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Relationship among Cyanide-Induced Encephalopathy, Blood Ammonia Levels, and Brain Aromatic Amino Acid Levels in Rats

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A recent study from our laboratory revealed that an injection of potassium cyanide (10 mg/kg) causes a severe hyperammonemia and an increase of brain aromatic amino acid levels in only mice with loss of consciousness (Yamamoto, 1989). In the present study, the correlation among loss of consciousness induced by cyanide, hyperammonemia and aromatic amino acid levels in blood and brain was further studied using rats. Although potassium cyanide (10 mg/kg, s. c.) caused loss of consciousness in all of the treated mice, potassium cyanide at same dose did not cause it in all of the treated rats. However, subcutaneous injection of potassium cyanide (20 mg/kg) caused loss of consciousness (loss of righting reflex) in all of the treated rats within 10 min after injection but did not kill those animals within 10 min after treatment.

Potassium cyanide (20 mg/kg, s. c.) elicited an increase of 202 μg N/dl in blood ammonia levels at 10 min after injection. However, injection of potassium cyanide (5 mg/kg) did not cause loss of consciousness in rats within 30 min after injection, though a small increase (38.4 μg N/dl) in the blood ammonia levels was observed at 10 min after injection.

On the other hand, subcutaneous injection of potassium cyanide (20 mg/kg) increased by 75 % and 150 %, respectively, in blood and brain tyrosine (Tyr) levels and by 25 % in brain phenylalanine (Phe) levels in rats with loss of consciousness. However, potassium cyanide (5 mg/kg, s. c.) administration did not elicit an increase of Tyr or Phe levels in blood and brain of rats 10 min after injection. The combined administration of ammonium acetate (AA; 5 mmol/kg two times per 15 min) and Phe (1 mmol/kg two times per 15 min) also caused loss of consciousness in 100 % of the treated rats, but neither AA alone, which produced only hyperammonemia nor Phe alone, which elicited only an increase of aromatic amino acid such as Phe and Tyr, caused loss of consciousness. These findings suggest that

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both increases of blood ammonia and brain aromatic amino acid levels may contribute to the induction of loss of consciousness induced by cyanide in rats.

Cyanide is a fatally poisonous substance and the toxic problems have been associated with ingesting cyanide-containing foods (Osuntokun 1980), and occupational hazards have arisen as the industrial use of cyanide has increased (Way 1984). It is believed that acute toxic effect of cyanide has been attributed to its production of cellular anoxia. Cyanide inhibits cytochrome oxidase a (cytochrome c oxidase), the terminal enzyme of the electron transport chain (Warburg 1931; Wikstrom et al., 1981; Jones et al. 1984). It has been found that cyanide decreases ATP content in liver but not in brain (Hoyer 1984; Yamamoto 1989). Cyanide also inhibits other enzymes related to amino acids metabolism requiring pyridoxal phosphate as a coenzyme (Tursky and Sajter 1962). In fact, it is suggested that tyrosine aminotransferase is inhibited by cyanide (*unpublished works*). Furthermore, our previous results suggested that cyanide elicits a tremendous increase of blood ammonia levels and brain aromatic amino acid, especially Tyr and Phe levels in mice with loss of consciousness (loss of righting reflex)(Yamamoto 1989). In addition, it is suggested that increase of blood ammonia levels induced by cyanide may be due to the inhibition of ATP-dependent urea cycle enzymes indirectly, by the inhibition of cytochrome c oxidase in mitochondria leading to the depletion of hepatic ATP content but it may be not due to the inhibition of glutamate dehydrogenase-dependent ammonia metabolism in liver (*unpublished works*). In present experiments, we carried out a further investigation on relationship among the increase of blood ammonia levels, brain aromatic amino acid levels, and the induction of loss of consciousness (loss of righing reflex) induced by cyanide.

MATERIALS AND METHODS

Male Wistar rats (body wt. 200-250 g) and male ddy mice (body wt. 20-22 g) were purchased from Ishikawa Pref. Animal Laboratory (Ishikawa, Japan). The animals were housed in a temperature (24 ± 1° C) and light (12 hr dark/light)- controlled room for a minimum of one week before use in these studies. They were given a diet of standard laboratory chow (oriental kobo) and water ad libitum. Rats received a subcutaneous or an oral injection of KCN (5, 10 or 20 mg /kg) in saline, an intraperitoneal injection of Phe (1 mmol/kg two times per 15 min) or alanine (Ala; 1 mmol/kg two times per 15 min) and/or AA (5 mmol/kg two times per 15 min) and/or α-ketoglutarate (AKG; 0.5 g/kg) and sodium thiosulfate (STS; 1 g/kg) in saline. Mice received a subcutaneous injection of KCN (10mg/kg) in saline. Blood samples were immediately taken when loss of consciousness was observed in the treated rats. When loss of consciousness was not observed, the blood samples 10 min after injection of chemicals (KCN:

5 mg/kg, s. c., AA : 5mmol/kg two times per 15 min, i, p. or Phe: 1 mmol/kg two times per 15 min, i. p. or KCN: 20 mg/kg, s.c., AKG: 0.5 g/kg, i. p. and STS: 1 g/kg,i.p.) were taken. Animals were kept under light ether anaesthesea and blood was obtained with a heparinized syringe from the inferior vena cava. The blood ammonia concentrations were immediately determined by the method of Okuda and Fujii (1966).

Blood was treated with a nine volume of 10 % trichloroacetic acid (TCA) and centrifuged at 1000 g for 15 min. The supernatant was used for the assay of blood amino acids.

Rats were killed immediately by decapitation when loss of consciousness (loss of righting reflex) was observed. When loss of consciousness was not observed, rats were killed by decapitation at 10 min after injection of chemicals. Brains were homogenized immediately in a nine volume of ice-cold distilled water. The homogenate was treated with a two volume of 10 % TCA and centrifuged at 1000 g for 15 min. The supernatant was used for the assay of brain amino acids. Blood and brain amino acids were determined by the HPLC Twincle System (Jasco, Japan) for a high performance liquid chromatography (HPLC) in a lithium buffer system (Yamamoto 1990a).

All data were compared by analysis of variance. When the analysis indicated that a significant difference existed, the means of selected groups were compared by Student's t-test.

RESULTS AND DISCUSSION

Although subcutaneous injection of potassium cyanide 20 mg/kg caused loss of consciousness (loss of righting reflex) in 100 % of the six treated rats within 10 min, its treatment did not kill those rats within 10 min. However, oral administration of potassium cyanide at same dose killed all of the treated rats within 10 min. Accompanying loss of consciousness was hyperammonemia. Blood ammonia levels (277.4 ± 22.3 μg N/dl) in the unconsciuosness-induced rats were increased by 268 % from that of the corresponding controls (75.4 ± 11.1 μg N/dl). On the other hand, subcutaneous injection of potassium cyanide (5 mg/kg) did not cause loss of consciousness (loss of righting reflex) within 30 min in all of the treated rats. The blood ammonia levels (113.8 ± 6.1 μg N/dl) at 10 min after injection were only increased by 51 % from that of controls. The combined administration of AA (5 mmol/kg two times per 15 min) and Phe (1 mmol/kg two times per 15 min) caused loss of consciousness (loss of righting reflex) in 100 % of the treated rats. The blood ammonia levels (2455.5 ± 721.2 μg N/dl) were tremendously increased from that of controls. However, intraperitoneal injection of AA (5 mmol /kg two times per 15 min) alone did not cause loss of consciousness, though the blood ammonia levels (1566.7 ± 119.8 μg N/dl) were tremendously increased. Intraperitoneal injection of Phe (1 mmol/kg

Table 1. Effects of cyanide, ammonium acetate (AA), phenylalanine (Phe), ammonium acetate plus phenylalanine (AA + Phe) and ammonium acetate plus alanine (AA + Ala) on blood ammonia levels in rats

Treatments	N	Blood Ammonia Levels (μg N/dl)	% of Coma Induced rats
Saline	4	75.4 ± 11.10	0
KCN (5 mg/kg)	6	113.8 ± 6.10*	0
KCN (20 mg/kg)	8	277.4 ± 22.31*	100
Phe (1 mmol/kg x 2)	8	75.7 ± 2.33	0
AA (5 mmol/kg x 2)	8	1566.7 ± 119.79*	0
AA (5 mmol.kg x 2) plus Phe (1 mmol/kg x 2)	8	2455.5 ± 721.24*	100
AA (5 mmol/kg x 2) plus Ala (1 mmol/kg x 2)	8	2185.5 ± 572.38*	0

Chemicals were treated as described under MATERIALS AND METHODS.
Values represent the mean ± S. E. M.
* Significantly increased from saline treated rats ($p<0.001$).

two times per 15 min) alone did not cause loss of consciousness and did not increase blood ammonia levels in rats. Furthermore, cotreatment with AA and Ala (1 mmol/kg two times per 15 min) did not cause loss of consciousness, though ammonia- , and blood and brain Ala levels (*data not shown*) were tremendously increased.

Subcutaneous injection of potassium cyanide (20 mg/kg) increased by 75 % in blood Tyr levels in rats with loss of consciousness as compared to that of the corresponding controls (Table 2). However, administration of potassium cyanide (5 mg/kg) did not change in blood Tyr and Phe levels at 10 min after injection in rats without loss of consciousness (loss of righting reflex). On the other hand, cotreatment with AA and Phe increased five-fold and eight-fold in blood Tyr and Phe levels, respectively in rats with loss of consciousness. In contrast, administration of AA (5 mg/kg two times per 15 min, i.p.) alone decreased by 26 % and 30 % in blood Tyr and Phe levels, respectively in rats without loss of consciousness. In addition, cotreatment with AA and Ala (1 mmol/kg two times per 15 min, i. p.) elicited a decrease of blood Tyr or Phe levels in rats without loss of consciousness. On the other hand, administration of Phe alone increased six-fold in blood Tyr and Phe levels, respectively in rats without loss of consciousness.

Subcutaneous injection of potassium cyanide (20 mg/kg) increased by 150 % and 25 % in brain Tyr and Phe levels, respectively in rats with loss of consciousness (loss of righting reflex)(Table 3). However, subcutaneous injection of 5 mg potassium cyanide/kg did not change in the brain Tyr and Phe levels in rats without loss of consciousness. On the other hand, the combined administration of AA (5

Table 2. Effects of cyanide, ammonium acetate (AA), phenylalanine (Phe), ammonium acetate plus phenylalanine (AA + Phe) and ammonium acetate plus alanine (AA + Ala) in blood tyrosine (Tyr) and Phe levels.

Treatments	N	Blood Tyr levels	Blood Phe levels
		nmol/ml	
Saline	4	95.4 ± 14.92	71.6 ± 4.91
KCN (5 mg/kg)	6	73.0 ± 7.33	72.5 ± 3.78
KCN (20 mg/kg)	8	167.2 ± 15.63*	73.6 ± 5.08
Phe (1 mmol/kg x2)	8	571.0 ± 78.65*	432.5 ± 86.46*
AA (5 mmol/kg x 2)	8	70.8 ± 4.84**	50.3 ± 4.28**
AA (5 mmol/kg x 2) plus Phe (1 mmol/kg x 2)	8	467.8 ± 55.65*	590.2 ± 140.25*
AA (5 mmol/kg x 2) plus Ala (1 mmol/kg x 2)	8	68.5 ± 5.85**	53.6 ± 3.64**

Chemicals were treated as described under MATERIALS AND METHODS.
Values represent the mean ± S. E. M.
* Significantly increased from saline treated rats ($p<0.001$).
** Significantly decreased from saline treated rats ($p<0.001$).

mmol/kg two times per 15 min, i. p.) and Phe (1 mmol/kg two times per 15 min, i.p.) increased by 322 % and 507 % in brain Tyr and Phe levels, respectively in rats with loss of consciousness. Furthermore, administration of Phe alone was increased by 260 % and 340 % in brain Tyr and Phe levels, respectively in rats without loss of consciousness. However, the combined administration of AA (5 mmol/kg two times per 15 min) and Ala (1 mmol/kg two times per 15 min) did not change in brain Tyr and Phe levels in rats without loss of consciousness.

The combined administration of AKG (0.5 g/kg, i. p.) and STS (1 g/kg, i. p.) completely blocked the induction of loss of consciousness induced by potassium cyanide (20 mg/kg). This treatment also completely abolished the increase of blood ammonia and brain Tyr and Phe levels induced by potassium cyanide (Table 4).

Our previous study revealed that treatment of mice with potassium cyanide caused a severe hyperammonemia, an increase of brain aromatic amino acid levels and loss of consciousness (Yamamoto 1989). Furthermore, we have suggested that hyperammonemia and increase of neutral and aromatic amino acid levels in brain may have an important role in the induction of hepatic coma induced by CCl_4 (Yamamoto et al. 1987 1988; Yamamoto 1990a). The present investigation was initiated to study in detail the relationship among cyanide-induced loss of consciousness, hyperammonemia and increase of blood and brain aromatic amino acid levels using rats. Potassium cyanide 10 mg/kg, s. c. did not cause loss of consciousness in all of six treated rats within 10 min after injection. However, potassium cyanide at same

Table 3. Effects of cyanide, ammonium acetate (AA), phenylalaine (Phe), ammonium acetate plus phenylalanine (AA + Phe) and ammonium acetate plus alanine (AA + Ala) on brain tyrosine (Tyr) and Phe.

Treatments	N	Brain Tyr Levels	Brain Phe Levels
		nmol/g wet brain	
Saline	4	71.2 ± 5.60	73.7 ± 4.91
KCN (5 mg/kg)	6	79.7 ± 5.65	79.5 ± 9.48
KCN (20 mg/kg)	8	178.0 ± 1.51*	92.1 ± 2.16*
Phe (1 mmol/kg x 2)	8	256.2 ± 30.80*	324.3 ± 23.91*
AA (5 mmol/kg x 2)	8	70.7 ± 5.65	79.5 ± 9.48
AA (5 mmol/kg x 2) plus Phe (1 mmol/kg x 2)	8	300.3 ± 17.67*	447.2 ± 20.59*A
A (5 mmol/kg x 2) plus Ala (1 mmol/kg x 2)	8	71.5 ± 4.75	75.6 ± 6.78

Chemicals were treated as described under MATERIALS AND METHODS.
Values represent the mean ± S. E. M.
* Significantly increased from saline treated rats ($p<0.001$).

dose caused loss of consciousness in all of the treated mice. This suggests that rats may be less sensitive to cyanide toxicity than mice. This result is similar to those results reported by Numata (1965).

Our present results clearly demonstrated that cyanide (20 mg/kg , s.c.) elicited a severe hyperammonemia, an increase of blood and brain Tyr levels, and an increase of brain Phe levels in only rats with loss of consciousness (loss of righting reflex). However, injection of AA alone did not cause loss of consciousness in rats, though a severe hyperammonemia was observed (Table 1). These results indicate that loss of consciousness (loss of righting reflex) induced by potassium cyanide may be not based on only increase of blood ammonia levels itself. In addition, intraperitoneal administration of Phe alone did not cause loss of consciousness, though high concentrations of Tyr and Phe in blood and brain were observed in rats without a severe hyperammonemia. This result suggests that loss of consciousness (loss of righting reflex) induced by cyanide may be not based on only increase of aromatic amino acid levels itself in blood and brain. On the other hand, the combined administration of AA and Phe caused loss of consciousness in 100 % of the treated animals and elicited a tremendous increase of Tyr and Phe levels in blood and brain in the rats with hyperammonemia. However, the combined administration of AA and Ala did not cause loss of consciousness, though a severe hyperammonemia and high levels of Ala in blood and brain were observed in all of the treated rats (*Data not shown*). These findings suggest that aromatic amino acids such as Tyr and Phe, but not aliphatic amino acid such as Ala may play an important role in the induction of loss of consciousness in rats with a

Table 4. Effects of α-ketoglutarate (AKG) and sodium thiosulfate (STS) on hyperammonemia and increases of aromatic amino acid levels in blood and brain induced by cyanide.

Treatments	Ammonia Levels	Tyr levels		Phe levels	
	(μg N/dl blood)	Blood (nmol/ml)	Brain (nmol/g)	Blood (nmol/ml)	Brain (nmol/g)
Saline	75.4±11.1	96.4±14.9	71.2±5.7	71.6±4.9	73.7±4.9
KCN	277.4±22.3*	167.2±15.6*	178.0±1.5*	73.6±5.1	92.1±2.2*
KCN plus AKG plus STS	72.4±10.2**	91.5±10.8**	70.5±4.8**	71.4±5.8	72.6±5.3**

Rats received potassium cyanide (20 mg/kg, s. c.) or potassium cyanide (20 mg/kg, s. c.), α-ketoglutarate (AKG: 0.5 g/kg, i. p.) and sodium thiosulfate (STS: 1.0 g/kg, i. p.). Control rats received saline. Blood ammonia levels and blood and brain Tyr and Phe levels were determined as described under MATERIALS AND METHODS. All data represent the mean ± S. E. M. (N = 4 - 8).
* Significantly increased from saline treated rats ($p<0.001$).
** Significantly decreased from KCN treated rats($p<0.001$).

severe hyperammonemia. Furthermore, the combined administration of AKG and STS, antagonists for cyanide poisoning (Isome and Way 1972; Chen and Rose 1952; Moore et al. 1986; Yamamoto 1989 1990b) completely blocked the induction of loss of consciousness induced by potassium cyanide and also blocked the increases of blood ammonia- and aromatic amino acid levels in blood and brain induced by potassium cyanide. These resluts suggest that the tremendous increase of blood ammonia levels and aromatic amino acids such as Tyr and Phe levels in brain may be critical event to the induction of loss of consciousness induced by potassium cyanide in rats. Since Ishikawa et al. (1985) have reported that a high ammonia concentration increases an uptake of neutral amino acid such as threonine into brain synaptosomes in man and rats, it is speculated that a severe hyperammonemia induced by potassium cyanide may increase the uptake of Tyr and Phe, neutral amino acids into synaptosomes in brain of rats. A high concentration of aromatic amino acid in synaptosomes may be possible to interfere with release of neurotransmitters such as norepinephirine and dopamine from synaptic terminals. The massive interference of neurotransmitters release may produce loss of consciousness in rats. However, further investigations are necessary to elucidate its hypothesis.

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