

A Fatty Neuropeptide

Potential Drug for Noninvasive Impotence Treatment in a Rat Model

Illana Gozes and Mati Fridkin*

Department of Chemical Pathology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; and *Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Abstract

Vasoactive intestinal peptide (VIP), a key penile neurotransmitter, induces erection after local injection in man. To augment the therapeutic potential of VIP for impotence treatment and circumvent difficulties of direct penile injections, a strategy was designed to increase peptide hydrophobicity. This was accomplished by the synthesis of a conjugate of VIP and stearic acid (stearyl-VIP). Upon penile topical application, stearyl-VIP, in contrast to native VIP, significantly increased sexual function as measured by copulatory activity and penile reflexes (erections) in testosterone-treated, castrated rats. In addition, stearyl-VIP penetrated the body in amounts severalfold greater than VIP. Pharmacokinetic studies demonstrated 10-fold higher penile concentrations of stearyl-VIP, as compared with that measured in the blood 15 min after application, with a gradual decrease thereafter. The peak of incorporation into peripheral tissues that was observed 30 min after administration was 1,000-fold less than that found in the penile tissue. Tissue extraction and chromatographic analysis revealed that stearyl-VIP remained essentially intact for ≥ 15 min and was cleared after 1 h. Thus, topically administered stearyl-VIP had increased bioavailability in comparison with VIP without apparent toxicity, suggesting significant therapeutic potential. (*J. Clin. Invest.* 1992. 90:810–814.) Key words: lipophilic peptides • penile reflexes • sexual behavior • vasoactive intestinal peptide

Introduction

The mechanism of penile erection requires functions of the endocrine, the nervous, and the vascular systems (1); failures in these systems may result in impotence. Current procedures of impotence treatment are surgery and implantation (2), as well as injection of smooth muscle relaxants (3). Since vasoactive intestinal peptide (VIP)¹ is important in erection formation (4–10), this neuropeptide is a rationale candidate for impotence therapy. VIP fulfills two important criteria for a neurotransmitter mediating penile erection: (a) it is present in nerve fibers innervating cavernous smooth muscle and blood vessels;

(b) it is elevated during erection (4, 5). VIP penile levels have been shown to be decreased in impotent men (6, 7). Moreover, penile injections of exogenous VIP induce erection in man (4) and systemic injections stimulate sexual behavior in rats with experimentally reduced masculine potential (8). Topical administration of such a drug is clearly advantageous as compared with direct penile injection. However, transdermal delivery of peptides is hindered because of their hydrophilic nature. Thus, the conjugation of lipophilic moieties to peptides might allow these poorly absorbed compounds to cross physiological barriers such as the skin.

As a model for the evaluation of sexual responses induced by VIP and VIP analogues, castrated male rats treated with testosterone have been employed (8, 11). After castration, the animals lose sexual activity in a time-dependent manner (11, 12) and eventually all sexual activities are blocked. In this paradigm, testosterone replacement treatment partially attenuates the blockade of sexual activity. In those rats, systemic injection of VIP increased sexual behavior (measured as the rate of copulation events). The VIP-induced increase was receptor mediated, as it was specifically inhibited by a potent VIP-receptor antagonist (8).

We now show that local topical application of a lipophilic derivative of VIP significantly stimulated sexual behavior (copulation rate) and penile reflexes (erections) in castrated rats. Similar stimulation was not achieved in the presence of topically administered native VIP. Using radioactively labeled VIP and VIP-derivatives, we have been able to assess the extent and time course of tissue penetration. The lipophilic VIP-derivative was incorporated into the body in amounts severalfold greater than VIP when identical doses of the two peptides were applied. Moreover, stearyl-VIP was cleared within 1 h. Thus, the newly described VIP analogue may be developed as a drug for impotence treatment in man. Moreover, similar lipophilic peptides may have clinical and basic significance enabling transdermal therapy.

Methods

Synthesis of fatty neuropeptides: stearyl-VIP. The VIP peptide chain was assembled manually, according to the general principles of the solid phase methodology (13), on a methyl benzhydryl amine resin (Nova, Laufelfingen, Switzerland) employing optimum side-chain protections. N-alpha amino acids functions were protected throughout the synthesis by t-butyloxycarbonyl group. Side chains were protected as follows: Ser, Asp, Thr with benzyl; Lys with 2-chlorobenzoyloxycarbonyl, and Arg with p-toluenesulfonyl. The synthesis was initiated by coupling the Boc-Asn to the methyl benzhydryl amine resin using as reagents N,N'-dicyclohexylcarbodiimide (Aldrich Chem. Co., Milwaukee, WI) and hydroxybenzotriazole (Nova). A molar ratio of 4:1 of Boc-amino acid 1-hydroxybenzotriazole ester and alpha amino group of growing peptide chain, respectively, was employed for all couplings. Each coupling cycle was repeated twice (the second cycle with one-half the amount of the added amino acid) to ensure complete reaction. As

Address reprint requests to Dr. Gozes, Department of Chemical Pathology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel.

Received for publication 23 July 1991 and in revised form 14 April 1992.

1. Abbreviations used in this paper: stearyl-VIP, stearic acid + vasoactive intestinal peptide; VIP, vasoactive intestinal peptide.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/92/09/0810/05 \$2.00

Volume 90, September 1992, 810–814

the last coupling step, the long chain aliphatic carboxylic acid, i.e., stearic acid, was combined, through formation of an amide bond to the NH_2 -terminus of the peptide chain. This was accomplished while the peptide was still bound to the polymeric support, using a combination of N,N' -dicyclohexylcarbodiimide and N -hydroxybenzotriazole, normally employed for peptide chain extension. The reaction proceeded for 45 min and was repeated twice. Deblocking of protecting groups and cleavage of the peptide from the resin was achieved by the anhydrous hydrofluoric acid techniques. The product was purified by gel chromatography on a Sephadex G-25 column, followed by reverse phase HPLC on a silica C_8 column (RP-8, 7 μm , 250 \times 25 mm; E. Merck, Darmstadt, FRG). The correct composition and sequence of the parent peptide (VIP) was verified using, respectively, amino acid and sequence analyses.

Animal model for impotence. Rats with reduced sexual potential due to castration were employed. Male rats (250–300 g, \sim 3 mo old) 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. Male rats were castrated and given partial testosterone replacement (4 μg /100 g of body wt) in the form of daily injection for 14–21 consecutive days (the duration of the experiment) (12, 13). Experiments were conducted on the second to third week after surgery.

Sexual behavior experiments. Before testing, each male was put in a separate cage for a period of \geq 1 h. A sexually receptive female was introduced to each male, and mounts plus intromissions were recorded over a period of 15 min. These experiments were performed for stearyl-VIP, VIP, and control vehicle after either intravenous injection, in the tail vein (5–10 μg drug in 5–10 μl saline/animal) or penile topical application [20 μl saline solution of 1 $\mu\text{g}/\text{ml}$ experimental drug mixed with 10 μl DMSO and directly applied on the sex organ].

Direct evaluation of penile reflexes (erections). We have concentrated on the final stages of the erection process (reddening of the penis accompanied by its distension and extension leading to complete erection) (14). For testing, each male was restrained in a supine position with the anterior portion of its body enclosed in a loosely fitting cylinder (7 cm diam). After a belt was secured around the torso, the glans penis was extruded from its sheath and gently held perpendicular to the abdomen by a thin metal applicator positioned at the posterior of the penis. The legs of the male were held by the observer and this position was maintained throughout the test period. The duration of the session was 45 min and the erection rate was measured.

Measurements of peptide permeation after topical application. VIP and stearyl-VIP were labeled with ^{125}I using the chloramine T based method (15). Each animal received, by topical application, 1.6×10^6 cpm of the labeled analogue, in a mixture containing 26 μl DMSO and 10 μl saline. To avoid oral absorbance, the animal mouth was sealed. At indicated times following topical application, animals were sacrificed and duplicate tissue samples were removed, weighed, and counted in a gamma counter. Tissues measured were lungs, heart, kidneys, liver, intestine, and penile tissue.

For monitoring systemic dispersion of the topically applied labeled stearyl-VIP, animals were cannulated in the carotid artery (under anesthesia with 10% chloral hydrate). Blood aliquots (0.5 ml) were removed 15, 30, and 60 min after application into heparin-containing test tubes. Samples were either counted for radioactivity or immediately applied onto Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA), followed by HPLC for peptide purification (see below).

Analytical peptide fractionation. The HPLC column used was a C_{18} column [Lichrospher RP-18 (5 μm) column (250 \times 10 mm); E. Merck]. The column was eluted by a linear gradient established between 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in 75% acetonitrile in water. The flow rate was 1 ml/min. Stearyl-VIP was eluted after 39.8 min at 55.5% of acetonitrile.

Results

Stearyl-VIP increases sexual activities in rats. Two experimental systems were employed to assess the biological activity of

stearyl-VIP. These were measurements of sexual behavior and evaluation of penile reflexes. For the behavior experiment, a sexually receptive female was introduced to each castrated testosterone-treated male rat and the rate of copulatory events (mounts plus intromissions/min) was recorded over a 15-min test period. These experiments were performed initially for stearyl-VIP and VIP after intravenous injection into the tail vein. Results show an almost twofold increase in copulation rates with VIP and a 50% increase with stearyl-VIP. Similarly, when the mean interval (latency) between events (mounts or intromissions) was calculated, an almost twofold greater interval was apparent when saline was injected as compared with either VIP or stearyl-VIP injection (Fig. 1). Thus, the lipophi-

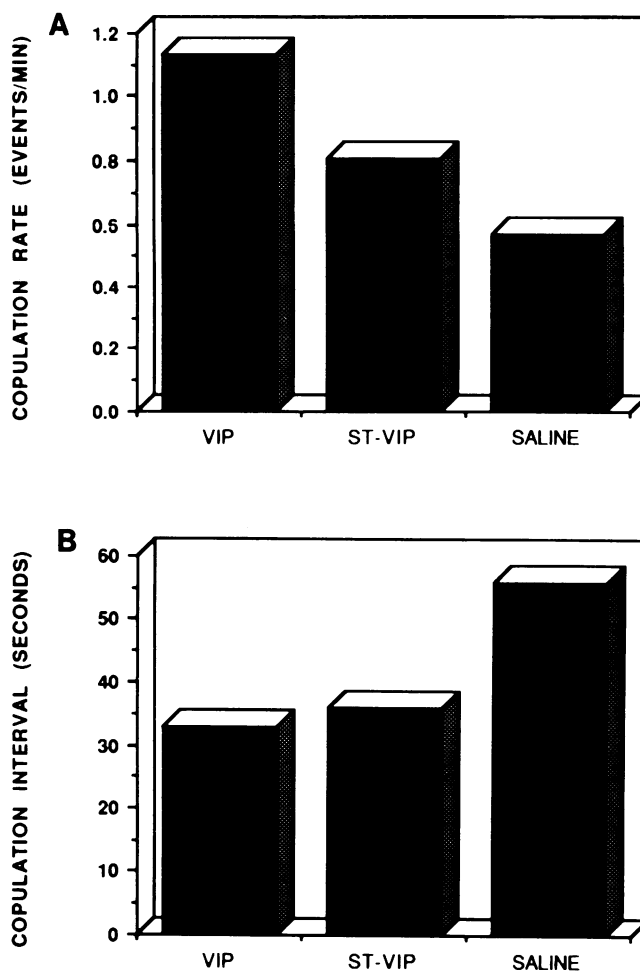


Figure 1. VIP stimulates sexual activity in castrated rats. A sexually receptive female was introduced to each male rat, and mounts plus intromissions were recorded over a period of 15 min. These experiments were performed for stearyl-VIP (ST-VIP) and VIP after intravenous injection (5–10 μg drug in 5–10 μl saline/animal). As a control we used saline injection. Six animals were used for each variable and the rate of copulatory events (mounts plus intromissions) per minute was recorded (A). The mean interval between copulatory events was recorded as well (B). SEM was 10–15%. An analysis of variance with a Student-Keuls multiple comparison of means test indicated that there was a significant increase in the rate of copulatory events ($P < 0.03$) and a significant decrease in the mean latency between copulatory events ($P < 0.008$) after either VIP or stearyl-VIP injection.

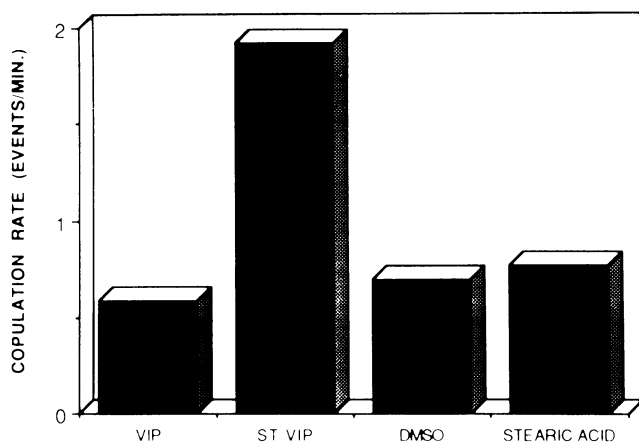


Figure 2. Topically applied stearyl-VIP stimulates sexual activity in castrated rats. The same experimental paradigm outlined in Fig. 1 was adapted, except that peptides were topically applied as follows: 20 μ l saline solution of 1 μ g/ml stearyl-VIP (or VIP or stearic acid) were mixed with 10 μ l dimethyl sulfoxide (DMSO) and were directly applied on the sex organ. We used 11 animals as vehicle (diluted DMSO)-applied controls and 11 animals for stearyl-VIP; 6 animals were used for VIP testing and 6 animals for stearic acid testing. The copulation rate was recorded. SEM was $\leq 10\%$. Analysis of variance indicated that there was a significant increase in the rate of copulation after stearyl-VIP application ($P < 0.01$) as compared with all the other treatments.

lic VIP derivative retained the ability to stimulate sexual behavior. The challenge was to discern if the lipophilic analogue can influence sexual activity when applied topically. Fig. 2 depicts a comparison in the rate of copulation after topical application of VIP or stearyl-VIP and as controls stearic acid and the vehicle of application (DMSO). In these experiments, only stearyl-VIP had a significant stimulatory activity, increasing the rate of copulation by two- to threefold as compared with DMSO, stearic acid, or VIP.

For direct evaluation of penile reflexes (erection), castrated rats treated with testosterone were employed. Results show a two- to threefold increase in the rate of erections when stearyl-VIP was topically applied as compared with vehicle application or tactile control (Fig. 3).

Pharmacokinetics. For measurements of penetration of 125 I-labeled VIP derivatives through the skin and their organ distribution, we compared topically applied stearyl-VIP to VIP. At least a fourfold better permeation of stearyl-VIP was observed 30 min after application (Fig. 4 A). Time course experiments indicated that the radioactive stearyl-VIP dissipated 1 h after drug administration (Fig. 4 B). To appraise the degree of penetration of stearyl-VIP to the penile tissue, in comparison with parenteral release, radioactive stearyl-VIP in the penile tissue (calculated per gram tissue, as percentage of total input) was compared to radioactive VIP in the blood circulation (calculated per 10 ml blood volume, as percentage of total input). Results shown in Fig. 4 C demonstrate that 5% of the applied material was found in the penile tissue 15 min after application, which decreased about twofold 0.5 h after application and fourfold 1 h after administration. In contrast, blood levels of VIP (15 min after application) were 0.6% of the applied material. Furthermore, by weight/volume comparison, there is an ~ 80 -fold enrichment in penile tissue versus blood.

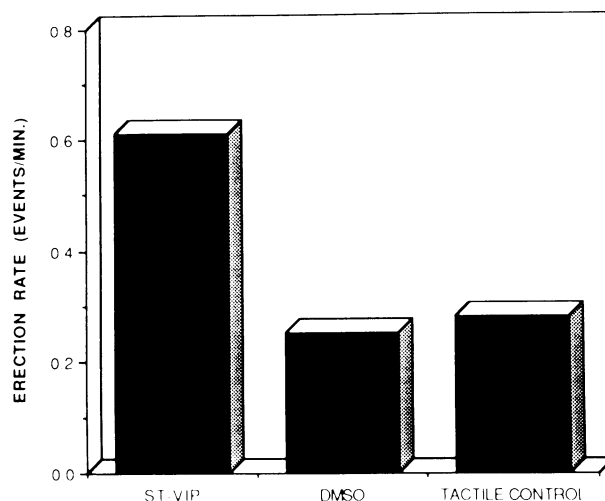


Figure 3. Stimulation of erection by topical application of stearyl-VIP. For testing, each male was restrained in a supine position with the anterior portion of its body enclosed in a loosely fitting cylinder (7 cm diam). The duration of the session was 45 min and the erection rate was measured. The stearyl-VIP (ST-VIP) was prepared and applied as in Fig. 2 and as a control we applied a diluted DMSO as in Fig. 2. SEM was $< 10\%$. We saw a significant stimulation of the erection rate when stearyl-VIP was used as compared with vehicle-treated control ($P < 0.05$, $n = 8$ for each group). No significant differences were obtained between vehicle-treated and tactile controls.

Finally, extraction of sample tissues, 15 min after application revealed an intact stearyl-VIP molecule, as demonstrated by HPLC, (Fig. 4 D). In contrast, 30 min after topical administration, the major radioactive peak observed in the intestinal tissue (Fig. 4 B) was degraded to metabolites as measured by HPLC fractionation (i.e., $< 0.1\%$ of the radioactivity observed in the intestinal tissue was associated with intact stearyl-VIP). Similarly, 1 h after topical administration, residual radioactivity found in the circulation was attributed to breakdown products.

Discussion

We have described above a novel strategy, "lipophilization," for modification of VIP, which leads to an enhancement of its passage through skin tissues, while preserving native function as neurotransmitter in penile erection.

In the nonerect penis, the trabecular smooth muscle cells of the cavernous body are continuously activated due to noradrenaline action; during tumescence the muscle cells relax and blood enters the sinuses. Although the cavernous body contains cholinergic fibers and muscarinic cholinergic receptors, atropine does not block erection formation, and therefore human penile erection can not be explained by the classic adrenergic and cholinergic concepts (16, 17). Hence, it was suggested that local release of a major neuropeptide, VIP, innervating cavernous smooth muscle and blood vessels, increases arterial flow, decreases venous flow, and induces sinusoidal relaxation (18). VIP's actions, coupled to withdrawal of the alpha-adrenoreceptor-mediated tonic supply of noradrenaline to the penis and activation of beta-adrenoreceptors, lead to penile erection in man (19). Intracavernosal (penile) injections of papaverine induce erection in man, although these

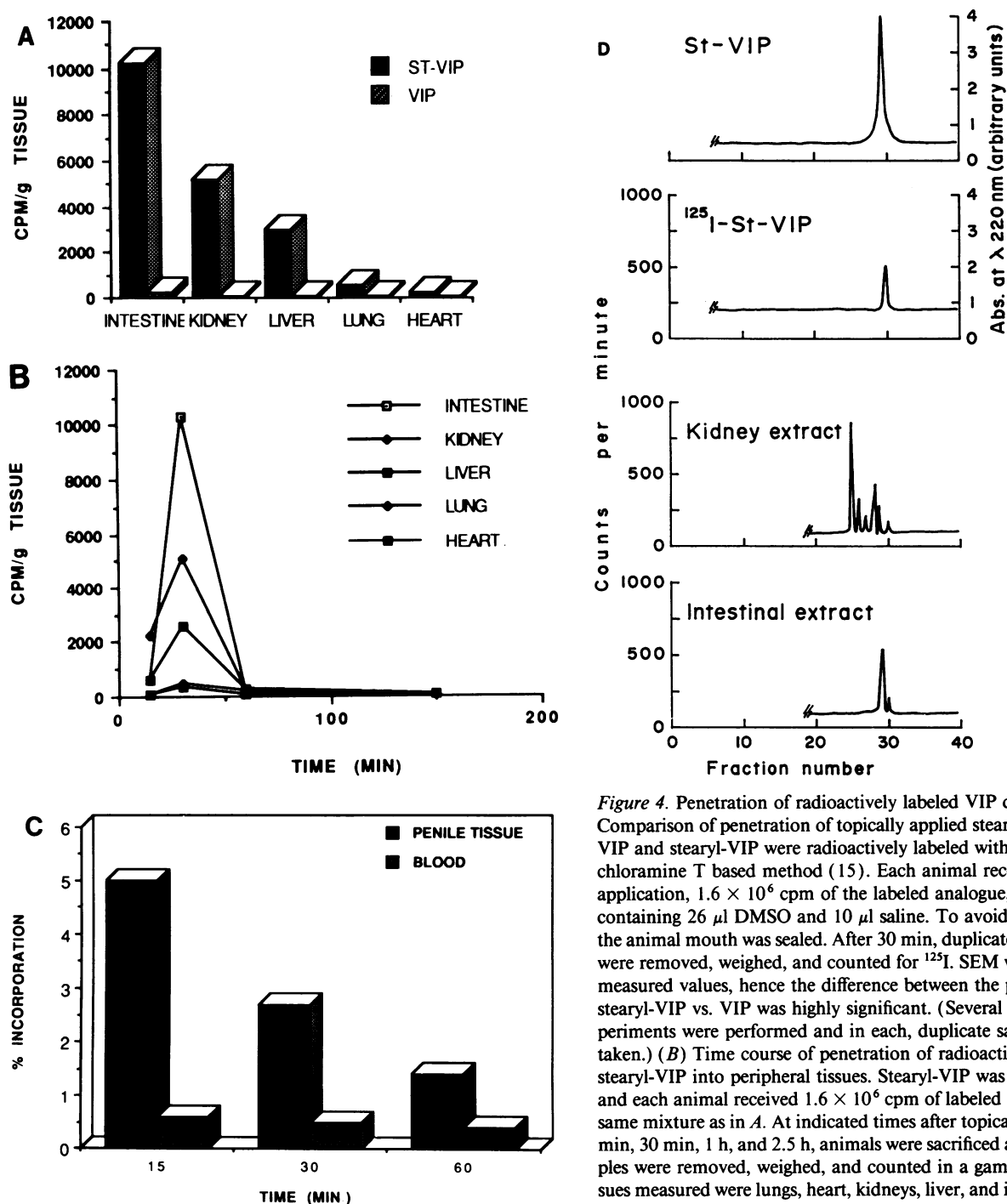


Figure 4. Penetration of radioactively labeled VIP derivatives. (A) Comparison of penetration of topically applied stearyl-VIP and VIP. VIP and stearyl-VIP were radioactively labeled with ^{125}I using the chloramine T based method (15). Each animal received, by topical application, 1.6×10^6 cpm of the labeled analogue, in a mixture containing $26 \mu\text{l}$ DMSO and $10 \mu\text{l}$ saline. To avoid oral absorbance the animal mouth was sealed. After 30 min, duplicate tissue samples were removed, weighed, and counted for ^{125}I . SEM was 10% of the measured values, hence the difference between the penetration of stearyl-VIP vs. VIP was highly significant. (Several independent experiments were performed and in each, duplicate samples were taken.) (B) Time course of penetration of radioactively labeled stearyl-VIP into peripheral tissues. Stearyl-VIP was labeled as in A and each animal received 1.6×10^6 cpm of labeled material in the same mixture as in A. At indicated times after topical application: 15 min, 30 min, 1 h, and 2.5 h, animals were sacrificed and tissue samples were removed, weighed, and counted in a gamma counter. Tissues measured were lungs, heart, kidneys, liver, and intestine. (The experiment was repeated three times and duplicate samples were removed each time; a representative experiment is depicted in the figure.) (C) Pharmacokinetics of VIP distributions in penile tissue as compared with systemic dispersion. Experiments were performed as above. A summary of three experiments is depicted. For measurements of systemic distribution of stearyl-VIP, animals were cannulated at the carotid artery (under anesthesia), blood aliquots were removed 15, 30, and 60 min after application and counted for radioactivity. Results are either given as incorporation per gram penile-tissue as a percentage of the total radioactivity applied or as incorporation per 10 ml blood as a percentage of the total radioactivity applied. (SEM was $\sim 15\%$.) (D) Extraction of stearyl-VIP from kidney and intestine after topical application. 15 min after topical application, tissues were removed, homogenized in PBS, centrifuged ($10,000 g$) and the postmitochondrial supernatant was injected onto HPLC C_{18} column. There was a good correlation between the migration of the native molecule and the tissue-extracted molecule.

removed each time; a representative experiment is depicted in the figure.) (C) Pharmacokinetics of VIP distributions in penile tissue as compared with systemic dispersion. Experiments were performed as above. A summary of three experiments is depicted. For measurements of systemic distribution of stearyl-VIP, animals were cannulated at the carotid artery (under anesthesia), blood aliquots were removed 15, 30, and 60 min after application and counted for radioactivity. Results are either given as incorporation per gram penile-tissue as a percentage of the total radioactivity applied or as incorporation per 10 ml blood as a percentage of the total radioactivity applied. (SEM was $\sim 15\%$.) (D) Extraction of stearyl-VIP from kidney and intestine after topical application. 15 min after topical application, tissues were removed, homogenized in PBS, centrifuged ($10,000 g$) and the postmitochondrial supernatant was injected onto HPLC C_{18} column. There was a good correlation between the migration of the native molecule and the tissue-extracted molecule.

erections may be prolonged (3). Intracavernosal injections of VIP also induce erection in man (4) and VIP's half-life in the blood has been shown to be less than 1 min (20). Our results have shown tissue stability of the fatty VIP-derivative for ≥ 15

min after topical application. Pharmacokinetics experiments indicated rapid incorporation into the penile tissue, followed by systemic release, incorporation into tissues (which measured up to 1,000-fold less radioactivity than that found in the

penile tissue), and decomposition as well as clearance of the lipophilic peptide 1 h later.

Preliminary toxicological studies in rats, employing single subcutaneous injections (to the area of the sex organ) of lipophilic VIP-derivatives (at doses used in this study for topical application) showed no evidence of side effects or toxicity over a 2-wk period. Side effects were monitored by daily inspections of body weight, food consumption, general appearance and behavior, incidence of diarrhea, and viability. In addition, at doses 1,000-fold greater than the amounts required to elicit a biological response, only 17% of the animals tested showed symptoms of transient diarrhea. Oral application of the drug at the same doses did not elicit any apparent toxic response (unpublished results). Furthermore, native VIP administered by local penile injection is now in clinical trials for treating sexual dysfunction (21, 22). The amounts of VIP that are used are 200–400 pmol/injection/patient, which are $\sim 1 \mu\text{g}$ per man, and it has been shown that these doses do not cause any local or systemic side effects in man (22). These doses are similar to the effective skin penetrating doses of stearyl-VIP (as extrapolated from the above study). The advantages of developing the stearyl-VIP analogue for impotence treatment in man are multiple: (a) stearyl-VIP has a preferred route of application in comparison to VIP; (b) stearyl-VIP's action may be prolonged (see half-life discussion above); (c) the bioavailability of stearyl-VIP is increased as compared with VIP; and (d) there is no apparent toxicity associated with stearyl-VIP.

Findings reported above may be of direct relevance even beyond the specific case of VIP and impotence. Firstly, VIP is a multifunctional neuropeptide (9) and hence other therapeutic aspects of this peptide should be investigated. Moreover, the concept of fatty peptides may be of general implication to other biologically active molecules of major clinical significance (23). Success in topical application of these compounds, e.g., as ointments, will result in circumvention of the need for oral or parenteral administration, which are markedly hampered by extensive proteolysis and hepatic first-pass elimination.

Acknowledgments

We thank Drs. Eyal Meltzer (Weizmann Institute) and Ben Sachs (University of Connecticut) for the help with the behavioral experiments and Dr. Sergei Zhukovsky (Tel Aviv University) for the help with measurements of systemic drug distribution. We thank Sara Rubinraut (Weizmann Institute) and Ayelet Reshef (Tel Aviv University) for excellent technical assistance.

This study was supported by Fujimoto Pharmaceutical Corp., Osaka, Japan.

References

1. Benson, G. S. 1981. Mechanism of penile erection. *Invest. Urol.* 19:65–69.
2. Light, J. K., and F. B. Scott. 1981. *Urology*. 17:341–343.
3. Virag, R. 1982. Intracavernous injection of papaverine for erectile failure. *Lancet*. 2:938.
4. Ottesen, B., G. Wagener, R. Virag, and J. Fahrenkrug. 1984. Penile erection: possible role for vasoactive intestinal polypeptide as a neurotransmitter. *Br. Med. J.* 288:9–11.
5. Dixon, A. F., K. M. Kendrick, M. A. Blank, and S. R. Bloom. 1984. Effects of tactile and electrical stimuli upon the release of vasoactive intestinal polypeptide in the mammalian penis. *J. Endocrinol.* 100:249–252.
6. Gu, J., J. M. Polak, M. Lazarides, R. Morgan, J. P. Pryor, P. J. Marangos, M. A. Blank, and S. R. Bloom. 1984. Decrease of vasoactive intestinal peptide in the penises from impotent men. *Lancet*. 2:315–317.
7. Crowe, R., J. Lincoln, P. F. Blacklay, J. P. Pryor, J. S. P. Lumley, and G. Burnstock. 1983. Vasoactive intestinal polypeptide-like immunoreactive nerves in diabetic penis. A comparison between streptozotocin-treated rats and man. *Diabetes*. 32:1075–1077.
8. Gozes, I., E. Meltzer, S. Rubinraut, D. E. Brenneman, and M. Fridkin. 1989. Vasoactive intestinal peptide potentiates sexual behavior: inhibition by novel antagonist. *Endocrinology*. 125:2945–2949.
9. Gozes, I., and D. E. Brenneman. 1989. VIP: molecular biology and neurobiological function. *Mol. Neurobiol.* 3:201–236.
10. Andersson, P.-O., S. R. Bloom, and S. Mellander. 1984. Hemodynamics of pelvic nerve induced penile erection in the dog possible mediation by vasoactive intestinal peptide. *J. Physiol. (Lond.)*. 350:209–224.
11. Bradshaw, W. G., M. J. Baum, and C. C. Awh. 1981. Attenuation by 5 alpha reductase of the activational effect of testosterone on penile erection in castrated male rats. *Endocrinology*. 109:1047–1051.
12. Beach, F. A., and A. M. Holtz. 1946. Mating behavior in male rats castrated at various ages and injected with androgen. *J. Exp. Zool.* 101:91–142.
13. Barany, G., and R. B. Merrifield. 1980. Solid phase peptide synthesis. In *The Peptides: Analysis, Synthesis, Biology*. Volume 2. E. Gross and J. Meienhofer, editors. Academic Press, Inc., New York. 1–284.
14. Sachs, B. D. 1983. Potency and fertility: hormonal and mechanical causes and effects of penile actions in rats. In *Hormones and Behavior in Higher Vertebrates*. J. Balthazart, E. Prove, and R. Gilles, editors. Springer-Verlag, Berlin and Heidelberg. 86–110.
15. Werner, H., Y. Koch, M. Fridkin, J. Fahrenkrug, and I. Gozes. 1985. Detection of elevated amounts of VIP in milk. *Biochem. Biophys. Res. Commun.* 133:228–232.
16. Hedlund, H., and K.-E., Andersson. 1985. Effects of some peptides on isolated human penile erectile tissue and cavernous artery. *Acta Physiol. Scand.* 124:413–419.
17. Schmalbruch, H., and G. Wagner. 1989. Vasoactive intestinal polypeptide (VIP)- and neuropeptide Y (NPY)-containing fibers in the penile cavernous tissue of green monkeys (*cercopithecus aethiops*). *Cell Tissue Res.* 256:529–541.
18. Juenemann, K.-P., T. F. Lue, J.-A. Luo, S. A. Jadallah, L. L. Nunes, and E. A. Tanagho. 1987. The role of vasoactive intestinal polypeptide as a neurotransmitter in canine penile erection: a combined in vivo and immunohistochemical study. *J. Urol.* 138:871–877.
19. Adaikan, P. G., S. R. Kottagoda, and S. S. Rratnam. 1986. Is vasoactive intestinal polypeptide the principal transmitter involved in human penile erection? *J. Urol.* 135:638–640.
20. Said, S. I., editor. 1982. Vasoactive intestinal peptide. In *Advances in Peptide Hormone Research Series*. Raven Press, New York. 1–512.
21. (Editorial). *Genet. Eng. News*. May 1991:9.
22. Roy, J. B., R. L. Petrone, and S. I. Said. 1990. A clinical trial of intracavernous vasoactive intestinal peptide to induce penile erection. *J. Urol.* 143:302–304.
23. Snyder, S. H. 1986. Virtuoso design of drugs. *Nature (Lond.)*. 323:292–293.