The action of physalaemin on the peristaltic reflex of guinea-pig isolated ileum

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Physalaemin is an endecapeptide which has been extracted from the skin of a South American amphibian *Physalaemus fuscumaculatus* (Anastasi, Erspamer & Cei, 1964). It belongs to the group of tachykinins (Bertaccini 1971, Erspamer, 1971) characterized by a prompt stimulant action on the extravascular smooth muscle (Bertaccini, Cei & Erspamer, 1965). Its structure has been elucidated and confirmed by synthesis (Bernardi, Bosisio & others, 1964).

We have recently shown it to enhance the cholinergic responses of the guinea-pig isolated ileum (Fontaine, Famaey & Reuse, 1977), we now present the analysis of its effects on the peristaltic reflex of the same organ.

The peristaltic reflex of the guinea-pig isolated ileum was examined using a modified Trendelenburg method as described previously (Van Nueten, Geivers & others, 1973). An ileum segment is suspended in Tyrode solution [(mg ml^{-1}): KCl, 0·2; $CaCl_2.2H_2O$, 0·264; $MgCl_2.6H_2O$, 0·212; $NaHCO_3$, 1·0; $Na_2H_2PO_4.H_2O$, 0·0575; NaCl, 8·0; glucose, 1·0] at 37° gassed with 5% CO_2 in oxygen.

The peristaltic reflex was induced by raising a pressure bottle from −5 to 20 mm H_2O for a period of 200 s (called a run) at 7·30 min intervals. The coordinated contractions of longitudinal and circular muscles were measured as an increase in longitudinal tension (Grass force transducer) and intraluminal pressure (Statham pressure transducer) respectively. At the same time, the fluid volume expelled was measured using an ultrasonic transit-time device (Geivers, Van Nueten & others, 1974).

In some experiments the passive intraluminal pressure of 20 mm H_2O was maintained throughout the experiment: under these conditions the preparation becomes "fatigued" and the peristaltic reflex is intermittent (Van Nueten, Janssen & Fontaine, 1976).

In other experiments the intraluminal pressure was raised to 5 mm H_2O which is too low to induce peristaltic waves.

To find if physalaemin was able to reverse an inhibition produced by various antagonists, it was added to preparations previously inhibited by the antagonists. It was always added to the bath either during the peristaltic activity or 20 s before the fourth run of an experiment.

Physalaemin at low concentrations (0·63–1·25 ng ml^{-1}), on the serosal side, is active only on the longitudinal response of the peristaltic reflex. When added to the bath during peristaltic activity (3 experiments per dose), it enhanced the slow phase of the longitudinal reflex (see Fig. 1). The longitudinal tension increases at a lower intraluminal volume (vt'_a) and the values reached when active intraluminal pressure develops (atp_0) are higher. The relaxations of the longitudinal muscle remained unchanged and the residual tone due to a direct muscular effect of physalaemin was very small. On the other hand, the circular muscle responses (measured by means of active intraluminal pressures) were slightly decreased (P) in its presence and this did not modify the expulsion wave (see Fig. 1).

When physalaemin, at the same concentrations, was added before the passive intraluminal pressure is applied, the first peristaltic waves appeared at the same time as in other runs of the experiment and only the longitudinal reflex was modified as described above (3 exp. per dose). There was no change in the duration or the frequency of the peristaltic activity and thus in the total volume expelled.

At higher concentrations (5 ng ml^{-1} or more) physalaemin has a marked influence on the basal longitudinal tone, which interferes non-specifically with the peristaltic waves in the same way as high doses of acetylcholine (Fontaine, Reuse & Van Nueten, 1973).

The effects of cumulative concentrations of physalaemin (from 0·63 to 5 ng ml^{-1}) were also analysed in some preparations in which the peristaltic reflex was spontaneously irregular and here it had no regulating or stimulatory effect. On a "fatigues" preparation, it increased the slow longitudinal reflex and this did not consistently regulate the peristaltic activity (regulation in 1 out of 3 exp.).

When the passive intraluminal pressure was too low (+5 mm H_2O) to induce the peristaltic activity, physalaemin up to 5 ng ml^{-1} did not trigger cyclic peristaltic waves but only increased the longitudinal tone (3 exp. per dose).

Physalaemin up to 20 ng ml^{-1} was unable to restore normal peristaltic activity previously inhibited by the antagonists hexamethonium, morphine, procaine, dopamine, atropine, adenosine.

From our experiments it appears that at low concentrations, which have no sustained direct effect on the longitudinal muscle of the guinea-pig, physalaemin has a stimulating effect only on the slow part of the longitudinal reflex. There is no evidence of any effect on the circular muscle (besides a slight inhibition) or on the intramural nervous plexus involved in the aboral expulsion of the intraluminal fluid. This is in agreement with the results of Mantovani & Vizi (1974).

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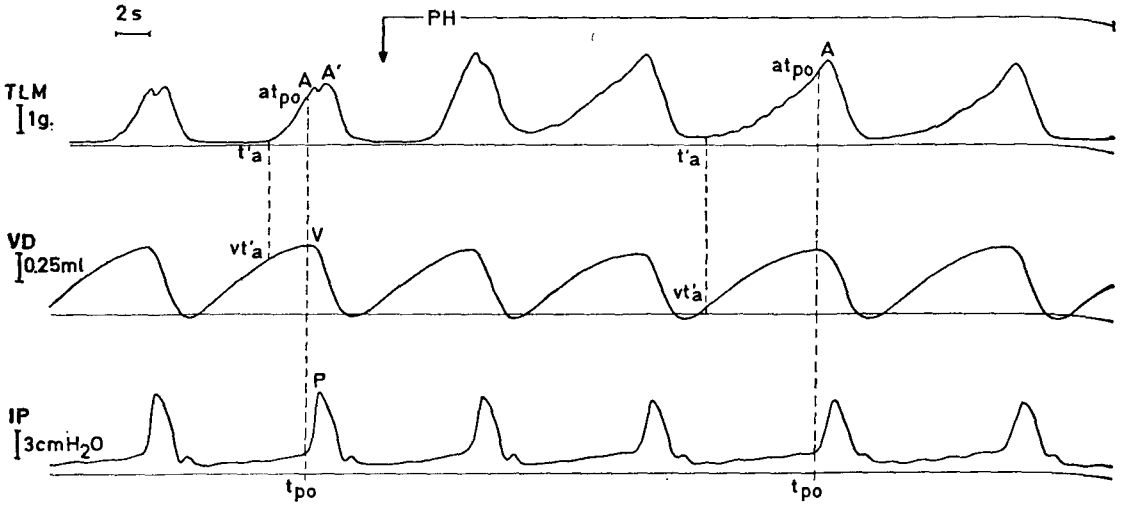

FIG. 1. Effect of physalaemin (PH) 1·25 ng ml^{-1} on the peristaltic reflex of the guinea-pig isolated ileum. TLM, tension of longitudinal muscle; VD, volume displacement, IP, intraluminal pressure. A and A', maximal TLM; V, maximal intraluminal volume; P, maximal active IP; tp_0, time when active IP develops; atp_0, TLM at tp_0; t'_a, time when TLM increases; vt'_a, intraluminal volume at t'_a.

Higher concentrations (5 ng ml^{-1} or more) produce a non-specific inhibition like that observed with any kind of longitudinal smooth muscle stimulant (Kosterlitz & Robinson, 1957; Fontaine, Reuse & Van Nueten, 1973).

The cholinergic nature of the slow longitudinal response of the peristaltic reflex is well known (Kosterlitz & Lees, 1964; Fontaine, Van Nueten & Janssen, 1973) and we have recently shown that physalaemin at low concentrations is able to increase the responses of the ileum to acetylcholine as well as to other indirect cholinergic stimuli (Fontaine & others, 1977). This cholinergic sensitization is not inhibited in the presence of tetrodotoxin, an aspecific inhibitor of nervous structures (unpublished results) and seems thus to be exerted at the muscular level itself.

A cholinergic sensitization has also been demonstrated for substance P, a tachykinin of mammalian origin, on the guinea-pig isolated ileum (Beleslin & Varagić, 1960) and more recently Euler & Hedqvist (1975) have shown that synthetic substance P is able to increase the responses of the ileum to coaxial stimulation.

We did not observe any consistent action of physalaemin on fatigued preparations as was described by Beleslin (1969) after serosal application of eledoisin, another structurally related polypeptide.

In conclusion, our observations have shown that, in the peristaltic reflex *in vitro*, the predominant effect of physalaemin is a stimulating action of the cholinergic component of the longitudinal muscle activity.

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The enterohepatic circulation of oxazepam-*O*-glucuronide in guinea-pigs

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Previous studies from this laboratory have shown that oxazepam given intravenously is present as a glucuronide in the bile of guinea-pigs in relatively large amounts (Bertagni, Marcucci & others, 1972). Since in this species oxazepam disappears at a relatively low rate from the blood, the existence of an enterohepatic circulation was suggested (Mussini, Marcucci & others, 1972; Garattini, Mussini & others, 1973b). This hypothesis can now be supported.

Twelve male albino guinea-pigs (400 g) were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p. as a solution of 20 mg ml^{-1}). After a midline incision the cystic bile duct was ligated and the bile duct was exposed and cannulated with two polyethylene tubes. One tube (external diameter 0·96 mm) was placed in the duodenum through the bile duct, the other tube (external diameter 1·27 mm) was directed towards the liver to facilitate bile collection. The two tubes were passed between muscle and skin, fixed at the tissues and joined by means of an external by-pass. Thus, the bile circulation could be either maintained or interrupted.

Oxazepam-*O*-glucuronide was purified from rabbit urine by two chloroform extractions and two passages through activated charcoal columns, followed by thin-layer chromatography on silica gel preparative plates as described in detail elsewhere (Marcucci, Bianchi & others, 1975). The indentity of the glucuronide was confirmed by means of gas chromatography-mass spectrometry (Marcucci & others, 1975).

For the determination of free and conjugated oxazepam in the tissues, bile and urine, a g.c. method previously described by Bertagni & others (1972) was used. The conjugated oxazepam is expressed as oxazepam released after incubation with β-glucuronidase.

The animals had a good recovery from the surgical procedure and their apparent normal behaviour had advantages over that of anaesthetized animals as anaesthesia reduces biliary flow (Roberts & Plaa, 1967), biliary excretion of a variety of compounds (Klaassen, 1970), intestinal peristalsis and the progression of intestinal contents. The last point was illustrated using a Velva Glo-Red 103-115 suspension as an indicator of the intestinal peristalsis (De Feo, Piccinelli & Silvestrini, 1971) (250 mg kg^{-1}) injected through the duodenum of 4 of the animals. By this means the progression of the intestinal contents in animals with the artificial biliary circulation was shown to be more than 4 times greater than in similarly prepared anaesthetized animals. After the surgical procedure the animals were kept in individual restraining cages with food and water freely available. Urine was collected during the experiments which lasted 3 h, after which the guinea-pigs were killed and the tissues taken for analysis. The guinea-pigs were injected through the cannula entering the duodenum with oxazepam-*O*-glucuronide at a dose corresponding to 5 mg kg^{-1} of oxazepam. After the injection, the animals were divided into two groups each of 4 animals. In one group (A) the circulation of bile was interrupted. The bile was collected from the liver side while normal bile, obtained from untreated animals, was infused toward the intestine at the usual rate of production (0·1 ml min^{-1}). In the other group (B) the bile circulation was normal.

Table 1 shows that during the 3 h of the experiment the guinea-pigs in Group A excreted in the bile 32% of the injected oxazepam-*O*-glucuronide while only about 15% was excreted in the urine. The animals with an intact biliary circulation (Group B) eliminated in the urine twice as much oxazepam-*O*-glucuronide (about 30%) as Group A. Consistent with this difference in the amount of oxazepam-*O*-glucuronide excreted is the

Table 1. *Percentage of the dose and concentration of oxazepam and oxazepam glucuronide 3 h after intraduodenal injection of oxazepam glucuronide (5 mg kg^{-1}) in 2 groups (A and B) of 4 guinea-pigs each.*

Oxazepam in	A (±s.e.)	B (±s.e.)
Bile % of dose conjugated	31·7 ± 1·0	—
Urine % of dose conjugated	15·7 ± 2·8	29·1 ± 3·5*
Blood μg ml^{-1} — free	0·21 ± 0·03	0·8 ± 0·03*
	0·08 ± 0·03	0·2 ± 0·08
Brain μg g^{-1} — conjugated	0·98 ± 0·03	4·57 ± 0·2*
Adipose tissue μg g^{-1} free	0·41 ± 0·07	2·12 ± 0·3*

* $P<0·01$ with respect to Group A.

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