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Intrathecal GABA, Glycine, Taurine or Beta-Alanine Elicits Dyskinetic Movements in Mice

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LARSON, A. A. *Intrathecal GABA, glycine, taurine or beta-alanine elicits dyskinetic movements in mice.* PHARMACOL BIOCHEM BEHAV 32(2) 505–509, 1989.—Dyskinetic, writhing-like movements, similar to those produced in mice after an intraperitoneal (IP) injection of acetic acid, were elicted by intrathecal (IT) injection of GABA, glycine, taurine or beta-alanine. Baclofen and muscimol failed to produce this behavior. While acetic acid-induced writhing is inhibited by narcotic and nonnarcotic compounds, GABA-induced writhing was found to be insensitive to pretreatment with either morphine or capsaicin. Moreover, acetic acid-induced writhing does not appear to involve GABAergic transmission as IT injections of nipecotic acid did not alter the intensity of response to IP acetic acid while it enhanced the response to IT GABA. Writhing induced by glycine was not inhibited by strychnine at subconvulsive doses, suggesting that it involves an action at strychnine-insensitive receptors. Together these data suggest that while the dyskinetic movements produced by inhibitory amino acids do not appear to reflect an alteration in nociception, they may mimic either the motor response to abdominal pain or spasticity.

Glycine GABA Beta-alanine Writhing Dyskinesia Pain Intrathecal Analgesia

A wide variety of evidence exists that both glycine and GABA act as inhibitory neurotransmitters in the brain and spinal cord. Areas such as the substantia gelatinosa and lamina V of the spinal cord, which are thought to be involved in the processing of pain perception, contain both glycine and GABA (3) as well as binding sites for these compounds (19,24).

Several studies have focused on the roles of glycine and GABA in pain transmission. These studies generally suggest that GABA and glycine may play a minor role in the modulation of pain perception. The exact role, however, is unclear as both agonists as well as antagonists of, e.g., GABA, have been shown to cause antinociception (21). Poisoning with strychnine is reported to produce intense pain in humans (4,9), however, both strychnine and glycine, when injected intrathecally (IT) in rats, enhance vocalization in response to light cutaneous stimulation (6). Intrathecally injected in mice, strychnine produces biting and scratching behavior at subconvulsive doses (15) which has been argued to reflect enhanced nociception. Morphine sulfate, however, a potent analgesic agent, has also been found to produce a similar motor response consisting of intense biting and scratching after IT injection (23). Also reported to elicit an antinociceptive effect in a variety of analgesic assays are baclofen (20,22), a GABA-B agonist, kojic amine, an analog of GABA (17), muscimol (10), a GABA-A agonist, and GABA itself (18).

Experiments designed to study the roles of GABA and glycine in the production of analgesia are complicated by the fact that GABA is too polar to cross the blood-brain barrier. In addition, direct IT injection of these inhibitory transmitters into the cerebral spinal fluid (CSF) leads to weakness and paralysis (5) which may be misinterpreted to reflect analgesia in assays that require motor responses to indicate nociception. On the other hand, experiments using antagonists of glycine or of GABA are further complicated by the tendency for such compounds to produce hyperactivity and convulsions.

During a series of experiments designed to examine the anticonvulsive effect of various inhibitory transmitters on the activity of convulsant compounds, we found that IT injections of some inhibitory transmitters caused a dyskinetic writhing response in mice at subparalytic doses. The present studies were designed to characterize these motor effects and compare them to those produced by the intraperitoneal (IP) injection of acetic acid which is thought to induce pain. Inhibition of acetic acid-induced writhing is commonly used to screen for analgesic compounds.

METHOD

Animals

Mice weighing between 17 and 22 g were injected intrathecally following the method of Hylden and Wilcox (12). Briefly, unanesthetized mice are held by the iliac crests while a 1 cm long, 30 gauge disposable needle mated to a 50 μl Hamilton syringe is inserted percutaneously between two lumbar vertebrae. All drugs injected IT, except capsaicin, were dissolved in saline for injection of a 5 microliter vol-

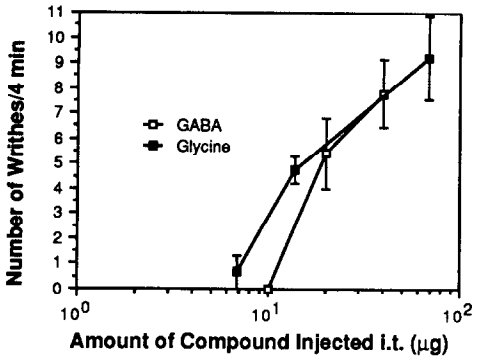

FIG. 1. The dose range over which GABA- and glycine-induced writhing are produced is narrow as higher doses tend to produce hindlimb paralysis. Each point represents the average value from 4 to 14 mice.

ume. Capsaicin was dissolved in a mixture (v:v) of 50% saline and 50% dimethylsulfoxide (DMSO) which was used as its vehicle control.

Drugs and Chemicals

Gamma-amino-n-butyric acid (GABA), glycine, beta-alanine, strychnine, taurine, nipecotic acid, muscimol and capsaicin were purchased from Sigma Chemical Co. (St. Louis, MO). Morphine sulfate was purchased from Merck & Co. (Rahway, NJ). Baclofen (Lioresal) was a gift from CIBA-Giegy Corp. (Summit, NJ) and naloxone hydrochloride was a gift from Endo Laboratories, Inc. (Garden City, NY).

Writhing Assay

Writhing was produced by the intraperitoneal (IP) injection of 0.3 ml of a 1% solution of acetic acid, as described previously by Hayashi and Takemori (11). The number of writhes were counted over a 15-min interval beginning 3 min after the injection of acetic acid.

Statistics

The average number of behavioral responses obtained after a single injection of an inhibitory compound was compared to that after coadministration or pretreatment with a modifying compound. The Student's *t*-test was used to determine whether changes in the average number of behavioral responses of mice were significantly altered ($p<0.05$) by any manipulation.

RESULTS

The IT injection of either GABA or glycine in mice resulted in the production of repeated dyskinetic movements resembling those following the IP injection of acetic acid (Fig. 1). Marked abdominal stretching occurred coincident with rotating of the head and forebody from side to side. Injections of less than 5 or 10 μg of glycine or GABA respectively did not elicit motor activity while doses larger than 55 μg of either amino acid produced hindlimb weakness and, at doses between 100 and 500 μg, hindlimb paralysis.

The time-course of the response to glycine was very similar to that of GABA, as shown in Fig. 2. The dyskinetic movements typically persisted for only 5 min after an IT injection, compared to the writhing following IP acetic acid which may last up to 30 min following injection.

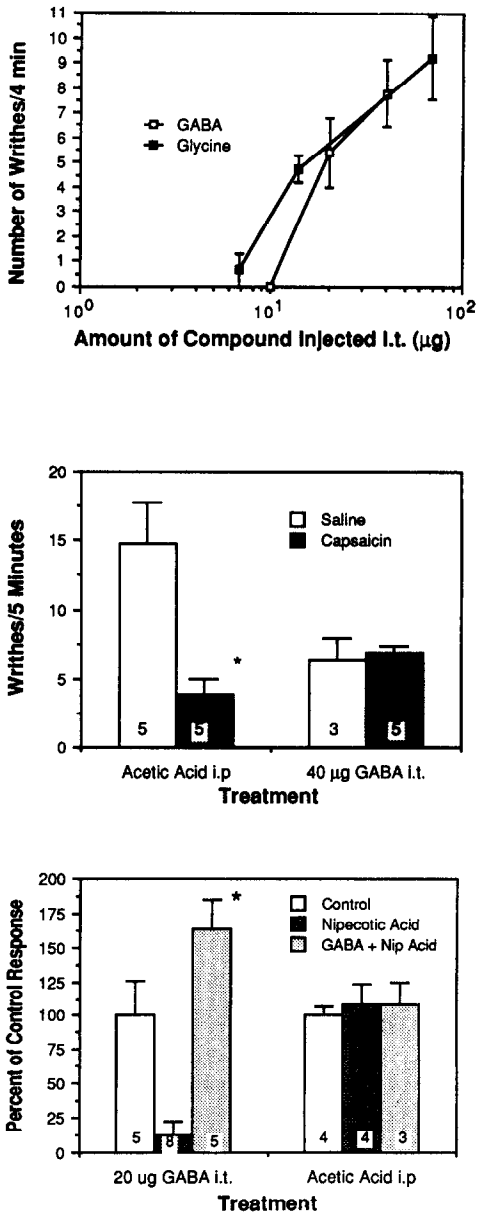

FIG. 2. The time-course of both GABA- and glycine-induced behaviors is relatively short, decreasing at a steady rate over a 5-minute period. The data in the upper figure represent consecutive values obtained over a 5-min period after injection of glycine to a group of 14 mice while that in the lower figure depict values obtained over a 5-min period from a single group of 10 mice after GABA.

To determine whether the production of writhing-like movements is common to all inhibitory hyperpolarizing compounds in the spinal cord, a variety of compounds that are thought to act at inhibitory receptors were injected IT to determine whether similar writhing-like movements were produced at subparalytic doses (Table 1). GABA, glycine, taurine and beta-alanine were found to elicit dyskinesias. Muscimol, an agonist at GABA-A receptors, and baclofen, an agonist at GABA-B receptors, both failed to elicit writhing-like movements at 0.1 to 160 μg and at 16 ng to 26.5 μg respectively. These dosage intervals included amounts sufficiently high to produce hindlimb weakness and paralysis

TABLE 1

ABILITY OF INHIBITORY COMPOUNDS TO ELICIT WRITHING IN MICE WHEN INJECTED INTRATHECALLY

Substance	Dose*	Writhes/5 min ± S.E.	
GABA	50 μg	8.5 ± 0.9	(n=14)
Glycine	50 μg	9.1 ± 1.3	(n=10)
Beta-alanine	46 μg	5.9 ± 0.8	(n=14)
Taurine	15 μg	4.6 ± 1.4	(n=5)
Muscimol	†	0	
Baclofen	†	0	

*All compounds were injected in a 5 microliter volume of saline.

†Injected at doses ranging between 0.1 to 160 μg of muscimol and 16 ng to 26.5 μg of baclofen. This range of doses was sufficiently wide to include doses that produce paralysis.

Only intrathecal injections of GABA, glycine, beta-alanine or taurine were found to elicit writhing movements when injected intrathecally. Muscimol, a GABA-A agonist, and baclofen, a GABA-B agonist, failed to produce a writhing response in mice at any dose tested.

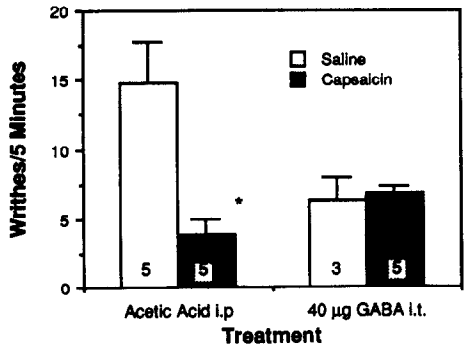

FIG. 3. While writhing produced by the IP injection of acetic acid is inhibited by pretreatment with capsaicin, those produced after the IT injection of 40 μg of GABA were not affected by identical treatment with capsaicin. These results would suggest that the release of substance P is not necessary for the GABA-induced writhing. The data shown above show the average values obtained from 3 to 5 mice. A dose of 0.8 μg of capsaicin or vehicle was injected intrathecally to mice 1 week prior to testing.

as well as doses sufficiently low that no observable effects were elicited.

Depletion of substance P in the spinal area by IT injection of 0.8 μg of capsaicin one week prior to testing mice significantly inhibited the number of writhing movements produced by the IP injection of acetic acid compared to similarly injected vehicle control mice (Fig. 3). In contrast, capsaicin pretreatment failed to alter the response to an IT injection of 40 μg of GABA compared to vehicle-pretreated control mice. The dyskinesia elicited by the IT injection of 20 μg of GABA was also found to be insensitive to inhibition by narcotic analgesics as pretreatment with 5 mg/kg of morphine failed to inhibit GABA-induced writhing in mice (Table 2).

While 40 μg of nipecotic acid, a GABA uptake inhibitor, had no propensity to elicit dyskinetic movements in mice when administered alone, coadministration of 20 μg of GABA with this dose of nipecotic acid resulted in a small but

TABLE 2

INSENSITIVITY OF GABA-INDUCED WRITHING TO OPIATES

Pretreatment*	Treatment	Writhes/5 min ± S.E.	
Saline	GABA	5.40 ± 1.44	(n=5)
SC	20 μg IT		
Morphine	GABA	4.50 ± 2.22	(n=4)
5 mg/kg SC	20 μg IT		
Naloxone	GABA	5.17 ± 1.45	(n=6)
5 mg/kg SC	20 μg IT		

*Pretreatment was administered 10 min prior to intrathecal injections.

While acetic acid-induced writhing is very sensitive to the inhibitory, antinociceptive effect of both narcotic and nonnarcotic analgesics, neither morphine nor naloxone pretreatment of mice altered the number of writhing responses produced by an intrathecal injection of GABA.

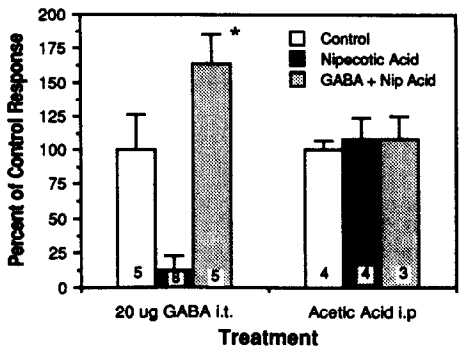

FIG. 4. GABA-induced writhing, but not acetic acid-induced writhing, was potentiated by coadministration of 40 μg of nipecotic acid, a GABA reuptake inhibitor that is believed to be especially effective at glial membranes.

significant potentiation of the number of GABA-induced behaviors (Fig. 4). In contrast, the injection of 40 μg of nipecotic acid, either alone or together with 20 μg of GABA, had no effect on the number of writhes produced by the IP injection of acetic acid.

The intensity of the writhing response to either 15 or 55 μg of glycine IT was not attenuated by coadministration of strychnine at 0.2 μg, a subconvulsive dose (Table 3). Coadministration of 0.4 or 0.8 μg of strychnine, doses which do elicit a short period of convulsive activity, did significantly inhibit the number of writhing movements produced by a dose of 55 μg of glycine.

DISCUSSION

The results of these studies reveal a novel behavioral response to the IT injection of a variety of inhibitory compounds. This behavioral response is of interest in light of the traditional concept that these inhibitory transmitters and/or modulators not only inhibit motor reflexes when applied directly to the spinal cord but in sufficiently high doses cause paralysis. While our results confirm the ability of these compounds to elicit paralysis, the doses required to produce dyskinetic movements were lower than those required for paralysis.

TABLE 3

EFFECT OF STRYCHNINE ON GLYCINE-INDUCED WRITHING MOVEMENTS

Intrathecal Injection	Number Writhes Per 10 min ± S.E. (n)
Glycine 55 μg	18.3 ± 1.0 (11)
Glycine 55 μg + Strychnine 0.2 μg	17.7 ± 1.1 (8)
Glycine 55 μg + Strychnine 0.8 μg	12.3* ± 0.4 (9)
Glycine 15 μg	2.0 ± 0.7 (5)
Glycine 15 μg + Strychnine 0.2 μg	2.4 ± 0.9 (5)

Coadministration of strychnine with an intrathecal injection of glycine failed to alter the number of writhing movements produced by the glycine until a dose of 0.8 μg of strychnine was injected. At this higher dose of strychnine, a period of convulsive behavior was produced in all animals tested.

The dyskinetic response to inhibitory compounds is behaviorally similar to the writhing response that results from the IP injection of acetic acid. Acetic acid-induced writhing is presumed to reflect pain and to be mediated by substance P as it is sensitive to pretreatment with capsaicin, a substance P depleter. The writhing response induced by inhibitory compounds, however, appears to be unique from that produced by IP injection of acetic acid as it was totally insensitive to capsaicin pretreatment (Fig. 3). Acetic acid-induced writhing is also sensitive to both narcotic and non-narcotic analgesics, while inhibitory amino acid-induced dyskinesia was insensitive to treatment with morphine (Table 2). It is therefore unlikely that the dyskinetic writhing response produced by the IT administration of inhibitory compounds is associated with perception of noxious stimulation, however it could mimic the motor component of the acetic acid-induced nociceptive response. On the other hand, endogenously occurring GABA is probably not involved in acetic acid-induced writhing as the GABA agonists muscimol and baclofen do not elicit writhing and nipecotic acid does not enhance acetic acid-induced writhing (Fig. 4). Nevertheless, other amino acids could be involved in producing this motor response.

It is possible that the dyskinetic movements reflect a simple disinhibition of motor activity. It has recently been reported, however, that two glycine receptors exist, i.e., the classic strychnine-sensitive inhibitory site (Gly_1) and a more recently characterized, strychnine-insensitive site (Gly_2) (8,14) The possible existence of a Gly_2 receptor is supported by autoradiographic studies which differentially localized [^{3}H]glycine and [^{3}H] strychnine binding sites in the CNS (7). Electrophysiological studies have also linked strychnine-insensitive glycine effects to N-methyl-D-aspartate (NMDA) receptor activity in cultured murine neuronal preparations (13). Our laboratory has shown that when injected together into the spinal area, glycine potentiates rather than inhibits the convulsive effect of strychnine, which appears to be due to an NMDA-linked Gly_2 receptor interaction (16).

It is thus possible that the dyskinetic response elicited by the IT injection of these putative inhibitory transmitters results from an action on Gly_2 receptors that are linked to excitatory effects in the CNS. The dyskinesia is frequently accompanied by intermittant biting and scratching behaviors, consistant with stimulation of NMDA receptors (1,2). The inability of strychnine to inhibit the dyskinetic effect argues against involvement of Gly_1 receptors, and the inability of either muscimol or baclofen to mimic the dyskinetic effect suggests that it is not mediated by a GABA receptor. Direct evidence of the Gly_2 receptor's involvement in dyskinesia awaits the development of a specific antagonist of that receptor.

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REFERENCES

1. Aanonsen, L. M.; Wilcox, G. L. PCP selectively blocks the action of N-methyl-D-aspartate in mice. Neurosci. Lett. 67:191–197; 1986.
2. Aanonsen, L. M.; Wilcox, G. L. Nociceptive action of excitatory amino acids in the mouse: Effects of spinally administered opioids, phencyclidine and sigma agonists. J. Pharmacol. Exp. Ther. 243:9–19; 1987.
3. Aprison, M. H.; Werman, R. The distribution of glycine in cat spinal cord and roots. Life Sci. 4:2075–2083; 1965.
4. Arena, J. M. Poisoning: Toxicology, symptoms, treatments. 4th ed. Springfield: Charles C. Thomas; 1979:177–179.
5. Beitz, A. J.; Larson, A. A. Inhibition of intrathecally administered picrotoxin- and bicuculline-induced convulsions in mice by pipecolic acid or GABA. Eur. J. Pharmacol. 114:181–187; 1985.
6. Beyer, C.; Roberts, L. A.; Komisaruk, B. R. Hyperalgesia induced by altered glycinergic activity at the spinal cord. Life Sci. 37:875–882; 1985.
7. Bristow, D. R.; Bowery, N. G.; Woodruff, G. N. Light microscopic localization of [^{3}H]glycine and [^{3}H]strychnine binding sites in rat brain. Eur. J. Pharmacol. 126:303–307; 1986.
8. DeFeudis, F. V.; Orsensanz-Munoz, L. M.; Fando, J. L. High-affinity glycine binding sites in rat CNS: Regional variation and strychnine sensitivity. Gen. Pharmacol. 9:171–176; 1978.
9. Gosselin, R. E.; Hodge, H. C.; Smith, R. P.; Gleason, M. N. Clinical toxicology of commercial products. 4th ed. Baltimore: Williams and Wilkins Co.; 1976:303–307.
10. Hammond, D. L.; Drower, E. J. Effects of intrathecally administered THIP, baclofen and muscimol on nociceptive threshold. Eur. J. Pharmacol. 103:121–125; 1984.
11. Hayashi, G.; Takemori, A. E. The type of analgesia-receptor interaction involved in certain analgesic assays. Eur. J. Pharmacol. 16:63–66; 1971.
12. Hylden, J. L. K.; Wilcox, G. L. Intrathecal morphine in mice: A mew technique. Eur. J. Pharmacol. 67:313–316; 1980.
13. Johnson, J. W.; Ascher, P. Glycine potentiates the NMDA response in clutured mouse brain neurons. Nature 325:529–531; 1987.
14. Kishmoto, H.; Simon, J. R.; Aprison, H. H. Determination of the equilibrium dissociation constants and the number of glycine binding sites in several areas of the rat central nervous system,

using a sodium-independent system. J. Neurochem. 37:1015–1024; 1981.
15. Kryzhanovsky, G. N. In: Bonica, J. J.; Albe-Fessord, D., eds. Advances in pain research and therapy. vol. 2. New York: Raven Press; 1976:225.
16. Larson, A. A.; Beitz, A. J. Glycine potentiates strychnine-induced convulsions: Role of NMDA receptors. J. Neurosci. 8:3822–3826; 1988.
17. Pelley, K. A.; Vaught, J. L. An antinociceptive profile of kojic amine: An analogue of gamma-aminobutyric acid (GABA). Neuropharmacology 26:301–307; 1987.
18. Roberts, L. A.; Beyer, C.; Komisaruk, B. R. Nociceptive responses to altered GABAergic activity at the spinal cord. Life Sci. 39:1667–1674; 1986.
19. Ryall, R. W. Amino acid receptors in CNS. I. GABA and glycine in spinal cord. vol. 2. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology, amino acid neurotransmitters. New York: Plenum Press; 1975:83–128.
20. Sawynok, J.; Reid, A. Role of ascending and descending noradrenergic pathways in the antinociceptive effect of baclofen and clonidine. Brain Res. 386:341–350; 1986.
21. Spaulding, T. C.; Little, J.; McCormack, K.; Fielding, S. The antinociceptive effects of GABA agonists and antagonists in mice. GABA neurotransmission. Brain Res. Bull. 5:415–419; 1980.
22. Wilson, P. R.; Yaksh, T. L. Baclofen is antinociceptive in the spinal intrathecal space of animals. Eur. J. Pharmacol. 51:323–330; 1978.
23. Yaksh, T. L.; Harty, G. J. The pharmacology of the allodynia in rats evoked by high dose intrathecal morphine. J. Pharmacol. Exp. Ther. 244:501–507; 1988.
24. Zarbin, M. A.; Wamsley, J. K.; Kuhar, M. J. Glycine receptor: Light microscopic autoradiographic localization with [^{3}H]strychnine. J. Neurosci. 1:532–547; 1981.