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Short communication

Non-synergistic relaxant effects of vasoactive intestinal polypeptide and SIN-1 in human isolated cavernous artery and corpus cavernosum

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Abstract

Since vasoactive intestinal peptide (VIP) and nitric oxide (NO) are considered to be non-adrenergic, non-cholinergic (NANC) inhibitory mediators in human penile erectile tissue, the goal of this study was to discover possible synergistic effects of exogeneous VIP and the NO donor 3-morpholino-sydnonimine (SIN-1) in human isolated cavernous arteries and cavernosal smooth muscle. In contrast to VIP, SIN-1 elicited complete and reproducible relaxant actions. Combined administration of VIP and SIN-1 revealed non-synergistic, independent relaxant effects in both investigated tissues. The results do not favour a combined administration of VIP and SIN-1 as a new therapeutic approach in the treatment of erectile dysfunction.

Keywords: Nitric oxide (NO); SIN-1 (3-morpholino-sydnonimine); VIP (vasoactive intestinal polypeptide); Cavernous artery; Smooth muscle, cavernosal; (Human)

1. Introduction

Non-adrenergic, non-cholinergic (NANC) neurotransmitters are believed to play an essential role in the relaxant mechanisms of human penile erectile tissue leading to tumescence (for a review see Andersson, 1993). Several reports suggest that the predominant NANC mediator, eliciting relaxation of the human cavernosal smooth muscle, is nitric oxide (NO) (Holmquist et al., 1991; Pickard et al., 1991). Besides its release by nerves, NO can also be synthetized and set free by cavernosal endothelial cells (Azadzoi et al., 1992; Kimoto et al., 1990). The putative importance of NO as a mediator of penile erection is further confirmed by encouraging clinical results after intracavernous injection of the NO donor 3-morpholino-sydnonimine (SIN-1) in patients suffering from erectile dysfunction (Stief et al., 1992).

A second candidate as NANC neurotransmitter in penile erectile tissue is vasoactive intestinal peptide (VIP), which has been discovered in nerves of human corpora cavernosa (Polak et al., 1981). Exogenous VIP evoked relaxant effects in isolated preparations of human corpus cavernosum tissue and human cavernous artery (Hedlund and Andersson, 1985; Pickard et al., 1993). However, Roy et al. (1990) saw weak clinical results when VIP was injected intracavernosally in impotent men, and in vitro studies (Pickard et al., 1993) seem to exclude VIP as the major inhibitory neurotransmitter in human erectile tissue. Since both NO and VIP are generated and colocalized in human cavernosal nerve fibres and since both transmitters act through different, but complementary intracellular effector pathways (Said, 1992), the aim of this study was to elucidate whether both relaxant compounds exert synergistic effects in human erectile tissue. This would explain minor effects of VIP in vivo, when administered alone, and it would suggest combined administration of NO donors and VIP as an interesting therapeutic approach. Thus, we examined the pharmacological interactions of SIN-1 and VIP on human isolated trabecular smooth muscle and cavernous artery.

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2. Materials and methods

Cavernosal tissue was obtained during operation of six patients with erectile dysfunction (veno-occlusive dysfunction; two patients suffered from diabetes mellitus) and transported to the laboratory in chilled buffer solution. The procedure was in accordance with the guidelines of the local ethics committee, patients having given informed consent. Cavernous arteries or proximal parts of helicine arteries (diameter of vessels ranged between 300 and 900 μm) were dissected out and cleaned of connective tissue. Arterial segments (length 2 ± 0.5 mm) were mounted on a myograph. A passive diameter-tension curve was constructed; the internal diameter and the corresponding pressure was calculated according to the Laplace equation. The experiments were performed at a diameter corresponding to 90% of the value reached at a calculated transluminal pressure of 100 mm Hg. After equilibration (60 min), during which two successive contractions to K^+ 40 mM were obtained, a cumulative concentration-response curve to phenylephrine was constructed. Thereafter, a submaximal contraction to phenylephrine 3×10^{-6} M (15 min) was established, which led to stable contractile responses. Then, concentration-response curves to SIN-1 or, in separate preparations, to VIP were constructed following contraction with phenylephrine 3×10^{-6} M. For comparison, concentration-response curves of SIN-1 or VIP in the presence of a threshold concentration of the respective other vasodilator were preceded and followed by control curves to SIN-1 or VIP alone, each separated by periods of about 30 min. In all experiments, we had to choose the cumulative concentration-response approach, since the construction of three successive single dose-response curves in one preparation would require at least 18 successive contractions to phenylephrine, each lasting more than 10 min. This procedure would not be possible due to the limited viability of an isolated preparation. Endothelial integrity was checked by application of carbachol 10^{-5} M to preconstricted vessels, and the induced relaxation exceeded 60% in each preparation (mean relaxation: $80 \pm 4\%$).

In another experimental setup, cavernosal tissue measuring approximately $3 \times 3 \times 5$ mm was suspended isometrically in 20 ml organ baths, applying a passive tension of 5–10 mN. During the equilibration period, two contractions to K^+ 70 mM were elicited. Then, a concentration-response curve to phenylephrine was constructed as well as the concentration-response curves to SIN-1 using the same protocol as descibed above.

Organ baths contained a Tyrode buffer with the following composition (mM): Na^+ 147, K^+ 2.7, Ca^{2+} 1.8, Mg^{2+} 1.1, Cl^- 145.5, HCO_3^- 12, $H_2PO_4^-$ 0.21, glucose 5.5, EGTA 0.01 and ascorbic acid 5.7. The buffer was bubbled with a mixture of 95% O_2 and 5% CO_2 to maintain a pH of 7.4, the temperature was kept at 37 ± 0.5° C. All substances were dissolved in water, stock solutions (10^{-2} M) were prepared daily. Data are expressed as percent of tone increase induced by the second contraction to K^+ (effects of phenylephrine) or induced by the preceding contractile effect of phenylephrine (effects of SIN-1 and VIP). Data represent the means $\pm$ S.E.M., E_{max} and EC_{50} values were established by non-linear regression using the Hill equation. Statistical comparisons were done using Student's t-test for paired or unpaired data.

3. Results

Phenylephrine evoked strong contractions both in cavernous artery ($pEC_{50} = 6.25 \pm 0.11$, $E_{max} = 137 \pm 8\%$ of tone increase induced by K^+ 40 mM, $n = 17$ preparations from five patients) and cavernosal smooth

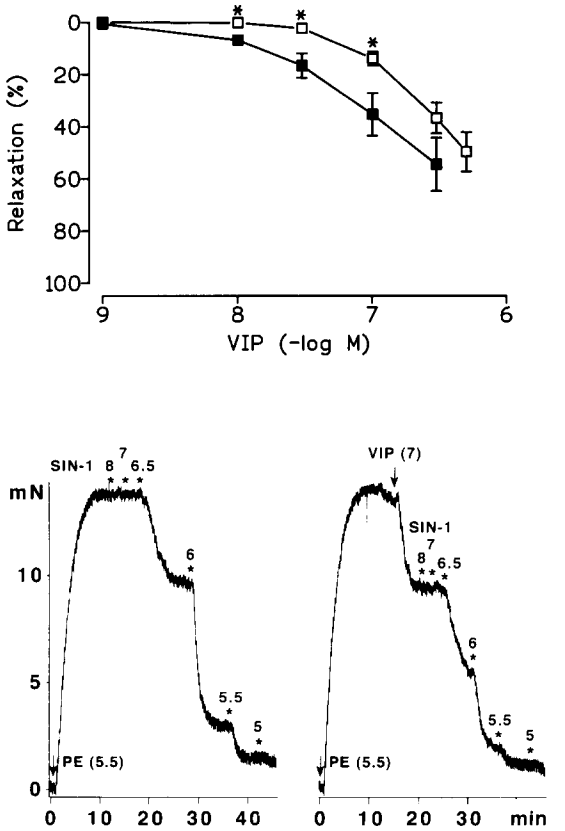

Fig. 1. Upper panel: First (filled squares) and second (unfilled squares) control concentration-response curve to VIP in cavernous arteries preconstricted by phenylephrine 3×10^{-6} M ($n = 7$ preparations from four patients). Data represent means $\pm$ S.E.M. $^*P < 0.05$. Lower panel: Tracings of a concentration-response curve to SIN-1 in a cavernous artery contracted by phenylephrine 3×10^{-6} M. Control condition and presence of VIP 10^{-7} M. Concentrations are presented as $-\log$ M.

muscle ($pEC_{50} = 6.10 \pm 0.05$, $E_{max} = 223 \pm 17\%$ of tone increase induced by K^+ 70 mM, $n = 17$ preparations from five patients). In both tissues, 3×10^{-6} M exerted submaximal and well reproducible effects with stable contractile plateaus.

The concentration-dependent relaxant responses to VIP in cavernous arteries decreased after repeated exposures to the peptide (Fig. 1). Furthermore, the relaxations were not stable but decreased after several minutes. Due to the development of tachyphylaxis, successive comparisons of the concentration-response curves to VIP under control conditions and in the presence of SIN-1 seemed not justified. Thus, combined administration could only be investigated applying SIN-1 in the presence of VIP, but not using VIP in the presence of SIN-1, since the control effects of VIP were not reproducible.

In contrast, SIN-1 induced complete and reproducible relaxations in both preparations, cavernous arteries being more sensitive ($pEC_{50} = 6.21 \pm 0.05$, $n = 8$ preparations from four patients) than cavernosal smooth muscle ($pEC_{50} = 5.42 \pm 0.15$, $n = 10$ preparations from four patients). There were no differences between the first (pEC_{50} as above) and second ($pEC_{50} = 6.17 \pm 0.09$, and 5.45 ± 0.12, respectively) control concentration-response curves to SIN-1 in both tissue types. Using the mean relaxation caused by VIP 10^{-7} M during concentration-response experiments in cavernous arteries (Fig. 1) and the mean pEC_{50} values and slopes from the first concentration-response curves to SIN-1 (Fig. 2, control responses to VIP and SIN-1 have been obtained in different preparations), a concentration-response curve to SIN-1 in the presence of VIP 10^{-7} M was calculated, assuming an independent, non-synergistic action of both vasodilators (Ariëns et al., 1956; Pöch, 1993). The measured concentration-response curve resembled the simulated curve, exhibiting a slight, but non-significant shift to the left (control and predicted curve: $pEC_{50} = 6.21 \pm 0.05$, measured pEC_{50} in the presence of VIP 10^{-7} M: 6.29 ± 0.06, Fig. 2). After normalization (i.e., after scaling by the relaxant effect of VIP 10^{-7} M) the concentration-response curve to SIN-1 in the presence of VIP equaled the control curve (normalized curve not shown). Thus, VIP and SIN-1 exert non-synergistic, independent relaxant effects. Comparable results were obtained in cavernosal smooth muscle, demonstrating the inability of VIP 10^{-7} M (which induced a relaxation of $18 \pm 4\%$) to significantly shift the concentration-response curve to SIN-1 to the left (control: $pEC_{50} = 5.42 \pm 0.15$, presence of VIP 10^{-7} M: $pEC_{50} = 5.55 \pm 0.14$, $n = 10$ preparations from four patients, Fig. 2).

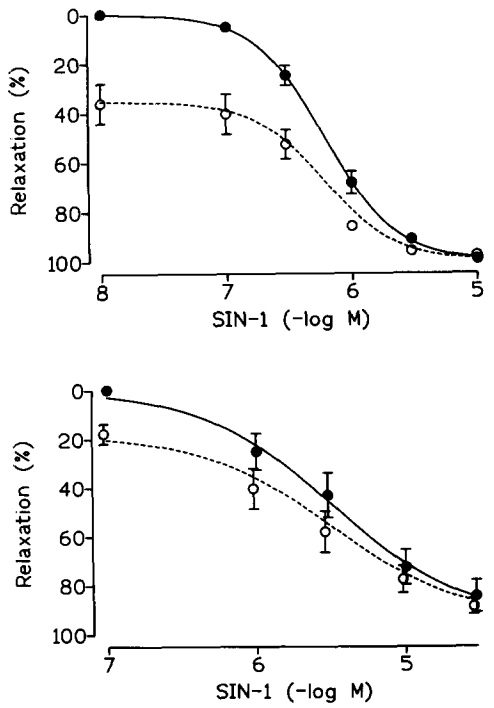

Fig. 2. Upper panel: Control concentration-response curve to SIN-1 in cavernous arteries ($n = 8$ preparations from four patients) precontracted by phenylephrine 3×10^{-6} M (filled circles, solid curve). Predicted concentration-response curve (dashed curve without symbols) to SIN-1 in the presence of VIP 10^{-7} M using the mean relaxant effect of VIP (10^{-7} M) in Fig. 1 and the mean pEC_{50} values and slopes of the control curve to SIN-1 in Fig. 2. Measured concentration-response data to SIN-1 in the presence of VIP (10^{-7} M) (unfilled circles without curve), the points are not significantly different from the predicted curve ($P > 0.05$). Lower panel: Same experiment and presentation as above in cavernosal smooth muscle ($n = 10$ preparations from four patients) precontracted by phenylephrine 3×10^{-6} M. As in cavernous arteries, in the presence of VIP 10^{-7} M there is no significant difference ($P < 0.05$) between the measured data (unfilled circles) and the predicted curve (dashed curve).

4. Discussion

The penile flaccid state is caused mainly by noradrenaline-induced contraction of the cavernous and helicine arteries as well as the sinusoidal smooth muscle cells, all of which are supplied by adrenergic nerves (Andersson, 1993). Recent pharmacological comparisons using different adrenergic agonists and antagonists suggested predominance of α_2 receptors in the human cavernous artery and of α_1 receptors in the cavernosal smooth muscle (Andersson, 1993; Hedlund and Andersson, 1985). However, in the present study the α_1 selective agonist phenylephrine elicited equally potent and well reproducible contractions in arteries and trabecular tissue, and it thus represents a useful tool for preconstriction of both tissues. Preconstriction by application of an adrenoceptor agonist seems more reasonable and physiological concerning the goal of our study than contraction by electrical field stimulation, since electrical field stimulation would also lead

to release of NO from nerves (Holmquist et al., 1991; Pickard et al., 1991). These unknown amounts of released NO would influence the combined effects of SIN-1 and VIP in an unpredictable way.

As demonstrated earlier (Hedlund and Andersson, 1985; Pickard et al., 1993), VIP exhibited an unusually low relaxant potency in human erectile tissue when compared with other smooth muscular preparations (Makhlouf, 1987). The observed tachyphylaxis in response to VIP might be one explanation for this, and for the discouraging clinical reports on intracavernous injections of VIP in impotent men (Roy et al., 1990). Another possibility could be inactivation of the peptide within the tissue. In any case, weak responses to exogeneous VIP in vivo or in vitro do not necessarily exclude the peptide as an important physiological relaxant transmitter. On the other hand, the pronounced effects and the lack of tachyphylaxis after administration of SIN-1 in cavernous arteries and cavernosal smooth muscle are in line with the good clinical results with NO donors (Stief et al., 1992).

The objective of this study was to elucidate whether the putative penile neurotransmitter VIP (Andersson, 1993; Polak et al., 1981) not only evokes direct dilatory effects but also partly elicits its relaxant actions by potentiating the responses to the most essential NANC mediator, NO (Pickard et al., 1991, 1993). However, our results demonstrate the inability of threshold concentrations of VIP to potentiate the effects of SIN-1 either in cavernous arteries and in trabecular smooth muscle from impotent men. Our data rather reveal independent, non-synergistic effects of both compounds.

In summary, the present study demonstrates that VIP and SIN-1 apparently exert non-synergistic, independent relaxant effects in human cavernous artery and cavernosal smooth muscle. One has to keep in mind that these results have been obtained in preparations from impotent patients. Thus, the independence of action of VIP and NO cannot be extrapolated, regarding either physiological release of these mediators, or healthy tissue. However, this study strongly argues against combined therapeutic administration of SIN-1 and VIP as a new approach for erectile dysfunctions. In addition, it should prompt further study of the mechanism of the weak and fading character of peptidergic relaxation.

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