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ENHANCEMENT EFFECT OF METHYLXANTHINES ON THE INTESTINAL ABSORPTION OF POORLY ABSORBABLE DYES FROM THE RAT SMALL INTESTINE

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Abstract—The effect of theophylline or caffeine on the absorption of phenol red (PR) or bromphenol blue (BPB) (poorly absorbable dyes) from the rat small intestine was investigated by using the *in situ* recirculation technique. The disappearance of PR and BPB from the recirculated solution was increased by pretreatment of the intestinal lumen with 5 mM theophylline and 5 mM caffeine. With BPB, increases in tissue accumulation and net absorption were observed in the presence of 5 mM theophylline or 5 mM caffeine. However, the administration of 15 μmoles theophylline or 25 μmoles caffeine into a femoral vein by a single injection failed to produce an increasing effect on the disappearance of PR or BPB from the recirculated solution. A possible mechanism of the enhancement effect of the methylxanthines on dye absorption was discussed.

We have investigated previously the absorption mechanism of some water-soluble dyes, which are highly ionized compounds with very low lipid solubilities, at the physiological pH range of the small intestine [1–3]. In those reports, it was suggested that the poor absorption of phenol red (PR) was due to its very low affinity to the intestinal mucosa, in addition to its poor lipid solubility. Although bromphenol blue (BPB) can bind rapidly to the brush borders, the following step, which is slower, i.e. the penetration through the lipid bilayer, seems to be the main cause of the poor absorption of BPB. This is consistent with the report that the epithelial border was found to be a critical anatomical barrier to the *in vitro* and *in situ* intestinal absorption of poorly lipid-soluble compounds [4].

Attempts have been made to enhance the absorption of poorly absorbable drugs by synthetic surfactants [5–8], bile salts [9–12], and chelating agents [10, 13]. However, the interaction of a surfactant with biological membranes may produce an accelerated loss of the structural integrity of the intestinal mucosa.

Recently, Briseid *et al.* [14] have shown that the addition of theophylline to the loop fluid increased the absorption of PR and pralidoxime. Beubler and Lembeck [15] further reported that the absorption of tritiated water, urea, antipyrine and salicylate was increased when blood flow was enhanced by theophylline and caffeine. However, all the effects of the methylxanthines on drug absorption cannot be explained solely through an action on blood flow.

The present study was undertaken to investigate the effects of the methylxanthines, theophylline and caffeine, on the intestinal absorption of the poorly absorbable water-soluble dyes, PR and BPB, and to clarify the mechanism of their action.

MATERIALS AND METHODS

Materials

PR, BPB, salicylate, theophylline, and anhydrous caffeine were reagent grade and were used without further purification. All other reagents used in these experiments were of the finest grade available.

Preparation of drug solution

The isotonic buffer solution of pH 6.5 was prepared from 0.123 M Na_2HPO_4 and 0.163 M NaH_2PO_4.

Apparent partition coefficient

The apparent partition coefficient was determined by the method described in a previous report from this laboratory [16].

Analytical methods

Spectrophotometric determination was made of all the drugs investigated.

PR in perfused solution. One ml of sample solution was alkalinized with 5 ml of 1 N NaOH and determined spectrophotometrically at 560 nm.

BPB in perfused solution. One ml of sample solution was diluted with 5 ml of pH 6.5 buffer solution and determined spectrophotometrically at 591 nm.

BPB in tissue. The small intestine was homogenized in twice its wet weight of pH 6.5 buffer solution. A mixture of 5 ml of the homogenate and 5 ml of acetone was shaken for 15 min and then centrifuged for 10 min at 3000 rev/min. The supernatant fraction was analyzed spectrophotometrically at 596 nm. A calibration graph was prepared from the spiked tissue homogenates. The amount of BPB in the tissue was calculated using the calibration graph.

Salicylate in perfused solution. Eight ml of chloroform and 0.1 ml of concentrated HCl were added to 2 ml of sample solution. The mixture was shaken for 15 min and then centrifuged for 10 min at 2500 rev/min. After aspirating the aqueous phase, 5 ml of the organic phase was added to 5 ml of 0.1 N NaOH. The mixture was shaken for 10 min and then centrifuged for 10 min at 2500 rev/min. The optical density of the aqueous phase was determined at 295 nm.

Procedure of absorption experiments

Male Wistar albino rats weighing 150–200 g were

used in all experiments. The procedure of the *in situ* absorption experiment in the rat small intestine was the same as that reported in a previous paper [17]. Animals were anesthetized with pentobarbital, given intraperitoneally, and the small intestine was cannulated for *in situ* recirculation. The entire length of the small intestine, from the proximal end of the duodenum to the distal end of the ileum, was used for the absorption experiments. The bile duct was ligated in all experiments. Forty ml of drug solution, kept at 37°, was recirculated through the intestine at a rate of 5 ml/min. Rectal temperature was maintained at 37 ± 1°. At the end of an absorption period, the perfused solution in the small intestine was withdrawn as completely as possible, and the intestinal lumen was washed with pH 6.5 buffer solution. The washings were combined with the perfused solution and made up to 100 ml with pH 6.5 buffer solution. The amount that disappeared from the lumen was calculated as the difference between the amounts of the drug in the initial and the final solutions. Determination of the accumulation of a dye in the intestinal tissues was carried out as follows. Immediately after washing, the entire small intestine was isolated by tearing off the mesentery and the serosal surface was blotted by paper. After weighing, the small intestine was cut into small pieces and determined as mentioned above. The net amount absorbed was calculated by the difference in amount of dye between the disappearance from the lumen and the tissue accumulation. Pretreatment was carried out as follows. The pH 6.5 buffer solution containing theophylline or caffeine was perfused for 10 min at a rate of 5 ml/min in the rat small intestine. At the end of a perfusion period, the small intestinal lumen was washed with 60 ml of pH 6.5 buffer solution as completely as possible, and the dye solution was perfused for 30 min. Results were compared statistically using Student's *t*-test.

RESULTS

The pH of the pH 6.5 buffer solution was not affected by the presence of 5 mM theophylline or 5 mM caffeine. In addition, no effect of 5 mM theophylline or 5 mM caffeine on the apparent partition coefficients (chloroform/water and benzene/water at pH 6.5) of dyes was found, which indicates that lipid soluble complexes did not form. From these results, it seems that the physico-chemical properties of the dye solution in the intestinal lumen were not influenced by 5 mM theophylline or 5 mM caffeine.

Absorption experiments on rat small intestine were carried out at pH 6.5 using the *in situ* recirculation technique; the results are summarized in Table 1 and Fig. 1. The effect of theophylline or caffeine on the disappearance of PR from luminal solution in 30 min is shown in Table 1. The disappearance of PR from the lumen was significantly increased in the presence of 5 mM theophylline or 5 mM caffeine. Similarly, increases in disappearance from luminal solution, in tissue accumulation and in net absorption of BPB were also observed, as shown in Fig. 1. These results indicate that transport of BPB from the intestinal lumen into the portal vein was increased in the presence of 5 mM theophylline or 5 mM caffeine.

Pretreatment experiments were carried out in order to examine, in more detail, the effect of theophylline or caffeine on the intestinal transfer of poorly absorbable dyes. The results are summarized in Figs. 2 and 3. The disappearance of PR from luminal solution was increased significantly after pretreatment with 1, 3 or 5 mM theophylline. The increase in the disappearance of BPB from luminal solution was also observed after pretreatment with 3 and 5 mM theophylline. As shown in Fig. 3, caffeine pretreatment similarly increased the intestinal transfer of both PR and BPB. From the

Table 1. Effects of theophylline and caffeine on intestinal transfer of PR in 30 min*

	Disappearance from lumen (%)	Significance level
Control	1.5 ± 0.7 (4)	
Theophylline 5 mM	4.8 ± 1.4 (8)	$P < 0.01$
Caffeine 5 mM	4.1 ± 1.1 (8)	$P < 0.01$

* Concentration of PR: 0.1 mM. Numbers in parentheses represent the number of experiments. Results are expressed as means ± S.D.

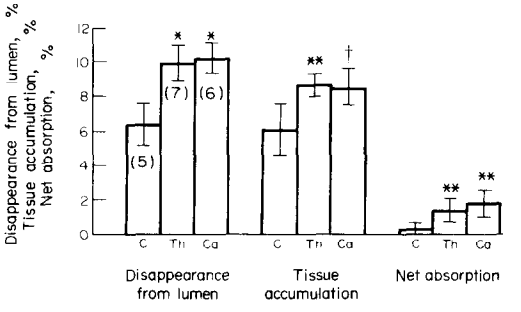

Fig. 1. Effects of theophylline and caffeine on the intestinal transfer of BPB in 30 min. Key: (C) control; (Th) 5 mM theophylline; and (Ca) 5 mM caffeine. Statistical significance: (*) $P < 0.001$; (**) $P < 0.01$; and (†) $P < 0.02$. Concentration of BPB: 0.1 mM. The amount of net absorption was calculated by the difference in amount of BPB between the disappearance from the lumen and the tissue accumulation. Numbers in parentheses represent the number of experiments. Vertical bars indicate ± S.D.

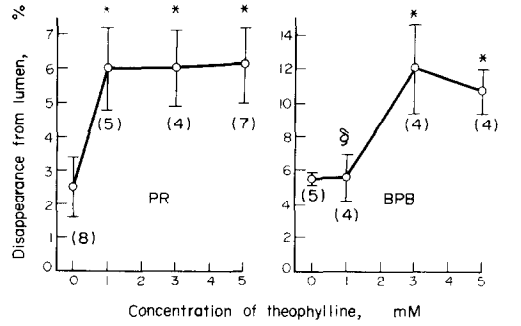

Fig. 2. Effects of pretreatment with theophylline on intestinal transfer of PR and BPB in 30 min. Statistical significance: (§) not significant; and (*) $P < 0.001$. Concentrations of PR and of BPB: 0.1 mM. Numbers in parentheses represent the number of experiments. Vertical bars indicate ± S.D.

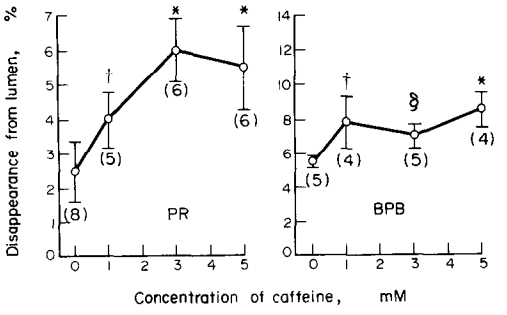

Fig. 3. Effects of pretreatment with caffeine on intestinal transfer of PR and BPB in 30 min. Statistical significance: (§) not significant; (*) $P < 0.001$; and (†) $P < 0.02$. Concentrations of PR and of BPB: 0.1 mM. Numbers in parentheses represent the number of experiments. Vertical bars indicate ± S.D.

results described above, it seems that the alteration of the permeability characteristics of the intestinal mucosa, by theophylline and caffeine, caused the enhancement of the absorption of poorly absorbable dyes.

The xanthines have important, clinically useful actions on the circulatory system. It is well known that theophylline and caffeine increase blood flow by vasodilation, and augment cardiac output [18]. Recently, Beubler and Lembeck [15] reported that the absorption of some model substances was increased when blood flow was enhanced by theophylline or caffeine. In order to examine the contribution of blood flow to the enhancement of the intestinal transfer of PR and BPB, theophylline or caffeine was administered into a femoral vein by a single injection. As shown in Fig. 4, the effect of intravenous administration of theophylline (15 μmoles) or caffeine (25 μmoles) was hardly observed. In this experiment, the disappearance of salicylate from luminal solution was increased significantly, as shown in Table 2, reflecting the increase in intestinal blood flow. Consequently, it seems reasonable to assume that the enhancement of intestinal transfer of poorly absorbable dyes by theophylline and caffeine is not due solely to an increase in blood flow.

DISCUSSION

PR and BPB were selected as models of poorly absorbable dyes. In a previous paper [1], we reported

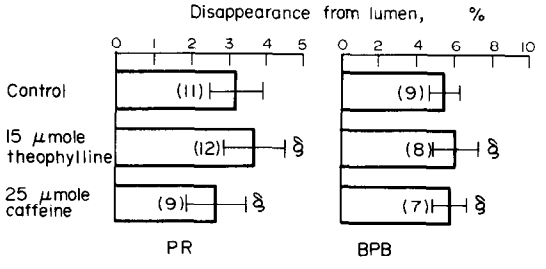

Fig. 4. Effects of theophylline or caffeine administered intravenously on intestinal transfer of PR and BPB in 30 min. Statistical significance: (§) not significant. Concentrations of PR and of BPB: 0.1 mM. Injection volume: 0.5 ml (30 mM theophylline, 50 mM caffeine). Both theophylline and caffeine were dissolved in distilled water. Numbers in parentheses represent the number of experiments. Horizontal bars indicate ± S.D.

Table 2. Effects of theophylline and caffeine administered intravenously on the intestinal transfer of salicylate in 30 min*

	Disappearance from lumen (%)	Significance level
Control	41.8 ± 3.9 (6)	
Theophylline 15 μmoles	50.8 ± 5.4 (8)	$P < 0.01$
Caffeine 25 μmoles	54.8 ± 5.1 (6)	$P < 0.001$

* Concentration of salicylate: 1 mM. Injection volume: 0.5 ml (30 mM theophylline, 50 mM caffeine). Theophylline or caffeine was dissolved in distilled water. Numbers in parentheses represent the number of experiments. Results are expressed as means ± S.D.

that the net absorption values of PR and BPB were 1.2 ± 0.5 and 2.4 ± 1.1 per cent in 1 hr respectively. Because a relatively larger accumulation in the intestinal tissue was noted in the case of BPB, not only the disappearance from luminal solution but also the amount accumulated in the intestinal tissue was examined.

There are some studies concerned with the enhancement of the absorption of poorly absorbable drugs by synthetic surfactants [5–8], bile salts [9–12], and chelating agents [10, 13]. The exact mechanism of the action of surfactants, which increases the permeability of biological membranes, has not been elucidated, although it has been postulated that the mechanism may involve changes in membrane structure, possibly by solubilizing lipid components of the membrane. Recently, Briseid *et al.* [14] reported a significant increase in the absorbed amount of PR and pralidoxime by theophylline. On the other hand, Beubler and Lembeck [15] have shown that theophylline and caffeine increased the absorption of tritiated water, urea, antipyrine and salicylate nearly parallel to the increase in intestinal blood flow.

In the present investigation, the disappearance of PR and BPB from luminal solution was increased significantly in the presence of either theophylline or caffeine (Table 1 and Fig. 1). No evidence of physico-chemical effects, such as formation of lipid soluble complexes, which lead to an enhanced absorption, was observed. Furthermore, the disappearance of PR and BPB from luminal solution was increased significantly by pretreatment of the intestinal lumen with these methylxanthines. These results suggest that the permeability characteristics of the intestinal mucosa could be changed by either theophylline or caffeine, and that the change was maintained for at least 30 min after the removal of the methylxanthines. However, it cannot be excluded that they affect the absorption of poorly absorbable dyes by a mechanism other than direct action on the intestinal mucosa. Therefore, an attempt was made to give a single injection of theophylline or caffeine into a femoral vein at the beginning of perfusion. As shown in Fig. 4, no effects of i.v. administration of the methylxanthines were observed. On the other hand, intravenously administered methylxanthines significantly enhanced the absorption of salicylate(Table 2), which is known to be influenced by intestinal blood flow [15]. Consequently, it seems reasonable to assume

that the enhancement of absorption of poorly absorbable dyes by theophylline or caffeine could not be due to an increase in intestinal blood flow. A recent report of Barnett *et al.* [19] demonstrated an increased salicylate permeability due to theophylline in isolated rat jejunum. They explained that the effect of theophylline on extracellular space increased passive diffusion of salicylate through the tight junction or shunt pathway of the epithelial tissue. If it is assumed that this is operative in our *in situ* system after i.v. administration of theophylline, the enhanced absorption of hydrophilic PR and BPB would not appear to be caused by an increase in their transport via the aqueous extracellular pathway.

Evidence has emerged that methylxanthines produce intracellular accumulation of cyclic AMP (cAMP) by inhibition of phosphodiesterase. Briseid *et al.* [14] have shown that dibutyryl cAMP causes significant increases in absorption of PR and pralidoxime. As shown in Table 3, the disappearance of either PR or BPB from luminal solution was increased significantly when the intestinal lumen was pretreated with 0.5 mM isoproterenol, which is known to produce intracellular accumulation of cAMP by activation of adenyl cyclase. This suggests the possibility that part of the enhancement effect on the dye absorption is dependent on an increase in the level of intestinal cAMP.

Also, methylxanthines have been shown to produce small intestine secretion [20–22] and intestinal smooth muscle relaxation [23]. Forstner *et al.* [24] reported that theophylline, isoproterenol and cAMP stimulate intestinal glycoprotein synthesis and may control the production of glycoproteins located at the intestinal surface. Further investigation is required to clarify its possible relation to the cAMP system.

Table 3. Effects of pretreatment with isoproterenol on the intestinal transfer of PR and BPB in 30 min*

	Disappearance from lumen (%)	
	PR	BPB
Control	2.5 ± 0.9 (8)	5.5 ± 0.3 (5)
Isoproterenol 0.5 mM	5.0 ± 1.7† (4)	8.9 ± 1.2‡ (4)

* Concentration of PR and of BPB: 0.1 mM. Numbers in parentheses represent the number of experiments. Results are expressed as means ± S.D.

† Statistical significance: $P < 0.01$.

‡ Statistical significance: $P < 0.001$.

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