Pharmacology Biochemistry and Behavior, Vol. 47, pp. 241–246, 1994
Printed in the U.S.A.

0091-3057/94 $6.00 + .00

Diazepam Potentiation by Glycine in Pentylenetetrazol Seizures Is Antagonized by 7-Chlorokynurenic Acid

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Received 17 May 1992

PETERSON, S. L. *Diazepam potentiation by glycine in pentylenetetrazol seizures is antagonized by 7-chlorokynurenic acid.* PHARMACOL BIOCHEM BEHAV 47(2) 241–246, 1994.—This study evaluated a possible mechanism by which glycine potentiates the activity of diazepam (DZP) and valproic acid (VAL) against the clonic seizures induced by pentylenetetrazol (PTZ) in rats. Neither 7-chlorokynurenic acid (7-CLKYNA) nor strychnine in doses of 10, 50, or 100 nmol ICV significantly altered the clonic seizure response to PTZ. However, 7-CLKYNA (100 nmol, ICV), but not strychnine (100 nmol, ICV), antagonized the anticonvulsant activity induced by coadministration of DZP (1.0 mg/kg, IP) and glycine (40 mmol/kg, PO). Neither 7-CLKYNA (100 nmol, ICV) nor strychnine (100 nmol, ICV) significantly altered the anticonvulsant activity induced by coadministration of VAL and glycine. 7-CLKYNA (100 nmol, ICV) had no effect on the anticonvulsant activity of DZP or VAL in the absence of glycine. These results provide evidence that the glycine potentiation of the anticonvulsant activity of DZP in clonic seizures induced by PTZ may be mediated by the strychnine-insensitive glycine receptor.

Glycine Diazepam Pentylenetetrazol 7-Chlorokynurenic acid Seizures Rats

GLYCINE modulates the NMDA receptor/ionophore complex by facilitating the opening of the NMDA-linked ion channel in a strychnine-insensitive manner (10). Glycine has been shown to potentiate NMDA-mediated activity as measured in electrophysiology (12), receptor binding (20), and in vivo studies (6). Because of the proposed stimulatory role for NMDA receptors in epilepsy, it has been hypothesized that glycine will induce proconvulsant effects in vivo (8).

In contrast, orally administered glycine has been shown to potentiate the activity of anticonvulsant drugs in the kindled amygdala (16), pentylenetetrazol (17), and maximal electroshock (19) models of epilepsy. In maximal electroshock, central administration of the selective strychnine-insensitive glycine receptor antagonist 7-chlorokynurenic acid (7-CLKYNA) (11) antagonized the glycine potentiation of phenobarbital and phenytoin (18). These findings are evidence that the glycine potentiation of phenobarbital and phenytoin anticonvulsant activity against the tonic extension component of maximal electroshock seizures may be mediated by the strychnine-insensitive glycine receptor. Similarly, in SC pentylenetetrazol (SC PTZ) seizures glycine had no anticonvulsant activity by itself but potentiated the anticonvulsant activity of diazepam (DZP) and valproic acid (VAL) administered by IP injection (17). In this same study, glycine did not alter the activity of ethosuximide in the SC PTZ seizures. The purpose of the present study was to evaluate the possible role of the strychnine-insensitive glycine receptor in the previously demonstrated (17) glycine potentiation of DZP and VAL anticonvulsant activity against the clonic seizures induced by PTZ.

METHOD

Animals

Male Sprague–Dawley rats (Harlan, Inc., Houston, TX) were maintained in a temperature-controlled vivarium on a 12 L : 12 D cycle and allowed free access to food and water. At the time of seizure testing, animals weighed in the range of 100–150 g, as is recommended for the SC PTZ model of epilepsy (22).

PTZ-Induced Convulsions

Convulsions were induced by injecting 80 mg/kg PTZ (SC) under a fold of skin over the scapula. After injection, animals were placed in a cage that was visually isolated from other animals being seizure tested and observed for 30 min for the occurrence of clonic and tonic seizures. Clonic seizures were defined as clonic activity of at least 5-s duration involving both the forelimbs and hindlimbs with or without the loss of the righting reflex. Tonic seizures were defined as loss of the

righting reflex with tonic forelimb extension and/or tonic forelimb and hindlimb extension. Animals treated only with the 80-mg/kg dose of PTZ showed no seizure response other than clonic seizures.

Cannula Implantation

Animals were anesthetized with Equithesin (mixture of choral hydrate, pentobarbital, magnesium sulfate, and ethanol) for the surgical implantation of the ICV guide cannulae. A 22-ga ICV injection guide cannula (Plastic Products, Inc., Roanoke, VA) was implanted 0.2 mm dorsal to the right ventricle using the stereotaxic coordinates of 1.8 mm lateral to bregma and 1.8 mm ventral from the surface of the cortex (15). A stylet was placed in the cannula to prevent clogging when not in use. Anchor screws were placed in the skull and the entire assembly was secured with dental acrylic cement. All animals were allowed 5–7 days for recovery before seizure testing. A 28-ga injection needle that extended 1 mm beyond the distal end of the guide cannula was inserted into the guide cannula for the ICV drug administration. Each rat received only one ICV infusion. Verification of the cannulae placements was made at the end of the experiments by localization of a dye that was infused into the ventricles (18).

Drug Treatment

All systemic drug doses, drug solutions, routes of administration, and times of administration were based on those used in a previous report (17) so that a direct comparison could be made with the present experiment. The doses of DZP and VAL when given in combination with glycine were chosen on the basis that they had previously been shown to be anticonvulsant (17) and would be ideal for demonstrating an antagonism of the anticonvulsant activity if it were to occur. Glycine (Sigma Chemical Co., St. Louis, MO) was administered orally in a dose of 40 mmol/kg as a 3-M solution in 0.9% saline 2 h prior to the seizure test. DZP (Hoffman-La Roche, Nutley, NJ) and VAL [Research Biochemicals, Inc. (RBI), Natick, MA] were administered IP in a 4-ml/kg volume of 2% carboxymethylcellulose (Sigma) 0.5 h prior to the seizure test. VAL doses were calculated as the free weight of the drug. PTZ (Sigma) was administered in 0.9% saline in a volume of 2 ml/kg. 7-CLKYNA (RBI) was dissolved in 0.5 N NaOH. The solution was adjusted to a pH of 7.4 with 0.1 M sodium phosphate buffer and brought to its final concentration with 0.9% saline. Strychnine HCl (Sigma) was dissolved in 0.9% saline and adjusted to a pH of 7.4 with sodium phosphate buffer. The vehicle control solution consisted of 0.9% saline adjusted to a pH of 7.4 with sodium phosphate buffer. Ten microliters of the final solutions were infused into the lateral ventricle at the rate of 5 μl/min. The cannula was left in place for 1 min after the end of the infusion to allow diffusion of the solution into the cerebrospinal fluid. PTZ was injected immediately after the protective stylet was replaced in the injection guide cannula.

Statistics

The quantal seizure response data were analyzed by probit regression. Estimation of parameters was performed through an integrative process using a maximum likelihood loss function. The significance of addition or deletion of an independent variable was tested by comparing the goodness of fit for models with or without the variable of interest. The result is reported statistically as the incremental maximum likelihood χ^2 with degrees of freedom and probability level (Complete Statistical System by StatSoft, Inc., Tulsa, OK).

RESULTS

Effects of 7-Chlorokynurenic Acid or Strychnine on PTZ Seizures

7-CLKYNA produced no significant effect on the SC PTZ seizures. The 80-mg/kg PTZ dose induced at least one episode of clonic seizures in 13 of 16 animals (81%) administered ICV vehicle control solution (Fig. 1). The 10-nmol, $\chi^2(1) = 0.2$, $p = 0.69$, 50-nmol, $\chi^2(1) = 0.7$, $p = 0.42$, and 100-nmol, $\chi^2(1) = 2.6$, $p = 0.10$, ICV doses of 7-CLKYNA induced no significant change in the incidence of the clonic seizure response (Fig. 1). None of the 7-CLKYNA-treated rats displayed tonic seizures. Although the 10-nmol 7-CLKYNA dose did not induce behavioral abnormalities in the rats, the 50-nmol dose produced ataxia and hypotonicity while those rats treated with 100 nmol 7-CLKYNA displayed splayed hindlimbs associated with retropulsion and randomly directed circling behavior. The 100-nmol dose was chosen for further study because it had no significant effect on the clonic seizures and would maximize the opportunity to observe the effect of selective strychnine-insensitive glycine receptor antagonism.

Strychnine altered the seizure response to PTZ. Administered alone, the 10-, 50-, and 100-nmol ICV doses of strychnine induced no clonic or tonic seizures in any of the animals tested ($n = 6$ for each dose, data not shown). Likewise, the 10-nmol, $\chi^2(1) = 1.8$, $p = 0.18$, 50-nmol, $\chi^2(1) = 0.1$, $p = 0.95$, and 100-nmol, $\chi^2(1) = 1.8$, $p = 0.18$, ICV doses of strychnine induced no significant effect on the incidence of clonic seizures in PTZ-treated animals (Fig. 1). However, strychnine induced a dose-related increase in the incidence of tonic seizures in PTZ-treated animals (Fig. 1). The 100-nmol strychnine dose was chosen for further study because it was pharmacologically active (induced tonic seizures in response to PTZ) and was the most appropriate comparison to the 100-nmol 7-CLKYNA dose.

7-Chlorokynurenic Acid Reversal of the Anticonvulsant Activity Induced by Diazepam and Glycine

The anticonvulsant activity induced by coadministration of 1.0 mg/kg DZP and 40 mmol/kg glycine was antagonized by ICV administration of 100 nmol 7-CLKYNA but not 100 nmol strychnine (Fig. 2). The group treated with 7-CLKYNA ICV responded with a significantly, $\chi^2(1) = 14.7$, $p = 0.001$, greater number of clonic seizures than the group treated with vehicle. In contrast, the number of animals responding with clonic seizures in the strychnine ICV-treated group did not differ significantly, $\chi^2(1) = 1.34$, $p = 0.25$, from the group treated with vehicle control (Fig. 2). Neither 7-CLKYNA, $\chi^2(1) = 1.35$, $p = 0.24$, nor strychnine, $\chi^2(1) = 0.28$, $p = 0.60$, altered significantly the anticonvulsant effect induced by 250 mg/kg VAL and 40 mmol/kg glycine (Fig. 3). No tonic seizures were induced in any of the animals used in these experiments.

The 100-nmol dose of 7-CLKYNA did not significantly alter the anticonvulsant activity of 1.5 mg/kg DZP, $\chi^2(1) = 0.75$, $p = 0.37$, or 350 mg/kg VAL, $\chi^2(1) = 0.57$, $p = 0.45$, in the absence of glycine (Fig. 4). Increased doses of DZP and VAL were chosen for these experiments because the doses used in combination with glycine (as shown in Figs. 2 and 3) are not sufficiently anticonvulsant in the absence of glycine to allow observation of a 7-CLKYNA-induced antagonism of the

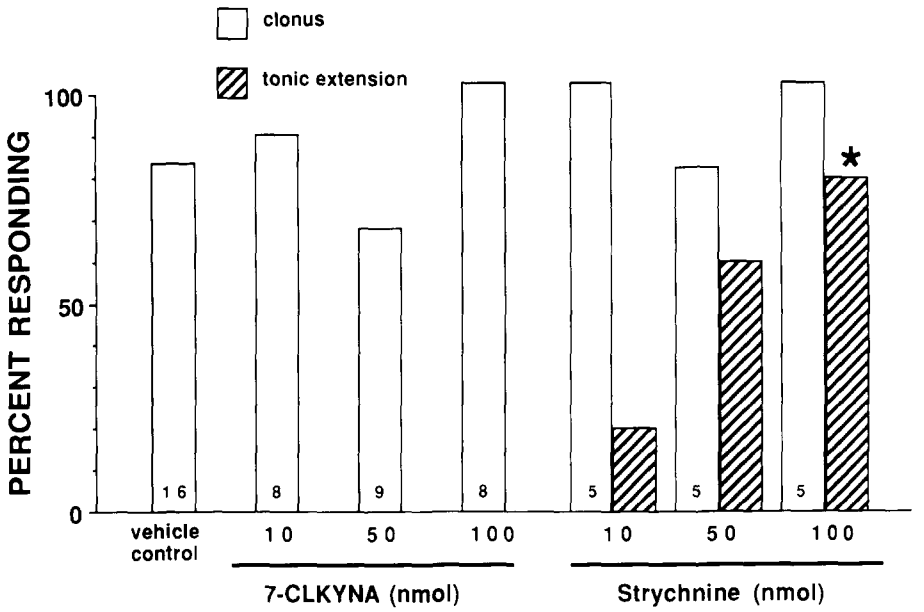

FIG. 1. Comparison of the effects of 10, 50, and 100 nmol 7-chlorokynurenic acid (7-CLKYNA) or strychnine ICV on the SC pentylenetetrazol (PTZ) (80 mg/kg) seizure response. Neither drug altered significantly the incidence of clonic seizures. Strychnine induced a dose-related increase in the incidence of tonic seizures. *Significant, $\chi^2(1) = 3.85$, $p = 0.05$, increase in tonic seizures as compared to the 10-nmol strychnine group. The number of rats in each group is indicated in the columns.

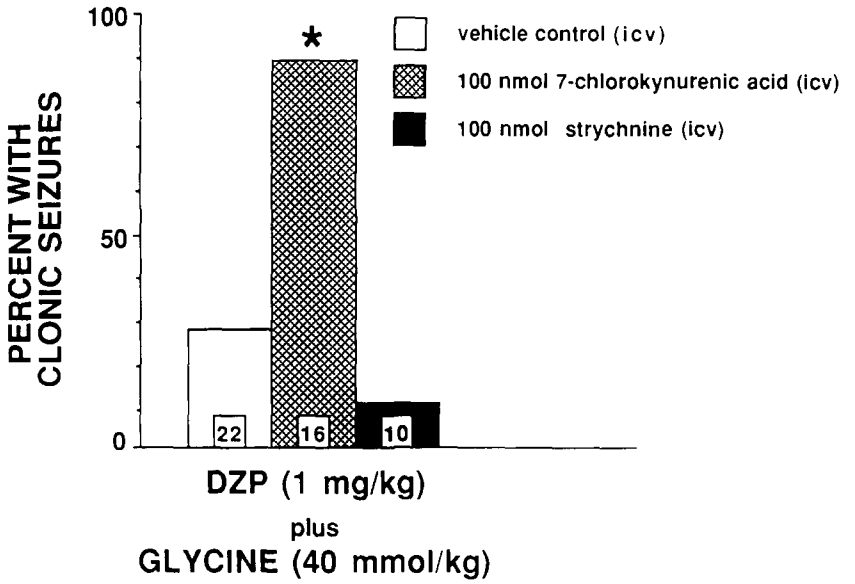

FIG. 2. Effects of vehicle control solution, 7-chlorokynurenic acid (7-CLKYNA) or strychnine ICV on the anticonvulsant activity induced by coadministration of glycine and diazepam (DZP) in SC pentylenetetrazol (PTZ)-induced clonic seizures. All animals were administered 1 mg/kg DZP (IP) and 40 mmol/kg glycine (PO) in addition to the vehicle control, 100-nmol 7-CLKYNA, or 100-nmol strychnine infusions. Glycine has been shown previously to potentiate the anticonvulsant activity of DZP in SC PTZ seizures (17). 7-CLKYNA increased significantly the incidence of clonic seizures as compared to vehicle control-treated animals. No tonic seizures were induced in any animals tested. *Significant, $\chi^2(1) = 14.7$, $p = 0.001$, increase in clonic seizures as compared to the vehicle control group. The number of animals in each group is indicated in the columns.

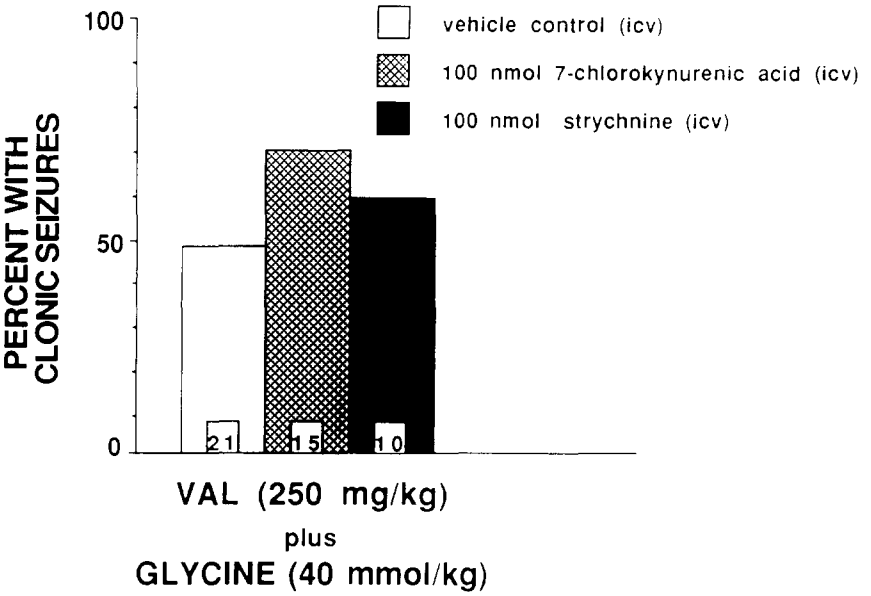

FIG. 3. Effects of vehicle control solution, 7-chlorokynurenic acid (7-CLKYNA) or strychnine ICV on the anticonvulsant activity induced by coadministration of glycine and valproic acid (VAL) in SC pentylenetetrazol (PTZ)-induced clonic seizures. All animals were administered 250 mg/kg VAL (IP) and 40 mmol/kg glycine (PO) in addition to the vehicle control, 100-nmol 7-CLKYNA, or 100-nmol strychnine infusions. Glycine has been shown previously to potentiate the anticonvulsant activity of VAL in SC PTZ seizures (17). No significant differences in the incidence of clonic seizures were detected. No tonic seizures were induced in any animals tested. The number of animals in each group is indicated in the columns.

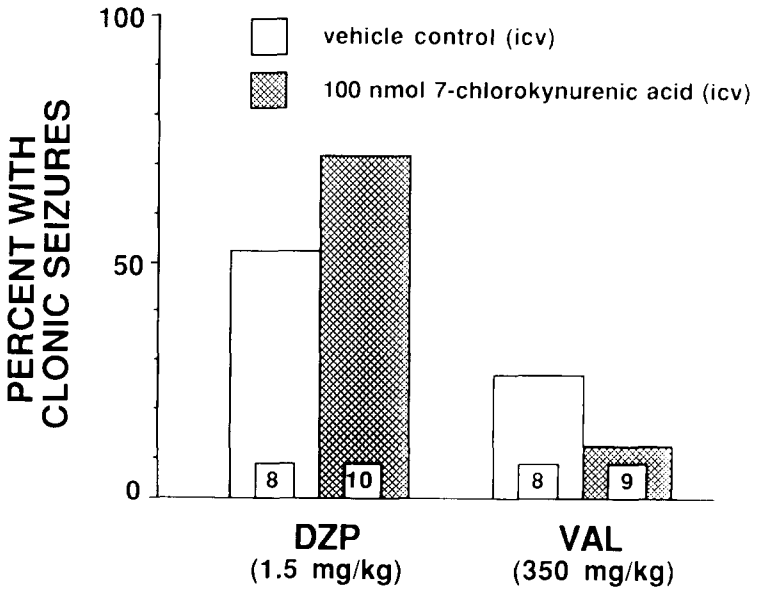

FIG. 4. Effects of vehicle control solution or 7-chlorokynurenic acid (7-CLKYNA) ICV on the anticonvulsant activity induced by diazepam (DZP) or valproic acid (VAL) in the absence of glycine in SC pentylenetetrazol (PTZ)-induced clonic seizures. Animals were administered either 1.5 mg/kg DZP (IP) or 350 mg/kg (IP) VAL in addition to the ICV infusion of either the vehicle control or 100-nmol 7-CLKYNA infusions. No significant differences in the incidence of clonic seizures were detected. No tonic seizures were induced in any animals. The number of animals in each group is indicated in the columns.

anticonvulsant activity if it occurred. No tonic seizures were induced in any of the animals used in these experiments.

DISCUSSION

It has been shown previously that the same dose of glycine as used in the present experiment has no anticonvulsant activity by itself but potentiates the anticonvulsant activity of DZP and VAL against the clonic seizures induced by SC PTZ (17). Using doses of 7-CLKYNA and strychnine that by themselves do not affect the clonic seizure response, the present findings demonstrate that the DZP potentiation by glycine is antagonized by 7-CLKYNA but not strychnine. 7-CLKYNA did not affect the activity of DZP in the absence of glycine (Fig. 4), indicating that 7-CLKYNA selectively antagonized the activity of glycine when glycine was coadministered with DZP (Fig. 2). Because 7-CLKYNA and strychnine are selective antagonists of the strychnine-insensitive and strychnine-sensitive glycine receptors, respectively (5), these data suggest that the glycine potentiation of DZP in SC PTZ seizures is mediated by the strychnine-insensitive glycine receptor. The present findings are similar to those of previous work in which 7-CLKYNA antagonized the glycine potentiation of phenobarbital and phenytoin in maximal electroshock seizures (18). Together, these results are evidence that the glycine potentiation of specific anticonvulsant drugs is active against the seizure mechanisms associated with both tonic seizures (maximal electroshock) and clonic seizures (SC PTZ).

In contrast, the glycine potentiation of VAL may be mediated by mechanisms other than the strychnine-insensitive glycine receptor because the anticonvulsant effect was not antagonized by 7-CLKYNA. It is interesting to note that glycine does not potentiate VAL in maximal electroshock seizures (18,19), which may indicate that VAL potentiation by glycine is specific to SC PTZ seizures and does not involve the strychnine-insensitive glycine receptor. One possible explanation may involve the observation that VAL and ethosuximide both interfere with γ-hydroxybutyrate catabolism and release from nerve terminals (23). However, possible interactions between glycine and the interference with γ-hydroxybutyrate catabolism are doubtful because glycine potentiates VAL but not ethosuximide in SC PTZ seizures (17). An alternative explanation involves the hypothesis that the anticonvulsant activity of VAL is mediated by an increase in nerve terminal GABA content (14). If this hypothesis is valid (7), then any glycine interaction with VAL-mediated GABA activity would be specific for SC PTZ seizures as glycine does not potentiate VAL in maximal electroshock seizures (19).

7-CLKYNA alone had no significant effect on the clonic seizure response induced by SC PTZ. In contrast, ICV 7-CLKYNA is anticonvulsant in audiogenic seizures (21) and NMDA-induced convulsions in mice (13,21). In rats, 7-CLKYNA administered into the amygdala inhibits the development of amygdala kindling (4) and 7-CLKYNA administered into the prepyriform cortex inhibits seizures induced by bicuculline infusion into the prepyriform cortex (24). However, ICV administration of 7-CLKYNA does not affect the tonic extension component of maximal electroshock seizures in rats (9,18). In the present experiment, ICV 7-CLKYNA also had no anticonvulsant effect on the clonic PTZ seizure response at doses that had induced a significant reduction in the clonic seizure response to bicuculline infusions into the prepyriform cortex in a previous study (24). Although no comprehensive explanation is possible at present, the differential activity of 7-CLKYNA in the various models of epilepsy may be related to the differential affinity of 7-CLKYNA to subtypes of the strychnine-insensitive glycine receptor (5). In that regard, the 7-CLKYNA antagonism of the glycine potentiation of DZP may have been mediated by strychnine-insensitive glycine receptors located in the forebrain. PTZ-induced clonic convulsions are proposed to be mediated in the forebrain (2), a site where a subtype of the strychnine-insensitive glycine receptor with high affinity for 7-CLKYNA is located (5).

Strychnine had no significant effect on the occurrence of clonic seizures induced by PTZ. However, doses of strychnine that had no convulsive activity by themselves produced a dose-related increase in the incidence of tonic seizures in response to the 80-mg/kg PTZ dose. Tonic seizures are part of a PTZ-induced convulsive continuum that occurs at higher doses of PTZ and is believed to involve the spread of seizure activity from the forebrain to the brainstem (2). Because the brainstem is proposed to mediate PTZ-induced tonic extension (3) and is a prominent site of strychnine-sensitive glycine receptors (1,5) it seems reasonable to hypothesize that strychnine acts in the brainstem to activate the brainstem components that mediate tonic seizures and convert the normally clonic seizures to tonic seizures. Strychnine would not be expected to influence the clonic seizures induced by PTZ that are mediated by the forebrain (2), an area lacking strychnine-sensitive glycine receptors (1,5), and in fact strychnine did not alter significantly the occurrence of clonic seizures in any of the present experiments. Strychnine did not induce tonic extension in animals treated with glycine and DZP or VAL (Figs. 2 and 3). This is probably a result of the failure of strychnine to antagonize the anticonvulsant activity of the coadministered drugs, which thereby continued to suppress the spread of seizure activity in the forebrain and prevented activation of the tonic seizure mechanisms in the brainstem.

ACKNOWLEDGEMENTS

The author thanks Laura Hinojosa and Karen Dwyer for their technical assistance. This work was supported by Public Health Service Grant NS24566.

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