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D-GALACTOSE ACCUMULATION IN RABBIT ILEUM
EFFECTS OF THEOPHYLLINE ON SEROSAL PERMEABILITY

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SUMMARY

The effects of theophylline and dibutyryl cyclic AMP, on in vitro unidirectional galactose fluxes across the mucosal and serosal borders of rabbit ileum have been studied.

1. When Ringer [galactose] = 2 mM, theophylline and dibutyryl cyclic AMP reduce both mucosal-serosal and serosal-mucosal galactose flux by approx. 50%. The K_I for theophylline inhibition of flux in both directions is 2 mM. 1 mM dibutyryl cyclic AMP elicits a maximal inhibitory response. Concurrent with the inhibition in transmural galactose fluxes, theophylline and dibutyryl cyclic AMP increase the tissue accumulation of [galactose] and the specific-activity ratio R of ^3H : ^{14}C-labelled galactose coming from the mucosal and serosal solutions respectively. It is deduced that theophylline and dibutyryl cyclic AMP are without effect on the mucosal unidirectional permeability to galactose but cause a symmetrical reduction in serosal entry and exit permeability.

2. Reduction in the asymmetry of the mucosal border to galactose by reducing Ringer [Na], raising Ringer [galactose] or adding ouabain reduces the theophylline-dependent increase in galactose accumulation.

3. Hypertonicity in the serosal solution increases the permeability of the serosal border to galactose and reduces tissue galactose accumulation. Serosal hypertonicity partially reverses the theophylline-dependent effects on galactose transport. Replacing Ringer chloride by sulphate abolishes the theophylline-dependent effects on galactose transport.

4. It is considered that the theophylline-dependent increase in galactose accumulation results from the reduction in serosal permeability. This is shown to be a quantitatively consistent inference.

5. Further support for the view that the asymmetric transport of galactose in rabbit ileum results from convective-diffusion is presented.

INTRODUCTION

Both theophylline, which inhibits cyclic AMP phosphodiesterase activity and consequently increases intracellular [cyclic AMP] in intestinal epithelium [1, 2], and

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dibutyryl cyclic AMP have been noted to increase organic solute accumulation in a number of epithelial tissues. α-Methyl D-glucoside accumulation in rabbit kidney cortex [3], L-leucine accumulation in rat jejunum [4] and L-methionine uptake [5] across the mucosal surface of Na-depleted rat ileum are all increased by theophylline.

Theophylline tends to reverse the normal direction of net NaCl absorptive movement across mammalian small intestine, possibly by reducing NaCl influx across the mucosal border [6–9]. Additionally, it has been noted that theophylline alters the relative passive mobility of Na : Cl across the intestine by increasing Cl conductance [10]. Since sugar accumulation is known to be a Na-linked process and theophylline has apparently opposing effects on Na flux and organic solute accumulation, it was considered of interest to further investigate the action of this drug, and of dibutyryl cyclic AMP on Na^+-dependent galactose transport across rabbit ileum. By using the method previously described [11, 12] it is possible to determine simultaneously the unidirectional fluxes and permeabilities of D-galactose across both the mucosal and serosal borders of rabbit ileum stripped of its serosal and outer muscle layer and hence accurately locate the site of action of the drug.

METHODS

The methods of measuring the transmural bidirectional fluxes, the specific activity ratio R and the tissue galactose concentration from which the unidirectional fluxes of ^{3}H- and ^{14}C-labelled D-galactose across the mucosal and serosal borders of rabbit ileum stripped of its serosa and outer muscle layers have been described previously [11, 12].

It was shown that the unidirectional fluxes of galactose across mucosal and serosal borders of strips of rabbit ileum can be estimated from groupings of two or three independently measured variables; the mucosal-serosal flux J_{13}, the serosal to mucosal flux J_{31}, and the ratio R to the specific activity of radioisotope within the tissue compartment 2.

When the concentrations of D-galactose in the mucosal and serosal compartments are identical

$$R = \frac{(\text{cpm})_2^T}{(\text{cpm})_2^C} \times \frac{(\text{cpm/ml})_3^C}{(\text{cpm/ml})_1^T}$$

Subscripts 1, 2, 3 refer to the mucosal, cell and serosal compartments respectively; superscripts T and C refer to ^{3}H- and ^{14}C-labelled D-galactose respectively. J_{ij} refers to flux from compartment i to j and $P_{ij} = J_{ij}/C_i$.

It was shown that:

$$J_{12} = J_{31}R + J_{13}$$
$$J_{21} = J_{31}(1+R)$$
$$J_{23} = J_{13}(1+1/R)$$
$$J_{32} = J_{31} + J_{13}/R$$

MATERIALS

Ouabain, galactose and theophylline were obtained from BDH Ltd, dibutyryl cyclic AMP from Sigma Chemicals Ltd.

Ringer solutions have the following compositions (concentration in mM).

(1) NaCl Ringer gassed with 95 % O_2/5 % CO_2 to bring pH to 7.4

140 NaCl, 10 $KHCO_3$, 0.4 K_2PO_4, 2.4 K_2HPO_4, 1.2 $CaCl_2$, 1.2 $MgCl_2$.

(2) Na_2SO_4 Ringer gassed with 95 % O_2/5 % CO_2 to bring pH to 7.4

70 Na_2SO_4, 1.2 $CaSO_4$, 1.2 $MgSO_4$, 72.4 mannitol, 10 $KHCO_3$, 0.4 K_2PO_4, 2.4 K_2HPO_4.

(3) 75 mequiv. NaCl Ringer+isosmotic replacement with mannitol or galactose gassed with 95 % O_2/5 % CO_2, pH 7.4

1.2 $CaCl_2$, 1.2 $MgCl_2$, 10 KHO_3, 0.4 K_2PO_4, 2.4 K_2HPO_4

(4) Choline Ringer gassed with 95 % O_2/5 % CO_2, pH = 7.4

140 NaCl replaced by 140 choline chloride, 10 $KHCO_3$, 0.4 K_2PO_4, 2.4 K_2HPO_4, 1.2 $CaCl_2$, 1.2 $MgCl_2$.

RESULTS

The effects of theophylline and dibutyryl cyclic AMP on the transmural fluxes, intracellular accumulation and specific activity ratio of D-*galactose present at 2 mM in Na-Ringer*

As [theophylline] is raised, both the mucosal-serosal and serosal-mucosal fluxes of D-galactose fall.

The concentration of theophylline giving half-maximal reductions in both transmural fluxes is the same, 2 mM. Since the absolute decrease in mucosal-serosal flux exceeds the reduction in serosal-mucosal flux, theophylline also reduces net galactose absorption, but the transmural flux ratio J_{13}/J_{31} remains unaffected (Fig. 1).

Concurrent with the fall in transmural galactose fluxes, theophylline increases the steady state level of [galactose] in the tissue water by approximately two-fold and

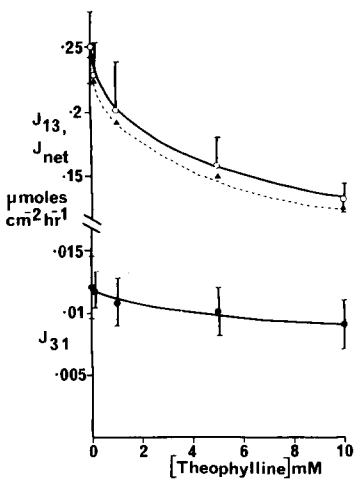

Fig. 1. The effect of varying [theophylline] on transmural galactose fluxes, J_{1-3} open circles, J_{3-1} closed circles (Ringer [galactose] is 2mM). J_{net} ($J_{1-3} - J_{3-1}$) is shown as the broken line. The bars represent the S.E.M. for 6 experiments. The significance levels for the theophylline effect on J_{1-3} ($P < 0.001$), J_{3-1} ($P < 0.01$) and J_{net} ($P < 0.001$) were assessed using one-way analysis of variance.

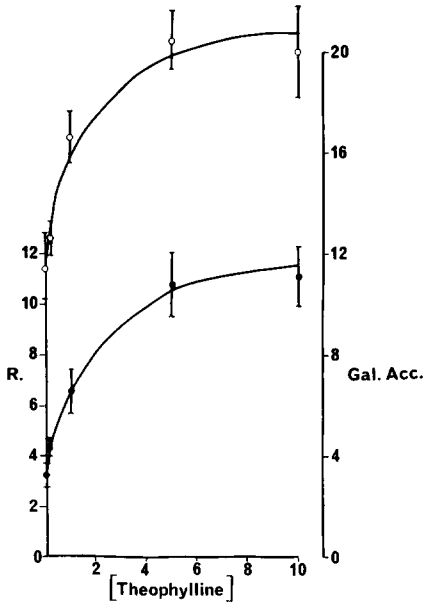

Fig. 2. The effect of varying [theophylline] on tissue accumulation of galactose (Gal. Acc.) (○—○) and on ^{3}H: ^{14}C specific activity ratio (●—●). Ringer [galactose] = 2 mM. The bars represent the S.E.M. for 4 experiments. The significance levels for the theophylline effect on accumulation ($P<0.001$) and R ($P<0.001$) were assessed using one way analysis of variance. [Theophylline] and Gal. Acc. scales are mM.

the tissue specific activity ratio R of ^{3}H : ^{14}C-labelled galactose, coming from the mucosal and serosal solutions respectively, is increased by approximately three-fold at maximum (Fig. 2).

1 mM dibutyryl cyclic AMP has effects on transmural galactose flux, tissue accumulation and the ratio R equivalent to the maximal response elicited by theophylline (Table I).

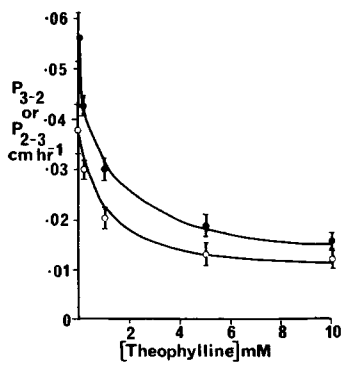

Fig. 3. The effect of varying [theophylline] on calculated entry and exit permeability of galactose across the serosal border of rabbit ileum. Ringer [galactcse] = 2 mM. Bars represent S.E.M. for 6 experiments. The significance levels for the theophylline effect were assessed using one way analysis of variance. Raising [theophylline] reduces P_{3-2} (●—●) $P<0.001$ and P_{2-3} (○—○) $P<0.001$.

TABLE I

A COMPARISON OF THE MAXIMAL EFFECT ON GALACTOSE TRANSPORT ACROSS RABBIT ILEUM PRODUCED BY 10mM THEOPHYLLINE WITH THAT PRODUCED BY 1mM THEOPHYLLINE AND 1mM DIBUTYRYL CYCLIC AMP

The significance levels (P) for the theophylline response were determined by one way analysis of variance. The reduction in transmural fluxes due to theophylline shown here are insignificant because of the small sample size. For analysis at several levels of [theophylline] see Fig. 1. The significance levels for the effect of Dibutyryl cyclic AMP were assessed using a Students 't' test (unpaired means solution).
NS, not significant. Ringer [galactose] = 2mM.

		μmol · cm^{-2} · h^{-1}					cm · h^{-1}				
	n	J_{13}	J_{31}	J_{net}	R	Accumulation (mM)	P_{12}	P_{21}	P_{23}	P_{32}	$\ln(P_{12}/P_{21})$
Control	4	0.294	0.013	0.259	3.52	11.51	0.131	0.0048	0.0362	0.055	3.51
		±0.034	±0.0047	±0.045	±0.47	±1.4	±0.02	±0.001	±0.0042	±0.0047	±0.306
Theophylline	4	0.255	0.0107	0.211	6.63	16.54	0.136	0.0043	0.0207	0.03	3.34
1.0 mM		±0.058	±0.0031	±0.073	±0.88	±1.02	±0.041	±0.0008	±0.0039	±0.0065	±0.34
Theophylline	4	0.144	0.0072	0.142	11.16	19.91	0.154	0.0054	0.0105	0.013	3.24
10.0 mM		±0.015	±0.0034	±0.0122	±1.29	±1.83	±0.019	±0.0019	±0.0021	±0.0034	±0.327
*P		NS	NS	NS	<0.001	<0.001	NS	NS	<0.001	<0.001	NS
Dibutyryl cyclic	3	0.18	0.0023	0.177	10.32	26.48	0.105	0.0011	0.007	0.0096	4.57
AMP 1.0 mM		±0.004	±0.0008	±0.0038	±0.54	±1.39	±0.005	±0.0004	±0.00001	±0.0003	±0.33
P		<0.05	NS	NS	<0.001	<0.001	NS	<0.05	<0.001	<0.001	NS

The effects of theophylline and dibutyryl cyclic AMP on D-*galactose entry and exit permeability at the brush-border and serosal border*

The data shown in Figs 1 and 2 can be used to calculate the unidirectional permeabilities of galactose across the brush and serosal borders. Table I shows that neither theophylline nor dibutyryl cyclic AMP significantly affect either the mucosal entry or exit permeabilities to galactose nor significantly reduce the mucosal entry: exit permeability ratio (ln P_{12}/P_{21}). However, Fig. 3 shows that with increasing [theophylline] in the range 0–10 mM both the serosal entry and exit permeabilities are reduced to 30 % of control values. 1 mM dibutyryl cyclic AMP has similar effects on serosal permeability to those observed with 10 mM theophylline (Table I). No significant change in the serosal entry: exit permeability ratio to galactose is observed with either theophylline or dibutyryl cyclic AMP.

The effect of varying Ringer [galactose] on the theophylline-dependent responses

It is known that the steady-state distribution of actively transported sugars falls towards unity as the Ringer [sugar] is increased [12]. This fall in distribution ratio was shown to result from a fall in the brush-border asymmetric permeability as [galactose] is raised. Fig. 4a shows that the theophylline-dependent increase in tissue accumulation of galactose is only seen when Ringer [galactose] is less than 20 mM. Above 5 mM no significant theophylline-dependent increase is seen. A secondary

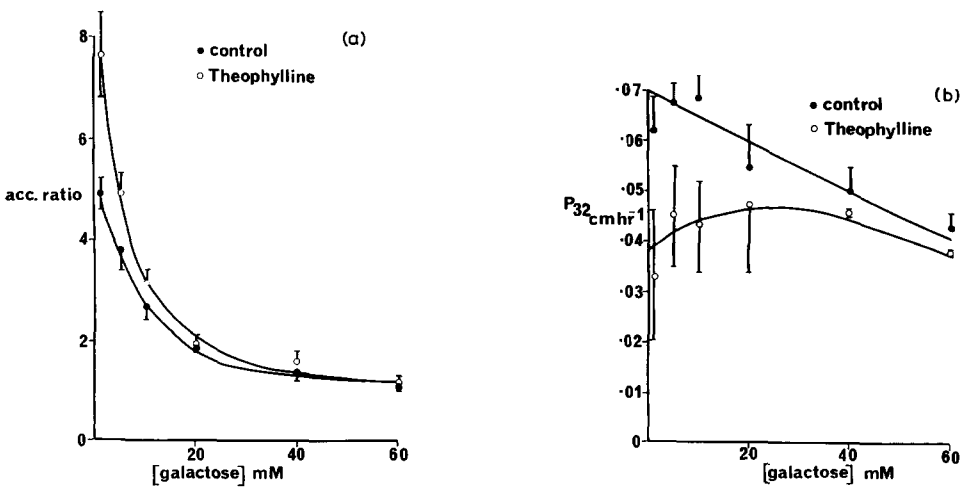

Fig. 4. (a) The effect of raising Ringer [galactose] on the galactose accumulation ratio in the presence (○—○) and in the absence (●—●) of 5 mM theophylline. Ringer [Na] is 75 mM with isotonicity maintained by the addition of galactose or mannitol. Bars represent S.E.M. of 6 control experiments and 3 experiments in the presence of theophylline. Using the '*t*' test (unpaired means solution) $P<0.001$ for a theophylline effect at 2—5 mM galactose. At 40—60 mM galactose the theophylline effect is insignificant. (b) The effect of raising Ringer [galactose] on serosal entry permeability P_{3_2} in the presence (○—○) and in the absence (●—●) of 5 mM theophylline. Ringer [Na] is 75 mM with isotonicity maintained by the addition of galactose or mannitol. Bars represent S.E.M. of 6 control experiments and 3 experiments in the presence of theophylline. Using Students '*t*' test (unpaired means solution) $P<0.001$ for a theophylline effect at 2—5 mM galactose. At 40—60 mM galactose the theophylline effect is insignificant.

cause of the reduced effect of theophylline at higher Ringer galactose concentrations can be seen in Fig. 4b. As the Ringer [galactose] is raised, the serosal permeability in theophylline-treated tissue increases to a maximum at 20 mM Ringer galactose and thereafter falls with the same concentration dependence as is found with control tissue. A fall in serosal galactose permeability due to saturation of the serosal galactose transport system has been previously described [11, 13]. The increase in serosal permeability of theophylline-treated tissue as Ringer [galactose] is raised from 2–5 mM is considered to result from an opening of the tissue spaces on stimulation of net transport, (see Effects of serosal hypertonicity).

Effects of replacement of Ringer Na with choline on the tissue galactose accumulation and serosal permeability

Fig. 5a indicates that replacement by choline of Ringer Na reduces the tissue accumulation of galactose both in control and theophylline treated tissues. With 25 mequiv. Na remaining in the Ringer, no significant difference exists between the accumulation level of galactose in control and theophylline treated tissues. Fig. 5b shows, as was previously demonstrated [13], that there is a small Na-dependent increase in serosal permeability to galactose as Ringer [Na] is raised from 25–140 mequiv. In theophylline-treated tissue there is an Na-dependent decrease in serosal permeability to galactose as Ringer [Na] is raised. This result suggests that the action of theophylline at the serosal border is contingent on the presence of Na in the bathing solution.

Effect of 0.1 mM ouabain on the theophylline-dependent galactose accumulation and reduction in serosal permeability

Addition of ouabain has been previously shown [11–13] to reduce both the

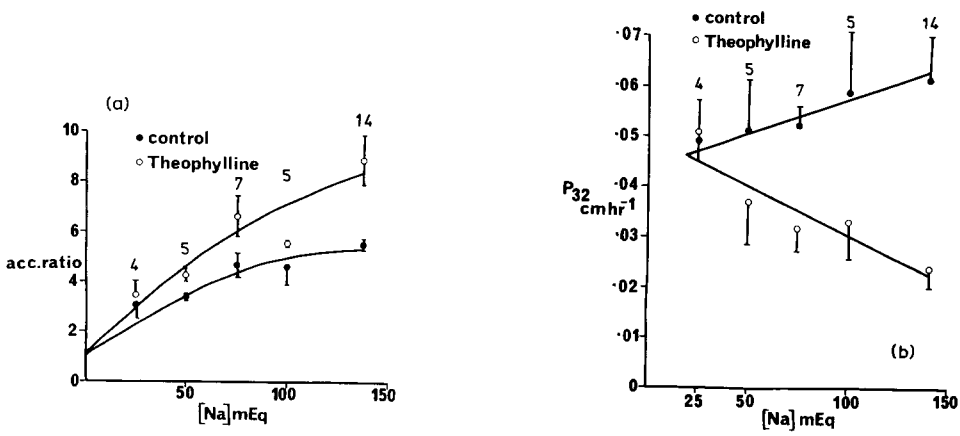

Fig. 5. (a) The effect of replacement of Ringer Na with choline on the tissue galactose accumulation ratio in the presence (○—○) and in the absence (●—●) of 5 mM theophylline. Bars represent S.E.M. of that number of experiments indexed. Using two-way analysis of variance (unequal group sizes) $P<0.001$ for a Na-dependent theophylline response. (b) The effect of replacement of Ringer Na with choline on the serosal entry permeability $P_{3\,2}$ in the presence (○—○) and in the absence (●—●) of 5 mM theophylline. Bars represent S.E.M. of that number of experiments indexed. Using two-way analysis of variance (unequal group sizes) $P<0.01$ for a Na-dependent theophylline response.

TABLE II

THE EFFECT OF THEOPHYLLINE ON GALACTOSE TRANSPORT IN RABBIT ILEUM IN CHLORIDE FREE RINGER'S SOLUTION AND IN THE PRESENCE OF OUABAIN

The significance levels (P) were assessed using a Students 't' test (unpaired means solution).

		μmol · cm^{-2} · h^{-1}					cm · h^{-1}					
	n	J_{13}	J_{31}	J_{net}	R	Accumulation (mM)	P_{12}	P_{21}	P_{23}	P_{32}	$\ln(P_{12}/P_{21})$	$\ln(P_{12}/P_{21})$
NaCl Ringer	13	0.435 ±0.059	0.0106 ±0.0019	0.425 ±0.0601	3.39 ±0.348	11.67 ±0.66	0.233 ±0.0291	0.0036 ±0.0004	0.0496 ±0.0059	0.0755 ±0.0096	4.16 ±0.235	0.392 ±0.0607
P		<0.05	NS	<0.05	<0.001	<0.001	NS	NS	<0.001	<0.001	NS	NS
+Theophylline 5 mM	13	0.283 ±0.032	0.008 ±0.0009	0.275 ±0.032	8.3 ±0.747	20.82 ±0.976	0.172 ±0.015	0.0033 ±0.0002	0.0153 ±0.0013	0.0225 ±0.0023	3.92 ±0.136	0.32 ±0.084
Na_2SO_4 Ringer	8	0.368 ±0.073	0.0103 ±0.002	0.358 ±0.075	3.58 ±0.662	11.3 ±1.37	0.199 ±0.0357	0.0041 ±0.0008	0.042 ±0.0044	0.058 ±0.0059	3.97 ±0.469	0.334 ±0.044
P		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05
+Theophylline 5 mM	8	0.253 ±0.0415	0.0135 ±0.0025	0.239 ±0.0432	3.46 ±0.444	9.37 ±1.03	0.146 ±0.0206	0.0064 ±0.0012	0.0391 ±0.0035	0.0437 ±0.0045	3.195 ±0.344	0.105 ±0.078
NaCl Ringer $+10^{-4}$ M Ouabain	6	0.046 ±0.0079	0.0205 ±0.0025	0.026 ±0.0098	0.41 ±0.076	4.09 ±0.334	0.027 ±0.0044	0.0068 ±0.0003	0.0401 ±0.0035	0.0681 ±0.0059	1.28 ±0.251	0.530 ±0.104
P		NS	NS	NS	NS	<0.05	NS	<0.01	<0.02	NS	NS	<0.05
+5 mM Theophylline $+10^{-4}$ M Ouabain	7	0.061 ±0.0124	0.0257 ±0.0028	0.035 ±0.014	0.519 ±0.108	3.14 ±0.247	0.0366 ±0.0069	0.0126 ±0.0015	0.0593 ±0.0054	0.0746 ±0.0076	0.981 ±0.289	0.224 ±0.059

Na-dependent permeability asymmetry of galactose across the mucosal border and to a small extent to reduce the entry permeability of galactose to the serosal border in Na Ringer. These changes in galactose fluxes account for the observed loss in tissue accumulation. The results shown in Table II indicate that ouabain reduces accumulation of galactose in controls and in theophylline-treated tissue. The effects of ouabain on galactose transport across the brush-border of control and theophylline-treated tissue are identical, entry permeability falls and exit permeability rises as has been reported previously [11, 12]. However, whereas in control tissue ouabain causes a small decrease in both serosal entry and exit permeability, in theophylline treated tissue, ouabain completely reverses the Na-dependent reduction in serosal permeability to galactose.

Effect of hypertonic mannitol in the serosal solution on the theophylline-dependent effects on galactose transport and accumulation

Since an increased intracellular level of cyclic AMP is known to decrease the width and volume of the paracellular and submucosal spaces in intestinal epithelium [14] it was considered possible that this action also decreases serosal permeability by reducing the area of lateral-basal membrane surface and consequently the number of sites accessible to galactose. Hypertonic serosal solutions have been previously shown to increase the width of the tissue spaces [11, 15] and hence to increase serosal permeability. Table III shows that a hypertonic serosal solution increases both serosal entry and exit permeability in both control and theophylline treated tissues; additionally

TABLE III

THE EFFECT OF HYPERTONIC SEROSAL MANNITOL ON GALACTOSE ACCUMULATION AND SEROSAL PERMEABILITY IN THE PRESENCE AND IN THE ABSENCE OF 5 mM THEOPHYLLINE

The significance levels (P) of the theophylline and serosal mannitol effects were assessed using two-way analysis of variance. The effect of serosal mannitol on the % change due to theophylline was assessed using a 't' test (unpaired means solution).

	n	Accumulation (mM)	% increase in accumulation	P_{2-3} (cm · h^{-1})	% inhibition of P_{2-3}	P_{3-2} (cm · h^{-1})	% inhibition of P_{3-2}
A_1 B_1 Control	4	12.15 ±1.45		0.045 ±0.0025		0.073 ±0.013	
A_1 B_2 Theophylline (5 mM)	4	21.72 ±1.77	128 ±19.7	0.0145 ±0.001	67.6 ±3.6	0.0185 ±0.0025	73.5 ±4.04
A_2 B_1 Serosal mannitol	4	10.21 ±1.408		0.0722 ±0.0022		0.1143 ±0.0114	
A_2 B_2 Serosal mannitol +theophylline (5 mM)	4	13.05 ±2.3	27.2 ±15.5	0.0434 ±0.0047	40.4 ±5.18	0.0623 ±0.0046	44.3 ±5.9
Serosal mannitol effect (A)		P <0.05		P <0.001		P <0.01	
Theophylline effect (B)		P <0.001		P <0.001		P <0.001	
Effect of serosal mannitol on theophylline response			P <0.01		P <0.01		P <0.01

there is a reciprocal fall in the level of galactose accumulation within the tissue as serosal permeability rises. Although serosal hypertonicity causes a larger relative change in serosal permeability in theophylline treated tissue, a significant theophylline-dependent decrease in serosal galactose permeability is observed even when the serosal solution is hypertonic.

Effect of chloride replacement by sulphate in Na Ringer on the action of theophylline on galactose transport

Nellans et al. [7] and Binder et al. [8] have demonstrated that the reduction in net Na absorption from rat and rabbit ileum following upon addition of theophylline is abolished when Ringer chloride is replaced by sulphate, despite a theophylline-dependent increase in tissue [cyclic AMP] in sulphate medium [1]. Hence, it was considered of some interest to determine if any theophylline action on galactose flux and accumulation is observed in sulphate Ringer. Table II indicates that whilst replacement of Ringer chloride with sulphate does not affect transport of galactose across the mucosal and serosal borders of control tissue, no theophylline-dependent increase in galactose accumulation or reduction in serosal permeability is observed in sulphate medium. This result indicates that the mechanism of action of theophylline on NaCl transport is similar to its action on galactose transport at the serosal border. Furthermore the result shows theophylline action also has a requirement for specific anions before it can be manifest.

TABLE IV

THE EFFECT OF THEOPHYLLINE ON TISSUE WATER AND [Na^+] AND [K]

Significance levels (P) were assessed using one-way analysis of variance, $P\star$, or a students 't' test. $P\star\star$, (unpaired means solution). NS, not significant.

	n	wt tissue water / dry wt	% reduction wt tissue water / dry wt	[Na+] mequiv/l tissue water	% reduction [Na^+]	[K^+] mequiv/l cell water
NaCl Ringer	6	7.55 ±1.1		54.8 ±6.7		89.6 ±4.4
+Theophylline 0.1 mM	6	6.7 ±0.88		51.7 ±7.6		92.3 ±4.4
1 mM	6	5.53 ±0.59		43.8 ±9.5		102.0 ±5.6
5 mM	6	3.48 ±0.55		37.4 ±7.7		100.8 ±9.6
10 mM	6	5.7 ±0.84	24.5	39.0 ±8.7	28.8	96.8 ±7.9
$P\star$		<0.01		<0.01		NS
Na_2SO_4 Ringer	6	6.98 ±0.65		45.4 ±2.9		86.5 ±3.2
+Theophylline 5 mM	6	7.4 ±0.89	–	35.6 ±3.0	21.5	77.1 ±5.5
$P\star\star$		NS		<0.05		NS

Effect of theophylline on tissue Na, K and water content

Table IV shows that in theophylline-treated tissue there is a reduction in the extracellular fluid content as is evident from the reduction in the wet:dry weight of the tissue and the proportionate reduction in the amount of Na within the tissue. No significant change is seen in the amount of K associated with the tissue. The control levels of Na and K resemble closely those determined by Knoopman and Schultz [16]. Since the fall in Na associated with the tissue is in proportion to the loss in fluid from the tissue, it is considered likely that most of this reduction in Na is due to extracellular loss rather than to a reduction in cell [Na]. These effects are being further investigated (in preparation). This reduction in extracellular volume in theophylline treated tissues is further evidence suggesting that theophylline reduces the paracellular and submucosal tissue spaces. No significant theophylline-dependent loss of tissue fluid is observed in sulphate Ringer.

DISCUSSION

The experimental results show that theophylline increases active accumulation of galactose and reciprocally reduces serosal permeability to galactose. In conditions where the asymmetric permeability of the mucosal border to galactose is reduced, i.e. with low Ringer [Na], high Ringer [galactose], or when ouabain is present, theophylline has either a much reduced or negligible effect on the tissue accumulation of galactose. Thus the action of theophylline is contingent on there being permeability asymmetry to galactose at the brush-border.

Since theophylline reduces both serosal entry and exit permeability without affecting the permeability asymmetry at either the serosal or mucosal border, a causal linkage between the theophylline-dependent increase in active galactose accumulation and reduction in serosal permeability is likely. This view is reinforced by observing that serosal hypertonicity increases the permeability of the serosal border and reduces tissue accumulation of galactose. Additionally, serosal hypertonicity partially reverses the effects of theophylline on both tissue galactose accumulation and serosal permeability.

The mechanism of theophylline action on the serosal border

As theophylline inhibits phosphodiesterase activity and increases intracellular levels of cyclic AMP, it is probable that the reduction of serosal permeability is triggered by some function which is critically dependent on the concentration of this substance.

The findings reported here indicate that there is a correlation between the action of cyclic AMP in reducing serosal permeability to galactose and in reversing the direction of net NaCl movement across the intestine [6–9]. However, in sulphate Ringer, although tissue cyclic AMP is increased by the action of theophylline [1], no theophylline-dependent effect on net NaCl flux [7, 8] or on serosal galactose transport is found. This evidence implies that control of net NaCl and serosal galactose transport stem from a common action of theophylline.

A possible explanation of these effects on Na and galactose transport is that both occur as a result of closure of the paracellular shunt pathway. This view is lent support on observing that there is a significant reduction in wet:dry weight ratio and

the amount of extracellular Na present within theophylline treated tissue. In sulphate Ringer these weight changes do not occur. The mechanism by which the paracellular shunt pathway closes and permeability changes occur in response to raised tissue [cyclic AMP] must also have specific requirements for anions and perhaps also Na-pump activity. (Further experiments concerning the nature of the relationship between the permeability of the paracellular pathway and NaCl and also non-electrolyte flux will be described in forthcoming papers.)

Quantitative support for the hypothesis that serosal permeability controls the tissue accumulation ratio

In order to sustain the hypothesis that reduction in serosal permeability causes the observed increase in galactose accumulation, it is necessary to demonstrate quantitatively that the reduction sufficiently accounts for the increase. In a previous paper [13] it was shown that the steady-state accumulation ratio of galactose could be predicted assuming that the reflexion coefficient for galactose at the serosal border was unity. Since, the K_m for serosal galactose transport is high and the serosal transport process is nearly symmetrical, it follows that

$$\frac{C_2}{C_1} = \frac{J_{23}}{J_{32}} = \frac{(1+1/R)}{J_{31}/J_{13}+1/R} = 1+\frac{J_{net}}{C_3 \cdot P_{32}} \tag{1}$$

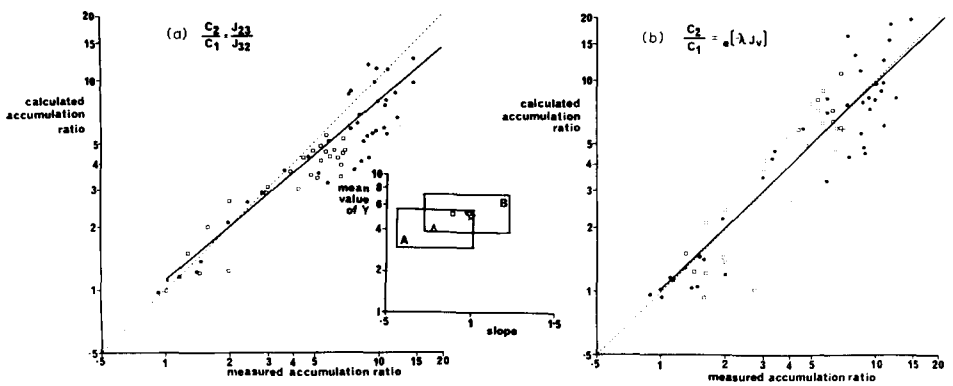

Fig. 6. Plots of calculated versus observed galactose accumulation ratio. The data are taken from experiments in which the accumulation ratio is varied by altering Ringer [Na] and also Ringer [galactose] in the absence (□—□) and presence (●—●) of 5 mM theophylline. The solid lines are the least-square linear regression line of y on x through all the data. The broken lines are the lines of identity between predicted and observed accumulation, i.e. $\bar{x} = \bar{y}$ with slope $y/x = 1$. (a) The calculated accumulation ratio is derived according to the relationship $C_2/C_1 = J_{23}/J_{32} = (1+1/R)/(J_{31}/J_{13}+1/R)$. The line of the logarithmically transformed and orthogonalized ($x_0 = x-\bar{x}$) data is $y = 1.38+0.79x$, $r = 0.917$, $n = 62$.(b) The calculated accumulation ratio is derived according to Eqn 3, where $Pe = \ln(P_{13}/P_{31})$. $y = 1.67+0.97x$, $r = 0.869$, $n = 62$. The mean value of x in both a and b is $\ln(5.12) = 1.63$. The 95 % confidence limits for the slopes and means of the lines are calculated according to the following formula: Confidence limit means $\bar{Y} = \sqrt{(2 \cdot SSR \cdot F)/(n(n-2))}$, Confidence limit slope $= \sqrt{(2 \cdot SSR \cdot F)/((n-2)Sxx)}$, where SSR = sum of squares of residuals and $= Syy - S(xy)^2/Sxx$ where for orthogonalized data $Sxx = \Sigma x^2$, $Sxy = \Sigma xy$ and $Syy = \Sigma y^2 - (\Sigma y)^2/n$. F is the 95 % critical value obtained from F tables for $F_{2,n-2} = 3.15$. Insert. The confidence area bounded by the rectangles are the 90 % confidence limits of the lines, slopes and means of y at mean of $x = 5.12$. Δ = line A; ● = line B; ★ = line of identity; □ = line for A obtained from regression line shown in Fig. 5 [13] for $\bar{x} = 5.12$.

This relationship indicates that the steady-state accumulation ratio is directly dependent on net transmural flux and inversely proportional to serosal permeability. As theophylline reduces both net transmural flux and serosal permeability it is important to determine whether the above relationship (Eqn 1) still holds in theophylline treated tissue. Fig. 6a shows a scattergram of the predicted galactose accumulation ratios in control and theophylline treated tissue plotted against the observed ratios. Since the variance in tissue accumulation is directly proportional to the accumulation ratio, the data was logarithmically transformed to normalise this Poisson distribution.

The predicted values of the accumulation ratio are not significantly different from the observed ratios in either control or theophylline treated populations. The regression line of the present series does not differ significantly from the line previously obtained despite the fact that the centre of the present distribution is 50 % higher than the previous series. Hence, this result indicates that the theophylline-dependent decrease in serosal permeability is quantitatively sufficient to account for the observed increase in tissue accumulation.

Further support for the convection-diffusion modes of intestinal sugar transport

The convective-diffusion hypothesis as previously described for [12, 13] asymmetric sugar transport and accumulation is a modification of earlier double membrane models for solvent flow across epithelia proposed by Curran and McIntosh [17] and Diamond and Bossert [18] and analysed by Patlak et al. [19] and Kedem and Katchalsky [20]. It was proposed that the observed asymmetric sugar permeability across the brush-border is caused by convective-diffusion flow via pores in the brush-border. The force causing the convective flow at the brush-border results from osmotic gradients generated by the action of the Na pump at the lateral-basal border of the intestinal cells. It was further proposed that in vitro intracellular accumulation of sugar results from concentration polarization at the lateral-basal border because the reflexion coefficient at this border to sugar is higher than at the brush-border.

Since, in vitro, the permeability asymmetry at the serosal border is small, it was assumed that the serosal reflexion coefficient is close to unity and hence sugars cross this border mainly by diffusion via low affinity transport sites.

Operationally, there are two differences in sugar transport as postulated by the convective-diffusion mechanism and the Na-gradient hypothesis. Firstly the convective-diffusion model predicts that the exit permeability of sugar across the brush-border should fall (as is observed in ref. 12), when Ringer [Na] is increased from 0 to 140 mequiv as a result of stimulation of the Na pump. In contrast, the Na-gradient hypothesis predicts that sugar exit permeability should increase with increasing cell [Na].

Hence the convective-diffusion model predicts that the main route for net sugar loss is via the lateral-basal border, whereas the Na-gradient hypothesis implies that a major component of sugar exit is via the brush-border. Nevertheless, the difference in the roles of the serosal border as envisaged by the convective-diffusion model and the Na-gradient hypothesis is only quantitative, thus, a demonstration that the flux ratio of galactose across the serosal border is identical to the tissue accumulation ratio is entirely consistent with both models.

A more rigorous test of the convective-diffusion hypothesis is to determine whether the observed accumulation ratio can be predicted according to this theory

from the flux ratios of galactose across the mucosal border when serosal permeability is a finite measurable variable. It was shown by Kedem and Katchalsky [20] that the solute concentration between a double membrane series array (which is analogous to the epithelial cell) [13] can be predicted as follows:

$$\frac{C_2}{C_1} = e^{(-\lambda J_v)} \tag{2}$$

where $\lambda = (\sigma_s - \sigma_m)/(P_m + P_s)$ and C_1 and C_2 the external and intermembrane solute concentrations respectively; J_v is the steady-state volume flow across the membrane array; (flow is considered positive when directed from mucosal-serosal compartments), σ_m, σ_s and P_m, P_s refer to the reflexion coefficients and passive permeability coefficients ($J_v = 0$) of the mucosal and serosal membrane respectively.

If it is assumed that the serosal membrane has a solute reflexion coefficient $\sigma_s = 1$, then it can be shown (see Appendix) that the accumulation ratio may be calculated as follows:

$$\frac{C_2}{C_1} = e^{\frac{Pe}{1+[Pe/R(1-e^{-Pe})]}} \tag{3}$$

where *Pe*, the Peclet number, is the dimensionless ratio of convective: diffusive velocity of solute across the mucosal membrane. It was shown previously [12] that *Pe* can be determined from the following expression:

$$Pe = \ln(P_{12}/P_{21})$$

R is the specific activity ratio of ^3H : ^{14}C-labeled galactose within the tissue fluid. The following approximation is made in order not to assume what has to be demonstrated:

$$Pe = \ln(P_{13}/P_{31}) \text{ (see Appendix)}$$

It can be seen in Fig. 6B that the observed distribution of galactose accumulation ratios in both control and theophylline treated tissue is not significantly different from that predicted according to Eqn 3. It may also be seen (Fig. 6, insert) that the 90 % confidence limits of the regression lines of both theoretical accumulations according to Eqns 1 and 3 versus observed data, overlap. Within this area of overlap lie the best-fit least-square linear regression lines, the line of identity between predicted and observed accumulation and the regression line obtained previously [12] according to Eqn 1.

Thus, the observed accumulation of galactose within the tissue is consistent with the hypothesis that the intestinal epithelial cell behaves like an asymmetric double membrane series array with a steady volume flow across it. Accumulation within the intracellular fluid results from convective diffusion of solute across the mucosal membrane with subsequent concentration polarization at the serosal membrane. In Fig. 6 where mucosal asymmetry is varied by altering Ringer [Na] and/or [galactose], theophylline increases accumulation merely by reducing the permeability of the serosal membrane.

A second operational difference between the Na-gradient and convective-diffusion models of intestinal sugar transport results from the prediction by the Na-gradient mechanism that before net absorption from mucosal to serosal solution can occur, sugar must first be accumulated within the tissue fluid to a higher concentration

than is present in the serosal solution. It can be deduced from Eqn 2 that with the convective-diffusion model, providing that the reflexion coefficient at the serosal border is less than unity, net absorption can take place with a higher sugar concentration in the serosal solution than is present within the tissue fluid ($C_3 > C_2, C_1$). If the reflexion coefficient at the serosal border is less than at the mucosal border, net absorption can occur with the steady-state tissue sugar concentration less than that in either the mucosal or serosal solutions ($C_3 > C_1 > C_2$).

Thus, the convective-diffusion model can explain the result, reported recently by Smirnova and Ugolev [23] and also found previously by Esposito et al. [24] that the in vivo steady-state concentration of glucose and 3-*O*-methyl glucose within the tissue fluid of rat small intestine is lower than the concentration within either the luminal fluid or in the circulating plasma. They suggest that in vivo a sugar pump is present at the serosal border of the cell which is inactivated when the tissue circulation is attenuated. However, if perfusion of the villar capillaries reduces the operational reflexion coefficient of the serosal border from unity to below that of the mucosal border, then convective diffusion would account for both the sugar accumulation seen within the tissue in vitro and the apparently wide variation in cell [sugar] observed in vivo [23–25].

APPENDIX

Kedem and Katchalsky [20] showed that the steady-state concentration of solute present within the intermembrane zone of a double membrane series array could be approximated by the following expression

$$\frac{C_2}{C_1} = e^{(-\lambda J_v)} \text{ where } \lambda = \frac{\sigma_m - \sigma_s}{P_m + P_s}$$

assuming $\sigma_s = 1$; then $(1-\sigma_m)J_v = V$ and $-\lambda \cdot J_v = V/(P_m+P_s)$ where σ_m, σ_s, P_m and P_s are the reflexion coefficients and passive permeability coefficients at the mucosal and serosal borders respectively; J_v is the volume flux across the membrane array and V is the convective velocity of solute across the mucosal membrane; C_2 is the solute concentration in the intermembrane zone and C_1 the solute concentration outside the intermembrane zone:

$$\text{hence} \quad \frac{C_2}{C_1} = e^{V/(P_m+P_s)}$$

$$\text{but} \quad J_{12} = \frac{VC_1}{1+e^{(-Pe)}} \text{ (see Appendix ref. 12)}$$

$$\text{hence} \quad V = \frac{J_{12}}{C_1}(1-e^{(-Pe)})$$

where Pe, the Peclet number, is the dimensionless ratio of convective: diffusive velocity of solute, across the mucosal membrane in this case.

It was previously shown [12] that $Pe = \ln(P_{12}/P_{21})$ and since the permeability ratio of galactose across the brush-border is approximately the same as across the whole tissue [12]

$\ln(P_{12}/P_{21}) \approx \ln(P_{13}/P_{31})$.

By definition $Pe = V/P_m$

hence $P_m = V/Pe$

$$\text{therefore } \frac{C_2}{C_1} = e^{\left\{\frac{\frac{J_{12}}{C_1}(1-e^{-Pe})}{\frac{J_{12}}{C_1}(1-e^{-Pe}) + \frac{J_{32}}{C_3}}\right\}}$$

from ref. 11, Eqn 8, $R = J_{12}/J_{32}$ and since in all the experiments described here sugar concentration in compartments 1 and 3 are identical, it follows that

$$\frac{C_2}{C_1} = e^{\frac{Pe}{1+[Pe/R(1-e^{-Pe})]}}.$$

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