Effects of natural analogues of substance P on the motility of human gastrointestinal tract *in vitro*

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Interest in the action of substance P on the gut stems from the finding that this peptide is uniformly distributed along the gastrointestinal mucosa from the stomach to the colon (Grossman, 1976). In a systematic investigation of the structure-activity relation of a group of some natural analogues of substance P (the so-called tachykinins; Bertaccini, 1976), we tested *in vitro* a variety of peptides for their stimulant activity on different segments of human gastrointestinal tract removed during surgery.

Approximately 70 strip preparations (Table 1) were used. The tissues were removed at operation and taken from macroscopically normal parts of the bowel. They were immediately put into Krebs–Henseleit solution (Everett, 1968) at room temperature (20°). Mucosa and submucosa were removed and strips of muscle measuring approximately 20 × 3 mm were cut parallel to the longitudinal or circular layer for the stomach, or longitudinally for the other tissues. The strips were suspended in an organ bath filled with nutrient solution at 37° and bubbled with 5% CO_2 in oxygen. After a resting period of about 2 h the strips were stimulated with acetylcholine: those that did not respond to acetylcholine (200 ng ml^{-1} for stomach, 20 ng ml^{-1} for small or large intestine) were discarded. Movements were usually recorded by means of an isotonic lever on a smoked drum; or occasionally with a isometric lever connected to a transducer, and a microdynamometer (Basile, Comerio) with a pen writer was used.

Drugs used were: substance P (Beckman), eledoisin, phyllomedusin, physalaemin, uperolein and phyllomedusin synthetized and kindly supplied by Farmitalia Laboratories (Milan, Italy), kassinin kindly offered by Professor V. Erspamer (Rome, Italy) as a purified extract from the skin of the African amphibian *Kassina senegalensis*. Other drugs were acetylcholine and atropine (Fluka) and verapamil (Isoptin, Knoll). The structures of the peptides are as follows:

Eledoisin: Pyr–Pro–Ser–Lys–Asp–Ala–Phe–Ile–Gly–Leu–Met–NH_2.

Phyllomedusin: Pyr–Asn–Pro–Asn–Arg–Phe–Ile–Gly–Leu–Met–NH_2.

Physalaemin: Pyr–Ala–Asp–Pro–Asn–Lys–Phe–Tyr–Gly–Leu–Met–NH_2.

Uperolein: Pyr–Pro–Asp–Pro–Asn–Ala–Phe–Tyr–Gly–Leu–Met–NH_2.

Substance P: Arg–Pro–Lys–Pro–Gln–Phe–Phe–Gly–Leu–Met–NH_2.

Kassinin: Asp–Val–Pro–Lys–Ser–Asp–Gln–Phe–Val–Gly–Leu–Met–NH_2.

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Table 1. *Spasmogenic potencies of tachykinins on the human gastrointestinal tract* in vitro.

	Stomach* (25)	Duodenum (3)	Ileum (16)	Taenia coli (24)
Eledoisin	100	100	100	100
Phyllomedusin	150	100*T*	75	65
Physalaemin	50*T*	20	50	20
Uperolein	100	190	40*T*	10*T*
Substance P	20	—	—	5

* In the different experiments longitudinal and circular muscle layers were examined, but the results obtained were similar and therefore have been considered together.

The activity of eledoisin was arbitrarily taken as 100. The activities of the other peptides were expressed as percentages of eledoisin (molar concentration). Each value represents the mean of the values obtained from 3 to 6 experiments. Comparisons were made at doses 2–3 times as high as the threshold doses.

T = presence of tachyphylaxis at the 2nd or 3rd consecutive administration.

All the compounds examined caused the appearance or reinforcement of rhythmic movements and an increase in tone in all the segments of the gastrointestinal tract from the stomach to the colon. After washing, the return to basal conditions usually took a few minutes. The most sensitive tissue was the ileum (threshold stimulant doses from 1 to 50 ng ml^{-1}, according to the different peptides) the least sensitive was the stomach (threshold doses from 10 to 300 ng ml^{-1}).

The ratios of activity obtained by comparing the potency of the different compounds on the various tissues to that of eledoisin taken as 100, are shown in Table 1. It is evident from Table 1 that some peptides had approximately the same potency in the different segments, whereas others showed wide variations: a typical example is uperolein which was about twice as active as eledoisin on the duodenum, but only 1/10th as active on the taenia coli. The compounds usually showed a good dose-response relation. Some exceptions are shown in Table 1. Tachyphylaxis was not specific to a single peptide and occurred with phyllomedusin (see Fig. 1), physalaemin and uperolein but only in some of the different gut segments.

Atropine (2×10^{-6} M) did not inhibit the responses to the peptides thus excluding a role of the cholinergic receptors in the contraction of the intestinal strips. By contrast verapamil (4×10^{-6} M) caused a complete disappearance of the spasmogenic effect of the peptides

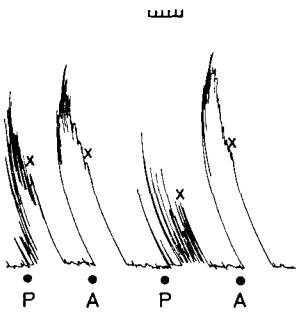

FIG. 1. Human duodenum: P = phyllomedusin (0·03); A = acetylcholine (0·03). Doses in μ g ml^{-1}. Note the tachyphylaxis showed by phyllomedusin. Horizontal scale: Time (min).

suggesting that tachykinins contracted the preparations by interfering with the transport of calcium ions.

The compound kassinin, recently isolated from the skin of the African amphibian *Kassina senegalensis* (Erspamer, unpublished), showed a spasmogenic effect qualitatively identical to that of eledoisin with no tachyphylaxis.

Two points emerge from the present investigation and from a comparison of our results with those of the recent literature:

(a) substantial quantitative differences in the ratios of activity of tachykinins are very common in *in vitro* and *in vivo* preparations. We reported conspicuous quantitative differences in the spasmogenic potencies of tachykinins on the longitudinal muscle of the guinea-pig ileum (Zséli, Molina & others, 1977) and also in the rat stomach *in vivo* (Bertaccini & Coruzzi, 1977; Bertaccini, 1977). Erspamer, Negri & others (1975) found even more remarkable differences in the activities of tachykinins on a series of smooth muscle preparations with a very high degree of dissociation of potencies;

(b) qualitative differences were also observed: a 'selective' tachyphylaxis was noted in the present investigation; a tachyphylaxis was observed by increasing the concentrations of uperolein, physalaemin and substance P together with cross tachyphylaxis among these peptides; but this was not true for phyllomedusin and kassinin in a study of the contraction of the circular muscle of the guinea-pig (Bertaccini & Impicciatore, unpublished); in the same preparation uperolein and physalaemin appeared to be atropine-sensitive.

This is, so far, an unique example for tachykinins which were always described as peptides acting directly on the smooth muscle (Bertaccini, 1976). While it is well known that the crucial part of the tachykinins molecule for the maintainance of the biological activity is the C-terminal tripeptide (Gly–Leu–Met–NH_2) and the phenylalanyl residue in position 5 from the C terminus (Bertaccini, 1976), the recent structure-activity studies seem to suggest that the N-terminal part, which is different in the natural tachykinins so far isolated, may play role not only in dissociating the ratios of activity in various experimental conditions but actually in determining qualitative differences. This situation seems to be peculiar to the tachykinin family and represents a point of discrimination with other gastrointestinal peptides like the gastrin and cholecystokinin group and the bombesin-like peptides in which only the C-terminal part of the molecule appears to be essential for biological activity.

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REFERENCES

BERTACCINI, G. (1976). *Pharmac. Rev.*, **28**, 127–177.

BERTACCINI, G. (1977). In: *Abstracts of Joint Meeting of German and Italian Pharmacologists*, Venezia, October 4–6, p. 95.

BERTACCINI, G. & CORUZZI, G. (1977). *Naunyn-Schmiedebergs Arch. Pharmac.*, **298**, 163–166.

ERSPAMER, V., NEGRI, L., FALCONIERI ERSPAMER, G. & ENDEAN, R. (1975). *Ibid.*, **289**, 41–54.

EVERETT, S. D. (1968). *Br. J. Pharmac.*, **33**, 342–365.

GROSSMAN, M. I. (1976). In: *5th International Congress of Endocrinology: Plenary Lecture*, Hamburg, Editor: James, V. H. J. Amsterdam: Exerpta Medica.

ZSÉLI, J., MOLINA, E., ZAPPIA, L. & BERTACCINI, G. (1977). *Eur. J. Pharmac.*, **43**, 285–287.