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ACETALDEHYDE-INDUCED FORMATION OF 1-METHYL-1,2,3,4-TETRAHYDRO-β-CARBOLINE-3-CARBOXYLIC ACID IN RATS

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Abstract—1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) is one of the metabolites of peak E substance, which, based on epidemiological studies, has been thought to be a possible causative agent of the tryptophan-induced eosinophilia-myalgia syndrome. Acute ethanol and L-tryptophan administration in rats pretreated with cyanamide resulted in the formation of MTCA. Concentrations of MTCA were estimated at 27 ng/g in blood and 33 ng/g in kidneys. Chronic treatment with a liquid diet containing ethanol as 36% of the total calories for 6 weeks increased these levels. MTCA was barely observed in rats that had received acute or chronic ethanol in the absence of cyanamide, or in the cyanamide–tryptophan controls. Cyanamide facilitation of ethanol-dependent MTCA biosynthesis may be due to a potentiation of the blood level of acetaldehyde derived from ethanol. The blood acetaldehyde level in rats that had been acutely treated with cyanamide, ethanol and L-tryptophan was 348 μM, and averaged 503 μM in rats that received the same treatment after chronic consumption of ethanol. In contrast to the above findings, L-tryptophan intake promoted the formation of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (TCCA) in rats. This is the first report of MTCA in mammalian tissue during tryptophan and ethanol metabolism.

Peak E is a contaminant from a manufacturing process which has been suggested to cause L-tryptophan-associated eosinophilia-myalgia syndrome (EMS)‡ [1]. Its chemical structure is reportedly 1-1′-ethylidenebis[tryptophan] [2, 3]. 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) is reportedly formed from peak E in artificial gastric juice *in vitro* [4, 5].

1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid (TCCA) and MTCA are considered to be condensation products of L-tryptophan with aldehydes, and metabolic intermediates to β-carboline, which is considered to be a benzodiazepine receptor inhibitor [6]. Moreover, the *N*-nitrosoamine of MTCA, which is formed by oxidation with nitric acid, has been reported to display strong mutagenicity [7]. Acute *in vitro* cytogenetic effects of MTCA on mouse bone marrow cells [8] or rat bone marrow cells, using the micronucleus test [9], have been observed. Therefore, it is important to understand the metabolic pathway from tryptophan to tetrahydro-β-carbolines.

In our previous study [10], urinary excretion of MTCA in humans was enhanced significantly by ethanol intake, suggesting the endogenous formation of MTCA without consumption of L-tryptophan being implicated in EMS. We showed, however, that various foodstuffs, especially fermented foods and alcoholic beverages, contain TCCA and MTCA [11]. Accordingly, it may be inappropriate to use humans as experimental subjects because it is difficult to limit the type of food and the amount of TCCA and MTCA consumed.

In the present study, rats were used as subjects to establish a diet to evaluate the intake of tetrahydro-β-carbolines. Daily urinary excretions of tetrahydro-β-carbolines were quantified to examine their endogenous formation in rats. Next, we administered L-tryptophan to rats to detect tetrahydro-β-carbolines in blood as well as in various organs. Finally, we investigated the involvement of ethanol and acetaldehyde, which were previously shown to play an important role in the endogenous formation of MTCA in humans [10].

MATERIALS AND METHODS

Materials. L-Tryptophan, formaldehyde, propionaldehyde and MTCA were all purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). MTCA was a 12:1 diastereoisomeric mixture of (−)-(1*S*,3*S*)-MTCA and (−)-(1*R*,3*S*)-MTCA of known absolute configuration [12] by ^{1}H-NMR spectrum analysis [11]. The synthesis of TCCA from L-tryptophan and formaldehyde, as well as the preparation of the internal standard, 1-ethyl-tetrahydro-β-carboline-3-carboxylic acid, from L-tryptophan and propionaldehyde were both based on a previously described procedure [13]. The methanol was of HPLC grade (Wako Pure Chemical Industries Ltd., Osaka, Japan). All other chemicals

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‡ Abbreviations: EMS, eosinophilia-myalgia syndrome; MTCA, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; and TCCA, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid.

were of analytical grade. Benzenesulfonic acid (SCX) bonded phase cartridges and the vacuum manifold (Vac-Elut®) were purchased from Varian (Harbor City, CA, U.S.A.). Liquid diets were purchased from the Oriental Yeast Co., Japan. The composition of the diets was the same as that described by Lieber and DeCarli [14].

Animals. The subjects were 101 male Wistar rats purchased from the Oriental Yeast Co., Japan. Four animals were housed in each standard stainless steel hanging cage. Their mean weight was 180 g at the start of these procedures. The animal room was maintained at 22 ± 2° and 60 ± 5% humidity with a 12-hr on/12-hr off light cycle. Purified diet MF containing 0.28% L-tryptophan was prepared by the Oriental Yeast Co., Japan. The diet and tap water were provided *ad lib.*

Procedure. The study consisted of two parts. In the first part, we attempted to obtain a rough picture of the endogenous formation of tetrahydro-β-carbolines by comparing food intake and urinary excretions of TCCA and MTCA. Rats that had been fed (N = 18) and rats that had been fasted overnight (N = 11) were divided into two groups: (1) control rats that received saline intragastrically; and (2) rats acutely treated with L-tryptophan given intragastrically as a single 160 mg/kg dose. After oral administration the rats were housed individually in stainless steel metabolism cages. Urine was collected separately in glass containers at the bottom of each cage for 4 or 24 hr. One milliliter of 0.6 M perchloric acid was added to 1 mL of each urine and centrifuged at 12,074 *g* for 30 min. The supernatant was frozen at −20° until later analysis.

In the second part of the study we examined the distribution of tetrahydro-β-carbolines in various organs. After a 17-hr fast, 72 rats were divided into nine groups and were given the following drugs intragastrically: (A) control rats, saline; (B) acutely tryptophan-treated rats, a single 160 mg/kg dose of L-tryptophan (corresponding to 12 mL of aqueous solution); (C) acutely ethanol-treated rats, a single 2 g/kg dose of ethanol; (D) acutely cyanamide–tryptophan-treated rats, cyanamide i.p. at a dose of 20 mg/kg 2 hr before 160 mg/kg of L-tryptophan; (E) acutely cyanamide–tryptophan–ethanol-treated rats, 20 mg/kg of cyanamide i.p. 2 hr before 160 mg/kg of L-tryptophan and 2 g/kg of ethanol; and (B′) chronically tryptophan-treated rats, 160 mg/kg of L-tryptophan per day for 6 weeks. The other three groups of rats were pair-fed nutritionally adequate liquid diets containing either ethanol (36% of total calories) or isocaloric carbohydrates as controls for 6 weeks before receiving the following drugs p.o. after an overnight fast: (A′) saline; (C′) 2 g/kg of ethanol; and (E′) 20 mg/kg of cyanamide i.p., 2 hr before 160 mg/kg of L-tryptophan and 2 g/kg of ethanol.

One hour after drug administration each rat was anesthetized with diethyl ether before being killed between 9:30 and 10:00 a.m. Blood was collected from cervical vessels following decapitation. The kidneys, liver, spleen, brain, heart, lung and gastrocnemius muscle were removed quickly and weighed. Approximately 2 g of each tissue was homogenized with 6 mL of 0.6 M ice-cold perchloric acid and centrifuged at 12,074 *g* for 30 min. The supernatant was frozen at −20° until later analysis.

Determination of MTCA and TCCA. The supernatant from the above procedure was poured into a test tube containing 30 ng of the internal standard. Bond Elut extraction cartridges of 3-mL capacity, containing benzenesulfonic acid-derivatized silica (SCX) packing material, were used to clean the sample, as reported previously [11]. Twenty microliters of eluate from the SCX cartridges was injected into HPLC, and a run was performed using a technique described in a previous study [11].

Determination of blood acetaldehyde and ethanol concentrations. Blood samples were collected from cervical vessels 1 hr after ethanol loading. Blood ethanol and acetaldehyde concentrations were measured by a Perkin–Elmer F45 Head Space Analyzer (Norwalk, CT, U.S.A.) according to a previously described method [15].

Statistical analysis. Statistical analysis was performed on the normalized data using one-way ANOVA.

RESULTS

Table 1 shows the amounts of intake and urinary excretions of TCCA and MTCA in rats. Daily consumption of food was regarded as 20 g. The intake of TCCA and MTCA, as estimated by measuring the concentrations of TCCA and MTCA in rat chow, was 8.2 and 1.2 μg, respectively. Urinary excretions of TCCA and MTCA over 24 hr were 78.5 and 0.7 μg, respectively. This result shows that the urinary excretion of TCCA over 24 hr was about ten times higher than the amount of intake. Table 2 shows the effects of fasting and L-tryptophan on 4-hr excretions of TCCA and MTCA in rat urine. TCCA excretion in the fed group was 15 and 9 times higher than that of the fasted rats in the control and L-tryptophan groups, respectively. In the L-tryptophan group, a significant increase, as compared to the control rats, was observed only in the fed rats. MTCA excretion did not increase with feeding; however, L-tryptophan administration produced a significant increase, as compared to the control rats, in both the fed and fasted groups.

Figure 1 illustrates high-performance liquid chromatograms of TCCA and MTCA in a standard mixture and in the liver, kidney or brain of rats that had received, p.o., L-tryptophan with and without cyanamide and ethanol. The chromatograms of rats that had received L-tryptophan, cyanamide and ethanol showed two distinct peaks of the MTCA diastereoisomer: (−)-(1*S*,3*S*)-MTCA and (−)-(1*R*,3*S*)-MTCA.

Table 3 shows an extremely high level of TCCA (363 ng/g) in kidney, and smaller, but still significant, levels of TCCA in blood (37 ng/g) and liver (41 ng/g) after a single p.o. dose of L-tryptophan. Significant elevations of TCCA were also observed in gastrocnemius muscle and brain of rats treated acutely with L-tryptophan. However, in the groups that received chronic L-tryptophan treatment, no significant increases in TCCA levels were observed in the blood or any of the tissues examined. In addition, no change in TCCA concentration was

Table 1. Intake and urinary excretions of TCCA and MTCA

	N	TCCA	MTCA
Concentrations (μg/g) in rat chow	3	0.41 ± 0.05	0.06 ± 0.01
Contents (μg) in 20 g of rat chow		8.2	1.2
Urinary excretions (μg) for 24 hr in fed rats	7	78.5 ± 12.9	0.7 ± 0.6

Abbreviations: TCCA = 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; and MTCA = 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. Values are means ± SD.

Table 2. Effects of fasting and L-tryptophan (160 mg/kg, p.o.) on 4-hr excretions of TCCA and MTCA in rat urine

Group		N	TCCA (μg)	MTCA (μg)
Control	17-hr Fasted	4	0.36 ± 0.11	± 0.04
	Fed	5	5.43 ± 2.04*	0.07 ± 0.01
L-Tryptophan	17-hr Fasted	7	1.55 ± 1.28	0.24 ± 0.04†
	Fed	6	14.63 ± 2.92*†	0.25 ± 0.07†

Abbreviations: TCCA = 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; and MTCA = 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. Values are means ± SD.

* Significantly greater than corresponding fasted value, $P < 0.05$.

† Significantly greater than corresponding control value, $P < 0.05$.

observed in groups where cyanamide-pretreated rats were given L-tryptophan with or without ethanol or in rats that received ethanol alone.

Table 4 shows a significantly high level of MTCA in the kidney (33 ng/g); blood (27 ng/g), and liver (11 ng/g) of rats treated acutely with cyanamide, tryptophan and ethanol (group E). The amount of MTCA was expressed as the sum of (−)-(1*S*,3*S*)-MTCA and (−)-(1*R*,3*S*)-MTCA. A significant increase was also observed in the lung, brain and muscle of this group, as compared with the control group. A similar increase in MTCA, except in brain tissue, was also observed in group E′, which had received the same treatment as the acute group after consuming a liquid diet containing ethanol for 6 weeks. No other chronic groups displayed any significant change in MTCA concentration.

Blood ethanol and acetaldehyde concentrations in ethanol groups and in cyanamide–tryptophan–ethanol groups are shown in Table 5. A significantly higher acetaldehyde level, but not a higher ethanol level, was observed in the chronic ethanol group (47.9 μM) than in the acute ethanol group (13.7 μM). In rats that had been acutely treated with cyanamide, ethanol and tryptophan, the acetaldehyde level was 348 μM, which was approximately 25 times higher than that of the acute ethanol group; however, the ethanol level was decreased significantly. In addition, the acetaldehyde level in rats that had received the same treatment after consuming a liquid diet containing ethanol for 6 weeks increased to an average of 503 μM, further revealing the accumulation of acetaldehyde in blood following chronic treatment with ethanol.

DISCUSSION

Since peak E substance reportedly is converted into MTCA *in vitro* [4, 5], we were interested in whether or not L-tryptophan itself is metabolized to a tetrahydro-β-carboline compound in mammalian tissue. Recent studies have shown the presence of β-carboline-related compounds in Japanese sake and soy sauce, i.e. MTCA, TCCA [11], harman-(1-methyl-β-carboline), norharman(β-carboline) [16], 1-(5-hydroxymethyl-2-furyl)-β-carboline-3-carboxylic acid (flazin) and 1-(5-hydroxymethyl-2-furyl)-β-carboline [17]. Therefore, it is likely that bacteria synthesize these β-carboline compounds. In the present study using HPLC we have detected for the first time MTCA and TCCA in the blood and various organs of an intact rat. We observed that the urine of a control rat contained more TCCA than MTCA, whereas in the previous study MTCA was predominant in human urine and human milk [11]. The amount of TCCA excreted in the urine was much larger than the amount of TCCA in rat chow, thus revealing the endogenous formation of TCCA in rats. We also observed that the administration of L-tryptophan did not induce an elevated level of MTCA in rat organs but did increase the 4-hr excretion of MTCA and caused a significant accumulation of TCCA in kidney, liver and blood of rats. These results reveal that in rats tryptophan is primarily metabolized to TCCA and to MTCA to a lesser extent, which does not agree with the previous results in human urine. Consequently, if MTCA is involved in EMS, then EMS may more readily occur in humans than in

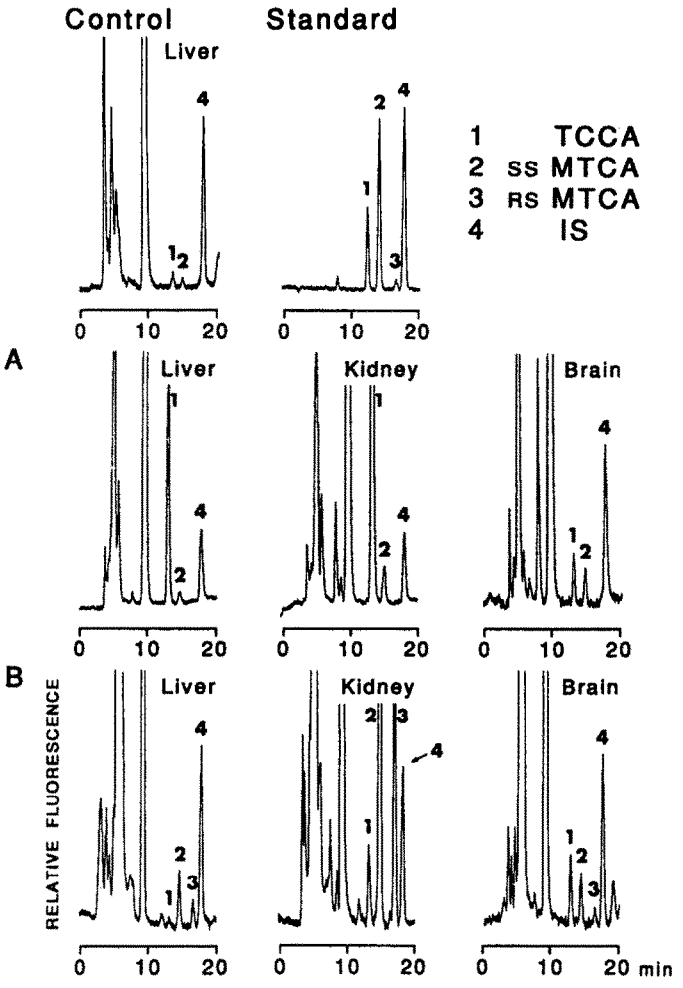

Fig. 1. High-performance liquid chromatograms of TCCA and MTCA. Key: TCCA = 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, MTCA = 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, *SS*-MTCA = (−)-(1*S*,3*S*)-MTCA, *RS*-MTCA = (−)-(1*R*,3*S*)-MTCA, and IS = internal standard. (A) L-Tryptophan was administered to rats (160 mg/kg, p.o.). (B) Cyanamide was given to rats (20 mg/kg, i.p.) 2 hr prior to ethanol (2 g/kg, p.o.) and L-tryptophan (160 mg/kg, p.o.).

rats. This may be one of the reasons why it is difficult to reproduce EMS using experimental animal such as rats.

Green *et al.* [18] reported that administration of L-tryptophan for 7 days in humans caused an increase of plasma kynurenine. This must be due to substrate induction of hepatic tryptophan 2,3-dioxygenase activity. Repeated doses of L-tryptophan could facilitate the oxidative pathway, so that chronic tryptophan administration might not produce the same elevation in TCCA as did a single dose of tryptophan, though the rats in the B′ group were killed at the same time after the last dose of tryptophan as were the rats in the B group after a single dose of tryptophan.

Formaldehyde may be a substrate of the reaction which forms TCCA from tryptophan in rats. If so, then TCCA should accumulate in the blood of rats that have been pretreated with cyanamide before receiving L-tryptophan, which is inconsistent with the present study. We have no explanation for this inconsistency. It is also unclear why the concentration of TCCA in kidney was the highest among those in the various rat organs and about 10 times higher than that in blood. The accumulation in the kidney may be due to the fact that TCCA is excreted into the urine, or because TCCA is synthesized in the kidney as well as in the liver.

Fukushima *et al.* [19] observed MTCA in rat brain but did not observe elevated MTCA levels in rats treated with cyanamide and acute ethanol. However, we found significantly increased MTCA levels in the peripheral organs, and in the brains of rats treated with cyanamide, L-tryptophan and ethanol. We also observed a significant increase of MTCA in blood and gastrocnemius muscle. Cyanamide can affect aromatic amino acid decarboxylase, so that a pathway from tryptophan to 5-hydroxytryptamine is inhibited by cyanamide treatment and the condensation reaction of tryptophan with acetaldehyde may readily occur.

Table 3. TCCA concentrations in blood, various organs and gastrocnemius muscle

Group*	No. of animals	TCCA (ng/g) Blood	Kidney	Liver	Spleen	Heart	Lung	Brain	Muscle
Acute									
(A) Control	11	1.2 ± 0.3	3.1 ± 0.8	0.6 ± 0.1	0.5 ± 0.4	1.0 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	2.5 ± 0.9
(B) Trp	10	37 ± 3.5†	363 ± 46†	41 ± 5.5†	0.9 ± 0.7	1.0 ± 0.6	0.8 ± 0.0	1.9 ± 0.2†	7.5 ± 0.8†
(C) EtOH	7	0.4 ± 0.1	1.6 ± 0.2	0.4 ± 0.1	1.2 ± 1.2	0.9 ± 0.6	0.3 ± 0.1	0.6 ± 0.1	0.8 ± 0.2
(D) Cy + Trp	6	1.5 ± 0.3	6.2 ± 1.1	2.0 ± 0.3				0.4 ± 0.1	0.9 ± 0.2
(E) Cy + Trp + EtOH	11	1.4 ± 0.7	4.9 ± 1.1	0.9 ± 0.2	0.6 ± 0.1	1.1 ± 0.2	1.3 ± 0.3	1.5 ± 0.7	1.9 ± 0.5
Chronic									
(A') Control	6	0.4 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.4		0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.1
(B') Trp	8	1.1 ± 0.1	4.4 ± 0.7	1.8 ± 0.2		0.9 ± 0.1		0.6 ± 0.0	1.0 ± 0.1
(C') EtOH	4	0.9 ± 0.7	1:0 ± 0.6	0.2 ± 0.0	0.4 ± 0.3	0.3 ± 0.1	0.7 ± 0.4	0.5 ± 0.2	0.5 ± 0.3
(E') Cy + Trp + EtOH	9	2.6 ± 1.7	3.3 ± 0.7	1.8 ± 0.8	2.5 ± 1.4	4.4 ± 2.2	7.7 ± 3.5	1.2 ± 0.8	2.6 ± 1.5

Values are means ± SEM for each group. TCCA = 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid.

* EtOH = ethanol 2 g/kg, p.o.; Trp = L-tryptophan 160 mg/kg, p.o.; Cy = cyanamide 20 mg/kg, i.p., 2 hr prior to L-tryptophan. Rats in the chronic group were fed for 6 weeks: (A') pair-fed ethanol-free diet, (B') Trp + standard diet, and (C' and E') liquid diet containing ethanol as 36% of total calories. Rats were starved overnight before the drug loading once (acute group) or the last administration of each drug as acute group (chronic group).

† Significantly different from other groups, $P < 0.05$.

Table 4. MTCA concentrations in blood, various organs and gastrocnemius muscle

Group*	No. of animals	MTCA (ng/g) Blood	Kidney	Liver	Spleen	Heart	Lung	Brain	Muscle
Acute									
(A) Control	11	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1		2.1 ± 0.8	2.9 ± 1.4	0.8 ± 0.4	1.3 ± 0.2
(B) Trp	10	1.0 ± 0.5	3.3 ± 0.8	1.2 ± 0.2	1.3 ± 0.8	0.6 ± 0.3	0.7 ± 0.3	1.5 ± 0.6	2.1 ± 0.7
(C) EtOH	7	1.0 ± 0.2	0.8 ± 0.1	0.7 ± 0.2		0.7 ± 0.4	0.5 ± 0.3	0.9 ± 0.1	0.5 ± 0.2
(D) Cy + Trp	6	1.3 ± 0.1	1.7 ± 0.3	1.4 ± 0.1				1.0 ± 0.1	1.0 ± 0.0
(E) Cy + Trp + EtOH	11	27 ± 2.8†	33 ± 5.9†	11 ± 1.0†	9.1 ± 1.3	2.7 ± 0.3	10.2 ± 3.1†	5.5 ± 0.6†	5.5 ± 0.5†
Chronic									
(A') Control	6	0.5 ± 0.1	2.2 ± 1.0	0.9 ± 0.3			2.9 ± 1.4	0.8 ± 0.2	3.1 ± 1.9
(B') Trp	8	0.9 ± 0.1	1.8 ± 0.2	1.2 ± 0.1		1.2 ± 0.0		2.1 ± 0.4	1.0 ± 0.1
(C') EtOH	4	1.2 ± 0.1	1.1 ± 0.4	0.8 ± 0.1	1.2 ± 0.6	1.0 ± 0.0	0.7 ± 0.2	0.6 ± 0.2	1.0 ± 0.2
(E') Cy + Trp + EtOH	9	48 ± 5.1‡	104 ± 13‡	16.7 ± 2.1‡	6.4 ± 0.5	6.7 ± 0.6‡	18.9 ± 2.4‡	5.3 ± 0.7	7.4 ± 0.8‡

Values are means ± SEM for each group. MTCA = 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid.

* EtOH = ethanol 2 g/kg, p.o.; Trp = L-tryptophan 160 mg/kg, p.o.; Cy = cyanamide 20 mg/kg, i.p., 2 hr prior to L-tryptophan. Rats in the chronic group were fed for 6 weeks: (A') pair-fed ethanol-free diet, (B') Trp + standard diet, and (C' and E') liquid diet containing ethanol as 36% of total calories. Rats were starved overnight before the drug loading once (acute group), or the last administration of each drug as acute group (chronic group).

† Significantly different from other acute groups, $P < 0.05$.

‡ Significantly different from the corresponding acute group, $P < 0.05$.

Table 5. Blood ethanol and acetaldehyde levels in rats 1 hr after ethanol (2 g/kg, p.o.) with and without cyanamide (Cy) and L-tryptophan (Trp)

Group*		N	Ethanol (mM)	Acetaldehyde (μM)
Ethanol	(C) Acute	5	41.3 ± 10.0	13.7 ± 6.9
	(C') Chronic	4	45.7 ± 4.4	47.9 ± 1.8†
Cy + Trp + Ethanol	(E) Acute	5	22.4 ± 6.9‡	348 ± 73‡
	(E') Chronic	9	37.4 ± 11.7†	503 ± 86†‡

Values are means ± SD.

* Group C received ethanol. Group C' consumed a liquid diet containing ethanol (36% of total calories) for 6 weeks, and then received ethanol after an overnight fast. Group E was pretreated with cyanamide (20 mg/kg, i.p.) 2 hr before ethanol and L-tryptophan (160 mg/kg, p.o.) administration. Group E' consumed a liquid diet containing ethanol for 6 weeks, and then received the same treatment as Group E.

† Significantly greater than the corresponding acute group, $P < 0.05$.

‡ Significantly different at $P < 0.05$ from the corresponding ethanol group.

Cyanamide-induced MTCA production is considered to be based on accumulation of acetaldehyde derived from ethanol, because cyanamide can inhibit aldehyde dehydrogenase (ALDH) [20] and because cyanamide pretreatment tremendously affects blood acetaldehyde levels following both acute and chronic ethanol treatment [21]. Accordingly, we have demonstrated for the first time that acetaldehyde is one of the factors affecting MTCA biosynthesis and one of the substrates which form MTCA in the rat. In the present study, the blood acetaldehyde level in rats that had received an acute administration of cyanamide, ethanol and L-tryptophan was 348 μM. However, in rats that had received the same treatment following chronic consumption of ethanol for 6 weeks, the blood acetaldehyde level was 503 μM. Therefore, more than 300 μM of the blood acetaldehyde level may be required in the formation of MTCA in the rat. We did not observe elevated MTCA levels in rats that had received a single 2 g/kg dose of ethanol after consuming a liquid diet containing ethanol for 6 weeks, while the blood acetaldehyde level was about 50 μM and significantly higher than in acutely treated rats, though the composition of the liquid diet used was the same as that used by Lieber and DeCarli [14]. This result was similar to that obtained in a previous study [10] in which we did not observe any difference in urinary MTCA levels between normal and ALDH-deficient human subjects.

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REFERENCES

1. Belongia EA, Hedberg CW, Gleich GJ, White KE, Mayeno AN, Loegering DA, Dunnette SL, Pirie PL, MacDonald KL and Osterholm MT, An investigation of the cause of the eosinophilia-myalgia syndrome associated with tryptophan use. *N Engl J Med* **323**: 357–365, 1990.
2. Mayeno AN, Lin F, Foote CS, Loegering DA, Ames MM, Hedberg CW and Gleich GJ, Characterization of "peak E," a novel amino acid associated with eosinophilia-myalgia syndrome. *Science* **250**: 1707–1708, 1990.
3. Smith MJ, Mazzola EP, Farrell TJ, Sphon JA, Page SW, Ashley D, Sirimanne SR, Hill RH and Needham LL, 1,1'-Ethylidenebis(L-tryptophan), structure determination of contaminant "97"—Implicated in the eosinophilia-myalgia syndrome (EMS). *Tetrahedron Lett* **32**: 991–994, 1991.
4. Analysis of L-tryptophan for the etiology of eosinophilia-myalgia syndrome New Mexico. *MMWR* **39**: 589–591, 1990.
5. Sakimoto K, The cause of the eosinophilia-myalgia syndrome associated with tryptophan use. *N Engl J Med* **323**: 992, 1990.
6. Braestrup C, Nielsen M and Olsen CE, Urinary and brain β-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. *Proc Natl Acad Sci USA* **77**: 2288–2292, 1980.
7. Wakabayashi K, Ochiai M, Saitô H, Tsuda M, Suwa Y, Nagao M and Sugimura T, Presence of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, a precursor of a mutagenic nitroso compound, in soy sauce. *Proc Natl Acad Sci USA* **80**: 2912–2916, 1983.
8. Fujie K, Nishi J, Wada M, Maeda S and Sugiyama T, Acute cytogenetic effects of tyramine and MTCAs on mouse bone marrow cells *in vivo* by the micronucleus test. *Mutat Res* **240**: 19–23, 1990.
9. Fujie K, Nishi J, Wada M, Maeda S and Sugiyama T, Acute cytogenetic effects of tyramine, MTCAs, NaCl and soy sauce on rat bone marrow cells *in vivo*. *Mutat Res* **240**: 281–288, 1990.
10. Adachi J, Yamamoto K, Ogawa Y, Ueno Y, Mizoi Y and Tatsuno Y, Endogenous formation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in man as the possible causative substance of eosinophilia-myalgia syndrome associated with ingestion of L-tryptophan. *Arch Toxicol* **65**: 505–509, 1991.
11. Adachi J, Mizoi Y, Naito T, Ogawa Y, Uetani Y and Ninomiya I, Identification of tetrahydro-β-carboline-3-carboxylic acid in foodstuffs, human urine and human milk. *J Nutr* **121**: 646–652, 1991.
12. Yamada S and Akimoto H, Reductive decyanization of α-amino nitriles with $NaBH_4$. A new synthetic approach to isoquinoline- and indole-alkaloids. *Tetrahedron Lett* 3105–3108, 1969.
13. Brossi A, Focella A and Teitel S, Alkaloids in mammalian tissues. 3. Condensation of L-tryptophan and L-5-hydroxytryptophan with formaldehyde and acetaldehyde. *J Med Chem* **16**: 418–420, 1973.
14. Lieber CS and DeCarli LM, The feeding of alcohol in

liquid diets: Two decades of applications and 1982 update. *Alcoholism Clin Exp Res* **6**: 523–531, 1982.

15. Okada T and Mizoi Y, Studies on the problem of blood acetaldehyde determination in man and its level after alcohol intake. *Jpn J Alcohol Drug Depend* **17**: 141–159, 1982.
16. Adachi J, Mizoi Y, Naito T, Yamamoto K, Fujiwara S and Ninomiya I, Determination of β-carbolines in foodstuffs by high-performance liquid chromatography and high-performance liquid chromatography–mass spectrometry. *J Chromatogr* **538**: 331–339, 1991.
17. Gessner WP, Brossi A, Bembenek ME and Abell CW, β-Carbolines from Japanese sake and soy sauce: Synthesis and biological activity of flazin and yellow substance YS (perlolyrine). *Arch Pharm (Weinheim)* **321**: 95–98, 1988.
18. Green AR, Aronson JK, Curzon G and Woods HF, Metabolism of an oral tryptophan load. I: Effects of dose and pretreatment with tryptophan. *Br J Clin Pharmacol* **10**: 603–610, 1980.
19. Fukushima S, Matsubara K, Akane A and Shiono H, 1-Methyl-tetrahydro-β-carboline-3-carboxylic acid is present in the rat brain and is not increased after acute ethanol injection with cyanamide treatment. *Alcohol* **9**: 31–35, 1991.
20. Kitson TM, Reactions of aldehyde dehydrogenase with disulfiram and related compounds. In: *Human Metabolism of Alcohol* (Eds. Crow KE and Batt RD), pp. 117–132. CRC Press, Boca Raton, 1989.
21. Johnsen J, Stowell A and Morland J, Clinical responses in relation to blood acetaldehyde levels. *Pharmacol Toxicol* **70**: 41–45, 1992.