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Effect of Theophylline and Phloretin on D-Galactose Transport in Guinea Pig Small Intestine

Theophylline and phloretin have been shown to reduce serosal sugar efflux in intact rat small intestine (1, 5) and in isolated chicken enterocytes (6). Theophylline also decreases serosal sugar permeability in intact rabbit ileum (3).

The present study shows that serosal sugar exit in guinea pig small intestine also responds to theophylline and phloretin.

Guinea pigs weighing about 250-300 g were anaesthetized with ether and killed by ether overdose. The distal small intestine was removed, rinsed free of intestinal contents with ice-cold Ringer's solution and stripped of its serosal and external muscle layers (9). Tissue galactose (2 mM) accumulation was studied by using the CRANE and MANDELSTAM technique (2). Transepithelial mucosal to serosal D-galactose (2 mM) fluxes were measured by mounting the tissue in Ussing-type chambers (3). The Ringer's solution contained, in mM: 140 NaCl, 10 $KHCO_3$, 0.4 KH_2PO_4, 2.4 K_2HPO_4, 1.2 $CaCl_2$ an 1.2 $MgCl_2$ and was continuously bubbled with 95 % O_2/5 % CO_2.

Galactose was evaluated by using ^{14}C-labelled galactose. The test agents were

Table I. *Effect of theophylline and phloretin on D-galactose (2 mM) transport in guinea pig small intestine.*

TFP: trifluoperazine. Values are the means ± S.E. Between brackets the number of independent determinations.

	J_{ms}^{gal} (μmol · h^{-1} · cm^{-2})			μmol galactose · g^{-1} tissue w.w.		
	Control	Phloretin (0.1 mM)	Theophylline (5 mM)	Control	Phloretin (0.1 mM)	Theophylline (5 mM)
No addition	0.52±0.02 (20)	0.31±0.01* (20)	0.35±0.01* (20)	4.02±0.06 (20)	7.23±0.08* (20)	7.33±0.10* (30)
TFP (0.1 mM)	—	0.59±0.02 (20)	0.54±0.03 (19)	—	3.78±0.06** (20)	3.72±0.09** (21)
RMI 12330A (0.1 mM)	—	0.52±0.02 (19)	0.54±0.02 (16)	—	3.13±0.05* (25)	2.85±0.10* (30)

* $p < 0.001$; ** $p < 0.01$. Comparisons between treated and control tissues (t-Student test).

Table II. *Dose-dependent effect of RMI 12330A and trifluoperazine (TFP) on the theophylline and phloretin-induced increase in tissue sugar accumulation.*

The concentrations of TFP and RMI 12330A used ranged from 10^{-9} to 10^{-4} M. The IC_{50} value is defined as the concentration of the drug required to produce a 50 % inhibition of either theophylline or phloretin-induced increase in tissue galactose accumulation.

	IC_{50} (M)	
	Phloretin (0.1 mM)	Theophylline (5 mM)
TFP	5×10^{-7}	5×10^{-7}
RMI 12330A	8×10^{-7}	5×10^{-7}

present in the bathing solutions from the start of the incubation period.

The results show (table I) that both theophylline (5 mM) and phloretin (0.1 mM) increased tissue galactose accumulation and decreased mucosal to serosal sugar fluxes. These results agree with previous reports on other intestinal epithelia (1, 3, 5, 6) and suggest that both modifiers may as well act as inhibitors of serosal sugar efflux in guinea pig small intestine.

The theophylline and phloretin action on intestinal sugar transport were abolished by the calmodulin antagonists RMI 12330A (4) and trifluoperazine (4, 7, 8) (table I), their IC_{50} being close to 10^{-6} (table II). These findings are in agreement with previous work on the affinity of trifluoperazine for purified calmodulin (4, 7, 8) and suggest that cytosolic free Ca^{2+} concentration is raised to approximately 6×10^{-7} M by both theophylline and phloretin.

The current study, therefore, suggests that calmodulin might be involved in the regulation of serosal sugar exit in guinea pig small intestine.

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