EFFECT OF PIPRADROL ON ENERGY METABOLISM OF THE BRAIN DURING PROLONGED MUSCULAR ACTIVITY

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UDC 612.822.1.013.7-06:
612.766.1].014.46:615.214

Under the influence of prolonged muscular work (swimming for 5 h) the content of ATP, ADP, AMP, and creatine phosphate in the brain of albino rats was reduced, while anaerobic carbohydrate breakdown was intensified. The lowered concentration of high-energy phosphates was due to a deficiency of their resynthesis because of disturbance of the coupling of oxidative phosphorylation and of the integrity of the mitochondrial membranes. Administration of pipradrol to the rats before swimming led to more severe disturbances of oxidative brain metabolism, reduced the generation of high-energy phosphates, and thus evidently led to exhaustion of the energy reserves of the brain.

The writers have previously shown [2] that the increased working capacity of rats produced by the psychostimulator pipradrol is achieved through increased utilization of the energy reserves of the body.

Since the stimulant effect of pipradrol is based on its effect on the central nervous system it was decided to study the action of this compound on energy metabolism of the brain in rats during prolonged muscular exertion leading to fatigue.

EXPERIMENTAL METHOD

By biochemical and histochemical methods described previously [3, 5] the concentrations of glycogen, glucose, lactic acid, creatine phosphate (CP), and adenine nucleotides, the activity of hexokinase and of the succinate-, cytochrome-, and NAD·H_2-oxidase systems, the intensity of oxidative phosphorylation, and the mechanochemical properties of the mitochondria were determined in the brain of albino rats weighing 120-140 g, in a state of relative rest and after swimming for 5 h in water at a temperature of 28-30°C.

Pipradrol was injected subcutaneously in a dose of 1 mg/kg immediately before the rats were placed in water. The control group of animals received an injection of the same volume of physiological saline.

EXPERIMENTAL RESULTS AND DISCUSSION

Injection of pipradrol into intact rats (Table 1) had no significant effect on oxidative phosphorylation or on the content of individual components of the adenosine system (ATP, ADP, AMP) in brain tissue; the CP concentration was reduced (by 10%), while the concentrations of lactic acid (by 15%) and glucose (by 70%) were increased. This increase evidently enabled the brain to obtain increased amounts of glucose from the blood, for injection of pipradrol is followed by a decrease in the liver glycogen content and by hyperglycemia [2].

To assess the effect of pipradrol on the energy metabolism of the brain in fatigued rats, the compound was injected before muscular exertion. Animals of the control group became lethargic after swim-

Tomsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 11, pp. 35-37, November, 1971. Original article submitted January 22, 1971.

TABLE 1. Effect of Pipradrol on Some Indices of Carbohydrate-Phosphorus and Oxidative Brain Metabolism of Albino Rats after Swimming for 5 h (M ± m; results of 10-15 determinations)

Indices studied	Control		Pipradrol	
	rest	swimming	rest	swimming
Oxidative phosphorylation substrate glutamic + malic acid				
ΔO (in μatoms/mg protein)	1.02 ± 0.11	0.93 ± 0.04	1.20 ± 0.09	1.18 ± 0.21
ΔP (in μatoms/mg protein)	2.02 ± 0.29	1.02 ± 0.12	2.23 ± 0.36	1.18 ± 0.14
P/O	1.94 ± 0.08	1.09 ± 0.11	1.79 ± 0.14	1.00 ± 0.10
Substrate: succinic acid				
ΔO (in μatoms/mg protein)	1.00 ± 0.09	0.94 ± 0.10	1.24 ± 0.06	1.07 ± 0.08
ΔP (in μatoms/mg protein)	1.58 ± 0.12	1.09 ± 0.04	1.99 ± 0.19	0.91 ± 0.08
P/O	1.59 ± 0.14	1.17 ± 0.10	1.60 ± 0.14	0.84 ± 0.06
Optical density of mitochondrial suspension (ΔΣ/mg protein)	0.430 ± 0.02	0.340 ± 0.03	0.460 ± 0.04	0.36 ± 0.02
NAD·H_2-oxidase system (10^{-7} mole/NAD·H_2/mg protein/min)	0.30 ± 0.03	0.17 ± 0.01	0.31 ± 0.02	0.17 ± 0.01
Succinate-oxidase system (in μl/100 mg moist tissue/h)	193.0 ± 11.0	182.1 ± 11.9	234.8 ± 12.4	192.5 ± 12.3
Cytochrome system (in μl/100 mg moist tissue/h)	58.9 ± 3.8	44.2 ± 3.2	71.3 ± 5.3	46.3 ± 3.3
Glycogen (in mg%)	68.3 ± 1.5	48.2 ± 2.4	69.7 ± 3.9	50.5 ± 2.7
Glucose (in mg%)	41.4 ± 1.5	57.9 ± 2.2	71.5 ± 4.4	57.8 ± 2.4
Lactic acid (in mg%)	29.8 ± 1.3	37.0 ± 1.7	34.3 ± 1.4	43.8 ± 2.9
Creatine phosphate (in mg%)	8.4 ± 0.1	7.6 ± 0.2	7.6 ± 0.2	6.7 ± 0.3
ATP (in μmoles/g moist tissue)	1.86 ± 0.07	1.34 ± 0.03	1.82 ± 0.05	1.30 ± 0.08
ADP (in μmoles/g moist tissue)	0.63 ± 0.03	0.52 ± 0.03	0.63 ± 0.05	0.56 ± 0.03
AMP (in μmoles/g moist tissue)	0.40 ± 0.02	0.36 ± 0.03	0.42 ± 0.02	0.46 ± 0.05
Hexokinase (in milliunits)	84.2 ± 5.1	121.1 ± 12.3	137.4 ± 9.5	163.9 ± 9.9

ming for 5 h, they developed dyspnea, and the balance between utilization and resynthesis of high-energy phosphates in the brain was upset, with, in particular, a decrease in the ATP and CP levels (by 28 and 10%, respectively); glycolysis was considerably intensified (the glycogen concentration fell by 29% while the lactic acid concentration rose by 27%), in agreement with results in the literature [1].

A definite uncoupling of oxidation and phosphorylation, and a decrease in activity of the NAD·H_2-oxidase and cytochrome systems were observed in mitochondria isolated from the brain of rats after swimming for 5 h. The decrease in the P/O ratio on account of a decrease in the esterification of inorganic phosphate took place with the use of a mixture of glutamic and malic acids and also of succinic acid as the oxidation substrate. This effect was evidently dependent on swelling of the mitochondria and an increase in the permeability of their membranes, because it was accompanied by a decrease in optical density of the mitochondrial suspension.

Cytochemical investigation of oxidative enzymes in the neurons of all layers of the cortex and subcortical nuclei revealed high activity of succinate dehydrogenase and of the cytochrome system, especially in layers II, III, and IV. With the onset of fatigue, the activity of succinate dehydrogenase and the cytochrome system in the cortical neurons fell, but it remained almost normal in the subcortical structures.

In brain sections stained by Nissl's method with toluidine blue moderate chromatolysis was present in some of the cortical pyramidal cells. Disappearance of the Nissl's substance was most conspicuous around the nuclei, and in some cells also at the points where the dendrites left the cell body. Pictures very similar to that described above, but illustrating the character of RNA distribution in the neurons, were observed in the cortex in sections stained by Brachet's method with methyl green – pyronine.

Pipradrol had no beneficial action on the brain energy metabolism during prolonged muscular work. Just as in the control group, in the rats receiving pipradrol before swimming the concentrations of glycogen,

ATP and, in particular, CP (to a greater degree than in the control; $P < 0.05$) in the brain were reduced. The lactate concentration and hexokinase activity were significantly increased ($P < 0.05$ compared with the control), indicating intensification of glycolysis. A significant decrease in the intensity of respiratory phosphorylation and in the optical density of the mitochondrial suspension was observed. The loss of mineral phosphate during oxidation of a mixture of glutamic and malic acids was equal to that in the control, while during oxidation of succinic acid it was actually greater ($P < 0.05$).

Pipradrol did not prevent depression of the activity of the oxidative enzyme system studied, as the cytochemical analysis confirmed: in all layers of the cortex, activity of succinate dehydrogenase and of the cytochrome system was significantly reduced. In sections stained by Nissl's method with toluidine blue, various forms of chromatolysis were observed in the overwhelming majority of cortical neurons, starting from central and ending with total (in some cells of the gangionic layer). The character of RNA distribution in the cytoplasm of these cells was considerably disturbed and its concentration greatly reduced.

During prolonged and intensive muscular exertion, pipradrol thus aggravates the disturbance of oxidative brain metabolism and reduces the generation of high-energy phosphate, thus evidently impairing the energy supply of the brain tissue. Evidently as a result of the reduced efficiency of respiratory phosphorylation, the compensatory activation of glycolysis mentioned above takes place, with the object of covering the energy requirements of the brain.

In the experiments in vitro, pipradrol in a final concentration of 1 : 20,000 had no effect on the indices of oxidative metabolism studied. This suggests that its action on brain energy metabolism is indirect in character. The increase in working capacity and the abolition of signs of fatigue produced by pipradrol are evidently due to weakening of the active inhibition in the central nervous system [5] which prevents exhaustion of the brain energy reserves.

LITERATURE CITED

1. L. G. Leshkevich, Fiziol. Zh. SSSR, No. 4, 475 (1951).
2. A. S. Saratikov et al., in: Proceedings of a Scientific Conference on the Action of Neurotropic Drugs on Trophic Processes and Tissue Metabolism [in Russian], Leningrad (1966), p. 76.
3. A. S. Saratikov, É. I. Voloshina, and S. A. Sakharova, Zh. Nevropat. i Psikhiat., No. 7, 995 (1970).
4. A. S. Sartikov et al., Izvest. Sibirsk. Otdel. Akad. Nauk SSSR. Seriya Biol.-Med. Nauk, No. 1, 108 (1968).
5. M. Steinbach, Pharmakopsychiatrie, 1, 303 (1968).