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Blockage of Substance P-Induced Scratching Behavior in Rats by the Intrathecal Administration of Inhibitory Amino Acid Agonists

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BEYER, C., C. BANAS, O. GONZALEZ-FLORES AND B. R. KOMISARUK. *Blockage of substance P-induced scratching behavior in rats by the intrathecal administration of inhibitory amino acid agonists.* PHARMACOL BIOCHEM BEHAV **34**(3) 491–495, 1989. —Intrathecal administration of 20 μg of substance P induced scratching behavior in most tested rats (80%). Scratching appeared in bouts of short latency and variable duration, intensity and frequency (range 1–60, mean number of scratching bouts in one hour test: 8.93 ± 1.86). Intrathecal administration of glycine (400 μg but not 66 μg) significantly decreased the effect of substance P on this behavior. Taurine, in dosages equimolar to glycine, abolished the response to substance P at the high dose level (700 μg), but did not significantly affect it at the lower level (120 μg). The $GABA_A$ agonist, muscimol, abolished the effect of substance P at the 3 μg dose level, but the 0.5 μg dose did not produce a significant effect. Baclofen, a $GABA_B$ agonist, was highly effective in significantly reducing the action of SP at 0.9 and 0.15 μg; only two of 8 rats receiving the low dose of baclofen (0.15 μg) exhibited scratching. The results suggest that the spinal inhibitory amino acids modulate nociceptive impulses generated by the action of substance P in dorsal horn neurons of the spinothalamic tract.

Glycine Baclofen Taurine Substance P Inhibitory amino acids Intrathecal administration Scratching Itch Rats, female

IT has been proposed that the inhibitory amino acids, GABA and glycine, participate in the regulation of nociceptive information at the spinal cord level (7, 33, 41, 44). The following observations support this: a) high concentrations of GABA and glycine and their receptors (2, 3, 18, 24, 27, 30, 43) occur in spinal cord regions involved in the transmission and processing of pain, i.e., substantia gelatinosa and lamina V; b) GABA and glycine inhibit the response of spinothalamic neurons to noxious stimulation (38); c) intrathecal GABA or glycine antagonists (7, 8, 31) decrease thresholds for certain types of nociceptive stimulation; and d) intrathecal GABA or glycine agonists exert analgesic actions (8, 12, 15, 34, 40).

The precise site and mechanism of action of the analgesic effect of these neurotransmitters is uncertain. It has been suggested for GABAergic drugs that they could act by modulating the action of substance P (1,34). This peptide is the major candidate for excitatory neurotransmitter in the primary afferent fibers mediating nociception (39, 41, 44).

Intrathecal administration of substance P produces a scratching, biting, licking syndrome (19, 28, 35). This response is consistent enough to be used as a criterion for assessing the antinociceptive effect of some drugs or neurotransmitters (35). Therefore, in the present study, we assessed the capacity of a series of drugs that have been shown to bind to GABA or glycine receptors to influence the behavioral response induced by the intrathecal injection of substance P.

METHOD

Experiments were performed with female Sprague-Dawley rats weighing 250–350 g. They were housed individually at 23 °C, and maintained on a reverse day-night cycle (dark from 10:00 to 20:00 hr). Food and water were supplied ad lib. All rats were ovariectomized through bilateral incisions. Ovariectomy was performed

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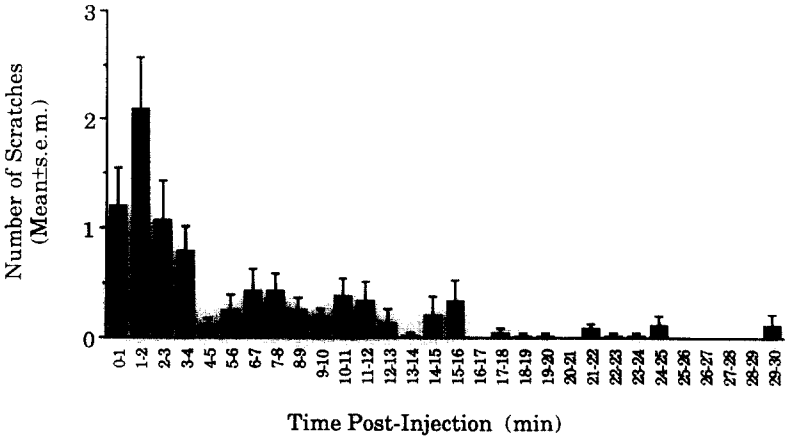

FIG. 1. Time course of a behavioral response to intrathecal administration of substance P (20 μg).

to avoid possible alterations in neural responsiveness due to gonadal steroid fluctuations. A catheter (Clay Adams PE-10 tubing, Fisher Chemical, Springfield, NJ) 7.5 cm length, was implanted in the subarachnoid intrathecal space, through an incision in the atlantooccipital membrane. Animals were anesthetized during surgery with ketamine (100 mg/kg IP) and xylazine (5 mg/kg IP) and treated once with terramycin (2.5 mg IM). At least 7 days of recovery were allowed before testing postsurgery. Rats showing neurological deficits were discarded from the observations.

Subjects (Ss) were observed in a circular Plexiglas cylinder for 5 min before intrathecal injection.

Drug Injections

Rats were injected intrathecally with one of the following solutions: group 1, 0.01 N acetic acid solution (vehicle) (n=5); group 2, substance P 20 μg (n=45); group 3, glycine 66 μg (n=5); group 4, glycine 400 μg (n=6); group 5, taurine 120 μg (n=5); group 6, taurine 700 μg (n=6); group 7, muscimol 0.5 μg (n=5); group 8, muscimol 3 μg (n=6); group 9, baclofen 0.15 μg (n=5); group 10, baclofen 0.9 μg (n=5); group 11, substance P 20 μg + glycine 66 μg (n=7); group 12, substance P 20 μg + glycine 400 μg (n=10); group 13, substance P 20 μg + taurine 120 μg (n=8); group 14, substance P 20 μg + taurine 700 μg (n=7); group 15, substance P 20 μg + muscimol 0.5 μg (n=7); group 16, substance P 20 μg + muscimol 3 μg (n=8); group 17, substance P 20 μg + baclofen 0.15 μg (n=8); group 18, substance P 20 μg + baclofen 0.9 μg (n=8). The dosage of substance P was selected from a pilot study in which various dose levels of the peptide were injected. The 20 μg was found to consistently induce a brief period of scratching and biting bouts in most Ss. Dosage of amino acids (glycine and taurine) that were equimolar to each other were selected from results obtained in previous studies (7,8). GABA and glycine agonist dosages were subthreshold to those producing gross motor disturbances such as hind limb paralysis.

All chemicals were dissolved in 6 μl of a 0.01 N solution of acetic acid (pH 3.0). Fresh solutions of substance P were prepared daily in order to avoid a possible loss of potency of the peptide. Drugs were delivered to the perispinal space with an additional 7 μl of saline flushed from the catheter. Injection duration was under 1 minute. Rostrocaudal diffusion following intrathecal injection at this volume is usually restricted to the spinal cord at least within the first 30 min postinjection (42). Behavioral tests were begun immediately after completion of the injection procedure.

Behavioral Testing

Rats were placed in a cylindrical Plexiglas cage and their behavior was recorded and registered. The following behavioral patterns were counted on a minute to minute basis: 1) bouts of scratching; 2) biting; 3) grooming; 4) licking the hind paws. Other unusual behavior or motor alterations that may occur with the administration of some of the drugs tested, e.g., hindlimb paralysis, were also recorded. After being released from the restrainer in which they were injected, the vehicle control rats exhibited prolonged periods of "face washing" and grooming of various parts of the body. Therefore, we consider grooming to be an inadequate criterion of disturbed cutaneous sensitivity and did not use it for analysis. In some animals, biting was observed clearly, but in many others it was difficult to distinguish it from grooming; therefore, we analyzed the scratching data exclusively, since it was the most valid measure. Observations continued for at least one hour following intrathecal injection. At the end of observations Ss were sacrificed and the placement of catheters checked by dissection. Ss with misplaced catheters were excluded from this study.

Statistical Analysis

The number of scratching bouts in each experimental group (substance P + drug) was compared with that obtained in the control group injected with only substance P. Dunnett's tests were used to compare each group with the substance P control (Fig. 2).

RESULTS

Administration of the vehicle did not induce significant scratching or biting behavior. Spontaneous scratching behavior occurred only rarely in the vehicle control rats or predrug condition. Substance P administration elicited in most Ss (80%) bouts of scratching and biting behavior initially directed toward the forelegs and lower abdomen. This behavior pattern appeared with a

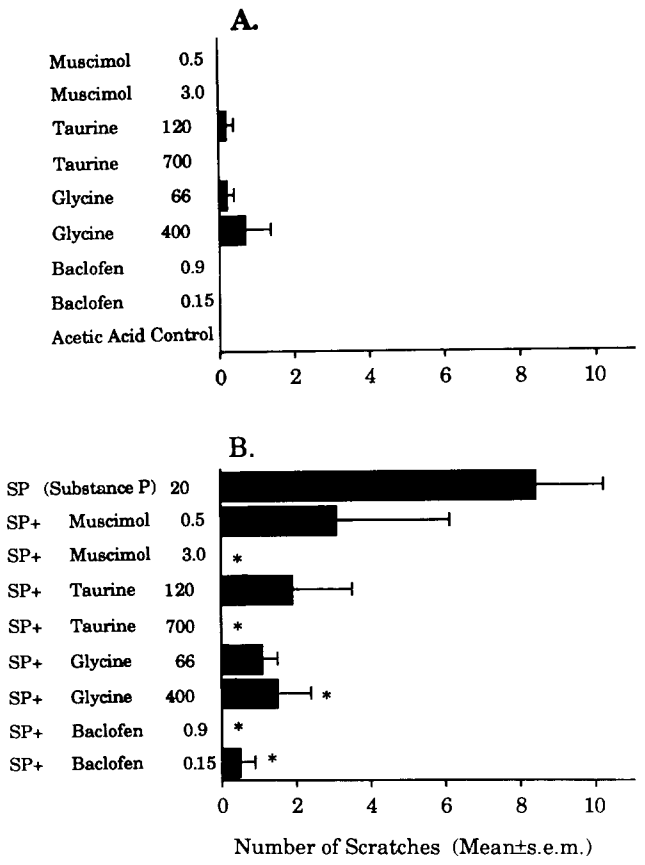

FIG. 2. (A) Behavioral effect of intrathecal administration of selected amino acids, without concurrent administration of substance P. None of the groups differed significantly from the acetic acid control, based on Dunnett's test (one-tailed). (B) Behavioral effects of the intrathecal administration of the above amino acids (same doses) administered concurrently with substance P (20 μg). *$p \leq 0.05$ compared to group receiving substance P only; Dunnett's test (one-tailed).

very short latency, frequently within the first minute after substance P injection (Fig. 1). The duration and intensity (frequency) of scratching bouts varied widely among Ss (range 1–60, mean: 8.93 ± 1.86 scratching bouts in the first hour). Figure 1 shows the temporal course of the scratching behavior obtained from 45 rats receiving substance P (20 μg). Notice that scratching occurred mainly within the first 15 min postinjection (peak value between 1–2 minutes), but some Ss occasionally showed this behavior even 30 minutes after substance P administration.

As shown in Fig. 2A, none of the groups receiving only the various amino acids intrathecally (i.e., in the absence of substance P) differed significantly from the acetic acid control group (Dunnett's test).

As shown in Fig. 2B, significant reductions in scratching, compared to the substance P-only group, were found when the higher of the two dosages of glycine (400 μg but not 66 μg), muscimol (3.0 μg but not 0.5 μg), or taurine (700 μg but not 120 μg) were administered in combination with substance P (Dunnett's test, t-values between 2.68 and 2.45; $p<0.05$, one-tailed). Baclofen at both doses (0.15 and 0.9 μg) combined with substance P produced a significant reduction in scratching compared with substance P alone. No motor effects, e.g., hindlimb paralysis, were observed as a result of these treatments.

DISCUSSION

The present results confirm that intrathecal administration of substance P is a nociceptive stimulus as evidenced by compulsive scratching and biting of the hindlegs and lower abdomen. This behavior suggests that substance P mediates nociceptive responses involving skin irritation resulting from mechanical or chemical stimulation of the skin, i.e., itch-like sensations. This interpretation is consistent with the reduction in the response induced by skin irritants (algiogenic chemicals) by neonatal or adult administration of capsaicin (20, 21, 23), a depletor of substance P in primary afferents (14, 20, 39, 41, 44).

Previous results have shown that occupation of either $GABA_A$ or $GABA_B$ spinal cord receptors by some GABA agonists (muscimol, THIP, baclofen) increases the nociceptive threshold for electrical and thermal stimulation (15, 31, 34, 40). The present data also indicate that the GABAergic system modulates transmission in the nociceptive system of neurons activated by substance P. Substance P primary afferent fibers mainly end in the external part of the dorsal horn (substantia gelatinosa), though some fibers may also terminate in lamina V (4, 5, 11, 13, 17, 26, 27). These areas also contain rich GABAergic innervation and both $GABA_A$ and $GABA_B$ binding sites (27). It has been proposed that baclofen exerts its analgesic action by presynaptically inhibiting the release of primary afferent neurotransmitters including substance P (1, 3, 12, 41). However, this mechanism of action, i.e., inhibition of release, cannot operate in the present study since substance P was directly administered. Therefore, GABAergic neurons probably establish inhibitory postsynaptic connections in these same neurons receiving substance P terminals, a suggestion consistent with some anatomical studies (18). In this neuronal arrangement, the hyperpolarization resulting from GABA postsynaptic action will counteract the slow depolarization produced by substance P in spinal cord neurons (45). Modulation of substance P neuronal responses by the GABAergic system has been reported on the motor side of the spinal cord. Thus, both muscimol, a $GABA_A$ agonist, and the $GABA_B$ agonist baclofen suppressed the depolarization of the ventral root produced by the application of substance P to the isolated rat spinal cord (1). Moreover, baclofen also depressed the depolarization of spinal motoneurons induced by the peptide (25). Interestingly, baclofen was relatively ineffective in counteracting the depolarization induced in these same neurons by glutamate (32), a finding suggesting that the $GABA_B$ agonists interacted in a specific manner with the postsynaptic action of substance P. However, the mechanism through which this interaction may occur is unclear since baclofen does not compete for ^{3}H-substance P binding sites (16). Comparison of the effective dosages for interfering with the behavioral actions of substance P revealed baclofen to be more potent than muscimol, a finding consistent with the higher density of $GABA_B$ than $GABA_A$ receptors in the areas of termination of substance P fibers (27).

Strychnine, a glycine antagonist, induces dramatic skin hyperalgesia (7) and high concentrations of glycine and its receptors exist in the dorsal horn (2,43). However, intrathecal glycine has no analgesic action in either the tail shock vocalization test or in the tail flick test (8). On the other hand, taurine, that is believed to act through the glycine receptor, elicits only at high dosages a mild analgesic effect in both of the above mentioned tests (8). In marked contrast, the present results show that both amino acids effectively counteracted the algesic action of substance P. Effec-

tive dose levels were much below those required to induce motor effects with glycine or analgesia to tail shock by taurine (8). The differential effect of glycine administration on the various modalities of nociception, and the present results, suggest that glycine primarily modulates information carried by substance P containing fibers. On the other hand, GABA appears to have a more general effect on nociception, since administration of some of its agonists interferes with pain originating from the stimulation of various types of nociceptors (thermal, etc.).

Recent findings indicate that glycine (9, 10, 22), but not taurine (9) facilitates excitatory amino acid transmission through activation of the NMDA receptor. This regulatory action can be exerted at very low concentrations of glycine, i.e., 10 nM (22). This effect appears not to be relevant to the inhibitory effect of glycine of substance P-induced scratching, since NMDA activation by either intrathecal glutamate or NMDA induces scratching in rats (29) and mice (37). However, it is possible that the scratching behavior induced by glycine in some Ss was due to its interaction with the NMDA receptor. Moreover, the fact that glycine apparently did not abolish the action of substance P may be due to the facilitatory action of this amino acid on L-aspartate activity.

In summary, the present results are indicative of the existence of a system of GABAergic and glycinergic neurons, tonically controlling information coming from the hairy skin through substance P-containing dorsal root ganglion neurons. Administration of the glycine and GABA receptor antagonists, strychnine and bicuculline, produce the same, though more intense, behavioral effects (i.e., scratching and biting) as does intrathecal administration of substance P (6,19). Furthermore, intrathecal administration of the glycine and GABA receptor agonists, glycine, taurine, or muscimol, attenuates or blocks the scratching produced by substance P. Since this behavior is indicative of a response to the perception of itch, and this sensation is thought to be mediated by polymodal receptors on C fibers (6,36), fluctuations in the activity level of the intraspinal glycine and GABA systems may determine whether cutaneous sensory input via the substance P system is perceived as itch.

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