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Decrease of serum alpha-tocopherol levels in rabbits after acute treatment with Δ^9-tetrahydrocannabinol[1]

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Summary. The i.v. administration of 10 mg of Δ^9-THC in rabbits produced, after 24 h, a 50% reduction of the initial values of serum alpha-tocopherol.

Our studies on chronic toxicity of Δ^9-THC (Δ^9-tetrahydrocannabinol) demonstrated the occurrence of a true muscular dystrophy in mice[2,3], and of a persistent alteration of bone marrow leucopoiesis in mice and rats[4,5] after 1-month treatment with Δ^9-THC at behavioral doses. Other authors reported sterility and infertility associated with testicular atrophy in experimental animals[6] and decreased serum testosterone levels in humans[7]. As these data are reconcilable with a relative alpha-tocopherol deficiency, the hypothesis of a possible interference of Δ^9-THC with serum alpha-tocopherol was taken into account.

Materials and methods. 10 male rabbits, weighing 2.5–3 kg, fed with a normal diet containing 14 mg/kg of alpha-tocopherol, were used. After 12 h fasting, 2–3 ml of blood was drawn from the auricular vein and about 1 ml of serum was obtained. Immediately after, rabbits were injected i.v. with 10 mg of Δ^9-THC suspended in the vehicle (1 ml of phosphate buffer pH 7.4 containing 2 drops of Tween 80). Samples of blood were drawn after 5 h and after 24 h. During this period, food was withheld. Control animals were injected with the vehicle alone.

Levels of alpha-tocopherol in serum were determined by gasliquid chromatography with solid injection, as described elsewhere[8].

Results and discussion. Results are summarized in the table and the figure. As can be seen, the injection of the vehicle and the food deprivation for 24 h have only a very limited influence on the alpha-tocopherol level, while the administration of Δ^9-THC produced a significant decrease of serum

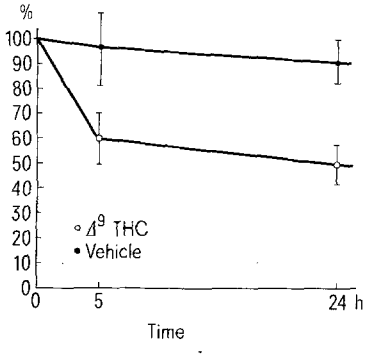
Number	Δ^9-THC			Vehicle		
	t_0	t_1	t_2	t_0	t_1	t_2
1	1.33	0.48	0.63	0.96	0.93	0.96
2	1.14	1.22	0.58	1.20	1.86	1.33
3	1.14	1.00	0.85	0.53	0.43	0.56
4	1.89	0.56	0.40	3.80	3.20	3.00
5	1.68	0.53	0.45	1.60	1.20	1.00
6	1.62	1.60	1.57			
7	1.30	0.70	0.60			
8	0.45	0.10	0.13			
9	1.98	1.51	1.27			
10	1.50	0.80	0.60			
$\bar{X}$	1.40	0.86	0.71	1.61	1.52	1.37
SE	0.14	0.15	0.13	0.57	0.47	0.42

t_0, Concentrations of alpha-tocopherol in serum (mg‰) before the treatment; t_1, concentrations 5 h after treatment; t_2, concentrations 24 h after treatment.

alpha-tocopherol after 5 h, and the reduction to about 50% of the initial value after 24 h.

No in vitro interactions of alpha-tocopherol with Δ^9-THC were observed in presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH·) according to the method of Boguth[9], at IR-spectrophotometry and by elution of the mixture of the 2 compounds on Sephadex LH-20 columns.

In experimental animals, however, the pertinent data of the literature and of our previous studies and the results of the present research seem to demonstrate that the normal intake of vitamin E with food is not sufficient to prevent losses following administration of Δ^9-THC. Studies on the effects of alpha-tocopherol given in excess to animals chronically treated with Δ^9-THC are in progress[10]. In humans no such lesions or biochemical related alterations have been reported, but an inconstantly reduction of testosterone levels and cases of gynaecomastia[11], which might possibly be consistent with a relative alpha-tocopherol-deficiency. We have no data at present to explain the interaction of alpha-tocopherol with Δ^9-THC. However, it has been stated that alpha-tocopherol, by interference with arachidonic acid, has a stabilizing action on the cellular membrane[12], while Δ^9-THC has an opposite action, as demonstrated by the alteration of membrane enzymes[13]. It is possible that alpha-tocopherol is firmly bound in plasma to Δ^9-THC or to Δ^9-THC-lipoprotein complex, or involved in the metabolism of Δ^9-THC.



See in abscissa the time (h) after treatment; in ordinate the percent of the initial values of serum alpha-tocopherol.

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Effects of lithium chloride on peripheral acetylcholine release and brain acetylcholine levels in the guinea-pig

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Summary. Lithium chloride administered acutely or chronically to guinea-pigs had no effect on brain level of acetylcholine or on peripheral release of acetylcholine from longitudinal muscle of the ileum. The results suggest differences between in vitro and in vivo action of lithium.

Acute in vitro application of lithium chloride (LiCl) has been shown to enhance the resting release of acetylcholine (ACh) while decreasing evoked release at neuromuscular junction[4,5], in ganglia[6], from brain cortical slices[7,8], and at smooth muscle neuroeffector junction[8,9].

When injected chronically, in vivo, LiCl has been shown to decrease the level of ACh in the medulla-oblongata area of rat brain, while producing no changes in any other brain area[10]. On the other hand, evoked release of ACh from cortical slices taken from rats treated chronically with LiCl was not different when compared with slices taken from controls[11].

It would appear that there are some differences between acute in vitro and chronic in vivo effects of LiCl. Since the clinical use of LiCl requires chronic in vivo application, it would be of interest to simultaneously study LiCl for both peripheral and central effect on ACh in the same animal. The guinea-pig provides the means for doing this. The purpose of the present work was to investigate the effect of acute and chronic LiCl in vivo on ACh release at the neuroeffector junction in guinea-pig ileum myenteric-plexus preparation and on levels of ACh in whole brain from the same animal.

Material and methods. Male albino guinea-pigs (250–300 g) were injected once daily with either 2.5 mEq/kg of LiCl or an equal volume of 0.9% saline (NaCl). Animals were sacrificed either 2 h following a single injection (acute treatment) or 2 h after the final injection of a series of between 5 and 10 injections (chronic treatment). Animals were sacrified by decapitation and blood samples were immediately taken for serum analysis of LiCl.

The ilea from these animals were removed, the longitudinal muscle stripped off and set up for electrical stimulation as previously described[12]. Acetylcholine output at rest and following electrical stimulation (0.4 msec, 0.3 Hz, supramaximal voltage) was measured using a bioassay technique[13]. All collection periods were 30 min, and unknown samples were matched against standard curves derived from assaying known quantities of ACh. Standards were run each day of the experiment. Pretreatment of the assay tissue with 3×10^{-7} M atropine sulfate completely eliminated the responses to the ACh standards and the unknown samples, confirming that contraction of the assay tissue to the unknown samples was the result of the presence of ACh.

The brains of these animals minus cerebellum were