Biochemical Pharmacology, Vol. 17, pp. 1207–1212. Pergamon Press. 1968. Printed in Great Britain

THE EFFECT OF PHENOLPHTHALEIN AND OTHER PURGATIVE DRUGS ON RAT INTESTINAL ($Na^+ + K^+$) ADENOSINE TRIPHOSPHATASE

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(*Received* 16 *November* 1967; *accepted* 11 *January* 1968)

Abstract—Phenolphthalein noncompetitively inhibited the ($Na^+ + K^+$)-adenosine triphosphatase (ATPase) activity of microsomes from the rat small intestine ($K_i = 6 \times 10^{-5}$ M). Quinone, hydroquinone and the purgative drugs, danthron and bisacodyl, were also inhibitory at 10^{-4} M, while phlorizin, phenolphthalein disulfate and phenolphthalein monoglucuronide were without effect. It is suggested that inhibition of intestinal sodium transport by purgative drugs results from inhibition of intestinal ($Na^+ + K^+$)-ATPase. Since danthron inhibited the intestinal ($Na^+ + K^+$)-ATPase, but did not affect glucose transport across the intestine, it appeared unlikely that ($Na^+ + K^+$)-ATPase was directly involved in glucose transport.

RECENTLY Phillips *et al.* have reported that both phenolphthalein and emodin block the active transport of sodium across the rabbit ileum and frog skin.[1] Phenolphthalein also inhibits the sodium-dependent uptake of 3-methyl-D-glucose by the hamster small intestine,[2] while phenolphthalein and bisacodyl reduce intestinal glucose absorption in the rat.[3] The question therefore arises as to what role inhibition of active sodium and sugar transport plays in the pharmacological effect of phenolphthalein and other purgatives. Perhaps even more important is an elucidation of the mechanism of inhibition, since this might lead to a better understanding of the active transport process itself.

The brush border cells of the rat small intestine contain high levels of microsomal ($Na^+ + K^+$) adenosine triphosphatase (ATPase),[4] an enzyme which is thought to be involved in the active transport of cations across cell membranes.[5] Csáky has suggested that ($Na^+ + K^+$)-ATPase might also be involved in the sodium-dependent active transport of sugars and amino acids by the small intestine.[6] We have therefore studied the effect of phenolphthalein and other purgative drugs on the microsomal ($Na^+ + K^+$)-ATPase from rat small intestine brush border (epithelial) cells.

MATERIALS AND METHODS

Danthron (1,8-dihydroxyanthraquinone) was obtained from Riker Laboratories. Bisacodyl [di-(4-acetoxyphenyl)-2-pyridyl-methane] was supplied by Geigy Research. ATP was purchased from Sigma; *p*-nitrophenyl phosphate was from Calbiochem. All other chemicals were of analytical grade. The phenolphthalein and bisacodyl were added as a solution in ethanol, the same amount of solvent being added to the control.

Microsomes were prepared from the small intestine epithelial cells of male Sprague–Dawley rats (200–250 g) by the method of Porteus and Clark,[7] and treated with sodium iodide according to the procedure of Nakao *et al.*[8]

Adenosine triphosphatase assay. This was carried out by the method of Chignell and Titus,[9] the final reagent concentrations being: ATP, 2 mM; $MgCl_2$, 5 mM; NaCl, 120 mM; KCl, 30 mM; Tris-HCl buffer (pH 7·4), 100 mM. Enzymatic activity observed in the presence of magnesium ions will be referred to as Mg^{2+}-ATPase, while the increment on the addition of both sodium and potassium ions will be designated $(Na^+ + K^+)$-ATPase.

p-*Nitrophenylphosphatase* (p*NPPase*) *assay.* Tubes containing approximately 0·2 mg microsomal protein in a final volume of 1·0 ml were shaken in a bath at 37° for 8 min. Each tube contained 2·0 mM pNPP, 5·0 mM $MgCl_2$ and 100 mM Tris buffer, pH 8·0. K^+-pNPPase was determined as the increase in rate of *p*-nitrophenol formation when 30 mM KCl was also included. Reactions were stopped by the addition of 0·5 ml cold 1·2 M $HClO_4$, followed by 0·5 ml cold 1·5 M KOH. After centrifugation of the precipitated $KClO_4$, the concentration of *p*-nitrophenol in the supernatant solution was estimated from the absorbancy at 410 mμ.

Protein assay. This was carried out by the method of Lowry *et al.*[10]

RESULTS AND DISCUSSION

A typical microsomal preparation hydrolyzed ATP at the rate of 30·8 μmole/hr/mg protein in the presence of magnesium alone, with an increase of 20·7 μmole/hr/mg protein on the addition of sodium and potassium ions (Table 1). After treatment with

TABLE 1. EFFECT OF VARIOUS PURGATIVE DRUGS AND OTHER COMPOUNDS ON THE $(Na^+ + K^+)$-ATPASE AND K^+-pNPPASE ACTIVITY OF INTESTINAL MICROSOMES

Drug (10^{-4} M)	$(Na^+ + K^+)$-ATPase* (μmoles/hr/mg protein)		Mg^{2+}-ATPase* (μmoles/hr/mg protein)		K^+-pNPPase* (μmoles/hr/mg protein)	
Control	20·7	(100)	30·8	(100)	5·35	(100)
Phenolphthalein disulfate	18·2	(87·9)	31·4	(101·9)	4·96)	(92·7)
Phenolphthalein monoglucuronide	21·2	(102·4)	34·8	(113·0)		
Danthron	7·0	(33·8)	33·4	(108·4)	2·91	(54·4)
Phlorizin	18·6	(89·8)	31·2	(101·3)	5·85	(109·3)
Phenol	21·0	(101·4)	31·0	(100·6)	6·27	(117·?)
Quinone	4·0	(19·3)	25·8	(83·8)	1·68	(31·4)
Hydroquinone	7·5	(36·2)	27·4	(89·0)	1·45	(27·1)
Control†	16·6	(100)	28·9	(100)	4·68	(100)
Phenolphthalein†	4·9	(29·5)	23·8	(82·4)	2·27	(48·5)
Bisacodyl†	9·9	(59·6)	27·1	(93·8)	3·26	(69·7)

* Figures in parentheses represent the value as a percentage of control.
† Activity measured in the presence of 1% ethanol.

sodium iodide, the corresponding activities were 32·5 μmole/hr/mg protein (Mg^{2+}-ATPase) and 42·1 μmole/hr/mg protein [$(Na^+ + K^+)$-ATPase]. Although the sodium iodide treatment of other microsomal preparations has resulted in a decrease in the Mg^{2+}-ATPase activity, the intestinal preparation appears to be somewhat resistant to this treatment.[8] The sodium iodide-treated microsomes were used only in the kinetic experiments (see Figs. 1 and 2, Table 3) where a higher $(Na^+ + K^+)$-ATPase activity permitted better estimation of the *Km* values for ATP, sodium and potassium.

From Table 1 it may be seen that all the purgative drugs tested inhibited the ($Na^+ + K^+$)-ATPase but not the Mg^{2+}-ATPase activity of the intestinal microsomes. Phenolphthalein (10^{-4} M) gave 70 per cent inhibition and was the most potent of all the purgatives examined. Quinone (*p*-benzoquinone) and hydroquinone, potent inhibitors of active sodium transport across the frog skin,[11] also inhibited the intestinal ($Na^+ + K^+$)-ATPase (Table 1). Danthron is the only purgative tested which inhibited the ($Na^+ + K^+$)-ATPase while having no effect on active sugar transport (Table 2).

TABLE 2. EFFECT OF VARIOUS PURGATIVE DRUGS AND OTHER COMPOUNDS ON ($Na^+ + K^+$)-ATPASE, SODIUM AND GLUCOSE TRANSPORT

Drug (10^{-4} M)	($Na^+ + K^+$)-ATPase (% control)	Sodium transport across frog skin*	Sodium transport across rabbit ileum† (mucosal→serosal flux) (μEquiv./g/15 min)	Glucose absorption by rat small intestine‡ (% glucose absorbed in 20 min)
Control	100	100	322	87·28
Phenolphthalein	29·0		196§	61·45
Quinone	19·2	21		
Hydroquinone	36·2	22		
Emodin (cascara)			162§	83·72
Danthron	34·0			90·92
Phlorizin	89·8			40·24
Bisacodyl	60·0			49·38

* Measured as the short circuit current with the value observed before the addition of the drug arbitrarily set at 100.[11]

† Data taken from ref. 1.

‡ Data taken from ref. 2.

§ Drug concentration, 10^{-3} M.

The related drug, emodin, also has no effect on sugar transport,[3] but does inhibit sodium transport in the rabbit ileum.[1] Phlorizin, on the other hand, does inhibit sugar transport, but has little effect on the ($Na^+ + K^+$)-ATPase (Table 2). This compound, however, does not inhibit other transport systems and appears to be specific for sugars.[12] It was not surprising to find that the intestinal K^+-pNPPase showed the same sensitivity towards the purgative drugs as did ($Na^+ + K^+$)-ATPase, since these enzymes appear to be closely related.[13]

The kinetics of the interaction between ($Na^+ + K^+$)-ATPase and phenolphthalein was further investigated. Fig. 1 clearly shows that this drug noncompetitively inhibits intestinal ($Na^+ + K^+$)-ATPase. The calculated K_i is 0·06 mM (Table 3), which is very close to the value of 0·07 mM found by Adamic and Bihler for phenolphthalein inhibition of sodium-dependent 3-methyl-D-glucose uptake by hamster intestinal strips.[2] The effect of phenolphthalein on the activation of ($Na^+ + K^+$)-ATPase by sodium and potassium ions was also studied. Inhibition was again found to be non-competitive (Fig. 2), while the calculated K_i values were similar to the value obtained with ATP as the variable substrate (Table 3).

Since phenolphthalein disulfate (Fig. 3) has little effect on the intestinal ($Na^+ + K^+$)-ATPase (Table 1), it might be concluded that the phenolic hydroxyl groups of phenolphthalein are necessary for inhibitory activity. However, phenolphthalein glucuronide,

in which one of the hydroxyls is still free, has no effect on enzyme activity (Table 1). Since phenol itself is also not an inhibitor (Table 1), it is obvious that the phenolic group *per se* is not responsible for inhibitory activity. However, from Table 1 it may be seen that both hydroquinone and quinone are good inhibitors of the intestinal

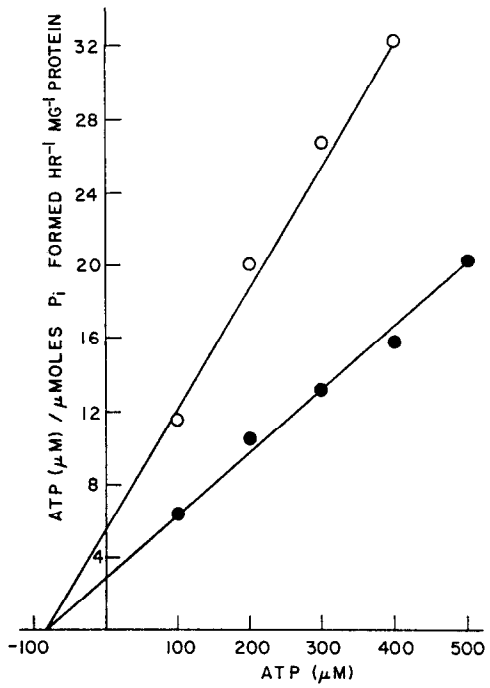


FIG. 1. Effect of phenolphthalein (5×10^{-5} M) on the relationship between ATP concentration and the ($Na^+ + K^+$)-ATPase activity of rat intestinal microsomes (–●–, control; –○–, phenolphthalein).

TABLE 3. KINETIC DATA FOR THE INTERACTION OF PHENOLPHTHALEIN WITH INTESTINAL ATPASE

Substrate	Enzyme	V_{max} (μmoles/hr/mg protein)		*Km* (mM)	K_i* mM)
		Control	Phenolphthalein (5×10^{-5} M)		
ATP	Mg^{2+}-ATPase	22·4	22·0	0·050	
ATP	($Na^+ + K^+$)-ATPase	28·8	15·1	0·087	0·06
KCl	($Na^+ + K^+$)-ATPase	34·9	21·2	3·5	0·08
NaCl	($Na^+ + K^+$)-ATPase	26·3	14·5	25·2	0·06

* For phenolphthalein.

($Na^+ + K^+$)-ATPase. Both of these compounds also inhibit active sodium transport across the frog skin.[11] The quinone structure also appears in emodin and danthron (see Fig. 3), and it has been suggested that at neutral pH even phenolphthalein exists in the quinone form (Fig. 4).[14] Perhaps it is the presence of a potential redox

system which confers inhibitory activity. Bisacodyl is the obvious exception to this generalization.

Brzhevskaya *et al.*[15] have studied rabbit myosin ATPase and found that propyl gallate is an inhibitor of this enzyme. These experiments together with some electron

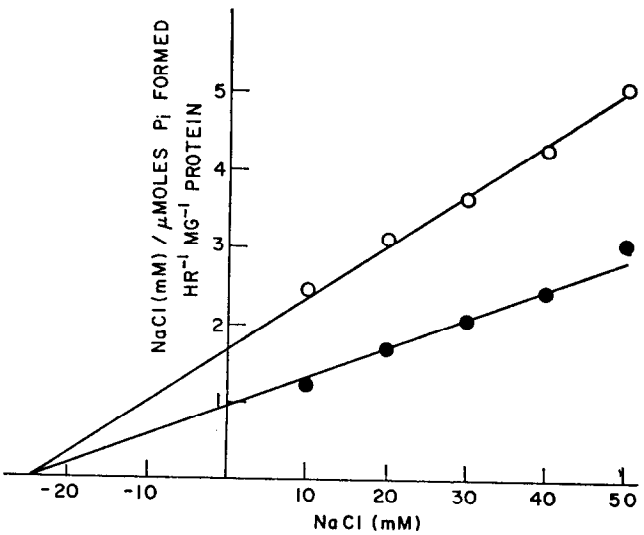
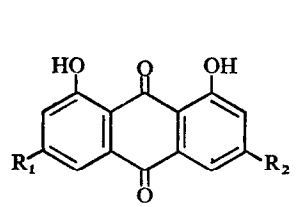
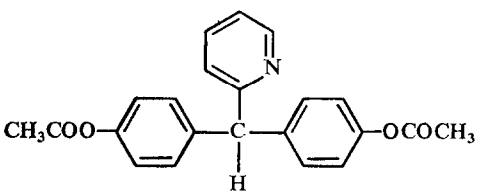


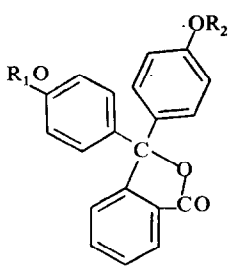
FIG. 2. Effect of phenolphthalein (5×10^{-5} M) on the relationship between NaCl concentration and the $(Na^+ + K^+)$-ATPase activity of rat intestinal microsomes (-●-, control; -○-, phenolphthalein).



Emodin $R_1 = CH_3$; $R_2 = OH$
Danthron $R_1 = R_2 = H$



Bisacodyl



Phenolphthalein $R_1 = R_2 = H$
Phenolphthalein disulfate $R_1 = R_2 = SO_3H$
Phenolphthalein monoglucuronide $R_1 = H$; $R_2 = C_6H_9O_6$

FIG. 3. Structure of emodin, danthron, bisacodyl, and phenolphthalein and its derivatives.

spin resonance data have led them to postulate the existence of free radicals during the hydrolysis of ATP by myosin ATPase. Perhaps therefore it is worth noting that hydroquinone and quinone are inhibitors of free radical reactions.[16]

Phenolphthalein, emodin, danthron and bisacodyl have been thought to promote peristalsis by local irritation of the mucosa or by a direct effect on the nerves or muscles

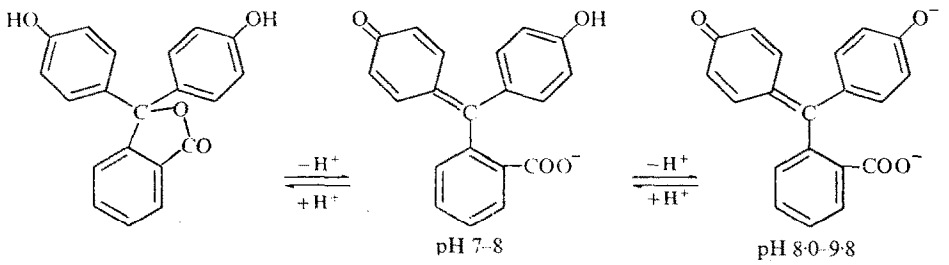


FIG. 4. Effect of pH on the structure of phenolphthalein.

of the small intestine.[17] The experiments presented here and elsewhere[1–3] would suggest a more complex mechanism of action. Inhibition of active sodium and sugar transport would result in water retention, an increased bulk in the lumen of the intestine, and a reflex stimulation of peristalsis. It also seems reasonable that inhibition of sodium transport by purgative drugs is the result of inhibition of the intestinal ($Na^+ + K^+$)-ATPase. Since danthron inhibits ($Na^+ + K^+$)-ATPase but does not affect intestinal glucose transport (Table 2), it appears unlikely that ($Na^+ + K^+$)-ATPase is directly involved in the transport of glucose.

Acknowledgements—The author is indebted to Mrs. D. K. Starkweather for expert technical assistance and to R. J. D'Agostino (Riker Laboratories) and M. Weiner (Geigy Research) for the supply of compounds used in this investigation.

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