investigated the influence of these two cyclic nucleotides on glycogen stores in brain slices of rats treated with propranolol, atropine, reserpine and chlorpromazine.

Materials and methods. The experiments were carried out on adult male Wistar rats. Brain slices were prepared according to the method already described[19] and were allowed 10 min in saline[19] at 37 °C. CAMP (10^{-3} μM/ml), dibutyryl-CAMP (10^{-3} μM/ml) or CGMP (10^{-3} μM/ml) alone and in combination with atropine (0.5 μ/ml) were added at the beginning of the incubation. Propranolol (10 mg/kg) or atropine (0.5 mg/kg) were injected i.p. 30 min before the animals were sacrified. Reserpine (2 mg/kg) was twice administered i.p.; the second administration was made 24 h after the first one and 12 h before the rats were sacrified. Chlorpromazine (2.5 mg/kg) was administrated 3 h before the animals were sacrified. Brain slices of rats treated before with propranolol, atropine, reserpine or chlorpromazine were incubated in the presence of CAMP, db-CAMP or CGMP. In all cases, after 10 min of incubation, from the brain tissue, glycogen was extracted[20] and estimated[21].

Results and discussion. The results obtained show that CAMP, db-CAMP, as well as CGMP in cerebral cortex, caudate and cerebellar cortex, decreased glycogen concentration (Table I). The glycogenolytic effect of CGMP is similar to the influence of db-CAMP. On the other hand, atropine in vitro prevented glycogenolytic effect of CGMP in cerebral cortex and caudate, but not the effects of CAMP, db-CAMP as well as the effect of CGMP in cerebellar cortex (Table II). Propranolol prevented the glycogenolytic influence of CGMP in cerebral cortex, caudate and cerebellar cortex, but not that of CAMP or db-CAMP (Table III). Atropine, on the other hand, prevented glycogenolytic effect of CGMP in cerebral cortex and caudate, but not in cerebellar cortex, as well as the effects of CAMP and db-CAMP (Table III). Reserpine (Table IV), which destroys noradrenaline stores in nerve cells, and chlorpromazine (Table V), which is known to block adrenergic receptor sites, also prevented the glycogenolytic effect of CGMP in rat cerebral cortex, caudate and cerebellar cortex, but not that of adenin cyclic nucleotides.

Physiological role of CGMP is still under consideration. A dissociation between CAMP and CGMP level was observed in the mouse brain following decapitation; CAMP level was elevated at the time when CGMP level was unaltered or slightly decreased[22]; after treatment with oxotremorine, CGMP content increased (this effect could be blocked by atropine) while CAMP level decreased in mouse brain[23]. Oxotremorine also decreased glycogen concentration in rat brain[24]; the effect could be blocked by atropine or propranolol[24].

The results obtained show that both CAMP and CGMP produced glycogenolysis in rat cerebral cortex, caudate and cerebellar cortex; drugs which are known to interact with adrenergic receptors could prevent the glycogenolytic effect of CGMP, but not that of adenosine cyclic nucleotide. The persistence of glycogenolytic influence of CAMP and db-CAMP, after treatment of animals with propranolol, reserpine or chlorpromazine, indicates the postsynaptic site of action of this cyclic nucleotide; at least our results suggest that the mechanisms of action of CAMP and CGMP in rat brain are different.

Table V. The influence of chlorpromazine (2.5 mg/kg) on glycogenolytic effects of cyclic nucleotides (10^{-3} μM/ml) in rat brain slices

Treatment of the tissue	Brain slices		
	Cortex cerebri	Caudate	Cortex cerebelli
Controls	33.5 ± 1.4	49.5 ± 1.3	69.5 ± 1.2
CAMP	26.7 ± 1.3[a]	35.4 ± 1.5[a]	55.4 ± 1.3[a]
db-CAMP	15.4 ± 1.9[a]	23.0 ± 1.3[a]	45.0 ± 1.3[a]
CGMP	30.4 ± 1.2	46.4 ± 1.7	71.5 ± 1.4

[a] $p < 0.01$ in comparison with the controls. The amount of glycogen is expressed in mg/100 ml of tissue. The numbers indicate the mean value (M) ± S.E.M.; 5 experiments in each group.

Résumé. Le propranolol et l'atropine empêchent l'effet glycogénolytique du GMP in vitro, bien qu'ils n'aient pas d'influence sur les actions du 3′,5′-AMP cyclique et son dérivé dibutirique. La rézerpine et la chlorpromazine empêchent l'effet glycogénolytique du GMP cyclique. Les résultats suggèrent une localisation présynaptique de l'action du GMP cyclique.

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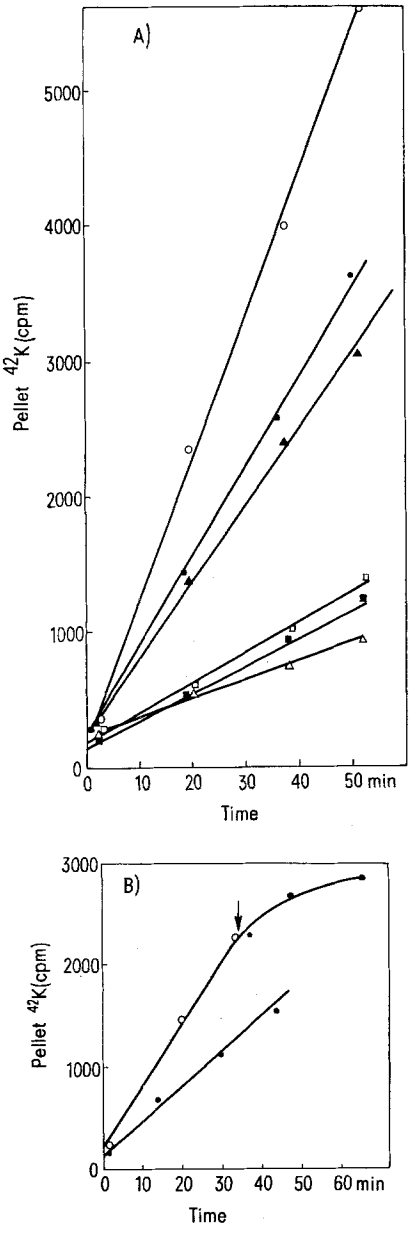
Effect of Δ^1-Tetrahydrocannabinol[1] on K^+ Influx in Rat Erythrocytes

Δ^1-Tetrahydrocannabinol (THC), the major psychoactive component of HASHISH[2], protects both rat and human erythroxytes against hypotonic hemolysis in vitro[3,4], at a concentration which may correspond to those leading to psychomimetic reaction in hashish smokers[5]. This protection is more prominent at pH 6 than at either pH 7 or 8, and at room temperature, 20 °C, more than at 37 °C[3]. It has recently been reported[6] that THC decreases ATPase at the THC concentration which causes maximum protection (0.02–0.1 mM). In view of these findings we decided to study the effect of THC on cation transport in red blood cells.

Materials and methods. THC was obtained from Dr. MECHOULAM of the Hebrew University, Jerusalem, and was used after solution in ethanol. Packed rat erythrocytes from freshly drawn blood in heparin, were washed twice and resuspended to 5% hematocrit in a solution consisting of: 10 mM sodium phosphate buffer of either pH 6 or 7, 4 mM Kcl, 4 mM $MgCl_2$ and 154 mM NaCl. Aliquots of 1 ml were transferred into 13 mm diameter tubes and

Effect of THC on K^+-uptake by rat erythrocytes

THC concentration [mM]	Inhibition of K^+ influx [%]			
	37 °C		20 °C	
	pH 6	7	6	7
0	0	0	0	0
0.06	46 ± 4	11 ± 3	36 ± 4	18 ± 3
0.01	35 ± 3	0 ± 5	10 ± 3	



Kinetics of ^{42}K uptake by treated and control rat erythrocytes. Samples were removed and the erythrocytes pellet separated and counted as described in the text. At time zero ^{42}K was added to the suspensions. A) pH = 6. ○, control; ▲, 0.06 mM THC; ●, 0.01 mM THC, at 37 °C; □, control; △, 0.06 mM THC; ■, 0.01 mM THC, at 20 °C. THC was added about 2 min before ^{42}K. B) pH = 6. ○, control; ●, 0.06 mM THC. ↑, THC was added.

incubated at either 20° or 37 °C with gentle shaking. After 3 min incubation, 10 µl THC was added to each tube, to a final concentration of either 0.01 mM or 0.06 mM. Ethanol (10 µl) without THC, served as control. About 2 min later, a 50 µl aliquot of a ^{42}K solution containing 10 mM KCl and 140 mM NaCl at pH 7.3, was added to each tube. The radioactivity was about 200 µCi/ml. The uptake of the radioisotope by the cells was determined by radioactivity measurements of erythrocyte pellets collected at various time intervals. The cells were separated from the extracellular medium by the differential flotation method described previously[7]. Separations were carried out in polyethylene tubes of about 0.4 ml. Each tube contained 0.05 ml of the separating fluid (density of 1.066 g/cm^3) to which 0.1 ml of the erythrocyte suspension was added.

Results and discussion. The results of typical experiments (Figure A) show that THC inhibits K^+ influx. The results obtained from a number of experiments (Table) indicate that the inhibitory effect of THC is more pronounced at pH 6 than at 7. Similar pH dependence has been reported for other anesthetics[8]. The K^+ influx in the control cells is not affected by the addition of ethanol to the cell suspension (1:100 v:v), nor by varying the pH value from 6 to 7.

Although no hemolysis of erythrocytes was observed during the experiments, it seemed necessary to ascertain that the reduced intracellular ^{42}K of THC-treated cells is a result of an inhibitory effect on K^+ influx. For this purpose an aliquot of THC was added to the red blood cell suspension 30 min after the addition of ^{42}K. In a similar experiment THC was added 60 min after ^{42}K. Following the addition of THC, the ^{42}K kinetics uptake function continued to behave as a monotonic function (Figure B). This is expected as long as the intracellular specific activity of ^{42}K is considerably lower than the extracellular one. Damage to the cells causing a leak of K^+ would have produced a transient decrease in the uptake of ^{42}K and a break in the monotonicity of the function. It is therefore concluded that THC is a K^+ influx inhibitor.

Zusammenfassung. Nachweis, dass Tetrahydrocannabinol als psychoaktive Haschischkomponente an der Membran den K^+-Eintritt in die Ratten-Erythrozyten mit Wirkungsoptimum bei pH 6,0 (in Konzentrationen, die bei Haschischrauchern zu psychomimetischen Reaktionen führen) hemmt.

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