr after the start of the drinking and assayed for norepinephrine, epinephrine and dopamine. On the day preceding the experiment with alcohol, each subject was requested to drink the same amount of water as "sake" for the control experiment. The urine samples were adjusted to pH 6.5 with phosphate buffer, poured onto the extraction column which was filled with the cation-exchange resin (Amberlite-CG-50 Type I), desorbed with 4 ml of 1 N hydrochloric acid and then treated by the same method as used for the analysis of catecholamine in the plasma sample.

**Determination of urinary VMA and MHPG:** Urine assays for VMA and MHPG were carried out with samples in 20 subjects for whom the phenotypes of ALDH could not be determined. Urine samples were collected at 30 min and 1, 2, 3, 4 and 5 hr after the start of alcohol ingestion or of water drinking in the control experiment. Urinary VMA and MHPG were assayed by the procedures reported by Pisano et al. (7) and Bigelow et al. (8), respectively.

**Statistical analysis:** The Student's t-test was used for a statistical comparison between mean values.

**Results**

1. **Phenotypes of ALDH:** The phenotypes of ALDH were evaluated in 36 out of 56 subjects: 18 out of 36 subjects had the usual ALDH and the other 18 subjects had the unusual ALDH. All of the subjects with unusual ALDH showed facial flushing from approximately 15 min after the drinking with restoration to normal color by 2–3 hr, while none of the subjects with usual ALDH showed facial flushing. Ten out of the remaining 20 subjects presented facial flushing, and the other 10 did not.

2. **Blood alcohol and acetaldehyde levels:** The mean values of blood alcohol and acetaldehyde are given in Tables 1 and 2, respectively. In both ALDH groups, the alcohol level reached a maximum 30 min after the start of the drinking, and the alcohol

| ALDH phenotype | 30 min (mM) | 1 hr (mM) | 2 hr (mM) | 3 hr (mM) | 4 hr (mM) |
|----------------|-------------|-----------|-----------|-----------|-----------|
| Usual (n=28)  | 10.02 ± 2.46| 9.56 ± 1.25| 6.46 ± 1.12| 2.79 ± 1.36| 0.51 ± 0.70 |
| Unusual (n=28)| 10.41 ± 1.73| 9.20 ± 1.55| 6.52 ± 1.22| 3.46 ± 1.16| 0.88 ± 0.83 |

±S.D.

| ALDH phenotype | Before (pM) | 30 min (pM) | 1 hr (pM) | 2 hr (pM) | 3 hr (pM) | 4 hr (pM) |
|----------------|-------------|-------------|-----------|-----------|-----------|-----------|
| Usual (n=18)  | 0.40 ± 0.18 | 2.07 ± 2.28 | 1.17 ± 0.38| 1.05 ± 0.46| 0.98 ± 0.47| 0.74 ± 0.36 |
| Unusual (n=18)| 0.41 ± 0.19 | 29.85 ± 24.08| 30.95 ± 30.10| 15.63 ± 12.96| 13.17 ± 12.74| 5.62 ± 7.48 |

±S.D.
gradually disappeared from the blood by 4 hr. There was no difference between the two groups in the blood alcohol levels at each time measured.

The mean blood acetaldehyde level of the usual ALDH group scarcely increased and remained about 2 \( \mu M \) at the maximum, while that of the unusual group was markedly increased and reached maxima, approximately 30 \( \mu M \), at 30 and 60 min after the drinking.

3. Plasma catecholamine levels: Table 3 shows the mean values of plasma norepinephrine and epinephrine. In the control experiment, there were modest variations among the subjects, but no difference between the mean values in both groups before the experiment, and water ingestion was found to have no effect. On the other hand, both the levels of plasma norepinephrine and epinephrine in the unusual ALDH group roughly doubled at 45 min after the alcohol intake and remained at a level 1.5 times as high as the control at 3.5 hr. However, in the usual ALDH group, neither the norepinephrine nor the epinephrine level showed remarkable increase after the alcohol ingestion.

4. Urinary excretion of catecholamine:

| Table 3. Plasma norepinephrine (NE) and epinephrine (E) levels (pg/ml) in the control and alcohol experiments |
|---------------------------------------------------------------|
|                  | Usual ALDH (n=18) | Unusual ALDH (n=18) |
|                  | Before | 45 min | 3.5 hr | Before | 45 min | 3.5 hr |
| NE Control       | 424±125 | 466±132 | 477±77 | 481±121 | 441±83 | 493±138 |
| Alcohol          | 382±108 | 432±97  | 443±111| 403±78  | 801±206| 716±214 |
| P value          | N.S.   | N.S.    | N.S.   | N.S.    | <0.005 | <0.005 |
| E Control        | 80±48  | 74±26   | 87±60  | 113±61  | 96±49  | 103±65  |
| Alcohol          | 66±15  | 70±41   | 72±27  | 125±66  | 220±124| 189±132 |
| P value          | N.S.   | N.S.    | N.S.   | N.S.    | <0.005 | <0.05  |

Urinary excretion of norepinephrine, epinephrine and dopamine in the usual and unusual ALDH groups in the control and alcohol experiments are shown in Table 4.

At 2 hr after the drinking, urinary excretion of norepinephrine in the unusual ALDH group was twice as much as the control value and that of epinephrine showed a value over twice the control. In the usual group, urinary excretion of neither norepinephrine nor epinephrine showed any significant difference between the control and alcohol experiments. In both groups, modest variation was shown on urinary excretion of dopamine in the control and alcohol experiments; and the urinary dopamine slightly increased after alcohol ingestion, but the elevation did not reach statistical significance.

5. Pulse rate and blood pressure: Changes of pulse rate and blood pressure in the usual and unusual ALDH groups are shown in Fig. 1. No difference was found in the control values of both pulse rate and blood pressure between the two groups.

After alcohol intake, pulse rate and blood pressure in the usual ALDH group did not increase. However, the mean pulse rate in the unusual ALDH group was elevated by 33.3% at 30 min, by 36.1% at 1 hr and still by
25% at 4 hr. The mean diastolic blood pressure decreased significantly in the unusual ALDH group, falling by 30.6% at 30 min, by 37.5% at 1 hr and still by 14.1% at 3 hr, while it was restored at 4 hr when facial flushing disappeared. The systolic blood pressure did not decline in any group.

6. Urinary excretion of VMA and MHPG:
Table 5 depicts the time-course of changes in urinary VMA and MHPG excretion. Both groups hardly displayed any changes, with no significant difference in the excretion between the two groups in the control experiment. After alcohol ingestion, nevertheless, the excretion of VMA in urine diminished conspicuously and reached the lowest level at 2 hr, followed by a gradual

Fig. 1. Changes of pulse rate and blood pressure in the control (○) and alcohol (●) experiments between the usual and unusual ALDH groups. Vertical bars represent the standard deviation. *P<0.005 and **P<0.025 compared with each control value.
restoration to the initial level by about 5 hr, in both groups. The urinary excretion of MHPG nearly doubled after ingestion of alcohol in the flushing group, while the increase of urinary MHPG in the nonflushing group remained approximately 40%; neither group showed any conspicuous time-course of change.

Discussion

The effect of alcohol on peripheral catecholamine was evaluated only by urinary excretion of catecholamine. After administration of alcohol to healthy subjects, increase of urinary norepinephrine was reported by Anton (9), and Perman (10) and Davis et al. (11) reported no change in it. Elevation of urinary epinephrine was found by Perman (10), while Anton reported no change (9). Ijiri (12) and Kijima (13) found that there were significant elevations of urinary norepinephrine and epinephrine in “flushers” and no change of them in “nonflushers” after intake of alcohol. The different results obtained in these experiments for healthy men about the influence of alcohol upon urinary catecholamine might be attributed to the difference in the quantity of alcohol ingested, the sensitivity of subjects to alcohol or the timing of sample collection.

In recent years, the development of high performance liquid chromatography with electrochemical detection using an internal standard has made it possible to determine catecholamine in 2–3 ml plasma accurately and specifically (14). With this reliable method, plasma catecholamine in man after alcohol intake was measured in the present study for the first time. We clarified that plasma catecholamine and urinary excretion of catecholamine were markedly elevated in the unusual ALDH subjects and not in the usual ALDH subjects. The fact shows that the alcohol sensitivity of each subject plays an important role in the elevation of plasma catecholamine as well as urinary catecholamine.

Since the disulfiram-alcohol reaction has been found, a great interest has been taken in the involvement of acetaldehyde, an intermediate metabolite of alcohol, on the cardiovascular symptoms after alcohol ingestion. That is, symptoms which result from ingestion of alcohol during disulfiram therapy, including facial flushing, palpitation and tachycardia have seemed to be at-
tributable to the action of acetaldehyde rather than alcohol (15). It is easy to consider that the change of catecholamine level in man is also involved in these autonomic symptoms. Akabane et al. (3) found in their animal experiments that acetaldehyde had stronger sympathomimetic action than alcohol and facilitated the release of catecholamine from the adrenal gland; therefore, they suggested that acetaldehyde induced the sympathetic signs by releasing catecholamine from sympathetic nerve endings.

In the present study, we found in the subjects with unusual ALDH the correlation between elevation of blood acetaldehyde level and cardiovascular symptoms, but there seems to be a difference between the symptoms observed in the unusual ALDH subjects and the disulfiram-alcohol reaction. In the disulfiram-alcohol reaction, overt facial flushing and significant decrease in systolic blood pressure occurred, and pulse rate increased conspicuously in the initial stage of the reaction (16). These symptoms are considered to be based on not only the elevation of blood acetaldehyde by the decrease of ALDH activity, but also the reduction of catecholamine synthesis by the inhibition of dopamine-β-hydroxylase (15) as the results of the action of disulfiram. In the present study, increase in pulse rate and decline in diastolic blood pressure were recognized in the subjects which were deficient in low Km ALDH even when blood acetaldehyde reduced to normal. Furthermore, in the previous paper (17), we found the dilated pattern in pulse wave and the rises in blood flow rate and arterial pressure in the common carotid arteries in the unusual ALDH subjects. In addition, the period of cardiovascular changes agreed with that of the elevation of plasma catecholamine. These phenomena may be presumed to be for the following reason: The elevation of plasma catecholamine level is a consequence of a regulating mechanism which acts against a decrease of blood pressure owing to the dilatation of peripheral blood vessels by the action of metabolic acetaldehyde. The increase of plasma catecholamine should result the elevation of heart rate, the rise of blood flow rate in carotid arteries and the avoidance of decrease in systolic blood pressure accompanied with the increase of cardiac output.

Nextly, Ogata et al. (18) reported that urinary dopamine was significantly elevated in alcoholics given a large quantity of alcohol. The present study showed that there was no change in urinary dopamine in both flushers and nonflushers. If a larger amount of alcohol had been given, peripheral dopamine would have been affected.

It has been demonstrated by a few investigators that acute administration of alcohol results in an alteration in the peripheral metabolism of catecholamine. Davis et al. (11, 19) reported that 3 times administration of 60 ml of alcohol to 2 healthy men caused a decrease of urinary excretion of VMA and an increase of MHPG. Giltow et al. (20) found an elevation of urinary MHPG and a decrease of VMA after administration of alcohol for 7 days to a normal man.

We found in the experiment using many healthy subjects that the alteration of catecholamine metabolism occurred even by the administration of a small amount of alcohol. That is, VMA decreased significantly in both flushers and nonflushers, but amount of urinary VMA was larger and elevation of MHPG was greater in the flushers than in the nonflushers. Elevation of the amount of catecholamine and its metabolites in urine of the flushers after alcohol intake may result from the rise in plasma catecholamine level.

It has been debated whether the shift in biogenic amine metabolism from an oxidative to a reductive pathway may be attributed to an increase in NADH/NAD ratio during
oxidation of alcohol or the result of competitive inhibition of aldehyde dehydrogenase by acetaldehyde, a metabolite of alcohol. We considered from the present results that the alteration was due to the increase in NADH/NAD ratio by alcohol oxidation because in both flushers and nonflushers, urinary VMA diminished conspicuously associated with elevation of blood alcohol level and followed to the initial level by a restoration when the blood alcohol declined to 0. Since alcohol oxidation may cause the increase of NADH, and this makes aldehyde reductase act with ease, urinary MHPG might be elevated after alcohol intake.

Acknowledgments: This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture in 1980. The authors wish to thank Prof. C. Tanaka and Dr. H. Fujiwara, Department of Pharmacology, Kobe University School of Medicine, for technical advice for the analysis of plasma catecholamine.

References
1) Hunt, W.A. and Majchrowicz, E.: Alterations in neurotransmitter function after acute and chronic treatment with ethanol. In Biochemistry and Pharmacology of Ethanol, Edited by Majchrowicz, E. and Noble, E.P., Vol. 2, p. 167-185, Plenum Press, New York and London (1979)
2) Mizoi, Y., Ijiri, I., Tatsuno, Y., Kijima, T., Fujiwara, S., Adachi, J., and Hishida, S.: Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. Pharmacol. Biochem. Behav. 10, 303-311 (1979)
3) Akabane, J., Nakanishi, S., Kohei, H., Asakawa, S., Matsumura, R., Ogata, H. and Miyazawa, T.: Studies on sympathomimetic action of acetaldehyde. II. Secretory responses of the adrenal medulla to acetaldehyde: Experiments with the perfused cat adrenals. Japan. J. Pharmacol. 15, 217-222 (1965)
4) Okada, T. and Mizoi, Y.: Studies on the problem of blood acetaldehyde determination in man and its level after alcohol intake. Japan. J. Alcohol and Drug Dependence 17, 141-159 (1982)
5) Harada, S., Agarwal, D.P. and Goedde, H.W.: Application of gel isoelectric focusing and electrophoresis in the study of alcohol dehydrogenase and acetaldehyde dehydrogenase iso-enzymes of human tissues and fibroblasts. In Electrophoresis. Edited by Catsimpooulas, N., p. 275-282, Elsevier North Holland, N.Y. (1978)
6) Keller, R., Oke, A., Mefford, I. and Adams, R.N.: Liquid chromatographic analysis of catecholamines routine assay for regional brain mapping. Life Sci. 19, 995-1004 (1976)
7) Pisano, J.J., Crout, J.R. and Abraham, D.: Determination of 3-methoxy-4-hydroxy-mandelic acid in urine. Clin. Chim. Acta 7, 285-291 (1962)
8) Bigelow, L.B., Neal, S. and Weil-Malherbe, H.: A spectrophotometric method for the estimation of 3-methoxy-4-hydroxyphenylglycol in urine. J. Lab. Clin. Med. 7, 677-683 (1965)
9) Anton, A.H.: Ethanol and urinary catecholamines in man. Clin. Pharmacol. Ther. 6, 462-469 (1965)
10) Perman, E.S.: Observations on the effect of ethanol on the urinary excretion of histamine, 5-hydroxyindoleacetic acid, catecholamines and 17-hydroxycorticosteroids in man. Acta Physiol. Scand. 51, 62-67 (1961)
11) Davis, V.E., Brown, H., Huff, J.A. and Cashaw, J.L.: Ethanol-induced alterations of norepinephrine metabolism in man. J. Lab. Clin. Med. 69, 787-799 (1967)
12) Ijiri, I.: Studies on the relationship between the concentrations of blood acetaldehyde and urinary catecholamine and the symptoms after drinking alcohol. Japan. J. Stud. Alcohol 9, 35-59 (1974) (Abs. in English)
13) Kijima, T.: Alcohol sensitivity and urinary catecholamines. Japan. J. Stud. Alcohol 14, 101-117 (1979) (Abs. in English)
14) Hjemdahl, P., Daleskog, M. and Kahan, T.: Determination of plasma catecholamines by high performance liquid chromatography with electrochemical detection: Comparison with a radioenzymatic method. Life Sci. 25, 131-138 (1979)
15) Faiman, M.D.: Biochemical pharmacology of disulfiram. In Biochemistry and Pharmacology of Ethanol, Edited by Majchrowicz, E. and Noble, E.P., Vol. 2, p. 325-348, Plenum Press, New York and London (1979)
16) Maruyama, J.: Studies on acetaldehyde formation from ethanol during the procedure of determination of blood acetaldehyde. Japan. J. Stud. Alcohol 15, 19-36 (1980) (Abs. in English)
17) Mizoi, Y., Tatsuno, Y., Adachi, J., Kogame, M., Fukunaga, T., Fujiwara, S., Hishida, S. and Ijiri, I.: Alcohol sensitivity related to polymorphism of alcohol-metabolizing enzymes in Japanese. Pharmacol. Biochem. Behav. 18, Supp. 1 (1983) (in press)

18) Ogata, M., Mendelson, J.H., Mello, N.K. and Majchrowicz, E.: Adrenal function and alcoholism. Psychosom. Med. 33, 159–180 (1971)

19) Davis, V.E., Cashaw, J.L., Huff, J.A., Brown, H. and Nicholas, N.L.: Alteration of endogenous catecholamine metabolism by ethanol ingestion. Proc. Soc. Exp. Biol. Med. 125, 1140–1143 (1967)

20) Gitlow, S.E., Dziedzic, L.M., Dziedzic, S.W. and Wong, B.L.: Influence of ethanol on human catecholamine metabolism. Ann. N.Y. Acad. Sci. 273, 263–279 (1976)