PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 565–568 (1978)

Effects of Synthetic Vasoactive Intestinal Peptide (VIP), Secretin and Their Partial Sequences on Gastric Secretion[1] (40097)

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VIP, secretin, glucagon and GIP (Gastric Inhibitory Peptide), in this order, constitute a series of peptides characterized by increasing structural homology with GIP (1). The first pair, VIP and secretin, exhibit considerable functional homology: they share effects on fat (2), liver (3), exocrine pancreas (4), and colonic mucosa (5) which appear to be mediated by the same set of receptor sites. Glucagon and GIP share few effects with VIP and secretin; even where the effects are shared they appear to be mediated by separate sets of receptor sites or intermediary mechanisms (6, 7). All four peptides inhibit gastric acid secretion (1) but have different effects on pepsin secretion; the mechanism(s) of inhibition remain unknown. Barbezat and Grossman (8) were first to report that natural porcine VIP inhibited histamine-stimulated acid secretion in dog Heidenhain pouches.

In the present study, we show that synthetic porcine VIP inhibits pentagastrin-stimulated acid and pepsin secretion in the dog. The inhibition differs in some respects from that produced by synthetic porcine secretin. Since these results were first reported (9), Konturek *et al.* (10) have shown that natural VIP inhibits histamine-stimulated acid and pepsin secretion in the dog and Villar *et al.* (11) have shown that natural VIP inhibits food-stimulated acid as well as gastrin secretion.

Materials and methods. Gastric secretion was measured in three dogs (15–20 kg body weight) equipped with gastric fistulae. Following an overnight fast, basal secretion was collected for a period of 30 min. Pentagastrin was then infused in a dose of 0.25, 1 or 4 $\mu g\ kg^{-1}\ hr^{-1}$ for 3 hr. Sixty to seventy-five min after the start of pentagastrin infusion, synthetic porcine VIP (0.5 $\mu g\ kg^{-1}\ min^{-1}$) or synthetic porcine secretin (0.05 $\mu g\ kg^{-1}\ min^{-1}$) was infused for 7.5 min. VIP was given once and secretin twice to each dog with each dose of pentagastrin. In control studies, pentagastrin was given alone without VIP or secretin (nine experiments).

The effect of carboxyl-terminal partial sequences of VIP (VIP_{14-28}, VIP_{15-28} and VIP_{18-28}) was examined on a background of 1 $\mu g\ kg^{-1}\ hr^{-1}$ of pentagastrin. The three fragments were infused sequentially for 7.5 min at 45-min intervals one hour after the start of pentagastrin infusion (three experiments). The dose of each was 1.8 $\mu g\ kg^{-1}\ min^{-1}$ corresponding to about 8 times the molar dose of VIP used in this study.

The effect of carboxyl-terminal partial sequences of secretin was also examined on a background of 1 $\mu g\ kg^{-1}\ hr^{-1}$ of pentagastrin. All secretin fragments were given once per session one hour after the start of pentagastrin infusion. $Secretin_{5-27}$ was given twice to each dog as a prompt intravenous injection of 5 $\mu g\ kg^{-1}$. A substituted analogue of $secretin_{5-27}$ in which the glutamic acid in position 9 is substituted by glutamine (9-Gln-$secretin_{5-27}$) was infused twice in each dog in a dose of 0.5 $\mu g\ kg^{-1}\ min^{-1}$ for 7.5 min. Finally, 9-Gln-$secretin_{5-27}$ was infused twice in each dog in a dose of 0.5 $\mu g\ kg^{-1}\ min^{-1}$ in combination with secretin 0.05 $\mu g\ kg^{-1}\ min^{-1}$ for 7.5 minutes. The doses of secretin fragments were about 12 times the molar dose of secretin used in this study.

Secretion was collected at 15-minute intervals throughout the experiments. Acid concentration was measured by electrometric titration to pH 7.5 and pepsin was measured by the method of Hunt (12) and expressed in mg of pepsin standard.

Synthetic porcine VIP and partial sequences of VIP and secretin were kind gifts of Dr. Miklos Bodanszky, Department of

[1] Supported by NIH Grant No. AM-15564 and HL-14187.

0037-9727/78/1574-0565$01.00/0

Chemistry, Case Western Reserve University. Synthetic porcine secretin was a kind gift of Dr. Miguel Ondetti, Squibb Institute for Medical Research, New Jersey.

Results. Effect of synthetic VIP and partial sequences of VIP. Infusion of synthetic VIP in a dose of 0.5 $\mu g\ kg^{-1}\ min^{-1}$ for 7.5 minutes produced a prompt inhibition of acid secretion which reached a nadir in the first 30 min (Fig. 1). The extent of inhibition was determined by comparing the rate of acid secretion in the 30-min periods before and after the start of infusion of VIP (Fig. 2). Inhibition at the lowest dose of pentagastrin (57 ± 7%) was significantly greater than inhibition at the highest dose (23 ± 7%) ($P < 0.01$). Dose-response analysis (response versus response/dose) indicated that inhibition was of the competitive type: the maximal acid responses for the period before (15.6 ± 0.8 meq/30 min) and after VIP (13.8 ± 2.2 meq/30 min) were not significantly different but the D_{50} for pentagastrin increased twofold from 0.47 ± 0.05 to 1.02 ± 0.22 $\mu g\ kg^{-1}\ hr^{-1}$ ($P < 0.05$) in the presence of VIP. An apparent inhibitor dissociation constant for VIP was calculated to be 0.43 $\mu g\ kg^{-1}\ min^{-1}$.

VIP inhibited also the maximal pepsin response by 65 ± 10% ($P < 0.01$) but it had no effect on the reversed pepsin response observed at higher doses of pentagastrin (Fig. 2).

None of the carboxyl-terminal partial sequences of VIP had any detectable effect on pentagastrin-stimulated acid or pepsin secretion.

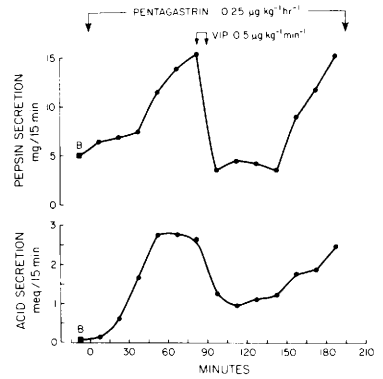

FIG. 1. Effect of infusion of 0.5 $\mu g\ kg^{-1}\ min^{-1}$ of synthetic porcine VIP for 7.5 min on pentagastrin-stimulated acid and pepsin secretion (mean of three experiments). B = basal secretion.

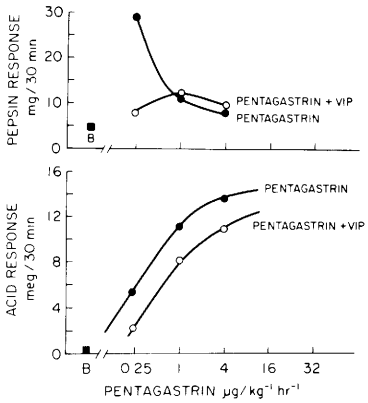

FIG. 2. Dose-response curves for the effect of pentagastrin infusion alone and in combination with VIP (0.5 $\mu g\ kg^{-1}\ min^{-1}$ for 7.5 minutes) on acid and pepsin secretion. Each point is the mean of three experiments. The highest pepsin response was attained at the lowest dose of pentagastrin (0.25 $\mu g\ kg^{-1}\ hr^{-1}$). B = basal response.

Effect of synthetic secretin and partial sequences of secretin. Infusion of synthetic secretin in a dose of 0.05 $\mu g\ kg^{-1}\ min^{-1}$ also produced a prompt inhibition of acid secretion which reached a nadir in the first 30 min (Fig. 3). Percent inhibition was the same at all dose levels of pentagastrin: 66 ± 3%, 60 ± 4% and 60 ± 8% at 0.25, 1 and 4 $\mu g\ kg^{-1}\ hr^{-1}$ of pentagastrin respectively. Accordingly, a linear relationship characteristic of inhibition of the non-competitive type prevailed between the responses before and after secretin (Fig. 4). From the slope in Fig. 4, an apparent inhibitor dissociation constant for secretin was calculated to be 0.03 $\mu g\ kg^{-1}\ min^{-1}$.

Neither secretin$_{5\text{-}27}$ given by prompt intravenous injection nor 9-Gln-secretin$_{5\text{-}27}$ given by intravenous infusion had any detectable effect on pentagastrin-stimulated acid secretion (Fig. 3). 9-Gln-secretin$_{5\text{-}27}$ infused in a dose twelve times the dose of secretin failed to block the inhibitory effect of secretin.

Discussion. VIP and secretin share a number of secretory (10), metabolic (13), motor (14) and hormone-releasing (13) or inhibiting (11) properties. These are thought to reflect similarities in the structure of the two peptides which in turn determine binding to common regulatory sites and activation of common intracellular effector (messenger) systems. Inhibition of gastric acid secretion in

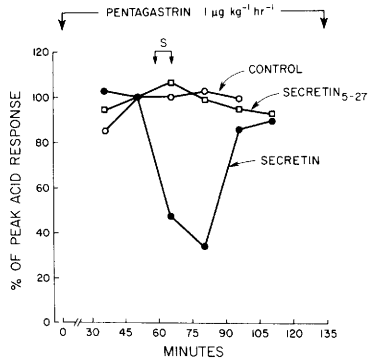

FIG. 3. Effect of infusion of 0.05 $\mu g\ kg^{-1}\ min^{-1}$ of synthetic porcine secretin (closed circles) or 0.5 $\mu g\ kg^{-1}\ min^{-1}$ of 9-Gln-secretin$_{5-27}$ (open squares) for 7.5 min on pentagastrin-stimulated acid secretion. Control secretion (open circles). Each point is the mean of six experiments. Responses expressed in percent of the response immediately preceding (i.e. 45–60 min) the infusion (S) of secretin of its analogue.

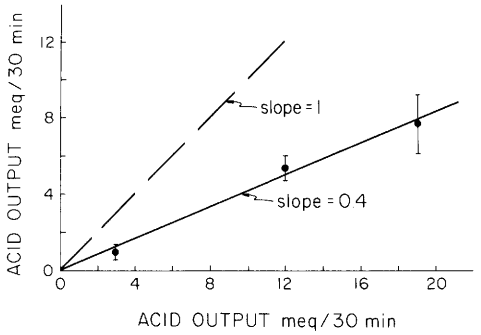

FIG. 4. Linear relation between the response to pentagastrin alone (horizontal axis) and the corresponding response to pentagastrin plus secretin (vertical axis). Each point is the mean of six experiments. A slope of 0.4 indicates 60% inhibition at all doses of pentagastrin. Slope = $1/(1 + i/K_i)$ where i = dose of secretin and K_i = inhibitor dissociation constant.

the dog appears to conform in some respects to this pattern; however, several differences emerge. First, as shown here for synthetic porcine VIP and secretin and elsewhere for the natural forms (8, 10, 11), both peptides inhibit stimulated acid secretion in the innervated stomach but only VIP inhibits histamine-stimulated acid secretion in a denervated pouch (8). Secondly, inhibition of acid secretion by VIP appears to be of the competitive type, whereas inhibition by secretin is of the noncompetitive type (15) (Fig. 4). Thirdly, inhibition by VIP extends to histamine- and pentagastrin-stimulated pepsin secretion.

In the present study, synthetic VIP appeared to be less effective than synthetic secretin, at least in terms of the doses required for an equivalent degree of inhibition. The difference is unlikely to be due to an artefact in the synthesis of either peptide. Konturek *et al.* (10) found a similar difference in potency between the natural forms of VIP and secretin. Furthermore, the inhibitor dissociation constant for synthetic VIP (0.4 $\mu g\ kg^{-1}\ min^{-1}$) was similar to that reported by Barbezat and Grossman (8) for natural VIP. Finally, the batch of synthetic VIP used in this study was tested against several target tissues and found to possess the spectrum of properties displayed by natural VIP (13). In particular, it was shown to be an effective intestinal secretory stimulant in the dog equipotent in this respect to natural porcine VIP (13).

A low potency in vivo precludes a role for VIP as an endocrine inhibitor of gastric acid secretion. The presence of VIP-secreting cells or nerves in the fundus and the antrum of the mammalian stomach (16, 17) argues in favor of a paracrine or neurocrine role for VIP in the control of gastric secretion, either directly by inhibition of gastric acid and pepsin secretion or indirectly by inhibition of antral gastrin secretion.

The decrease in gastric secretion which is a common feature of the "watery diarrhea" syndrome has been attributed to high levels of circulating VIP (18, 19). This attribution remains to be validated by simultaneous immunometric and secretory measurements and by the exclusion of other causes, such as dehydration and hypokalemia, for the decrease in gastric acid secretion.

The synthetic partial sequences of VIP and secretin used in this study are known to be active on or to retain binding to some tissues such as the exocrine pancreas (20) and the colonic mucosa (5) in some species. However, at the relatively small doses used, none showed an inhibitory effect on gastric acid secretion.

Summary. The effects of synthetic porcine VIP and secretin on pentagastrin-stimulated gastric secretion were compared in dogs equipped with gastric fistulae. VIP inhibited both acid and pepsin secretion. Percent inhi-

bition of acid secretion by VIP varied with the dose of pentagastrin: dose-response analysis showed inhibition to be of the competitive type. Percent inhibition of acid secretion by secretin did not vary with the dose of pentagastrin and was consistent with inhibition of the noncompetitive type. Carboxyl-terminal partial sequences of VIP and secretin, at approximately 10 times the molar doses of the parent peptides, had no detectable inhibitory effect.

1. Makhlouf, G. M., Gastroenterology **67,** 159 (1974).
2. Desbuquois, B., Laudat, M. H., and Laudat, P. H., Biophys. Biochem. Res. Commun. **53,** 1187 (1973).
3. Bataille, D., Freychet, P. and Rosselin, G., Endocrinology **95,** 713 (1974).
4. Gardner, J. D., Conlon, T. P., and Adams, T. D., Gastroenterology **70,** 29 (1976).
5. Waldman, D. B., Gardner, J. D., Zfass, A. M., and Makhlouf, G. M., Gastroenterology **73,** 518 (1977).
6. Brown, J. C., Dryburgh, J. R., Moccia, P., and Pederson, R. A., *in* "Gastrointestinal Hormones" (J. C. Thompson, ed.), p. 537. University of Texas Press, Austin (1975).
7. Dupre, J., Brown, J. C., Greenidge, N., McDonald, T. J., Ross, S., and Rubinstein, D., Clin. Res. **23,** 420A (1975).
8. Barbezat, G. O., and Grossman, M. I., Science **174,** 422 (1971).
9. Schorr, B. A., Said, S. I., and Makhlouf, G. M. Clin. Res. **22,** 23A (1974).
10. Konturek, J. S., Konturek, P. T., Dembinski, A., and Krol, R., *in* "Gastrointestinal Hormones" (J. C. Thompson, ed.) p. 611. University of Texas Press, Austin (1975).
11. Villar, H. V., Fender, H. R., Rayford, P. L., Ramus, N. I. and Thompson, J. C., *in* "Gastrointestinal Hormones (J. C. Thompson, ed.) p. 467. University of Texas Press, Austin (1975).
12. Hunt, J. N., Biochem. J. **42,** 104 (1948).
13. Makhlouf, G. M., and Said, S. I., *in* "Gastrointestinal Hormones" (J. C. Thompson, ed.) p. 599. University of Texas Press, Austin (1975).
14. Jaffer, S. S., Farrar, J. T., Yau, W. M., and Makhlouf, G. M., Gastroenterology **66,** 716 (1974).
15. Johnson, L. R., and Grossman, M. I., Amer. J. Physiol. **217,** 1401 (1969).
16. Bryant, M. G., Bloom, S. R., Polak, J. M., Albuquerque, R. H., Modlin, I., and Pearse, A. G. E., Lancet **1,** 991 (1976).
17. Larsson, L. I., Fahrekrug, J., Schaffalitzky de Mucadell O., Sundler, F., Hakanson, R., and Rehfeld, J. F., Proc. Nat. Acad. Sci. **73,** 3197 (1976).
18. Bloom, S. R., Polak, J. M., and Pearse, A. G. E. Lancet **2,** 14 (1973).
19. Said, S. I., and Falloona, G. R., N. Engl. J. Med., **293,** 155 (1975).
20. Robberecht, P., Conlon, T. P., and Gardner, J. D., J. Biol. Chem. **251,** 4635m (1976).

Received September 19, 1977. P.S.E.B.M. 1978, Vol. 157.