Effect of 6-Hydroxydopamine on the Duration of Hexobarbital Sleep in Rats

The aim of this study was to investigate the duration of hexobarbital sleep in rats after chemical sympathectomy of the central nervous system (CNS).

Methods. Experiments were carried out on 33 male Wistar rats, weighing approximately 200–230 g and divided into 3 groups. Chemical sympathectomy of CNS was induced in 13 rats. 250 µg of 6-hydroxydopamine – HCl (6-OHDA) (Kistner Lab. Göteborg) in 20 µl of the artificial cerebrospinal fluid[1] with 0.1 % ascorbic acid was injected under the aether anesthesia into the both lateral brain ventricles, according to HERMAN[2], twice in 2 days interval. 6-OHDA was dissolved immediately before injections. Separate samples were prepared for each injection. The control animals (11 rats) were injected with the equal quantity of the solvent in the same manner. 9 intact animals, as the separate control group, were injected neither 6-OHDA nor solvent.

After 28–30 days following the second injection of 6-OHDA, the hexobarbital-Na in the dose of 60 mg/kg was administered into the caudal vein of rats and the duration of the time of sleep was measured according to COURVOISIER et al.[3].

Results. The marked prolongation of the time of hexobarbital sleep after an i.v. administration of 6-OHDA was established in comparison to both control groups (see Table). No differences was noted in the time of sleep between the 2 control groups.

Discussion. The administration of 6-OHDA causes in animals the evident depletion of catecholamines (CA) and degeneration of adrenergic terminals defined as a chemical sympathectomy[4]. Application of 6-OHDA into the lateral brain ventricles causes a long-lasting reduction of CA level in CNS and degeneration of adrenergic neurons[5,6]. In our laboratory we have confirmed these observations previously[7]. Therefore, in the present experiments we have not determined CA level in the brain, assuming that the mode of application of 6-OHDA and the dose applied are sufficient to induce chemical sympathectomy of CNS.

There are data showing that CA take part in the mechanisms of physiological sleep[8,9]. The reduction of the level of CA in CSN by injection of 6-OHDA prolongs the duration of paradoxical sleep in rats[10]. The role of CA in the mechanism of barbital sleep is not defined unequivocally. Barbiturates induce the changes of the CA distribution of in CNS and influence the turn-over of noradrenaline in animal brain as well the dopamine synthesis[11,12]. On the other hand, it was shown that barbiturates does not change the CA level in animal brain[13]. Barbital anesthesia weakens the effect of 6-OHDA on noradrenaline level in the rat brain[14]. An increase of noradrenaline level in the CNS by exogenous administration prolongs the duration of hexobarbital sleep[15–17]. On the contrary, our results show that the reduction of CA level in the CNS by means of 6-OHDA injections prolongs the duration of hexobarbital sleep.

These results confirm the role of CA in hypnotic action of hexobarbital. But their role in this mechanism is complex and it is difficult to explain in the light of the present experiments.

Duration of hexobarbital sleep after central chemical sympathectomy in rats

Time of sleep (sec) hexobarbital – Na 60 mg/kg i.v.		
Control I intact rats	Control II 2 × artificial cerebro-spinal fluid	6-OHDA 2 × 250 µg
667 ± 28 $n = 9$	675 ± 14 $n = 11$	845 ± 25 $n = 13$ $p < 0.001$[a]

[a]Compared to both control groups.

Zusammenfassung. Nachweis einer Verlängerung des Hexobarbitalschlafes bei Ratten durch zentrale Anwendung von 6-Hydroxydopamin, was auf die Bedeutung der Katecholamine bei der Hexobarbitalwirkung hinweist.

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Cyclic Nucleotides and Brain Glycogen

A fair amount of evidence now exists indicating that neurotransmitter agents exert their biological actions by altering the amount of adenosine 3′,5′-monophosphate (CAMP) in target cells[1,2]. Recently it was shown that biogenic amines, such as noradrenaline, dopamine, histamine and serotonin, which increased the CAMP levels in cerebral cortical slices[3,4], showed glycogenolytic effects in rat brain slices[5–7]. In view of published data,

Table I. The effect of cyclic nucleotides (10^{-3} μM/ml) on glycogen concentration in rat brain slices

Treatment of the tissue	Brain slices		
	Cortex cerebri	Caudate	Cortex cerebelli
Controls	28.4 ± 1.3	45.7 ± 1.4	63.5 ± 1.8
CAMP	19.4 ± 1.4[a]	31.3 ± 1.3[b]	40.1 ± 1.4[b]
db-CAMP	12.4 ± 1.7[b]	20.1 ± 1.0[b]	28.9 ± 1.5[b]
CGMP	11.5 ± 1.5[b]	18.5 ± 1.6[b]	25.6 ± 1.3[b]

[a] $p < 0.05$ in comparison with the controls. [b] $p < 0.01$ 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.

Table II. In vitro influence of atropine (0.5 μ/ml) on glycogenolytic effect of cyclic nucleotides (10^{-3} μM/ml)

Treatment of the animals	Brain slices		
	Cortex cerebri	Caudate	Cortex cerebelli
Atropine (controls)	31.4 ± 1.6	47.6 ± 1.3	67.8 ± 1.4
CAMP + Atropine	22.4 ± 1.2[a]	30.6 ± 1.4[b]	43.4 ± 1.3[b]
db-CAMP + Atropine	10.4 ± 1.4[b]	23.4 ± 1.4[b]	30.1 ± 1.4[b]
CGMP + Atropine	30.4 ± 1.2	44.5 ± 1.3	23.4 ± 1.3[b]

[a] $p < 0.05$ in comparison with the controls. [b] $p < 0.01$ 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.

guanosine 3′,5′-monophosphate (CGMP) may possibly play a significant role and function in animal tissue. First found in urine[8], it is still, beside CAMP, only the other 3′,5′-cyclic nucleotide known to occur in nature.

Table III. The influence of propranolol and atropine on glycogenolytic effect of cyclic nucleotides (10^{-3} μM/ml) in rat brain slices

Treatment of the tissue	Brain slices		
	Cortex cerebri	Caudate	Cortex cerebelli
A) Treated with propranolol (10 mg/kg)			
Controls	30.2 ± 1.3	45.4 ± 1.3	65.2 ± 1.4
CAMP	17.5 ± 1.4[a]	30.8 ± 1.2[a]	42.5 ± 2.1[a]
db-CAMP	7.4 ± 1.3[a]	22.4 ± 1.4[a]	31.8 ± 2.0[a]
CGMP	28.5 ± 1.8	47.5 ± 1.8	62.4 ± 1.5
B) Treated with atropine (0.5 mg/kg)			
Controls	29.5 ± 1.3	46.5 ± 1.2	62.4 ± 1.8
CAMP	18.0 ± 1.2[a]	31.5 ± 1.4[a]	40.5 ± 1.2[a]
db-CAMP	7.8 ± 1.3[a]	24.5 ± 1.4[a]	32.5 ± 2.0[a]
CGMP	28.0 ± 1.1	49.4 ± 1.8	29.5 ± 1.5

[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.

Table IV. The influence of reserpine (2 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	38.4 ± 1.3	58.4 ± 1.2	79.5 ± 1.5
CAMP	25.4 ± 1.3[a]	33.5 ± 1.4[a]	52.4 ± 1.4[a]
db-CAMP	18.5 ± 1.2[a]	28.5 ± 1.2[a]	40.5 ± 1.3[a]
CGMP	35.4 ± 1.4	59.4 ± 1.8	81.5 ± 1.3

[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.

CGMP has been found in tissues and fluids of several species[9–11] and the enzymes have been found that catalyze the synthesis[12] and breakdown[13] of CGMP. Nevertheless, little is known about the factors that regulate the concentration of CGMP in tissue or about the nature of the biological effects that may be regulated by CGMP. According to the some suggestions, CGMP may behave, like CAMP, as a 'second messenger' in metabolic regulation[14] and may in some way be involved in the expression of some cholinergic effects[15,16]. Also, CGMP, as well as CAMP, showed the ability to produce a decrease of glycogen concentration in liver[17,18] and diaphragm[18].

Taking into account the possibility that the mechanisms of actions of CAMP and CGMP are different and that the glycogenolytic effect of CGMP was similar to the effect of physostigmine in diaphragm and liver[18], it was of interest to compare the effect of cyclic nucleotides on glycogen concentration in rat brain slices. On the other hand, in order to confirm the hypothesis that CAMP and CGMP have independent functional roles in the CNS, we

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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