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5-Hydroxytryptamine Induces Electrogenic Secretion in the Duodenum of Gerbil (*Gerbillus cheesmani*)

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Abstract: The effect of serosally added 5-hydroxytryptamine (5-HT 100 μM) on the short circuit-current (Isc) across duodenum taken from fed, starved (4 days, water ad lib) and undernourished (50% control food intake for 21 days) gerbils (G*erbillus cheesmani*) were investigated. The effect of the neurotoxin, tetrodotoxin (TTX 10 μM) and atropine (100μM) on the maximum increase in Isc induced by 5-HT were also studied. The 5-HT-induced Isc were higher in unstripped than in the stripped sheets in the three feeding conditions. TTX reduced the maximum increase in Isc induced by 5-HT across stripped and unstripped sheets taken from fed, starved and undernourished gerbils. Atropine decreased the 5-HT-induced Isc of stripped sheets in the three feeding conditions and it also decreased the 5-HT-induced Isc in unstripped sheets in fed duodenum. Therefore, the duodenal response to 5-HT occur partly by activation of a nonneural pathway and partly by activating electrogenic ion transport via muscarinic neural mechanism. It also showed that the 5-HT-induced Isc was chloride-dependent in fed duodenum and were chloride and bicarbonate dependent in the duodenum taken from starved and undernourished gerbil The results also showed that the increase in 5-HT-induced Isc as a results of starvation and undernourishment were TTX-sensitive and both chloride and bicarbonate dependent.

Key words: Short circuit-current, starvation, tetrodotoxin, undernourishment

INTRODUCTION

5-hydroxytryptamine and its receptors were found both in the central and peripheral nervous system, as well as in a number of nonneural tissues such as gastrointestinal tract. 5-HT has many possible sites of action within the intestine[1] and there were regional variations in the way the intestine respond to 5-HT challenge[2,3]. The intestinal secretion in response to luminal distension and feeding were mediated at least in part by the ENS, involving the neurotransmitters substance P and acetylcholine and also the release of 5-HT from enterochromaffin cells[4,5]. In two previous studies we investigated the effect of 5-HT on the Isc in both the small[6] and the large intestine of gerbil[7]. Therefore, we thought that it may be interesting to investigate the effect of 5-HT on the Isc across the gerbil duodenum.

MATERIALS AND METHODS

Animals and diets, Gerbils (*Gerbillus cheesmani*) of both sexes, body weight 36-40 g, were captured from the desert in the State of Kuwait and kept in the animal house for at least three weeks before use. Three nutritional groups were used. The fed groups had free access to water and food (SDS rodent diet, Essex, England) and were held in rooms maintained at 27 ± 2°C. The lights were on from 5 am until 5 pm and the humidity was 50%. For the starved groups, water was given ad lib but the food was removed 4 days before the animals were used. The chronically undernourished group was housed in individual cages and was fed 50% of the control food intake for 21 days. Animals were housed routinely in plastic cages with wired mesh bottoms to reduce coprophagy. Animal procedures were approved by the Kuwait University Research Administration.

Methods On the day of use, animals were anaesthetised with thiopentone sodium (30 mg/kg body weight, ip). When surgical anesthesia was achieved, a mid-line incision was made along the abdomen and the entire duodenum (3cm) was removed and flushed with 0.9% NaCl. The duodenum was used either intact (unstripped preparation) or with the outer smooth muscle layers removed (stripped preparation).Stripping removes the myenteric plexus as well as the muscle coat but leaves intact the submucosal and mucosal plexus[8]. The duodenum was then cut open and mounted as a flat sheet between two plates over an aperture creating an exposed tissue area of approximately 0.42 cm^2. The plates were clamped

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between two Perspex chambers and the measurements of the short circuit-current (Isc in microamps) across the tissue was obtained as described in[9]. The chambers (7.5 mL) were filled with bicarbonate saline[10] (pH7.4) which contained (mM) 143Na, 125.7 Cl^-, 24.9 HCO_3^-, 5.9 K^+, 2.5 Ca^{2+}, 1.2 H_2PO^-, 1.2 Mg^{2+}. The medium was maintained at 38°C and gassed with humidified 95% O_2, 5% CO_2. The serosal fluid contained initially 10mM glucose and the mucosal 10mM mannitol (to maintain osmotic balance). The mounted tissue was to stabilize for 10 min before experiments were conducted.

5-HT (100 μM) was added to the serosal solution and then the maximal increase in the Isc was monitored. In some experiments tetrodotoxin (10 μM) or atropine (100 μM) was added to the serosal solution 10 min before the addition of the 5-HT with the control sheets receiving an equivalent volume of vehicle. In experiments investigating the ion replacement, all the chloride ions in the bicarbonate saline were replaced with isoosmolar solutions of sodium, potassium and calcium gluconate. In the bicarbonates free conditions, Krebs phosphate buffer oxygenated with 100% O_2 was used on both sides of the tissue. All gerbils were killed by thoracotomy at the end of the experiments. All chemicals were purchased from Sigma Chemical Co Ltd.

Statistical analysis: All data are expressed as means ± SE . The data were assessed for statistical analysis using ANOVA. Asignificant difference was considered at $P < 0.05$ (*post hoc* Student-Newman-Keuls test).

RESULTS

Effect of starvation and undernourishment on the maximum response in Isc generated by 5-HT: The maximum increase in Isc which resulted from addition of 5-HT (100μM) to the serosal bathing solution are shown in Fig. 1. The maximum increase in Isc generated by 5-HT was significantly ($P<0.01$) higher using unstripped sheets under the three feeding conditions. Starvation and undernourishment increased significantly the maximum increase in Isc induced by 5-HT in duodenum using stripped sheets only.

Effect of replacing chloride by gluconate on the maximum response in Isc generated by 5-HT: Replacing chloride in the bathing buffer by gluconate decreased significant the maximum increase in Isc induced by 5-HT in the duodenum taken from fed, starved and undernourished gerbils (Fig. 1). Moreover, the increase in 5-HT-induced Isc which resulted from starvation and undernourishment in the duodenum disappeared in absence of chloride.

Effect of removing bicarbonate from bathing buffer on the maximum response in Isc generated by 5-HT: Removing bicarbonate from the bathing buffer decreased significantly the maximum increase in Isc induced by 5-HT in the duodenum taken from starved

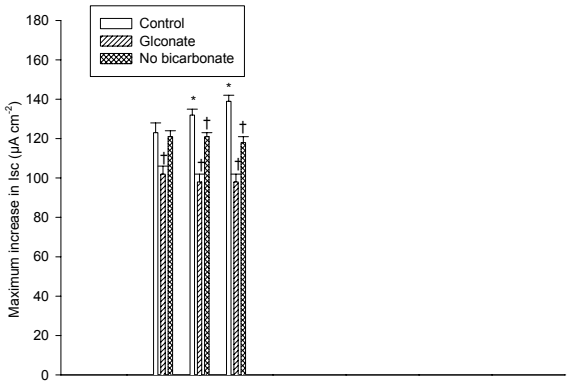

Fig. 1: Effects of replacing chloride ions by gluconate or removing bicarbonate ions from bathing buffer on the maximum increase in Isc induced by 5-HT across stripped sheets of duodenum taken from fed, starved and undernourished gerbils. Results are shown as the mean ± SE. * Compared Isc of starved and undernourished with fed. † Compared replacing chloride ions by gluconate or removing bicarbonate from bathing buffer with normal buffer. Numbers animals were 5 for test group and 6 for control

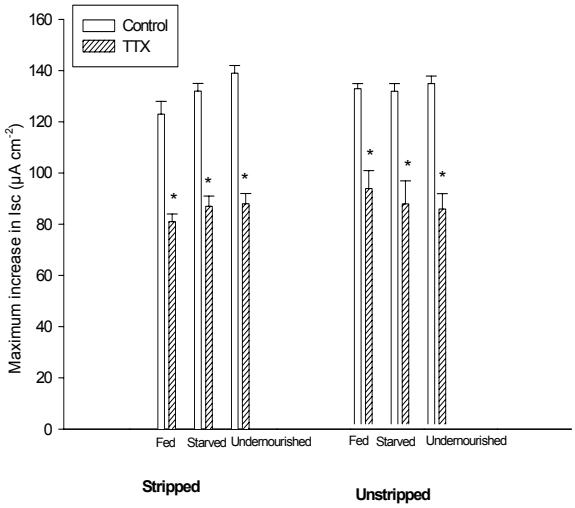

Fig. 2: Effects of TTX (10μM) on the maximum increase in Isc induced by 5-HT across stripped and unstripped sheets of duodenum taken from fed, starved and undernourished gerbils. Results are shown as the mean ± SE. * Compared Isc in presence with absence of TTX in the same feeding condition. † Compared unstripped with the stripped sheets taken from fed animal. Numbers of animals were 5 for test group and 6 for control

and undernourished gerbils (Fig. 1). Moreover, the increase in 5-HT-induced Isc which resulted from starvation and undernourishment in the duodenum disappeared in absence of bicarbonate.

Effect of TTX on the maximum responses in Isc generated by 5-HT: The effect of TTX (10μM) placed in the serosal bathing solution, 10 min before the addition of 5-HT on the maximum Isc generated by 5-HT is shown in Fig. 2. TTX reduced significantly the maximum increase in Isc induced by 5-HT both using stripped and unstripped sheets in duodenum under the three feeding conditions. In the presence of TTX the increase in 5-HT-induced Isc as a result of starvation and undernourishment disappeared.

Effect of atropine on the maximum response in Isc generated by 5-HT: Atropine (100μM) in the serosal side reduced significantly the maximum increase in Isc generated by 5-HT in fed animal using both stripped and unstripped sheets and the percentage decrease using unstripped sheets were double that of the stripped ones. Moreover, the increase in 5-HT-induced Isc which resulted from starvation and undernourishment disappeared in the presence of atropine.

DISCUSSION

The 5-HT-induced Isc was significantly higher in the unstripped sheets, indicating that in the gerbil duodenum in addition to the submucosal plexus, there is a contribution of myenteric plexus in the 5-HT-induced Isc. This disagreed with the result in the small[7] and large[8] intestine where 5-HT-induced Isc where not significantly different in stripped and unstripped sheets. It is of interest to mention here that, Fujimiya *et al.*[11] showed that serotonin-immunoreactive (5HT-IR) neurons located in the myenteric plexus projected fibers widely in the rat GI tract. In fed duodenum TTX reduced significantly the maximum increase in Isc induced by 5-HT both using stripped and unstripped sheets. Replacing chloride in the bathing buffer by gluconate decreased significantly the maximum increase in Isc induced by 5-HT in the fed duodenum. In fed jejunum and ileum[7] replacing chloride by gluconate had no significant effect while only in proximal colon, but not in the mid and distal colon, replacing chloride by gluconate decreased the 5-HT-induced Isc[8]. It seems that the gerbil duodenum behaves more or less like proximal colon. On the other hand, removing bicarbonate from bathing buffer had no significant effects on 5-HT-induced Isc. This is in agreement with the results from the small and large intestine. Therefore, the response of fed duodenum to 5-HT was the function of both submucosal and myenteric plexus and it was TTX-sensitive and chloride-dependent.

Atropine reduced significantly the maximum increase in Isc generated by 5-HT and the percentage decrease using unstripped sheets were double that of stripped one. Although TTX and atropine reduced 5-HT-stimulated duodenal Isc responses, they did not completely abolish them. This indicates that the duodenal response to 5-HT occur partly by activation of a nonneural pathway, probably involving a direct

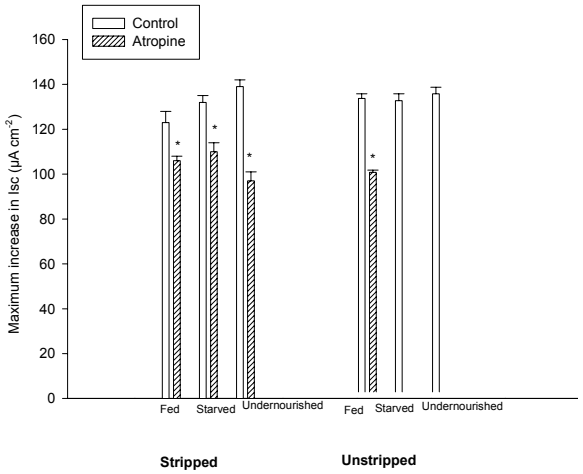

Fig. 3: Effects of atropine on the maximum increase in Isc induced by 5-HT across stripped and unstripped sheets of duodenum taken from fed, starved and undernourished gerbils. * Compared Isc in presence with absence of atropine in the same feeding condition. Results are shown as the mean ± SE. Numbers animals were 5 for test group and 6 for control

interaction with duodenocytes and by activating electrogenic ion transport via muscarinic neural mechanism. This is in agreement with results of Tuo & Isenberg[12] who found that 5-HT stimulated duodenal Isc in a dose-dependent manner and pretreatment with TTX and atropine reduced 5-HT-stimulated duodenal Isc.

Similar to the fed condition the 5-HT-induced Isc in duodenum taken from starved and undernourished gerbil were significantly higher in the unstripped sheets. TTX and atropine cause a significant reduction in 5-HT-induced Isc in the duodenum taken from starved and undernourished animals. Replacing chloride in the bathing buffer by gluconate decreased significantly the maximum increase in Isc induced by 5-HT in the duodenum taken from starved and undernourished

gerbils. However, different from that in the fed duodenum removing bicarbonate from bathing buffer decreased significantly the 5-HT-induced Isc in starved and undernourished gerbils. This indicates that the 5-HT-induced Isc in the duodenum taken from starved and undernourished gerbil were chloride and bicarbonate dependent.

Starvation and undernourishment increased significantly the maximum increase in Isc induced by 5-HT in duodenum using stripped sheets only. Partial stripping (mucosa-muscularis / submucosa intact) allows a better access of metabolic substrates and agonist/antagonists to the basolateral aspect of the epithelium[13]. Tanaka *et al.*[14] demonstrated the release of 5-HT into the intact duodenum during the fasting state in conscious dogs. In the presence of the both TTX and atropine, the increase in 5-HT-induced Isc as a result of starvation and undernourishment disappeared indicating that such increase was TTX-and atropine sensitive. Furthermore, the maximum increase in Isc induced by 5-HT as a results of starvation and undernourishment disappeared in the absence of chloride and in the absence of bicarbonate indicating that such increase was both chloride and bicarbonate dependent.

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