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SHORT COMMUNICATION

Effects of dimethylsulfoxide on metabolism of isolated perfused rat brain

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There is a considerable amount of evidence indicating that dimethylsulfoxide (DMSO) alters the permeability of cells and facilitates the transport of substances across membranes [1]. DMSO has been shown to enhance the transport across the blood–brain barrier of the drug, pemoline [2], of L-dopa [3], and of the catecholamines [4]. This property of DMSO makes it a potentially valuable agent for the study of psychopharmacologic agents and their effects on the metabolism of the brain. Such studies require a knowledge of the effects of DMSO itself on the brain and its metabolism. We have investigated the changes in the metabolism of the isolated rat brain during perfusion with fluid containing DMSO. We found that DMSO caused an increase in the rate of glycolysis, a slight decrease in the energy reserves of the brain, and a definite shift to a more reduced state in the brain tissue.

Unanesthetized male rats of the Long–Evans strain, weighing about 200 g, were rendered deeply hypothermic (rectal temperature 18°) as described previously [5]. The isolated brain preparation, consisting of the detached skull and its contents, was made without lapse of circulation from the hypothermic rat and was perfused through the internal carotid arteries as described previously [6]. The perfusion fluid [7] contained the perfluoro compound FC-80 (3M Co., St. Paul, Minn.) as an erythrocyte substitute, which was dispersed ultrasonically in an 8% (w/v) solution of bovine serum albumin (fraction V powder, Sigma Chemical Co., St. Louis, Mo.) in Krebs–Ringer bicarbonate buffer. The concentration of glucose in the perfusion fluid was 2 mg/ml (11 mM). Closed circuit perfusion was performed at 25° with continuous oxygenation of the perfusion fluid with 5% CO_2–95% O_2. The venous drainage during the first 10 min was discarded, recirculation was started, and then DMSO was added to the perfusion fluid. Biopolar electroencephalograms (EEG) were recorded from electrodes placed in depressions made in the bone of the frontal and parietal regions on both sides. If the EEG was not normal during the first 10 min of perfusion, the preparation was discarded. Samples of perfusion fluid were collected at periodic intervals from the venous drainage directly into pipettes. The perfusion fluid samples were analyzed for glucose by a glucose oxidase method using the commercial reagent Glucostat (Worthington Biochemical Co., Freehold, N. J.), for lactate with lactate dehydrogenase spectrophotometrically [8], and for potassium by flame photometry.

At the end of the perfusion period, the calvarium was removed while perfusion continued and the brain preparation was rapidly crushed between the jaws of heavy tongs which had been immersed in liquid nitrogen. This caused the cerebral tissue to be extruded from the skull. The whole preparation was immediately immersed in liquid nitrogen. The extruded frozen cerebral tissue was separated from bone and stored in liquid nitrogen until analysis was performed. Extracts of the brain tissue were made and analyzed for glycolytic intermediates, creatine phosphate and adenine nucleotides by enzymic fluorometric methods described previously [9].

In the experiments [4] in which DMSO was shown to facilitate the transport of catecholamines into the brain, the dose injected was 2.75 g/kg. In other experiments [2, 3] in which DMSO was injected, the dosage was similar or lower. Since DMSO penetrates most cells rapidly, it is reasonable to assume that it becomes distributed in all of the body water; this would result in a concentration in blood and body fluids which is about 0.4 g/100 ml or 0.05 M. Therefore in our experiments, we used concentrations of 0.01 and 0.1 M DMSO in the perfusion fluid.

Isolated rat brains were perfused for 40 min with fluid containing either 0.01 or 0.1 M DMSO or no DMSO (controls). The EEG (recorded from electrodes on the skull) of the brains perfused with DMSO contained spikes and occasional bursts of spikes which were more frequent in the brains perfused with 0.1 M than with 0.01 M DMSO. The EEG of the controls showed a relatively constant amplitude for the duration of the perfusion period whereas those perfused with DMSO showed decreasing amplitudes, with the effect more marked in the brains perfused with 0.1 M DMSO.

Table 1. Effects of DMSO on metabolic rates of isolated, perfused rat brains*

	Control	Concn of DMSO in perfusion fluid	
		0.01 M	0.1 M
Glucose consumption (μmoles/hr)	23.0 ± 4.0	30.5 ± 6.1	47.3 ± 3.4
Lactate production (μmoles/hr)	20.0 ± 3.2	25.0 ± 3.4	27.8 ± 4.0
K^+ efflux (μmoles/hr)	16.7 ± 2.6	24.2 ± 2.2	32.5 ± 3.9
Lactate/pyruvate ratio	15.8 ± 3.7	17.5 ± 2.6	20.8 ± 2.2

* The values given are the mean ± standard deviation of ten control experiments and of four experiments for each of the two concentrations of DMSO. Closed-circuit perfusion was done at 25° for 40 min with an artificial blood equilibrated with 95% O_2 5% CO_2.

Table 2. Effects of DMSO on levels of glycolytic intermediates and energy reserves in cerebral tissue of isolated, perfused rat brains*

	Control	Conc of DMSO in perfusion fluid	
		0.01 M	0.1 M
Glucose 6-phosphate	73.0 ± 8.4	85.0 ± 2.6	63.8 ± 3.9
Fructose 6-phosphate	20.0 ± 4.0	17.0 ± 2.2	15.8 ± 3.1
Mannose 6-phosphate	228 ± 36	251 ± 42	311 ± 37
Fructose 1,6-diphosphate	93 ± 11	75.8 ± 5.1	75.0 ± 9.6
Triose phosphate	41.2 ± 4.6	42.0 ± 2.2	44.5 ± 7.1
3-Phosphoglycerate	39.8 ± 5.2	53.3 ± 8.1	47.8 ± 6.2
2-Phosphoglycerate	14.8 ± 1.9	14.3 ± 3.1	9.8 ± 1.7
Phosphoenolpyruvate	7.7 ± 1.2	10.3 ± 2.1	12.0 ± 3.4
Pyruvate	60.8 ± 6.4	60.3 ± 7.0	62.8 ± 7.0
Lactate	1850 ± 400	3600 ± 1000	4330 ± 1400
Creatine phosphate	2550 ± 230	2370 ± 220	2450 ± 530
ATP	1990 ± 140	1850 ± 140	1700 ± 110
ADP	720 ± 50	690 ± 70	690 ± 50
AMP	240 ± 40	330 ± 100	430 ± 50
ATP + ADP + AMP	2950 ± 130	2860 ± 130	2820 ± 70
Lactate/pyruvate	31 ± 7	60 ± 15	72 ± 28
ATP/ADP	2.93 ± 0.37	2.71 ± 0.52	2.48 ± 0.38

* All concentrations are in nmoles/g (wet wt) of tissue. All values are mean ± standard deviation of ten control experiments and of four experiments for each of the two concentrations of DMSO. Closed-circuit perfusion was done at 25° for 40 min with an artificial blood equilibrated with 95% O_2 5% CO_2.

From changes in the concentrations in the known volume of perfusion fluid, the rates of glucose utilization, lactate production and efflux of potassium were computed. These values and the lactate/pyruvate ratios in the perfusion fluid at the end of the perfusion period are listed in Table 1. These results indicate that DMSO caused a modest increase in the rate of glycolysis and in the rate of efflux of potassium from the brain into the perfusion fluid. At the same time there was an increase in the lactate/pyruvate ratio in the perfusion fluid, suggesting that the brain tissue was moving to a more reduced state under the influence of DMSO.

The concentrations of the intermediates of glycolysis and of the adenine nucleotides and creatine phosphate in the cerebral tissue of the perfused brains are listed in Table 2. The most pronounced changes from control values were the approximately 100 per cent increases in lactate concentration and in the lactate/pyruvate ratio in the brains perfused with 0.01 M DMSO; in the brains perfused with 0.1 M DMSO there were somewhat larger increases in these values. The other significant changes in the concentrations of the glycolytic intermediates were a decrease in fructose diphosphate, and increases in phosphoenolpyruvate and lactate without significant changes in pyruvate. The changes in the levels of fructose diphosphate and phosphoenolpyruvate indicate that there were alterations in the regulation of glycolysis at both phosphofructokinase and pyruvate kinase steps with the resulting increase in glycolytic flux which occurred. This does not mean that the changes observed were due to a direct action of DMSO on these glycolytic enzymes, although this possibility is not excluded. The large change in the redox state of the tissue and the decrease in ATP level suggest that the changes in regulation of glycolysis were more likely secondary to an effect on the mitochondrial enzymes.

The creatine phosphate levels were not significantly changed but the ATP levels were reduced and the AMP levels were increased in the cerebral tissue of the brains which were perfused with DMSO. Thus, the energy reserves of the brain appear to have been slightly reduced by the presence of DMSO in the perfusion fluid. It is also noteworthy that, although there was a small change in the ATP level, the total adenine nucleotides (ATP + ADP + AMP) of the tissue were not changed significantly in the brains perfused with DMSO. This suggests that DMSO, in the concentrations used, did not exert an irreversible deleterious effect on the tissue of the perfused rat brain.

In our experiments, the principal effects of DMSO on the cerebral tissue of perfused rat brains were: (1) an increase in the rate of glycolysis, (2) an increase in lactate/pyruvate ratio indicating a marked shift to a reduced state in the tissue, and (3) a small decrease in the tissue energy reserves (i.e. in the concentrations of creatine phosphate + ATP). These findings are similar to the effects of acute hypoxia on cerebral tissue of mice observed by Duffy *et al.* [10]. In our experiments, there was no lack of oxygen and it seems unlikely that DMSO could have blocked the delivery of oxygen to the tissue. Therefore, it is likely that DMSO reduced the utilization of oxygen, probably by an inhibitory effect on mitochondrial function. Presumably, the energy loss due to inhibition of oxidative activity was largely compensated by the increase in glycolytic activity. The effects of DMSO on mitochondrial activity have not been studied, but Conover [11] observed that DMSO inhibited ATPase activity and ^{32}P-ATP exchange in submitochondrial particles from bovine heart muscle.

Our results (Table 1) showed that both glucose consumption and lactate production increased in the brains perfused with DMSO but the increase in lactate production did not account for all of the increased glucose utilized. It seems unlikely that there was increased oxidation of glucose during perfusion with DMSO since the brain tissue became more reduced. It also seems improbable, although not excluded, that there was increased synthesis of glycogen: usually an increase in glycolysis in a tissue is accompanied by a decrease in glycogen content [10]. The other possibility is that DMSO caused an increase in the normally very low level of activity of the pentose phosphate pathway [12]. Various experimental stresses have been found to cause marked changes in the levels of the intermediates of this pathway [13] in brain tissue.

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