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Vasoactive Intestinal Peptide Potentiates Sexual Behavior: Inhibition by Novel Antagonist*

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ABSTRACT. Vasoactive intestinal peptide (VIP) has been suggested as a neurotransmitter mediating penile erection. We now show that VIP can stimulate sexual behavior in rats with reduced masculine potential due to pituitary grafting or castration. This effect was attenuated in the presence of a novel VIP antagonist, devised by a hybrid peptide strategy. Thus, we have synthesized a molecule combining a portion of VIP with a portion of neurotensin, peptides of opposite pharmacological action on cAMP formation and smooth muscle relaxation. The hybrid peptide markedly inhibited VIP's effect on sexual behavior. This inhibition was manifested by a significant increase in the mean interval between copulatory events (> 3-fold change) coupled with a blockade of VIP-stimulated ejaculation. Other putative VIP antagonists were not as effective in blocking these activities. Thus, our results imply that VIP is not only associated with penile erection, but is involved in sexual behavior as well. Furthermore, the hybrid antagonist was shown to inhibit VIP binding in glial cell cultures. The availability of highly potent VIP antagonists may offer a route to study the possible multiple VIP receptors as well as help delineate other biological activities attributable to VIP. (*Endocrinology* **125:** 2945–2949, 1989)

VASOACTIVE intestinal peptide (VIP) fulfills several criteria for a neurotransmitter mediating penile erection. It is present in nerve fibers innervating cavernous smooth muscle and blood vessels, and it is elevated during erection (1, 2). Injection of exogenous VIP induces erection in man (1) and penile VIP levels have been shown to be decreased in impotent men (3). Since VIP appears to be important in erection formation (4), its administration might help in relieving penile dysfunction.

In this paper we have shown that the neuropeptide VIP can stimulate sexual behavior in rats with experimentally reduced masculine potential (5–9). This effect was attenuated in the presence of a novel VIP antagonist, a molecule composed of a portion of VIP and a portion of neurotensin. The rationale of this design resides in the observation that VIP is a potent vasodilator (10) and neurotensin causes contraction of smooth muscle cells (11). Moreover, VIP produces significant increases in cAMP levels in a variety of tissues, including reproductive and brain tissue (12–14). In contrast, neurotensin inhibits cAMP formation through an interaction of the peptide's receptor with the regulatory GTP-binding protein, N_i (15). The hybrid VIP antagonist markedly inhibited VIP's effect on sexual behavior. Furthermore, this novel antagonist competitively displaced the binding of VIP to glial cells.

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Materials and Methods

Antagonist synthesis

The peptide chain was assembled according to the solid phase strategy employing optimum side-chain protections (16). The product was purified by gel chromatography on Sephadex G-25, followed by reverse phase HPLC on a C_{18} column. The pure peptide showed the desired molar ratios of the constituent amino acids. The sequence of the antagonist is: Lys-Pro-Arg-Arg-Pro-Tyr-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2.

Behavioral tests

Male rats were sexually experienced Wistar-derived animals (250–300 g), approximately 3 months old, from the Department of Hormone Research, Weizmann Institute of Science. All rats were kept in a 12-h light, 12-h dark cycle. Experiments were always conducted within the dark period, 2–6 h after the onset

of darkness. Before testing, each male was put in a separate cage for a period of at least 1 h. A sexually receptive female (as tested by vaginal smearing) was introduced to each male. We define latencies as the intervals between copulatory behaviors (mounts and intromissions). These were recorded over a 15-min period. The latencies to the first copulatory event are reported separately.

The first model of sexually inhibited rats was pituitary-grafted gonadally intact males (7). This procedure results in hyperprolactinemia and a decrease in sexual potency in male rats (7). Pituitary-transplanted male rats (3 weeks after transplantation) were injected with 5 μg VIP, iv. As controls, we injected saline iv at identical volumes. The peptide was injected into the tail vein, restraining the animal by hand (two people were involved in the actual injection). The animals were subjected to the behavioral tests immediately after the injection. At least 24 h elapsed between two consecutive tests per animal (*i.e.* between saline injection and VIP injection). The doses of VIP were deduced from those used to induce penile erection in man (1). The statistical analysis involved a single test measurement per animal.

The second model of sexually inhibited rats was castrated rats. After castration, the animals lose sexual activity in a time-dependent manner (8, 9), and eventually all sexual activities are blocked. Rats were castrated and injected with testosterone (4 μg/100 g BW) daily for 14 consecutive days. VIP (5 μg/animal) and the antagonists (30 μg/animal) were injected ip in a volume of 0.25 ml. Initial experiments were conducted using iv injections; however, as no significant differences were observed between iv and ip administration, we chose to employ ip injection in this paradigm. When comparisons were made, the same group of rats was injected with one peptide and, after a period of at least 24 h, with another peptide. The other parameters used for the experiment are described above.

Cell culture and VIP binding experiments

Rat cortical astrocytes cultures were prepared by previously described methods (17, 18). VIP binding studies were conducted on intact cells at 4 C, using PBS containing 0.1% BSA. The cultures (1 mg protein/35-mm culture dish) were incubated with either VIP or the antagonist (10 μM to 1 pM) for 30 min before the addition of 50 pM [^{125}I]VIP at Tyr-22 (2000 Ci/mmol; Amersham Corp., Arlington Heights, IL). Labeled VIP was incubated with the cultures for 1 h; the medium was then removed, and cells were washed three times by the addition and rapid removal of 1 ml PBS (at 4 C). The labeled cells were dissolved in 0.02 N NaOH and transferred for radioactivity counting.

Results and Discussion

The first model of sexually inhibited rats involved pituitary-grafted gonadally intact males (7). Sexual behavior was monitored after iv injection of 5 μg VIP in saline. For controls, the same rats were used before injection and after sterile saline injection at identical volumes. A significant reduction in the mean interval between copulatory events coupled to a significant increase in the rate of copulation were observed after the administration of VIP (Fig. 1). A 2- to 3-fold decrease in the latency to the first intromission was observed after VIP injection. Thus, the mean latency to the first mount and intromission was 76 ± 27 sec in control animals, 76 ± 30 sec after saline injection, and 23 ± 4 sec after VIP injection. The latter was significantly decreased compared to that in the saline-injected controls. While only 10–20% of the rats tested ejaculated before VIP treatment, all of the rats ejaculated after administration of the peptide, which is highly significant for VIP. A similar effect was seen in an additional independent experiment which included eight animals.

The second paradigm was the castrated rat model. When we tested animals 14 days after castration, the ip administration of 5 μg VIP resulted in a 2- to 4-fold drop in the intervals between copulatory events (including the latency to the first intromission); thus, the intromission latency dropped from 90 ± 16 to 35 ± 2.4 sec in the six animals tested.

Another model was one in which the decrease in sexual behavior after castration was partially reversed by the daily injection of testosterone (4 μg/100 g BW as indicated). About a 2-fold increase in the rate of intromissions before ejaculation or the end of test period was observed after ip VIP injection compared to that after saline injection (Fig. 2). Thus, VIP increased the number of intromissions per a given test period and induced ejaculations. This was coupled to a 2-fold decrease in intervals (intromission latencies) between copulatory events after VIP injection. The latency to the first intromission decreased from 74 ± 10 to 40 ± 9 sec in nine animals tested ($P < 0.05$). Similar results were obtained with iv VIP injection in a group of 6 additional animals. Using this model, we found that 80–100% of the animals ejaculated after VIP treatment (15 animals were tested in this paradigm). In contrast, when the model without testosterone supplementation was used, only 1 of 6 animals tested ejaculated after VIP treatment. All subsequent experiments were conducted using the castrated rat model supplemented with testosterone.

To further demonstrate the specificity of the VIP effects, we used a VIP receptor binding inhibitor, an octamere of the following sequence: Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys (Peninsula, Belmont, CA) (10). Simultaneous injection of VIP in the presence of excess (20-fold when expressed as molar ratio) of the octamere specifically inhibited VIP effects on sexual behavior (Fig. 2).

Additional VIP analogs were then designed and synthesized. One analog, a VIP-neurotensin hybrid (see *Materials and Methods* for structure), blocked sexual activity after ip injection. Coinjection of this analog with VIP at a 3-fold molar excess of the former resulted in a

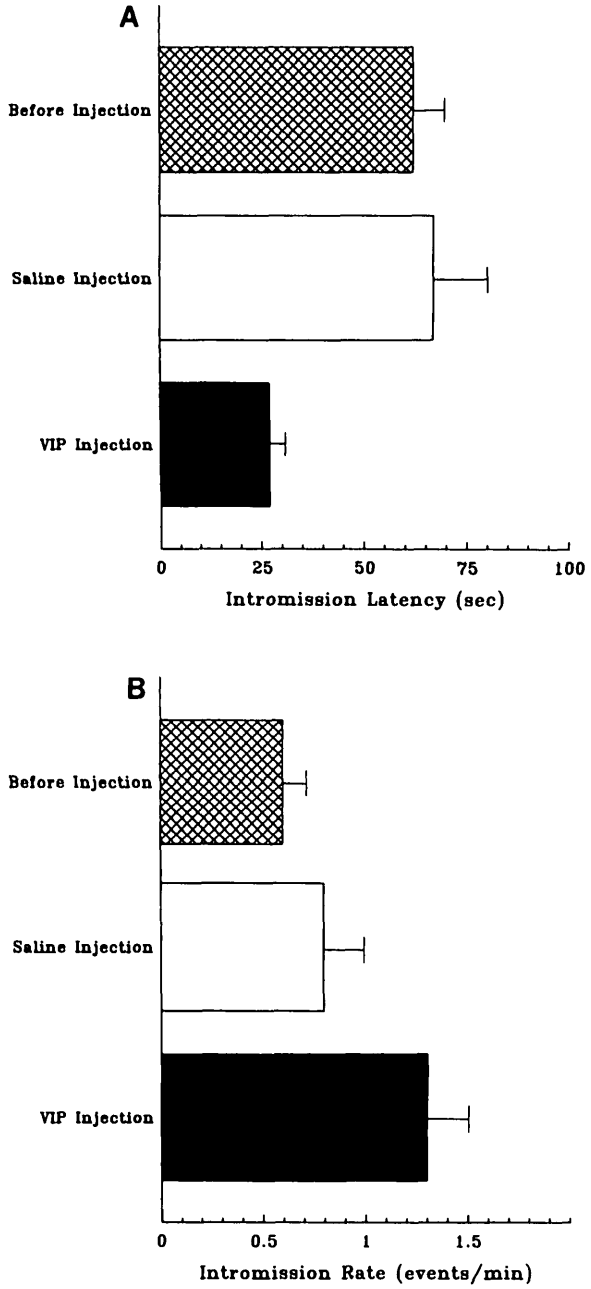

FIG. 1. VIP-stimulated sexual activity in pituitary-transplanted rats. Pituitary-transplanted male rats were injected with 5 μg VIP, iv. As controls, we injected saline iv at identical volumes. Eight animals were tested for each variable. The mean intromission latency to the first ejaculation or end of the test period (15 min) is depicted for all animals in A. As the mounts without intromissions represented only a very minor fraction (10–20%) of the copulatory events, they were grouped together with the intromissions. Thus, the data presented show the mean interval between copulatory events. An analysis of variance with a Student-Keuls multiple comparison of means test indicated that there was a significant difference in intromission latency (interval between copulatory events) after VIP injection ($P < 0.01$). B, The rate of intromissions (copulatory events) per group measured over a 15-min test period. An analysis of variance indicated a significant increase in the VIP-injected animals ($P < 0.02$; n = 8). All results are the mean ± SEM.

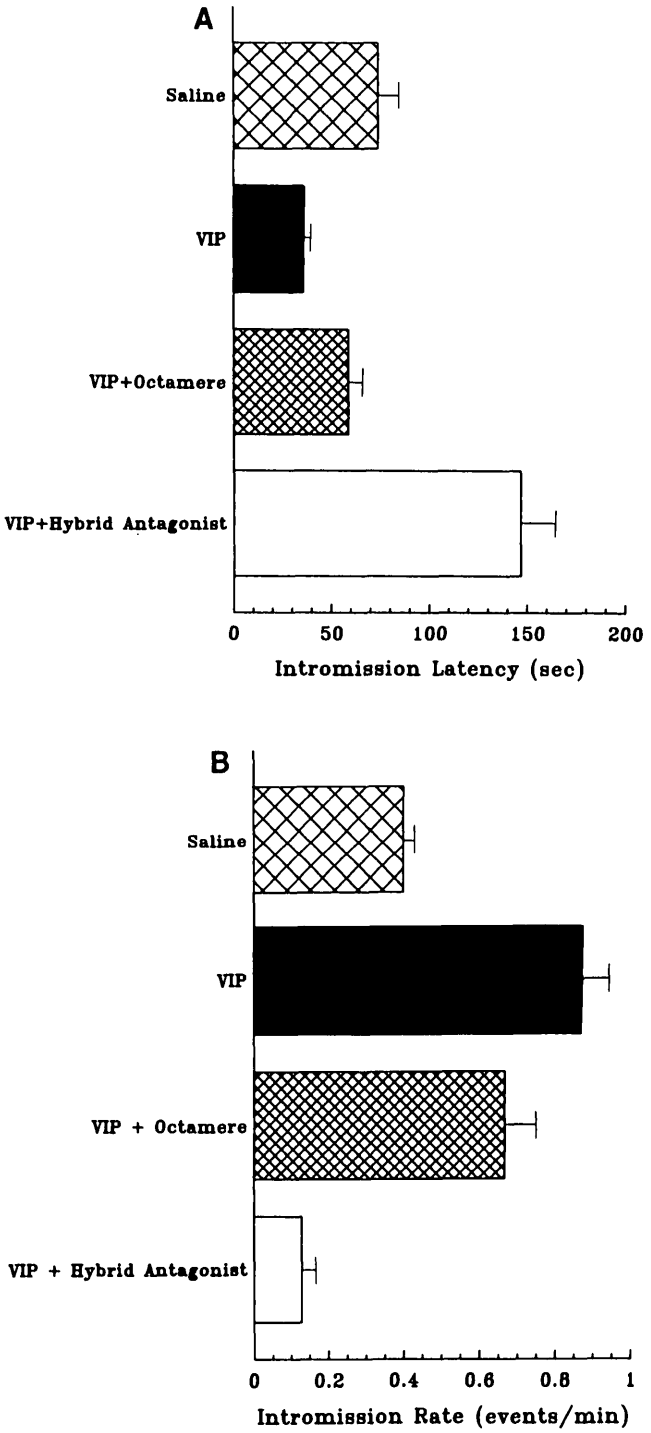

FIG. 2. VIP-stimulated sexual activity in castrated rats: inhibition by specific antagonists. Rats were castrated and injected with testosterone daily for 14 consecutive days. VIP (5 μg/animal) and the antagonist (30 μg/animal) were injected ip in a volume of 0.25 ml. The other parameters used for the experiment are described in *Materials and Methods*. A, The mean intromission latency (interval between copulatory events) is shown for saline injection (controls), VIP injection, VIP plus octamere injection, and VIP plus hybrid antagonist injection. Nine animals were tested for each variable. An analysis of variance indicated a significant decrease in the mean intromission latency after VIP injection, which could be prevented by both antagonists used ($P < 0.005$). B, The rate of intromissions (copulatory events) is depicted. The mean values for nine animals per group tested over a 15-min period are shown. A significant increase was observed after VIP injection, which could be prevented by the hybrid antagonist ($P < 0.001$).

complete blockade of VIP activity, with values 2-fold lower than the control values (Fig. 2). This was also reflected in the latency to the first copulatory event, which was 92 ± 20 sec compared to 40 ± 9 sec with VIP injection by itself. Thus, the VIP-neurotensin hybrid may serve as a novel VIP antagonist (Fig. 2). As expected, neurotensin by itself inhibited rat sexual behavior, decreasing the number of copulatory events per test period by 2-fold and increasing the intervals by 2-fold ($P < 0.01$ events/min, which is significant according to Student's t test). The hybrid antagonist when injected by itself decreased the rate of copulatory events from 1.66 ± 0.12 to 1.16 ± 0.11 and increased the intromission interval from 36.9 ± 2.4 to 53.4 ± 3.6 sec, in 10 animals ($P < 0.01$). When injected with VIP, the hybrid antagonist decreased activity to below basal levels. One explanation is that the antagonist blocked endogenous VIP, which may contribute to basal sexual activity. As the octamere antagonist had much less of an effect, our results suggest that the hybrid antagonist is more potent. Together, the data above indicate that both neurotensin and VIP can contribute to sexual activity and that the inhibition produced by the antagonist may be only partially due to blockade of the VIP receptor.

We have also examined the hybrid VIP antagonist in another well characterized biological system to substantiate its pharmacological activity and begin assessing the generality of these effects. The new molecule was also tested in cell cultures derived from the central nervous system (17, 18). The hybrid antagonist (10 μM) displaced 85–90% of VIP binding to glial cell cultures. This displacement curve was biphasic, indicating two binding sites, one with an IC_{50} of 50 pM and another with an IC_{50} of 0.1 μM. Moreover, under the same experimental conditions, unlabeled VIP (10 μM) produced 75% displacement.

As controls for specificity, we investigated peptides that exhibit structural similarity to VIP (10, 19). While glucagon did not have any effect (data not shown), peptide histidine isoleucine amide (PHI), which is cosynthesized with VIP on the same protein precursor (19, 20), apparently inhibited rat sexual behavior and VIP-induced rat sexual behavior (Fig. 3). Accordingly, the measured average latency to intromissions and rate of copulatory events in the presence of either PHI by itself or VIP plus PHI in equimolar concentrations were similar and 2-fold different from the values obtained with VIP by itself (Fig. 3). The same was found for the latency to the first intromission. Only one of nine animals ejaculated in the presence of PHI, which is similar to the saline control group. In cell culture, PHI has been shown to produce significant neuronal cell death, which can be antagonized by VIP (21). Thus, the two peptides originating from the same protein precursor may modulate

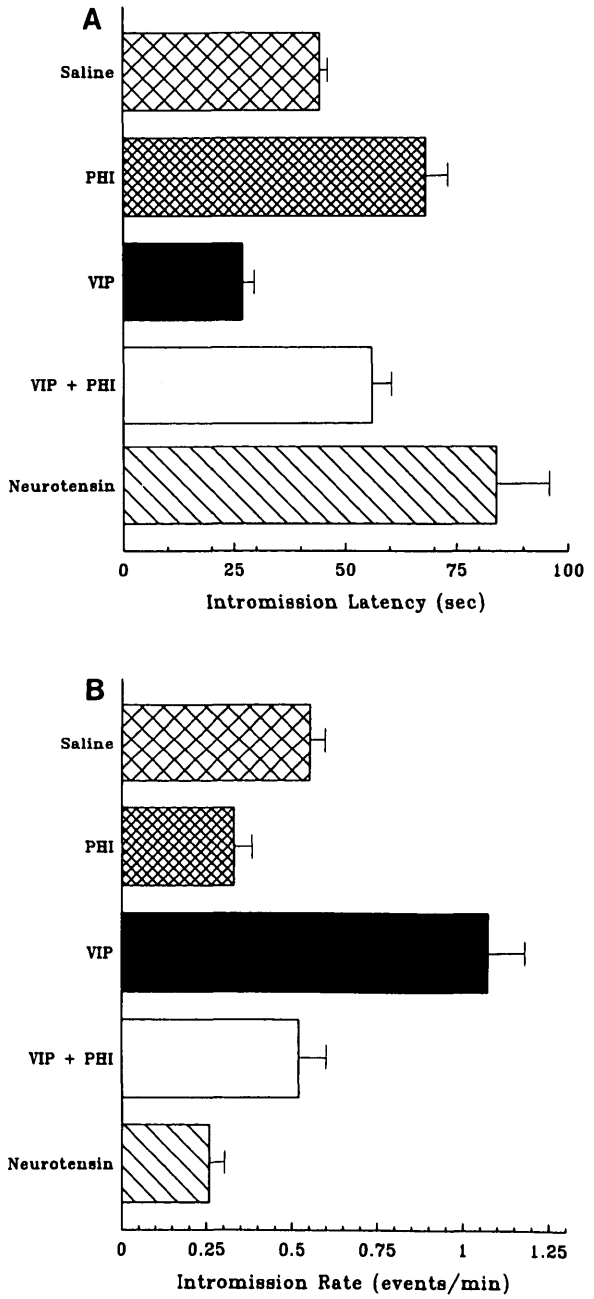

FIG. 3. PHI inhibits VIP-stimulated sexual behavior. Castrated rats (as in Fig. 2) were tested. Injections were 0.25 ml saline iv. Five micrograms of each peptide were injected per animal. Nine animals were tested for each peptide; at least 24 h elapsed between tests on the same animal. A, The effects of PHI, VIP, VIP plus PHI, and neurotensin on the mean intromission latency. Experiments were performed as described in Fig. 1. VIP significantly decreased the mean intromission latency, which was prevented by PHI ($P < 0.001$). B, The mean rate of intromission (copulatory events per min), which was measured over a 15-min test period. Analysis of variance indicated again that VIP injection significantly improved sexual function ($P < 0.001$), as measured by intromission rates. Injection of PHI or neurotensin caused a decrease in sexual performances ($P < 0.001$ and $P < 0.003$, respectively, compared to the saline control).

their respective actions on target cells. These two peptides can antagonize each other, as shown above, or act in concert to increase PRL release from anterior pituitary cells (22, 23) and stimulate cAMP formation in diverse cell types (24). Similarly, neurotensin stimulates PRL release, although independently of the cAMP system (25).

We have demonstrated that VIP simulates sexual behavior in sexually inhibited rats. This effect is consistent with a receptor-mediated process which was inhibited by two peptide antagonists, an octamere (10, 26) and a novel VIP analog, both shown to inhibit VIP binding to its receptor. Although the physiological mechanism for VIP's effects on sexual activity is not addressed in the present studies, previous work has indicated that VIP has marked effects on vasodilatation which could contribute to the above responses. As VIP is involved not only in sexual behavior but in other basic functions such as bronchodilation, digesting, neural function, and endocrine homeostasis (10, 12, 19), the availability of highly potent analogs may prove to be of clinical significance and offer a route to study possible multiple receptor sites.

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