drug metabolizing enzymes[10], but also with acute ethanol administration[11], which is known to inhibit microsomal drug metabolism[10,12]. Since no metabolic changes have been demonstrated for Amanita toxins, it is unlikely that these considerations apply to the mechanism by which ethanol affords protection against death cap toxicity. It is also not possible to conclude from a study assessing in mice the influence of ethanol given as an oral dose of 4800 mg/kg 16 h earlier on a variety of hepatotoxic agents including carbon tetrachloride and α-amanitin[13], that the observed prevention of the rise of serum enzymes due to α-aminitin was caused by a metabolic interference involving microsomal enzymes.

As chronic application of ethanol with a last dose at 32 or 56 h before the APL did not reduce its toxicity[3], the protection seems to be due to an acute effect of ethanol on the APL-induced liver damage. One possibility is that ethanol interferes with the uptake of the mushroom toxins into liver cells, either by blocking receptors or a transport system[14], or by causing structural disorders of membrane lipids[15,16]. The fact that the toxins are normally taken up by liver cells within 15 min would explain why ethanol given 30 min after APL did not ameliorate survival. Finally, acute ethanol administration may affect the disturbances in cellular calcium homeostasis caused by the toxins[17].

According to our finding, simultaneous ethanol interferes with the toxicity of the toadstool. This observation could contribute to explaining why the mortality of death cap intoxication in children below 10 years of age is much higher (51.3%) than in adults (16.5%)[2], although the dose/b.wt relation has to be considered. So far, a) age and b) latency period between the meal and appearance of the first symptoms have been ascertained as prognostic variables in death cap poisoning[2]. Although blood ethanol levels in ordinary meals comprising mushrooms are likely to be lower than those observed in our experiments, the present observation clearly suggests that the ingestion of alcoholic beverages with the meal should also be taken into account when therapeutic measures in clinical *Amanita phalloides* intoxication are evaluated.

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Forskolin supresses seizures induced by pentylenetetrazol in mice

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Summary. Forskolin, an adenylate cyclase activator when injected s.c. (1 mg/kg) in mice, caused an elevation of cAMP in the forebrain and cerebellum of up to 170% and 130%, respectively. The treatment was found to prevent seizures induced by pentylenetetrazol. This suppression had subsided 30 min after the injection, when cAMP level was again normal in the cerebellum but still elevated in the forebrain.

Key words. Mouse brain; convulsions; forskolin; anti-convulsant drugs; cAMP, cerebral.

Forskolin, a diterpene, is isolated from a plant (*Coleus forskohlii*[1]). The *Coleus* sp. have been described in ancient medical texts as having medicinal properties against various diseases including central nervous system diseases like insomnia and convulsions[2]. Recently, forskolin was shown to be a highly potent and specific activator of various mammalian adenylate cyclase systems in intact cells, membranes and detergent-dispersed membranes[3–5]. Forskolin can mimic biological actions of hormones through an increase of cAMP content[6,7]. It has been demonstrated that experimental seizures alter the cyclic nucleotide level in the brain; electroconvulsive shock[8,9] and several convulsant drugs[10,11] can elevate the level of cAMP after the onset of the seizures in the mouse brain. Ferendelli hypothesized that this elevation may be involved in mechanisms inhibiting the seizure activity since he found that animals pretreated with cAMP-lowering agents (reserpine, propranolol or aminophylline) showed an enhanced sensitivity to seizures induced by pentylenetetrazol, while cAMP or its derivatives depress the electrical activity of neurons[12,13].

In the present investigation, we wanted to see whether forskolin is able to affect seizures induced by the convulsant drug, pentylenetetrazol.

Materials and methods. Experiments were performed on male ICR mice weighing 35 to 37 g, 2 months old, which were maintained on a 12:12 h light-dark cycle. Forskolin, purchased from Calbiochem-Behring Corp., was disolved in ethanol and diluted with Ringer solution to 8.3% ethanol (V/V) just before its use, and was injected s.c. in 0.1 to 0.125 ml. Pentylenetetra-

zol was disolved in Ringer solution and 0.1 ml of solution was injected i.p., and behavioral changes were observed for 30 min. To determine cAMP levels in various tissues, the whole animal was frozen by rapid immersion in liquid N_2 and stored at −80 °C. Subsequently, brain and other organs were dissected out at −20 °C, and homogenized with 6% trichloroacetic acid followed by centrifugation. The trichloroacetic acid extracts were neutralized with $CaCO_3$ according to the method of Tihon et al.[14]. Cyclic AMP was measured using the radioimmunoassay method described by Steiner et al.[15]. The materials for the assay were purchased from Yamasa Chemical Corp. (Choshi, Japan). Protein was determined by the method of Lowry et al.[16] with bovine serum albumin as standard.

Results and discussion. As shown in figure 1a and 1b, forskolin effectively prevented tonic seizures induced by 50 mg/kg pentylenetetrazol when injected 2 or 10 min, but not 30 min prior to the convulsant. In another series of experiments, when the higher dose of pentylenetetrazol (70 or 80 mg/kg) was used, the forskolin-treatment could not prevent tonic seizures, but reduced seizure intensity and decreased mortality of the animals (fig. 1c). The injection of forskolin (tested up to 7 mg/kg) made the animal obviously quiet a few minutes after the administration. However, no animal exhibited neurotoxicity for the observation period of 30 min. The animals remained apparently healthy for at least 3 weeks after the injection of forskolin.

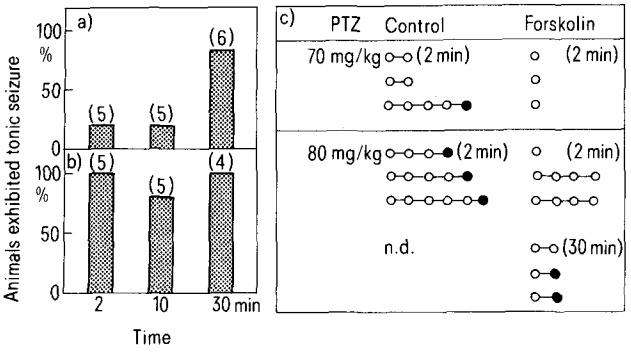

Figure 1. Effect of forskolin on seizures induced by pentylenetetrazol. Pentylenetetrazol (50 mg/kg) was injected i.p. at the time indicated after the s.c. injection of forskolin at a dose of 1.25 mg/kg (a) or an equivalent volume of ethanol-Ringer solution mixture (b). The percentage animals exhibiting tonic seizure(s) over 30 min was counted. The number of mice tested is indicated in parenthesis. Following the injection of forskolin, the effect of the higher dose of pentylenetetrazol (70 or 80 mg/kg) was also examined (c). Number of tonic seizures (○) or seizures with ensuing death (●) observed for 30 min, are illustrated. Data represent duplicate experiments.

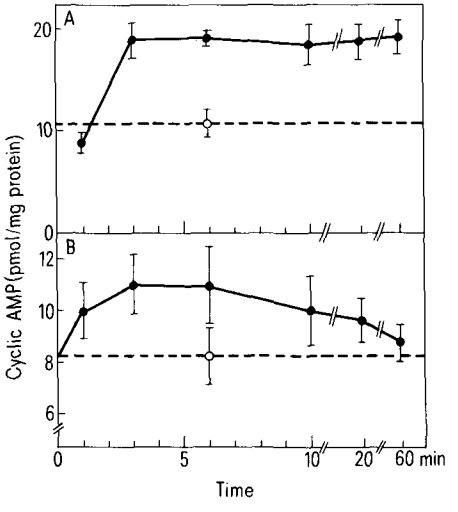

Figure 2. Time course of changes in cAMP levels in the mouse forebrain (A) and cerebellum (B). Animals received forskolin (1 mg/kg) (●) or an equivalent volume of ethanol-Ringer solution mixture (○) and were sacrificed at the time intervals indicated. Cyclic AMP levels were determined as described in the text. Data are means ± SD from 3 animals.

Forskolin also caused an elevation of cerebral and cerebellar cAMP levels (up to 170% and 130%, respectively) 6 min after administration (fig. 2). Forskolin in doses between 0.1 to 1.0 mg/kg dose-dependently raised the cerebral cAMP content when measured 6 min after the injection (data not shown). Forskolin had little effect on the cAMP content of other organs such as heart, liver or lung in mice (not shown).

These results, showing that forskolin prevented or lowered the seizure activity induced by pentylenetetrazol and, at the same time, increased the cAMP level in the brain but little in other organs, suggest that forskolin may change seizure activity by the elevation of the cAMP level in the brain, supporting the hypothesis of Ferendelli[12].

It is important to note that the responsiveness to pentylenetetrazol had already recovered 30 min after the injection of forskolin as shown in figure 1. At that time, cAMP was again down to the normal level in the cerebellum but was still elevated in the cerebrum. In spite of the limited number of mice used, the coincidence of loss of the anticonvulsant activity of forskolin and the return to normal of the diterpene-induced cAMP accumulation in the cerebellum, suggests the possibility that an elevation of cerebellar cAMP may be responsible for the anti-pentylenetetrazol activity of forskolin.

It is not known whether an elevation of cAMP in the brain affects pentylenetetrazol-induced seizure. Nevertheless, the present study is the first demonstration that administration of forskolin has an inhibitory effect on pentylenetetrazol-induced seizure. Further investigations should be made in the evaluation of forskolin as a candidate for a new type of anticonvulsant.

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