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Relative potencies of porcine bombesin-like heptacosapeptide (PB-27), amphibian bombesin (B-14) and litorin, and bombesin C-terminal nonapeptide (B-9) on in vitro and in vivo smooth muscle preparations

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The occurrence of bombesin-like peptides in avian and mammalian tissues, first demonstrated by Erspamer & Melchiorri (1975) and Erspamer et al (1979), has been widely confirmed. Recently two bombesin-like heptacosapeptides have been isolated from porcine non-antral gastric tissue and from chicken proventriculus, respectively (McDonald et al 1979, 1980). They possess the whole spectrum of activity of bombesin and alytesin and, with unimportant differences, have in common with these amphibian peptides the C-terminal decapeptide

-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
amphibian bombesin decapeptide
-Gly-Thr-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
amphibian alytesin decapeptide
-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
porcine bombesin decapeptide
-Gly-Ser-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
avian bombesin decapeptide
(homologous residues are underlined)

Previous studies (Broccardo et al 1975) have shown that the biological activity of bombesin resides in its C-terminal nonapeptide (the C-terminal octapeptide and even the heptapeptide possess some activity) and in accordance with these results it has been found that the essential requirement for the activity of the porcine heptacosapeptide is located in its C-terminal octapeptide fragment (Taché et al 1980).

Before its exact structure was recognized, the porcine bombesin-like peptide was named 'gastrin releasing peptide (GRP)', but throughout this paper it will be more properly indicated as porcine bombesin-27 (PB-27) in conformity with the nomenclature generally accepted for hormonal peptides.

In this study the relative potency of PB-27 synthesized by Yajima et al (1980) was compared with that of amphibian bombesin (B-14). Comparison was with nine in vivo and in vitro smooth muscle preparations. The bombesin C-terminal nonapeptide (B-9) and litorin were also included in the study.

Materials and methods

The bombesins were assayed in parallel on the following test preparations.

(a) Isolated smooth muscle preparations: rat uterus and large intestine (Tyrode solution at 32 °C); rat urinary bladder, guinea-pig large intestine and gall bladder, kitten small intestine (Tyrode solution + 0·1% glucose at 37 °C).

(b) Guinea-pig gall bladder in situ (urethane anaesthesia, 2 g kg^{-1} subcutaneously), guinea-pig urinary bladder and rat urinary bladder in situ (urethane anaesthesia, 1·5 g kg^{-1} intraperitoneally).

The contractions of isolated and in situ smooth muscle preparations were recorded isometrically by a strain-gauge transducer (DY 2, Basile, Milan; force up to 10 g) and displayed on a recording microdynamometer (Basile, Milan).

The porcine bombesin-27 was a gift of Prof. Yajima, Kyoto. The samples of bombesin, C-terminal nonapeptide of bombesin and litorin used were prepared by synthesis at the Farmitalia Carlo Erba Research Laboratories, Milan.

Results

In this series of experiments the threshold concentrations and doses of bombesin were as follows: rat uterus 0·12–0·3 nM, rat large intestine 0·6–1·8 nM, rat urinary bladder (isolated) 0·3–1·2 nM, rat urinary bladder (in situ) 0·03–0·12 nmol kg^{-1}, guinea-pig large intestine 0·12–0·6 nM, guinea-pig urinary bladder (in situ) 0·02–0·06 nmol kg^{-1}, guinea-pig gall bladder (isolated) 3–6 nM, guinea-pig gall bladder (in situ) 0·015–0·06 nmol kg^{-1}, kitten small intestine 0·6–2·4 nM.

Table 1 shows the potency of PB-27 on various smooth muscle preparations expressed as a percentage of that of

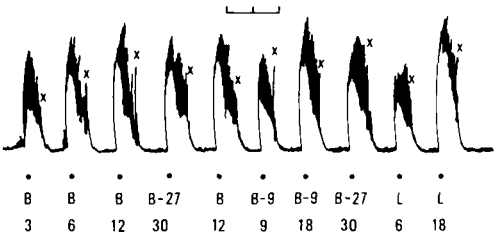

FIG. 1. Kitten isolated small intestine suspended in 10 ml Tyrode solution at 37 °C. The effects produced by different concentrations (nM) of porcine bombesin-27(B-27), amphibian bombesin (B), bombesin C-terminal nonapeptide (B-9) and litorin (L). In this experiment B-27 showed approximately 30–35% of the activity of B. Time marks, 5 min.

* Correspondence.

Table 1. The relative potency, on a molar basis, of porcine bombesin-27 (PB-27), expressed as a percentage of that of bombesin (taken as 100), on isolated and in situ smooth muscle preparations. Relative potencies of bombesin C-terminal nonapeptide (B-9) and litorin are shown for comparison.

Smooth muscle	No. of preps	Relative potencies PB-27	B-9	Litorin
Rat uterus	5	14–20	100–180	120–360
large intestine	4	20–40	60–80	180–480
urinary bladder (isolated)	4	8–12	100–180	100–300
urinary bladder (in situ)	4	10–15	60–120	60–120
Guinea-pig large intestine	4	10–18	60–80	120–300
urinary bladder (in situ)	5	20–40	100–150	60–120
gall bladder (isolated)	4	10–20	40–60	30–60
gall bladder (in situ)	4	20–30	60–80	120–180
Kitten small intestine	4	25–40	70–140	90–120

bombesin-14, taken as 100. Relative potencies of the C-terminal nonapeptide of frog bombesin (B-9) and of litorin are also shown.

It may be seen that PB-27 was invariably less potent than B-14, B-9 and litorin on all in vitro and in vivo smooth muscle preparations tested (Fig. 1).

In general the response to PB-27 was somewhat less rapid in onset than that to B-14, and even more so than that to litorin. Furthermore, upon washing with fresh physiological solution, relaxation was less prompt. A similar response was observed in the guinea-pig and rat urinary bladder in situ (Fig. 2). Tachyphylaxis frequently occurred, but was more intense for PB-27 than for B-14, B-9 or litorin.

Discussion

The present results corroborate the conclusion by Brown et al (1980) that PB-27 must be considered a mammalian counterpart of amphibian bombesin, and that the various pharmacological effects described using bombesin are relevant to mammalian physiology.

PB-27 was clearly less potent than B-14, B-9 and litorin on all smooth muscle preparations examined in this study and tachyphylaxis was more frequent. These data are in agreement with those of Brown et al (1980) and Taché et al (1980, 1981) showing PB-27 to be 10 to 15 times less active than B-14 in inhibiting gastric acid output, and 5 to 10 times less active in lowering body temperature and producing hyperglycaemia. The same investigators, on the other hand, reported that the C-terminal octapeptide of PB-27 had full activity in inhibiting gastric acid secretion.

The greater potency of the smaller bombesin peptides in comparison with the larger, is not unlike analogous observations concerning cholecystokinin-33 (CCK-33) compared with its C-terminal octapeptide (CCK-8), and raises the question of whether the active bombesin molecule released in birds and mammals by peptidergic nerves and by bombesin epithelial cells is actually bombesin-27 and not instead a smaller molecule.

In fact, it is generally accepted that bombesin is present in birds and mammals in various forms, of differing molecular size. In chicken proventriculus extracts, for example, bombesin-like peptides may occur as inactive pro-bombesin and as several active forms, one of which corresponding to the recently isolated heptacosapeptide, and another probably displaying a similar molecular size to that of the amphibian nonapeptide litorin (Erspamer et al 1979).

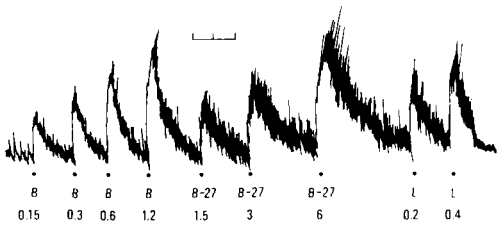

FIG. 2. Urinary bladder in situ of a guinea-pig anaesthetized with urethane (1·5 g kg^{-1}, intraperitoneally). The effects produced by different doses (nmol kg^{-1}) of porcine bombesin-27(B-27), amphibian bombesin (B), and litorin (L) given by intravenous route. In this experiment B-27 showed approximately 20% of the activity of B. Note, for B-27, the slower onset and the longer duration of action. Time marks, 5 min.

On the other hand, it has been found that trypsin digestion of ovine hypothalamic bombesin (of ca 32 amino acids) generates a bombesin-like peptide approximately equal in size to bombesin (Villareal & Brown 1978), and that a human pulmonary oat-cell carcinoma grown in nude mice contained an immunoreactive bombesin which may have a structure equivalent to that of the amphibian peptide (Erisman et al 1981).

It is, therefore, tempting to suggest that PB-27 is a precursor molecule from which a smaller, more agile peptide is split off when actions on the target organ are required.

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