EVIDENCE OF ASCENDING RELEASE OF ACETYLCHOLINE FROM THE LOCALLY DISTENDED GUINEA PIG ILEUM

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In the previous study (1), radial distension of isolated guinea pig ileum induced an augmented release of acetylcholine (ACh) from the distended part and its continuing anal part, but not from its oral part. Based on this study, we suggested the existence of long descending cholinergic fibers within the myenteric plexus and then proposed the significance of such cholinergic pathways in maintaining the polarity of peristaltic waves. Hirst and McKirdy (2) have shown the existence of descending cholinergic pathways by an electrophysiological technique. We cannot exclude, however, the possible existence of a short orally directed pathway since neither technique has allowed observations to be made to obtain evidence at a distance less than 1.5 cm from the stimulated region of the intestinal segment. When the intestinal wall was subjected to a local radial distension, contraction just oral to the distended region of the intestinal segment can be generally observed, and this was blocked by atropine; the contraction is, therefore, likely to be mediated by ACh (3, 4). In the present study, we examined whether evoked ACh release can be detected from the region just oral to the distended part.

Male guinea pigs weighing 500 to 600 g were killed by a blow on the head and bled. A piece of intestine about 6 to 4 cm long was taken from the distal part of the small intestine excluding its most distal 10 cm portion and mounted horizontally in a thermostatically controlled (36–38°C) organ bath which was separated into two compartments with a rubber diaphragm. Each compartment contained 5 to 10 ml of Mg-free Tyrode solution (composition, mM: NaCl, 136.9; KCl, 2.68; CaCl₂, 1.80; NaH₂PO₄, 0.41; NaHCO₃, 11.90; and glucose, 5.55; aerated with 95% O₂, 5% CO₂). Five μM eserine sulfate was added to the medium. The intestinal segment was threaded through a small hole which was made in the center of the diaphragm and then set up in such a position so that the oral half of the intestinal segment was in one compartment of the organ bath and the anal half was in the other (Fig. 1). Before obtaining a sample of bath fluid for estimation of ACh output, the intestine was left in the bath for 20 min. Thereafter, the bath fluid was changed with fresh medium containing eserine and incubation was further carried out for 10 min. During this period, both the anal and oral part of the intestinal segment was in the resting
(not distended) state. After collecting the fluid in the two compartments separately, each compartment was washed out quickly, fresh medium was added, and the preparation left in it for another 10 min. At the beginning of this period, the anal part of the intestinal segment was radially distended by inserting a glass rod of proper diameter, usually 7 mm. After 10 min, the glass rod was withdrawn, and all of the fluid in each compartment was collected separately. All the samples collected were kept ice cold until biological estimation of ACh. ACh was assayed on a longitudinal muscle strip of guinea pig terminal ileum as described previously (5). At the end of the experiments, the segment was divided into the distended and non-distended parts by cutting the segment at the position of the rubber diaphragm. Each part of the segment was weighed after blotting both sides of the wall with filter paper. The ACh release was expressed in ng/g tissue/min or as a percentage of the resting state of the corresponding region of the intestinal segment before local distension (resting release). The results were analyzed using the Student's t-test for paired and unpaired data. Where probability values are not given, “significant” means P>0.05.

In the first series of experiments, a small intestine of about 6 cm long was used and the length of the oral and anal part was set to about 3 cm. When any part of the intestinal segment was not subjected to radial distension throughout the incubation, the intestinal segment released fairly constant amounts of ACh over 40 min. The resting release of ACh during two runs of sequential 10 min (i.e. 20 to 30 min and 30 to 40 min period after the initiation of incubation) were 39.2±1.5 and 42.0±5.6 ng/g tissue (mean ±S.E. n=6) in the oral half, 32.5±5.7 and 39.2±4.1 ng/g tissue (n=6) in the anal half. When the anal part of the intestinal segment was distended, the release of ACh from the distended part increased to 152.1±7.1% of the resting release, and that from the continuing oral half was only 108.0±4.1% (Fig. 2A). This is consistent with the results obtained in the previous experiments (1). In another series of experiments in which an intestinal segment of about 4 cm long was used and the length of the oral part was set to 1 cm, however, the release of ACh from the oral part during anal distension significantly increased (125.0±3.7% of resting release, P<0.05) (Fig. 2B). This value is also significantly greater (P<0.02) than the 108.4% obtained in the above mentioned experiments in which the length of the oral part was set to about 3 cm. When tetrodotoxin (1.5 μM) or hexamethonium (180 μM) was applied exclusively on the oral part at 10 and 20 min before distension, respectively, the augmented ACh release at the site oral to the distended part was abolished in both cases (Fig. 2B).
Previously, we have demonstrated that distension of the intestinal wall excited the myenteric plexus and thus increased the release of ACh (6). In the present study, the augmented release of ACh in the oral part induced by anal distension may reflect the excitation of cholinergic pathways which extend in the oral direction in the myenteric plexus. At least one nicotinic synapse may be involved in each pathway since the augmented oral release of ACh was blocked by hexamethonium. The evidence that the augmented release of ACh was clearly observed when the length of the oral part was about 1 cm, but not clear when the length was about 3 cm suggests that the synaptic pathways directing orally are excited within a very limited region from the radially distended part of the intestine. This idea is supported by the electrophysiological studies of conduction in the Auerbach's plexus of guinea pig ileum (7) in which the excitatory potentials were recorded only when the distance between the stimulating and recording electrodes were less than 10 to 15 mm. The next question is why the orally directed cholinergic synaptic pathways are functional within a very short distance compared with the anally directed one. There are at least two possible explanation for this: a) in the myenteric plexus, short cholinergic synaptic pathways extend in the oral direction and long pathways in the anal direction and b) the existence of inhibitory modulation of transmission by adrenergic nervous elements. With respect to the first explanation, we can not discuss its likelihood since, to our knowledge, there has been no study in which a morphological difference of the anally and orally directed myenteric neurons was demonstrated. It is conceivable that ascending release of ACh during the local distension may be modulated presynaptically through α-adrenergic receptors (8–11). The investigation of this possibility, however, need further experiments.

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