Br. J. Pharmac. (1977), **59**, 599–601

EFFECTS OF Δ^9-TETRAHYDROCANNABINOL AND CANNABIDIOL ON A Mg^{2+}-ATPase OF SYNAPTIC VESICLES PREPARED FROM RAT CEREBRAL CORTEX

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1 Δ^9-Tetrahydrocannabinol and cannabidiol both exhibited a concentration-related inhibition of Mg^{2+}-ATPase of vesicles prepared from synaptosomes isolated from rat cerebral cortex. Cannabidiol was about 3 times more potent than tetrahydrocannabinol.

2 These results were similar to those obtained previously using drugs with well established anticonvulsant activity.

3 Tetrahydrocannabinol at a sub-inhibitory concentration (1 μM) increased the activity of the Mg^{2+}-ATPase relative to values obtained with vehicle controls.

Introduction

The effects of a wide range of drugs on the activity of the magnesium-activated adenosine triphosphatase (Mg^{2+}-ATPase) of synaptic vesicles prepared from rat cerebral cortex have previously been studied (Gilbert & Wyllie, 1975; 1976). Of the drugs tested only those known to have anticonvulsant activity were found to inhibit this ATPase. Δ^9-Tetrahydrocannabinol (tetrahydrocannabinol) and cannabidiol, both constituents of cannabis, exhibit anticonvulsant activity in rats (Izquierdo, Orsingher & Berardi, 1973) and mice (Karler, Cely & Turkanis, 1973) and it was therefore of interest to investigate the effect of these cannabinoids on the activity of the vesicular Mg^{2+}-ATPase.

Methods

Mitochondrial and synaptosomal fractions were prepared from cerebral cortices of male Sprague–Dawley rats (body wt. 150 to 350 g) by a modification of the method of Gray & Whittaker (1962) as described by Gilbert & Wyllie (1976). Synaptic vesicles were prepared by osmotic disruption of synaptosomes and subsequent sucrose density gradient centrifugation as described by Whittaker, Michaelson & Kirkland (1964). The fractions were stored at −23°C.

For ATPase assays, vesicle-containing fractions were resuspended in 50 mM imidazole/HCl buffer pH 7.4 containing $MgCl_2$ (5 mM). The assays were carried out with 0.9 ml samples of this mixture, each sample containing 0.1 to 0.2 mg protein.

The samples were pre-incubated for 15 min at 37°C before the reactions were started by addition of 0.1 ml Tris-ATP solution (4 mM final concentration). Sodium dodecyl sulphate (DDS 1 ml, 0.8% w/v in water) was used to stop the reactions after 10 minutes. The phosphate contents of the clear solutions were determined by the method of Bonting, Simon & Hawkins (1961). Blanks were prepared by addition of DDS after the pre-incubation period, before the addition of the ATP.

The activity of Mg^{2+}-ATPase was calculated by subtraction of the phosphate released in the blank from that in the Mg^{2+}-ATPase reaction mixture.

The protein contents of solutions were determined by the method of Lowry, Rosenbrough, Farr & Randall (1951).

Drugs were added to the pre-incubation medium in aliquots of 5 μl. Tetrahydrocannabinol and cannabidiol were added in absolute ethanol. Appropriate control experiments with ethanol were included. Drug effects were expressed as the percentage change in the activity of the enzyme in the absence of ethanol or any other drug. Limits of error were expressed as standard error and differences between means were evaluated by Student's *t* test (paired).

Results

The results are summarized in Table 1. Ethanol (78 mM) produced a small but statistically significant degree of inhibition of the Mg^{2+}-ATPase. Cannabidiol also inhibited the enzyme. This inhibition was significantly greater than ethanol control values at

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concentrations of 1 μM and above. The effect of tetrahydrocannabinol appeared to be biphasic. At concentrations ranging upwards from 3 μM tetrahydrocannabinol was inhibitory, but at a drug concentration of 1 μM the activity of the vesicular Mg^{2+}-ATPase was 14.5 ± 1.4% greater than it was in the presence of ethanol alone. The degree of inhibition produced by both cannabinoids reached a maximum at and above concentrations of 0.1 mM. This maximum effect consisted of a 69 to 70% inhibition of the Mg^{2+}-ATPase activity. Experiments with sub-maximal concentrations of the cannabinoids showed the inhibitory effect of the vesicular Mg^{2+}-ATPase to be concentration-related and the relative inhibitory potency of cannabidiol and tetrahydrocannabinol to be about 3.

Because of the low solubility of the cannabinoids in water, the question as to whether or not inhibition of vesicular Mg^{2+}-ATPase by ethanol and by the cannabinoids is additive was not investigated. Such an investigation was however carried out with the more water soluble phenytoin. In the absence of ethanol, phenytoin inhibited the ATPase by 36.5 ± 1.1% at a concentration of 1 μM and by 71.3 ± 0.5% at a concentration of 20 μM. In the presence of ethanol, inhibition by these concentrations of phenytoin was respectively 49.5 ± 3.7% and 78.7 ± 0.6%. In these experiments, ethanol produced an inhibition of 8.6 ± 2.3%. The results confirm that phenytoin can inhibit vesicular Mg^{2+}-ATPase and suggest that the inhibitory effects of phenytoin and of ethanol are indeed additive.

Discussion

The results show that tetrahydrocannabinol and cannabidiol can both inhibit vesicular Mg^{2+}-ATPase. Since both drugs have been reported to exhibit anticonvulsant activity *in vivo* (Izquierdo *et al.*, 1973; Karler *et al.*, 1973) the results add to the evidence (Gilbert & Wyllie, 1976) for the concept that the capacity to inhibit the vesicular Mg^{2+}-ATPase is a characteristic property of anticonvulsant drugs.

There are similarities between the effects of the cannabinoids reported in this paper and the effects (reported elsewhere, Gilbert & Wyllie, 1976) of other anticonvulsant drugs. The concentrations at which tetrahydrocannabinol and cannabidiol inhibited vesicular Mg^{2+}-ATPase are of the same order as those at which other anticonvulsant drugs produce inhibition. As with the other anticonvulsants so far studied, the inhibition of vesicular Mg^{2+}-ATPase produced by the cannabinoids at maximal concentrations was not complete. This incomplete inhibition may result from the heterogeneity of the nerve terminal content of the synaptosome preparation. This point has been discussed elsewhere (Gilbert & Wyllie, 1976).

A sub-inhibitory concentration of tetrahydrocannabinol was found to increase Mg^{2+}-ATPase activity relative to values observed in the presence of ethanol, the vehicle in which the drug was dissolved. Further experiments are needed to determine whether this observation reflects direct activation of the enzyme by tetrahydrocannabinol or antagonism of the inhibitory effect of ethanol. In addition it would be interesting to determine whether, at sub-inhibitory concentrations, cannabidiol increases the activity of Mg^{2+}-ATPase.

Finally, it is of interest that the relative potency of tetrahydrocannabinol and cannabidiol in inhibiting vesicular Mg^{2+}-ATPase is of about the same value as the relative potency of these cannabinoids in decreasing the susceptibility of rat hippocampus to seizure discharges caused by afferent stimulation (Izquierdo *et al.*, 1973). However, without further experimental data it would not be profitable to speculate about this correlation which could well have arisen by chance and the question of whether there is a 'cause-effect' relationship between the inhibition of vesicular Mg^{2+}-ATPase and anticonvulsant activity still remains to be answered.

We thank Dr M.C. Braude of NIMH for supplies through the MRC of Δ^9-tetrahydrocannabinol and Professor R. Mechoulam for a gift of cannabidiol.

Table 1 Effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) on vesicular Mg^{2+}-ATPase

Cannabinoid concentration (μM)	*Mean % inhibition of Mg^{2+}-ATPase (± s.e.)* Δ^9*THC*	P	*CBD*	P	*Ethanol control*
1	3.8 ± 1.9 (4)	<0.01	19.3 ± 2.1 (4)	<0.02	10.7 ± 2.4 (4)
3	19.3 ± 2.6 (2)	<0.02	39.6 ± 2.1 (2)	<0.09	8.6 ± 2.3 (2)
10	43.2 ± 1.4 (4)	<0.001	57.5 ± 1.7 (4)	<0.001	10.7 ± 2.4 (4)
100	68.8 ± 4.3 (5)	<0.001	65.1 ± 1.2 (5)	<0.001	11.8 ± 2.0 (5)
1000	67.7 ± 0.5 (2)	<0.06	69.6 ± 0.6 (2)	<0.06	12.9 ± 4.4 (2)

Number of experiments are given in parentheses. Student's *t* test (paired) was used to evaluate (*P*) differences between drug treatment and ethanol controls. A typical value for vesicular Mg^{2+}-ATPase activity (in the absence of ethanol) was 5.25 ± 0.17 (4) μmol Pi mg protein^{-1} h^{-1}.

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(Received August 11, 1976. Revised October 12, 1976.)