Further observations on the relaxant effect of caerulein on the guinea-pig ileum

Caerulein, a polypeptide similar to cholecystokinin in structure and action (Erspamer, 1970; Bertaccini, 1971), has been shown (Mantovani, 1970) to relax guinea-pig isolated ileum previously contracted by histamine. However, no attempt was made to study the mechanism of the relaxation. Vizi, Bertaccini & others (1972, 1973) presented direct evidence that motor effect of caerulein on guinea-pig ileum is mediated via acetylcholine release but they failed to observe any relaxant effect of caerulein in the longitudinal muscle strip from the guinea-pig ileum. Therefore, it seemed worth investigating the possible relaxant effect of caerulein on the guinea-pig ileum previously contracted by histamine.

The technique of Trendelenburg (1917) with considerable modification was used to examine the effect of caerulein both on longitudinal and circular muscle layers of the same preparation. A sensitive pressure transducer connected to an electrical recording system of a polygraph was used to measure intraluminal pressure changes produced by the contractions of the circular muscle.

The responses of the longitudinal muscles were recorded by means of an isometric strain-gauge system connected to the second channel of the recording system.

A segment of ileum was set up in an organ bath of 10 ml capacity in Krebs solution at 36° and bubbled with 5% carbon dioxide in oxygen. In some experiments responses to "field" stimulation (Paton & Vizi, 1969) were recorded. Supramaximal (10 V cm^{-1}) square-wave pulses of 1 ms duration at a frequency of 10 Hz were delivered for 1 s every minute by stimulator.

The following drugs were used: acetylcholine chloride, morphine hydrochloride, synthetic caerulein and physalaemin, hexamethonium bromide, atropine sulphate, histamine dihydrochloride, eserine sulphate, tetrodotoxin. Except for caerulein, physalaemin and tetrodotoxin, the concentrations refer to the salts.

When the intraluminal pressure of the ileum was less than that needed to produce

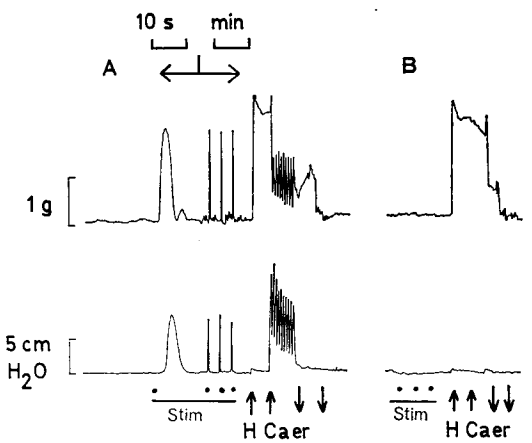

FIG. 1. Contractions of the longitudinal muscle (upper tracing) and intraluminal pressure (lower tracing) of the guinea-pig ileum in response to stimulation and to administration of caerulein. Organ bath, 10 ml Krebs solution; 95% O_2 + 5% CO_2.Stim., "field" stimulation (10 Hz for 1 s, 1 ms, 10 V cm^{-1}). Two different paper-speeds were used as indicated by time calibration marks. A. H, histamine (50 ng ml^{-1}); Caer, caerulein (1 ng ml^{-1}). Note that caerulein increased intraluminal pressure and relaxed the longitudinal muscle when the longitudinal layer was contracted by histamine. B. Tetrodotoxin, 500 ng ml^{-1}, is present in the bath. H, histamine (50 ng ml^{-1}); Caer, caerulein (1 ng ml^{-1}). Note that tetrodotoxin completely inhibited the effect of caerulein.

peristaltic reflex (20 mmH_2O), histamine (100–300 ng ml^{-1}), acetylcholine (10–50 ng ml^{-1}) or physalaemin (5–20 ng ml^{-1}), produced contractions of the longitudinal layer without affecting the intraluminal pressure. However, caerulein induced coordinated motor responses in both muscular layers of the ileum; there was an alternating increase in tension of the longitudinal muscle and in intraluminal pressure. The effective concentrations of the peptide, 0·1–1·0 ng ml^{-1} (7·5 to 75 $\times$ 10^{-11}M) were in the same range as those found by Vizi & others (1972, 1973) in longitudinal muscle strips of the guinea-pig ileum. Caerulein, administered into the bath while the longitudinal muscle layer was contracted by histamine (50 ng ml^{-1}), produced an increase in intraluminal pressure and the longitudinal muscle relaxed instead of contracting (Fig. 1A).

Tetrodotoxin (0·5–1 μg ml^{-1}; 5 expts) blocked the effect of caerulein (Fig. 1B). Atropine (6 expts), at a concentration of 2 μg ml^{-1}, was unable to abolish the effect of the peptide on circular muscle, however, the contractions of the longitudinal muscle layer were completely inhibited. Eserine (2–5 ng ml^{-1}; 6 expts) potentiated the motor responses to caerulein.

In conclusion, the results of the present experiments show that caerulein is very active in eliciting motor activity in the circular muscle of the guinea-pig ileum. The relaxant effect of caerulein (Mantovani, 1970) observed in whole ileum previously contracted by histamine may be related to the contraction of the circular muscle, and this contraction may cause a purely mechanical lengthening of the segment. A similar observation was made by Kosterlitz (1968) who showed that when a segment of guinea-pig ileum is shortened by acetylcholine or histamine and is then stimulated transmurally, the longitudinal muscle relaxes. However, an additional role of inhibitory nerves to the longitudinal muscle (Kosterlitz & Lydon, 1969; Kottegoda, 1969; Frigo, Lecchini & others, 1971; Del Tacca, Soldani & Crema, 1971) in relaxation cannot be excluded. The fact that tetrodotoxin prevented the effect of caerulein both on longitudinal and circular muscle layers provides evidence for excluding a direct action of this peptide on smooth muscle, a view already presented by Vizi & others (1972, 1973).

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