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Vasoactive Intestinal Polypeptide (VIP) Potentiates the Behavioral Effect of Substance P Intrathecal Administration

CARLOS BEYER,*†[1] MARIO CABA,†‡ CYNTHIA BANAS* AND BARRY R. KOMISARUK*

**Institute of Animal Behavior, Rutgers University, Newark, NJ 07102*
†Centro de Investigación en Reproducción Animal, CINVESTAV, Universidad Autonoma de Tlaxcala, Mexico
‡Centro de Investigaciones Biologicas, Universidad Veracruzana, Mexico

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BEYER, C., M. CABA, C. BANAS AND B. R. KOMISARUK. *Vasoactive intestinal polypeptide (VIP) potentiates the behavioral effect of substance P intrathecal administration.* PHARMACOL BIOCHEM BEHAV **39**(3) 695–698, 1991.—Intrathecal (IT) injection of 20 μg substance P (SP) induced a behavioral syndrome consisting of scratching and biting the flanks (83% and 57%, respectively, of 48 rats), and distress-like vocalization (42% of 26 rats tested) in response to a previously innocuous tactile stimulus with a von Frey fiber (allodynia). These behavioral events following SP were of short latency (1–2 min) and duration (around 10 min). Injection IT of 5 μg, but not 1 μg, of vasoactive intestinal polypeptide (VIP), concurrently with SP, significantly increased the frequency of both scratching and biting bouts over that produced by SP alone. VIP IT alone (1 or 5 μg) did not stimulate scratching-biting, but induced allodynia in a significant proportion of rats.

Vasoactive intestinal polypeptide Substance P Allodynia Itch Scratching Biting Rats Females Hyperalgesia Pain

SUBSTANCE P (SP), a putative neurotransmitter mediating nociception [for review see (30)], is colocalized with several other neuropeptides, e.g., calcitonin gene related peptide, CGRP (29), enkephalins (6) and vasoactive intestinal peptide, VIP (12,13) in dorsal root ganglia neurons of the sacral region. The functional significance of this colocalization has not been fully established, but behavioral studies suggest that these neuropeptides modulate the action of SP on second order spinal cord neurons.

Intrathecal (IT) administration of SP to rodents produces scratching, biting and licking of flanks and lower limbs (2, 9, 10, 18, 22). Concurrent administration of calcitonin gene related peptide (CGRP), one of the neuropeptides colocalized with SP, potentiates and prolongs the action of SP (28) although CGRP alone has no behavioral effect.

The possible interaction of VIP and SP in the spinal cord has not been explored, despite the colocalization of these two peptides in neurons of the sacral ganglia (12,13) and the overlapping in the distribution of their afferent terminals in the dorsal horn (16). Therefore, we studied the behavioral effects of the concurrent IT administration of VIP and SP.

METHOD

Subjects

Female Sprague-Dawley rats (250–300 g) were supplied with food and water ad lib. They were housed individually at 23 °C on a reversed light:dark cycle (dark 1000–2000 h).

Surgery

Ovariectomy was performed to avoid possible sensory alterations due to sex steroid secretion. Under ketamine anesthesia (100 mg/kg IP) and xylazine (5 mg/kg IP) a 7.5 cm catheter (Clay Adams PE-10 tubing, Fisher Chemical, Springfield, NJ) was introduced into the subarachnoid IT space through an incision in the atlanto-occipital membrane. At least one week of recovery was allowed before testing (7 to 14 days of recovery).

Treatment Groups

Rats were injected IT with one of the following solutions: group 1: 0.01 N acetic acid (vehicle), n = 5; group 2: SP 20 μg, n = 48; group 3: VIP 1 μg, n = 10; group 4: VIP 5 μg, n = 10; group 5: SP 20 μg + VIP 1 μg, n = 10; group 6: SP 20 μg + VIP 5 μg, n = 14. Rats were injected twice in a balanced design, in which half of the rats initially received SP and the other half were allotted at random to the experimental groups. Seven to ten days later the treatment schedule was inverted. The dose of 20 μg SP was selected from a pilot study in which an IT injection of 1 μg, 5 μg or 20 μg was administered to groups of 4 rats each. Only with the 20 μg dose of SP was scratching

[1]Requests for reprints should be addressed to Dr. Carlos Beyer, Centro de Investigación en Reproducción Animal, A.P. No. 62 Tlaxcala, 90000, Tlaxcala, Mexico.

behavior induced consistently in our rats. Dosages of VIP were selected from previous studies in this laboratory. All substances were dissolved in 6 μl 0.01 N solution of acetic acid at pH 3. During the performance of the IT injection rats were kept in a Stoelting animal holder. Drugs were delivered to the perispinal space with an additional 7 μl saline flushed from the catheter. The injection duration was less than one minute. Previous studies have shown that with this injection procedure (32), drugs are restricted to the spinal cord at least for the first 30 min postinjection.

Behavioral Testing

Rats were observed in a circular Plexiglas cylinder before injection. Immediately after the injection they were placed back in the cylinder where they remained throughout the observation period. Rats showing signs of motor or sensory disturbances after surgery were excluded from the study.

Vocalization response to cutaneous stimulation was determined by briefly applying pressure alternatively to the lower half of the dorsum, flanks and hind legs with a medium diameter von Frey fiber (5.5 g force; Stoelting Co., Chicago, IL). In each test ten stimuli were applied at approximately 5-s intervals and the occurrence of vocalization, scratching-biting or other motor responses was noted. This stimulus rarely, if ever, induces vocalization in normal rats (1).

All observations were performed by two observers, which were blind to the treatment. The number of bouts of 1) scratching and 2) biting were counted on a minute-to-minute basis for 5 min before the injection and for 60 min after the injection. The effect of tactile stimulation with the von Frey fiber was tested immediately before injection, 1 minute following the IT injection and every 5 min subsequently.

Statistics

Data were analyzed by ANOVA and subsequent paired-comparisons (Tukey's Protected T) using the GB-STAT Professional Statistics and Graphics Version 2.0 computer program package (Dynamic Microsystems, Silver Springs, MD). Statistical comparisons for von Frey fiber stimulation between preinjection and postinjection conditions were made using the Wilcoxon test. Statistical comparisons for proportion of responsive subjects (scratching, biting) were made using a χ^2 test.

RESULTS

Administration of the vehicle induced no overt behavioral alterations, i.e., no scratching or biting. Spontaneous scratching behavior occurred only rarely in the predrug condition. Tactile stimulation with the von Frey fiber had no effect on vocalization in any of the five vehicle-injected rats. Similarly, as shown in Fig. 1, vocalization to von Frey fiber stimulation was rarely observed in the predrug condition (range from 1% to 4% of vocalization response in the various treatment groups).

SP administration elicited significant spontaneous scratching in 83% ($p<0.01$, SP vs. vehicle) and biting in 58% ($p<0.5$, SP vs. vehicle) of the rats (Figs. 1 and 2). Usually, spontaneous scratching was directed towards a skin area but in some cases stereotypic waving of the hind paw occurred without contacting the skin. SP-induced scratching was not accompanied by signs of overt distress, such as vocalization or abrupt motor changes. The scratching bouts were usually interspersed with periods of grooming or gentle biting of the paws or flanks. Scratching-biting episodes appeared within the first 2 min of the injection, peaked at 3 min and subsided in most rats by 20 min postinjection. Substantial individual variations in scratching frequency in response to SP were observed; thus 72% of the rats that manifested scratching showed fewer than 10 bouts of scratching during the first 20 min postinjection, and only 8% displayed more than 25 bouts during this period. Vocalization in response to von Frey fiber stimulation (i.e., "allodynia") was noted in 22 out of 26 tested rats injected with SP. Only two of these rats vocalized (each only once) to this stimulus before SP administration (Fig. 3). In five out of 26 rats injected with SP, tactile stimulation with the von Frey fiber consistently induced scratching directed to the stimulated point. This response to tactile stimulation (reflex scratching) was not observed under any other control or predrug condition.

VIP only, at either the 1 or 5 μg dose, failed to significantly stimulate scratching or biting (Figs. 1 and 2). However, as shown in Fig. 3, the high dose of VIP induced significant allodynia compared to values obtained before treatment.

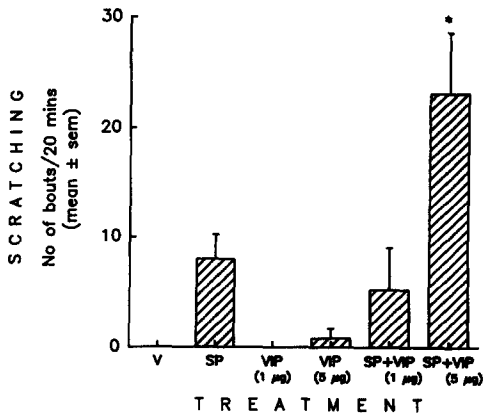

FIG. 1. Effect of IT injection of two dose levels of VIP (1 and 5 μg) with or without the concurrent injection of SP (20 μg) on the number of scratching bouts performed by the rats during the 20-min period immediately following injection. Note the synergy between SP and VIP (5 μg). *$p<0.05$ compared to group receiving only SP. V = vehicle (n = 5); SP (n = 48); VIP 1 μg (n = 10); VIP 5 μg (n = 10); SP + VIP 1 μg (n = 10); SP + VIP 5 μg (n = 14).

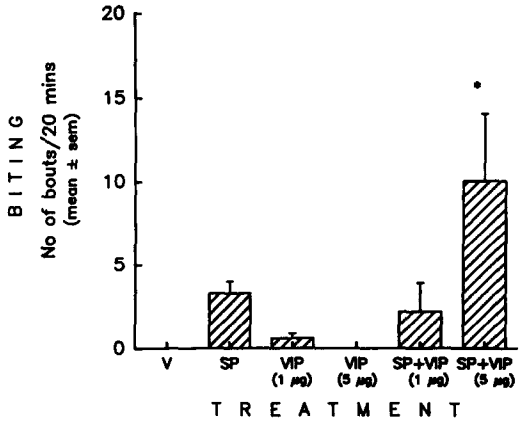

FIG. 2. Effect of IT injection of two dose levels of VIP (1 and 5 μg) with or without the concurrent injection of SP (20 μg) on the number of bites performed by the rats during the 20-min period immediately following injection. *$p<0.05$ compared with substance P alone. V = vehicle; number of rats as in Fig. 1.

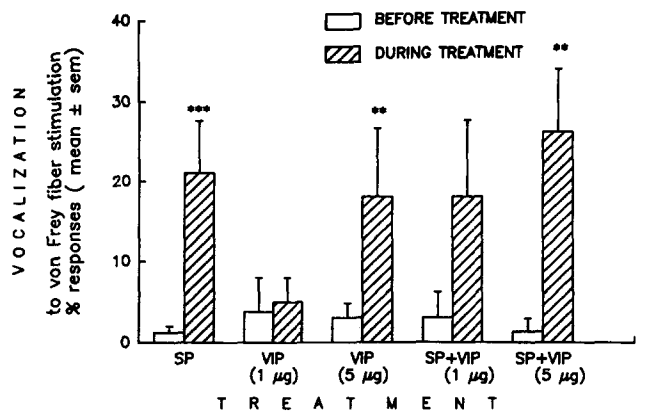

FIG. 3. Effect of IT injection of two dose levels of VIP (1 and 5 μg) with or without the concurrent injection of SP (20 μg) on the percentage of vocalization induced by von Frey fiber stimulation. Values obtained before treatment (open bars) were means of the percentage of times the individual rats vocalized in response to a test consisting of 10 trials with the von Frey fiber. Values obtained during treatment (hatched bars) indicate the mean percentage of times the rats vocalized in response to 4 tests, i.e., 40 trials with the von Frey fiber, performed at 5, 10, 15 and 20 minutes following the IT injection. Values were pooled since no significant differences in vocalization response were noted between these 4 postinjection tests. Note that von Frey fiber stimulation induced vocalization only rarely before the IT injections (less than 5% in any group). Administration of substance P and the higher dose of VIP significantly increased vocalization response to the mechanical stimulus. The vocalization response to von Frey fiber stimulation in the groups receiving VIP and substance P together did not significantly differ from values obtained with the peptides alone. $^{**}p<0.02$, $^{***}p<0.01$. Number of rats as in Fig. 1.

The concurrent administration of the high dose of VIP (5 μg) but not the low dose (1 μg) significantly increased the effect of SP on spontaneous scratching (Fig. 1). This effect was due to both an increase in the frequency and duration of the periods during which the scratching bouts were manifested. Thirteen of 14 rats injected with both SP and VIP displayed scratching, and 42% of these rats (versus 8% in SP rats) showed more than 20 scratching bouts within 20 min postinjection. Biting was also significantly increased in this group when compared with SP only (Fig. 2). By contrast, no synergism between VIP and SP was noted for the vocalizations induced by von Frey fiber stimulation (Fig. 3).

DISCUSSION

The present results are consistent with the concept that SP intrathecal administration is perceived as a noxious stimulus that mediates itch-like sensations (1). SP also produces mild allodynia, since indifferent tactile stimulation induced vocalization in a significant proportion of SP-injected rats, a finding in agreement with previous observations (5). The mechanisms through which SP stimulates allodynia are not clear, though it may involve an indirect effect. Thus SP increases the release of both aspartate and glutamate in the dorsal cord (28). These excitatory amino acids at its turn act on NMDA receptors which have been implicated in the production of allodynia, since NMDA antagonists prevent the intense allodynia induced by IT strychnine (31). It has been suggested (3) that SP produces scratching by acting directly on motoneurons rather than by stimulating second order sensory neurons. Yet, the fact that SP-treated rats frequently bit and licked the hind limbs or flanks is not consistent with this interpretation, since these actions involve motoneurons located in neural levels above the area of diffusion of the injected SP (32). Moreover, some SP-treated rats consistently responded to the brief von Frey fiber stimulation with scratching oriented to the site of the stimulus, suggesting that transmission in the afferent limb of the scratching reflex arc was facilitated by the peptide. Reflex scratching was not observed in control rats or during predrug testing.

The finding that combined IT administration of VIP and SP elicited biting directed to the same site as scratching implies that the peptides produced a sensory state to which the rats responded with an organized behavior pattern. A parsimonious interpretation is that combined intraspinal VIP and SP administration generated a sensation of itch, whose site of origin is the spinal cord and not the brain or periphery. In this case, the spinal cord appears to be a site of origin of "motivated" behavior, in which a specific afferent state (e.g., "itch") is produced by an interaction between two primary afferent peptides, to which the rat responds by generating a specific adaptive motor pattern (e.g., scratching).

It is only possible to speculate on the mechanism involved in the SP-VIP synergy. The synergistic effect of VIP on SP behavioral actions could be simply due to a summation effect, since VIP also excites dorsal horn neurons driven by noxious stimulation (11). However, the finding that VIP did not stimulate scratching does not support this interpretation, but rather suggests that it acted as a positive neuromodulator of SP postsynaptic action. VIP could modulate the action of SP at the synaptic level by increasing the binding affinity and/or number of SP receptors. To our knowledge, no information exists on the possible regulation of SP receptors by VIP, but it has been reported that VIP modifies the binding of serotonin to brain 5-HT receptors (26), and enhances muscarinic ligand binding in the salivary glands (19). It is known that SP acts at the cellular level through phospholipase-C-coupled receptors [see (15)], while VIP stimulates adenylate cyclase-coupled receptors [see (24)]. Although no specific studies on SP-VIP interactions on intracellular signal transduction have been made, several reports support this possibility (8, 20, 21). Thus synergistic interactions between SP and various drugs increasing cyclic AMP levels, e.g., isoprenoline and forskolin, have been reported in rat parotid glands (23). Moreover, in rat cerebral cortical slices, extremely low concentrations of VIP enhance the potency of carbachol (a muscarinic agent using the same biochemical effector as SP) in inducing inositol phosphate accumulation (25). Recently, an alternative mechanism for peptide interactions has been suggested by the observation that one peptide may potentiate the action of another by inhibiting its breakdown. Thus CGRP, despite not having structural affinity with SP, enhances and prolongs the behavioral action of IT SP (29) by inhibiting its degradation (17). Although the effect of VIP on SP breakdown has not been studied, it is probable that the synergistic effect can be explained by a similar, competitive, mechanism since in some tissues the same proteases, i.e., mast cell chymases, cleave both SP and VIP (4). It is also possible that VIP indirectly potentiates SP action by increasing local blood flow in the spinal cord, an idea supported by the existence of VIP-containing fibers innervating spinal cord blood vessels (7). This vasomotor mechanism has been shown to participate in the potentiation of the SP induced neurogenic inflammatory response by concurrent administration of VIP (14). Obviously, further studies should be done to evaluate these possible mechanisms of SP-VIP behavioral interactions at the spinal cord level.

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