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Occurrence of an Eledoisin-Like Polypeptide (Physalaemin) in Skin Extracts of *Physalaemus fuscumaculatus*[1]

Acetone and methanol extracts of wet or dry skin of *Physalaemus fuscumaculatus*, a South-American amphibian (Tucuman, Argentine), contain a principle which exerts a powerful stimulant action on several smooth-muscle preparations and potently lowers the systemic blood pressure in dogs and rabbits.

The active principle, which we call *physalaemin*, is in all probability a polypeptide. In fact, it was completely inactivated both by chymotrypsin and trypsin digestion (extract corresponding to 0.1 g fresh skin plus 100 μg chymotrypsin or 1 mg trypsin; incubation for 30 min at pH 7.5–7.7 and 38°C), and acid hydrolysis of active chromatographic spots yielded a mixture of amino acids.

Physalaemin may be distinguished in parallel assays from all other known polypeptides, including eledoisin[2]. However, eledoisin is the polypeptide to which physalaemin seems so far to be most similar in its pharmacological properties.

Like eledoisin, physalaemin potently stimulates movements and tonus of the isolated rabbit large intestine (threshold concentration: extract corresponding to 1–2 μg fresh tissue per ml nutrient liquid), the isolated guinea-pig ileum (2–6 μg fresh tissue/ml), and other preparations of gastro-intestinal smooth muscle as well (rat stomach, rat duodenum, frog stomach, etc.); like eledoisin, physalaemin is not very active on the oestrus uterus of the rat.

The blood pressure of the anaesthetized dog and rabbit is potently lowered by the intravenous injection of *Physalaemus* extracts. Intensity and duration of hypotension are proportional to the injected dose of physalaemin and there is no sign of tachyphylaxis. A short-lived but evident fall of blood pressure may be produced in the dog by the intravenous injection of the extract corresponding to 5 μg fresh skin per kg body weight.

The accompanying Table gives some approximate activity equivalencies for the extract corresponding to 1 g fresh *Physalaemus* skin.

	Approximate equivalencies for 1 g fresh *Physalaemus* skin			
	Eledoisin	Substance P	Bradykinin	Histamin
Dog blood pressure	300–400 μg	20000 to 30000 μ	>20 mg	> 20 mg
Rabbit large intestine	150–200 μg	50000 μ	>15 mg	>100 mg
Rat uterus	30–50 μg	50–200 μ	< 1 μg	inhibition
Rat duodenum	100 μg	- -	inhibition	—
Guinea-pig ileum	100–200 μg		0.5–2 mg	—

Rabbit large intestine, guinea-pig ileum and dog or rabbit blood pressure are particularly suitable for the quantitative bioassay of physalaemin, owing to their high sensitivity and the excellent dose/response relationship.

Crude *Physalaemus* extracts do not apparently contain other active substances with the possible exception of small amounts of a bradykinin-like polypeptide. A biologically pure physalaemin preparation may be obtained by absorption of the crude *Physalaemus* material dissolved in 95% ethanol on an alkaline alumina column and subsequent elution with descending concentrations of ethanol, followed by ion-exchange chromatography on a column of Amberlite CG-50.

The occurrence of physalaemin is now being investigated in other *Physalaemus* species, as well as in numerous other amphibians gathered throughout the world.

A full report on the pharmacological properties of physalaemin will be published elsewhere. The isolation of the polypeptide and the elucidation of its structure is in progress.

Riassunto. Gli estratti di pelle fresca o secca di *Physalaemus fuscumaculatus* contengono una sostanza attiva di natura polipeptidica, la *physalaemina*, dotata di potente azione ipotensiva e di intensa azione stimolante su alcuni muscoli lisci extravasali. La physalaemina è facilmente distinguibile, mediante saggi paralleli, da tutti gli altri polipeptidi biogeni finora noti, compresa la

eledoisina che alla physalaemina s'accosta per parecchie delle sue azioni farmacologiche.

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[2] V. ERSPAMER and A. ANASTASI, Exper. *18*, 58 (1962). – A. ANASTASI and V. ERSPAMER, Brit. J. Pharmacol., in press. – V. ERSPAMER and G. FALCONIERI ERSPAMER, Brit. J. Pharmacol., in press.