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Administration of AP5, a Glutamate Antagonist, Unmasks Glycine Analgesic Actions in the Rat

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BEYER, C., B. R. KOMISARUK, A. M. LÒPEZ-COLOMÈ AND M. CABA. *Administration of AP5, a glutamate antagonist, unmasks glycine analgesic actions in the rat.* PHARMACOL BIOCHEM BEHAV **42**(2) 229–232, 1992.—The effect of intrathecal (IT) injection of glycine alone or in combination with 2-amino-5-phosphonopentanoate (AP5) on two nociceptive tests—the vocalization threshold to tail-shock (VTTS) and the tail-flick latency (TFL)—was studied in ovariectomized Sprague-Dawley rats. IT injection of 400 μg glycine induced a nonsignificant decrease, that is, in comparison with saline, in both nociceptive thresholds. IT AP5 (10 μg) provoked a slight but significant increase in both nociceptive thresholds within the first 15 min postinjection. Combination of both glycine (400 μg) and AP5 (10 μg) produced marked and prolonged analgesia in both tests, which was significantly different from that obtained with AP5 alone. The results suggest that IT glycine acting through the strychnine-sensitive Gly_1 receptor produces analgesia provided its effect on the Gly_2 receptor linked to the NMDA receptor is prevented by an antagonist.

Glycine NMDA receptor Glycine receptors Inhibitory amino acid Glutamate antagonist
AP5 Pain Analgesia Hyperalgesia

SEVERAL studies suggest that glycine participates in the modulation of nociceptive information (4,29). Thus, intrathecal (IT) administration of strychnine, a glycine antagonist, induces hyperalgesia in the vocalization threshold to tail-shock test (VTTS) (3), scratching (3,4,10), and aversive reactions to innocuous cutaneous stimulation, that is, allodynia (3,4,26). From these data, it could have been anticipated that glycine administration would produce analgesia. However, in rats IT glycine produced hyperalgesia in the VTTS test (4). This result was interpreted as due either to the activation of glycine autoreceptors diminishing glycinergic tone (4) or to the inhibition of GABAergic neurons modulating afferent nociceptive impulses from the periphery (3). However, a more compelling explanation has emerged from the observation that glycine enhances the excitatory effect of glutamate and aspartate by acting on the NMDA receptor (12,14,15,22). Since both glutamate and aspartate intrathecal injection induce nociceptive responses [scratching and biting (20,21,28)], it appears likely that the hyperalgesic effect of glycine was due to its interaction with the NMDA receptor. This effect on the NMDA receptor would counteract the analgesia expected from the interaction of glycine on strychnine-sensitive Gly_1 receptors. To explore this possibility, we studied the effect of IT administration of 2-amino-5-phosphonopentanoate (AP5), a specific NMDA receptor antagonist (6,9), on the action exerted by perispinal glycine on nociceptive thresholds in the rat.

METHOD

Subjects

Subjects were virgin, Sprague-Dawley rats (200–300 g), housed individually at 23°C in a room maintained on a reverse day-night cycle (dark from 10:00 to 20:00 h). Food and water were supplied ad lib. Subjects were ovariectomized to exclude possible alterations in pain thresholds related to fluctuations in ovarian secretion during the estrous cycle.

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Surgery

Ovariectomy was performed under ketamine. One week later, Ss were anesthetized with ketamine (20–25 mg, IP) and xylazine (1.2 mg, IP) and a catheter (Clay Adams PE-10 tubing, 7.5-cm insertion length) was implanted permanently in the subarachnoid intrathecal space, its tip lying at the lumbosacral level of the spinal cord, through an incision into the atlanto-occipital membrane (27). At least 7 days of recovery were allowed before testing. Subjects showing motor or sensory alterations after surgery were not included in the experiment.

Drug Treatments and Behavioral Testing

Before drug infusion, all Ss were tested to determine their VTTS and tail-flick latency (TFL). Rats were placed in a Plexiglas restrainer and two stainless steel electrodes were taped to the tail, after applying conductive gel. Electrical shocks (100-ms train of 60-Hz sine waves) with an intershock interval of 5 s were delivered from a constant-current shock generator (Coulbourn Instruments Programmable Shocker, Leigh Valley, PA) via tail electrodes. The shock amperage was increased in 100-μA steps until vocalization was elicited and then decreased stepwise (also in 100-μA steps) until no longer elicited. This procedure was repeated three times, and the upper and lower shock levels were averaged to provide an estimate of vocalization threshold. Tail-flick latencies were measured with an IITC Model 33 Analgesimeter (at 80% beam intensity). Rats were placed in a Plexiglass restrainer with the tail exposed to a radiant heat lamp. Latency from tail flick was measured automatically by activation of a photocell upon tail withdrawal. A cutoff time of 15 s was employed to avoid tissue damage.

Infusion Procedure and Treatment Groups

Rats received IT injections of one of the following drugs or combination of drugs: group 1—saline solvent (n = 10); group 2—glycine, 400 μg (n = 11); group 3—AP5, 10 μg (n = 12); group 4—glycine, 400 μg + AP5 10 μg (n = 13). The glycine dosage was selected from previous studies indicating that 400 μg glycine causes no analgesia (4). The dose of AP5 was chosen from a pilot study in which various dosages (up to 54 μg) were injected in groups of four rats. The dose of 10 μg AP5 was selected as one inducing no or weak analgesia and no overt motor effects.

Drugs were dissolved in 5 μl saline and delivered IT with an additional 7 μl saline flushed from the catheter. Rostrocaudal spread of drugs following IT injection is generally limited to the spinal cord with this volume (27). Duration of IT injection was around 1 min. During this period, subjects were kept in a Stoelting animal holder. Immediately after infusion, subjects were placed in a clear Plexiglas cage and observed for behavioral or motor alterations. Observers did not know the nature of the injected material. Testing for both VTTS and TFL was performed at 6, 10, 20, 30, 40, and 60 min postinjection.

Statistical Analysis of Data

Baseline values for both VTTS and TFL were established for each subject (0 time). Control (saline) and experimental values (various drugs) at all testing intervals were expressed as percent variations of the baseline values. A variance analysis (Kruskal–Wallis) was initially made and subsequently values obtained with the different experimental treatments were compared at each interval using the Mann–Whitney U-test. An overall response, that is, including all interval values, was determined for each subject by measuring the area under the

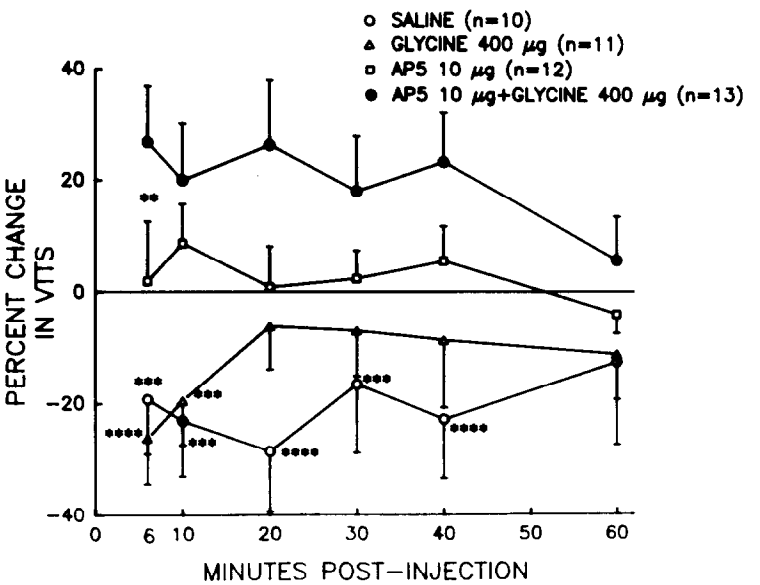

FIG. 1. Effect of IT administration of saline, glycine (400 μg), AP5 (10 μg), or AP5 (10 μg) + glycine (400 μg) on VTTS. Values are percent changes of preinjection VTTS values (0 level). Note that the combined administration of a nonanalgesic dose of glycine and only a mild analgesic dose of AP5 resulted in significant and prolonged analgesia. Statistical comparisons included were made between AP5 + glycine vs. the other three groups, that is, saline, AP5, and glycine. Results of other statistical comparison are presented in the text. $^{**}p < 0.025$, $^{***}p < 0.01$, $^{****}p < 0.001$.

response curve. Resulting overall values were expressed as the proportion of a theoretical maximal response (defined as the curve resulting from a 100% increase in the nociceptive threshold at all intervals). Comparisons between groups were made using the Student's *t*-test.

RESULTS

Figures 1 and 2 summarize the results obtained with the control (saline) and various drug treatments on the VTTS and TFL tests. IT injection of saline decreased both VTTS and TFL values when compared with baseline values. This decrease in nociceptive thresholds (VTTS and TFL) was similar to that reported in previous studies following saline injections (4,16,19) or holding of the rat (23). The IT injection of 400 μg glycine also resulted in values below the preinjection values in both the VTTS and TFL tests (except at the 60-min interval). Values following glycine in both the VTTS and TFL tests did not significantly differ, at any time interval, from those obtained with saline. Comparison of the response curves also failed to show any significant difference between the saline and glycine groups. AP5 (10 μg) induced in the VTTS test a moderate analgesia that was significantly different from saline values at the 20- and 40-min postinjection intervals ($p < 0.05$). A similar analgesic response was observed in the TFL test following AP5 (values at 6, 10, and 40 min being significantly different from saline values, $p < 0.05$). The overall response to AP5 was also significantly different from saline in both the VTTS and TFL tests ($p < 0.05$). No overt motor effects were associated with the analgesic effect obtained with AP5. Combined administration of 10 μg AP5 and 400 μg glycine produced intense and prolonged analgesia in both nociceptive tests (Figs. 1 and 2). Values following AP5 + glycine were significantly greater ($p < 0.01$) than saline values at all intervals tested except at 60 min in the VTTS. Similarly, as shown in Figs. 1 and 2, AP5 + glycine resulted in values significantly higher than those observed following either AP5 alone or glycine alone at various intervals in both tests (see Figs. 1 and 2). Moreover, the overall responses obtained with the combined treatment were significantly different from those obtained following separate administration of these drugs (AP5 + glycine vs. AP5 $p < 0.05$ in both VTTS and TFL; AP5 + glycine vs. glycine $p < 0.01$ in both VTTS and TFL).

DISCUSSION

Two types of glycine receptors exist in the CNS (5,7,8,13). The strychnine-sensitive Gly_1 receptor mediates the inhibitory effects of the amino acid, while the strychnine-insensitive (Gly_2) receptor is related with the NMDA receptor, where it facilitates the excitatory action of glutamate or aspartate (12,14,22). Activation of the NMDA receptor results in algesic responses since the IT injection of glutamate or aspartate produces signs of pain (biting and scratching), as well as hyperalgesia, measured by tail-flick and tail-pressure tests in rats and mice (1,20). The fact that the Gly_2 excitatory receptor has a higher affinity for glycine than the Gly_1 inhibitory receptor explains why IT glycine, particularly at low dose levels, produces hyperalgesia rather than analgesia (4). The present results show that when the action of glycine at the NMDA receptor is prevented by a specific glutamate antagonist, AP5 (6,9), a significant analgesia in both VTTS and TFL ensues. This effect is most likely mediated by the inhibition of spinothalamic neurons induced by glycine (11,25) through its activation of Gly_1 receptors. The idea that activation of spinal Gly_1 receptors decreases pain perception is supported by the finding that IT taurine, which acts on the Gly_1 but not on the Gly_2 receptor, produces analgesia (3,21).

The fact that glycine together with AP5 induced analgesia in both the VTTS and TFL tests suggests that it inhibits nociceptive information carried by both Aδ and C fibers. The

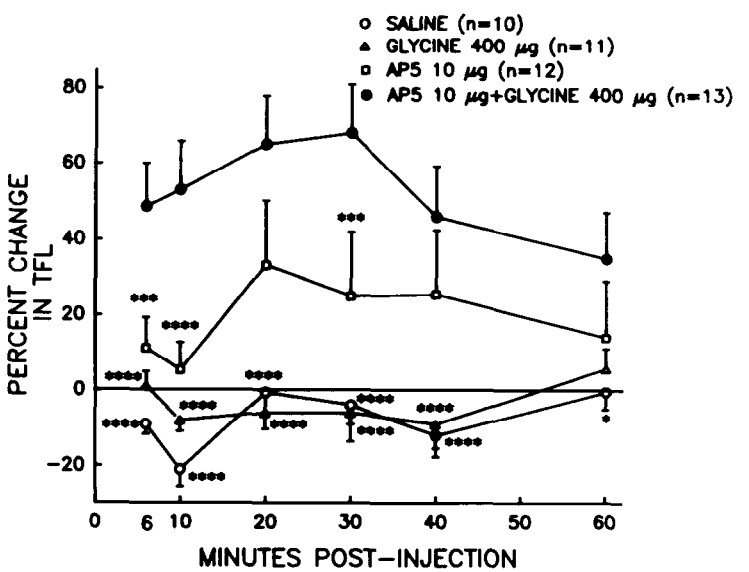

FIG. 2. Effect of IT administration of saline, glycine (400 μg), AP5 (10 μg) or AP5 (10 μg) + glycine (400 μg) on TFL. Values are percent changes of preinjection TFL values (0 level). Note that glycine significantly enhanced and prolonged the action of AP5 on TFL measures. Statistical comparisons included were made between AP5 + glycine vs. the other three groups, that is, saline, AP5, and glycine. Results of other statistical comparison are presented in the text. *$p < 0.05$, ***$p < 0.01$, ****$p < 0.001$.

increase in TFL was surprising considering that IT strychnine does not produce hypersensitivity to noxious thermal stimulation (3,4). However, the lack of a hyperalgesic effect by IT strychnine in the TFL test was probably due to the stress condition induced by the neurotoxin. Stress lengthens the latency for the tail-flick response to radiant heat (24). Blockage of Gly_1 spinal receptors by IT strychnine induces allodynia, a condition in which innocuous mechanical stimuli produce aversive behavior (3,26). This finding suggests that glycine also controls afferent inflow from Aβ fibers carrying information from low-threshold mechanical receptors. Interestingly, some of these fibers use glutamate as a spinal neurotransmitter since strychnine allodynia is blocked by IT injection or glutamate receptors antagonist (26). Therefore, the possible participation of the NMDA receptor in this response may explain why glycine diminishes but does not suppress strychnine-induced allodynia (3). Complex effects of glycine administration due to its simultaneous action on two receptors have also been seen in other neural systems beside pain. Thus, IT glycine potentiates the convulsant effect of IT strychnine by acting through the NMDA receptor (Gly_2 type receptor), while it inhibits IT NMDA-induced convulsions by acting at the Gly_1 receptor (15).

In conclusion, the present results support the idea that some glycinergic interneurons depress nociceptive information by inhibiting second-order neurons in the dorsal horn through Gly_1 receptor. This is consistent with anatomical and biochemical data showing that high concentrations of glycine and Gly_1 receptors (2,29), as well as high-affinity uptake mechanisms for this amino acid (17,18), exist in the substantia gelatinosa and lamina V, spinal cord regions related to the processing of pain signals.

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