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# Effects of Betaine on Seizures in the Rat

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GHOZ, E. H. AND W. H. FREED. *Effects of betaine on seizures in the rat.* PHARMACOL BIOCHEM BEHAV 22(4) 635–640, 1985.—The ability of betaine to block homocysteine, pentylenetetrazol, and electroshock induced seizures in mice has previously been observed. In this study, betaine administered IP and intraventricularly to rats blocked pentylenetetrazol-induced seizures, but IP betaine did not block audiogenic seizures. Intraventricular betaine was about 1000-fold more potent than IP betaine in blocking PTZ-induced seizures. Glycine, a component of the betaine molecule, was ineffective. It is concluded that betaine has an appreciable but selective effect in controlling experimental seizures in rats. This effect is mediated directly by the brain, and is not due to metabolism of betaine to glycine.

Seizures Betaine Pentylenetetrazol Audiogenic seizures Glycine

BETAINE (N,N,N-trimethylglycine) is a substance widely distributed in plant and animal tissues [11]. In the mammal, betaine is an oxidation product of choline [1]. It has a well-documented biological role as a methyl donor in the transformation of homocysteine to methionine, catalyzed by the enzyme betaine-homocysteine methyl transferase [9,21].

The possibility that betaine has anticonvulsant properties was originally suggested by studies of the pharmacology of homocysteine-induced seizures [25]. Homocysteine is a convulsant substance [8, 12, 25] which has been implicated as a cause of the seizures which accompany homocystinuria. Betaine antagonizes homocysteine induced seizures in mice [7,25], blocks neuronal excitations induced by glutamate and homocysteine [28], and has been reported to reverse some of the clinical manifestations of homocystinuria, including seizures in two patients [23].

In addition to the specific antagonism of homocysteine induced seizures, betaine may possess more general anticonvulsant properties. It has been found that betaine antagonizes electroshock and pentylenetetrazol (PTZ) induced seizures in mice [7]. Recently, Roach and Carlin [18] reported control of seizures in one developmentally-retarded patient by dimethylglycine, a metabolite of betaine.

The aims of the present study were: (1) to further examine the possible anticonvulsant effects of betaine in rats, (2) to determine whether betaine has an anticonvulsant effect when administered directly into the brain, and (3) to determine whether the underlying mechanism for this anticonvulsant effect can be attributed to glycine, a component of the betaine molecule. Two acute experimental models of epilepsy were used; pentylenetetrazol (PTZ), and audiogenic seizures in susceptible rats.

## GENERAL METHOD

### *Materials and Procedure*

Male Sprague Dawley rats weighing 150–280 g were used in the PTZ experiments. Each rat was used once only. Female rats weighing 230–370 g were used in the audiogenic seizure experiments. The latter were bred from an inbred strain of rats susceptible to audiogenic seizures maintained by Phillip C. Jobe, Louisiana State University School of Medicine, Shreveport, LA. Betaine and PTZ were obtained from the Sigma Chemical Co. The betaine solutions were prepared fresh before use. Betaine monohydrate was prepared as a 20 ml/kg solution for intraperitoneal injections containing either 338, 676, or 1352 mg/kg. For intracerebral administration, betaine monohydrate was prepared in concentrations of 112 to 900 $\mu$g per 20 $\mu$l. The betaine solutions were adjusted to approximately normal osmolarity and neutral pH. PTZ was prepared as a 70 mg/2 ml solution in saline and given intraperitoneally in a fixed dose of 70 mg/kg. Saline controls were used in all experiments. The experimenter was blind to the contents of all betaine solutions.

### *Observations*

For PTZ-induced seizures the time of onset of seizures and any other abnormal phenomena were recorded. Seizures occurred as clonic seizures or clonic seizures with tonic components. Since full tonic extension was not frequently seen at the dosage of PTZ that was used and the varying degrees of tonic components were difficult to distinguish, all were scored simply as "seizures" for purposes of data analysis. Extended seizures were defined as continuous repetitive seizure activity lasting for more than two minutes. Single, isolated non-repetitive contractions of much or all of the body musculature not associated with a seizure were defined as "twitches." Clonus was distinguished from twitches by the repetitive nature of the muscular contractions.

### *Statistical Analysis*

Nonparametric statistical tests [20] were used in all cases. Kruskal-Wallis, Mann-Whitney U tests, or Friedman's two way ANOVA for repeated measures were used for ordinal variables, and for nominal data the Fisher Exact Probability test or the Cochran Q tests were used as appropriate.

TABLE 1

INTRAPERITONEAL BETAINE IN AUDIOGENIC SEZIURES

| Betaine Monohydrate (mg/kg) | No. with Seizures* | No. with Extensor Response† | Mean Score (0–9)‡*** | Latency to 1st Run (sec)§** | Duration of Seizures (Sec)¶** |
|---|---|---|---|---|---|
| 0 | 15/15 | 6/15 | 5.7 ± 0.8 | 8.7 ± 2.2 | 19.2 ± 3.0 |
| 338 | 15/15 | 7/15 | 6.1 ± 0.8 | 9.7 ± 3.2 | 14.6 ± 1.7 |
| 676 | 15/15 | 6/15 | 5.7 ± 0.8 | 6.7 ± 1.3 | 15.2 ± 2.5 |
| 1352 | 13/15 | 7/15 | 5.7 ± 0.9 | 12.6 ± 5.0 | 12.9 ± 2.2 |

No statistically significant differences.

*$p=0.13$, Cochran Q test.

†$p=0.75$, Cochran Q test.

‡$p=0.9$, Friedman's two way ANOVA.

§$p=0.29$, Friedman's two way ANOVA.

¶$p=0.23$, Friedman's two way ANOVA.

**Means ± SEM.

††Scoring System: 0: no response; 1: running only; 2: two running phases plus clonus; 3: one running phase plus clonus; 4: two running phases plus tonus of forelimbs and neck, conus of hindlimbs; 5: same as 4 with one running phase; 6: same as 4 but with partial hindlimb tonus; 7: same as 6 but with one running phase; 8: same as 4 but complete hindlimb tonus, maximal convulsion; 9: same as 8 but with one running phase (for more details see [13]).

### EXPERIMENT I: BETAINE MONOHYDRATE IN AUDIOGENIC SEIZURES

Like some human epilepsies audiogenic seizures in the rat are hereditary [13]. Seizures in humans are sometimes evoked by auditory stimuli [13]. The anticonvulsant effects of betaine were therefore tested with this model of epilepsy.

*Procedure*

After screening for audiogenic seizure susceptibility, 15 adult female rats were selected for further testing. Each rat was tested 10 times, once a week, and for the last four tests the animals received either saline or 338, 676, and 1352 mg/kg betaine monohydrate (20 ml/kg solution) IP in random order until all four dosages had been administered. Thirty minutes after injection, the animal was placed in a closed cage. After 15 sec an electric doorbell attached to the wall which delivered a sound of 95 dB was sounded for one minute. In the event of seizure onset, the bell was turned off when tonic extension of the hindlimbs started, or otherwise 10 seconds after seizure onset. Seizures were scored according to the method described by Jobe [13], and the latencies in seconds to the onset of running and seizure onset, and duration of the seizure were measured by a stopwatch.

*Results and Discussion*

Betaine had no effect on audiogenic seizures (Table 1). There was no significant effect on the number of animals with seizures, latency to the first run, the duration of seizures, or any of the other measures (Table 1). Thus intraperitoneally administered betaine monohydrate was ineffective in controlling seizures induced by audiogenic stimuli in rats.

### EXPERIMENT II: INTRAPERITONEAL BETAINE VERSUS PENTYLENETETRAZOL

The purpose of this experiment was to examine the effects of intraperitoneal betaine on PTZ-induced seizures in the rat.

*Method*

Betaine monohydrate solutions were prepared as described above. One hundred and twenty rats were randomly assigned to receive either saline or 338, 676, and 1352 mg/kg of betaine IP and tested in groups of five. Seventy mg/kg PTZ was injected IP 10 minutes after betaine injection. Each group of five animals was observed continuously for one hour after injection.

*Results and Discussion*

Betaine monohydrate decreased the severity of PTZ-induced seizures but there was no significant effect on the number of rats with seizures (Table 2). The mean number of seizures per rat showed a significant decrease ($p<0.046$). Also, the latency of onset of the first seizure was significantly increased by betaine ($p<0.042$). The incidence of extended seizures was significantly reduced by betaine ($p<0.03$). Also, the number of twitches per animal was significantly reduced by betaine administration ($p<0.0025$). There was a significant decrease in the number of deaths between the low and high dosages of betaine ($p<0.025$); but the difference from saline controls on the same variable was not significant ($p<0.10$). Thus IP betaine monohydrate is capable of decreasing the severity of PTZ-induced seizures but not of preventing seizures from occurring.

### EXPERIMENT III: INTRACEREBROVENTRICULAR BETAINE VERSUS PENTYLENETETRAZOL

The purpose of this experiment was to determine whether betaine has an anticonvulsant effect when administered directly into the brain in small dosages. It is quite possible that the effects of betaine or the interaction between betaine and PTZ might take place in the periphery rather than in the brain. If the effects of betaine are central rather than peripheral, it is possible that betaine has a limited ability to cross the blood-brain barrier and its effects might be greater after direct intraventricular injection. These possibilities were investigated in the present experiment.

TABLE 2
INTRAPERITONEAL BETAINE VERSUS PENTYLENETETRAZOL

| Betaine Monohydrate (mg/kg) | No. with Seizures | Latency to 1st Seizure (min)*** | No. of Seizures†** | No. of Twitches‡** | Extended Seizures§ | Death |
|---|---|---|---|---|---|---|
| 0 | 30/30 | 2.22 ± 0.36 | 1.7 ± 0.14 | 4.6 ± 0.58 | 8/30 | 6/30 |
| 338 | 28/30 | 6.06 ± 2.69 | 1.3 ± 0.14 | 3.6 ± 0.60 | 9/30 | 8/30 |
| 676 | 27/30 | 7.93 ± 3.23 | 1.3 ± 0.15 | 2.4 ± 0.50 | 6/30 | 6/30 |
| 1352 | 28/30 | 7.27 ± 2.66 | 1.3 ± 0.13 | 2.2 ± 0.47 | 1/30 | 1/30 |

*$p=0.042$, Kruskal-Wallis Test.
†$p=0.046$, Kruskal-Wallis Test.
‡$p=0.0025$, Kruskal-Wallis Test.
§$p=0.03$, Fisher's Exact Probability Test.
**Means ± SEM.

*Method*

This experiment was performed in two steps: first a presumably high dose of betaine was tested against PTZ, and subsequently a dose response curve was established. In the first part betaine monohydrate was prepared fresh as a 45 μg/μl solution in saline. PTZ was prepared as usual (70 mg/2 ml in saline). Forty rats weighing between 150–200 g were divided in two equal groups and given either saline or betaine in a fixed dose of 20 μl (900 μg) into the lateral cerebral ventricle by the freehand method of Noble *et al.* [16] under light ether anesthesia. PTZ (70 mg/kg) was injected IP 30 minutes later, and the animals observed in groups of six for one hour. In obtaining the dose response curve the same procedure was followed, but betaine monohydrate was prepared in four dosages (112, 225, 450 and 900 μg/20 μl), and each dose and saline were administered to 12 rats on a random basis.

*Results and Discussion*

The administration of betaine directly into the lateral ventricle substantially inhibited PTZ-induced seizures (Table 3). Table 3a illustrates that the number of animals developing seizures was reduced by the high dosage of betaine (17/20, and 8/20 respectively, $p<0.008$). Tha latency to the first seizure was prolonged form a mean of 15.6 minute to 40.0 minutes ($p<0.005$), the number of seizures was reduced from 1.3 to 0.45 ($p<0.005$), and the mean number of twitches was reduced from 5.4 to 2.2 ($p<0.001$).

Intracerebroventricular betaine produced a significant dose-related effect in the control of PTZ-induced seizures (Table 3b). The number of animals developing seizures decreased from 10/12 to 4/12 ($p=0.0014$), the latency to the 1st seizure increased from 13.6 minutes to 56.3 minutes ($p=0.0002$), the mean number of seizures decreased from 1.5/rat to 0.5/rat ($p=0.007$), the number of twitches decreased from 7.9/rat to 1.58/rat ($p=0.011$), and lastly the number of animals with extended seizures was significantly reduced from five to zero ($p=0.015$). It should, however, be noted that the baseline seizure severity was decreased as compared to Experiment II, possibly due to the administration of ether for anesthesia.

The 225 μg/rat dosage of intraventricular betaine was more effective than the largest (1352 mg/kg) dosage of IP betaine. As the 1352 mg/kg dosage translates to roughly 300 mg/rat, it can be concluded that betaine is more than 1000-fold more potent when administered intracerebrally as compared to I.P. As the brain: body weight ratio is on the order of 1:100, it can tentatively be concluded that (1) the brain is the site of action for the anticonvulsant effect of betaine, and (2) after IP administration, betaine has a limited (on the order of 10%) ability to enter the brain.

EXPERIMENT IV: GLYCINE IN PENTEYLENETETRAZOL INDUCED SEIZURES

Glycine has been demonstrated to be an inhibitory neurotransmitter, especially in the spinal cord [24,27]. It is possible that betaine owes its anticonvulsant properties to the glycine part of the molecule. The last experiment was carried out to study the possibility that glycine can inhibit PTZ-induced seizures after intracerebral administration.

*Method*

Forty-eight male Sprague Dawley rats weighing 150–200 g were divided into four groups receiving either saline or one of three dosages of glycine (125,250 and 500 μg per 20 μl in saline). Solutions were prepared fresh before use. A fixed amount (20 μl) was injected intraventricularly as in Experiment III. PTZ (70 mg/kg) was injected IP 30 minutes later. Testing was carried out on six animals at a time, and each animal was observed continuously for one hour after PTZ injection.

*Results and Discussion*

Glycine did not have a significant effect on PTZ-induced seizures (Table 4). The number of animals that had seizures was not significantly decreased by glycine. Although there was a tendency for the latency to the onset of the first seizure to be increased (Table 4), this effect was not statistically significant. The mean number of seizures was not significantly reduced. The mean number of twitches was significantly decreased (5.25 for saline, and 2.42 for the largest dose of glycine; $p<0.02$). It is not clear, however, in what manner these twitches are related to seizures and a decrease in twitches is probably insufficient to conclude that there was an anticonvulsant effect. Note, however, that there was a difference in the baseline seizure severity as compared to Experiment III, and that glycine did tend to slightly suppress seizures although the effects were not significant.

TABLE 3

INTRACEREBROVENTRICULAR BETAINE VERSUS PENTYLENETETRAZOL

| Betaine Monohydrate ($\mu$g) | No. with Seizures* | Latency to 1st Seizure (Min)‡** | No. of Seizures‡** | No. of Twitches§** | Extended Seizures | Death |
|---|---|---|---|---|---|---|
| | | a. Single Dosage | | | | |
| 0 | 17/20 | 15.6 ± 5.26 | 1.3 ± 0.33 | 5.4 ± 0.8 | 2/20 | 1/20 |
| 900 | 8/20 | 40.0 ± 6.09 | 0.4 ± 0.14 | 2.2 ± 0.6 | 2/20 | 2/20 |

*$p$=0.008, Fisher's Exact Probability Test.
‡$p$=0.005, Mann Whitney U Test.
§$p$=0.001, Mann Whitney U Test.

| Betaine Monohydrate ($\mu$g) | No. with Seizures* | Latency 1st Seizure (Min)†** | No. of Seizures‡ | No. of Whole Body Twitches§** | Extended Seizures¶ | Death¶ |
|---|---|---|---|---|---|---|
| | | b. Dose-response Curve | | | | |
| 0 | 10/12 | 13.6 ± 6.5 | 1.5 ± 0.3 | 7.9 ± 1.4 | 5/12 | 5/12 |
| 112 | 11/12 | 8.1 ± 5.0 | 1.2 ± 0.3 | 3.3 ± 1.0 | 3/12 | 3/12 |
| 225 | 3/12 | 48.2 ± 6.6 | 0.3 ± 0.2 | 2.7 ± 0.8 | 0/12 | 0/12 |
| 450 | 6/12 | 33.6 ± 8.0 | 1.0 ± 0.4 | 3.9 ± 1.1 | 1/12 | 1/12 |
| 900 | 4/12 | 56.3 ± 13.3 | 0.5 ± 0.2 | 1.6 ± 0.8 | 0/12 | 0/12 |

*$p$=0.0014, Fisher's Exact Probability Test.
†$p$=0.001, Kruskal-Wallis Test.
‡$p$=0.007, Kruskal-Wallis Test.
§$p$=0.011, Kruskal-Wallis Test.
¶$p$=0.015, Fisher's Exact Probability Test.
**Means ± SEM.

TABLE 4

INTRACEREBROVENTRICULAR GLYCINE VERSUS PENTYLENETETRAZOL

| Glycine Dose ($\mu$g) | No. of Animals with Seizures* | Mean Latency to 1st Seizure (Min)†** | Mean No. Seizures‡ | Mean No. of Whole Body Twitches§** | Extended Seizures¶ | Death†† |
|---|---|---|---|---|---|---|
| 0 | 8/12 | 22.2 ± 8.1 | 0.8 ± 0.2 | 5.2 ± 1.6 | 1/12 | 1/12 |
| 125 | 6/12 | 36.2 ± 8.7 | 0.6 ± 0.2 | 3.2 ± 0.6 | 0/12 | 0/12 |
| 250 | 9/12 | 32.4 ± 13.6 | 1.1 ± 0.3 | 1.9 ± 1.2 | 2/12 | 1/12 |
| 500 | 6/12 | 40.4 ± 7.8 | 0.5 ± 0.1 | 2.4 ± 1.2 | 2/12 | 1/12 |

*$p$=0.57, Fisher's Exact Probability Test.
†$p$=0.20, Kruskal-Wallis Independent ANOVA.
‡$p$=0.36, Kruskal-Wallis Independent ANOVA.
§$p$=0.02, Kruskal-Wallis Independent ANOVA.
¶$p$=0.74, Fisher's Exact Probability Test.
††$p$=1.00, Fisher's Exact Probability Test.
**Means ± SEM.

## GENERAL DISCUSSION

Our findings confirm that betaine can antagonize PTZ-induced seizures. Betaine, however, failed to control audiogenic seizures in susceptible rats. This failure might be due to differences in the mechanisms of audiogenic and PTZ induced seizures. The genetic audiogenic seizure susceptibility has been postulated to be the result of a cochlear abnormality rather than a primary CNS abnormality [13]. Again, the running component of this type of seizures, and their dependence upon certain stimulus intensities and frequencies, distinguish these seizures from the classic clonic or tonic-clonic seizure induced by PTZ. Nonetheless, these data suggest that betaine has a limited spectrum of activity since a number of other broad spectrum anticonvulsants have been shown to control the extensor component of audiogenic seizures [3].

Betaine was about 1000-fold more effective in controlling PTZ seizures when given intraventricularly than when given IP. As the brain:body weight ratio is only on the order of 1:100, this suggests that the brain is the site of action of peripherally-administered betaine. Betaine might not cross the blood brain barrier readily, although it is also possible that the 10 minute interval between intraperitoneal injections of betaine and PTZ in Experiment II was insufficient for maximal penetration of betaine into the brain. The anticonvulsant dosage level for centrally-administered betaine would be expected to translate to anticonvulsant IP dosages about 10-fold smaller than those which were actually observed if betaine crossed the blood-brain barrier readily. Thus, substances similar to betaine which readily cross the blood-brain barrier might be found to have greatly increased anticonvulsant potency.

The lack of effect of intracerebral glycine on PTZ-induced seizures agrees with the findings of a similar study [26]. The anticonvulsant potential of glycine is slight at most and even when strychnine is used as the convulsant stimulus glycine has been reported to have only a modest anticonvulsant effect [14,19]. These data do not tend to suggest that the anticonvulsant effect of betaine is due to a glycine agonist effect. This conclusion is also consistent with previous reports that dimethylgylcine does not act as a glycine or $\gamma$-aminobutyric acid agonist [4]. The mode of action of betaine as an anticonvulsant thus remains obscure. In order to suggest that betaine acts by enhancing the methylation of homocysteine, a demonstration that homocysteine is implicated in the genesis of seizures of varying etiology would be required. Again, the absence of appreciable amounts of the enzyme betaine-homocysteine methyl transferase in the brain [6,15] argues against this proposition. It is interesting, however, that betaine can induce the activity of this enzyme in cultured murine cells originally devoid of such activity [10], and it is conceivable that this mechanism could come into effect with prolonged intracerebral betaine administration.

Betaine is a surface-active substance affecting the cell membrane, enhancing cellular respiration and protecting against high sodium concentrations in the extracellular fluid [17,22]. PTZ may produce seizures through reducing chloride-conductance and probably blocking GABAergic inhibitory transmission [5]. Betaine might act directly on these phenomena. A relationship between betaine and GABAergic transmission is further suggested by the demonstration that gamma-butyro betaine inhibits GABA uptake [2].

Thus IP betaine has an anticonvulsant effect for PTZ-induced seizures but not for seizures induced by audiogenic stimuli. This anticonvulsant effect appears to be mediated by a direct effect of betaine on the brain. Betaine, but not glycine, was a potent antagonist of PTZ-induced seizures when administered directly into the cerebral ventricles. This suggests that betaine has a direct inhibitory effect on the central nervous system which is not simply a result of degradation to glycine.

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