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EFFECT OF SUBSTANCE P AND ITS NATURAL ANALOGUES ON GASTRIC EMPTYING OF THE CONSCIOUS RAT

G. BERTACCINI, R. DE CASTIGLIONE* & C. SCARPIGNATO

Institute of Pharmacology, University of Parma, Italy and *Farmitalia-Carlo Erba Research Laboratories, Milan, Italy

1 Substance P and its natural analogues were tested for their effect on gastric emptying in the rat.

2 Substance P and kassinin were virtually inactive even at the maximum dose tested.

3 The other tachykinins significantly delayed gastric emptying, their effect being quite remarkable with 100 μg/kg. The lowest doses (30 μg/kg) caused a slight and non-significant increase in emptying rate.

4 The effect on gastric emptying was most probably correlated with a spasmogenic effect on the gastroduodenal junction, as pointed out in previous work.

Introduction

In a thorough investigation concerning the structure-activity relationship of the naturally occurring tachykinins (Bertaccini, 1976) it was observed that these peptides possessed a remarkable stimulatory effect on the stomach (both the proximal and the distal part) of the anaesthetized rat (Bertaccini & Coruzzi, 1977). In the present study we wanted to examine the possible effects of these peptides on gastric emptying of conscious rats, a parameter which is strictly connected with the activity of the smooth muscle of the stomach. Together with the tachykinins examined in the previous study (substance P, eledoisin, physalaemin, phyllomedusin and uperolein), kassinin, the newest member of this peptide family (Yajima, Sasaki, Ogawa, Fujii, Segawa & Nakata, 1978) was also used.

Methods

Male Wistar rats weighing approximately 200 g and fasted 24 h prior to experiments were used. The test meal consisted of a solution of 50 mg phenol red in 100 ml aqueous methylcellulose (1.5%) given by oral intubation, 1.5 ml per rat. Drugs were injected intraperitoneally in a constant volume (1 ml/kg) 5 min before the administration of the meal. Animals were killed 20 min after the test meal. In each experiment a group of 4 animals was killed immediately after the administration of the meal and considered as a standard (100% phenol red) to avoid errors connected with contraction of the stomach during terminal convulsions. The stomach was then exposed by laparotomy, quickly ligated at the pylorus and the cardia, and removed. The stomach and its contents were homogenized in a Waring blender with 100 ml of NaOH 0.1N. The analytical procedure for the assay of phenol red was described in a previous paper (Scarpignato, Capovilla & Bertaccini, 1980). It involves precipitation of proteins with 20% trichloracetic acid, realkalinization with NaOH and colorimetric assay at 560 nm. Gastric emptying (GE) for each rat was calculated according to the following formula:

$$GE(\%) = \left[1 - \frac{\text{amount of phenol red recovered from the test stomach}}{\text{average amount of phenol red recovered from the standard stomachs}}\right] \times 100.$$

Under our experimental conditions, in control rats (receiving only physiological saline) the meal leaving the stomach after 20 min was 53.4% ± 4.2 in comparison with the standards. The results are presented as percentage changes in comparison with controls, taken as 0. Student's *t* test for unpaired data, was used for determining statistical significance.

Heart rate and blood pressure were measured through the arteries of the tail by means of a W + W BP recorder 8005 according to Gerold & Tschirky (1968). The peptides used, the structure of which is shown below, were synthesized by one of us (R. De Castiglione) with the exception of substance P and kassinin which were purchased from Peninsula Laboratories (California, U.S.A.).

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Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Kassinin	= Asp-Val-Pro-*Lys*-Ser-Asp-*Gln*-*Phe*-Val-*Gly*-*Leu*-*Met*-NH_2
Eledoisin	= Pyr-*Pro*-Ser-Lys-Asp-Ala-*Phe*-Ile-*Gly*-*Leu*-*Met*-NH_2
Physalaemin	= Pyr-Ala-Asp-*Pro*-Asn-Lys-*Phe*-Tyr-*Gly*-*Leu*-*Met*-NH_2
Phyllomedusin	= Pyr-Asn-*Pro*-Asn-Arg-*Phe*-Ile-*Gly*-*Leu*-*Met*-NH_2
Uperolein	= Pyr-*Pro*-Asp-*Pro*-Asn-Ala-*Phe*-Tyr-*Gly*-*Leu*-*Met*-NH_2

The amino acid residues which are in the same position as in the substance P molecule are in italics.

Results

The results are summarized in Figure 1. It is evident from the figure that within the range of the doses employed, substance P and kassinin (the two most closely related peptides from a structural point of view) were virtually ineffective, inducing erratic and insignificant changes. The other peptides caused generally a delay of gastric emptying which varied according to the different compounds and the doses employed. The smallest dose (30 μg/kg) appeared to induce an increase in emptying but the change was never statistically significant. The two most effective compounds were phyllomedusin and physalaemin, with a maximum effect (approx. 75% delay in comparison with controls) elicited with 100 μg/kg. With the exception of eledoisin, a dose of 300 μg/kg was apparently supramaximal since it slowed gastric emptying to a lesser extent than 100 μg/kg.

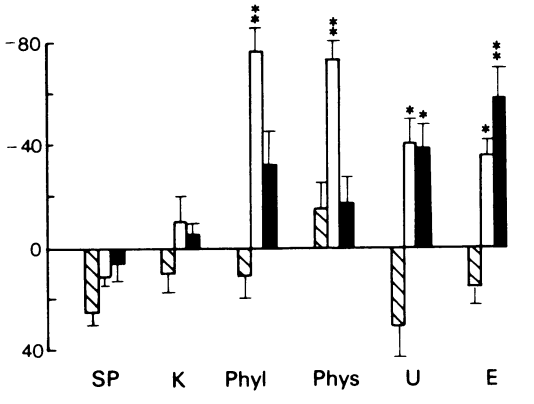

Figure 1 Gastric emptying in conscious rats. Ordinate scale % changes in comparison with controls (n = 12) taken as zero. SP = substance P; K = kassinin; Phyl = phyllomedusin; Phys = physalaemin; U = uperolein; E = eledoisin; Hatched columns = 30 μg/kg; open columns = 100 μg/kg; solid columns = 300 μg/kg. Height of column indicates mean value from 5 to 10 rats; vertical lines show s.e. mean. * = $P < 0.02$; ** = $P < 0.001$.

Discussion

Our results are substantially in agreement with those previously described (Bertaccini & Coruzzi, 1977) for the *in situ* stomach of the anaesthetized rat; the order of potency in provoking the contraction of the pylorus was: eledoisin > phyllomedusin > physalaemin > uperolein > substance P, which is similar to that found in the present experiments and suggests that contraction of the pyloric sphincter plays a major role in determining the delay in gastric emptying. However, in our previous study (Bertaccini & Coruzzi, 1977), a spasmogenic effect of these peptides on the whole stomach excluding the pylorus was also demonstrated and this action could counteract the effect on the pylorus as far as gastric emptying is concerned. Thus, we suggest that the effect on gastric emptying may represent the net effect of different actions in the stomach and gastro-duodenal junction. The doses used in the present experiments were noticeably higher than those used in the anaesthetized rat: however, an analogous difference was noted in similar experiments carried out with caerulein and bombesin both of which were found to contract the pylorus in the anaesthetized rat (Bertaccini, Impicciatore & De Caro, 1973; Bertaccini & Impicciatore, 1975) and to delay gastric emptying in the conscious rat (Scarpignato *et al.*, 1979; Scarpignato & Bertaccini, 1980). The fact that the two most potent peptides, namely phyllomedusin and physalaemin, were decidely less effective at 300 than at 100 μg/kg may be explained by the spasmogenic effect on the whole gastric muscle which overwhelmed that of the pylorus thus allowing an easier emptying of the stomach. While other systemic effects of the peptides cannot be excluded, it should be mentioned that cardiovascular effects of these peptides were very modest in conscious rats: we observed a maximum decrease in blood pressure of approximately 30 mmHg and a maximum increase in heart rate of approx. 20%. The great difference in the potency of these natural tachykinins, which share a common C-terminal part (same C-terminal tripeptide Gly-Leu-Met-NH_2 and a phenylalanyl residue in position 5 from the C-terminus), once again emphasizes that the different

amino acid sequence at the N-terminal part of the molecule may also play an important role in determining the degree of potency in different experimental conditions. This situation, which seems to be peculiar to the tachykinin family, was already predicted from experiments performed in our laboratory (Zséli, Molina, Zappia & Bertaccini, 1977; Zappia, Molina, Sianesi & Bertaccini, 1978; Bertaccini & Zappia, unpublished observations).

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