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Effects of 5-Hydroxytryptamine on the Short-circuit Current Across the Small Intestine of the Gerbil (*Gerbillus cheesmani*) in Different Dietary States

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Summary

The effects of serosally added 5-hydroxytryptamine (5-HT, 100 μM) on the short circuit-current (Isc) across jejunum and ileum taken from fed, starved and undernourished (G*erbillus cheesmani*) were investigated. The effects of the neurotoxin, tetrodotoxin (TTX, 10 μM) on the basal Isc as well as on the maximum increase in Isc induced by 5-HT were also studied. There were regional variations in the basal Isc as well as in the way by which the small intestine responds to 5-HT. The basal Isc was greater in jejunum than in ileum and such differences were TTX-sensitive. The maximum increase in Isc, which results from addition of 5-HT, was higher in jejunum than in ileum under all three feeding conditions. TTX reduced the maximum increase in Isc induced by 5-HT across stripped and intact intestine of the two regions in the three nutritional states. The 5-HT-induced Isc in the jejunum of both starved and undernourished gerbils and in the ileum of starved animals was the function of both submucosal and myenteric plexus. In jejunum and ileum taken from starved and undernourished gerbils the 5-HT-induced Isc was both chloride- and bicarbonate-dependent. Thus the results indicated that both starvation and undernourishment increase that response and such increases were TTX-sensitive and both chloride- and bicarbonate-dependent.

Key words

Gerbil • 5-hydroxytryptamine • Short circuit-current • Starvation • Tetrodotoxin • Undernourishment

Introduction

Dietary deprivation in the form of starvation or chronic undernourishment have been shown to induce intestinal hypersecretory activity in the small (Al-Balool 2005a) and large intestine (Al-Balool 2002) of the gerbil G*erbillus cheesmani* for a variety of secretagogues and toxins (Al-Balool 2003, 2004a,b, 2005b). The major source of 5-HT in the body is the intestinal tract, where this amine is found predominantly in enterochromaffine cells of the mucosa, although it is also present in neural and immune elements of subepithelial tissue (McKay and Perdue 1993).

The role of 5-HT in the gut is very complicated due to the presence of multiple 5-HT receptor subtypes. 5-HT receptors in the gut are now classified into four main subclasses with various locations in the mucosa, smooth muscle and neurons. 5-HT receptors appear to be present in enteric neural pathways that trigger mucosal protective mechanisms, such as active anion secretion

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(Brown 1996). There is some evidence that 5-HT has a direct action on the transporting cells. In the chicken small intestine a 5-HT-induced rise in cytoplasmic calcium levels has been reported in isolated enterocytes (Hirose and Chang 1988). Intestinal secretion induced by 5-HT stimulation is complex, with both neural and non-neural components contributing to the response (McKay and Perdue 1993, Cooke 1994, Franks *et al.* 1996). 5-HT has many possible sites of action within the intestine (Cooke 1994) and there are regional variations in the way the intestine responds to 5-HT challenge (Ayton *et al.* 1995, Hardcastle and Hardcastle 1997a) and there are also species differences (McLean *et al.* 1995).

The aim of the present study was to show the effects of starvation and undernourishment on basal Isc as well as on the 5-HT-induced electrogenic secretion across stripped and intact sheets of jejunum and ileum and to examine whether the altered Isc under basal (non-stimulated) conditions is in part due to activity of enteric neurons. The effects of the neurotoxin TTX on such changes were also investigated. Such studies have been performed in different mammals. From a comparative point of view, it will be very interesting to use a desert mammal like the gerbil as it will adapt to the lack of water.

Materials and Methods

Animals and diet

Gerbils (*Gerbillus cheesmani*) of both sexes (body weight 36-40 g) were captured in the desert of the state of Kuwait and kept in the animal house for at least three weeks before use. Three nutritional groups were used. The fed group of gerbils 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 5:00 h until 17:00 h and the humidity was 50 %. In the starved groups water was given *ad libitum*, but the diets were 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 a wired mesh floor to reduce coprophagy.

Tissue preparation

On the day of use, animals were anesthetized with thiopentone sodium (30 mg/kg body weight, i.p.). When surgical anesthesia was achieved, a midline incision was made along the abdomen and the entire small intestine (28-30 cm) was removed and flushed with 0.9 % NaCl. Jejunal sheets were taken just distal to the ligament of Treitz and ileal sheets from the region proximal to the terminal centimeter of the small intestine. The intestine was used either intact or with the outer smooth muscle layers removed (stripped preparation). Stripping removes the myenteric plexus as well as the muscle layer but leave intact the submucosal and mucosal plexus (Andres *et al.* 1985).

Voltage-clamp measurements

Each segment 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 between two perspex chambers and the measurements of the short circuit-current (Isc in μA) across the tissue was monitored by an automatic voltage clamp (DVC 200, WPI Inc., Stevenage, UK). The chambers (7.5 ml) were filled with bicarbonate saline, pH 7.4 (Krebs and Henseleit 1932) which contained (mM) 143 Na^+, 125.7 Cl^-, 24.9 HCO_3^-, 5.9 K^+, 2.5 Ca^{2+}, 1.2 $H_2PO_4^-$, 1.2 SO_4^{2-}, 1.2 Mg^{2+}. The medium was maintained at 38 °C and gassed with humidified 95 % O_2 and 5 % CO_2. All chemicals were purchased from Sigma Chemical Company Ltd.

The short-circuit current (Isc in μA cm^{-2}), was measured by a previously published standard *in vitro* technique (Baldwin and Levin 1985). The mounted tissue was allowed to stabilize for 10 min and the Isc was measured (basal reading). 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) was added to the serosal solution 10 min before the addition of 5-HT, with control sheets receiving an equivalent volume of vehicle. All gerbils were killed by thoractomy as soon as the intestine had been removed.

Statistical Analysis

All data are expressed as means ± S.E.M. The data were evaluated statistically using ANOVA. A significant difference was considered at $P<0.05$ (*post hoc* Student-Newman-Keuls test).

Results

Effects of starvation and undernourishment on basal Isc across jejunum and ileum

In the stripped and intact intestine the basal Isc was greater in the jejunum than in the ileum taken from fed, starved or undernourished gerbils (Fig. 1). Removal

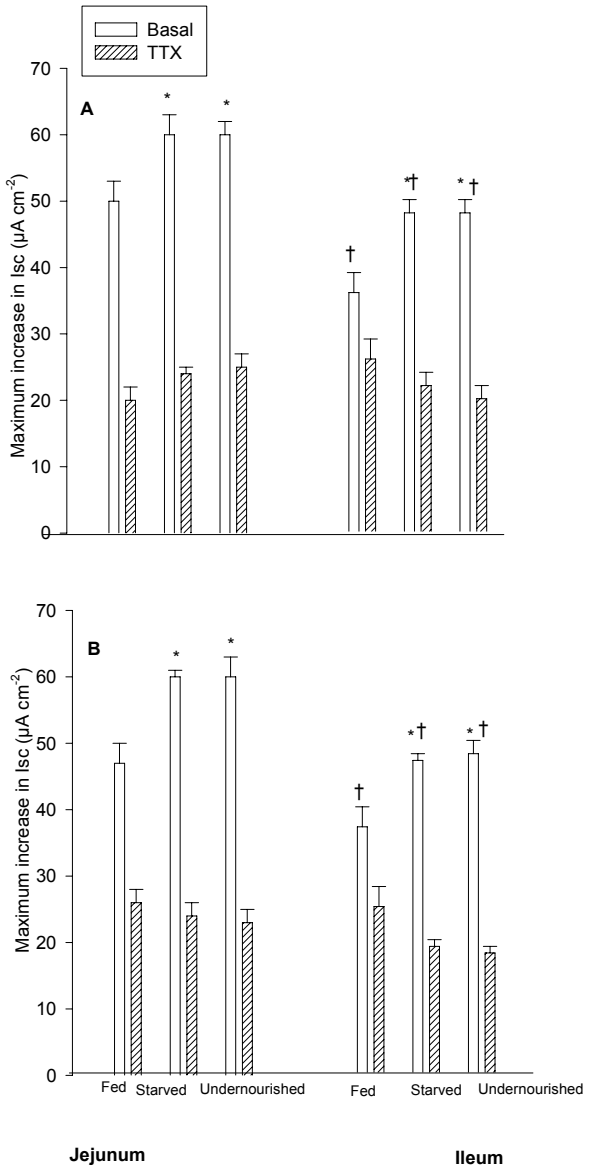

Fig 1. Effects of TTX (10 μM) on the basal Isc across stripped (A) and unstripped (B) sheets of jejunum and ileum taken from fed, starved or undernourished gerbils. Results are shown as means ± S.E.M. Significant effects ($p<0.05$): * Compared to the fed in the same region. † Compared jejunum with the ilem under the same feeding condition. There were 5-6 animals per group.

of the muscle layers and myenteric plexus (Fig. 1A) had no significant effects on the basal Isc across jejuna and ilea taken from the animals under the three feeding conditions. Starvation of gerbils for four days increased significantly the basal Isc of stripped and intact intestine (Fig. 1B) of the two regions. Reduction of food intake to one half for 21 days produced similar effects.

Effects of TTX on basal Isc across jejunum and ileum

In order to investigate if there is any neural mediation of the basal Isc, TTX (10 μM) was added to the serosal bathing solution and the subsequent changes in basal Isc were recorded 10 min later. TTX reduced significantly the basal Isc of stripped or intact jejunum and ileum taken from fed, starved or undernourished gerbils. In the presence of TTX the differences in the basal Isc between jejunum and ileum disappeared under all three feeding conditions. Moreover, the increase in the basal Isc which results from starvation and undernourishment disappeared (Fig. 1).

Effects of 5-HT on Isc across jejunum and ileum

The maximum increases in Isc, which resulted from addition of 5-HT (100 μM) to the serosal bathing solution, are shown in Figure 2. Basal Isc in fed jejunum were 50±3 and 47±3 μA cm^{-2} in stripped and intact sheets, respectively. After 5-HT addition the maximum increase in Isc were 108±4 and 111±5 μA cm^{-2} in stripped and intact sheets of fed jejunum. In fed jejunum and ileum the maximum increase of Isc generated by 5-HT was not significantly different in stripped and intact intestine (Fig. 2). Comparing two regions of the small intestine, the maximum increase in Isc induced by 5-HT was significantly higher in jejunum than ileum both using stripped or intact intestine under the three feeding conditions. Starvation increases significantly the maximum increase of Isc induced by 5-HT in both jejunum and ileum using stripped and intact intestine. The 5-HT-induced Isc was significantly ($P<0.05$) higher in the intact intestine than in stripped sheets across the two regions of the small intestine. Reduction of food intake to one half for 21 days increased significantly the maximum Isc generated by 5-HT in both jejunum and ileum using either stripped or intact intestine. Similar to the starved jejunum the 5-HT-induced Isc increase was significantly higher ($P<0.05$) in the intact than stripped sheets, but in the ileum there were no significant differences between the two preparations.

Effects of TTX on maximum responses in Isc generated by 5-HT across jejunum and ileum

The effects of TTX (10 μM) placed in the serosal bathing solution, 10 min before the addition of 5-HT on the maximum Isc generated by 5-HT are shown in Figure 2. TTX reduced significantly the maximum increase in Isc induced by 5-HT in both stripped and intact intestine in both regions of the small intestine under the three feeding conditions. The enhanced increases of Isc induced by 5-HT, which resulted from starvation, were significantly inhibited by TTX in

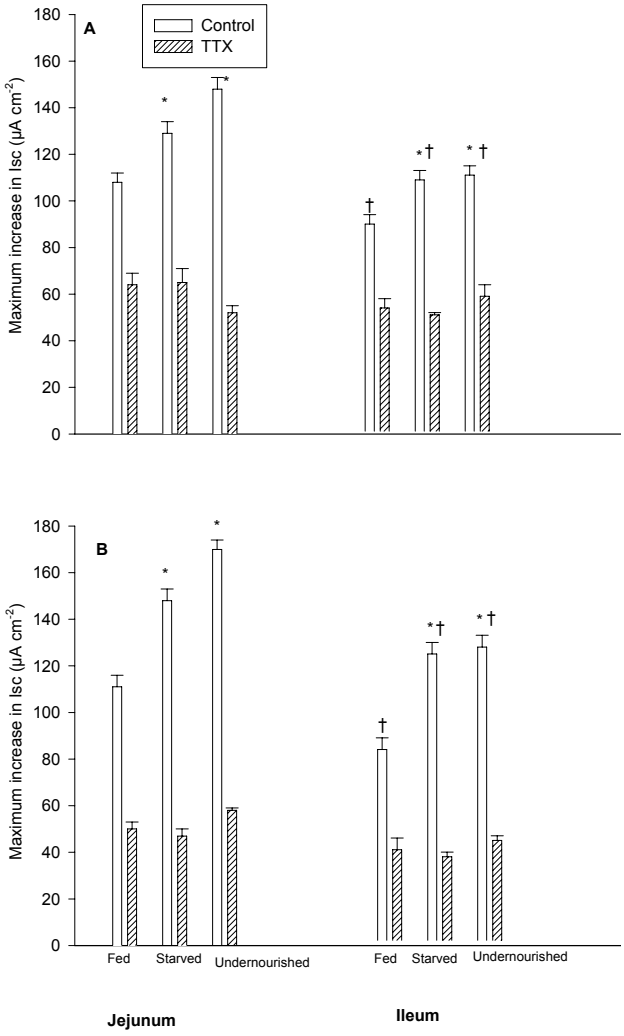

Fig. 2. Effects of TTX (10 μM) on the maximum increase in Isc induced by 5-HT (100 μM) across stripped (A) and unstripped (B) sheets of jejunum and ileum taken from fed, starved or undernourished gerbils. Results are shown as means ± S.E.M. Significant effects ($p<0.05$): Compared to the fed in the same region. † Compared jejunum with the ileum under the same feeding condition. There were 5-6 animals per group.

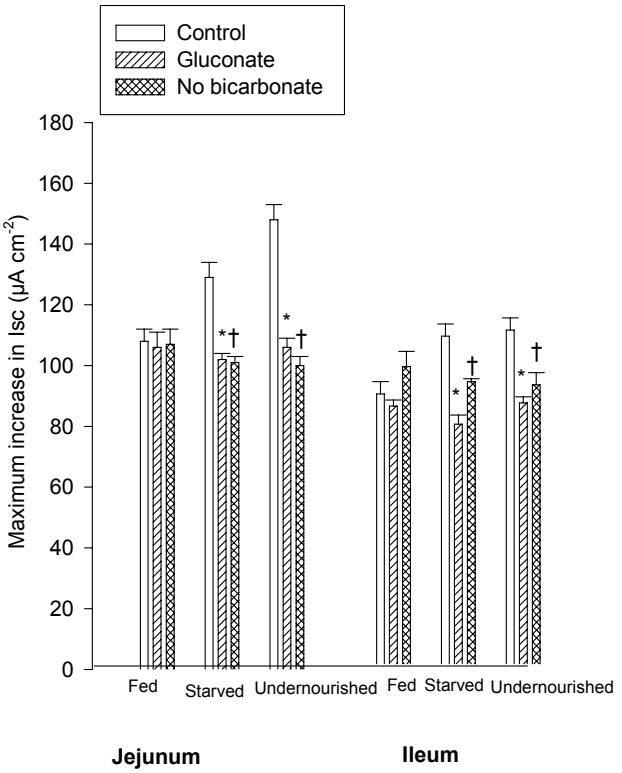

Fig. 3. Effects of replacing chloride ions by gluconate or removing bicarbonate ions from bathing buffer on the maximum increase in Isc induced by 5-HT across stripped sheets of jejunum and ileum taken from fed, starved or undernourished gerbils. Results are shown as means ± S.E.M. Significant effects ($p<0.05$): * Compared replacing chloride ions by gluconate with the control in the same feeding condition, † Compared removing bicarbonate with the control in the same feeding condition. There were 5-6 animals per group.

stripped and intact intestine of both the jejunum and ileum. Similarly in the jejuna and ilea taken from undernourished gerbils TTX reduced significantly the maximum increase in Isc generated by 5-HT so that the effect of undernourishment disappeared. In the presence of TTX the 5-HT-induced Isc was significantly lower in the intact intestine in both regions taken from starved and undernourished gerbils.

Effects of replacing chloride by gluconate on maximum responses in Isc generated by 5-HT

Replacing chloride in the bathing buffer by gluconate had no significant effects on the maximum increase in Isc induced by 5-HT in the two regions of fed gerbils, but significantly reduced the 5-HT-induced Isc increase across jejunum and ileum taken from starved and undernourished gerbils (Fig. 3). Moreover, the increases in 5-HT-induced Isc increase, which resulted from starvation or undernourishment in the two regions of the small intestine were disappeared in absence of chloride. However, the 5-HT-induced Isc increase in the jejunum was still higher than that in the ileum even in the absence of chloride.

Effects of removing bicarbonate from bathing buffer on the maximum responses in Isc generated by 5-HT

The effects of removing bicarbonate from bathing buffer on the maximum increases in Isc induced by 5-HT across stripped intestine taken from fed, starved or undernourished animals are shown in Figure 3. In fed animal there were no significant effects in the two regions of the small intestine. Nevertheless, in the jejunum and

ileum taken from starved or undernourished gerbil the removal of bicarbonate from the bathing buffer decreased significantly the 5-HT-induced Isc increase. The differences between the two regions of the small intestine disappeared in the absence of bicarbonate.

Discussion

Using stripped sheets only, Al-Balool (2005a) showed that in the fed gerbil the basal Isc was significantly higher in the jejunum than ileum. The present study showed that using the intact intestine such differences are still present. This is in agreement with the results in rats where the basal Isc has been shown to be greater in the jejunum than in the ileum (Hardcastle and Hardcastle 1997a). The present study also showed that there were no significant differences in the basal Isc between stripped and intact intestine. This indicates that the contribution of myenteric plexus in the basal Isc is minimal in the gerbil small intestine. TTX reduced significantly the basal Isc across jejunum and ileum in both stripped and intact intestine. This is in disagreement with the results of Rolfe and Levin (1998) who found that pretreatment of unstripped sheet of rat ilea with TTX did not affect the basal Isc. In the presence of TTX the differences in the basal Isc between jejunum and ileum disappeared indicating that such difference was TTX-sensitive. TTX has been found to cause a reduction in basal Isc of small intestinal tissue of many species (Hubel 1978, Binder *et al.* 1984, Keast *et al.* 1985, Perdue and Davison 1986, Carey and Cooke 1989, Sheldon *et al.* 1989). In a previous study (Al-Balool 2005a) the replacement of sodium chloride with lithium chloride decreased the basal Isc of jejunum and ileum by the same magnitude in the three feeding conditions while replacing chloride by gluconate or removing bicarbonate from bathing buffer decreased the basal Isc across jejuna and ilea taken from starved and undernourished gerbils only.

Similarly to the stripped sheets (Al-Balool 2005a) the use of intact intestine in the present study has shown that starvation increases the basal Isc in jejunum and ileum. Undernourishment produced the same effect and the increases in Isc, which resulted from starvation and undernourishment disappeared in the presence of TTX, indicating that such increases were TTX-sensitive. Hayden and Carey (2000) found that fasting enhances enteric neural control of basal ion transport and short-term fasting enhances reflex activity within the submucosal plexus that maintains basal ion transport. Thus in the gerbil small intestine, food deprivation enhanced enteric neural control of basal ion transport especially those parts, which are TTX-sensitive.

Using stripped sheets only, the increases in the basal Isc across gerbil small intestine, which resulted from starvation and undernourishment, were found to be both chloride- and bicarbonate-dependent (Al-Balool 2005a). Therefore, it can be suggested that in the gerbil small intestine, as in the piglet jejunum, the increase in Isc was due to alteration in ion transport characteristics of the small intestinal epithelium secondary to its reduced exposure to luminal contents. Similarly to fed intestine, the basal Isc of jejunum were higher than that of the ileum using both stripped and intact intestine taken from starved or undernourished gerbils**.**

In the present study, 5-HT induced electrogenic secretion, expressed as an increase in the maximum Isc generated by 5-HT in the gerbil small intestine using both stripped and intact intestine under all three feeding conditions. In fed animals, similarly to basal Isc, the maximum increases in Isc induced by 5-HT were significantly higher in the jejunum than ileum both using stripped and intact intestine. There were no significant differences in the 5-HT-induced Isc between stripped and intact intestine, indicating that similarly to the basal Isc the contribution of myenteric plexus to the 5-HT-induced Isc are minimal. The neurotoxin TTX decreased significantly the maximum Isc generated by 5-HT in stripped and intact intestine of both regions taken from fed, starved or undernourished gerbils. Earlier studies showed that although 5-HT-induced secretion is inhibited by the neurotoxin, it is not abolished (Cooke and Carey 1985, Baird and Cuthbert 1987, Siriwardena *et al.* 1991, Hardcastle and Hardcastle 1997a,b, 1998) suggesting that a non-neural mechanism contributes to the response. In isolated rat ileum, Rolfe and Levin (1998) found that 5-HT induced electrogenic Cl^- ion secretion partly *via* a neural pathway (probably involves sensory afferent C-fibers) and partly *via* a non-neural mechanism (probably involving a direct interaction with enterocytes).

5-HT causes stimulation of electrogenic chloride secretion and inhibition of electroneutral sodium chloride absorption (Hardcastle *et al.* 1981). i.e. the changes that lead to fluid secretion (Kisloff and Moore 1976, Donowitz *et al.* 1977). This is in disagreement with the present study, which showed that replacing chloride by gluconate or removal of bicarbonate from bathing buffer had no significant effect on the 5-HT-induced Isc. One explanation for such a difference could be based upon

species differences. Another explanation could be that they measured ion flux while in the present study chloride replacement was assessed.

In the two regions of the small intestine taken from starved gerbil, the 5-HT-induced Isc were significantly higher using intact sheets than using stripped sheets. Thus in contrast to fed animals, the 5-HT-induced Isc across the starved small intestine is a function of both submucosal and myenteric plexus. Moreover, in the presence of TTX the differences between stripped and unstripped were still remarkable which indicates that TTX affected both plexus. Replacing chloride by gluconate or removing bicarbonate from bathing buffer decreased significantly the 5-HT-induced Isc across the two regions of the small intestine taken from starved animals. This is in agreement with the results of Green and Brown (2002) in porcine fed ileum where they found that responses to 5-HT were reduced in tissue bathed in Cl^- -or HCO_3^- deficient media. Thus in the gerbil, different from porcine, the starved small intestine was more sensitive to the reduction of Cl^- or HCO_3^- than the fed intestine. The 5-HT-induced Isc across starved small intestine is the function of both submucosal and myenteric plexus and it is both chloride- and bicarbonate-dependent. Starvation increases significantly the maximum Isc generated by 5-HT in stripped and intact small intestine of gerbils. Moreover, the increases in the 5-HT-induced Isc resulting from starvation disappeared in the absence of chloride and bicarbonate, indicating that such increase were both chloride- and bicarbonate-dependent.

Only in the jejunum taken from undernourished gerbils, the 5-HT-induced Isc were significantly higher using intact sheets. Therefore, in the jejunum of undernourished gerbils both submucosal and myenteric plexus contributed in the 5-HT-induced Isc. In the absence of chloride or bicarbonate the 5-HT-induced Isc across both regions of the small intestine were significantly reduced, indicating that, similarly to starving conditions, 5-HT-induced Isc found in undernourished gerbils was both chloride- and bicarbonate-dependent.

Therefore, the present study showed that in the gerbil (*Gerbillus cheesmani*) there were regional variations in the basal Isc as well as in the way by which the small intestine responds to 5-HT. It was also showed that the intestinal secretion induced by 5-HT stimulation is complex, with neural and non-neural components contributing to the response. The 5-HT-induced Isc in the jejunum of both starved and undernourished gerbils and in the ileum of starved animals were the function of both submucosal and myenteric plexus. It wasalso showed that both starvation and undernourishment increase the response and such increases were TTX-sensitive and both chloride and bicarbonate dependent.

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