Pharmacology Biochemistry & Behavior, Vol. 9, pp. 673–676. Printed in the U.S.A.

Activity of VIP, Somatostatin and Other Peptides in the Mouse *Vas Deferens* Assay

ABBA J. KASTIN, DAVID H. COY, ANDREW V. SCHALLY AND CHESTER A. MEYERS

Veterans Administration Hospital and Tulane University School of Medicine, New Orleans, LA 70146

(Received 21 August 1978)

KASTIN, A. J., D. H. COY, A. V. SCHALLY AND C. A. MEYERS. *Activity of VIP, somatostatin and other peptides in the mouse* vas deferens *assay*. PHARMAC. BIOCHEM. BEHAV. 9(5) 673–676, 1978.—Non-opiate peptides such as vasoactive intestinal peptide (VIP) and somatostatin were tested for their effects on electrically induced contractions of the *vas deferens*. VIP ($ED_{50}=2.7\times10^{-8}$ M) and to a lesser extent somatostatin ($ED_{50}=5.2\times10^{-8}$ M) were found to be in the same general range of activity as enkephalin and the endorphins in this system. Human pancreatic polypeptide (HPP) exerted a biphasic effect, inhibiting the contractions at high concentrations but enhancing them at lower concentrations. A number of other naturally occurring brain peptides were ineffective at concentrations of 1×10^{-6} M. Several somatostatin analogues were tested and their activity on the *vas deferens* was found to more closely parallel their potency to inhibit the release of gastric acid than of growth hormone. In contrast to the brain opiates, however, the inhibitory effects of VIP, somatostatin and its analogues, and HPP were not reversed by the opiate antagonist naloxone. The results suggest that the *vas deferens* can be readily used for evaluation of analogues of VIP, somatostatin, and other peptides.

Gastrointestinal hormones Somatostatin Smooth muscle Peptides Bioassay
Vasoactive intestinal peptide *Vas deferens* Human pancreatic polypeptide

THE BIOASSAY of opiate activity involving inhibition of electrically induced contractions of the mouse *vas deferens* has been very useful in the testing of enkephalins and endorphins. We thought that it also might provide a simple method for measuring the activity of non-opiate peptides such as somatostatin. The recent demonstration by immunohistochemical techniques of the abundant presence of VIP nerves in the *vas deferens* suggested the possibility that VIP also might exert biological effects there [1]. Therefore, we examined the feasibility of using the *vas deferens* for the rapid evaluation of analogues of VIP, somatostatin, and other peptides.

METHOD

Male albino mice (25–30 g), obtained from Charles River Laboratories, were killed and the *vasa deferentia* removed and placed in a solution of Krebs Ringer's bicarbonate-glucose (KRBG). After the semina were gently expressed from the lumen, an individual *vas deferens* was suspended vertically between platinum wire electrodes in a 5 ml bath of KRBG, maintained at 37°C, through which 5% CO_2–95% O_2 was gently bubbled. The tissue was subjected to 0.5 g applied tension and allowed to equilibrate an hr before use. Electrical stimulation of the *vas deferens* was provided by a Grass S-4 stimulator delivering rectilinear pulses of 1.0 msec duration at a frequency of 0.1 Hz and 80 V. Contractions were isometrically recorded by a force displacement transducer (Grass Model FT .03) on a polygraph (Grass Model 7).

Peptides were added in a volume of 50 μl; 10 min were allowed between successive additions. The effective molar dose which inhibited the amplitude of the contractions by 50% was obtained from the resulting dose-response curves and termed the ED_{50}. At least three different tissues were used to determine the mean for each active peptide. The responsiveness of every tissue was confirmed with D-Ala^2-Met-enkephalin-NH_2. Reversal of the inhibition caused by each effective peptide was tested by pretreatment of the *vas deferens* with naloxone (Endo Laboratories) at a dose of 40 ng/ml of bath fluid.

Most of the peptides were synthesized by solid phase methods and then highly purified. A pentagastrin derivative (Peptavlon) was purchased from Ayerst Laboratories. The following abbreviations were used: VIP (vasoactive intestinal peptide), HPP (human pancreatic polypeptide), MSH (melanocyte-stimulating hormone), MIF-I (MSH-release inhibiting hormone=Pro-Leu-Gly-NH_2), DSIP (delta sleep-inducing peptide), TRH (thyrotropin releasing hormone), and LH-RH (luteinizing hormone-releasing hormone).

RESULTS

The mean concentration (±SEM) of VIP found to inhibit the electrically induced contractions of the *vas deferens* by 50% (ED_{50}) was 2.7 (±0.35)$\times10^{-8}$ M (Table 1). Greater concentrations of VIP (1×10^{-6}M, 1×10^{-7} M), although tested on fewer tissues, also inhibited the contractions. Weaker inhibition was seen with 1×10^{-9} M VIP. Addition of naloxone (40 ng/ml) immediately before addition of VIP had no effect on the inhibitory action of VIP. In contrast, the same dose of naloxone completely reversed the effects of the D-Ala^2-Met-enkephalin-NH_2 ($ED_{50}=1.5$ (±0.07)$\times10^{-9}$ M). These effects are illustrated in Fig. 1.

The actions of somatostatin were similar to those of VIP. The ED_{50} was 5.2 (±0.43)$\times10^{-8}$ M (Table 1). Greater con-

TABLE 1

MEAN (±SEM) MOLAR CONCENTRATION OF PEPTIDES INHIBITING ELECTRICALLY INDUCED CONTRACTIONS OF THE *VAS DEFERENS* BY 50% (ED_{50})

Peptide	ED_{50} (M)
VIP	$2.7\ (\pm 0.35) \times 10^{-8}$
somatostatin	$5.2\ (\pm 0.43) \times 10^{-8}$
HPP	$5.6\ (\pm 0.87) \times 10^{-7}$
D-Trp^8, D-Cys^{14}-somatostatin	$1.2\ (\pm 0.13) \times 10^{-8}$
Ala^2, D-Trp^8, D-Cys^{14}-"	$1.4\ (\pm 0.53) \times 10^{-8}$
L-5-F-Trp^8-"	$2.0\ (\pm 0.33) \times 10^{-8}$
D-Trp^8-"	$2.2\ (\pm 0.90) \times 10^{-8}$
D-Cys^{14}-"	$2.4\ (\pm 0.95) \times 10^{-8}$
Ala^2, D-Cys^{14}-"	$3.9\ (\pm 1.2\ ) \times 10^{-8}$
D-Ala^2, D-Trp^6-"	$4.2\ (\pm 1.2\ ) \times 10^{-8}$
α-MSH	$>1 \times 10^{-6}$
MIF-I	"
DSIP	"
TRH	"
LH-RH	"
pentagastrin (Peptavlon)	"
bombesin	"
neurotensin	(stimulatory)
substance P	"
HPP_{22-36}	"

centrations (1×10^{-6} M, 1×10^{-7} M) inhibited the contractions, a weaker concentration (1×10^{-9} M) had the same inhibitory tendency, and naloxone (40 ng/ml) did not prevent the inhibition. Addition of somatostatin (1×10^{-8} M and 1×10^{-9} M) failed to alter the response of the stimulated *vas deferens* to the ED_{50} dose of VIP.

HPP had some of the same effects as VIP and somatostatin in that there was an ED_{50} ($5.6\ (\pm0.87)\times10^{-7}$ M) for inhibition of the contractions of the *vas deferens* not reversed by naloxone and inhibition was still evident at a higher concentration (1×10^{-6} M). However, at 1×10^{-7} M and 1×10^{-8} M concentrations, addition of HPP resulted in stimulation of the electrically induced contractions. The 22–36 fragment of HPP increased the electrically stimulated contractions at all three doses tested (1×10^{-6} M, 1×10^{-7} M, and 1×10^{-8} M), with the strength of the stimulation decreasing as the concentration of the peptide decreased. Stimulation rather than inhibition of the electrically induced contractions were also seen with substance P and neurotensin at the dose (1×10^{-6} M) used for initial testing. Unlike the peptides mentioned above, addition of substance P and neurotensin resulted in contractions of the unstimulated *vas deferens*.

At the dose at which all peptides were initially tested, 1×10^{-6} M, no effects on the electrically induced stimulation of the *vas deferens* were observed after addition of MIF-I, DSIP, LH-RH, TRH, pentagastrin (Peptavlon), or bombesin. α-MSH caused a slight inhibition of the contractions at this dose.

Several analogues of somatostatin were tested in the same system. Since the effective dose of somatostatin was about 5×10^{-8} M, these analogues were initially tested at 1×10^{-8} M. Their mean ED_{50} ± SEM appear in Table 1 and their relative potencies in Table 2. Naloxone (40 ng/ml) did not reverse the inhibition induced by any of these somatostatin analogues.

DISCUSSION

The usefulness of the *vas deferens* assay for opiate activity has recently been emphasized. The *vas deferens* is more sensitive to the enkephalins than the guinea pig ileum [13] and is more predictable for following the relative potencies of enkephalin analogues [15]; its correlations with opiate receptor affinities are high [15]. However, the correlations between these *in vitro* tests and *in vivo* measures of analgesia are not so reliable [6], and even greater discrepancies can be seen between any of these opiate tests and behavior [9, 11, 12]. The present results suggest new uses of this assay for other peptides.

VIP was found to be very active in inhibiting electrically induced contractions of the mouse *vas deferens*. The ED_{50} of 2.7×10^{-8} M for VIP and 5.2×10^{-8} M for somatostatin were in the same broad range as those of Met-enkephalin [5] as well as α-, β-, and γ-endorphins [2]. HPP was less active in this test ($ED_{50}=5.6\times10^{-7}$ M). In contrast, many other peptides found in the brain or exerting effects there [10] were inactive in preliminary tests at the single dose of 1×10^{-6} M. These included MIF-I, DSIP, LH-RH, TRH, pentagastrin (Peptavlon), bombesin, substance P, neurotensin, and to some extent α-MSH. Substance P, HPP at concentrations $\leq1\times10^{-7}$ M, HPP fragment 22–36, and neurotensin exerted the opposite type of effect, stimulating the contractions of the *vas*

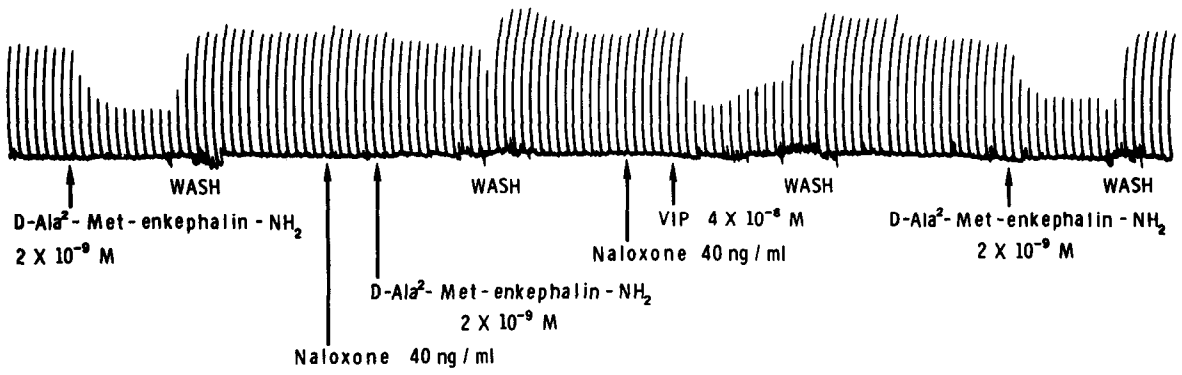

FIG. 1. Inhibition of electrically-induced contractions of mouse *vas deferens* by VIP. Naloxone blocked the effect of D-Ala^2-Met-enkephalin-NH_2 but not that of VIP.

TABLE 2

RELATIVE POTENCIES OF SOMATOSTATIN AND ITS ANALOGUES ON INHIBITION OF ELECTRICALLY STIMULATED CONTRACTIONS OF THE *VAS DEFERENS*, GASTRIC ACID SECRETION, AND GROWTH HORMONE RELEASE

Peptide	*Vas Deferens*	Gastric Acid Secretion*	Growth Hormone Release*†
somatostatin	1	1	1
D-Ala^2, D-Trp^{8}-"	1.2	3.3	10–20
Ala^2, D-Cys^{14}-"	1.3	1.9	2.3
D-Cys^{14}-"	2.2	1.6	1–3
D-Trp^{8}-"	2.4	1.6	6–8
L-5-F-Trp^{8}-"	2.6	1.4	4.8
Ala^2, D-Trp^{8}, D-Cys^{14}-"	3.7	4.3	5
D-Trp^{8}, D-Cys^{14}-"	4.3	2.9	10

*See reference 3
†See reference 14

deferens both in the basal state and after electrical stimulation. At their ED_{50}, as well as at 1×10^{-6} M, VIP, somatostatin, and HPP did not exert any effect on the resting state of the unstimulated tissue. The results illustrate another experimental condition in which only some peptides are active [10]. Further investigations of why some peptides inhibit, some stimulate, some do both, and some do neither may provide productive clues to the study of their differing functions.

The effects of the enkephalins and endorphins in the *vas deferens* bioassay can be prevented or reversed by naloxone. Addition of naloxone to the fluid bathing the *vas deferens* had no observable effect upon the ability of VIP, HPP, somatostatin, or any of the active analogues to suppress the electrically stimulated contractions. This indicates that the actions of these peptides on the *vas deferens* do not involve opiate receptors.

In addition to the inhibition of contractions found after addition of HPP at relatively greater concentrations, stimulation of the electrically induced contractions was found at lower concentrations ($\leq1\times10^{-7}$ M). This biphasic type of response was not observed with VIP and somatostatin; the 22–36 fragment of HPP stimulated but did not inhibit the contractions at concentrations $\leq1\times10^{-6}$ M. Haloperidol (5 μg/ml) slightly increased the electrically induced contractions of the *vas deferens*, but did not seem to block the inhibitory effects of VIP or somatostatin even though it has been reported to totally abolish the inhibitory actions of norepinephrine and dopamine, the presumed motor transmitters of this tissue [19]. This suggests the possibility of a different mechanism of action for the peptides on smooth muscle.

Somatostatin has been detected in cells throughout the body [7], and its actions are numerous. No cellular elements containing somatostatin have been identified in the testis or epididymis [7], but there is a brief mention in the literature that somatostatin produced an inhibition of the guinea pig ileum which was not blocked by naloxone [4]. Despite the apparent absence of somatostatin in the *vas deferens*, its release in the pancreas after VIP [8] makes it conceivable that inhibition in the *vas deferens* by VIP may simply be due to the release of endogenous somatostatin. Even though VIP was twice as active as somatostatin, the greater activity of VIP than somatostatin on the *vas deferens* is only evident when the concentrations are compared on a molar basis, as is customary in this assay. Since the molecular weight of VIP (3326) is about twice as great as that of somatostatin (1639), the absolute amounts of these two peptides required for an ED_{50} based on weight of peptide would be almost the same.

The potency of the somatostatin analogues in inhibiting the electrically induced stimulation of the *vas deferens* tended to parallel their effects in inhibiting the release of gastric acid better than in inhibiting the release of growth hormone. This method provides a new and rapid assay for assessment of analogues of somatostatin.

The excitatory actions of substance P and neurotensin on the *vas deferens* were opposite to the inhibitory actions of VIP and somatostatin. Moreover, after addition of substance P and neurotensin the contractions occurred regardless of whether or not the tissue was electrically stimulated. The effects of substance P were expected, since its stimulant actions on various smooth muscles including the *vas deferens* of the guinea pig are well known [16,20]. The stimulant effects of neurotensin on the mouse *vas deferens* would not be expected from the reported lack of effect of this peptide on the guinea pig *vas deferens*, although it can contract guinea pig ileum [17]. It is not known whether the use of tissue from different species of animals is sufficient to explain the different findings. Substance P resembles the enkephalins and endorphins in exerting similar effects on analgesia [18], but the effects on the *vas deferens* are completely different.

It remains to be established whether peptides are involved in the normal functioning of smooth muscle. With the demonstration of the presence of gastrointestinal hormones in the brain and their anticipated effects there, as well as the effects of somatostatin on the gastrointestinal system, the *vas deferens* assay may help in the evaluation of these peptides.

ACKNOWLEDGEMENTS

The authors appreciate the excellent technical assistance of Barbara Kuzemchak-Barry. This study was supported in part by funds from the Medical Research Service of the Veterans Administration, NIDA (01806), NIAMD (18376), and Mrs. Louise Dunagan Kramer.

REFERENCES

1. Alm, P., J. Alumets, R. Hakanson and F. Sundler. Peptidergic (vasoactive intestinal peptide) nerves in the genito-urinary tract. *Neuroscience* **2:** 751–754, 1977.
2. Britton, D. R., R. Fertel, D. H. Coy and A. J. Kastin. The effect of enkephalin and endorphin analogs on receptors in the mouse *vas deferens*. *Biochem. Pharmac.*, in press.
3. Brown, M. P., D. H. Coy, A. Gomez-Pan, B. H. Hirst, M. Hunter, C. Meyers, J. D. Reed, A. V. Schally and B. Shaw. Structure-activity relationships of eighteen somatostatin analogues on gastric secretion. *J. Physiol.* **277:** 1–14, 1978.
4. Cox, B. M., K. E. Opheim, H. Teschemacher and A. Goldstein. A peptide-like substance from pituitary that acts like morphine. 2. Purification and properties. *Life Sci.* **16:** 1777–1782, 1975.
5. Coy, D. H., A. J. Kastin, A. V. Schally, O. Morin, N. G. Caron, F. Labrie, J. M. Walker, R. Fertel, G. G. Berntson and C. A. Sandman. Synthesis and opioid activities of stereoisomers and other D-amino acid analogs of methionine-enkephalin. *Biochem. Biophys. Res. Commun.* **73:** 632–638, 1976.
6. Coy, D. H., A. J. Kastin, M. J. Walker, R. F. McGivern and C. A. Sandman. Increased analgesic activities of a fluorinated and a dimeric analogue of [D-Ala-2]-methionine enkephalinamide. *Biochem. Biophys. Res. Commun.* **83:** 977–983, 1978.
7. Hokfelt, T., S. Efendic, C. Hellerstrom, O. Johansson, R. Luft and A. Arimura. Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A_1-cells of the pancreatic islets and to the hypothalamus. *Acta endocr.* Suppl. 200 **80:** 1–41, 1975.
8. Ipp, E., R. E. Dobbs and R. H. Unger. Vasoactive intestinal peptide stimulates pancreatic somatostatin release. *FEBS Lett.* **90:** 76–78, 1978.
9. Kastin, A. J., D. H. Coy, R. D. Olson, J. Panksepp, A. V. Schally and C. A. Sandman. Behavioral effects of the brain opiates enkephalin-endorphin. In: *Central Nervous System Effects of Hypothalamic Hormones and Other Peptides*, edited by R. Collu. New York: Raven Press, 1978, in press.
10. Kastin, A. J., D. H. Coy, A. V. Schally and L. H. Miller. Peripheral administration of hypothalamic peptides results in CNS changes. *Pharmac. Res. Commun.* **10:** 293–312, 1978.
11. Kastin, A. J., E. L. Scollan, M. G. King, A. V. Schally and D. H. Coy. Enkephalin and a potent analog facilitate maze performance after intraperitoneal administration in rats. *Pharmac. Biochem. Behav.* **5:** 691–695, 1976.
12. Kastin, A. J., E. L. Scollan, A. V. Schally and D. H. Coy. Enkephalin and other peptides reduce "passiveness". *Pharmac. Biochem. Behav.*, in press.
13. Kosterlitz, H. W. Endogenous opioid peptides: historical aspects. In: *Centrally Acting Peptides*, edited by J. Hughes. Baltimore: University Park Press, 1978, pp. 157–178.
14. Meyers, C. A., D. H. Coy, W. Y. Huang, A. V. Schally and T. W. Redding. Highly active position eight analogues of somatostatin and separation of peptide diastereomers by partition chromatography. *Biochemistry* **17:** 2326–2331, 1978.
15. Miller, R. J., K. J. Chang and P. Cuatrecasas. Distribution and pharmacology of the enkephalins and related opiate peptides. In: *Centrally Acting Peptides*, edited by J. Hughes. Baltimore: University Park Press, 1978, pp. 195–213.
16. Otsuka, M. and T. Takahashi. Putative peptide neurotransmitters. *Ann. Rev. Pharmac. toxic.* **17:** 425–439, 1977.
17. Rokaeus, A., E. Burcher, D. Chang, K. Folkers and S. Rosell. Actions of neurotensin and (gln^4)-neurotensin on isolated tissues. *Acta Pharmac. tox.* **41:** 141–147, 1977.
18. Stewart, J. M., C. J. Getto, K. Neldner, E. B. Reeve, W. A. Krivoy and E. Zimmermann. Substance P and analgesia. *Nature* **262:** 784–785, 1976.
19. Tayo, F. M. Further evidence for dopaminoceptors in the *vas deferens*. *Br. J. Pharmac.* **59:** 511P–512P, 1977.
20. Von Euler, U. S. and P. Hedqvist. Effects of substance P on the response of guinea pig *vas deferens* to transmural nerve stimulation. *Acta physiol. scand.* **90:** 651–653, 1974.