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Possible Involvement of Kinins in Cardiovascular Changes After Alcohol Intake

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HATAKE, K., T. TANIGUCHI, H. OUCHI, N. SAKAKI, S. HISHIDA AND I. IJIRI. *Possible involvement of kinins in cardiovascular changes after alcohol intake.* PHARMACOL BIOCHEM BEHAV 35(2) 437–442, 1990.—Japanese healthy male subjects were divided into two groups, i.e., a normal aldehyde dehydrogenase (ALDH) group with a low Km isozyme of ALDH for acetaldehyde, and a deficient group without it. After intake of 0.4 g/kg alcohol, the deficient group showed high levels of blood acetaldehyde, facial flushing including an increased pulse rate and a fall in diastolic blood pressure, while the normal group did not manifest these changes. In the deficient group, the total kininogen concentration gradually decreased after alcohol intake due to a reduction in low molecular weight kininogen, and plasma prekallikrein remained unchanged. The normal group showed no significant changes in any of these values after alcohol intake. In an in vitro study with pooled plasma, the low concentrations of urinary kallikrein caused a decrease in the low molecular weight kininogen only. These results suggest that kinins released by acetaldehyde-induced activation of glandular kallikreins are associated with the changes in cardiovascular symptoms in deficient group which display flushing after alcohol intake.

Alcohol sensitivity   Kinins   Blood acetaldehyde   Cardiovascular changes   Facial flushing

MONGOLOID people frequently display facial flushing, palpitation, tachycardia and blood pressure depression during alcohol intake (6,32). It has been suggested that higher blood acetaldehyde (AcH) levels after alcohol intake, which are observed in individuals deficient in the low-Km isozyme of liver aldehyde dehydrogenase (ALDH), play a major role in producing the cardiovascular symptoms (13, 14, 17). Also, AcH is known to evoke catecholamine release from adrenal glands in experimental animals (2, 25, 30, 39). Elevated plasma catecholamines and urinary excretion of catecholamines are observed in individuals showing facial flushing associated with higher blood AcH levels (1, 18, 19). Therefore, cardiovascular responses occurring during alcohol intake may be considered to result from the sympathomimetic action of AcH. Though dilatation of peripheral blood vessels and diastolic blood pressure depression due to the accumulation of AcH have been observed after the intake of alcohol (24), disulfiram-alcohol reaction (15), cyanamide-ethanol reaction (29) and nitrefazole-ethanol interaction (34) by human subjects, intravenous infusion of AcH in animals has been reported by many investigators (8, 22, 26) to induce a pressor response. Altura *et al.* (3) have also reported AcH-produced constrictions of both arterioles and muscular venules in the rat mesenteric vasculature. These differences between humans and animals raise the question of whether the cardiovascular symptoms after drinking result only from the effects of AcH-induced catecholamine release and AcH itself. The possible involvement of vasoactive factors such as kinins, histamine and prostaglandins in the symptoms following alcohol intake should be considered.

Kinins (bradykinin and kallidine) are a group of polypeptides released from plasma kininogen by kininogenases in plasma or exocrine glands. Kinins are potent vasodilators. Intravenous injection of bradykinin in humans produces marked flushing over the face, neck and upper chest (10,20). A similar effect following intra-arterial infusion has also been described by Burch and De Pasquale (4). Moreover, when injected intravenously, bradykinin induces a decrease in blood pressure due to arterial vasodilatation and a resulting decrease in peripheral resistance (7, 9, 33). Its effect on blood vessels is always accompanied by an increase in heart rate. We focused our attention on kinins as a vasoactive factor which may induce symptoms similar to those produced after alcohol intake. In general, there are two kallikrein-kinin systems: one in plasma involving plasma kallikrein (PK) and one in exocrine glands involving glandular kallikreins (GKs). Both PK and GKs are proteolytic enzymes which specifically split circulating plasma kinin precursors, i.e., high molecular weight (HMW) and low molecular weight (LMW) kininogens. The very short half-life (17 sec) (37) and lability of free kinins in the circulation has led most investigators to use the plasma kininogen assay as a tool for determining the degree of activation of the kallikrein-kinin system. A fall in plasma kininogen levels has been widely interpreted to indicate the formation of plasma kinins. Plasma kininogen had been considered to be total kininogen, but a method

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for assaying HMW and LMW kininogens separately was developed by Uchida *et al.* (35,36) making it possible to determine whether circulating kinins are formed by either PK or GKs by measuring plasma prekallikrein (PreK) which is a proenzyme of PK, HMW and LMW kininogens. In the present study which measured PreK and plasma kininogen levels, we examined the relationship between the kallikrein-kinin system and the cardiovascular symptoms following the intake of a small amount of alcohol.

METHOD

Experimental Procedures

Twenty-two healthy Japanese male students, aged 18–25 years, volunteered to participate in this investigation. The subjects who had the ALDH I isozyme with a low Km for AcH were assigned to the normal group, while the subjects deficient in ALDH I isozyme were placed in the deficient group. Thirteen and 9 subjects belonged to the deficient and the normal groups, respectively. For estimation of the ALDH phenotypes of the subjects, about 20–30 hair roots were used for isoelectric focusing in the range of pH 3.5–10.0 according to the method of Harada *et al.* (16). The two groups classified by ALDH phenotypes were used in the following alcohol experiments.

The 22 subjects did not eat breakfast on the day of the experiment, and each was given sake (Japanese rice wine with 16% v/v alcohol content), 0.4 g of alcohol per kg of body weight, with a simple relish (30 g of rice crackers) between 10:30 and 10:40 a.m. and then made to rest on a sofa. They were allowed to eat a light lunch following blood sampling 2 hours after alcohol intake.

Determination of Blood Alcohol and AcH

Blood samples were drawn from the antecubital vein of each subject prior to alcohol intake and at 30, 60, 120, 180 and 240 minutes afterward. Alcohol and AcH levels were measured by head-space gas chromatography. The blood alcohol concentration was measured using a Shimadzu gas chromatograph 4BM according to the method of Mizoi *et al.* (23). The blood AcH level was determined using a Perkin Elmer F45 gas chromatograph by the PCA (perchloric acid) method (28). Widmark's factor r, elimination rate and disappearance rate ($\beta 60$) were calculated from the blood alcohol concentration curve for each subject according to Widmark's method as described by Wallgren and Barry (38).

Skin Temperature

The forehead skin temperature was determined by a thermister-type digital thermometer with a sensitivity of 0.1°C.

Blood Pressure and Pulse Rate

Blood pressure was measured with a sphygmomanometer in the brachial artery and the pulse rate was counted at the time of each blood sampling.

Determination of PreK and Kininogens

Blood samples were collected from the antecubital vein of each subject with a disposable plastic syringe containing 1/10 volume of 3.8% sodium citrate. The samples were transferred immediately to plastic tubes and centrifuged at $1000 \times g$ at 25°C for 15 minutes. The plasma was stored at −70°C until use. All contact with glass or "negatively" charged surfaces was carefully avoided during the entire procedure.

Kininogens were assayed as HMW and LMW kininogens, as well as total kininogen by the method of Uchida and Katori (35,36). Bradykinin converted from plasma kininogen using trypsin was compared with standard synthetic bradykinin (Peptide Institute Inc., Osaka) on an isolated estrous rat uterus smooth muscle preparation. The plasma kininogen levels are expressed as ng bradykinin equivalents per mg protein. The protein content in plasma was estimated by the method of Lowry *et al.* (21). Assay of PreK was carried out by the method of Oh-ishi and Katori (27). Briefly, the PK activity generated by activation with acetone and kaolin was measured using a peptidyl-fluorogenic substrate, Z-Phe-Arg-MCA (Peptide Institute Inc., Osaka). The PreK levels are expressed in terms of arbitrary units. One unit is defined as the amount of enzyme which releases 1×10^{-7} M 7-amino-4-methylcoumarin (AMC)/10 minutes.

Experiment 1. A preliminary study for PreK and total kininogen concentrations was performed a week before Experiment 2 using the 22 subjects. Blood samples were taken before alcohol intake and at 30, 60, 120, 180 and 240 minutes thereafter. As shown in Table 3, both PreK and total kininogen concentrations showed time-dependent reductions with maximum reductions 4 hours after alcohol intake.

Experiment 2. Based on the results of Experiment 1, we carried out a further study using the 22 subjects. Blood samples for the determination of PreK, total, HMW and LMW kininogen concentrations were taken before the alcohol intake and 4 hours thereafter.

Experiment 3. An in vitro experiment was also conducted with blood from 5 of the 13 subjects in the deficient group. The plasma was obtained as described above. The plasma samples were pooled and 950 µl of the mixed plasma was incubated with urinary kallikrein (Green Cross Corp., Osaka, Japan) in concentrations between 0.05×10^{-3} and 2.0×10^{-3} PNA units in a volume of 50 µl for 45 minutes at 37°C, and then the total HMW and LMW kininogen concentrations were assayed.

Statistical Analysis

The statistical significance of differences was determined by paired and unpaired *t*-test. Differences were considered significant if $p<0.05$. The results are expressed as means ± SD.

RESULTS

Facial Flushing

All of the 13 subjects in the deficient group exhibited facial flushing from approximately 15 minutes after alcohol intake and it was most apparent after 0.5–1 hour, with the subjects returning to normal color in 2–4 hours. Facial flushing varied from marked to slight among the subjects. In contrast, none of nine subjects in the normal group exhibited facial flushing. In the deficient group, the forehead skin temperature rose by an average of 1.5 ± 0.4°C at 30 minutes after drinking. The temperature was scarcely found to rise in the normal group.

Blood Alcohol and AcH Concentrations

Table 1 shows the mean blood alcohol and AcH levels in the two groups. The mean alcohol level reached peak values of 11.0 and 10.4 mM in the deficient and normal groups, respectively, 30 minutes after alcohol intake. There were no significant differences in alcohol levels at any of the measurement points between the two groups. The mean AcH level reached peak values of 30.4 and 2.5

TABLE 1

BLOOD ALCOHOL AND ACETALDEHYDE LEVELS, PULSE RATE AND BLOOD PRESSURE IN DEFICIENT AND NORMAL GROUPS BEFORE AND AFTER ALCOHOL INTAKE

		Before	30	60	120	180	240 (min)
Blood Alcohol (mM)							
Normal Group		0	10.4 ± 2.1	9.7 ± 1.7	6.2 ± 1.9	3.0 ± 1.7	0.5 ± 0.5
Deficient Group		0	11.0 ± 2.0	9.2 ± 1.1	5.9 ± 1.5	2.7 ± 1.5	0.7 ± 0.8
Blood Acetaldehyde (μM)							
Normal Group		0.4 ± 0.2	2.5 ± 1.0	2.1 ± 1.2	1.8 ± 1.2	0.6 ± 0.4	0.3 ± 0.2
Deficient Group		0.4 ± 0.1	30.4 ± 20.3†§	28.7 ± 16.5†§	15.3 ± 10.3†§	10.7 ± 8.3†§	3.3 ± 3.0
Pulse Rate (beats/min)							
Normal Group		73.7 ± 9.2	81.3 ± 8.2	80.1 ± 9.9	78.4 ± 10.0	81.8 ± 9.7	80.3 ± 9.4
Deficient Group		70.4 ± 8.4	91.1 ± 13.8*	95.3 ± 12.3†‡	90.1 ± 10.7†‡	90.1 ± 12.5*	85.3 ± 15.4
Blood Pressure (B.P., mmHg)							
Normal Group	Systolic B.P.	114.8 ± 6.7	112.7 ± 9.3	108.7 ± 7.2	107.2 ± 8.5	108.3 ± 8.7	109.0 ± 8.1
	Diastolic B.P.	73.7 ± 5.1	70.5 ± 7.1	63.3 ± 11.3	66.3 ± 10.0	61.7 ± 8.2	61.3 ± 4.7
Deficient Group	Systolic B.P.	121.8 ± 11.4	119.8 ± 12.0	114.7 ± 11.9	110.1 ± 10.0	113.8 ± 12.9	116.2 ± 9.5
	Diastolic B.P.	76.6 ± 6.4	54.2 ± 17.1*‡	49.5 ± 16.4†‡	57.2 ± 12.1†	57.9 ± 9.3†	65.9 ± 7.5

Normal Group (n = 9), Deficient Group (n = 13).
Values are mean ± SD.
*$p<0.05$ and †$p<0.01$ compared to the preintake values.
‡$p<0.05$ and §$p<0.01$ compared to the corresponding normal group.

μM in the deficient and normal groups, respectively, 30 minutes after alcohol intake. While the highest levels in all the subjects in the normal group were less than 4 μM, the values ranged from 16 to 97 μM in the deficient group. The degree of facial flushing in the deficient group seemed to depend on the degree of the elevated AcH levels. AcH concentration was significantly higher in the deficient group than in the normal group.

Table 2 shows the mean values of ages, body weights, the r value and β60, and rates of alcohol elimination in both groups. As can be seen, there were no significant differences in any of these values between the two groups.

Cardiovascular Changes

The pulse rate in the normal group showed no change, while the rate in the deficient group was significantly increased at 30–180 minutes after alcohol intake. No change of systolic blood pressure was seen in either group, while the diastolic blood pressure markedly decreased only in the deficient group until 180 minutes after alcohol intake. An elevation of the pulse rate (at 60 and 120 minutes, $p<0.05$) and a decrease of the diastolic blood pressure (at 30 and 60 minutes, $p<0.05$) in the deficient group after alcohol intake were also observed when compared to the normal group.

TABLE 2

AGE, BODY WEIGHT, β_{60}, r AND RATE OF ALCOHOL ELIMINATION

	Deficient (n = 13)	Normal (n = 9)
Age (year)	22.3 ± 3.0	22.4 ± 1.9
Body weight (kg)	62.8 ± 8.6	65.0 ± 8.1
β60 (mg/ml/hr)	0.15 ± 0.03	0.16 ± 0.03
r	0.68 ± 0.05	0.66 ± 0.12
Elimination (mg/kg/hr)	104.1 ± 15.1	105.9 ± 13.7

Values are mean ± SD.

Plasma Kininogen and PreK Concentrations

The results of the preliminary study (Experiment 1) for the involvement of kallikrein-kinin system in the two groups are presented in Table 3. The total kininogen concentration in the deficient group started to decrease from 1 hour after alcohol intake in a time-dependent fashion and the minimum levels were reached after 4 hours. However, the PreK concentration showed no significant changes after alcohol intake. The normal group showed no significant changes in these two values. There were no significant differences in PreK concentrations at any of the measurement points between the two groups. However, the total kininogen level was significantly decreased at each of the measurement points between 60–240 minutes in the deficient group compared with the normal group. Based on the results of this preliminary study, we also performed a differential assay of the total kininogen concentration. In Experiment 2, the mean values of PreK, total, HMW and LMW kininogens did not significantly differ before and 4 hours after alcohol intake in the normal group (Table 4). In the deficient group, no significant changes were found in the PreK and HMW kininogen concentrations, but there was a decrease in the total and LMW kininogen concentrations at 4 hours after alcohol intake. The deficient group showed significantly less total and LMW kininogen concentrations at 4 hours after alcohol intake when compared with the normal group. However, there were no significant differences in the PreK or HMW kininogen levels 4 hours after alcohol intake between the two groups (Table 4). Table 5 (Experiment 3) shows the effects of various concentrations of urinary kallikrein on plasma kininogens obtained from five subjects in the deficient group. Total and LMW kininogen levels started to decrease upon incubation with a low concentration of 0.05×10^{-3} PNA unit urinary kallikrein, while HMW kininogen levels decreased on incubation with concentrations higher than 1×10^{-3} PNA unit. Also, the results performed

TABLE 3

TIME CHANGES IN PLASMA KININOGEN AND PREKALLIKREIN LEVELS IN THE NORMAL GROUP AND DEFICIENT GROUP AFTER ALCOHOL INTAKE

		Before	30	60	120	180	240 (min)
Total Kininogen (ng BK equivalent/mg protein)							
Normal Group	(n = 9)	55.1 ± 2.0	54.7 ± 1.3	54.6 ± 1.5	54.7 ± 1.9	54.0 ± 1.7	53.8 ± 1.9
Deficient Group	(n = 13)	55.6 ± 1.8	54.0 ± 1.2	52.0 ± 1.3*‡	50.7 ± 1.3†§	49.8 ± 1.9†§	49.3 ± 1.7†§
Prekallikrein (arbitrary units)							
Normal Group	(n = 9)	2.50 ± 0.19	2.53 ± 0.22	2.48 ± 0.16	2.45 ± 0.16	2.51 ± 0.18	2.47 ± 0.17
Deficient Group	(n = 13)	2.48 ± 0.20	2.52 ± 0.23	2.47 ± 0.12	2.50 ± 0.23	2.46 ± 0.14	2.50 ± 0.20

BK, bradykinin. Values are mean ± SD.
*$p<0.05$ and †$p<0.01$ compared to the preintake values.
‡$p<0.05$ and §$p<0.01$ compared to the corresponding normal group.

with blood from five subjects in the normal group were the same as those obtained from the deficient group (data not shown).

DISCUSSION

Symptoms such as facial flushing, increase in pulse rate and decrease in diastolic blood pressure upon alcohol intake are frequently manifested by Mongoloid peoples (6,32). It has been considered that persons deficient in ALDH I would have elevated levels of blood AcH after drinking which would cause flushing and cardiovascular responses (13, 14, 17). In the present study, the elevation of blood AcH concentration after alcohol intake accompanied by changes in cardiovascular responses, such as pulse rate and diastolic blood pressure, were recognized in the deficient group, but not in the normal group. As reported previously (24), these results confirm that the cardiovascular responses associated with facial flushing are produced by the elevated AcH levels, because there were no significant differences in the peak alcohol levels and alcohol pharmacokinetic variables between the two groups.

The mechanism by which the cardiovascular changes occur in the deficient group has not been clarified. The involvement of AcH itself and/or AcH-induced catecholamine release has been suggested (1, 18, 19, 34). As shown by the significant reductions of the total kininogen levels in the deficient group in our preliminary experiment for the kallikrein-kinin system, it is suggested that kinins are released in the deficient group. If PK, which acts on HMW kininogen alone, is produced in plasma after alcohol intake, the reductions of HMW kininogen and PreK, a proenzyme of PK, must be recognized. However, it was demonstrated that the decrease in the total kininogen concentration in the deficient group in the assay for HMW and LMW kininogens was due to consumption of LMW kininogen, but not that of HMW kininogen. In addition, there were no changes in the PreK level in the deficient group. These results suggest that the kinins released are not produced by the activation of the PK-kinin system. It is likely that kinins are released from LMW kininogen by activation of the GK-kinin system and that the kinins released play a role in the development of cardiovascular symptoms appearing in the deficient group. However, GKs are known to act on both LMW and HMW kininogens. Our present results showed a decrease in LMW kininogen alone in the deficient group. Therefore, we speculated that there may be a concentration at which GKs act on LMW kininogen, but not on HMW kininogen. To test this possibility, we carried out an in vitro study using urinary kallikrein as one of GKs. As shown in Table 5, lower concentrations of urinary kallikrein act only on LMW kininogen, but higher concentrations act on both LMW and HMW kininogens. Thus, GKs preferentially act on LMW kininogen. Since urinary kallikrein showed the same effect on plasma kininogen obtained from each group, this suggests that the reduction of LMW kininogen in the deficient group is not due to a difference in sensitivity of LMW

TABLE 4

LEVELS OF KININOGENS AND PREKALLIKREIN IN PLASMA OF NORMAL AND DEFICIENT GROUPS BEFORE AND 4 HOURS AFTER ALCOHOL INTAKE

		Kininogens (ng BK equivalent/mg protein)			
		HMW	LMW	Total	Plasma Prekallikrein (Arbitrary Unit)
Normal Group (n = 9)	before	12.2 ± 0.8	41.0 ± 1.4	53.9 ± 2.1	2.41 ± 0.30
	after	12.4 ± 1.1	40.2 ± 1.6	55.4 ± 2.9	2.37 ± 0.25
Deficient Group (n = 13)	before	12.5 ± 1.6	41.3 ± 1.9	54.5 ± 2.0	2.57 ± 0.28
	after	11.8 ± 1.3	35.7 ± 2.3*†	48.3 ± 2.9*†	2.53 ± 0.32

HMW, high molecular weight. LMW, low molecular weight. BK, bradykinin.
Values are mean ± SD.
*$p<0.01$ compared to the preintake values.
†$p<0.01$ compared to the corresponding normal group.

TABLE 5

EFFECT OF URINARY KALLIKREIN VARYING IN CONCENTRATIONS BETWEEN 0.05×10^{-3} AND 2.0×10^{-3} PNA UNITS ON TOTAL, HMW AND LMW KININOGENS

Kininogens (ng BK equivalent/ ml plasma)	Urinary Kallikrein ($\times 10^{-3}$ PNA unit)						
	0	0.05	0.1	0.2	0.5	1.0	2.0
HMW	0.73	0.73	0.74	0.73	0.73	0.71	0.69
LMW	2.65	2.42	2.17	1.90	1.51	1.24	1.10
Total	3.48	3.30	2.92	2.71	2.28	1.85	1.64

HMW, high molecular weight. LMW, low molecular weight. BK, bradykinin.

kininogen to GKs between the two groups. Therefore, in the deficient group, activation of in vivo GKs after alcohol intake was considered.

GKs are found in exocrine organs such as the major salivary glands, the pancreas, and the kidney and in the secretion from these organs. GKs probably function within the organ in which they are synthesized either by exerting a local vasodilatory effect and/or possibly a direct effect on cells active in electrolyte transport. GK has also been isolated from human sweat (12). Fox *et al.* (11) demonstrated that bradykinin formation in human eccrine sweat glands produced periglandular vasodilatation. Therefore, the GK involving cardiovascular changes seen after alcohol intake may be released from the sweat glands. In other words, the cardiovascular response in the deficient group after alcohol intake may be due to the kinins released into the peripheral circulation from LMW kininogen by GK in sweat glands.

The mechanism by which GKs are activated remains unknown. Castania *et al.* (5) noted the reduction of kininogen in the plasma of rats injected with catecholamines. Rabito *et al.* (31) have also reported that the sympathetic nervous system, through activation of α_1-adrenoceptor, controls GK secretion from rat submandibular glands into the circulation. Thus, adrenergic control of kininogen concentrations has been suggested. Perhaps the activation of GKs is mediated by the sympathomimetic action of increased AcH. In the present study, cardiovascular changes such as facial flushing, increased pulse rate and decreased diastolic blood pressure were found together with elevated AcH levels after alcohol intake. These changes in the signs as mentioned above were conspicuous 0.5–1 hour after alcohol intake. However, as shown in Table 3, the total kininogen concentration started to decrease from 1 hour after alcohol intake and the minimum levels were reached only after 4 hours. This time difference between the cardiovascular changes and the minimal kininogen level may occur because consumption of kininogen continues with the release of kinin, which is rapidly metabolized. Therefore, the maximum decrease of the total kininogen concentration 4 hours after alcohol intake results from the cumulative consumption of kininogen over 4 hours.

We concluded that the symptoms occurring after alcohol intake may reflect the combined effects of vasoactive substances such as catecholamines, kinins and other agents, including AcH. The phenomena act as a homeostatic mechanism for the dispersal of AcH accumulating in blood in the living body. Thus, the kinins released by kallikreins in exocrine glands such as sweat glands may play a role in discharging the noxious substance.

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