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CYTOCHALASIN B DOES NOT STIMULATE SUGAR UPTAKE INTO SMALL INTESTINE OF *NECTURUS* OR CHICK

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Summary

3-*O*-Methylglucose accumulation by *Necturus* intestine was studied in the presence and absence of cytochalasin B. The effect of this agent on 3-*O*-methylglucose-induced changes in transepithelial potential difference was also investigated. Cytochalasin B (50 μM) blocked the rise in potential difference induced by 4 mM 3-*O*-methyglucose. 3-*O*-Methylglucose accumulation by *Necturus* intestine was measured; it was inhibited by cytochalasin B at concentrations of 50 and 100 μM. These results indicate that, in *Necturus* intestine, sodium-dependent sugar transport across the apical cell membrane is blocked by cytochalasin B.

Cytochalasin B inhibits facilitated sugar transport into different cell types, e.g., fibroblasts [1], HeLa cells [2] and hepatoma cells [2,3]. Recently, Kimmich and Randles [4] reported a 30—40-fold 3-*O*-methylglucose concentration gradient across the cellular membrane of isolated enterocytes from chicken small intestine in the presence of cytochalasin B compared to a 10-fold gradient under control conditions. This enhancement of the ability of the intestinal cell to establish a gradient for 3-*O*-methyglucose was attributed to an inhibition by cytochalasin B of 3-*O*-methylglucose exit from the cell via a facilitated diffusion system in the absence of any effect of this agent on sodium-dependent 3-*O*-methylglucose transport across the apical cell membrane [4].

This 3—4-fold increase in the cell-to-medium accumulation ratio of the sugar analog, 3-*O*-methylglucose, raises intriguing questions concerning the energetic adequacy of the transapical Na^+ gradient to effect intestinal sugar accumulation

in the presence of cytochalasin B [5]. As a first step in a systematic study of this problem, we measured 3-*O*-methylglucose accumulation by isolated *Necturus* intestine in the presence and absence of cytochalasin B.

Necturus (*Necturus maculosus*) were kept in a large aquarium at 4°C. They were killed by a blow on the head and a segment of the intestine was rapidly excised. The segment was opened along the mesenteric border, stripped of its external muscle layers and was mounted at 23°C in an Ussing chamber [6]. The composition of the bathing medium (in mM) was: sodium gluconate, 100; KH_2PO_4, 0.8; K_2HPO_4, 2.3; calcium gluconate, 1.8 and mannitol, 21; pH was 7.2. Gluconate was substituted for Cl^- in order to avoid possible complications arising from the presence of coupled transapical Na-Cl transport in these experiments. When 3-*O*-methylglucose was present in the medium an osmotically equivalent amount of mannitol was omitted. The bathing solutions were continuously bubbled with O_2.

Addition of 3-*O*-methylglucose to the mucosal bathing medium elicited an increase in the transepithelial potential difference (V) of *Necturus* intestine. This rise in V was abolished by 10^{-6} M phlorizin. Different concentrations of 3-*O*-methylglucose were assayed and the increase in V (ΔV) as a function of 3-3-*O*-methylglucose concentration was analyzed by means of double-reciprocal and Augustinnson [7] plots. The apparent Michaelis constant (K_t) of the system determined by these two methods was 2.3 ± 0.4 and 2.2 ± 0.3 mM (S.E., $n = 4$), respectively. The maximum increase in V (ΔV_{max}) was 2.1 ± 0.6 and 2.0 ± 0.6 mV.

Cytochalasin B was dissolved in ethanol and was added to both bathing media at a final concentration of 50 μM. Preliminary experiments showed that ethanol, at the final concentration employed (0.1%), did not alter the electrical parameters of the tissue. In three experiments in which the tissue was preincubated for 1 h with 50 μM cytochalasin B, steady-state V averaged 0.7 ± 0.1 mV. Addition of 4 mM 3-*O*-methylglucose, and subsequent addition of 10^{-6} M phlorizin, to the mucosal medium did not change this value. In another set of three experiments, steady-state V under control conditions averaged 1.3 ± 0.5 mV. After the addition of 4 mM 3-*O*-methylglucose to the mucosal medium, V increased significantly ($P < 0.05$) to 2.3 ± 0.5 mV. When 50 μM cytochalasin B was added to both bathing media, V fell to 1.5 ± 0.9 mV, a significant ($P < 0.05$) decrease. From these results we concluded that cytochalasin B blocked the rise in V elicited by the presence of 3-*O*-methylglucose in the mucosal medium.

To measure the cell-to-medium 3-*O*-methylglucose concentration ratio and the effect of cytochalasin B on this parameter, the following procedure was adopted. Two segments of stripped intestine (about 50 mg wet wt.) were prepared from each animal. One was incubated at 23°C in 5 ml of normal Ringer solution containing 100 μM 3-*O*-methylglucose, and the other was incubated in 5 ml of the same solution containing cytochalasin B at a concentration of 50 μM. A trace amount of ^{14}C-labelled 3-*O*-methylglucose (New England Nuclear) was also added to the bathing medium. 3-*O*-methylglucose accumulation was determined following 2-h incubation at room temperature. Following incubation, the wet weight of the tissue was obtained. The tissue was dried at 100°C overnight and the dry weight was determined. The dry tissue was extracted in 0.1 M

HNO_3, as previously described [8]. Aliquots of the extract and of the bathing medium were taken for counting in a Packard series 2660 liquid scintillation counter.

Apparent extracellular space was estimated in a parallel set of experiments using poly(ethylene glycol) ($M_r \simeq 4000$), labelled with trace amounts of poly-([^{14}C]ethylene glycol) (New England Nuclear). Tissues were incubated for 30, 60, 90 and 120 min.

Apparent extracellular space reached a steady state after 1 h. Its value was 19 ± 2% ($n = 6$) under control conditions and in the presence of 50 μM cytochalasin B. Under control conditions, the cell-to-medium 3-*O*-methylglucose accumulation ratio was 1.8 ± 0.6 ($n = 6$); when 50 μM cytochalasin B was present in the bathing medium, the accumulation ratio fell to 1.0 ± 0.3 ($n = 6$). This decrease was significant at the 0.05 confidence level.

To test the possibility of a non-specific effect of ethanol on 3-*O*-methylglucose accumulation, a different set of experiments was performed. In these experiments, cytochalasin B was dissolved in dimethylformamide as in the experiments of Kimmich and Randles [4] and was added to the bathing medium at a final concentration of 100 μM. Incubation was for 1 h. Under control conditions, the cell-to-medium 3-*O*-methylglucose accumulation ratio was 2.4 ± 0.8 ($n = 5$). In the presence of 100 μM cytochalasin B, this ratio decreased significantly ($P < 0.01$) to 1.1 ± 0.1 ($n = 5$).

These results and the fact that, as described above, the electrical response to the presence of 3-*O*-methyglucose in the mucosal medium was blocked by cytochalasin B suggest that cytochalasin B inhibits sodium-dependent sugar transport [9] across the apical membrane of *Necturus* intestine.

Since these results are strikingly different from those reported for isolated chicken enterocytes by Kimmich and Randles [4], experiments were performed with the chicken intestine. These experiments were performed under conditions that closely approximated those used by Kimmich [10]. Male White Leghorn chickens, 4-week-old, were used. The chickens were killed by decapitation and two segments of small intestine were excised. The experimental procedure was as described above. The incubation medium had the following composition, (in mM): NaCl, 120; Tris-HCl, 20; K_2HPO_4, 3, $MgCl_2$, 1; $CaCl_2$, 1 and bovine serum albumin, 1 mg/ml. 3-*O*-Methylglucose was present at 100 μM concentration. Incubations were performed at 38°C. In these experiments, the tissue-to-medium accumulation ratio of 3-*O*-methylglucose was measured. The average value was 3.4 ± 0.9 ($n = 6$) under control conditions and 2.8 ± 0.9 ($n = 6$) in the presence of 100 μM cytochalasin B (dissolved in dimethylformamide). These values did not differ significantly.

From the results presented in this paper, it may be concluded that cytochalasin B, in the concentrations used, inhibits Na-dependent transapical sugar transport in *Necturus* intestine. The difference between this result and those reported by Kimmich and Randles [4] might be attributed to a special sensitivity of *Necturus* intestine to cytochalasin B. However, when cytochalasin B is present, the 3-*O*-methylglucose accumulation reported in this paper for the intact chicken intestine is very different from that obtained with an isolated cell suspension from the same species [4]. In agreement with the results presented in this communication, it has been reported [11] that cytochalasin B

failed to alter the uptake of 3-*O*-methylglucose by hamster small intestinal everted rings. It seems possible that, when a whole tissue preparation is used, cytochalasin B does not reach the site of action where it inhibits facilitated sugar exit across the basolateral membrane of the cell. This would explain the lack of effect of cytochalasin B on 3-*O*-methylglucose accumulation by chicken intestine in the present experiments and the essentially similar result reported by Mak et al. [11].

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