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# CYCLIC NUCLEOTIDES IN THE BRAIN OF MICE AND RATS SUBMITTED TO THE CONVULSANT, METHIONINE SULFOXIMINE

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**Abstract**—Regional cyclic AMP and cyclic GMP levels were measured in the rat and mouse brain following intraperitoneal injection of the convulsant methionine sulfoximine (MSO), at a dose of 100 mg/kg body weight. No change in the cyclic AMP content is noticeable in the mouse cerebral cortex and in the rat cerebral cortex, striatum, hypothalamus and cerebellum during the preconvulsive, convulsive and postconvulsive periods following administration of MSO. The cyclic GMP content increases in the mouse cerebral cortex and brain stem, but it does not change in the cerebellum, during the period of MSO-induced seizure activity. At the time of recovery from seizures, the increase of cyclic GMP content persists only in the brain stem. During the whole periconvulsive period cyclic GMP level rises in the brain stem. Administered at a dose of 50 mg/kg body weight, MSO induces a relatively reduced increase of cyclic GMP content in the brain stem only. It is suggested that the increase of the level of cyclic GMP may develop simultaneously in neuronal and glial cells, primarily in the brain stem, during some periods following administration of the convulsant MSO.

The mechanisms of the convulsant and glycogenic effects of methionine sulfoximine (MSO) in the central nervous system are not yet elucidated [1–4]. MSO administered to mice and rats induced an activation and probably a biosynthesis *de novo* of the key gluconeogenic enzyme fructose-1,6-biphosphatase (EC 3.1.3.11), in the cerebral cortex, brain stem and cerebellum, during the preconvulsive and convulsive periods, and also on recovery from seizures at the time when the animals appear normal [5, 6]. These data suggest that the subsequent intracerebral accumulation of glycogen which occurs during the period of seizures induced by administration of MSO, and persists for up to 72 hr after injection of the convulsant, results from an activation of the gluconeogenic pathway. Since it has been shown that glycogen accumulates prominantly within swollen astrocytes in the cerebral cortex and subcortical white matter [3], it is tempting to consider that the gluconeogenic pathway develops in astroglia from a precursor which may be glutamate originating from activated neurons and subsequently taken up by astroglia [3, 4]. Glutamate would be quantitatively available for gluconeogenesis since its conversion into glutamine is prevented due to the inhibition of glutamine synthetase acitivity by MSO [7, 8].

Recent data indicate that seizure activity induced by pentylenetetrazol affects adenosine 3′, 5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) levels differently in the cerebral cortex, cerebellum, striatum, thalamus and hippocampus of mice, and that regional changes of cyclic nucleotide concentrations are dependent upon the type of seizure activity [9]. Moreover, the regulation of regional cAMP and cGMP levels appear to be independent of each other [10].

It has been claimed that the excessive neuronal discharge which occurs during seizure activity may, by itself, lead to neuronal damage [4]. Since it has been postulated that the ultrastructural damages occurring in the mouse and rat brain following administration of MSO [11–13] do enhance gluconeogenesis [1, 5, 6], probably in astrocytes, it seems relevant to the study of the relationship between neuronal depolarization and glial glycogenesis to follow the regional changes of the levels of cAMP and cGMP in the mouse and rat brain.

## MATERIALS AND METHODS

Male Swiss mice (OF1 strain) of about 30 g body weight and male Wistar rats (laboratory inbred strain) of about 300 g body weight were used throughout the experiments. MSO (L-methionine-DL-sulfoximine, Sigma, St. Louis, MO.) (50–200 mg/kg body wt.) dissolved in 0.2 ml (mice) or 1.0 ml (rats) of 0.9% NaCl was injected intraperitoneally; control animals received the same volume of 0.9% NaCl. During the experiments the animals were placed in individual cages at 22°. The mice were frozen intact in liquid nitrogen and the rats were decapitated and their head instantaneously immersed in liquid nitrogen, at different periods of seizure activity. The frozen animals and heads were stored at −30° until the brain was removed. The chilled cerebral cortex, striatum, hypothalamus, brain stem and cerebellum were dissected out in a refrigerated chamber and rapidly weighed. Each brain region was homogenized in 6 vol. (w/v) of 10% trichloroacetic acid, and the homogenate was centrifuged at 18,000 *g* for 15 min at 4°. The supernatant

Table 1. Recovery of cAMP and cGMP and effect of cyclic nucleotide phosphodiesterase on assay for cAMP and cGMP

| Tissue | cAMP (nmoles/g wet weight) | Recovery (%) |
|---|---|---|
| Cerebral cortex (control mouse) | 1.66 ± 0.13 | |
| + phosphodiesterase | 0 | |
| + 1.20 nmoles of cAMP | 2.75 ± 0.12 | 96 |
| Cerebral cortex (MSO-injected mouse) | 1.46 ± 0.10 | |
| + phosphodiesterase | 0 | |
| +1.20nmoles of cAMP | 2.46 ± 0.14 | 92 |
| | **cGMP (pmoles/g wet weight)** | **Recovery (%)** |
| Cerebral cortex (control mouse) | 77.15 ± 6.93 | |
| + phosphodiesterase | 0 | |
| + 150 pmoles of cGMP | 213.94 ± 7.51 | 94 |
| Cerebral cortex (MSO-injected mouse) | 110.10 ± 11.29 | |
| + phosphodiesterase | 0 | |
| + 150 pmoles of cGMP | 241.63 ± 13.77 | 93 |
| Brain stem (control mouse) | 43.42 ± 5.81 | |
| + phosphodiesterase | 0 | |
| + 150 pmoles of cGMP | 179.68 ± 8.64 | 93 |
| Brain stem (MSO-injected mouse) | 105.65 ± 21.24 | |
| + phosphodiesterase | 0 | |
| + 150 pmoles of cGMP | 244.80 ± 27.64 | 96 |
| Cerebellum (control mouse) | 168.68 ± 8.89 | |
| + phosphodiesterase | 0 | |
| +150 pmoles of cGMP | 296.52 ± 14.63 | 93 |
| Cerebellum (MSO-injected mouse) | 180.31 ± 9.18 | |
| + phosphodiesterase | 0 | |
| + 150 pmoles of cGMP | 314.26 ± 7.52 | 95 |

For the recovery experiments 1.20 nmoles of cAMP and 150 pmoles of cGMP were added to the tissue extracts. Equal volumes of tissue extracts with no added cyclic nucleotide were used in parallel to determine the basal concentrations of cAMP and cGMP. The MSO-injected mice were sacrificed in the convulsive period about 8 hr after an intraperitoneal injection of MSO (100 mg/kg body wt) as described in the text. Values are means ± S.D. for three animals.

was washed five times with 4 vol. of water-saturated diethyl ether; diethyl ether remaining in the solution was evaporated at 80° for 1 min. The content of cyclic nucleotides was estimated in this brain tissue fraction. cAMP was measured by the Gilman competitive protein binding assay [14] modified [15], with an adsorption of the cyclic nucleotides on charcoal [16, 17]. cGMP was estimated by the radioimmunoassay described by Steiner *et al.* [18]. The materials for the cyclic nucleotide determinations were purchased from the Radiochemical Centre, Amersham, Buckinghamshire and they were checked for possible interference from other nucleotides, divalent metal ions and the non-dialysable compound reported by Weller *et al.* [15]. The standard curves were plotted with the standard cyclic nucleotides dissolved in 10% trichloroacetic acid washed five times with water-saturated diethyl ether [16]. Blank values were carried on aliquots of the tissue extract incubated for 60 min at 37° in an equal volume of 100 mM Tris–HCl buffer, pH 7.5, containing cyclic nucleotide phosphodiesterase (Boehringer Mannheim France, Meylan) (10 μg per ml). The reaction was stopped by heating at 100° for 2 min; the samples were centrifuged and the resulting supernatants were used as blanks. Cyclic nucleotide phosphodiesterase hydrolyzed 100 per cent of the reactive cAMP and cGMP in extracts from cerebral cortex, brain stem and cerebellum from control and MSO-injected mice (Table 1). Statistical comparisons were established using Student's *t* test.

## RESULTS

### *Effect of MSO on brain cyclic AMP*

The cAMP level determined in the control rat cerebral cortex, striatum, hypothalamus and cerebellum, appears to be relatively homogeneous (Table 2). After intraperitoneal injection of MSO, at a dose of 100 mg/kg body weight, no change in the cAMP content is noticeable in the mouse cerebral cortex

Table 2. Effect of MSO on cAMP content in mouse cerebral cortex and rat cerebral cortex, striatum, hypothalamus and cerebellum

| Tissue | cAMP (nmoles/g tissue wet weight) | |
|---|---|---|
| | Convulsive period | Postconvulsive period |
| Cerebral cortex (mouse) | | |
| control | 1.53 ± 0.10 (5) | |
| MSO-injected | 1.32 ± 0.07 (5)* | |
| Cerebral cortex (rat) | | |
| control | 0.94 ± 0.13 (4) | |
| MSO-injected | 1.11 ± 0.05 (4) | 1.10 ± 0.02 (4) |
| Striatum (rat) | | |
| control | 0.99 ± 0.14 (4) | |
| MSO-injected | 0.72 ± 0.11 | 0.83 ± 0.02 (4) |
| Hypothalamus (rat) | | |
| control | 1.06 ± 0.04 (4) | |
| MSO-injected | 1.07 ± 0.02 (4) | 1.01 ± 0.06 (4) |
| Cerebellum (rat) | | |
| control | 0.81 ± 0.03 (4) | |
| MSO-injected | 0.92 ± 0.04 (4)† | 0.82 ± 0.01 (4) |

The animals were sacrificed in the convulsive and postconvulsive periods respectively about 8 and 24 hr after intraperitoneal injection of MSO (100 mg/kg body wt.) Results are expressed as nanomoles cAMP/g tissue wet weight (mean value ± S.D. for number of animals in parentheses). Statistically significant differences between MSO-injected and control animals are indicated.

* $P < 0.05$.

† $P < 0.01$.

and in the four rat brain regions studied too, during the convulsive and postconvulsive periods (Table 2). Even administered at a dose of 400 mg/kg body weight, MSO does not influence cAMP level in the rat brain structures during the preconvulsive and convulsive periods.

*Effect of MSO on brain cyclic GMP*

The cGMP content in cerebral cortex of control mice is about 22 times smaller (Table 3) than that of cAMP (Table 2), and its regional distribution is heterogeneous, the concentration in the cerebellum being two and three times higher than that in the cerebral cortex and brain stem respectively (Table 3). During the preconvulsive period, 4 hr after administration of MSO (100 mg/kg body wt), the concentration of cGMP does not change significantly in the three brain regions studied; 8 hr after administration of MSO, the animals being in the period of intense tonic and clonic seizure activity, the concentration of cGMP increases approximately by 44 per cent and 100 per cent respectively in the cerebral cortex and brain stem, without any alteration of this concentration in the cerebellum (Table 3). During the post-convulsive period at the time of recovery from seizures, 24 hr after administration of MSO, the concentration of cGMP in the brain stem is only increased by 27 per cent (Table 3).

In order to investigate the possible relationship between the state of seizure activity induced by MSO and the regional cGMP content in the mouse brain, we have performed the estimation of the cyclic nucleotide, on the one hand during the periconvulsive period using MSO at a dose of 100 mg/kg body weight, and on the other hand during the convulsive period using the drug at an increasing dose of 50–200 mg/kg body weight. From 4 to 7 hr after injection of MSO, when the animals exhibit an increasing syndrome of ataxia, the concentration of cGMP augments by 40 per cent in the brain stem (Table 3). When the mice are killed just after the onset of the first spontaneous clonic seizure, the cGMP content in the cerebral cortex and brain stem reaches respectively 87 and 82 pmoles/g tissue wet weight. Although these latter values are significantly higher than those determined in the control mice, they do not reach the values determined in the mouse brain during the period of intense seizure activity. During this intense tonic and clonic seizure activity, approximately 8 hr after administration of MSO, the concentration of cGMP in the cerebral cortex and brain stem increases respectively by 50 per cent and 137 per cent, without any change in the cerebellum (Table 3). At the end of the period of seizure activity, about 9 hr after administration of MSO, the cGMP content in the brain stem is still 56 per cent higher than that determined in the control mice (Table 3). The animals submitted to MSO at a dose of 50 mg/kg body weight exhibit a slight syndrome of ataxia and no seizure activity, and 8 hr after the administration of the drug only the concentration of cGMP in the brain stem increases by 30 per cent (Table 4). When the mice are killed 8 hr after an intraperitoneal injection of MSO at a dose of 200 mg/kg body wt, the concentration of cGMP does not change in the cerebral cortex and brain stem, but it decreases by 33 per cent in the cerebellum (Table 4). The animals submitted to this high dose of MSO exhibit either a strong syndrome of ataxia followed by an akinesia or a seizure activity, but they all die within 24 hr. For the mice exhibiting a seizure activity, the cGMP content in the cerebral cortex, brain stem and cer-

*Table 3. Effect of MSO on cGMP content in mouse cerebral cortex, brain stem and cerebellum*

| Time after MSO administration (hr) | Cerebral cortex | | Brain stem | | Cerebellum | |
|---|---|---|---|---|---|---|
| | Controls | MSO-injected | Controls | MSO-injected | Controls | MSO-injected |
| 4 | 73.44 ± 12.81 (5) | 70.94 ± 17.44 (6) | 47.12 ± 6.86 (5) | 46.55 ± 7.23 (6) | 153.80 ± 12.58 (3) | 151.62 ± 14.30 (5) |
| 4–7 | 70.71 ± 15.78 (8) | 74.43 ± 8.88 (3) | 48.18 ± 6.26 (10) | 67.23 ± 15.45* (4) | 158.35 ± 9.52 (7) | 151.41 ± 11.24 (3) |
| 8 | | 102.09 ± 15.09‡ (5) | | 95.03 ± 29.66† (7) | | 165.65 ± 25.94 (7) |
| 9 | | 90.16 ± 16.62 (4) | | 74.96 ± 9.31‡ (4) | | 155.79 ± 11.44 (4) |
| 24 | 71.62 ± 14.67 (5) | 67.30 ± 17.17 (10) | 48.16 ± 6.73 (6) | 61.11 ± 15.11* (10) | 155.56 ± 12.37 (4) | 148.84 ± 32.97 (6) |

The animals were killed 4 hr (preconvulsive period), between 4 and 7 hr (period of increasing state of ataxia), 8 hr (period of intense tonic and clonic seizure activity), 9 hr (period of ending of seizure activity) and 24 hr (postconvulsive period) after intraperitoneal injection of MSO (100 mg/kg body weight). Results are expressed as picomoles cGMP/g tissue wet weight (mean value ± S.D. for number of animals in parentheses). Statistically significant differences between MSO-injected and control animals are indicated.

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.005$.

Table 4. Effect of an increasing dose of MSO on cGMP content in mouse cerebral cortex, brain stem and cerebellum

| Dose of MSO (mg/kg body weight) | Cerebral cortex | Brain stem | Cerebellum |
|---|---|---|---|
| 0 | 70.71 ± 15.78 (8) | 48.18 ± 6.26 (10) | 158.35 ± 9.52 (7) |
| 50 | 79.86 ± 10.64 (4) | 62.49 ± 14.77* (4) | 144.14 ± 18.90 (3) |
| 100 | 106.40 ± 13.42† (4) | 113.89 ± 25.13† (4) | 170.72 ± 26.94 (4) |
| 200 | 63.83 ± 16.82 (8) | 49.06 ± 9.46 (8) | 106.24 ± 15.25† (4) |

The animals were killed 8 hr after intraperitoneal injection of MSO (50–200 mg/kg body wt.). Results are expressed as pmoles cGMP/g tissue wet weight (mean value ± S.D. for number of animals in parentheses). Statistically significant differences between MSO-injected and control animals are indicated.

* $P < 0.05$.

† $P < 0.005$.

ebellum is respectively 81, 58 and 118 pmoles/g tissue wet weight.

## DISCUSSION

The possible role of cyclic nucleotides in specific neuronal function is not clearly elucidated, and it seems likely that the cyclic nucleotide-mediated metabolic events in the brain are compartmentalized [19, 20]. Recent experimental data have shown that the concentrations of cyclic nucleotides in the brain *in vivo* vary according to the level of excitability. Both cAMP and cGMP levels in mouse brain *in vivo* increase following electroconvulsive shock [21, 10] and administration of pentylenetetrazol [9]. The results of our study do not demonstrate that MSO has such a widespread effect on both cAMP and cGMP levels in mouse and rat brain. During the period of intense tonic and clonic seizure activity, which takes place approximately 8 hr after intraperitoneal injection of MSO, at a dose of 100 mg/kg body wt, we fail to detect any changes in the regional cAMP concentration, except for a slight decrease in the mouse cerebral cortex and the rat cerebellum. On the contrary, the concentration of cGMP increases in the mouse brain stem during the period of the onset of the clonic seizures, particularly during the period of tonic and clonic seizure activity, and still remains elevated 24 hr after administration of MSO at the time of recovery from seizures, when the animals appeared normal. The content of cGMP in the cerebral cortex augments only during the period of intense seizure activity and it does not change in the cerebellum. It has previously been shown that seizures induced by convulsant drugs elevate cerebellar cGMP levels [22], and that this effect on cerebellar cGMP is antagonized by anticonvulsant [10]. Our present results are at variance with this previous data, since MSO-induced seizures have no influence on cerebellar cGMP content. The percentage of increase of cGMP concentration in the brain stem and cerebral cortex during the period of tonic and clonic seizure activity, is markedly lower than that obtained in the various brain areas during clonic seizures induced by pentylenetetrazol [9]. The chronology of development of seizure activity state is different between mice submitted to MSO and pentylenetetrazol, since with the latter the frequency of clonic-type seizures is maximal approximately 15 min after its intraperitoneal injection [9], whereas with MSO the state of long-lasting tonic seizures with intermittent clonic seizures is maximal only about 7–8 hr after its intraperitoneal injection. Our present results seem to be in agreement with recent data showing that during all the phases of focal epileptic activity induced by penicillin in the cat cerebral cortex, cGMP levels were significantly higher in the epileptic focus than in corresponding contralateral cortex or the occipital cortex; cGMP levels in the focus did not change [23]. It was concluded from these data that alterations of cAMP level may not be essential for epileptogenesis.

At a dose of 50 mg/kg b. wt., MSO induces a relatively low increase of cGMP content in the brain stem only, but at a dose of 200 mg/kg body weight, which appears to be lethal within 24 hr for all the animals thus treated, the drug induces a significant decrease in cerebellar cGMP level 8 hr after its administration. In this last experimental condition, the mice exhibiting a severe syndrome of ataxia, the cerebellum seems to be specifically sensitive to MSO. Although being a convulsant drug, MSO induces an accumulation of glycogen in the rodent brain [24, 2]. After administration of MSO, at a dose of 100 mg/kg body wt, glycogen content in the mouse cerebral cortex rises by 65 per cent during the preconvulsive period and by 59 per cent during the convulsive period, with a maximum of 470 per cent 24 hr later at the time of recovery from seizures [1]. This MSO-induced cerebral glycogenesis seems to originate from an activation of gluconeogenic pathway, since the drug induces in the brain an increase in activity and probably a biosynthesis *de novo* of the key gluconeogenic enzyme fructose-1, 6-biphosphatase, during the preconvulsive and convulsive periods and also at the time of recovery from seizures; this enhanced gluconeogenesis seems to be particularly located in the brain stem [5, 6]. It has been suggested that changes in cGMP levels in all regions of the brain directly reflect alterations in neuronal activity [22]. The elevated concentration of cGMP observed in the brain during seizure activity originates from depolarization of neurons following the action of a specific neurotransmitter. However, in our present

results, as in those previously obtained by Raabe *et al.* [23] in focal penicillin epilepsy, it seems unlikely that cGMP levels increase only in association with neuronal depolarization, since an increase in cGMP content occurs at the time of recovery from MSO-induced seizures and in the postictal depression in focal penicillin epilepsy. At the present time no data allow us to explain how cGMP may play a well-defined effector role in the central nervous system, as was recently postulated [25]. The cGMP-mediated metabolic events in the brain are compartmentalized and it is not excluded that the increase of the level of the cyclic nucleotide may develop simultaneously in neuronal and glial cells during some periods following administration of MSO. The brain stem appears to be a region of rat and mouse brain in which MSO or its metabolite(s) primarily exert their stimulating effect on some specific neuronal cells, followed by an induction of gluconeogenesis probably in glial cells.

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## REFERENCES

1. A. Bérel, P. R. Lehr and J. Gayet, *Brain Res.* **128**, 193 (1977).
2. J. Folbergrova, J. V. Passonneau, O. H. Lowry and D. W. Schulz, *J. Neurochem.* **16**, 191 (1969).
3. C. G. Phelps, *J. Neurocytol.* **4**, 479 (1975).
4. B. K. Siesjö, *Brain Energy Metabolism*. Wiley, New York (1978).
5. T. Hevor and J. Gayet, *Brain Res.* **150**, 210 (1978).
6. T. Hevor and J. Gayet, *Biochem. Soc. Trans.* **6**, 1029 (1978).
7. C. Lamar and O. Z. Sellinger, *Biochem. Pharmac.* **14**, 489 (1965).
8. J. Pace and E. E. McDermott, *Nature, Lond.* **169**, 415 (1952).
9. J. A. Ferrendelli and D. A. Kinscherf, *Epilepsia* **18**, 525 (1977).
10. W. D. Lust, H. J. Kupferberg, W. D. Yonekawa, J. K. Penry, J. V. Passonneau and A. B. Wheaton, *Molec. Pharmac.* **14**, 347 (1978).
11. E. DeRobertis, *Triangle* **10**, 93 (1971).
12. E. DeRobertis, O. Z. Sellinger, G. R. DeLores Arnaiz, M. Alberici and L. M. Zieher, *J. Neurochem.* **14**, 81 (1967).
13. N. Rizzuto and N. K. Gonatas, *J. Neuropath. exp. Neurol.* **33**, 237 (1974).
14. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
15. M. Weller, R. Rodnight and D. Carrera, *Biochem. J.* **129**, 113 (1972).
16. J. D. M. Albano, G. D. Barnes, D. V. Maudsley, B. L. Brown and R. P. Etkins, *Analyt. Biochem.* **60**, 130 (1974).
17. K. C. Tovey, K. G. Oldham and J. A. M. Whelan, *Clin. chim. Acta* **56**, 221 (1974).
18. A. L. Steiner, C. W. Parker and D. M. Kipnis, *J. biol. Chem.* **247**, 1106 (1972).
19. J. W. Daly, *Int. Rev. Neurobiol.* **20**, 105 (1977).
20. J. A. Nathanson, *Physiol. Rev.* **57**, 157 (1977).
21. W. D. Lust, N. D. Goldberg and J. V. Passonneau, *J. Neurochem.* **26**, 5 (1976).
22. J. A. Ferrendelli, in *Advances in Cyclic Nucleotide Research* (Eds. W. J. George and L. J. Ignarro), Vol. 9, p. 453. Raven Press, New York (1978).
23. W. Raabe, S. Nicol, R. J. Gumnit and N. D. Goldberg, *Brain Res.* **144**, 185 (1978).
24. J. Folbergrova, *J. Neurochem.* **20**, 547 (1973).
25. N. D. Goldberg and M. K. Haddox, *A. Rev. Biochem.* **46**, 823 (1977).