for 48 h. The lowest concentration of the agent inhibiting visible growth was regarded as the MIC.

Results and discussion

Tables 1 and 2 show the antimycotic activity of HY-1 compared with the other agents. Several isolates resistant to mycostatin, canesten and daktarin were obtained. About 5% of the isolates cross-resistant to mycostatin, canesten and daktarin were inhibited by 4% HY-1. H-115 and Jadit did not select any resistant strains.

The results indicated that all the test agents were effective against *Candida albicans*. HY-1 from this study seemed promising because about 90% of the *Candida albicans* isolates tested were inhibited by 2% v/v HY-1. All strains resistant to antimycotic agents were inhibited by 4% v/v HY-1.

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Parallel bioassay of physalaemin and hylambatin on smooth muscle preparations and blood pressure

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Hylambatin, a novel tachykinin endecapeptide isolated from the skin of the African frog *Hylambates maculatus*, must be ascribed to the physalaemin subfamily. It differs structurally from all other known tachykinins mainly in having a methionine residue replacing the usual leucine residue at position 2 from the C-terminus. In parallel bioassay on a number of in-vitro and in-vivo test objects, hylambatin and physalaemin were nearly indistinguishable from each other, with few moderate quantitative differences.

Methanol extracts of the skin of the South African rhacophorid frog *Hylambates maculatus* contain, in addition to a kassinin-like peptide, Glu^2,Pro^5-kassinin, a physalaemin-like dodecapeptide, hylambatin (Yasuhara et al 1981). This differs from physalaemin by substitution of several amino acids in the *N*-moiety of the molecule and the presence, at position 2 from the *C*-terminus, of a Met residue, replacing the usual Leu residue, common to all other known tachykinins, including substance P and neuromedin K (Kangawa et al 1983).

However, considering the primary structures of physalaemin and hylambatin it may be seen that, leaving apart the *N*-terminal asparagyl residue, six of the amino acids (in italic) constituting the molecule of hylambatin are found in an equivalent region of physalaemin, while the remaining five amino acids may represent simple base exchanges

Asp-Pro-Pro-*Asp-Pro*-Asp-Arg-*Phe-Tyr-Gly*-Met-*Met-NH₂* Hylambatin

Pyr-Ala-*Asp-Pro*-Asn-Lys-*Phe-Tyr-Gly*-Leu-*Met-NH₂* Physalaemin

We describe the result of parallel bioassay of hylambatin and physalaemin on a number of in-vitro and in-vivo test objects. It will be seen that the two peptides were nearly indistinguishable from each other by parallel bioassay, with few moderate quantitative differences.

Materials and methods

Hylambatin and physalaemin were assayed in parallel on the following test preparations.

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

(b) Rat urinary bladder in-situ (urethane anaesthesia, 1·2–1·5 g kg^{-1}, intraperitoneally).

(c) Blood pressure of the rabbit and the rat (urethane, 1·5 g kg^{-1}, intraperitoneally).

(d) Salivary secretion of the rat anaesthetized with urethane (1·2 g kg^{-1}, intraperitoneally) as described Lembeck & Starke (1968).

* Correspondence.

Table 1. The relative potency, on a molar basis, of hylambatin, expressed as a percentage of that of physalaemin (taken as 100) on isolated and in-situ preparations.

Preparation	No. of preparations	Potency of hylambatin
(a) Isolated preparations		
Rat large intestine	4	17–35
urinary bladder	4	85–105
Guinea-pig ileum, intact	4	75–105
ileum, longitudinal muscle	3	70–95
large intestine	4	100–110
urinary bladder	4	140–160
Rabbit large intestine	4	90–110
urinary bladder	4	45–80
(b) In-situ preparations		
Rat urinary bladder	4	80–110
blood pressure	3	80–110
salivary secretion	*	80–100
Rabbit blood pressure	4	40–130

* 16 groups of 3 animals each. In each animal two successive i.v. injections were administered.

In-vivo, peptides were always administered by rapid intravenous injection.

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}).

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).

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

Hylambatin (mol. wt 1457) was the natural peptide isolated as decribed by Yasuhara et al (1981), physalaemin (mol. wt 1265) was a synthetic product prepared at the Farmitalia Carlo Erba Research Laboratories, Milan.

Results

In this series of experiments the threshold concentrations and doses of physalaemin were as follows: rat urinary bladder 2·5–4 nM, rat colon 4–8 nM, guinea-pig ileum, intact, 0·15–0·3 nM, longitudinal muscle-myenteric plexus preparation, 0·25–0·4 nM, guinea-pig large intestine 1–4 nM, guinea-pig urinary bladder 1·8–8 nM, rabbit large intestine 0·15–0·4 nM, rabbit uterus 25–40 nM; rat urinary bladder in situ 25–70 pmol, rat blood pressure 15–30 pmol, rabbit blood pressure 1–3 pmol, rat salivary secretion 150–250 pmol.

Table 1 shows the potency of hylambatin on various isolated and in-situ preparations expressed as a percentage of that of physalaemin (taken as 100).

The relative potency has been determined in each

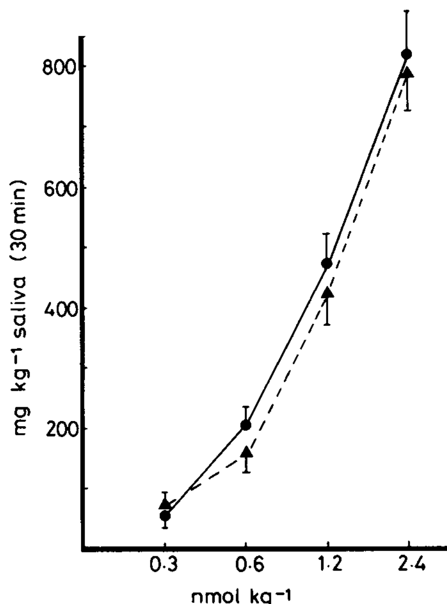

FIG. Salivary secretion of rats anaesthetized with urethane (1·2 g kg^{-1}, intraperitoneally). Dose-response curves of salivary secretion (in mg per 30 min) induced by physalaemin (●) and hylambatin (▲). Vertical bars represent s.e.m. of 6 experiments.

preparation using different doses of the two peptides. This was possible because hylambatin and physalaemin had the same efficacy, i.e. they produced always the same maximum response. In the case of desensitization of the preparation or appearance of moderate tachyphylaxis (guinea-pig and rat urinary bladder) the response was reduced to the same extent for the two peptides.

Fig. 1 presents a dose-response curve of salivary secretion induced, during 30 min, by physalaemin and hylambatin.

Discussion

Parallel bioassay of physalaemin and hylambatin on smooth muscle preparations, blood pressure and salivary secretion demonstrated a virtually complete superimposition of the activity spectrum of the two peptides, both from a qualitative and quantitative point of view. Exceptions were the rat colon, in which hylambatin was 3 to 5 times less active, and the guinea-pig urinary bladder in which it was 50% more potent than physalaemin.

Thus, hylambatin, like uperolein and Lys^5,Thr^6-physalaemin, should be included in the physalaemin subfamily of the tachykinins.

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The use of phenothiazines to enhance the rectal absorption of water-soluble compounds

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The ability of phenothiazines to enhance the rectal absorption of sodium cefoxitin and gentamicin sulphate from aqueous formulations was examined in rats. In the absence of absorption-promoting adjuvants, sodium cefoxitin and gentamicin sulphate bioavailabilities from the rectal compartment were less than 5% of the corresponding intravenous administration. In aqueous microenemas containing 20 mg ml^{-1} phenothiazine, sodium cefoxitin bioavailability increased to 16–62%, while gentamicin sulphate bioavailability increased to 74–146%. The absorption-promoting potential of chlorpromazine and perphenazine was concentration-dependent, with significant increases in gentamicin sulphate absorption occurring with 1 mg ml^{-1} chlorpromazine or 2·5 mg ml^{-1} perphenazine. Maximal gentamicin sulphate bioavailability and serum concentrations were achieved with 10 mg ml^{-1} chlorpromazine or 20 mg ml^{-1} perphenazine. The findings indicate that the phenothiazines, which are well absorbed rectally, also significantly enhance the rectal absorption of water-soluble, poorly absorbed compounds.

The use of the rectal compartment as a site for systemic drug absorption has been largely limited to well-absorbed compounds whose oral use is precluded for a variety of reasons. In recent years, considerable effort has been directed toward identifying agents which increase the rectal absorption of poorly absorbed compounds. Surface-active agents (George et al 1977; Yamasaki et al 1981), chelating agents (Cassidy & Tidball 1967), salicylates (Nishihata et al 1980, 1981a, b), anti-inflammatory drugs (Yaginuma et al 1981a) and basic amino acid salts (Yaginuma et al 1981b) have been shown to increase gastrointestinal permeability to a variety of compounds.

Although the mechanisms of action of these absorption promoters is unknown, direct effects on the biological barrier membrane is a distinct possibility. As such, compounds whose pharmacological activity involves alterations in cellular membrane function may be candidates as promoters of drug absorption across biological membranes.

The phenothiazines, a class of antipsychotic and antiemetic compounds, are well absorbed rectally (Moolenaar et al 1981) and are available in a number of rectal dosage forms (Byck 1975). Chlorpromazine, a classical phenothiazine, has been shown to modulate various membrane functions, including alterations in hepatocyte membrane permeability (Tsao et al 1982) and reversal of cholera toxin induced intestinal secretions (Holmgren et al 1978). Because of the observed membrane effects of various phenothiazines, the potential of chlorpromazine and related compounds to enhance the rectal absorption of poorly absorbed compounds has been examined.

Gentamicin sulphate and sodium cefoxitin were used as target drugs. Both are water-soluble antibiotics which exhibit negligible rectal absorption. Eight phenothiazine compounds were examined for absorption-promoting potential and the concentration-dependence of two representative phenothiazines was determined.

Materials and methods

Animal preparation

Adult male Sprague-Dawley rats (200–250 g) were fasted overnight with free access to water. Anaesthesia was induced by intramuscular injections of 0·5 ml 43% (w/v) ethylcarbamate per 100 g.

Table 1. Effect of phenothiazines (20 mg ml^{-1}) on the rectal absorption of sodium cefoxitin and gentamicin sulphate.

	Per cent bioavailability (mean ± s.d.)			
	Gentamicin sulphate		Sodium cefoxitin	
Phenothiazine	Bio-availability %	Serum peak ($\mu g\ ml^{-1}$)	Bio-availability %	Serum peak ($\mu g\ ml^{-1}$)
None	1 ± 0·5	0·8 ± 0·2	3 ± 2·1	1·4 ± 0·7
Perphenazine	100 ± 14·3	16·6 ± 1·3	62 ± 8·1	16·6 ± 2·2
Chlorpromazine	107 ± 10·7	20·1 ± 4·4	—[a]	—[a]
Thioridazine	115 ± 19·9	18·9 ± 1·7	38 ± 6·6	14·4 ± 3·2
Triflupromazine	146 ± 25·8	23·7 ± 3·6	58 ± 13·9	19·8 ± 4·4
Fluphenazine	143 ± 8·0	25·6 ± 2·7	49 ± 8·7	14·0 ± 2·4
Promazine	76 ± 6·0	11·7 ± 1·0	41 ± 11·7	13·5 ± 3·7
Trifluoperazine	74 ± 11·5	14·3 ± 1·9	68 ± 1·1	18·2 ± 0·3
Prochlorperazine	76 ± 18·9	18·2 ± 4·7	16 ± 6·4	6·7 ± 2·7

[a] Insoluble.
n = 3 animals.

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