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The Effects of Various Peptides on Human Isolated Gut Muscle

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Abstract—The effects of eleven peptides of gastrointestinal origin have been studied on the contraction, relaxation and spontaneous activity of circular and longitudinal muscle strips from different regions of the human gastrointestinal tract. The effects varied with the peptides and sometimes with the region and muscle layer. There was either contraction, no effect, or relaxation and/or inhibition of an acetylcholine-induced contraction. Responses to some peptides are consistent with the possibility that they may contribute directly to the control of motility: galanin, neurotensin and substance P might be involved in contraction, and vasoactive intestinal peptide, peptide histidine isoleucine and peptide histidine methionine might be inhibitory transmitters.

Various peptides produced by the alimentary tract have pharmacological activity on gut muscles and nerves, but in many cases it is not known if these substances have roles in normal or disordered gut motility. We have examined this by studying the responses of human isolated gastrointestinal muscle to peptides. Since responses may differ amongst species, regions of the gut, and different muscle layers from the same tissue, we have determined the effects on circular and longitudinal muscle from human stomach, small intestine and colon.

Previous studies of peptides on human isolated gut muscle include substance P (Liljedahl et al 1958), bradykinin (Fishlock 1966), gastrin (Bennett et al 1967), angiotensin (Fishlock & Gunn 1970), secretin (Cameron et al 1970), glucagon (Cameron et al 1970), bombesin (Bertaccini et al 1974), cholecystokinin (see D'Amato et al 1990 for references), galanin (Maggi et al 1989), and neuromedin (porcine neuromedin U-8 (Maggi et al 1990)). Our studies mainly concern other peptides, nine of which do not seem to have been examined pharmacologically in previous studies on human gastrointestinal tissues. The main objectives were to determine the peptide effects and the extents to which responses were similar with peptides of the same family. Human tissues are difficult to obtain and to study pharmacologically, and we do not yet have sufficient data on all peptides and tissues to report on the sites and mechanisms of action.

Materials and Methods

Peptides

The following peptides (all >95% pure) were supplied as powders: synthetic calcitonin gene-related peptide (both human and rat; respectively HCGRP and RCGRP); peptide histidine methionine (PHM); peptide histidine isoleucine (PHI); porcine galanin (GAL); and vasoactive intestinal peptide (VIP) were obtained from Peninsular Laboratories (Merseyside, UK). Acetylcholine chloride (ACh), substance P, neurotensin, somatostatin, leucine enkephalin (leu-enkephalin), and methionine enkephaline (met-enkephalin) were obtained from Sigma UK. The peptides were reconstituted in 154 mM NaCl, except for one sample of GAL that was prepared in 10% human serum albumin (HSA). The appropriate solvents were used as the controls. Tissue from one patient constituted one experiment each for longitudinal and circular muscle (when there was sufficient specimen for both), regardless of the number of strips. Most peptides were studied on tissues from several patients, but this was not always possible because of the shortage of peptides. Tissues that were used soon after resection or after overnight storage at 4°C responded similarly, and the results have been pooled.

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Tissue experiments

Human tissues obtained at operation for benign or malignant gastrointestinal disease were transported to the laboratory in Krebs solution at ambient temperature, and used either immediately or after overnight storage at 4°C. Specimens were taken at least 5 cm from any macroscopically visible lesion, and were bathed in Krebs solution while the layer of mucosa/submucosa was carefully cut away and discarded. Strips about 2 cm long and 2–4 mm wide were cut through the combined muscle layer parallel to either the circular or longitudinal fibres (except for colonic longitudinal muscle where strips of taenia were used). After suspension under a load of 1 g in tissue baths (5–8 mL) in Krebs solution at 37°C, isotonic responses were measured at a magnification of 14–18 for different specimens, using transducers linked to pen recorders.

Except for some of the experiments on colon, which used a different method, consistent submaximal contractions were obtained to ACh (range 0·5–2·4 μM) in contact with the tissue for 30 s, with a cycle time that was constant in each experiment but varied from 5–8 min between tissues depending on the relaxation time after washing out the ACh. Some of the peptides (VIP, HCGRP, RCGRP, GAL, PHM and PHI) were added to the bathing fluid to give final concentrations of 1·2–120 nM, followed 2–14 min later by the standard submaximally effective concentration of ACh added while the peptide was present in the bath. Contact times (min)

were: VIP 2–11, HCGRP 2–14, RCGRP 2–6, GAL 2–14, PHM 2–9 and PHI 2–8.

The other 5 peptides (substance P, somatostatin, neurotensin, leu- and met-enkephalin) were studied only on colonic muscle in final bath concentrations of 1·5 pM–8·7 μM; responses to ACh were not obtained. Contact times were 10 min for somatostatin and the enkephalins, 5 min for neurotensin and 30 s for substance P.

The Krebs bathing fluid containing (mM) NaCl 121·5, $CaCl_2 \cdot 6H_2O$ 2·51, KH_2PO_4 1·18, KCl 4·7, $MgSO_4 \cdot 7H_2O$ 1·17, $NaHCO_3$ 25 and dextrose 5·55 was maintained in the baths at 37°C, and bubbled with 95% O_2–5% CO_2.

Statistics

Fisher's exact test was used for statistical comparisons between circular and longitudinal muscles.

Results

Positive results are recorded fully in the text and in Table 1. Details of the experiments which showed no response to the peptides are mentioned only briefly below, and are listed in Table 2.

Stomach

VIP 12-53 nM usually reduced the submaximal contraction to ACh (by 10–37% in 3/4 experiments on longitudinal muscle, and 12–94% in 3/3 gastric circular muscle experiments; Fig. 1). There was no effect on tone or spontaneous activity. PHM 17–54 nM sometimes lowered the tone (1/2 circular, 2/4 longitudinal gastric muscles), but the spontaneous activity and the submaximal contraction to ACh were unaffected.

PHI 30 nM reduced the contraction to ACh of both circular

Table 1. Peptide-evoked changes in muscle tone, spontaneous activity and contractions to acetylcholine.

		Spontaneous activity		Tone		Acetylcholine	
Peptide	Concn (nM)	Long	Circ	Long	Circ	Long	Circ
Stomach							
VIP	12–53	—	—	—	—	3/4	3/3
PHM	17–54	—	—	2/4	1/2	—	—
PHI	30	—	—	—	—	1/1	2/2
Colon							
VIP	1·2–60	1/5	—	1/5	5/5	1/5	4/5
PHM	13–67	—	—	1/4	3/7	2/3	3/7
PHI	30–67	—	—	—	2/5	1/7	3/5
GAL	12–39	1/2	1/4	1/2	—	—	—
HCGRP	11–32	—	—	—	—	1/5	1/7
Neurotensin	0·12–1·2	—	—	3/3	3/3		
Substance P	0·0015–150	—	—	5/5	4/4		
Leu-enkephalin	0·36–3600	—	—	1/2	2/2		

The numbers represent experiments in which responses were seen and the effective peptide concentration ranges (nM). Long, longitudinal muscle; Circ, circular muscle.

Table 2. Numbers of experiments in which the peptides had no effect on the variables shown.

		Spontaneous activity		Tone		Acetylcholine	
Peptide	Concn (nM)	Long	Circ	Long	Circ	Long	Circ
Stomach							
VIP	12–53	4/4	3/3	4/4	3/3	1/4	0/3
PHM	17–54	4/4	2/2	2/4	1/2	4/4	2/2
PHI	13–30	1/1	2/2	1/1	2/2	0/1	0/2
GAL	12–120	4/4	3/3	4/4	3/3	4/4	3/3
RCGRP	0·4–105	2/2	1/1	2/2	1/1	2/2	1/1
Colon							
VIP	1·2–120	4/5	5/5	4/5	0/5	4/5	1/5
PHM	13–67	4/4	7/7	3/4	4/7	1/3	4/7
PHI	13–67	7/7	5/5	7/7	3/5	6/7	2/5
GAL	12–56	1/2	3/4	1/2	4/4	2/2	4/4
HCGRP	11–58	5/5	7/7	5/5	7/7	4/5	6/7
RCGRP	11–54	4/4	5/5	4/4	5/5	4/4	5/5
Neurotensin	0·12–1·2	3/3	3/3	0/3	0/3		
Substance P	0·0015–150	5/5	4/4	0/5	0/4		
Leu-enkephalin	0·36–3600	2/2	2/2	1/2	0/2		
Met-enkephalin	0·036–8700	3/3	3/3	3/3	3/3		
Somatostatin	0·12–300	3/3	2/2	3/3	2/2		

The numbers represent experiments. Underlined numbers are those in which a response occurred (see description in text and Table 1). Long, longitudinal muscle; Circ, circular muscle.

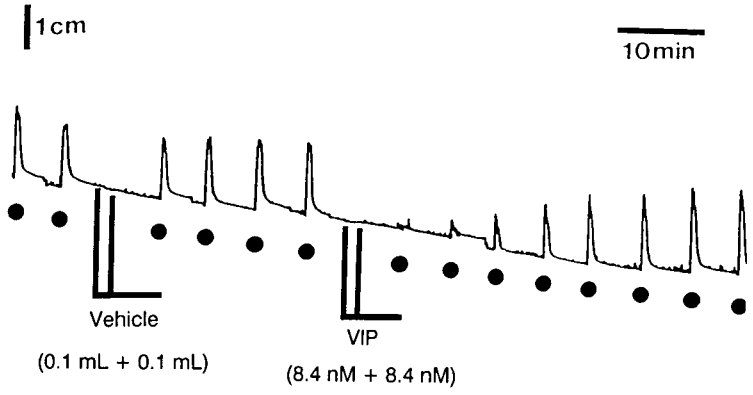

FIG. 1. Vasoactive intestinal peptide (VIP) did not alter stomach circular muscle tone, which continued to fall throughout the experiment, but it did reduce the subsequent contractions to acetylcholine 0·8 μM administered at ●.

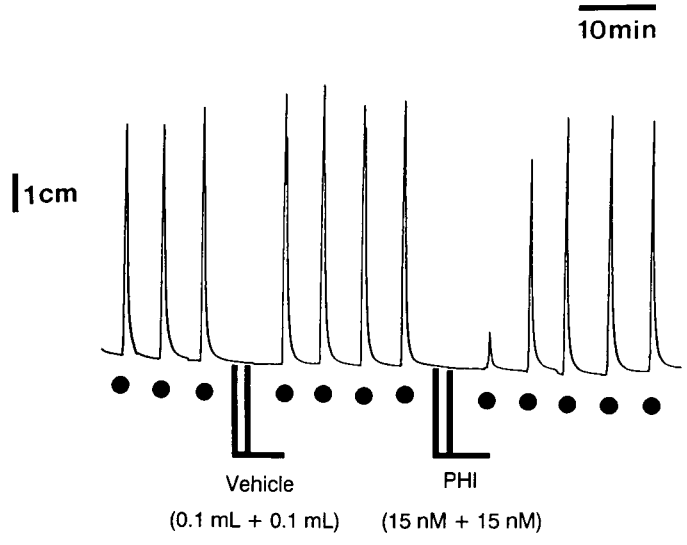

FIG. 2. Peptide histidine isoleucine (PHI) did not alter the tone of human stomach longitudinal muscle but it did reduce the subsequent contractions to acetylcholine 0·5 μM administered at ●

muscle strips and one longitudinal strip (by 86, 47 and 82%, respectively; Fig. 2); another longitudinal muscle showed no change in the ACh response with half the concentration of PHI. The tone and spontaneous activity were unaffected.

Neither GAL 12–120 nM nor RCGRP 0·40–105 nM altered the gastric tone, spontaneous activity or contraction to ACh.

Colon

VIP effects mainly differed on the two muscle layers (Fig. 3). In the circular muscle, VIP 1·2–17 nM lowered the tone in all 5 experiments (2 sigmoid, 2 transverse and 1 ascending colon), and reduced the contraction to ACh by 22–80% in 4/5 experiments (the exception being the ascending colon). However, the spontaneous activity was unchanged. In contrast, with VIP 12–120 nM, the taeniae usually showed little change in the tone, spontaneous activity or the contraction to ACh (4/5 specimens including 2 transverse colons), but in one specimen of transverse colon the tone, spontaneous activity and response to ACh were less with 48 nM VIP.

In the circular muscle, PHM 13–67 nM caused relaxation in 3/7 cases including the single specimen of transverse colon. PHM 13–67 nM reduced the contractions to ACh by 10–92% in 3/7 circular muscle strips (only 1 of these relaxed with PHM), but 24–54 nM had no effect on the others. In the taeniae, PHM 13–30 nM sometimes reduced the contraction to ACh (by 14 and 20% in 2/3 specimens from the 1 ascending and 1 sigmoid specimen; the ascending colon from another patient was not affected). The peptide did not alter the tone of taeniae from the ascending (n = 2) and sigmoid (n = 1) colon, but 54 nM relaxed the taenia from the transverse colon (n = 1). Spontaneous activity was not changed in the circular or longitudinal muscle strips.

With PHI, the circular muscle tone fell with 30 nM in 2/5 tissues (Fig. 4). The response to ACh was 14–92% less with PHI 13–59 nM in 3/5 tissues (only one of these tissues did not relax with PHI); with the other 2/5 experiments, PHI 67 nM had no effect on the subsequent ACh contraction in one case, and in the other experiment (transverse colon) the pronounced lowering of tone by PHI 30 nM presumably explained the 60% greater ACh response. PHI did not alter the longitudinal muscle tone (n = 7) or, usually, the submaxi-

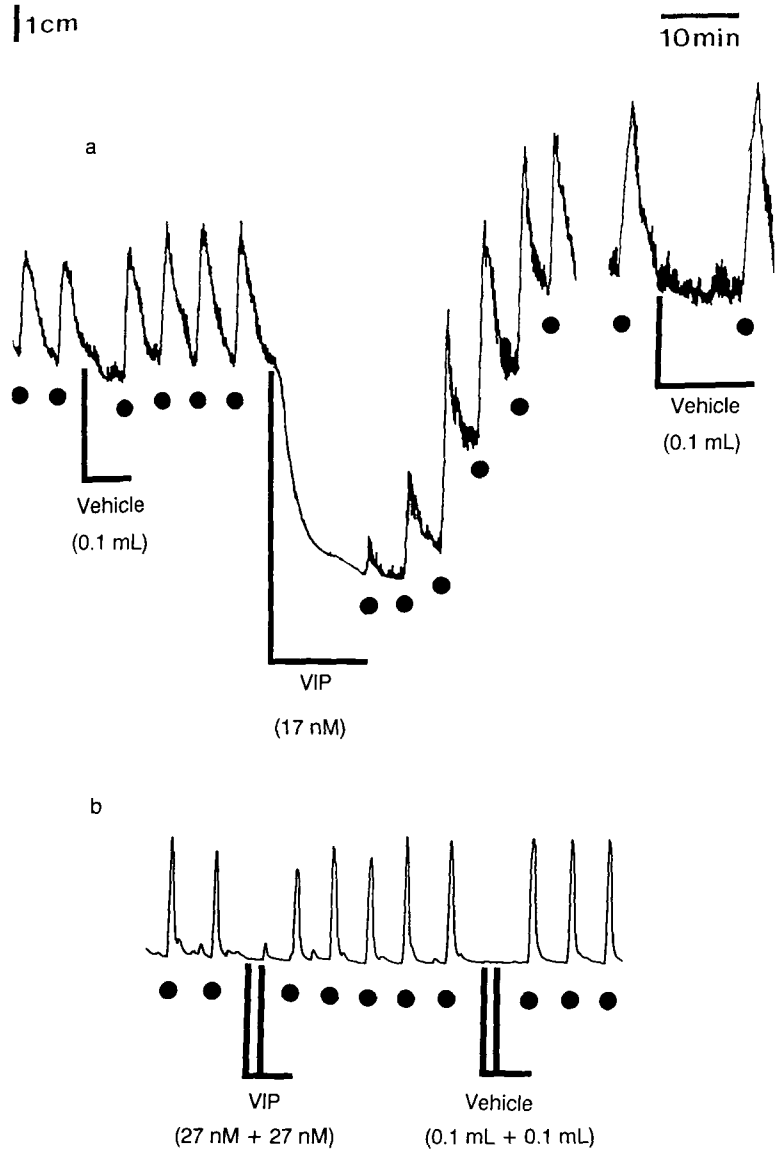

FIG. 3. Vasoactive intestinal peptide (VIP) relaxed human transverse colon circular muscle (a) and reduced subsequent contractions to acetylcholine 44 nM (●). VIP did not affect the tone of the transverse taenia coli (b) and had little or no effect on acetylcholine 0·48 μM (●).

mal contraction to ACh (n = 6), but in another experiment at the slightly higher concentration of 67 nM the response to ACh was halved. PHI did not change the spontaneous activity in either circular or longitudinal muscles.

GAL showed little activity overall. With GAL 19–56 nM in 10% HSA, the small decrease of tone in 2/4 specimens of circular muscle was mimicked by the vehicle (0·2 mL 10% HSA). GAL 12–56 nM, either in saline or HSA (both n = 3), did not alter the spontaneous activity except in the specimen of transverse colon which showed increased tone of both the circular and longitudinal muscles with GAL in saline. GAL had little or no effect on the contraction to ACh (4 circular muscles from 2 ascending, 1 transverse and 1 sigmoid colon; 2 taeniae from 1 ascending and 1 transverse colon).

HCGRP did not affect the tone or spontaneous activity (5 circular muscle; 7 taeniae), and did not alter the contraction to ACh except for a fall of 25% in one circular muscle strip of sigmoid colon (HCGRP 11 nM) and of 42% in a taenia (32 nM) from a different specimen of sigmoid colon.

RCGRP 11–54 nM did not alter the tone, spontaneous activity or response to ACh (5 circular, 4 longitudinal muscles).

Neurotensin, substance P, leu-enkephalin, met-enkephalin, and somatostatin were studied at a different time by sequential addition; the effect on the response to ACh was not examined. None of these peptides affected the spontaneous activity.

Neurotensin caused a concentration-dependent contraction of both muscle layers (3 taeniae, 3 circular; Fig. 5), with a threshold response at 0·12–1·2 nM.

Substance P also caused a concentration-dependent contraction of both muscle layers (5 taeniae, 4 circular muscle).

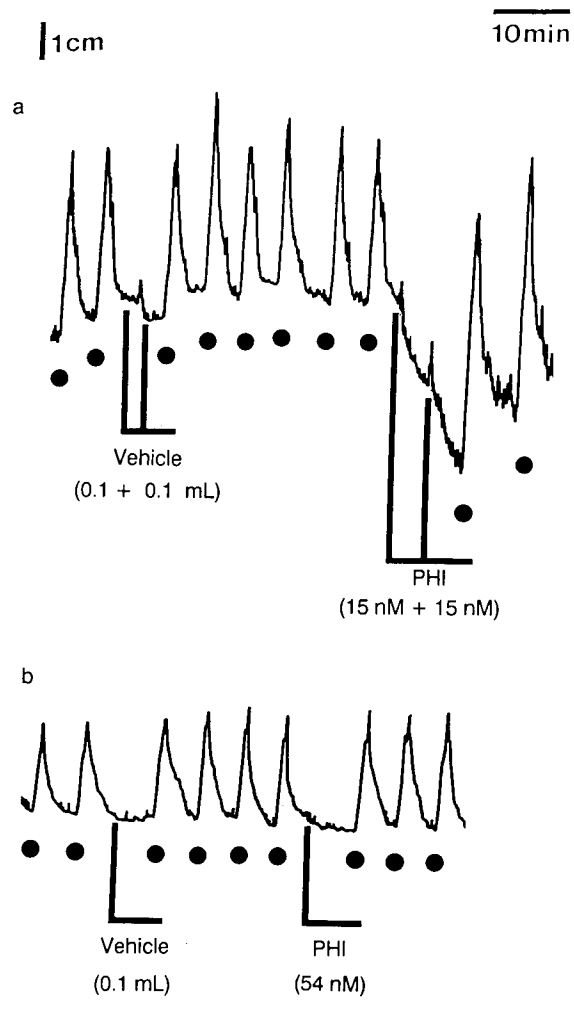

FIG. 4. (a) Peptide histidine isoleucine (PHI) relaxed human transverse colon circular muscle. ● acetylcholine 0·24 μM. (b) PHI was ineffective on sigmoid colon longitudinal muscle. ● acetylcholine 1·1 μM.

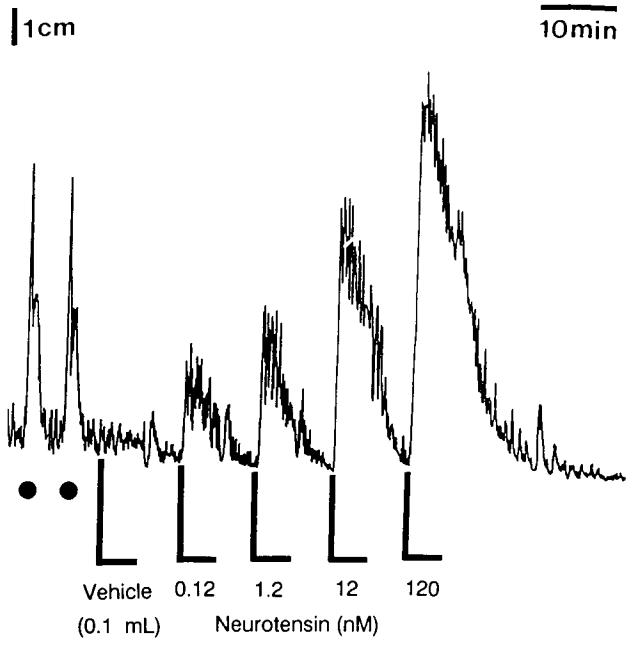

FIG. 5. Neurotensin caused a concentration-dependent contraction of human sigmoid colon circular muscle. ● acetylcholine 1·1 μM.

The effect on the circular muscle was much greater, and occurred at a lower threshold concentration (0·0015–1·5 nM) than in the taeniae (15–150 nM).

Leu-enkephalin 3·6 μM weakly contracted both specimens of circular muscle studied, but had no effect on 1 taenia, although another contracted weakly with 360 nM. Met-enkephalin 0·36 nM–8·7 μM had no effect (3 taeniae, 3 circular muscle).

Somatostatin 0·12–300 nM was also inactive (3 taeniae, 2 circular muscle from 1 transverse and 2 sigmoid colons).

Terminal ileum

This tissue is less frequently obtained at operation, and only a few peptides were tested. All of the following studies examined the effect on tone, spontaneous activity and the response to ACh.

VIP 12–60 nM had no effect on tone (2 longitudinal, 2 circular muscle strips), but in one of these experiments on the circular muscle with VIP 12 nM the spontaneous activity fell, and the response to ACh was 66% lower.

With PHM 15–20 nM, the longitudinal muscle tone and spontaneous activity were unaltered in 2/3 experiments but fell in another. In the only specimen of ileal circular muscle, PHM 2·7–13 nM caused a concentration-dependent lowering of the tone but did not alter the spontaneous activity. PHM did not affect the response of any preparation to ACh.

PHI 27–57 nM did not change the tone, spontaneous activity, or submaximal contraction to ACh (1 circular, 2 longitudinal muscle strips).

HCGRP 11–45 nM did not alter the tone or spontaneous activity (2 longitudinal and 2 circular muscles), but the response to ACh was 29–33% lower in both circular muscles, and 22% less in 1 of the longitudinal muscle strips (HCGRP 20 nM).

RCGRP 20–40 nM showed no activity on the longitudinal muscle (n = 2).

GAL was used in only 1 experiment; 14–28 nM caused the ileal longitudinal muscle to contract markedly, whereas the vehicle (HSA 10%) had no effect.

Jejunum

This tissue is rarely removed at surgery, and only 2 longitudinal muscle strips were obtained from one jejunal specimen. Three of the peptides (PHM 50 nM, RCGRP 21 nM and VIP 30 nM) reduced the spontaneous activity but did not affect the tone or submaximal contraction to ACh. In contrast, GAL (1·2–6·2 nM) contracted the muscle, and increased the spontaneous activity, tone, and the subsequent contraction to ACh (Fig. 6). Cumulative addition of GAL caused a concentration-dependent contraction with a threshold response at 1·2 nM.

Grouping of the results with VIP, PHI, and PHM

This grouping has been done because these peptides are related. VIP, PHI and PHM were either inhibitory or ineffective on our gut specimens. In the stomach, PHM

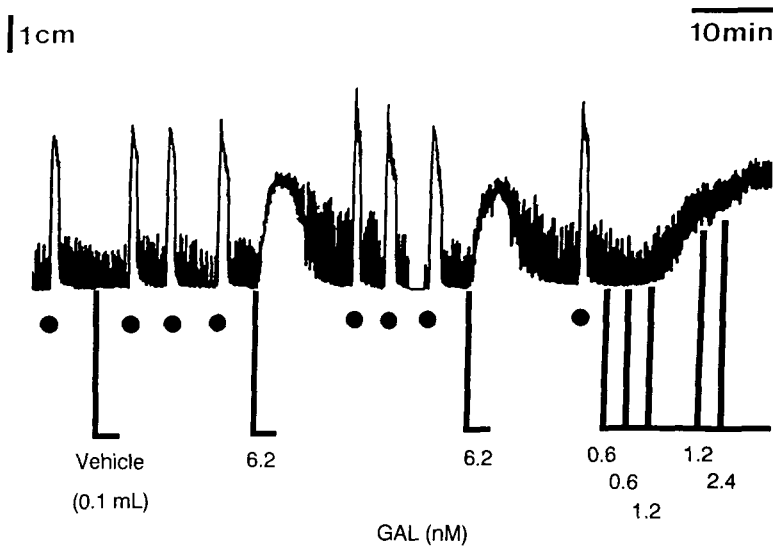

FIG. 6. Galanin 6·2 nM (GAL) contracted human jejunal longitudinal muscle (threshold response at 1·2 nM) and slightly increased the subsequent spontaneous activity and response to acetylcholine (●; 0·11 μM).

relaxed half the circular and longitudinal muscle strips, whereas both VIP and PHI had no effect. The ACh-induced contraction was reduced by both VIP and PHI in all circular, and in 75 and 50% of the longitudinal muscle specimens, but PHM had no effect. In the colon the ACh-induced contraction of the circular muscle was reduced in 80% of specimens by either VIP or PHI, but this effect occurred less often in the taeniae (VIP 20%; PHI 14%; $P=0{\cdot}01$, circular vs longitudinal muscle for both peptides analysed together, but $P<0{\cdot}1$ for either alone). In colon circular muscle, approximately 80, 40 and 40% of the specimens relaxed with VIP, PHI and PHM, respectively; relaxation also occurred in 25% of taenia strips with PHM (in contrast to none with PHI) and 20% with VIP. The pooled PHI and PHM results for stomach and colon show an inhibition of contraction to ACh in 67% of circular and 27% of longitudinal muscle specimens ($P=0{\cdot}057$ for the comparison between the two layers for VIP, PHI and PHM combined). Both muscle layers of the small intestine were unaffected by these peptides.

Discussion

This discussion is almost exclusively confined to human gastrointestinal muscle and its innervation, since these are the subject of the study and there are often great species differences. Except for CGRP and PHM, the peptides used are thought to occur in human gastrointestinal nerves, as shown in some cases by immunohistochemistry, chromatography, amino acid sequence determination or radioimmunoassay (see Furness & Costa 1987). Their presence raises the possibility of local roles in gastrointestinal functions.

For the purpose of discussion, we have grouped together VIP, PHI and PHM which are related and have similar actions; the other peptides are discussed separately. The formation of VIP, PHI and PHM depends on the same gene and occurs from the same precursor (Dockray 1987). VIP is one of the most abundant and potent transmitters in the human gut. With regard to the mucosa, there are high amounts in the proximal oesophagus, moderate amounts in the pylorus, and less in the gastric antrum and fundus. The gastric muscle contained smaller amounts of VIP, with less in the antrum than in the pylorus (Ferri et al 1989). In sigmoid colon, VIP was found in the muscle (Ferri et al 1983, 1988), and the highest amounts were in the myenteric plexus (Milner et al 1990). PHI is almost entirely localized in the muscle layers (Christofides et al 1983). In general the levels increased gradually from the fundus to the colon, although most occurred in the duodenum (Christofides et al 1984).

The projection pathways of enteric nerves containing VIP in colon indicate differences for circular and longitudinal muscle; those in the circular layer originate in both the submucous and myenteric ganglia, whereas projections in the taenia coli originate only in the myenteric ganglia (Domoto et al 1990).

Disease may change the amounts of colonic VIP, its levels in sigmoid colon being below normal in idiopathic constipation, and increased in diverticular disease (Milner et al 1990). Although Koch et al (1990) reported a bimodal distribution of VIP in Crohn's disease, their seven results were distributed approximately evenly above and below the mean normal value, and we believe that they merely show similarly variable levels in health and disease.

VIP relaxed strips of lower oesophageal sphincter (Domschke et al 1978) and colon (Couture et al 1981) and has been proposed as a mediator of gut inhibitory nerves. We found that VIP and PHI always inhibited the gastric circular muscle contractions to ACh, and usually inhibited the longitudinal layer as did PHM; VIP also inhibited most strips of colonic circular muscle, but fewer of the taeniae. PHM sometimes relaxed or inhibited colon circular muscle contractions to ACh, but was less effective on the longitudinal muscle; PHI had similar effects to PHM but was usually inactive on longitudinal muscle.

Immunoreactive met-enkephalin was located in human sigmoid colonic muscle (approx. 70 pmol g^{-1}) with decreas-

ing amounts towards the anal canal; much less occurred in the mucosal and submucosal layers (Ferri et al 1987). Highest amounts (100 pmol g^{-1}) were found in the muscle and submucosal layers in the pyloric sphincter region. Since leu-enkephalin only weakly contracted both muscle layers of colonic muscle, and met-enkephalin usually had no effect, these peptides seem likely to have no important roles as direct enteric neurotransmitters in the colon. However, we have not examined the possibility that they affect the release or activity of other substances.

There is immunohistochemical evidence that the 29-amino acid peptide GAL occurs throughout the gut, with most in stomach circular muscle, least in small intestine circular muscle, and moderate amounts in the longitudinal muscle of all regions (Bishop et al 1986). These findings were supported by Bauer et al (1988) who found, in addition, that GAL occurs in neurons, muscle and submucosa of the jejunum and colon but not in the epithelium. Hoyle & Burnstock (1989) found GAL-immunoreactive nerve fibres in colonic muscle, with denser innervation in the circular than in the longitudinal muscle; they also found GAL in the submucosa, with most present in the neurons of the submucosal plexus.

Porcine GAL potently contracted the longitudinal muscle of isolated small intestine (Maggi et al 1989). We confirmed this in our single experiment (the circular muscle from the small intestine was not studied), but found that the effect of GAL varied regionally; both layers of the transverse colon contracted, but other regions of the colon and the gastric muscle were unaffected.

Substance P, an 11-amino acid peptide which belongs to the tachykinin family, was located by radioimmunoassay in gut muscle (Holzer et al 1982) and was later found almost exclusively in nerves of the gut non-epithelial layer (Ferri et al 1983). Colon circular and longitudinal muscle was found to contain many immunoreactive nerve fibres (Wattchow et al 1988), consistent with the high levels of substance P in sigmoid colon (Ferri et al 1988). Most of the immunoreactive nerve fibres occurred in the myenteric plexus, with moderate amounts distributed in Meissner's plexus, Schabadasch's plexus and the middle layers of the submucous plexus (Milner et al 1990). Levels in the submucosal plexus were higher than in the muscle layers (Milner et al 1990).

Mammalian intestinal nerves containing substance P generally have similar distributions and activities, and in many species this peptide may be a gut muscle excitatory transmitter. The presence of these nerves in human gut muscle layers, mucosa and around blood vessels (Llewellyn-Smith et al 1984) suggests a contribution to the control of gastrointestinal function and blood flow. In agreement with the studies of Liljedahl et al (1958) and of Zappia et al (1978) on taenia coli and stomach, we obtained a concentration-dependent contraction with substance P in all specimens of colonic circular and longitudinal muscle.

We have not found any reports concerning the detection of CGRP in the human gastrointestinal tract. Of all the peptides studied, both the human and rat CGRP were amongst the least active. These peptides did not affect the ileal and colonic muscle tone and spontaneous activity, and only occasionally decreased the colonic responses to ACh; only HCGRP reduced the ileal circular and longitudinal muscle response to ACh.

Holzer et al (1982) found immunoreactive neurotensin mainly in the wall of the terminal ileum, with much less in the jejunum, duodenum and descending colon, whereas Ferri et al (1983) found that neurotensin was confined to endocrine cells in the ileal epithelium (other regions were not studied). We found that neurotensin caused a concentration-dependent contraction of both colonic muscle layers, but other regions were not examined.

There are at most a few somatostatin-containing nerves around gastrointestinal muscle (Keast et al 1984; Ferri et al 1988, 1989; Wattchow et al 1988). The peptide appears to be located mainly in endocrine cells in the mucosa, but it does not seem to occur in every region (Ferri et al 1983, 1988, 1989; Keast et al 1984). Somatostatin-immunoreactive nerves were found throughout the gut except in the gastric body. In-vivo, somatostatin relaxed the rectal musculature (Lerebours et al 1987), a tissue which contains small amounts of the peptide (Ferri et al 1988). However, somatostatin lacks activity on the muscle layers of the sigmoid or transverse colon in-vitro, and is therefore unlikely to affect this region by a direct action on the muscle, although it might act via nerves or by altering the response to other substances.

In the light of the results reported here, the peptides that may contribute directly to the control of human gastrointestinal motility are VIP and PHI for inhibition/relaxation, and GAL, substance P and neurotensin for contraction. However, these conclusions must be treated with caution since not all of the peptides were of the human type, and for various reasons the responses of human tissues are not always the same in-vitro as in-vivo (Bennett 1968). This may be due, at least in part, to the way that the peptide reaches the tissue. In dog stomach, motilin, pentagastrin and substance P increase cholinergic activity when injected through the vascular system in-vivo, but not when added to the solution bathing isolated strips (Fox et al 1983).

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