Experiments on the Mechanism of Action of Caerulein at the Level of the Guinea-Pig Ileum and Colon

by M. Del Tacca, G. Soldani and A. Crema
Department of Pharmacology, University of Pisa, Pisa, Italy

Abstract
The action of caerulein in guinea-pig ileum and colon was studied in vitro and in vivo. The action appears to be mediated mainly through nervous pathways, inasmuch as tetrodotoxin is able to abolish more than 90% of the response. One of the mediators liberated by caerulein seems to be acetylcholine, since caerulein gives rise to increased acetylcholine release associated with stimulation. Furthermore, it enhances the responses to pelvic nerve stimulation, whilst atropine causes a reduction in the responses. At present no data are available for determining the identity of the other mediator(s); however, serotonin and histamine seem not to be involved.

Caerulein (I) is a decapeptide present in the skin of Hyla caerulea and other Australian hylid frogs [1, 2], of Leptodactylus pentadactylus labyrinticus and related South American leptodactylid frogs, and of the South African amphibian Xenopus laevis [3]. The amino acid sequences reported below clearly show the striking resemblance in chemical structure existing between the caeruleins, the C-terminal octapeptide of porcine cholecystokininpancreozymin (II) and the C-terminal hexapeptide of gastrin II (III).

(I) Pyr-Gln-Asp-Tyr(SO$_3$H)-Thr-Gly-Trp-Met-Asp-Phe-NH$_2$
(II) -Asp-Tyr(SO$_3$H)-Met-Gly-Trp-Met-Asp-Phe-NH$_2$
(III)-Tyr(SO$_3$H)-Gly-Trp-Met-Asp-Phe-NH$_2$

Extensive pharmacological investigations have demonstrated that the chemical similarity is accompanied by a close resemblance in the pharmacological effects displayed by the caeruleins and the gastro-duodenal hormones (quoted by Erspamer [4]).

The action of caerulein on many parts of the intestinal tract has been investigated by Bertacchi et al. [5]. These authors concluded on the basis of the antagonistic activity demonstrated by atropine in some organs that the cholinergic system may be involved in some way in the action of caerulein. No further information is available on the mechanism of action of caerulein at the level of the gut. The aim of this study was to investigate, in the guinea-pig, the site and the mode of action of caerulein on the longitudinal muscle of ileum and colon.

Methods
Hukovic's preparation (quoted by Rand and Ridehalgh [6]) of the isolated guinea-pig colon with both the sympathetic and parasympathetic extrinsic nerves intact and similar to the preparation of the rabbit colon described by Garry and Gillespie [7] was used.

The terminal colon and a piece of ileum (length 2-3 cm) were removed from adult female guinea-pigs weighing 250-300 g. Both preparations were suspended vertically in a 10 ml bath containing oxygenated Tyrode solution at 35°C. Movements of the colon and ileum were recorded using a frontal isotonic lever with a magnification of 10 and exerting a tension of 2.5 g for the colon and 1.0 g for the ileum.

Bipolar electrodes made from rings of silver wire (2 mm apart) were placed around the nerves of the colon. Transmural stimulation of both organs was performed by inserting a silver wire into the lumen and by placing the reference electrode in the bath.

The preparations were left in the bath for about 120 minutes before beginning the experiments.

A few colons (4 experiments) were obtained from guinea-pigs which had been surgically denervated 4 days before the experiments. The inferior mesenteric ganglion was removed and the periarterial nerves frozen as described by Del Tacca et al. [8]. For measurement of acetylcholine release, guinea-pig ileum was incubated for 60 minutes with eserine sulphate (1 x 10$^{-5}$ g/ml) in organ baths containing 5 ml of oxygenated Tyrode solution. The experiments were carried out as follows: during the first period the preparation was stimulated for 3 minutes...
at the frequency of 20 c/sec, 1 msec duration and at supramaximal voltage. After an additional 3 minutes at rest without stimulation, the Tyrode solution was removed for the bioassay of acetylcholine. After washing, the preparation was left at rest for 6 minutes (2nd period), then the Tyrode solution was removed and its acetylcholine content reestimated. The first and second periods were taken as a trial cycle which was repeated three times for the measurement of acetylcholine release in the presence of caerulein (1 × 10⁻⁸ g/ml). The amount of acetylcholine released during 6 minutes at rest (as calculated from the second period of each cycle) was subtracted from the amount obtained during the first period of each cycle to calculate the actual release of acetylcholine associated with 3 minutes of stimulation. The acetylcholine content in the samples (diluted 1:1.4) was assayed on eserinized dorsal leech muscle against suitable standards according to MURNAGHAM [9]. The responses were abolished after boiling the samples at pH 10 for 3 minutes, or in the presence of (+)-tubocurarine (5 × 10⁻⁶ g/ml), confirming that the active substance released from the ileum was acetylcholine or a similar compound. The presence of interfering substances in the bath fluid was tested and excluded by adding a known amount of acetylcholine to the samples and by repeating the assay.

Four experiments were carried out on guinea-pig colon in situ according to the method described by SODANI et al. [10]. Briefly, female guinea-pigs (300 to 400 g) first anesthetized with ether, then with urethane (1 g/kg) and chloralose (10 mg/kg) in saline solution injected into the cannulated jugular vein, were laparotomized and the colon with attached pelvic nerves exposed. The proximal end of the colon was cut and injected into the cannulated jugular vein, were laparotomized and the colon with attached pelvic nerves exposed. The proximal end of the colon was cut and connected to an isometric transducer exerting a tension of 2.5 g. Pelvic nerves were stimulated by means of bipolar electrodes as described above. To avoid cooling and drying the exposed colon was superfused by a continuous flow of warm (37°C) oxygenated Tyrode solution.

The following drugs were used: acetylcholine chloride, hemicholinium bromide, triethylcholine chloride, choline chloride, nicotine acid tartrate, caerulein (Farmitalia), bradykinin (Sandoz), prostaglandins E₁, E₂ (Upjohn), atropine sulphate, hexamethonium bromide, histamine dihydrochloride, 5-hydroxytryptamine- creatinine sulphate, chlorpheniramine maleate, tetrodotoxin (Sankyo), propranolol hydrochloride, phenoxybenzamine hydrochloride, eserine sulphate, pentamethyipiperidine hydrochloride, +(-) tubocurarine chloride, methysergide maleate.

Except for tetrodotoxin, caerulein, bradykinin, and prostaglandins E₁, E₂, the concentrations refer to the salts.

Results

Isolated ileum

Caerulein produced a pronounced and sustained contraction of the longitudinal muscle, which rapidly relaxed after washing. The threshold concentration differed from one organ to another, but in all the experiments we used caerulein at 5 × 10⁻⁸ g/ml.

To avoid tachyphylaxis, a subsequent dose of caerulein was not added before at least 30 minutes had elapsed.

As reference drug, bradykinin was employed because it has been demonstrated to have a direct action on the muscle [11, 12, 13].

(a) Action of atropine

The antagonistic activity of atropine greatly varies from one organ to another. For this reason and for the easy development of tachyphylaxis to caerulein, we used atropine only at maximal concentrations supposed to be specifically antimuscarinic. In fact at 5 × 10⁻⁷ to 1 × 10⁻⁶ g/ml atropine reduced the response to caerulein. The mean degree of inhibition was 55.4 ± 8.1% (11 experiments) ranging from 22 to 89. PGE₁ and PGE₂ were inhibited by atropine by 32% ± 7.5 (4 experiments) while bradykinin was hardly affected.

(b) Action of eserine

After incubation with eserine (5 × 10⁻⁹ to 2 × 10⁻⁸ g/ml) the response to caerulein was potentiated by a mean value of 23.5% ± 6.1 (6 experiments), while the effect of bradykinin was unchanged. The residual response to caerulein after tetrodotoxin was also potentiated by eserine (Fig. 1).

(c) Action of tetrodotoxin

After tetrodotoxin (5 × 10⁻⁷ to 1 × 10⁻⁶ g/ml in 7 experiments) the response to caerulein was reduced by 94.7% ± 1.2 (Fig. 1). In 6 experiments the residual positive response was preceded by a small and short-lasting relaxation. Tetrodotoxin added in the presence of atropine caused a further large reduction of the response to 5% of the contraction obtained before atropine. However, the response to bradykinin was not significantly affected.

(d) Action of hemicholinium and triethylcholine

In presence of hemicholinium and triethylcholine (5 × 10⁻⁹ to 1 × 10⁻⁴ g/ml) the response to a single shock (0.5 msec duration, supramaximal voltage, 12 c/min) declined gradually to a near complete abolition in the course of 4 hours. At this point caerulein was no longer active. However both drugs (5 × 10⁻⁵ to 1 × 10⁻⁴ g/ml), after a contact period of 30–60', reduced the