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Action of Substance P and Its Natural Analogs on the Circular Muscle of the Guinea Pig Ileum

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BERTACCINI, G. AND L. ZAPPIA. *Action of substance P and its natural analogs on the circular muscle of the guinea pig ileum.* PEPTIDES 2: Suppl. 2,205–208, 1981.—Substance P and its natural analogs were tested for their activity on the circular muscle of the guinea pig ileum taking into account the possible role of these peptides in the regulation of intestinal peristalsis. All of the peptides examined were found to be less potent (from 2 to 10 times) in contracting the circular than the longitudinal muscle. The order of "potency" was the following: kassinin = eledoisin > uperolein > physalaemin > phyllomedusin = substance P. The order of "efficacy" was: kassinin > eledoisin > uperolein > phyllomedusin > physalaemin > substance P. Thus considering both the threshold dose (potency) and the maximum effect (efficacy) substance P appeared to be the less effective peptide. The hypothesis may be suggested that guinea pig substance P, so far not identified, is more closely related from a chemical and a pharmacological point of view to one of the above tachykinins than to bovine substance P used in our experiments. Since all these peptides possess a common C-terminal part in their molecules, the importance of the N-terminal part (which is different in the various peptides) must be emphasized.

Guinea pig ileum Circular muscle Substance P Tachykinins

SEVERAL investigations from our and other laboratories raised the question of the structure-activity relation in the field of substance P (SP) natural analogs (tachykinins). Differences in the ratio of potency among the various peptides were reported (for reviews see [2,8]) but we wanted to check if also qualitative differences could be demonstrated: circular muscle layer of the guinea pig ileum was selected because data on this tissue are not so abundant as those on the longitudinal ileum and because of the role which circular muscle may have in the physiological peristalsis as it was recently pointed out by Holzer and Lembeck [10].

METHOD

Contractions of the circular muscle layer were recorded by connecting several (4 to 7) rings obtained by cutting the ileum from freshly killed guinea pigs (300 g) in the direction of the circular muscle. Isometric recording was made by using a strain gauge transducer (average tension 0.25 g) and a direct writing microdynamometer. To obtain a full dose-response curve peptides were administered at intervals of 3–4 min and washed out soon after the development of maximum effect. When atropine was used it was added to the bath in the amount of 0.1 μg/ml 10 min prior to administration of the peptides. In a few preliminary experiments the intraluminal pressure in the whole ileum during longitudinal isometric contractions was also evaluated as an index of the circular muscle contractions. The structure of the peptides used in our experiments is shown in Table 1 which points out the analogies among the various tachykinins.

All the peptides used in our experiments were purchased from the Peninsula Lab (California). Atropine (Fluka) was also used.

RESULTS

Results obtained are summarized in Table 2. It is evident from the table that differences among the various tachykinins were quite remarkable. Not only the potency of the different peptides varied considerably (up to 10 times) but also the efficacy and other important parameters like the occurrence of tachyphylaxis and the sensitivity to atropine were markedly at variance. Examples of the good concentration-response curves obtained with eledoisin, uperolein and kassinin are shown in Figs. 1, 2, and 3.

The figures show that both tone and phasic movements increased dose-dependently with the tachykinins in contrast with observations concerning the circular muscle of rabbit intestine in which the tone was not affected at all [7].

DISCUSSION

Our data showed that tachykinins possess a stimulant action on the circular muscle of the guinea pig ileum. The potency of these compounds on the circular muscle was approximately ten times lower than that on the longitudinal

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TABLE 1

SUBSTANCE P-RELATED PEPTIDES*

12	11	10	9	8	7	6	5	4	3	2	1	
											Pyr-Ala-Asp-Pro-Asn-Lys-*Phe*-Tyr-*Gly-Leu-Met-NH$_2$*	Physalaemin
											Pyr-Asn-Pro-Asn-Arg-*Phe*-Ile-*Gly-Leu-Met-NH$_2$*	Phyllomedusin
											Pyr-Pro-Asp-Pro-Asn-Ala-*Phe*-Tyr-*Gly-Leu-Met-NH$_2$*	Uperolein
											Pyr-Pro-Ser-Lys-Asp-Ala-*Phe*-Ile-*Gly-Leu-Met-NH$_2$*	Eledoisin
											Asp-Val-Pro-Lys-Ser-Asp-Gln-*Phe*-Val-*Gly-Leu-Met-NH$_2$*	Kassinin
											Arg-Pro-Lys-Pro-Gln-Gln-*Phe*-Phe-*Gly-Leu-Met-NH$_2$*	Substance P

*The amino acid residues which are common to the different peptides are in italics. The numbering of amino acids residues starts unconventionally from the C-terminus in order to point out the structural analogies of the tachykinins.

TABLE 2

EFFECTS OF TACHYKININS ON THE CIRCULAR MUSCLE OF GUINEA PIG ILEUM

Compounds	Relative Activity*	Efficacy	Tachyphylaxis	Sensitivity to Atropine
Physalaemin	3	1.18	+	++
Phyllomedusin	1	1.31	−	−
Uperolein	9	1.40	−	++
Eledoisin	10	1.42	−	+
Kassinin	10	1.44	−	−
Substance P	1	1.00	+	+

*Calculated on the basis of the threshold doses.

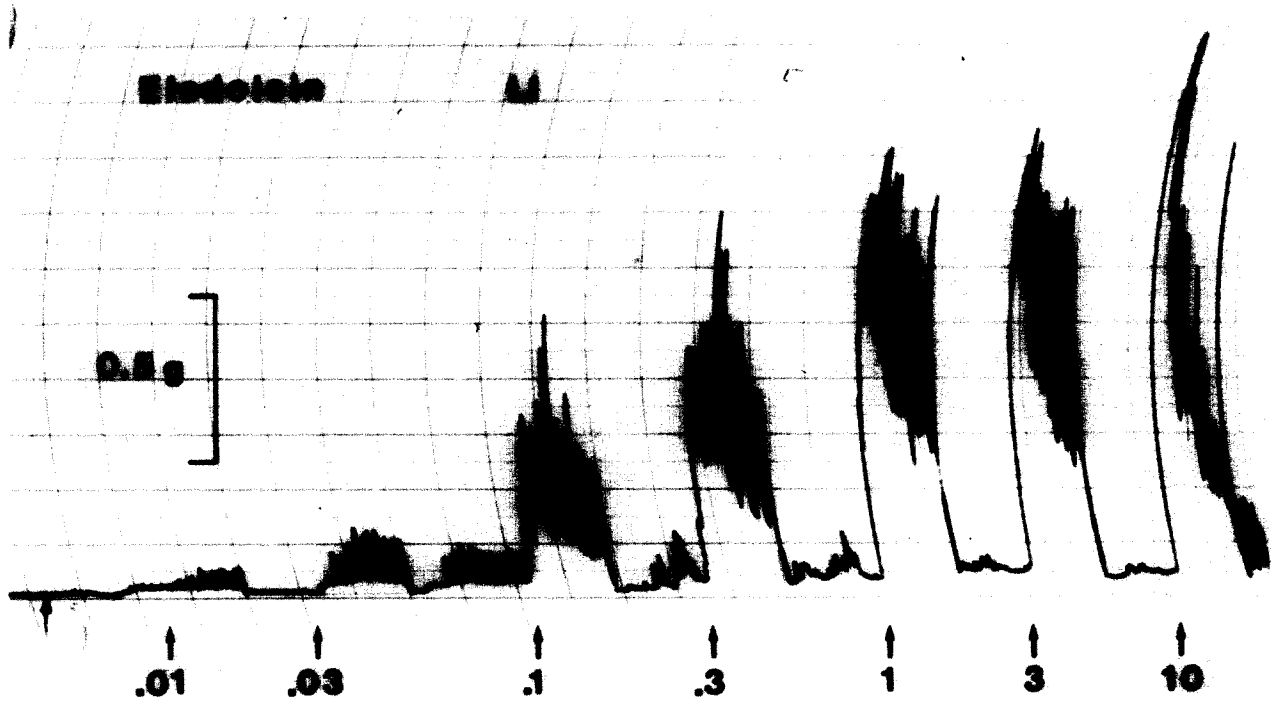

FIG. 1. Circular muscle of the guinea pig ileum: dose-response curve to eledoisin; doses are in μg per bath (10 ml); on the ordinate tension of the transducer; time marks 1 min.

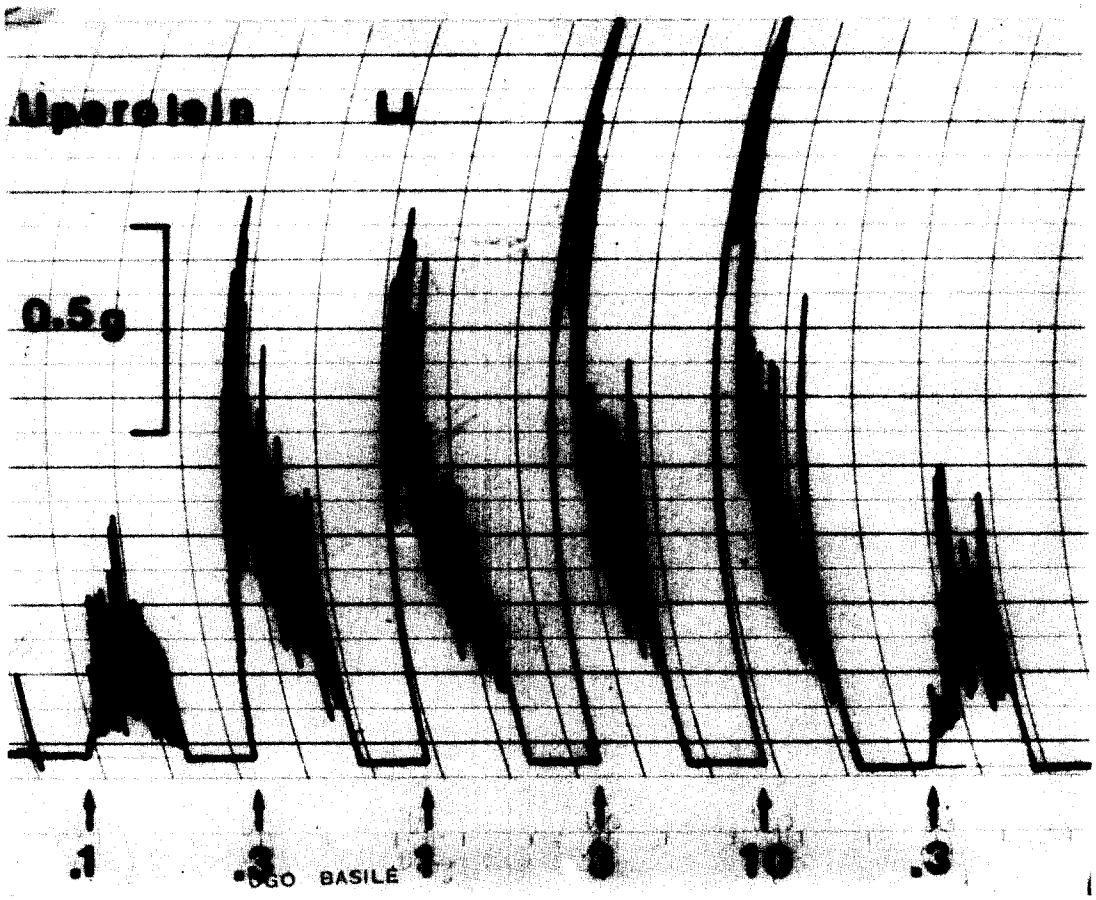

FIG. 2. Circular muscle of the guinea pig ileum: dose-response curve to uperolein; doses are in μg per bath (10 ml); on the ordinate tension of the transducer; time marks 1 min.

muscle previously reported from our laboratory [16]. However this is probably connected with the tissue or the species because in the opossum duodenum, Anuras and Nowak [1] found a much smaller difference in the sensitivity of the two muscle layer (1.5–2 times). Moreover in our laboratory [15] we found no difference at all in the response to the tachykinins, examining circular and longitudinal muscle of different segments of the human gastrointestinal tract.

The erratic occurrence of tachyphylaxis was already observed [3, 6, 15]; also the sensitivity to atropine which was not noted in some of our studies [5, 6, 16], was pointed out for substance P by other authors in different tissues [9,11].

On the whole the qualitative differences among the different tachykinins (efficacy, erratic occurence of tachyphylaxis and different sensitivity to atropine which of course suggests different mechanism of action) are consistent with the idea that the N-terminal portion of the molecule is important to characterize the biological effects of the tachykinins which are chemically similar in the C-terminal portion of their molecules. The importance of the C-terminal portion was emphasized in several investigations and also in quite recent reports [4,12]. Our data suggests therefore that results obtained with one peptide cannot be extrapolated to the other members of the family as previous experiments had pointed out.

Substance P was in our experiments less effective (especially in terms of efficacy) than other tachykinins but it is possible that substance P of the guinea pig is more closely related to other tachykinins than to bovine SP used in our experiments. It is worth mentioning here that Lazarus and coworkers [13,14] described in the gastrointestinal tract of mammals a substance with an immunoreactivity resembling that of physalaemin. The antiserum against physalaemin was specific for the N-terminal region of this peptide (the cross-reactivity with uperolein was 2% whereas absolutely negligible recognition was shown for SP).

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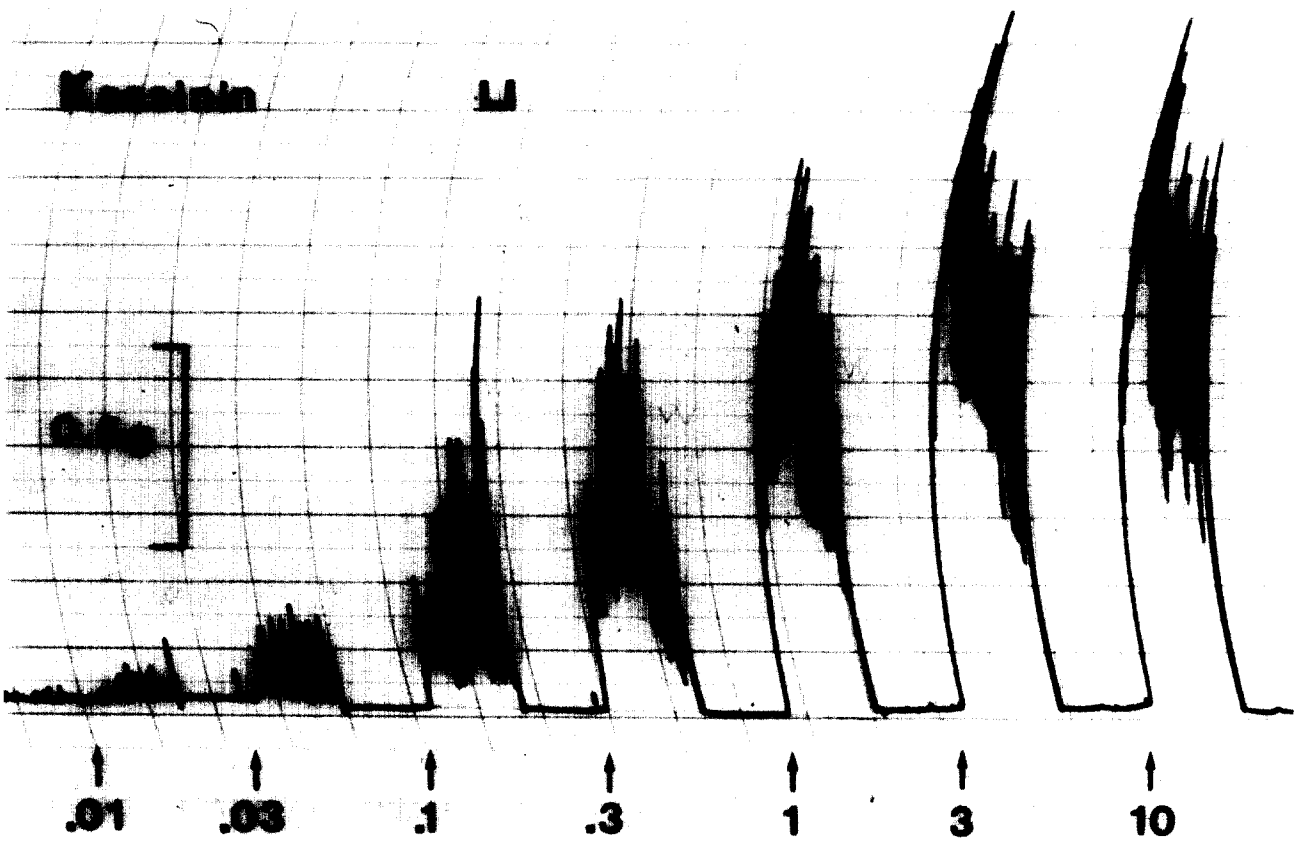

FIG. 3. Circular muscle of the guinea pig ileum: dose-response curve to kassinin; doses are in μg per bath (10 ml); on the ordinate tension of the transducer; time marks 1 min.

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