Pharmacology Biochemistry and Behavior, Vol. 61, No. 1, pp. 45–48, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00083-5

Analgesic Synergism Between AP5 (an NMDA Receptor Antagonist) and Vaginocervical Stimulation in the Rat

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Received 29 August 1997; Revised 5 February 1998; Accepted 9 February 1998

CABA, M., B. R. KOMISARUK AND C. BEYER. *Analgesic synergism, between AP5 (an NMDA receptor antagonist) and vaginocervical stimulation in the rat.* PHARMACOL BIOCHEM BEHAV **61**(1) 45–48, 1998.—Vaginocervical stimulation (VS) releases multiple neurotransmitters into superfusates of the spinal cord; these can stimulate both nociceptive (e.g., glutamate, and glycine acting at the NMDA site), and antinociceptive (e.g., GABA, norepinephrine, 5-HT, and glycine acting at the strychnine-sensitive receptor) systems. Although the balance between these two opposing systems can determine the nature, magnitude, and duration of the response to VS, the characteristic prevailing response to VS is analgesia. We hypothesized that by counteracting the nociceptive component of this system, the magnitude and duration of the response to VS would be augmented. In the present study, the NMDA receptor antagonist AP5 [10 μg injected intrathecally (IT)] significantly increased the magnitude and duration of the analgesia (measured as tail flick latency to radiant heat) produced by VS (200 g force). At several time points the analgesic effect of AP5 combined with VS was greater than the sum of the effects of AP5 and VS separately, suggesting that they act synergistically. We propose that AP5 potentiates the analgesic effect of VS by two mechanisms: (a) antagonizing the putative pain-producing action of glutamate and glycine acting jointly at the NMDA receptor, and consequently, (b) permitting the unimpeded expression of the analgesic action of inhibitory neurotransmitters released by VS (e.g., glycine at the strychnine-sensitive receptor, and GABA). © 1998 Elsevier Science Inc.

Pain Nociception Analgesia NMDA receptor Glutamate Vaginal stimulation Glycine receptors Intrathecal Spinal cord

MECHANICAL vaginocervical stimulation (VS) in rats elevates pain thresholds, i.e., produces analgesia in a variety of tests [e.g., (11,12)]. Although VS-produced analgesia is a robust and reliable phenomenon, the magnitude of the analgesia decreases with prolonged repeated stimulation (6). Under certain conditions [e.g., neonatal capsaicin treatment; (16)] VS actually reduces pain thresholds (i.e., produces hyperalgesia). This may be due to the VS-produced release of glutamate and aspartate (17), which are primary afferent excitatory neurotransmitters for nociception in the spinal cord (1,5,19). The analgesic effect of VS is mediated by monoaminergic (7,20), opiate (9), and peptidergic (14) mechanisms. It is likely that glycine also contributes to VS-produced analgesia because IT strychnine, a glycine 1 (GLY1) antagonist, greatly decreases VS-produced analgesia (18). It may seem contradictory that administration of glycine itself IT produces hyperalgesia (2). However, this action can be accounted for on the basis that glycine acts as a cotransmitter at the NMDA receptor (10,21), thereby permitting and enhancing the excitatory hyperalgesia-produced action of glutamate and aspartate. In support of this interpretation, we have demonstrated that after blocking the action of glycine at the GLY2 receptor by administration of the specific NMDA receptor antagonist, AP5 [2-amino-5-phosphonopentanoate; (4,8)] administration of glycine IT produced not hyperalgesia, but instead, a strong, persistent analgesia (3). VS releases glycine, aspartate, and glutamate into the spinal cord (17). Furthermore, it was recently reported (15) that ACEA-1021, a competitive NMDA

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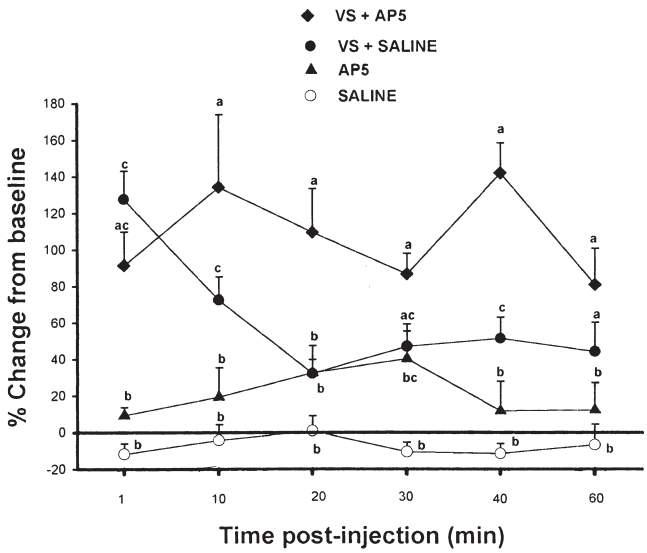

FIG. 1. Effect of the intrathecal administration of AP5 (10 μg) alone or in combination with vaginocervical probing. Values are mean ± standard errors. At any given time period, groups that do not share the same letter differ significantly from each other ($p < 0.05$). Thus, at 20 min, the VS + AP5 group is significantly greater than all other groups, and those groups do not differ significantly among themselves. At 30 min the VS + AP5 group was significantly greater than the AP5 group and the saline group, and the VS + saline group was significantly greater than the saline group, but no other pairs of groups differed significantly from each other.

receptor/glycine site antagonist, injected IT in mice attenuated the nociceptive response on the formalin test, which led the authors to suggest that antagonism of NMDA through blockade of glycine coagonist sites could produce antinociceptive effects.

It is possible that glycine, aspartate, and glutamate, interacting at the NMDA receptor are responsible, at least in part, for the gradual diminution of the effectiveness of repeated VS, as well as for the hyperalgesia that can be observed under certain conditions (i.e., neonatal capsaicin administration) in response to this stimulus (16).

In the present study, we hypothesized that AP5 administered IT would block the nociceptive effects of aspartate and glutamate, as well as glycine, all acting at the NMDA receptor. This would "unmask" the purely inhibitory, analgesia-producing effect of the glycine that is released into the spinal cord in response to VS, thereby increasing the magnitude of VS-produced analgesia.

METHOD

Subjects (Ss)

Ss were virgin female Sprague–Dawley rats (Charles River, Kingston, NY) weighing 250–300 g, housed individually at 23°C under a controlled light–dark environment (lights from 2000 to 1000 h). Food and water were provided ad lib. The subjects were tested during the dark phase of their light cycle.

Surgery

Rats were ovariectomized under Ketamine anesthesia (100 mg/kg IP) to avoid possible alterations in pain thresholds related to fluctuations in ovarian secretion during the estrous cycle. Two weeks later, under Ketamine anesthesia, a catheter (Clay Adams PE-10 tubing; Fisher Chemical, Springfield, NJ) was implanted chronically in the subarachnoid intrathecal space through an incision into the atlanto-occipital membrane (22). The tip of the catheter (7.5 cm insertion length) reached the thoraco-lumbar level of the spinal cord. Only rats with no motor or sensory disfunction after surgery were used in the experiment. At least 1 week of recovery was allowed before testing. At the completion of the study, Ss were anesthetized with an overdose of Chloropent, perfused through the heart with 4% formalin, and the spinal cord was dissected to verify correct placement of the catheter. Only Ss with catheters situated appropriately in the intrathecal space were included in the experiment.

Nociception Test

Before IT injection, all Ss were tested to determine their tail-flick latency (TFL) using an IITC Model 33 Analgesy meter (IITC Corp., Landing, NJ, at 90% intensity). Ss 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 cut-off time of 10 s was employed to avoid tissue damage. Each Ss was pretested one time, and the average TFL based on three successive trials with 10-s intertrial intervals was calculated and used as the preinjection baseline level; they were taken immediately before the actual test.

Vaginocervical Probing

Ss received VS, which consisted of inserting a force-calibrated glass probe (6) against the cervix of a lightly restrained animal, and aplying 200 g force during the measurement of the TFL.

TABLE 1

EFFECT OF THE INTRATHECAL ADMINISTRATION OF AP5 (10 μg) ALONE OR IN COMBINATION WITH VAGINOCERVICAL PROBING ON THE TAIL-FLICK LATENCY (MEANS ± STANDARD ERRORS; SECONDS)

			Minutes Postinjection					
Treatment	*n*	Preinjection	1	10	20	30	40	60
Saline	10	4.3 ± 0.9	3.7 ± 0.5	4.1 ± 0.8	4.4 ± 0.8	3.8 ± 0.5	3.8 ± 0.6	4.0 ± 0.9
VS + Saline	10	4.0 ± 0.5	9.1 ± 1.5	6.8 ± 1.2	5.2 ± 0.7	5.8 ± 1.2	6.1 ± 1.1	5.7 ± 1.6
AP5	9	5.2 ± 0.7	5.6 ± 0.4	6.2 ± 1.5	6.9 ± 1.4	7.3 ± 1.5	5.8 ± 1.5	5.8 ± 1.4
VS + AP5	9	4.0 ± 0.8	7.6 ± 1.8	9.3 ± 2.8	8.3 ± 2.2	7.4 ± 1.1	9.6 ± 1.6	7.2 ± 1.7

Infusion Procedure and Treatment Groups

Two weeks after implantation of the catheter, preinjection baseline values of each rat were obtained and they were randomly assigned to one of four groups: group 1—saline (n = 10); group 2—AP5 10 μg (n = 9); group 3—vaginocervical probing + saline (n = 10); group 4—vaginocervical probing + 10 μg AP5 (n = 10). AP5 was dissolved in saline. The selected dose of 10 g AP5 was based on a previous report (4) and 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 on the basis that it induced weak or no analgesia and no overt motor effects. Higher doses produced strong persistent analgesia and motor problems.

Ss were injected IT with saline or AP5 in a 5-μl volume delivered to the subarachnoid space, flushed with an additional 7 μl of saline. During IT injections (delivered over a period of approximately 1 min) Ss were restrained in a Stoelting animal holder. Immediately after injection, subjects were placed in a clear Plexiglas cage and observed for any abnormal motor activity. After confirming Ss did not show motor disturbances, they were restrained in the Plexiglas restrainer for the entire 60 min. Observers were blind as to the drug treatment. To determine the time course of drug-induced effects, TFL was measured at 1, 10, 20, 30, 40, and 60 min postinjection.

Statistics

The effect of each treatment was assessed by obtaining baseline values for each subject before IT injection or VS. Control (saline) and experimental values (AP5, VS + saline, AP5 + VS) at all testing intervals were expressed as percent change from the baseline values. A two-way ANOVA for repeated measures was performed using GB-STAT for MacIntosh (Dynamic Microsystems, Silver Spring, MD). Subsequent paired comparisons were performed using Fisher's Protected *t*.

RESULTS

The results are summarized in Fig. 1. A two-way ANOVA for repeated measures yielded a significant interaction for groups × time, $F(3, 5) = 3.61$, $p < 0.0001$, and a significant between-groups effect, $F(3) = 17.70$, $p < 0.0001$. The time effect was not significant, $F(5) = 1.89$, $p = 0.10$. Using the measurement of percent change of TFL from baseline, the VS + AP5 group was significantly greater than all other groups, including the VS group, at 10, 20, and 40 min postinjection. The VS + AP5 group was significantly greater than the saline group and AP5 group at all times. The VS group was significantly greater than the saline group at all times except at 20 min, and significantly greater than the AP5 group at all times except 20 and 30 min. The absolute mean time values for baseline and test groups are shown in Table 1.

DISCUSSION

The present results confirm earlier findings that vaginocervical probing induces a marked but relatively transient analgesia in rats (6,12,14). In support of our hypothesis, administration of AP5 significantly increased both the magnitude and duration of vaginocervical probing-induced analgesia. It is likely that this effect is due, at least in part, to blockage of the NMDA receptor by AP5. This blockage would reduce the postsynaptic activation of spinal nociceptive pathways in response to glutamate and glycine, which are released into the spinal cord by VS (17). This would unmask the analgesic effect of glycine, thereby enhancing the analgesia produced by VS. The release of primary afferent excitatory neurotransmitters (e.g., glutamate) by VS suggests a mechanism by which it could produce nociception. However, the observation that VS characteristically produces antinociception rather than nociception, indicates that the concomitant release of inhibitory neurotransmitters [e.g., GABA, Norepinephrine, 5-HT; (17,20)] supersedes the action of the excitatory neurotransmitters. A change in balance of excitatory and inhibitory neurotransmitters released by repeated VS could account for a gradual change from analgesic to algesic effects of VS under conditions of prolonged stimulation.

There are multiple parallels between the neural pathways and transmitters that mediate VS-produced analgesia and those that mediate other forms of analgesia, for example, stress-produced analgesia (13). Consequently, it is possible that NMDA antagonists may potentiate not only VS-produced analgesia, but also other forms of sensory stimulation-produced analgesia, a mechanism that could be of therapeutic significance.

ACKNOWLEDGEMENTS

Contribution number 695 from the Institute of Animal Behavior. This work was supported by a grant from the National Institute of Health-MBRS Grant 5 S06 GM 08223 (B.R.K.) and the Charles and Johanna Busch Foundation (B.R.K.). We gratefully acknowledge the excellent technical assistance of Ms. Cindy Banas.

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