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Effects of Somatostatin and Vasoactive Intestinal Peptide on the Contractile Activity of Vas Deferens

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Elucidation of the role and mechanisms of action of neuropeptides in male reproductive tract is under intensive investigation lately. We examined and compared the ability of somatostatin and vasoactive intestinal peptide (VIP) to modulate contractile activity in guinea-pig vas deferens. The contractions elicited by electrical field stimulation (0.3 to 0.5 ms, 8 Hz, 40 V) consisted of first rapid "twitch" component followed by a second slower "tonic" component. Somatostatin (0.1 μM to 1 μM) and VIP (0.001 μM to 0.1 μM) concentration-dependently decreased the amplitude of both components, VIP being more powerful inhibitory agent than somatostatin by 43 and 22 times for the first and second component, respectively. Somatostatin (1 μM) and VIP (0.01 μM) also significantly inhibited the adenosine triphosphate (100 μM)- induced contractile responses. However, exogenous application of 0.01 μM VIP enhanced noradrenaline (10 μM)- evoked contractions of guinea-pig vas deferens, while somatostatin (1 μM) had no effect.

Key words: neuropeptides, somatostain, vasoactive intestinal peptide, vas deferens.

Introduction

The mammalian vas deferens is innervated by postganglionic nerve fibres originating primarily from neurons in pelvic ganglia and, to a lesser extent, from neurons in the inferior mesenteric ganglion and sympathetic chain ganglia as well as by sensory nerve fibres arising from dorsal root ganglia. Three major populations of nerve terminals innervating the organ can be distinguished: (1) noradrenergic fibres; (2) cholinergic fibres containing vasoactive intestinal peptide (VIP), neuropeptide Y, nitric oxide synthase, and somatostatin; and (3) non-noradrenergic, presumably sensory fibres, containing calcitonin gene-related peptide and/or substance P [5].

Intensive investigations are carried out lately in order to elucidate the role and mechanisms of action of neuropeptides in male reproductive tract. Both VIP and somatostatin belong to so called group of "brain-gut" neuropeptides. Vasoactive intestinal peptide seems to be involved in penile erection in men. Dysfunction of the VIP nerves can lead to erectile failure and VIP in combination with phentolamine can be successfully used as self-injection therapy of impotence [10]. VIP-related peptides have been shown to suppress the electrically evoked twitches in the isolated rat vas deferens, while VIP was without significant effect [4, 8]. Somatostatin acts both locally and distally

through one or a combination of four different mechanisms: i) paracrine – via diffusion; ii) endocrine – via blood flow; iii) transmission – like the classical neurotransmitters; iv) modulation – modulates the synthesis and release of neurotransmitters. We have demonstrated that exogenous application of somatostatin exerted inhibition on the electrically-induced, neurogenically mediated contractions in both rat and guinea-pig vas deferens. Some of these results have been already published in an abstract form [12].

In the present study we compare the action of somatostatin and VIP on the contractile activity in isolated prostatic portions of guinea-pig vas deferens. The aim of these experiments was to elucidate:

1. The effects of somatostatin and VIP on both components of the electrical field stimulation (EFS)- induced contractile responses of vas deferens.

2. The effects of somatostatin and VIP on the adenosine triphosphate (ATP)- or noradrenaline (NA)- induced contractions of vas deferens.

Material and Methods

Male guinea-pigs (300–400 g) were stunned and sacrificed by a cervical dislocation. The prostatic part of vas deferens was dissected from adjacent tissue, leaving the surrounding tissue sheath. The lumen was washed with Krebs buffer. Segment of 12 mm was then cut out from the prostatic end of every vas deferens, and was suspended under 1 g tension in 10 ml organ bath containing Tyrode solution in mM: Na^+ 149; K^+ 2.9; Ca^{2+} 1.8; Mg^{2+} 0.5; Cl^- 194; HCO_3^- 11.9; $H_2PO_4^-$ 0.4, glucose 5.5; pH 7.4; aerated with 95% O_2 and 5% CO_2 at 37°C. There was a 60-min equilibration period before any measurements were made. The mechanical activity was recorded by means of mechanoelectrical force transducers (Swema SG 4-90) and a direct recorder (Linseis-6).

Electrical field stimulation (duration of 0.3 to 0.5 ms, frequency of 8 Hz, supramaximal voltage of 40 V, 80 pulses) was achieved by platinum wire electrodes placed 10 mm apart, current being supplied from a Grass S-44 stimulator. This method allows to mimic neurogenically-induced excitation in the peripheral tissues, and also to examine the effects and mechanisms of action of neuropeptides and other substances. Square pulses were applied before and after somatostatin or VIP administration. Guanethidine (1 μM, contact time 30 min) was administered to block the sympathetic neurons, while tetrodotoxin (1 μM, contact time 15 min) was applied to block the neuronal input into the smooth muscle cells in order to differentiate neurogenic from direct myogenic response. Somatostatin or VIP were added in a single concentration to the organ baths before stimulation of the contractile activity by exogenous ATP or noradrenaline. The changes in the amplitude of the contractions were measured in grams. Percentage difference in the contractile effects were determined before somatostatin or VIP administration (taken to be 100%) and in the presence of the two neuropeptides. Experimental data were expressed as mean value ± S.E.M. The data were assessed for statistical differences using Student's *t*-test, and considered significant if *P* value was lest than 0.05.

Results

Effects of somatostatin and VIP on electrically-induced contractions

No spontaneous changes in the mechanical activity have been recorded in the prostatic part isolated from guinea-pig vas deferens, but all preparations responded to EFS with a first transient phasic "twitch" component followed by a second, slower "tonic" compo-

nent (Fig. 1). Amplitude of phasic component in the controls was usually higher than the amplitude of tonic component by 39.46 ± 9.67% ($P < 0.001$, $n = 23$).

The addition of somatostatin (0.01 to 1 μM) or VIP (0.001 to 0.1 μM) reduced both components (Fig. 2 – A and B) of neurogenically-evoked contractions in a concentration-dependent manner. The effect of both neuropeptides was more pronounced on the first (twitch) component as compared to the second (tonic) component. As it is shown in Fig. 2 – A, the first component of the response was reduced by 45.12 ± 8.91 % ($P < 0.001$, $n = 6$) at VIP 0.01 μM, and by 32.65 ± 10.36 % ($P < 0.01$, $n = 5$) at somatostatin 1 μM. The second component was reduced by 23.39 ± 8.62 % ($P < 0.05$, $n = 6$) at VIP 0.01 μM, and by 25.15 ± 5.37 % ($P < 0.001$, $n = 5$) at somatostatin 1 μM (Fig. 2 – B).

VIP appeared to be more potent inhibitory agent of the neurogenically-induced contractions as compared to somatostatin. As shown in Table 1, IC_{50} of VIP for the first component was only 0.05 μM as compared to IC_{50} of somatostatin equal to 2.16 μM. Respectively, for the second component IC_{50} of VIP was 22 times lower as compared to IC_{50} of somatostatin (Table 1). Inhibitory effect of VIP was more prolonged and lasted throughout the experiment (up to 40 min) without changes in parameters. Unlike VIP, the inhibitory effect of somatostatin was pronounced during the first 8 min, and the basic contractile activity was restored after 15 min.

Guanethidine or tetrodotoxin (data not shown) abolished EFS-evoked contractions, indicating that the neurogenically-induced responses in guinea-pig vas deferens are realized via noradrenergic innervation. On the other hand, both guanethidine and tetrodotoxin did not influence and even potentiated the amplitude of contractile responses of

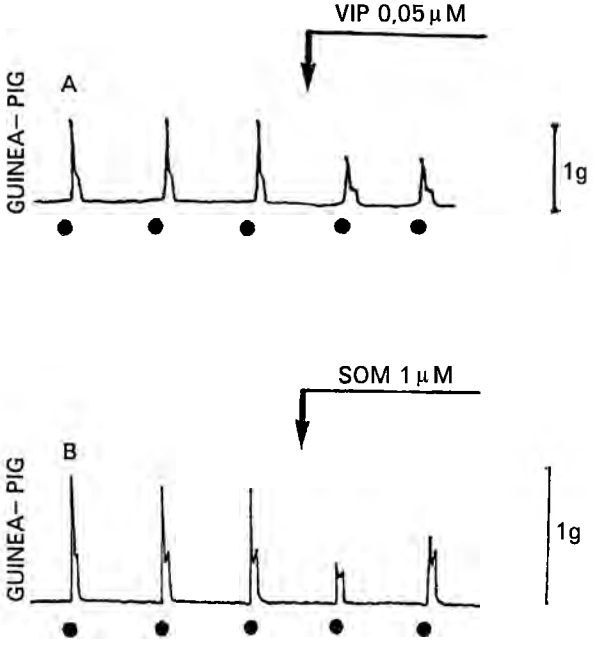

Fig. 1. Effects of VIP (A) and somatostatin (B) on the first and second component of the contractile responses elicited by electrical field stimulation (duration of 0.5 ms, frequency of 8 Hz, supramaximal voltage, 80 pulses) as indicated by (•) in guinea-pig vas deferens. Administration of VIP (0.05 μM) and somatostatin (1 μM) is indicated by arrows

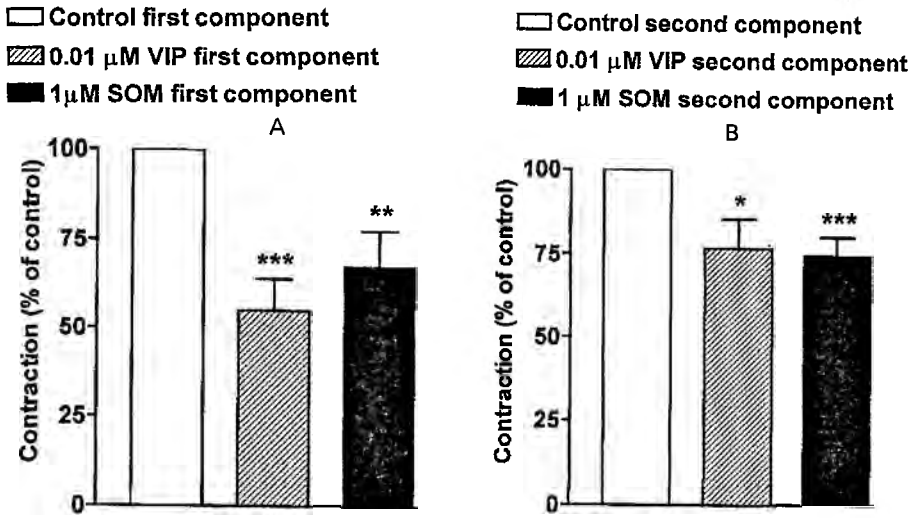

Fig. 2. Effects of VIP and somatostatin on the first (A) and second (B) component of the contractile responses elicited by electrical field stimulation in guinea-pig vas deferens. Bars represent contractile response in controls (taken to be 100%) and in the presence of VIP (0.01 μM) or somatostatin (1 μM). Each bar represents mean ± S.E.M of at least 5 experiments. Statistical analysis was performed using Student's *t*-test. Significant differences from the control group are indicated; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

T a b l e 1. Inhibition of neurogenically-evoked contractions of guinea-pig vas deferens

NEUROPEPTIDE	IC_{50} (μM) FOR FIRST COMPONENT	IC_{50} (μM) FOR SECOND COMPONENT
Vasoactive intestinal peptide	0.05	0.09
Somatostatin	2.16	2.02

vas deferens to exogenously applied ATP and NA, suggesting that myogenic mechanisms are involved in these responses.

Effects of somatostatin and VIP on ATP-induced contractile responses

Adenosine triphosphate is considered putative transmitter of the first component of neurogenically-induced contraction [5, 7]. In our experiments exogenous application of ATP exerted rapid and monophasic contractions. As it is shown in Fig. 3 – A, both somatostatin (1 μM) and VIP (0.01 μM) significantly inhibited the ATP (100 μM)-evoked contractile responses. Somatostatin reduced the ATP-induced contractions by 23.98 ± 12.19 % ($P < 0.05$, $n = 6$), while VIP inhibited them by 20.99 ± 10.19 % ($P < 0.05$, $n = 14$).

Effects of somatostatin and VIP on noradrenaline-induced contractile responses

Noradrenaline is considered putative transmitter of the second component of the neurogenic contraction [13]. In our experiments exogenous application of NA markedly increased and prolonged the tonic response. As it is shown in Fig. 3 – B, somatostatin (1

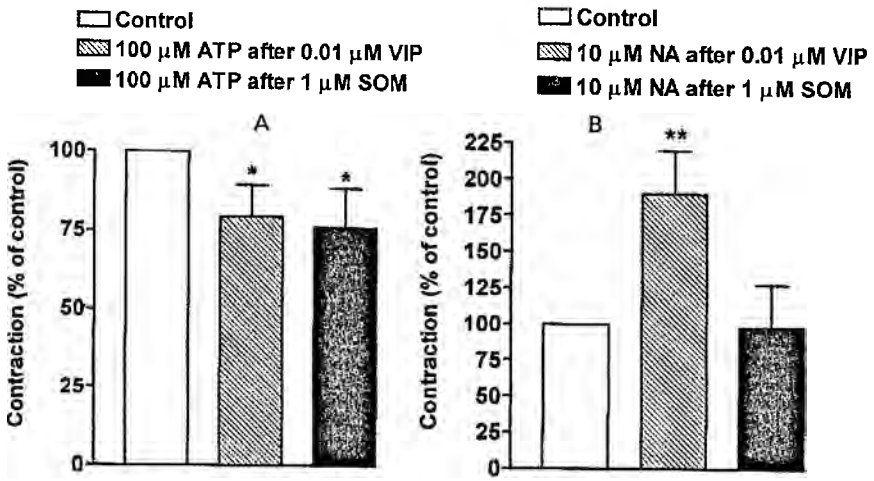

Fig. 3. Effects of VIP (0.01 μM) and somatostatin (1 μM) on the contractile responses of guinea-pig vas deferens exerted by exogenously applied ATP (100 μM – A) or NA (10 μM – B). Contractile response induced by exogenous application of ATP or NA is taken to be 100%. Each bar represents mean ± S.E.M of 6 to 16 experiments. Statistical analysis was performed using Student's *t*-test. Significant differences from the control group are indicated; $*P < 0.05$, $**P < 0.01$.

μM) failed to influence NA (10 μM)-induced contractile responses of guinea-pig vas deferens, while VIP (0.01 μM) enhanced them by 89.86 ± 30.08 % ($P < 0.01$, $n = 16$).

Discussion

In the present study we demonstrated the ability of somatostatin and VIP to modulate neurotransmission in guinea-pig vas deferens. The present results are in agreement with the data of K a s t i n and co-workers [6], that somatostatin and VIP could decrease the neurogenically-evoked contractile responses with different potency. However, inhibitory effect of both neuropeptides has been more pronounced on the first component of EFS-induced contractile responses, VIP being 43 times more powerful than somatostatin.

There is evidence for a modulatory action of somatostatin and VIP on sympathetic transmitter release in different smooth muscle organs and animals [9, 11, 3] realized probably by different prejunctional receptors. However, in the literature there is no evidence for existence of specific prejunctional receptors for somatostatin or VIP in guinea-pig vas deferens, but there are enough data for existence of other prejunctional receptors that affect neurotransmitter release in this tissue in various species [14, 1]. It has been shown that inhibitory effects of somatostatin on chemical neurotransmition elicited by EFS in rat vas deferens is indirect, partly mediated via prejunctional α_2 adrenoceptors, and is due to the released noradrenaline [15]. In this study somatostatin and VIP decreased the ATP-evoked contraction in guinea-pig vas deferens. On the other hand, VIP increased the NA-evoked contractions while somatostatin did not influence these contractions. The different action of the two neuropeptides on contractions elicited by exogenous ATP and NA could be possibly explained with involvement of different kind of pre- and post-junctional receptors [16]. However, further investigations are needed to specify which receptors underlie different modulatory action of somatostatin and VIP on vas deferens contractile activity.

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