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BRIEF COMMUNICATION

Prevention of Strychnine-Induced Seizures and Death by the N-Methylated Glycine Derivatives Betaine, Dimethylglycine and Sarcosine

WILLIAM J. FREED

Preclinical Neurosciences Section, Neuropsychiatry Branch, National Institute of Mental Health Saint Elizabeths Hospital, Washington, DC 20032

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FREED, W. J. *Prevention of strychnine-induced seizures and death by the N-methylated glycine derivatives betaine, dimethylglycine and sarcosine.* PHARMACOL BIOCHEM BEHAV **22**(4) 641–643, 1985.—Betaine (N,N,N-trimethylglycine) and N,N-dimethylglycine have been reported to have anticonvulsant properties in animals. The purpose of the present study was to determine whether these compounds can antagonize strychnine-induced seizures when administered intraperitoneally and to compare their effects with those of sarcosine (N-methylglycine) and glycine. Betaine, N,N-dimethylglycine and sarcosine were equipotent in decreasing the incidence of seizures and death, causing a 38 to 72 percent decrease in the incidence of seizures and death at a dosage of 5 mmole/kg. Glycine had no effect. Thus anticonvulsant activity is conferred to glycine by a single N-methylation.

Strychnine Betaine Glycine betaine N,N-dimethylglycine Sarcosine Convulsions Seizures Mice

BETAINE, or N,N,N-trimethylglycine, is a normal constituent of mammalian tissues and is found in rat kidney and liver in a concentration of about 1 mg/g [10]. The primary function of betaine in mammals is as a methyl donor for the remethylation of homocysteine to form methionine [9]. In the brain, however, betaine does not serve this function [6,16] and is not present in detectable amounts [10].

Betaine was first reported to have an anticonvulsant effect by Sprince and his colleagues [14,15], who found that homocysteine-induced seizures could be blocked by the administration of betaine. Subsequently betaine was found to have more general anticonvulsant properties, and to decrease seizures induced by pentylenetetrazol and electroconvulsive shock [8]. Betaine has recently been reported to have beneficial effects, including alleviation of seizures, in patients with homocystinuria [13,18].

In the process of donating a methyl group for the methylation of homocysteine, betaine loses one methyl group and becomes N,N-dimethylglycine (DMG). Recently, Roach and Carlin [11] reported a striking decrease in seizure frequency in a patient with developmental retardation who was administered DMG obtained from a health-food store. DMG has also been reported to block pentylenetetrazol-induced seizures in mice [7]. Since DMG is not capable of donating a methyl group for homocysteine remethylation, these findings suggest that some property of betaine and DMG unrelated to methylation reactions may be responsible for the anticonvulsant effect.

Glycine is a putative inhibitory amino acid neurotransmitter primarily in the spinal cord [1–3, 5, 17, 20]. It has been suggested that DMG might mimic or enhance the central effects of glycine, accounting for its anticonvulsant effect [11]. Although Curtis *et al.* [3] found that DMG neither reduced nor potentiated the synaptic inhibitory effect of either γ-aminobutyric (GABA) or glycine, it is still possible that DMG influences glycine-containing neurons indirectly.

The glycine antagonist strychinine causes convulsions primarily by blocking neuronal inhibition at the spinal cord level [3, 4, 20]. If the anticonvulsant activity of betaine or of DMG is due to a direct central inhibitory effect related to glycine-mediated spinal cord inhibition, it would be predicted that betaine or DMG would be capable of blocking strychnine-induced convulsions. The purposes of the present study were (1) to test this hypothesis, and (2) to compare the effects of betaine with those of DMG, glycine, and sarcosine (N-methylglycine).

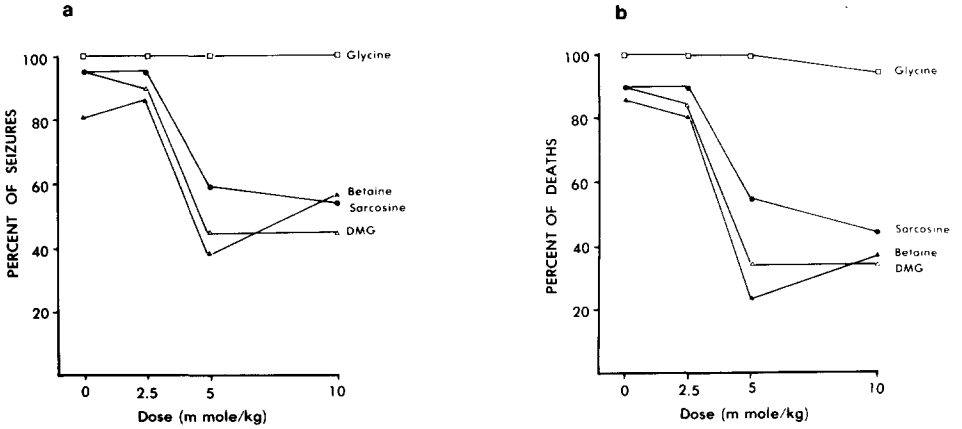

FIG. 1. Percentages of animals manifesting seizures and death following administration of 2.5 mg/kg of strychnine as a function of betaine, DMG, sarcosine, and glycine dosage. (a) Percentage of animals with seizures. For the 5 mM/kg dosage, the decrease in seizure frequency as compared to saline (0 mM/kg) was statistically significant for betaine ($p=0.011$), DMG ($p=0.001$) and sarcosine ($p=0.009$), but not for glycine (Fisher's exact test). (b) Percentage of deaths. For the 5 mM/kg dosage, the decrease in percentage of deaths was statistically significant for betaine ($p=1.27\times10^{-4}$), DMG ($p=7.72\times10^{-4}$), and sarcosine ($p=0.016$), but not for glycine. Between 20 and 22 mice were tested at each dosage point.

METHOD

Animals

Subjects were adult female Swiss-Webster mice weighing between 25 and 40 g. A total of 334 mice were tested. Animals were housed in groups of four to ten.

Drugs and Injections

Betaine HCl, N,N-dimethylglycine HCl, sarcosine HCl, and glycine (Sigma Chemical Co.) were administered IP in dosages of 2.5, 5 and 10 millimoles per kg in a volume of 10 ml/kg. The highest dosage was dissolved in distilled water and the osmolarity of the lower dosages was adjusted to equal that of the highest dosage using NaCl. The solution used for the zero dose group was 2 osmolar NaCl. Strychnine HCl (Sigma) was dissolved in normal saline and administered IP in a volume of 10 ml/kg.

Procedure

Effects of each drug (betaine, dimethylglycine, sarcosine, and glycine) were tested in a separate experiment. For each experiment, drug solutions were coded and administered to the mice on a random basis. The mice were tested eight at a time, so that two mice received each dose of the drug being tested for any one run. Ten minutes after drug administration, each mouse received strychnine HCl (2.5 mg/kg) and was subsequently observed for one hour. The times of occurrence of any seizures (clonic or tonic) and death were recorded by an observer who was "blind" to the drug treatment.

RESULTS AND DISCUSSION

Sarcosine, DMG, and betaine all inhibited strychnine-induced seizures at a dosage of 5 mM/kg. No further inhibition was produced by the higher dosage (10 mM/kg). The percentage of animals that manifested seizures or death was decreased (Fig. 1) and the latency to the onset of seizures

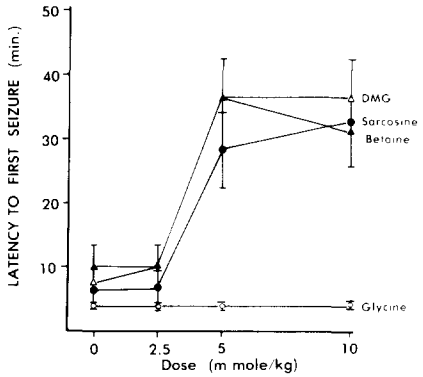

FIG. 2. Latency to the onset of seizures (means±S.E.M.) following administration of strychnine for animals pretreated with varying dosages of betaine, DMG, sarcosine, and glycine. For each compound the data were analyzed by one-way analysis of variance. Significant treatment effects were obtained for betaine, $F(3,80)=8.42$, $p<0.001$, DMG, $F(3,76)=10.89$, $p<0.001$, and sarcosine, $F(3,82)=9.68$, $p<0.001$, but not for glycine, $F(3,80)=0.93$, $p=0.434$.

was increased (Fig. 2) similarly by all three compounds. Glycine had no effect at any dosage level.

Thus a single N-methylation of glycine is sufficient for an anticonvulsant effect after peripheral administration. Additional N-methylations do not result in increased potency. Thus mono- di- and tri-methylglycine were equipotent in inhibiting seizures. For all three compounds, the anticonvulsant effect was not a linear function of log dosage; in each case, a maximal effect was produced by 5 mM/kg. Smaller dosages had no effect while larger dosages produced no additional effect.

Curtis and his colleagues [3] found that DMG does not have a simple postsynaptic glycine-agonistic effect on spinal cord neurons, nor did DMG potentiate the action of glycine and

GABA. The similarity of the dose-response curves for DMG, betaine, and sarcosine suggests a common mechanism of action for each of the three compounds, and thus it is probable that a simple glycine agonistic effect cannot explain their anticonvulsant properties. The reason for the anticonvulsant effect is therefore unknown but could be due to any of numerous effects, including an indirect influence on one-carbon metabolism [12], a glutamate-antagonistic effect of betaine (cf., [19]), a non-specific effect on biological membranes, or an indirect potentiation of glycine-mediated inhibition.

Although betaine, dimethylglycine, and sarcosine are normal body constituents, there is little information regarding side effects of the large doses which would be required to inhibit convulsions. In addition, the anticonvulsant and metabolic effects of these compounds during chronic administration have not been explored. Thus any direct application to seizures in humans should be approached cautiously.

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