radicals), and in this way it stops the free radical cascade which leads to radiation damage.

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Possible involvement of indolamines in the glycogenic effect of the convulsant methionine sulfoximine in rat brain

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Summary. The aim of the present investigation was to look for the mechanisms causing disturbances in carbohydrate metabolism during the action of the epileptogenic agent methionine sulfoximine. The levels of glucose, glycogen, and indolamines were measured in seven different regions of rat brain. Methionine sulfoximine induced a decrease in serotonin level which was roughly dose-dependent. There were no obvious changes in tryptophan and 5-hydroxyindoleacetic levels in any area. Methionine sulfoximine induced the known increase in glucose and glycogen levels. The direct precursor of serotonin, 5-hydroxytryptophan, and benserazide (a decarboxylase inhibitor) were then injected into rats in association with methionine sulfoximine. In this case, methionine sulfoximine failed to induce seizures. Moreover, the serotonin level was unchanged and the carbohydrate content did not significantly increase. There was only a rise in 5-hydroxyindoleacetic acid level. This work shows a striking parallelism between serotonin decrease and glycogen increase.
Key words. Methionine sulfoximine; epileptogenesis; serotonin; glycogen; glucose.

Methionine sulfoximine (MSO) is a potent epileptogenic agent in a variety of laboratory animals. Its particular interest is the existence of a long period of latency before the seizure onset. MSO has been described as a glycogenic agent in the central nervous system of rodents during the preconvulsive, convulsive, and post-convulsive periods [1 – 3]. In our laboratory, we looked for the mechanism responsible of glycogen accumulation in brain induced by this convulsant. We found that MSO increased the activity of the glycogenic enzyme fructose-1,6-diphosphatase [4]. The quantity, the de novo biosynthesis, and the immunostaining of this enzyme notably increased under the influence of MSO [5, 6]. Since glycogen particles and fructose-1,6-diphosphatase are exclusively localized in the same cells, we concluded that the gluconeogenesis induced by the enzyme may account for glycogen accumulation [7]. A decrease in glycogen level induced by indolamine has been reported by Quach et al. [8], Pennington and Pentreath [9], and Magistretti [10]. The selective stimulation of serotonergic pathways increases glucose utilization in rat brain [11]. Thus, it seemed interesting to investigate whether the indolamine neurotransmitter is involved in the mechanism of action of MSO on carbohydrate metabolism. Sellinger and Dietz [12] and Blizard and Balkoski [13] have already studied the effect of MSO on the indolamine system, with a particular interest in seizure onset. We pursued and extended this work essentially looking at the glycogenic effect; we used increasing doses of MSO in order to correlate changes in indolamine level and glycogen level. We reinforced serotonin synthesis by using its precursor, 5-hydroxytryptophan, in association with benserazide, a decarboxylase

inhibitor which prevents the peripheral degradation of the precursor, thus enhancing the serotonin level in the central nervous system.

Materials and methods

The following compounds were purchased from Sigma Chemical Company (St-Louis, Missouri): L-methionine-D,L-sulfoximine, amyloglucosidase, glucose oxidase, peroxidase, and serotonin and its derivatives. Sodium octyl sulfate was purchased from Kodak (Eastman Kodak, Rochester). Benserazide (DL-serine 2-(2,3,4-trihydroxybenzyl)-hydrazide hydrochloride) was a gift from Hoffmann-La Roche (Basel). The other reagents used throughout the experiments were of the best grade available.

The animals used were rats of the Sprague-Dawley strain weighing about 300 g. They were housed in a temperature-controlled room with a standard light regimen (12 h light, 12 h darkness). The schedule of administration of the compounds was as follows: the animals were divided into groups given either NaCl 0.9 % (controls), or MSO at the doses of 50, 100, or 200 mg/kg b.wt, and benserazide (10 mg/kg). In a second experiment, MSO (100 mg/kg) was associated with benserazide (10 mg/kg) plus L-5-hydroxytryptophan (100 mg/kg). All the compounds were dissolved in 1 ml NaCl 0.9 % and were administered i.p. To minimize the influence of circadian rhythm, all the experiments were performed in the morning between 09.00 h and 10.00 h. The rats were decapitated and the heads were immediately frozen in liquid nitrogen. Afterwards, the encephalons were dissected out using the method of Glowinski and Iversen [14]. The midbrain was reduced to the thalamus. Each weighed region was immersed in cold 0.2 mol/l perchloric acid and was homogenized by sonication.

One part of the homogenate of each region was spun for 30 min at 20 000 × g (4 °C). The indolamines were measured in the supernatants by high-performance liquid chromatography (HPLC) with electrochemical detection using the method of Mefford [15]. The HPLC system comprised an isocratic pump (Beckman model 112 solvent delivery module, Berkeley, California) coupled with a glassy carbon electrochemical detector with an amperometric unit (Tacussel, Lyon). The mobile phase was 0.1 mol/l disodium phosphate, 0.1 mol/l citric acid, and 8 % methanol, at a flow rate of 1 ml/min. The results were expressed as ng/mg protein.

The glucose level was determined directly in the supernatant described above, using the method of Bergmeyer and Bernt [16]. The concentrations were expressed as µg of glucose per mg protein. The glycogen level was measured in the original homogenates according to the method of Keppler and Decker [17]. The homogenates were neutralized with $KHCO_3$ and the pH was adjusted to 4.8 with sodium acetate buffer. Amyloglucosidase was used to hydrolyze glycogen to glucosyl units, and the resulting glucose was measured as before. The results were expressed as µg of glucosyl units per mg protein.

The protein levels were measured using the method of Lowry et al. [18] modified by Miller [19]. All the results were analyzed with Student's t-test. Comparisons were made with the unpaired and small sample test. The two-tailed procedure was used, the level of confidence being at least 95 %.

Results

At a dose of 100 mg/kg, MSO induced the known convulsive activity in rats by 8 h after injection [2, 6, 20]. The serotonin level significantly decreased in all the areas of brain investigated 8 h after drug administration (table). In controls, the level of the serotonin precursor, tryptophan, was high as compared to that of serotonin in all the areas (the lowest level was recorded in cerebral cortex, 20.80 ± 3.50 ng/mg protein, the highest in pons and medulla oblongata, 24.80 ± 5.90 ng/mg protein). This level did not significantly change when the rats were given MSO (100 mg/kg). In controls, the highest concentration of the main metabolite of serotonin, 5-hydroxyindoleacetic acid, was measured in the hypothalamus,

Table 1. Effect of methionine sulfoximine (MSO, 100 mg/kg b.wt by i.p. injection) on the levels of glucose, glycogen and serotonin, in different regions of rat brain at variable times after drug administration. Each value is the mean ± SEM of 5 or more independent determinations. The labeled values (*) differ from the controls for at least $p < 0.05$ (Student's t-test).

Time after MSO administration	Cerebral cortex	Hypothalamus	Hippocampus	Striatum	Thalamus	Cerebellum	Pons and medulla oblongata
Controls							
– glycogen (µg/mg protein)	4.90 ± 0.90	4.40 ± 0.70	6.00 ± 0.90	3.00 ± 0.40	5.30 ± 1.10	7.10 ± 1.00	6.10 ± 1.20
– glucose (µg/mg protein)	0.24 ± 0.07	0.84 ± 0.15	0.33 ± 0.10	0.38 ± 0.09	0.47 ± 0.10	0.67 ± 0.20	1.00 ± 0.20
– serotonin (ng/mg protein)	3.03 ± 0.60	7.61 ± 1.70	2.97 ± 0.80	5.67 ± 1.20	4.53 ± 1.10	0.18 ± 0.07	5.00 ± 1.10
8 h							
– glycogen	9.80 ± 1.30*	7.00 ± 0.80*	9.70 ± 1.00*	7.50 ± 3.80*	7.10 ± 0.70*	11.20 ± 2.60*	7.40 ± 2.00
– glucose	1.27 ± 0.30*	1.15 ± 0.14*	1.68 ± 0.31*	0.94 ± 0.20*	0.37 ± 0.08	0.77 ± 0.40	1.97 ± 0.53
– serotonin	2.23 ± 0.50*	3.80 ± 1.00*	1.90 ± 0.50*	2.90 ± 0.80*	2.80 ± 0.60*	0.09 ± 0.02	2.90 ± 0.93*
24 h							
– glycogen	10.60 ± 2.30*	6.10 ± 1.10*	10.20 ± 1.80*	5.90 ± 1.20*	5.30 ± 0.70	10.60 ± 2.10*	7.60 ± 2.10
– glucose	0.60 ± 0.12*	1.15 ± 0.18*	1.34 ± 0.24*	0.61 ± 0.15*	0.47 ± 0.09	0.84 ± 0.15	1.36 ± 0.30
– serotonin	2.70 ± 0.80	5.10 ± 1.30	2.41 ± 0.80	5.10 ± 1.70	3.20 ± 0.80	0.07 ± 0.02	3.10 ± 0.80

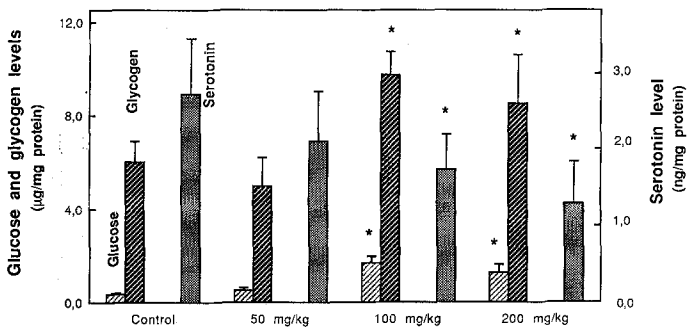

Figure 1. Changes in the contents of glucose, glycogen and serotonin in the hippocampus of rat brain 8 h after the intraperitoneal injection of different doses of methionine sulfoximine (MSO). Each value is the mean ± SEM of 5 or more independent determinations. The labeled values (*) differ from the controls for at least $p < 0.05$ (Student's t-test).

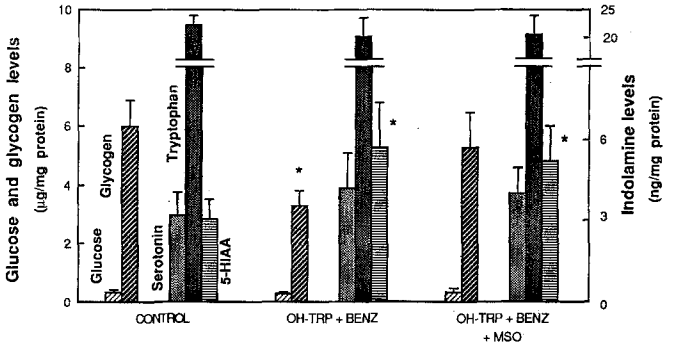

Figure 2. Glucose, glycogen, serotonin, tryptophan, and 5-hydroxyindoleacetic acid (5-HIAA) contents in the hippocampus of rat brain 8 h after the intraperitoneal administration of 5-hydroxytryptophan (OH-TRP, 100 mg/kg b.wt plus benserazide (BENZ, 10 mg/kg) plus methionine sulfoximine (MSO, 10 mg/kg). Each value is the mean ± SEM of 5 or more independent determinations. The labeled values (*) differ from the controls for at least $p < 0.05$ (Student's t-test).

5.90 ± 1.80 ng/mg protein. The convulsant at a dose of 100 mg/kg had almost no effect on the level of this metabolite in any of the areas.

MSO induced the known glycogenic effects in the nervous tissue, as shown in the table. Moreover, we showed that this effect is widespread in the central nervous system of the rat. The glycogen level was significantly increased, in all the areas except in the medulla oblongata, 8 h and 24 h after drug administration. The glucose level also increased significantly in the cerebral cortex, hypothalamus, hippocampus, and striatum at the same times.

At the lower dose of 50 mg/kg, MSO-treated animals displayed apparently normal behavior. At this dose, the convulsant induced a mild decrease in serotonin content, which was significant only in the hypothalamus and striatum. In other experiments, with 200 mg/kg of MSO, the rats displayed violent tonic and clonic convulsions. Then, there was a deep decrease in serotonin level (a typical pattern is shown in fig. 1). In many areas, tryptophan and 5-hydroxyindoleacetic acid concentrations were a little lower than in controls, but their decreases were generally insignificant. The effects of 50 mg/kg of MSO on glucose and glycogen levels were insignificant. But, at 200 mg/kg, there was an important glycogenic effect in most areas.

Benserazide (10 mg/kg) did not change the behavior, the amine levels or the carbohydrate levels in the rats 8 h after administration (data not shown). The direct precursor of serotonin, 5-hydroxytryptophan, and benserazide were injected together in some rats. When 5-hydroxytryptohan (100 mg/kg), benserazide (10 mg/kg) and MSO (100 mg/kg) were simultaneously injected, there was no convulsion. At the same time, the serotonin and tryptophan levels were unchanged (example in fig. 2). There was only an increase in the 5-hydroxyindoleacetic acid level, except in the striatum and pons and medulla oblongata.

In the preceding experiments, the glycogen content did not change in any of the areas under the effects of benserazide plus 5-hydroxytryptophan plus MSO. The changes in glucose content were different in different areas.

Discussion

Among the convulsants, MSO is known to be a potent glycogenic agent in the central nervous system[1,2,21]. However, it is important to know that during the convulsions induced by MSO in mice, there was a transient sharp and brief decrease in current glycogen level followed again by an increase, suggesting a probable increase in energy utilization during this period[21]. This property of increasing glucose utilization during the seizures is observed in most convulsants. Nevertheless, the general result of MSO treatment in nervous tissue is glycogenesis. How can that occur?

The role of serotonin in glycogen metabolism has recently been investigated. Using leech segmental ganglia comprising only neuronal and glial cells, Pennington and Pentreath[9] showed that serotonin induced glycogenolysis in glial cells. Quach et al.[8] have already observed the glycogenolytic effect of serotonin. In our experiment, MSO induced a widespread decrease in serotonin level, the magnitude of which depended on the area of the central nervous system being considered. The results showed a striking parallel between decreases in serotonin level and increases in the glycogen level: a) the subconvulsive dose of 50 mg/kg of MSO induced little change either in serotonin or in glycogen content; b) the higher dose of 200 mg/kg of the convulsant, which depleted the serotonin content, was the most glycogenic. So. if we assume that indolamine neurotransmitter directly reacts with glycogen metabolism, as shown by Pennington and Pentreath[9], it could possibly be involved in the glycogenic effect of MSO. The reason for the lack of an obvious increase in glucose content in thalamus and cerebellum, regions in which glycogen content increased, is

unclear. Perhaps, the glycogen synthetase is more active in these areas, converting an appreciable quantity of glucose into glycogen. In the cerebellum, this contingency can be related to the low level of cyclic AMP[22].

The preceding hypothesis is also supported by the experiments with 5-hydroxytryptophan. In contrast to Blizard and Balkoski[13], who used tryptophan to prevent the MSO effect, we used 5-hydroxytryptophan, which can be easily converted to serotonin, the rate-limiting enzyme being tryptophan hydroxylase. This compound was associated with benserazide, a decarboxylase inhibitor, to prevent its peripheral degradation. In this case, MSO failed to induce a decrease in serotonin content. In the same time, the glycogen content did not increase.

Blizard and Balkoski[13] have suggested that a restriction in tryptophan availability can explain the decrease in serotonin level induced by MSO. In our hands, and as stated by Sellinger and Dietz[12], the decrease in tissue tryptophan level was insignificant and the remaining amount was very large as compared to that of serotonin in all the areas. MSO must rather exert its action on the synthesis of serotonin. In agreement with Sellinger and Dietz[12], we have observed that MSO alone had a little effect on 5-hydroxyindoleacetic acid content, some significant decrease being observed only with the high dose of 200 mg/kg in two areas. But, the treatment with 5-hydroxytryptophan led to a significant increase in the level of 5-hydroxyindoleacetic acid, suggesting a possible increase in the turnover of serotonin. This possible high turnover may be the sign of an augmented liberation of serotonin by neurons, even if the actual content of the amine did not appreciably increase. This effect may explain the decrease in glycogen content observed when using 5-hydroxytryptophan plus benserazide, since the astrocytes, which contain most polysaccharide[7], would be in a medium rich in serotonin. This idea is supported by the results in the striatum where there was no change in the levels of either 5-hydroxyindoleacetic acid or glycogen. Moreover, when MSO was associated with the two preceding drugs, 5-hydroxyindoleacetic acid levels were generally less high, and the glycogen decreases were very moderate and insignificant.

Further experiments may make it possible to describe more precisely the exact correlation between serotonin and glycogen metabolism under the effect of methionine sulfoximine.

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Retention of topical liposomal formulations on the cornea

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Summary. The ability of liposomes composed of different kinds of phospholipid materials to adhere to the surface of the cornea was studied in the rabbit. The liposomes were labelled with tracer amounts of an I^{125}-labelled phosphatidylethanolamine derivative and were instilled in 10 µl drops onto the cornea. The retention of radioactivity was monitored. The results show that liposomes containing positively charged phospholipids are better retained than an albumin control. Thus, it may be possible to develop a drug delivery liposome system which would permit long-term sustained release of ophthalmic drugs onto the cornea.
Key words. Rabbit cornea; retention; liposomes; drug delivery; ophthalmic drugs.