J. Pharm. Pharmacol. 1980, 32: 497

COMMUNICATIONS

Parallel bioassay of litorin and Glu(OMe)^2-litorin on smooth muscle preparations and blood pressure

G. FALCONIERI ERSPAMER*, D. PICCINELLI, *Istituto di Farmacologia e Farmacognosia dell'Università di Roma, Città universitaria, 00100 Rome, Italy*

Methanol extracts of the skin of the Australian frog *Litoria aurea* contain, in addition to litorin, a second bombesin-like peptide which was identified as Glu-(OMe)^2-litorin. This differed from litorin only by the replacement of the glutamine residue present at position 2 in the litorin sequence with the γ-methyl-ester of glutamic acid (Anastasi et al 1977).

Pyr-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2 Litorin

Pyr-Glu(OMe)-Trp-Ala-Val-Gly-His-Phe-Met-NH_2 Glu(OMe)^2-litorin

Soon after elucidation of its structure Glu(OMe)^2-litorin was reproduced by synthesis (Mazzoli & de Castiglione 1977).

We describe the result of a parallel bioassay of litorin and Glu(OMe)^2-litorin on a number of in vitro and in vivo test-objects. It will be seen that Glu(OMe)^2-litorin closely mimicked litorin in its pharmacological effects, although there were some notable quantitative differences.

Materials and Methods. Litorin and Glu(OMe)^2-litorin were assayed in parallel on the following test preparations.

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

(b) Blood pressure of the dog (pentobarbitone anaesthesia, 30 mg kg^{-1} intravenously), the rabbit and the rat (urethane anaesthesia, 1·5 g kg^{-1} intraperitoneally);

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

The longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum was prepared as described by Gyang & Kosterlitz (1966), using the same modified Krebs bathing solution at 36 °C and the same electric stimulation (supramaximal, rectangular pulses of 0·5 ms duration at a frequency of 6 min^{-1}).

* Correspondence.

The motility of the isolated and in situ smooth muscle preparations was recorded on a smoked drum by means of an isometric microdynamometer (7001, U. Basile, Milan). The strain-gauge transducer most frequently used was DY2 (force up to 10 g).

More details on methods have been presented earlier (Erspamer et al 1972a, b, 1975; Endean et al 1975).

The samples of litorin and Glu(OMe)^2-litorin used were prepared by synthesis at the Farmitalia Carlo Erba Research Laboratories, Milan.

Results. In this series of experiments the threshold dose of litorin, taken as criterion of absolute potency, was as follows: rat uterus, 0·05–0·2 ng per ml bathing solution; rat colon, 0·3–1 ng ml^{-1}; rat stomach, 0·5–3 ng ml^{-1}; rat urinary bladder, 0·5–2 ng ml^{-1}; guinea-pig ileum, intact, 3–10 ng ml^{-1}; guinea-pig ileum, longitudinal muscle-myenteric plexus preparation, 20–40 ng ml^{-1}; guinea-pig large intestine, 0·3–1 ng ml^{-1}; guinea-pig urinary bladder, 0·05–0·3 ng ml^{-1}; guinea-pig gall bladder, 0·5–2 ng ml^{-1}; kitten small intestine, 0·2–0·5 ng ml^{-1}; kitten urinary bladder, 3–10 ng ml^{-1}; rabbit large intestine 0·2–2 ng ml^{-1}; man urinary bladder, 2–5 ng ml^{-1}; rat urinary bladder in situ, 20–50 ng kg^{-1} intravenously; guinea-pig gall bladder in situ, 3–10 ng kg^{-1}; rat blood pressure 5–10 ng kg^{-1}; rabbit blood pressure 10–40 ng kg^{-1}; dog blood pressure, 10–30 ng kg^{-1}.

Table 1. The activity of Glu(OMe)^2-litorin expressed as a percentage of that of litorin (taken as 100) on isolated smooth muscle preparations.

Smooth muscle	No. of preparations	Activity of Glu(OMe)^2-litorin
Rat uterus	7	90–150
colon	5	10–15
stomach	5	12–15
urinary bladder	9	90–130
Guinea-pig ileum, intact	5	spike contractions
ileum, longitudinal muscle	4	300–600
large intestine	5	5–15
urinary bladder	5	10–50
gall bladder	5	5–12
Kitten small intestine	9	2–6
urinary bladder	3	15–20
Rabbit large intestine	4	2–13
Man urinary bladder	5	6–22

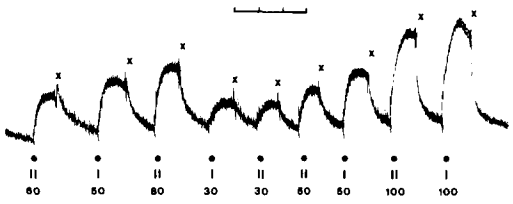

Fig. 2

FIG. 1. Isolated rat urinary bladder suspended in 10 ml Tyrode solution at 32 °C. The effects produced by graded doses (in ng) of litorin(I) and Glu(OMe)²-litorin (II). In this experiment II showed approximately 80% of the activity of I. Time marks, 5 min.

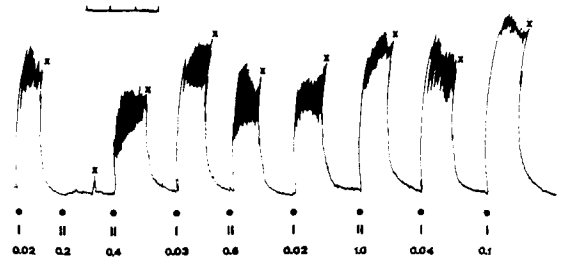

Fig. 1

FIG. 2. Kitten isolated small intestine suspended in 10 ml Tyrode solution at 32 °C. The effects produced by different doses (in μg) of litorin(I) and Glu(OMe)²-litorin(II). In this experiment II showed approximately 3–4% of the activity of I. Time marks, 5 min.

Table 1 shows the activity of Glu(OMe)²-litorin on various isolated smooth muscle preparations expressed as a percentage of that of litorin (taken as 100).

It may be seen that whereas Glu(OMe)²-litorin was as potent as litorin on the rat uterus and urinary bladder, it appeared considerably less potent on all other preparations tested. The guinea-pig ileum responded, as usual, with repeated spike contractions, often accompanied by increase in tone, but no comparison with litorin was possible, owing to tachyphylaxis.

However, when instead of the intact ileum a preparation of longitudinal muscle-myenteric plexus was used, tachyphylaxis was apparently less striking and Glu-(OMe)²-litorin was 3 to 6 times as potent as litorin in causing increase in tone. Atropine produced a sharp fall of tone when introduced into the bath during the action of Glu(OMe)²-litorin, and reduced the response to subsequent doses of the peptide, by 60–80% when given before it.

Figs 1 and 2 illustrate the effects of litorin on the rat isolated urinary bladder and on the kitten small intestine. On the in situ urinary bladder of the rat the activity of Glu(OMe)²-litorin was 70–100% of that of litorin (5 preparations), on the in situ guinea-pig gall bladder barely 5–15% (7 preparations).

Owing to the rapid appearance of tachyphylaxis, data on blood pressure are qualitative. In the dog, Glu-(OMe)²-litorin, unlike litorin which was hypertensive, caused a moderate fall. In the rat, Glu(OMe)²-litorin produced usually frank hypotension, but in some cases, like litorin, it caused a short-lived hypertension usually followed by hypotension. The rabbit responded to both peptides with hypotension. Both in the rabbit and the rat Glu(OMe)²-litorin displayed less than 30% of the potency of litorin.

Discussion. Parallel bioassay of litorin and its natural analogue Glu(OMe)²-litorin on smooth muscle preparations and blood pressure shows that apparently small changes in the peptide structure may bring about remarkable dissociations in the spectrum of activity. Most frequently the substitution of the GluOMe residue for the Gln residue at position 2 caused a reduction of potency, which was particularly evident for preparations of intestinal smooth muscle and for the in situ gall bladder. However, on the isolated longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum Glu(OMe)²-litorin was 3 to 6 times as active as litorin Of interest also is the fact that in all three species examined Glu(OMe)²-litorin was frankly hypotensive, which is at partial variance with litorin.

It has been recently shown that skin extracts of another Australian leptodactylid frog, *Uperoleia rugosa*, contain, in addition to Glu(OMe)²-litorin, another analogue, Glu (OEt)²-litorin (Nakajima et al 1979). This peptide has not yet been studied, but it appears probable that it possesses a spectrum of activity similar to that of Glu(OMet)²-litorin.

This study was supported by a grant from the Consiglio Nazionale delle Ricerche, Rome.

December 1, 1979

REFERENCES

Anastasi, A., Montecucchi, P., Angelucci, F., Erspamer, V., Endean, R. (1977) Experientia 33: 1289

Endean, R., Erspamer, V., Falconieri Erspamer, G., Improta, G., Melchiorri, P., Negri, L., Sopranzi, N. (1975) Br. J. Pharmacol. 55: 213–219

Erspamer, V., Falconieri Erspamer, G., Inselvini, M., Negri, L. (1972a) Ibid. 45: 333–348

Erspamer, V., Melchiorri, P., Sopranzi, N. (1972b) Ibid. 45: 442–450

Erspamer, V., Negri, L., Falconieri Erspamer, G., Endean, R. (1975) Naunyn-Schmiedeberg's Arch. Pharmacol. 289: 41–54

Gyang, E. A., Kosterlitz, H. W. (1966) Br. J. Pharmacol. 27: 514–527

Mazzoli, M., de Castiglione, R. (1977) Experientia 33: 990–991

Nakajima, T., Yasuhara, T., Falconieri Erspamer, G., Negri, L., Endean, R. Chem. Pharm. Bull. in the press