Suppression of Pentylenetetrazol-Induced Seizures and c-fos Expression in Mouse Brain by L-Carnitine

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Department of Environmental Toxicology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health—Seizure development in ddY mice was recorded on a videotape for 20 min after injection of pentylenetetrazol (PTZ) (50 mg/kg) and analyzed in detail. In addition, expression of the c-fos gene in the brain was semi-quantified by reverse transcription-polymerase chain reaction analysis. When saline, L-carnitine (10 mmol/kg), or D-carnitine (10 mmol/kg) was administered 30 min prior to PTZ, the seizures were suppressed in mice given L-carnitine; the seizure scores were significantly lower in mice treated with L-carnitine at several time points, and the overall seizure scores were 31.43 ± 2.49, 10.57 ± 2.86 and 24.71 ± 3.05 (expressed as the mean ± S.E.M., n=7), in saline, L-carnitine and D-carnitine groups, respectively. The latency to the first clonic-tonic seizure was also prolonged in mice treated with L-carnitine. The level of c-fos mRNA in the brain was lower in the animals treated with L-carnitine than in those treated with saline or D-carnitine. Thus, L-carnitine shows not only anticonvulsive effects in one of the most widely used animal models of chemically induced seizures but also the potential to suppress the seizure-associated expression of an immediate early gene in the brain.

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Key words: Seizures, Carnitine, Pentylenetetrazol, Immediate early genes, c-fos

Materials and Methods

PTZ, L-carnitine (inner salt) and D-carnitine (inner salt) were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and dissolved in 0.85% NaCl.
Male ddY mice weighing 28–33 g were administered with chemicals intraperitoneally. PTZ (50 mg/kg) was injected 30 min after the administration of saline, L-carnitine (10 mmol/kg) or D-carnitine (10 mmol/kg). In the controls, saline was injected 30 min after the first administration of saline.

The behavior of the mice was recorded with a Sony CCD-TR850 video recorder for 20 min after PTZ injection and subsequently analyzed. The seizures were classified according to Erdtmann-Vourliotis et al. as follows: stage 0, no response; stage 1, ear and facial twitching; stage 2, myoclonic jerks without upright position; stage 3, myoclonic jerks, upright position with bilateral forelimb clonus; stage 4, clonic-tonic seizures; stage 5, generalized clonic-tonic seizures, loss of postural control. The time to the first occurrence of clonic seizure was also determined.

Twenty min after the injection of PTZ or the second saline, the mice were killed by decapitation and immersed quickly in liquid nitrogen. The frozen brain was dissected out and homogenized with a Polytron PT 3000. The isolation of total RNA and RT-PCR analysis were carried out as described previously. The c-fos and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were obtained from Clontech (Palo Alto, CA, USA). After electrophoresis on an agarose gel containing ethidium bromide, the density of each band was quantified. For mRNA analyses, the density of c-fos product was normalized to that of GAPDH.

Statistical analyses of seizure activities (seizure stage, overall seizure stage scores, and latency to the first clonic-tonic seizure) and the c-fos mRNA level in different treatment groups were done by the Kruskal-Wallis test followed by Dunn’s test and by one-way analysis of variance (ANOVA) followed by Bonferroni’s test, respectively. Differences at p<0.05 were considered statistically significant.

**Results**

In mice given saline plus PTZ, the mean value for the seizure stage peaked 2 min after PTZ injection, and thereafter lowered gradually (Fig. 1A). On the other hand, the pretreatment with L-carnitine delayed the peak to 4 min, and also lowered the seizure stage scores significantly at 2, 6 and 7 min when compared to the saline plus PTZ group (Fig. 1B). In contrast, the pretreatment with D-carnitine did not affect the seizure scores significantly at any time (Fig. 1C).

Overall seizure stage scores during the observation period (20 min) (Fig. 2) differed (p<0.01) for mice pretreated with saline and L-carnitine but not for those treated with saline and D-carnitine. When the scores in each group are expressed as the mean ± S.E.M., they are 31.43 ± 2.49, 10.57 ± 2.86 and 24.71 ± 3.05 (n=7) in saline, L-carnitine and D-carnitine groups, respectively.

The latency to the first clonic-tonic seizure (Fig. 3) also differed (p<0.05) for the saline and L-carnitine groups but not for the saline and D-carnitine groups.

In the semi-quantitative RT-PCR analysis, PTZ had increased the c-fos mRNA level in the brain (p<0.05) when examined 20 min after its administration (Fig. 4). The PTZ-induced increase in the c-fos mRNA level was noticeably suppressed by the pretreatment with L-carnitine but not with D-carnitine (Fig. 4).

**Discussion**

In our previous study, in order to compare with the beneficial effects of carnitine in hyperammonemia, we...
observed animal behavior for 10 min after PTZ, with 70 mg/kg PTZ and mainly with 20 mmol/kg carnitine. In the present experiment, we employed a smaller dose of PTZ (50 mg/kg) and analyzed seizures over 20 min in detail recorded on a videotape. As a result, we found that the pretreatment with L-carnitine clearly suppressed the PTZ-induced seizures even at 10 mmol/kg. On the other hand, the same dose of D-carnitine, a physiologically inactive form of carnitine, failed to suppress seizures. The anticonvulsive effects of L-carnitine therefore seem due to the inherent action of L-carnitine rather than non-specific actions.

It has been reported that a single administration of PTZ (45 mg/kg) to mice induced c-fos expression in several brain regions including the cerebral cortex and hippocampus10). In good agreement with this finding, we observed that PTZ (50 mg/kg) increased the c-fos mRNA level in the mouse brain. Moreover, the pretreatment with L-carnitine decreased the c-fos mRNA level but that with D-carnitine did not. The decrease in c-fos mRNA expression might simply be due to the suppression of seizures by L-carnitine but intraventricular or intrahippocampal administration of antisense oligodeoxynucleotides against c-fos mRNA has been reported to suppress neuronal damage caused by N-
methyl-d-aspartate (NMDA) or hippocampal partial seizures elicited by electrical stimulation in the rat. These findings suggest that the induction of c-fos gene expression might play an important role in the development of seizures as well as in the excitatory amino acid-induced neurotoxicity. Since PTZ has been reported to evoke seizures depending on the stimulation of excitatory amino acid receptors, the suppression of c-fos gene expression in the brain by l-carnitine alone might underlie its anticonvulsive effects.

L-Carnitine is widely distributed among tissues including the brain. It can be synthesized in the brain, but the physiological function of carnitine in the brain is not known. Not only myopathy but hyperammonemia and hypoketotic hypoglycemic encephalopathy may be seen in primary systemic carnitine deficiency. In this regard, it is of interest that carnitine treatment in mice intoxicated with ammonia suppressed seizures, ameliorated energy metabolism of the brain and moreover lowered the ammonia level in the brain.

As shown in the present experiments, l-carnitine can suppress PTZ-induced seizures clinically and it also suppresses a gene expression in the brain caused by PTZ, though the mechanism of these beneficial effects of carnitine is still not clear. Activity in the PTZ model often indicates that a drug can affect GABAergic systems, either by enhancing brain GABA levels or by altering the sensitivity of postsynaptic GABA receptors. It was reported that GABA competitively inhibits carnitine transport in rat brain slices, suggesting that the same system mediates the transport for GABA and carnitine in the brain. On the other hand, carnitine and GABA did not compete in the OCTN2-mediated transport expressed in HEK293 cells. In addition, the brain has low expression of OCTN2. These findings suggest that the anticonvulsive effects of carnitine seen in the present experiments might not involve the transport of carnitine through OCTN2 in the brain.

Previous experiments indicate that l-carnitine may protect the brain from ischemia and hyperammonemia in animals. Patients with Alzheimer’s disease treated for one year with acetyl-l-carnitine had a slowed rate of deterioration in neuropsychological tests and in the performance of every day activities. They also suggest that l-carnitine and its analogues may have the potential to protect the cerebral functions in humans as well as in animals. To understand its mechanism, it remains to be determined whether the levels of carnitine in the brain and other tissues are affected by chemicals especially by convulsants including PTZ.

References
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