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Prevention of the Convulsant and Hyperalgesic Action of Strychnine by Intrathecal Glycine and Related Amino Acids

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BEYER, C., C. BANAS, P. GOMORA AND B. R. KOMISARUK. *Prevention of the convulsant and hyperalgesic action of strychnine by intrathecal glycine and related amino acids.* PHARMACOL BIOCHEM BEHAV **29**(1) 73–78, 1988.— Intrathecal administration of 25 μg strychnine induced consistent sensory and motor behavioral events in rats. Sensory events included scratching and biting the lower half of the body, spontaneous vocalizations and skin hyperalgesia, evidenced by vocalization and reflex scratching in response to stimulation with a 5.5 g von Frey fiber. This mild stimulus failed to elicit vocalizations in the preinjection condition. Strychnine induced two types of motor seizures: (1) falling over with tail whipping and (2) convulsions. The effect of equimolar doses of glycine (G) and some related amino acids: β-alanine (A), taurine (T) and betaine (B) on the strychnine syndrome was tested by administering them (intrathecal route) along with strychnine. T and G but not B significantly decreased most of the sensory events triggered by strychnine. All amino acids significantly decreased the incidence and duration of convulsions; T and B abolished them. A decreased vocalizations and skin hyperalgesia but synergized with strychnine to facilitate scratching and self biting. These results are consistent with findings that G, A and T displace strychnine from its binding sites in the CNS.

Glycine β-Alanine Taurine Betaine Strychnine syndrome Sensorimotor action of strychnine Inhibitory amino acids Glycine inhibition of strychnine action Scratching Self-biting Convulsions Antagonism of strychnine by inhibitory amino acids Skin hyperalgesia

STRYCHNINE is a potent glycine antagonist that induces intense motor disturbances including convulsions [2, 5, 7, 11]. Therefore, it is not surprising that most studies on the action of strychnine have focused on processes related to effects on motoneurons or interneurons involved in motor control [28]. However, strychnine acts also on neuronal mechanisms related to the perception of itch and pain [5, 11, 15, 18]. Thus, perispinal administration of strychnine to rats induces skin hyperalgesia, scratching, and a decrease in the threshold to induce vocalization by tail shock [5]. These findings, together with the observation that glycine inhibits the discharge of spinothalamic neurons [24], support the idea that glycinergic neurons modulate afferent transmission related to nociception [5, 27, 28].

Since glycine and other related amino acids (β-alanine and taurine) displace labeled strychnine [6, 26, 27] it appears likely that their administration will counteract all or some of the effects induced by strychnine. This prediction has not been fulfilled since glycine administration has failed to prevent or to decrease strychnine induced convulsions [13,23], though it exerts anticonvulsant actions in other chemical models of convulsions [23]. Therefore, it has been proposed that glycine acts as an anticonvulsant by decreasing the brain concentration of the excitatory neurotransmitter glutamic acid rather than by enhancing agonist action on glycine receptors [23].

Some properties of glycine and glycine-like amino acids [8], however, may explain their failure to counteract strychnine actions: (1) glycine and β-alanine are rapidly removed from their site of action in the spinal cord (the postsynaptic membrane) by highly efficient uptake systems [10,16] and (2) these amino acids penetrate very slowly into the brain [22]. This last point is relevant to the assessment of the possible anticonvulsant action of glycine since in all the studies designed to test this property, it has been administered systemically, i.e., intragastrically or intravenously.

Therefore, in the present study we decided to study the capacity of glycine, β-alanine, taurine or trimethylglycine (betaine) to antagonize the motor and sensory effects of intrathecal strychnine when each amino acid was adminis-

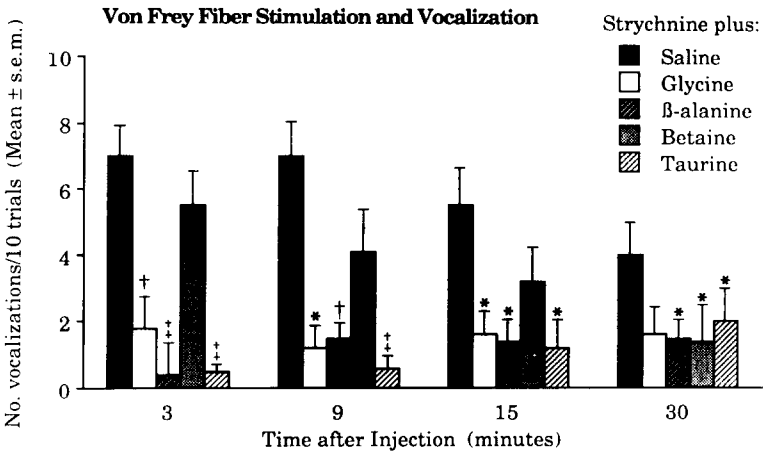

FIG. 1. Effect of the combined intrathecal injection of strychnine (25 μg) and equimolar doses of various amino acids on the incidence of vocalizations induced by von Frey fiber stimulation (10 stimuli to the back and flanks). Note that this stimulus did *not* elicit vocalizations before intrathecal injection.

tered in combination with strychnine. In addition, the effects were determined of each amino acid administered without strychnine.

METHOD

Subjects (Ss) were adult Sprague Dawley rats (250 to 350 g), housed individually in a light controlled room (dark from 10.00 to 20.00 hr) and maintained at 23°C. Food (Purina) and water were supplied ad lib. Rats were ovariectomized to exclude possible alterations in neuronal excitability due to gonadal hormone secretion. A 7.5 cm catheter (Clay Adams PE-10 tubing; Fisher Chemical) was inserted permanently in the subarachnoid intrathecal space through an incision in the atlantooccipital space according to the technique of Yaksh and Rudy [25]. The catheter extended to the lumbar level of the spinal cord. Animals were anesthetized during surgery with Ketamine (Bristol Laboratories, 20–25 mg, IP) and Xylazine (Haver Lockhart, 1.2 mg, IP) and treated once with Terramycin (5 mg, IM). At least 7 days of recovery were allowed before testing.

Preinjection Testing

Before intrathecal injections Ss were observed in a circular Plexiglas cylinder for 5 min. Rats having motor problems as a result of the catheter implantation were discarded from these experiments. The response to cutaneous stimulation with a von Frey fiber (5.5 g force; Stoelting Co., Chicago, IL) was determined by counting the number of vocalizations elicited by 10 successive applications of the stimulus (approximately 3 sec intervals) to alternate parts of the lower half of the dorsum and flanks. Since strychnine has been reported to lower the vocalization threshold in response to tail shock [5], this test and the radiant heat test (tail flick test) were used to establish a possible analgesic effect of the amino acids. Vocalization threshold to tail shock was determined by placing the Ss in a restrainer and taping two stainless steel electrodes to the tail after applying conductive gel. Electrical shocks (100 msec train of 60 Hz symmetrical, biphasic square waves) with an intertrain interval of 3 sec, were delivered by a constant current shock generator (Coulbourn Instruments Programmable Shocker, Lehigh Valley, PA). The current was increased in 10 μA steps until vocalization was elicited and then decreased stepwise until no longer elicited; this was repeated three times. Upper and lower shock levels were averaged to provide an estimate of vocalization threshold according to the method described previously [5]. Tail flick latency was determined by using an IITC Model 33 analgesimeter (Landing, NJ, at 90% intensity). Rats were placed in a Plexiglas restrainer with the tail exposed to a radiant heat lamp. Tail flick latencies were measured automatically by activation of a photocell upon tail withdrawal. A heat cutoff time of 15 sec was employed to avoid tissue damage.

Injection Procedures

After control observations, rats were injected intrathecally with one of the following solutions: strychnine, 25 μg (n=10); glycine, 400 μg (n=9); alanine, 480 μg (n=10); betaine, 800 μg (n=9); taurine, 700 μg (n=10); strychnine+glycine (n=10); strychnine+alanine (n=10); strychnine+betaine (n=10); strychnine+taurine (n=10). Dosages of amino acids were practically equimolar. All chemicals were dissolved in 5 μl of saline and were obtained from Sigma Chemicals (St. Louis, MO). Drugs were delivered to the perispinal space with an additional 7 μl of saline flushed from the catheter. Injection duration was 1 to 2 minutes. Rostrocaudal diffusion following intrathecal injection at this volume is usually limited to the spinal cord at least within the first 30 minutes post-injection [25]. Post injection tests began immediately after completion of the injection.

Post Injection Behavioral Testing

Rats were replaced in the cylindrical Plexiglas cage and their behavior was recorded and registered. From previous studies of intrathecal strychnine administration [5] the following behavioral parameters were measured on a minute to minute basis: (1) grooming, (2) spontaneous bouts of scratching, (3) spontaneous bouts of skin biting, (4) spontaneous vocalizations, (5) hopping, (6) falling over with tail whipping, and (7) convulsions. Additionally, the response to cutaneous stimulation with a von Frey fiber was determined at 3, 6, 9, 12, 15, 20, 30, 45 and 60 min post injection as described

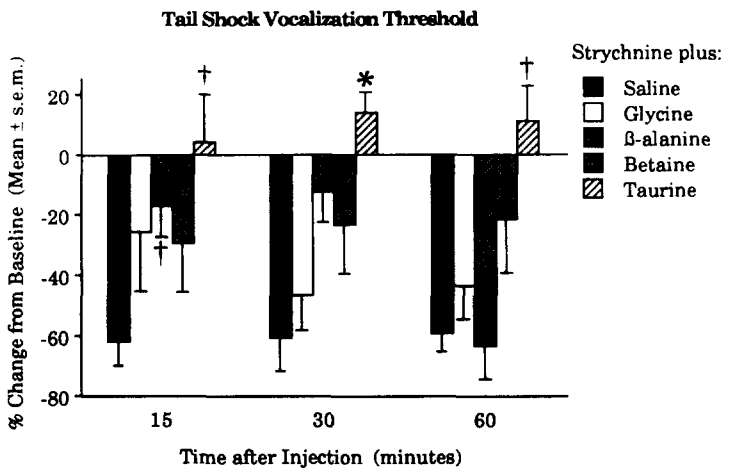

FIG. 2. Effect of the combined intrathecal injection of strychnine (25 μg) and equimolar doses of various amino acids on the values obtained in the tail shock vocalization test. Note that strychnine *per se* elicits hyperalgesia, evidenced by a marked and persistent decrease in the threshold current to induce vocalization. Taurine, and to a lesser extent β-alanine, significantly counteracted this hyperalgesic effect.

above. Other types of motor or aversive reactions were recorded with video equipment (Panasonic Co., Secaucus, NJ). Observations continued for at least one hour. The vocalization to tail shock and the tail flick latency tests were made at 15, 30, 45, and 60 minutes post intrathecal injection.

RESULTS

Behavioral Effects of Strychnine, Glycine, β-Alanine, Taurine and Betaine

Effects of strychnine alone. The perispinal administration of 25 μg of strychnine induced a series of sensory and motor events which showed a highly predictable temporal pattern. Only one out of ten Ss failed to respond to the toxin. Within the first two minutes strychnine induced intense and prolonged scratching and biting directed to the lower half of the body. Spontaneous vocalizations frequently signalled the onset of scratching bouts. Stimulation with the von Frey fiber (starting at 3 min) elicited intense distress vocalization (Fig. 1) and often triggered scratching or biting directed towards the stimulated skin point. Stimulation of the skin of the tail had no effect, indicating that the skin hyperalgesia is restricted to the hairy skin. Hyperresponsiveness to skin stimulation lasted for more than 30 minutes. Strychnine also produced hyperalgesia, i.e., it significantly reduced the tail shock vocalization threshold when the Ss were tested 15 min after the perispinal injection (Fig. 2). No significant effects on tail flick latencies were induced by the toxin. Motor effects (hopping, falling over with tail whipping, and convulsions) appeared later and subsided earlier than the sensory responses. Ss treated with strychnine showed a group mean of 3 convulsions that lasted a mean of two minutes (Fig. 4a, b). Convulsions were observed in 80% of the Ss. Convulsions usually appeared 5 to 10 minutes post injection.

Effects of the amino acids alone. Perispinal administration of glycine, betaine or taurine did not produce any overt sensory or motor manifestations. β-Alanine induced prolonged scratching bouts in 50% of the Ss. None of the amino acids tested had a significant effect on the tail shock vocalization threshold. Taurine produced a significant increase in the tail flick latency test (60% increase, $p<0.01$) 15 minutes after its administration.

Behavioral Effects of Glycine and Related Amino Acids in Combination With Strychnine

Glycine significantly decreased the number of vocalizations in response to stimulation with the von Frey fiber (Fig. 1) but failed to significantly reduce the hyperalgesic effect of strychnine as measured by vocalization threshold (Fig. 2). On the other hand, glycine significantly reduced the number of convulsions induced by strychnine (Fig. 4a).

β-Alanine was similar in potency to glycine in significantly decreasing vocalizations induced in response to von Frey fiber stimulation (Fig. 1). Moreover, β-alanine significantly counteracted the hyperalgesic effect of strychnine as measured by vocalization threshold at 15 min after injection (Fig. 2). Similarly, β-alanine inhibited convulsions (Fig. 4). On the other hand, β-alanine differed from the other amino acids in that it synergized with strychnine to facilitate scratching and self biting (Fig. 3a, b).

As shown in Fig. 3a, b, taurine effectively antagonized all actions of strychnine except scratching. This behavior pattern was not significantly inhibited by any of the amino acids employed. Taurine not only significantly antagonized the decrease in vocalization threshold produced by strychnine, it increased the vocalization threshold above baseline control values (Fig. 2). Taurine significantly reduced the number and duration of convulsions and occurrences of falling over (Fig. 4). Indeed, this behavioral effect i.e., falling over with tail whipping, was displayed only once by two rats receiving taurine + strychnine.

Betaine failed to prevent or diminish most of the sensory effects of strychnine (Figs. 2, 3), only significantly decreasing the duration of spontaneous vocalizations during intoxication. On the other hand, betaine had a powerful action on the motor effects of strychnine since it significantly reduced the number and duration of episodes of falling over and con-

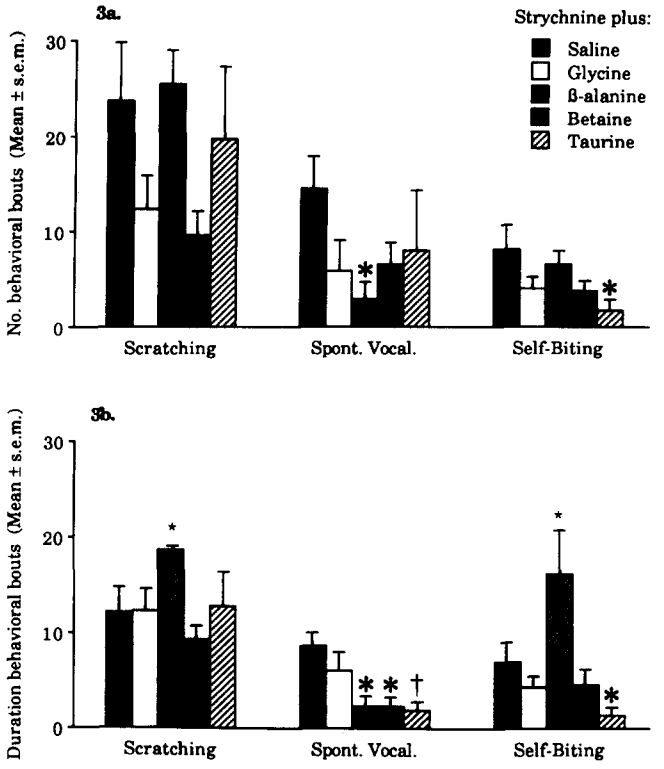

FIG. 3. Effect of the combined intrathecal injection of strychnine (25 μg) and equimolar doses of various amino acids on "sensory" events (number and duration) occurring within the following 30 minutes. Note that β-alanine increased the duration of the period during which strychnine-treated rats displayed scratching and self-biting. *: $p<0.05$, †: $p<0.01$, Mann-Whitney U test

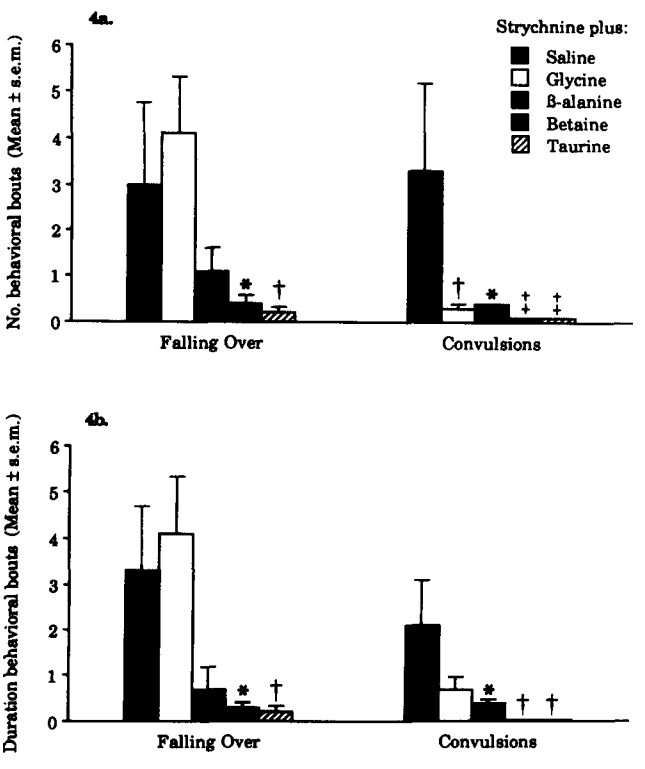

FIG. 4. Effect of the combined intrathecal injection of strychnine (25 μg) and equimolar doses of various amino acids on motor seizures (falling over with tail whipping, convulsions) occurring within the following 30 minutes. Note the striking inhibition exerted by the amino acids on the motor effects induced by strychnine.

vulsions (Fig. 4a, b).

For comparative purposes, Table 1 summarizes the capacity of the various amino acids used in this study to modify the various sensory and motor responses to strychnine.

DISCUSSION

Our observations confirm the previously reported characteristics of the intoxication resulting from the perispinal administration of strychnine to the rat [5]. Using this route, highly predictable sensory and motor effects are induced by this toxin. Sensory responses, such as itch and skin hyperalgesia, occurred continuously, appeared earlier and subsided later than motor responses (convulsions) which were sporadic and disappeared within ten minutes of the intrathecal injection of strychnine. These observations may indicate that glycine receptors in sensory interneurons are more accessible to perispinal strychnine than those located in motoneurons. Alternatively, inhibition of sensory neurons may require binding of a substantially smaller number of glycine receptors than that required to inhibit motoneurons.

Our present results show that all the amino acids tested except betaine attenuated or even abolished skin hyperalgesia produced by intrathecal strychnine, as measured by vocalization and reflex scratching in response to von Frey fiber stimulation. None of the amino acids significantly reduced the "spontaneous" scratching produced by strychnine. However, Kryzhanovsky [15] succeeded in blocking scratching (induced in the rat by topical application of strychnine to the spinal cord) by injecting glycine directly into the dorsal horns of the spinal cord.

The finding that glycine and related amino acids influenced nociceptive responses in strychnine intoxicated rats supports the possibility that they can act as analgesics. However, glycine failed to prevent or to decrease the hyperalgesic effect exerted by strychnine as measured by vocalization threshold. This result is consistent with previous observations showing that perispinal glycine not only failed to exert an analgesic action but induced a strong hyperalgesic effect on the tail shock vocalization test [5]. This paradoxical observation may be explained by the fact that glycine inhibits GABAergic neurons which control, through presynaptic receptors, the traffic of afferent impulses from the periphery [3]. This action may mask or obliterate a possible inhibitory effect of this neurotransmitter on neurons pertaining to the nociceptive pathway. Taurine, and to a lesser extent β-alanine, antagonized the hyperalgesic effect of strychnine as measured by vocalization threshold. Moreover, taurine alone showed a relatively transient but significant effect of increasing the tail flick latency, a result consistent with the report that this amino acid depresses the sensitivity to noxious stimulation [1].

Several investigators have failed to counteract convulsions induced by strychnine by administration of glycine [13,23]. These data may be explained by relatively low bioavailability, since in the studies glycine was administered systemically and it penetrates very slowly into the brain [22].

TABLE 1

EFFECT OF PERISPINAL ADMINISTRATION OF GLYCINE, β-ALANINE, BETAINE OR TAURINE ON THE SENSORY AND MOTOR EFFECTS OF PERISPINAL STRYCHNINE (25 μg)

	Sensory Effects				Motor Effects	
Amino Acid	Skin* Hyperalgesia	Spontaneous Vocalization	Self-Biting	Tail Shock Vocalization	Fall Over Tail Whip	Convulsions
Glycine	++	0	0	0	0	++
β-alanine	++	+	0	+	0	++
Betaine	0	+	0	0	+	+++
Taurine	++	++	++	+++	++	+++

*Distress vocalization in response to von Frey fiber contact.
0=no significant difference from strychnine.
+, ++=partial blockage of response to strychnine.
+++=complete blockage of response to strychnine.

When this problem was circumvented by using intrathecal spinal administration, glycine significantly decreased motor seizure activity in strychnine intoxicated rats. Similarly, β-alanine and particularly taurine counteracted the motor effects of strychnine.

No correlation was found between the reported relative capacity of the amino acids to displace strychnine from its binding site [20,27] and their efficacy in counteracting strychnine-induced sensory and motor effects. Thus, taurine, which was the most effective amino acid in antagonizing strychnine actions, has only approximately one-third of the efficacy of glycine in displacing strychnine binding in the spinal cord [20,27]. This discrepancy between *in vitro* and *in vivo* results may be partially accounted for by the rapid removal of glycine and β-alanine from the post synaptic region by their highly efficient uptake systems [10,16]. Taurine, in turn, may act for a more prolonged period at the glycine receptor than either glycine or β-alanine since its specific uptake system is much slower than that of GABA or glycine [21]. It is also possible that taurine exerted its effect by mechanisms other than the displacement of strychnine from the glycine receptor. Thus, taurine can modulate GABA actions [17] and prevent calcium uptake into neurons [14,19], both effects tending to decrease neuronal excitability and therefore the production of seizures. Indeed, taurine has been reported to exert anticonvulsant actions on several experimental models which are not directly mediated by alterations in glycinergic transmission [19,23].

The present results confirm previous findings that betaine protects against the occurrence of convulsions in response to strychnine [13]. However, the lack of effect of betaine in strychnine-induced skin hyperalgesia indicates that it acts through mechanisms different from the other amino acids. This interpretation agrees with the observation that dimethyl glycine, another methylated glycine derivative like betaine, which has also been found to counteract strychnine convulsions [12], does not mimic or enhance the effects of glycine on spinal cord neurons [9].

β-Alanine is generally considered to act through its interaction with the glycine recognition site of the glycine receptor [4] and in the present study, it exerted effects quantitatively similar to those of glycine. Additionally, it induced, in a significant proportion of subjects, intense scratching and synergized with strychnine to produce scratching and self-biting. This suggests that β-alanine may interact also with the strychnine domain of the glycine receptor [4].

ACKNOWLEDGEMENTS

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REFERENCES

1. Ahtee, L., H. Auvinon, A. R. Maenna, M. L. Vahala, M. Lehtinen and J. Halmekoski. Comparison of the central nervous system actions of taurine and N-pivoloytaurine. *Acta Pharmacol Toxicol* **57**: 96–105, 1985.
2. Aprison, M. M. and N. S. Nadi. Glycine: inhibition from the sacrum to the medulla. In: *Amino Acids as Chemical Transmitters*, edited by F. Fornum. New York: Plenum Press, 1977, pp. 531–570.
3. Bagust, J., K. A. Green and G. A. Kerkut. Strychnine sensitive inhibition in the dorsal horn of mammalian spinal cord. *Brain Res* **217**: 425–429, 1981.
4. Betz, H. Biology and structure of the mammalian glycine receptor. *Trends Neurosci* **10**: 113–117, 1987.
5. Beyer, C., L. A. Roberts and B. R. Komisaruk. Hyperalgesia induced by altered glycinergic activity at the spinal cord. *Life Sci* **37**: 875–882, 1985.
6. Bristow, D. R., N. G. Bowery and G. N. Woodruff. Light microscopic localisation of ^{3}H-glycine and ^{3}H-strychnine binding sites in rat brain. *Eur J Pharmacol* **126**: 303–307, 1986.
7. Curtis, D. R., A. W. Duggan and G. A. R. Johnston. The specificity of strychnine as a glycine antagonist in the mammalian spinal cord. *Exp Brain Res* **12**: 541–565, 1976.
8. Curtis, D. R., L. Hosli and G. A. R. Johnston. A pharmacological study of the depression of spinal neurons by glycine and related amino acids. *Exp Brain Res* **6**: 1–18, 1968.
9. Curtis, D. R., L. Hosli, G. A. R. Johnston and I. H. Johnston. The hyperpolarization of spinal motoneurons by glycine and related amino acids. *Exp Brain Res* **5**: 235–258, 1968.
10. Curtis, D. R. and G. A. R. Johnston. Amino acid transmitters in the mammalian central nervous system. *Ergeb Physiol* **69**: 97–188, 1974.
11. Dusser de Barenne, J. G. The mode and site of action of strychnine on the nervous system. *Physiol Rev* **13**: 325–335, 1933.
12. Freed, W. J. N-dimethylglycine, betaine and seizures. *Arch Neurol* **41**: 1127, 1984.

13. Freed, W. J. Prevention of strychnine-induced seizures and death by the N-methylated glycine derivatives betaine, dimethylglycine and sarcosine. *Pharmacol Biochem Behav* **22:** 641–643, 1985.
14. Izumi, K., H. Igisu and T. Fukuda. Effects of edetate on seizure suppressing actions of taurine and GABA. *Brain Res* **88:** 576–579, 1975.
15. Kryzhanovsky, G. N. Experimental central pain and itch syndromes: modeling and general theory. In: *Advanced Pain Research Therapy,* vol 1, edited by J. J. Bonica and D. Albe-Fessard. New York: Raven Press, 1977, pp. 225–230.
16. Logan, W. J. and S. H. Snyder. High affinity uptake systems for glycine, glutamic and aspartic acids in synaptosomes of rat central nervous tissues. *Brain Res* **42:** 413–431, 1972.
17. Medina, J. H., M. L. Noras and E. De Robertis. Regulation of the benzodiazepine receptor: *in vivo* and *in vitro* experiments. In: *GABAergic Transmission and Anxiety,* edited by G. Biggio and E. Costa, New York: Raven Press, 1986, pp. 107–120.
18. Panchin, Y. V. and R. N. Skryma. Scratch reflex evoked by strychnine applied to the spinal cord. *Neirofiziologiya* **10:** 622–625, 1978.
19. Pasantes-Morales, H. and M. E. Arzate. Effect of taurine on seizures induced by 4-aminopyridine. *J Neurosci Res* **6:** 465–474, 1981.
20. Pfeiffer, F., D. Graham and H. Betz. Purification by affinity chromatography of the glycine receptor of rat spinal cord. *J Biol Chem* **257:** 9389–9393, 1982.
21. Schmid, R., W. Sieghart and M. Karobath. Taurine reuptake in synaptosomal fractions of rat cerebral cortex. *J Neurochem* **25:** 5–9, 1975.
22. Toth, E. and A. Lajtha. Elevation of cerebral levels of nonessential amino acids in vivo by administration of large doses. *Neurochem Res* **6:** 1307–1317, 1981.
23. Toth, E., A. Lajtha, S. Sarhan and N. Seiles. Anti-convulsant effects of some inhibitory neurotransmitter amino acids. *Neurochem Res* **8:** 291–302, 1983.
24. Willcockson, W. S., J. M. Chung, Y. Mori, K. M. Lee and W. O. Willis. Iontophoretic study of biogenic amine and peptide effects on primate spinothalamic tract cells. *Soc Neurosci Abstr* **9:** 1, 1983.
25. Yaksh, T. L. and T. A. Rudy. Chronic catheterization of the subarachnoid space. *Physiol Behav* **17:** 1031–1036, 1976.
26. Young, A. B. and S. H. Snyder. Strychnine binding associated with central nervous glycine receptors. *Proc Natl Acad Sci USA* **70:** 2832–2836, 1973.
27. Zarbin, M. A., J. K. Wamsley and M. J. Kuhar. Glycine receptor: light microscopic autoradiographic localization with ^{3}H-strychnine. *J Neurosci* **1:** 532–547, 1981.
28. Zieglgansberger, W. Central control of nociception. In: *Handbook of Physiology, Sect. I, The Nervous System,* vol IV, edited by F. E. Bloom. Bethesda, MD: American Physiological Society, 1986, pp. 581–645.