LETTERS TO THE EDITOR

Enhancement by physalaemin of the contractions induced by cholinomimetics in the guinea-pig ileum

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Physalaemin is an undecapeptide present in the skin of the South American amphibian *Physalaemus fuscumaculatus* (Anastasi, Erspamer & Cei, 1964). Its biological and chemical properties closely resemble those of the decapeptide phyllomedusin (Anastasi & Erspamer Falconieri, 1970) and the undecapeptides eledoisin (Anastasi & Erspamer, 1963) and substance P (Chang, Leeman & Niall, 1971). These substances are potent smooth muscle stimulants and have been termed collectively the tachykinins (Erspamer, 1971). At low concentrations crude preparations of substance P selectively enhance the contractile responses of guinea-pig isolated ileum to parasympathomimetic agents (Beleslin & Varagić, 1960), whilst the pure synthetic peptide potentiates the responses of this tissue to transmural electric field stimulation (Hedqvist & von Euler, 1975). Thus substance P may have important post- and prejunctional actions on the cholinergic system in addition to a direct spasmogenic effect.

We now present evidence that physalaemin also enhances the effects of cholinomimetics on intestinal smooth muscle. Four cm lengths of guinea-pig ileum were suspended in Krebs-Henseleit solution maintained at 37° and gassed with a mixture of 5% CO_2 in oxygen. Control contractions were elicited with acetylcholine (2 ng ml^{-1}), histamine (3 ng ml^{-1}), nicotine (1 μg ml^{-1}), 5-hydroxytryptamine (30 ng ml^{-1}) and with coaxial electrical stimulation (pulse width 0·5 ms, pulse strength 5–25V, frequency 0·1 Hz; Paton, 1955). After obtaining at least three constant contractions to an agonist, or constant responses to a 5 min period of electrical stimulation, a small concentration of physalaemin was added to the bath and the stimulus repeated 5 min later.

Significant reversible increases (median of 10 experiments, Wilcoxon test) compared to controls were observed (Figs 1 and 2) for contractions induced by acetylcholine (65·1%), nicotine (57·0%), 5-hydroxytryptamine (5-HT) (25·9%) and electrical stimuli (19·3%) in the presence of a low concentration of physalaemin (0·16 ng ml^{-1} $\simeq$ 1·3 10^{-10} M) which had only a very slight effect on the intestinal basal tone.

A further enhancement of these contractions was not observed by increasing the bath concentration of

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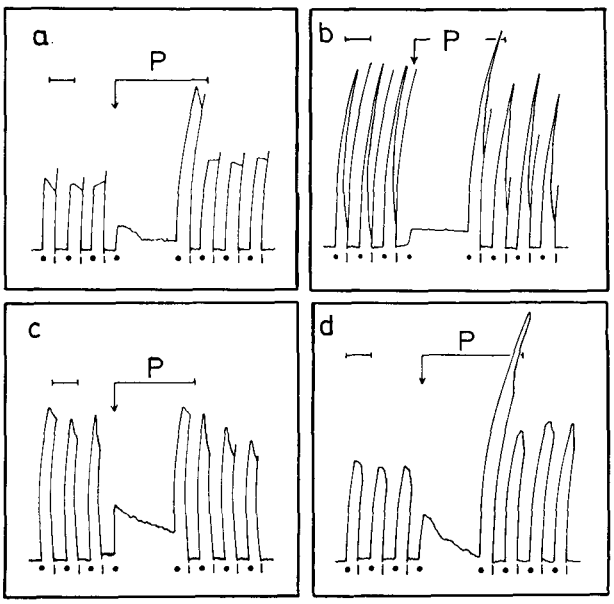

FIG. 1. The effect of physalaemin (P) (0·16 ng ml^{-1}) on guinea-pig ileum contractions to a-acetylcholine (2 ng ml^{-1}), b-5-HT (30 ng ml^{-1}), c-histamine (3 ng ml^{-1}), d-nicotine (1 μg ml^{-1}) added at ● and washed out at I. Time bar indicates 2 min.

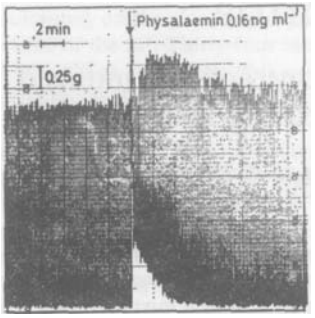

FIG. 2. The effect of physalaemin on guinea-pig ileum contractions to coaxial stimulation.

physalaemin. However, at these higher concentrations physalaemin caused marked contraction of the muscle by itself which masked the normal responses to electrical stimulation and drugs. At 1·25 ng ml^{-1} ($\simeq$ 0·9 10^{-9} M) of physalaemin, reversible increases were still observed for contractions to acetylcholine (14·6%) and nicotine (47·5%) but not to 5-HT.

Contractions to histamine were not potentiated by physalaemin at any of the concentrations tested (Fig. 1).

These observations show that physalaemin behaves like substance P and sensitizes ileal smooth muscle to cholinomimetics. The small increase in the size of the contractions produced by 5-HT was at variance with the depression of contractions of that agonist described by Beleslin & Varagić (1960) with crude substance P. Thus whether the mechanism of this sensitization observed with physalaemin is similar to that described for substance P remains to be determined.

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Evaluation of copper complexes as potential anti-arthritic drugs

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Recently copper aspirinate and copper-salicylate were compared with aspirin and several salicylates to evaluate the potential use of copper complexes as oral anti-inflammatory drugs (Rainsford & Whitehouse, (1976). Because it was felt that reported anti-inflammatory activity of a variety of Cu complexes (Sorenson, 1974) was at least partially due to irritation following subcutaneous administration, the anti-inflammatory and irritant activities of Cu aspirinate and Cu salicylate were determined after subcutaneous or oral administration. Results of those studies were interpreted as indicating that orally administered Cu salicylates may be no more effective than aspirin or salicylate and that irritation accounted for increased activity following subcutaneous dosing. However, I wish to caution against these interpretations in the light of the following considerations.

Anti-inflammatory studies. The use of the oral route and use, as a suspending agent, of acacia, which is acidic and also forms complexes with metals (The Merck Index), leaves open the possibility of partial or total Cu complex destruction before absorption and peripherial distribution. It was anticipated that gastric acid alone would cause at least partial destruction of these complexes so that the subcutaneous route was favoured (Sorenson, 1974). Acacia may have additionally prevented complex absorption by forming a quaternary complex or removing Cu from the complex, bringing about a release of the original complexing agent, i.e. aspirin or salicylic acid. If the existence of as much as possible of the free and intact complex in the dosage form is not assured it may not be possible to optimize the biological activity and adequately evaluate the hypothesis that Cu complexes are more active than the