Creatine kinase activities in brain and blood: possible neurotoxic indicator of acrylamide intoxication

Masato Matsuoka, Hirohiko Matsumura, Hideki Igisu

Abstract

***Objectives*—To examine whether the activities of creatine kinase (CK) correlate with neurological disturbances caused by acrylamide.**

***Methods*—The activities of CK and other enzymes reported to be inhibited by acrylamide in the brain and plasma, and landing foot spread (LFS) were measured in mice and rats intoxicated with acrylamide.**

***Results*—Activity of CK was suppressed by acrylamide in the brain of mice in parallel with the neurological dysfunction measured by LFS. No clear alterations were found in glyceraldehyde-3-phosphate dehydrogenase, neuron-specific enolase, and lactate dehydrogenase activities over the experimental period (eight days for the exposure and 43 days for the recovery). In rats, among the plasma enzymes examined, suppression of CK activity was most notable, but thyroid activity was not affected.**

***Conclusions*—Among the enzymes so far examined, the CK activities in the brain and blood seem to be the most sensitive indicators of acrylamide intoxication.**

(*Occup Environ Med* 1996;53:468–471)

Keywords: creatine kinase; acrylamide; neurotoxic indicator

Department of Environmental Toxicology, University of Occupational and Environmental Health, 1–1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807, Japan
M Matsuoka
H Matsumura
H Igisu

Correspondence to:
Dr Masato Matsuoka, Department of Environmental Toxicology, University of Occupational and Environmental Health, 1–1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807, Japan.

Accepted 21 February 1996

Acrylamide is a potent neurotoxin which can cause neuropathy and encephalopathy in humans and in animals.[1 2] Although inhibition of several enzyme activities by acrylamide have been found,[2–5] and postulated as the possible pathogenesis of its neurotoxicity, the mechanism of toxicity of acrylamide has not been clarified. We have found that acrylamide inhibits creatine kinase (CK, enzyme commission number (EC) 2·7·3·2) activities in the rat brain.[6 7] Creatine kinase is present in neurons, astrocytes, and oligodendrocytes,[8] and seems to play an important part in continuously replenishing ATP from phosphocreatine in these cells,[9] catalysing the reaction:

phosphocreatine + ADP ↔ ATP + creatine.

To explore the involvement of brain CK in the genesis of neurotoxicity of acrylamide, we examined whether activities of CK in the brain correlate with the neurological disturbances in mice given acrylamide, along with other enzyme activities which have been reported to be inhibited by acrylamide.[3–5] If a clear correlation between activities of CK in the brain and neurological deficits is found, it seems interesting to examine whether activity of CK in the blood, which is measured frequently in the blood chemistry, can be a peripheral marker of acrylamide intoxication. In the second experiment with rats, we therefore measured the activities of CK in the plasma.

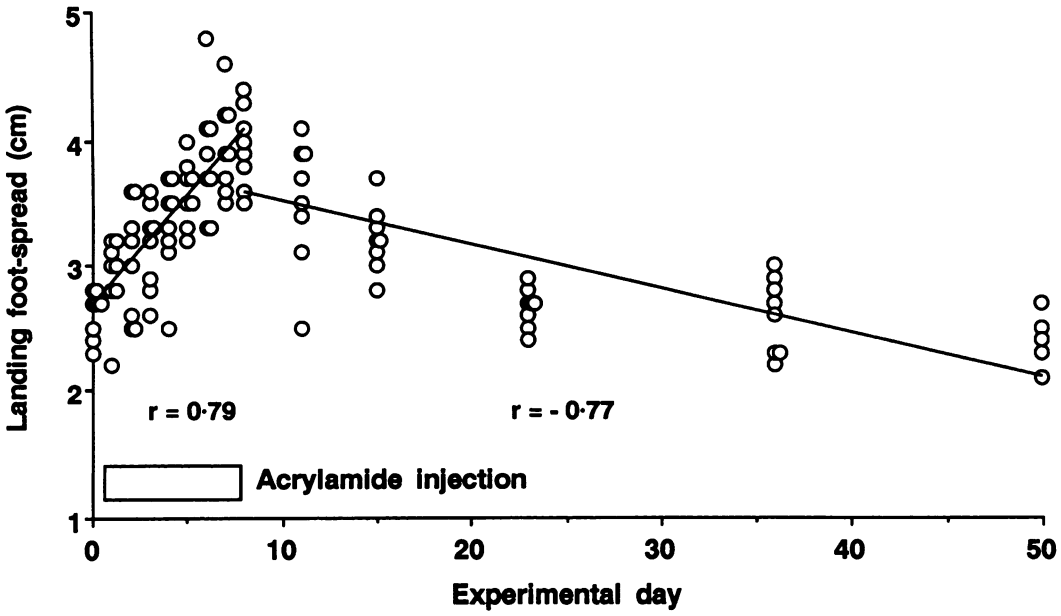
Method

FIRST EXPERIMENT

Male ddy mice weighing 26–30 g were obtained from Kyudo (Kumamoto, Japan). Animals had free access to food and water throughout the experimental period. Acrylamide was dissolved in 0·85% NaCl and injected intraperitoneally (50 mg/kg/day) from day 0 to 7 (total dose; 400 mg/kg). After eight injections, all mice were allowed to recover from day 8 to 50. Animals given acrylamide were killed on days 5, 8, 11, 15, 23, 36, and 50. Two control groups were prepared as follows. On day 0, mice without any injections were killed by decapitation (control 1). As the experiments were done over 51 days, mice given isotonic saline (2·0 ml/100 g/day) from day 0 to 7 were killed on day 50 (control 2) to evaluate possible effects of aging. After decapitation, the forebrain was removed and kept at −40°C until use.

The body weight was measured every day and the behaviours of each mouse were carefully observed. To evaluate the neurological deficit, the distance between toe pads of bilateral hind limbs was measured when the mouse landed from the height of 30 cm (landing foot spread, LFS).[10] This measurement was performed before each injection (day 0–7) and on appropriate days during the recovery with eight (five on day 50) mice which were selected randomly each day.

Frozen tissues were thawed at room temperature for 20 minutes. Subsequent procedures were all carried out at 0–4°C. Each tissue was homogenised in five volumes of ice cold 50 mM sodium phosphate buffer, pH 7·4 containing 1 mM EDTA and 1 mM dithiothreitol, with an all glass Potter-Elvehjem homogeniser. Part of the homogenates (350 µl) was centrifuged at 1200 *g* for 10 minutes and supernatants without nuclei were obtained. Remaining homogenates were centrifuged at 15 000 *g* for 10 minutes, and the resulting supernatants were further centrifuged at 100 000 *g* for 60 minutes to obtain the soluble fractions. These supernatants were kept at −120°C until analysis. The super-



Landing foot spread (LFS, cm) in mice intoxicated with acrylamide. Mice were injected with acrylamide from day 0 to 7 (total dose; 400 mg/kg), and then allowed to recover from day 8 to 50. The number of mice examined by LFS was eight except for day 50 when five mice were examined. Regression lines were drawn with a linear regression analysis, separating the experimental period into days 0–8 ($Y = 0{\cdot}18X + 2{\cdot}67$, $r = 0{\cdot}79$, $P < 0{\cdot}0001$) and days 8–50 ($Y = -0{\cdot}04X + 3{\cdot}87$, $r = -0{\cdot}77$, $P < 0{\cdot}0001$).

natants without nuclei were used to measure the activities of CK, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1·2·1·12) and lactate dehydrogenase (LDH, EC 1·1·1·27), and the soluble fractions were used for CK, GAPDH, enolase (EC 4·2·1·11), and neuron specific enolase (NSE).

Activities of GAPDH, enolase, and NSE were measured by the method of Howland *et al.*[5] When NSE activity was measured, the thermolabile non-neuronal enolase was inactivated at 50°C for 60 minutes.[11] The CK and LDH activities were measured as described previously.[6] Protein was measured by the method of Lowry *et al.*[12]

SECOND EXPERIMENT

To obtain enough blood to measure the activities of plasma enzymes, rats were used in this experiment. Male Wistar rats weighing 220–260 g were obtained from Kyudo (Kumamoto, Japan). Either acrylamide or isotonic saline was given to the rats, with the same procedure, in the first experiment except for the volume of solutions injected (0·33 ml/100 g/day). Twenty four hours after the last injection (total dose; 400 mg/kg), the animals were anaesthetised with diethyl ether, and blood was collected by cardiac puncture into the heparinised tube. The activities of plasma aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, LDH, cholinesterase, and CK were measured by Hitachi H-736 autoanalyser. The concentrations of tri-iodothyronine and thyroxine in the plasma were measured by radioimmunoassay.

STATISTICS

All values are expressed as means (SD). The relation between LFS and experimental day (day 0–8 and 8–50) was assessed by linear regression analysis. When LFS values of two groups were compared, a Mann-Whitney *U* test was used. The other values were examined by one way analysis of variance (ANOVA) and Bonferroni's test, unless otherwise stated. When two groups were compared, Student's or Welch's *t* test was also used. In all cases, differences at $P < 0{\cdot}05$ were considered as significant.

Results

ACTIVITY OF CK IN THE BRAIN AND NEUROLOGICAL DEFICIT IN MICE

There was no significant difference in the body weight between the acrylamide and control groups at any time during the experiment. Only those injected with acrylamide developed ataxia and weakness of the hind limbs on day 6 or 7. These clinical signs were not found one week after the last injection of acrylamide. The LFS increased from day 0 to 8 ($Y = 0{\cdot}18X + 2{\cdot}67$, $r = 0{\cdot}79$, $P < 0{\cdot}0001$) and peaked on days 7 and 8, and gradually returned to the control value after the end of the injections (from day 8 to 50, $Y = -0{\cdot}04X + 3{\cdot}87$, $r = -0{\cdot}77$, $P < 0{\cdot}0001$, fig).

There was no difference in any enzyme activities between controls 1 and 2 (tables 1 and 2). Thus, aging of mice did not affect those enzyme activities.

When simply compared with Student's *t* test, the activities of GAPDH on days 5, 8, and 36 in the supernatants without nuclei (table 1) and those on days 8 and 11 in the soluble fractions (table 2) seemed lower than that of the control 1 (day 0). However,

Table 1 Mean (SD) enzyme activities in the supernatants without nuclei from brains of mice intoxicated with acrylamide

		CK		GAPDH		LDH
Day	n	Activity	%†	Activity	%†	Activity
Control 1	6	4·72 (0·32)	100·0	1·17 (0·05)	100·0	1·12 (0·10)
5	6	3·32 (0·11)*	70·3	1·07 (0·08)	91·5	1·05 (0·02)
8	6	2·98 (0·26)*	63·1	1·03 (0·04)	88·0	1·05 (0·08)
11	5	3·39 (0·27)*	71·8	1·10 (0·12)	94·0	1·09 (0·08)
15	6	4·01 (0·22)*	85·0	1·09 (0·10)	93·2	1·09 (0·07)
23	6	4·49 (0·18)	95·1	1·09 (0·10)	93·2	1·03 (0·06)
36	5	4·95 (0·33)	104·9	1·07 (0·06)	91·5	0·99 (0·07)
50	5	5·11 (0·21)	108·3	1·18 (0·11)	100·9	1·07 (0·07)
Control 2	6	4·83 (0·46)	102·3	1·15 (0·13)	98·3	1·04 (0·03)

*$P < 0{\cdot}001$ *v* control 1.
†Percentage of the control 1 value.
Activities of enzymes are expressed in terms of μmol/min/mg protein.

Table 2 Mean (SD) enzyme activities in the soluble fractions from brains of mice intoxicated with acrylamide

Day	n	CK Activity	CK %†	GAPDH Activity	GAPDH %†	Enolase activity	NSE activity
Control 1	6	7·30 (0·24)	100·0	1·47 (0·05)	100·0	1·08 (0·05)	0·59 (0·05)
5	6	4·90 (0·42)*	67·1	1·41 (0·22)	95·9	1·08 (0·05)	0·58 (0·06)
8	6	4·46 (0·34)*	61·1	1·29 (0·10)	87·8	1·11 (0·05)	0·59 (0·05)
11	5	5·13 (0·37)*	70·3	1·32 (0·06)	89·8	1·10 (0·03)	0·62 (0·03)
15	6	6·09 (0·32)*	83·4	1·41 (0·11)	95·9	1·15 (0·07)	0·61 (0·05)
23	6	6·95 (0·22)	95·2	1·38 (0·19)	93·9	1·14 (0·08)	0·59 (0·05)
36	4	7·56 (0·17)	103·6	1·54 (0·09)	104·8	1·14 (0·08)	0·62 (0·07)
50	4	7·44 (0·20)	101·9	1·43 (0·19)	97·3	1·12 (0·08)	0·60 (0·05)
Control 2	6	7·40 (0·28)	101·4	1·48 (0·13)	100·7	1·16 (0·06)	0·61 (0·06)

*P < 0·001 *v* control 1.
†Percentage of the control 1 value.
Activities of enzymes are expressed in terms of μmol/min/mg protein.

Table 3 Blood chemistry in rats intoxicated with acrylamide (400 mg/kg)

	Control Mean	Control (SD)	Control n	Acrylamide Mean	Acrylamide SD	Acrylamide n
Aspartate aminotransferase (U/l)	92	(12)	12	92	(20)	13
Alanine aminotransferase (U/l)	39	(5)	12	49	(16)	13
Alkaline phosphatase (U/l)	722	(131)	12	610	(107)*	13
Lactate dehydrogenase (U/l)	388	(117)	12	406	(121)	13
Cholinesterase (U/l)	103	(14)	12	91	(13)*	13
Creatine kinase (U/l)	399	(102)	12	250	(69)***	13
Tri-iodothyronine (ng/dl)	85	(10)	12	84	(17)	12
Thyroxine (μg/dl)	5·1	(0·5)	8	5·3	(0·8)	8

*P < 0·05; ***P < 0·0005 *v* controls.

ANOVA did not show any significant difference (tables 1 and 2). The LDH (table 1), enolase, and NSE (table 2) activities did not show any significant changes during the experimental period in either fraction.

On the other hand, CK activities were significantly suppressed from day 5 to 15 in both supernatants without nuclei and soluble fractions, and the suppression was most notable on day 8. The activities did not differ from the controls after day 23. Although the number of mice in each day was less than six, the present results agree well with our previous findings in which CK activities in the rat brain were suppressed by acrylamide.[6 7]

ACTIVITIES OF PLASMA ENZYMES IN RATS

A significant difference ($P < 0.0001$) in the increase of body weight during the experimental period (eight days) was found between the controls (47 (8) g, n = 12) and acrylamide group (22 (9) g, n = 13). On day 6 or 7, the acrylamide group developed ataxia and weakness of the hind limbs. There was a significant difference ($P < 0.0005$) in the LFS on day 8 between the controls (62 (20) mm, n = 9) and acrylamide group (110 (11) mm, n = 10).

Among the plasma enzymes examined on day 8, CK activity was most notably suppressed (table 3). The activities of CK, alkaline phosphatase, and cholinesterase were lowered by 37%, 16%, and 12%, respectively. No alterations were found in the concentrations of tri-iodothyronine and thyroxine.

Discussion

The LFS was originally devised to evaluate neurological deficits caused by acrylamide in rats.[10] The method is simple and yet sensitive and quantitative when the neurological abnormalities are not too severe.[10] Although the significant suppression of the gain in body weight, an apparent sign of systemic intoxication, was found only in rats, LFS was obvious in both rats and mice, and we could measure the neurological dysfunction during and after acrylamide exposure.

In the mouse brain, GAPDH and NSE activities which had been reported to be inhibited by acrylamide in cats or rats,[3–5] showed no significant changes throughout the experimental period over 51 days. Although the exact cause of the discrepancy between those studies[3–5] and the present one is not clear, it may be due to species difference. Thus, GAPDH and NSE activities may not be so sensitive to acrylamide in mice as in cats or rats, although clinical signs of neurotoxicity of acrylamide were found in all of these species. This suggests that inhibition of GAPDH and NSE is not important in the genesis of neurotoxicity of acrylamide.

In contrast, activity of CK in the brain was clearly suppressed by acrylamide both in rats[6 7] and in mice, as shown in the present study. Moreover, its suppression was most notable on day 8 when the neurological dysfunction measured by LFS was most severe. Also, as the neurological dysfunction became less apparent after the end of giving acrylamide, the suppression of activities of CK in the brain became less obvious until it returned to normal when there was no neurological dysfunction. Thus, inhibition of brain CK seems to be the most sensitive indicator of acrylamide intoxication among the enzymes so far examined.

With the non-invasive nuclear magnetic resonance (NMR) technique of magnetisation transfer, linear relations were found between the rate constant of the brain CK forward reaction (phosphocreatine + ADP → ATP + creatine) and the intensity of electroencephalograms in rats when general brain suppressant (thiopentone sodium) or stimulant (bicuculline) was given.[13] This indicates that activity of CK is closely linked to the brain function. Considering this and the importance of substrates catalysed by CK, the inhibition of activity of CK in the brain might underlie the genesis of the neurotoxicity of acrylamide, especially that of encephalopathy, and it may be a good indicator of acrylamide intoxication. It should be noted that activities of CK in the brain could be measured in living animals by NMR.[13] When this non-invasive technique becomes available to humans, the present results might provide reference data.

In the plasma of rats intoxicated with acrylamide, several enzymatic activities were decreased. Those may reflect the systemic effects of acrylamide. However, activity of CK was more suppressed than other activities. Moreover, CK activity was suppressed more than NSE activity (and only CK was inhibited in the mouse brain by acrylamide). It is well known that activity of CK in the blood is affected by thyroid status; the activity

decreases in hyperthyroidism and increases in hypothyroidism. Nevertheless, involvement of thyroid function in acrylamide intoxication was unlikely, as the levels of tri-iodothyronine and thyroxine were not changed.

Ethylene oxide, another neurotoxin which can cause encephalopathy and central-peripheral distal axonopathy as acrylamide can, also suppressed CK activity in the blood as well as in the brain.[14] Thus, plasma CK activity may be used as a peripheral marker of intoxication with these neurotoxic chemicals. Obviously, more studies are necessary before CK activities are used to monitor human exposure to those neurotoxins.

We thank Professor M Ikeda for his comments on the statistical analysis. This work was supported in part by a Special Research Grant from the Ministry of Labor, Japan.

1 Igisu H, Goto I, Kawamura Y, Kato M, Izumi K, Kuroiwa Y. Acrylamide encephaloneuropathy due to well water pollution. *J Neurol Neurosurg Psychiatry* 1975;**38**:581–4.
2 O'Donoghue JL. Acrylamide and related substances. In: O'Donoghue JL, ed. *Neurotoxicity of industrial and commercial chemicals, vol 2*. Florida: CRC Press, 1985: 169–77.
3 Howland RD. The etiology of acrylamide neuropathy: enolase, phosphofructokinase, and glyceraldehyde-3-phosphate dehydrogenase activities in peripheral nerve, spinal cord, brain, and skeletal muscle of acrylamide-intoxicated cats. *Toxicol Appl Pharmacol* 1981;**60**:324–33.
4 Howland RD, Vyas IL, Lowndes HE. The etiology of acrylamide neuropathy: possible involvement of neuron specific enolase. *Brain Res* 1980;**190**:529–35.
5 Howland RD, Vyas IL, Lowndes HE, Argentieri TM. The etiology of toxic peripheral neuropathies: in vitro effects of acrylamide and 2,5-hexanedione on brain enolase and other glycolytic enzymes. *Brain Res* 1980;**202**:131–42.
6 Matsuoka M, Igisu H, Lin J, Inoue N. Effects of acrylamide and *N,N'*-methylene-bis-acrylamide on creatine kinase activity. *Brain Res* 1990;**507**:351–3.
7 Kohriyama K, Matsuoka M, Igisu H. Effects of acrylamide and acrylic acid on creatine kinase activity in the rat brain. *Arch Toxicol* 1994;**68**:67–70.
8 Manos P, Bryan GK, Edmond J. Creatine kinase activity in postnatal rat brain development and in cultured neurons, astrocytes, and oligodendrocytes. *J Neurochem* 1991;**56**: 2101–7.
9 Wyss M, Smeitink J, Wevers RA, Wallimann T. Mitochondrial creatine kinase: a key enzyme of aerobic energy metabolism. *Biochim Biophys Acta* 1992;**1102**: 119–66.
10 Edwards PM, Parker VH. A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats. *Toxicol Appl Pharmacol* 1977;**40**:589–91.
11 Marangos PJ, Parma AM, Goodwin FK. Functional properties of neuronal and glial isoenzymes of brain enolase. *J Neurochem* 1978;**31**:727–32.
12 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;**193**:265–75.
13 Sauter A, Rudin M. Determination of creatine kinase kinetic parameters in rat brain by NMR magnetization transfer. Correlation with brain function. *J Biol Chem* 1993;**268**:13166–71.
14 Matsuoka M, Igisu H, Inoue N, Hori H, Tanaka I. Inhibition of creatine kinase activity by ethylene oxide. *Br J Ind Med* 1990;**47**:44–7.