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Activation of submucosal but not myenteric plexus of the gastrointestinal tract accompanies reduction of food intake by camostat

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ABSTRACT

It has been shown in the rat that endogenous cholecystokinin (CCK), released in response to the non-nutrient trypsin inhibitor camostat, reduces food intake at meals and increases Fos-like immunoreactivity (Fos-LI; a marker for neuronal activation) in the dorsal vagal complex (DVC) of the hindbrain but not the myenteric plexus of the duodenum and jejunum. *Experiment 1:* We examined Fos-LI in the myenteric and the submucosal plexuses of the gut in response to orogastric gavage of camostat in rats. As we reported previously, camostat failed to increase Fos-LI in the myenteric plexus. We show here that camostat increased Fos-LI in the submucosal plexus of the duodenum and jejunum. Camostat also increased Fos-LI in the DVC. *Experiment 2:* Pretreatment with devazepide, a specific CCK_1 receptor antagonist abolished camostat-induced Fos-LI in the submucosal plexus and the DVC. *Experiment 3:* Bilateral subdiaphragmatic vagotomy reduced camostat-induced Fos-LI in the submucosal plexus approximately 40% and abolished it in the DVC. *Conclusions:* Activation of the submucosal plexus by cholecystokinin at the CCK_1 receptor accompanies stimulation of the dorsal vagal complex of the hindbrain and inhibition of food intake. Unlike the submucosal plexus, activation of the myenteric plexus is not necessary for cholecystokinin's influence on the dorsal vagal complex and food intake. The lack of activation in the myenteric plexus after camostat stimulation, in contrast to nutrient releasers of CCK such as oleate, suggests that intestinal stimulants can either release different amounts of CCK or cause release of CCK from I cells with different molecular forms of CCK. This would suggest that CCK-8 is released by camostat and is not able to travel to the myenteric plexus while a more stable form of CCK such as CCK-58 can travel to this site that is further away from the I cell.

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1. Introduction

We have investigated a possible role for the enteric nervous system (ENS) of the gut in the reduction of food intake by cholecystokinin (CCK). The ENS consists of two plexuses, myenteric and submucosal. We recently reported that camostat, a non-nutrient trypsin inhibitor that releases endogenous CCK, activates the dorsal vagal complex (DVC) of the hindbrain, but fails to activate the myenteric plexus of the gut [1]. This is unlike oleate, a nutrient that releases endogenous CCK and stimulates both myenteric and submucosal plexuses [2,3]. Here, we test whether endogenous CCK released by camostat stimulates the submucosal plexus of the intestine.

The currently accepted scheme for the reduction of food intake by CCK states that following the ingestion of a meal luminal nutrients cause the endocrine I cell of the upper small intestine to secrete cholecystokinin (reviewed in [4]). After its release, CCK activates vagal afferents, which terminate in food control areas in the dorsal vagal complex of the hindbrain such as area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV) to reduce food intake. Ritter and colleagues [5] postulated further that CCK first activates the ENS of the gastrointestinal tract, which in turn activates the vagal afferents that terminate in the DVC.

The role of the vagal afferents in the stimulation of the DVC and the reduction of food intake by CCK is well-established [6,7]. For the ENS, however, the role of the submucosal plexus in the reduction of food intake by CCK has not been differentiated from that of the myenteric plexus. In the past, this differentiation did not seem important because oleate (a nutrient releaser of endogenous CCK) and exogenous CCK-8 activate both myenteric and submucosal plexuses [7,8].

This is the first report to examine activation of the submucosal plexus by the CCK releaser camostat. To define this activation, we quantified Fos-like immunoreactivity (Fos-LI), an activation marker [9,10], in the submucosal plexus of the duodenum and jejunum. The results may support a role for the submucosal plexus in the reduction of food intake by endogenous CCK. They suggest that endogenous CCK released by camostat acts in a paracrine fashion, stimulating CCK_1 receptor in the submucosal plexus, but not in the myenteric plexus.

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2. Materials and methods

2.1. Experimental procedures

Sprague Dawley rats (250–350 g) were housed individually in wire-mesh cages in a controlled environment: lights were on from 0600 to 1800 h, temperature was maintained at 21.5 °C, and rats were given free access to water and pelleted rodent chow (*Teklad, WI*). In order to adapt the animals to the experimental protocol and laboratory environment, each rat was handled daily for 10 min, and orogavaged with double distilled water (ddH_2O) twice a week for two weeks.

Rats were fasted beginning at 1800 h on the day prior to all experiments. All injections were made in a volume of 0.5 ml of vehicle (saline or 1% dimethyl sulfoxide [DMSO]) and given intraperitoneally (i.p.). All orogastric gavages were made in a volume of 3.5 ml of ddH_2O.

2.2. Experiment 1

2.2.1. Effect of camostat on Fos-LI of myenteric and submucosal plexuses and DVC

Sixteen rats were divided into two groups (n=8 rats per group). One group received camostat (Camostat mesilate, [FOIPAN: *N*,*N*-dimethylcarbamoylmethyl-4-(4-guanidinobenzoyloxy) phenylacetate monomethanesulfonate], *Ono Pharmaceuticals Japan*, 200 mg/kg) by orogastric gavage, and the other group received ddH_2O. The two groups were sacrificed with an overdose of sodium pentobarbital (100 mg/kg, i.p.) at 105 min post gavage, the optimal time point for producing maximum Fos expression in response to camostat [1].

2.3. Experiment 2

2.3.1. Effect of devazepide, a CCK_1 receptor antagonist, on Fos-LI of submucosal plexus and DVC induced by camostat

Sixteen rats were assigned to four treatment groups (n=4 per group). Two groups received the CCK_1 receptor antagonist, devazepide (*ML laboratories, Leicester, England*, 1000 μg/kg in 0.5 ml of 1% DMSO) followed 15 min later by an orogastric gavage of camostat (200 mg/kg) or ddH_2O. The remaining two groups received 1% dimethyl sulfoxide (DMSO) i.p. followed 15 min later by an orogastric gavage of camostat or ddH_2O. For maximum Fos expression, based on previous experience [1], all rats were euthanized with an overdose of sodium pentobarbital (100 mg/kg, i.p.) 105 min following the gavage of camostat or ddH_2O.

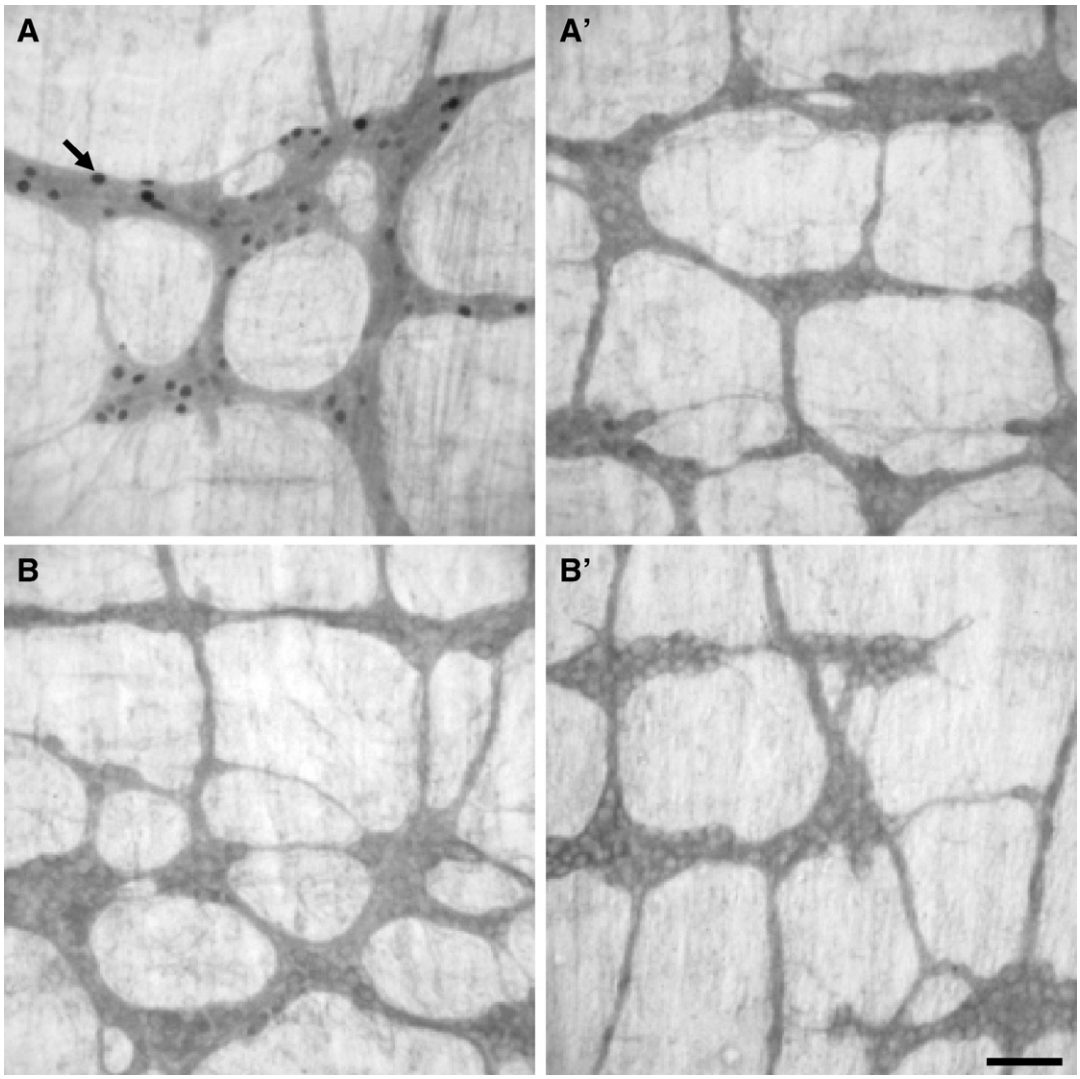

Fig. 1. Photomicrographs of the myenteric plexus of the duodenum from CCK-8-treated (A), saline-treated (A′), camostat-treated (B) and ddH_2O-treated animals (B′). CCK-8 increased Fos-like immunoreactivity in the myenteric plexus compared to saline vehicle; camostat did not. Scale bar=20 mm.