Biochemical Pharmacology, Vol. 27, pp. 1961–1965

0006-2952/78/0801-1961$02.00/0

EFFECT OF DEOXYCHOLATE ON Ca^{2+}-ATPase OF INTESTINAL BRUSH BORDER MEMBRANE

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(*Received* 25 *October* 1977; *accepted* 12 *January* 1978)

Abstract—The effect of deoxycholate on the activity of intestinal brush border membrane Ca^{2+}-ATPase has been studied. The activity of Ca^{2+}-ATPase at 0.1 mM Ca^{2+} was stimulated by deoxycholate ranging from 0.1 to 0.4 mM but at 1 mM Ca^{2+} was depressed by the same concentrations of deoxycholate. A deoxycholate concentration exceeding 0.6 mM inhibited Ca^{2+}-ATPase at any concentration of Ca^{2+}. Deoxycholate affected the activity of Ca^{2+}-ATPase at lower concentrations than caused turbidity of Ca^{2+}-ATPase. It lowered the K_m value of Ca^{2+}-ATPase for Ca^{2+}. An affinity component of Ca^{2+}-ATPase for ATP separated into low and high affinity components in the presence of deoxycholate. Sodium and potassium ions decreased Ca^{2+}-ATPase activity in the presence of deoxycholate. It is concluded that the ionic environment influences the effect of deoxycholate on Ca^{2+}-ATPase. These results suggest that the low dose of deoxycholate stimulates Ca^{2+}-ATPase by improving the accessibility of Ca^{2+} to ATPase molecules but the high dose inhibits Ca^{2+}-ATPase by perturbing membrane lipid–ATPase interactions.

Bile acids inhibit intestinal active transport of sugar [1, 2] and amino acids [3, 4]. They also produce a reduction of net sodium and water absorption [3, 5–9] and an immediate increase in calcium absorption [10]. Lengemann and Dobbins [11] have observed that an increased bile flow enhances calcium absorption. Many workers have found that bile acids increase the permeability of intestine to solutes such as cholesterol [12], fat [13], salicylate [14], sulfaguanidine [15] and phenol red [16]. Further, bile acids cause histological alterations of mucosal epithelia [2, 4, 17–19], stimulate the transmural potential difference across rat ileum [20, 21] and reduce the slow wave frequency in rabbit jejunum [22]. The mechanism of the above-mentioned effects of bile acids which exist in the intestinal lumen at more than 5 mM [23, 24] has not been established.

Some workers have investigated the effect of bile acids on intestinal ATPase. First of all, doxycholic acid has been shown to cause an inhibition of Na^+–K^+-ATPase [25]. Gracey *et al.* [26] also have found that deoxycholate inhibits small intestinal Na^+–K^+-ATPase. Administering deoxycholate to rats produces a reversible inhibition of jejunal Na^+–K^+-ATPase [27]. Cholylglycine reduces ATPase activity in small intestines [28] and in intestinal brush borders [3]. In contrast, Faust and Wu [29] have observed that cholylglycine and cholyltaurine stimulate intestinal mucosal Na^+–K^+-ATPase and Mg^{2+}-ATPase. Deoxycholate (5 mM) has been reported to stimulate Mg^{2+}-ATPase of semipurified brush borders [30]. Thereafter, Guiraldes *et al.* [31] confirmed that 5 mM deoxycholate stimulates Mg^{2+}-ATPase, whereas 1 mM deoxycholate inhibits Na^+–K^+-ATPase and Mg^{2+}-ATPase. However, it is unsettled whether deoxycholate influences the activity of intestinal brush border Ca^{2+}-ATPase.

Deoxycholate is used at greater than critical micellar concentration for the solubilization of membrane-bound enzymes which are held as integral protein in the membranes by hydrophobic interactions with lipids [32, 33]. The proteins released are virtually free from phospholipid [32]. It is generally accepted that lipid–ATPase interactions alter properties of membrane-bound ATPase activity, such as pH dependence, temperature dependence, drug sensitivity and K_m value [34–37]. Therefore, characterization of perturbed brush border Ca^{2+}-ATPase by surface-active deoxycholate may give some indications of the mechanism of its action on membrane lipid–ATPase interactions. The present paper describes the effect of deoxycholate on Ca^{2+}-ATPase of intestinal brush border membrane.

MATERIALS AND METHODS

Preparation of Ca^{2+}-ATPase of intestinal brush border membrane. Male Wistar rats weighing about 250 g were used after overnight fasting. They were killed by decapitation. The mesenteric artery was perfused with ice-cold saline to remove blood. The small intestine was immediately isolated and everted. The brush border membrane fractions I and II were prepared from the scraped mucosa by the method of Forstner *et al.* [38]. They were used as a purified brush border fraction from which most of the fibrillars were removed. The purified brush border fraction contained negligible amounts of DNA, succinate dehydrogenase, NADPH-cytochrome C reductase and Na^+–K^+-ATPase. Judging from the results of marker enzyme and component assays, these brush border fractions were free from nuclear, mitochondrial, microsomal and baso-lateral plasmic membranes. The purified brush border fraction (10 mg protein) was frozen, thawed and sonicated in 30 ml of 2.5 mM EDTA–NaOH (pH 7.4) for a total of 25 sec. After 20 min, the mixture was centrifuged at 10^5 *g* for 60 min to remove the EDTA-soluble peripheral proteins from the brush border membranes. The resulting pellet was resuspended in 2.5 mM EDTA-NaOH (pH 7.4). After recentri-

fugation of this suspension, the resulting pellet which contained the most ATPase activity [39] was separated on a continuous ficoll density gradient (12–40 per cent ficoll in 50 mM Tris–Cl, pH 7.4) into low and high density fractions by a centrifugation at 1.5×10^4 *g* for 90 min. A low density fraction contained a higher specific activity of Ca^{2+}-ATPase and alkaline phosphatase than a high density fraction. The low density fraction was collected and suspended in 50 mM Tris–Cl (pH 7.4). This suspension was centrifuged at 10^5 *g* for 60 min. The resulting pellet was resuspended. These procedures were repeated three times to remove ficoll. The resulting pellet was suspended in 50 mM Tris–Cl (pH 7.4) to use as a brush border membrane ATPase fraction. Enzymatic protein was determined by the method of Lowry *et al.* [40]. All the steps of the procedure were carried out at 0–4°.

ATPase assay. Ca^{2+}-ATPase activity was measured by the amount of inorganic phosphate released from ATP during incubation at 37° in shaking bath. A basic reaction mixture consisted of: 2.5 mM $CaCl_2$, 1 mM ATP, 50 mM Tris–Cl (pH 7.4) and approximately 15 μg protein of ATPase in a total volume of 1 ml. Calcium ions were omitted in blank determinations. The reaction was started at 37° by adding ATP and stopped by adding 1 ml of ice-cold 10% trichloroacetic acid. The released inorganic phosphate of the supernatant fraction was analyzed [41].

RESULTS

Calcium ions changed the effect of deoxycholate on Ca^{2+}-ATPase activity in intestinal brush border membranes, as shown in Fig. 1. The rate of Ca^{2+}-ATPase activity in the presence of 0.1 mM $CaCl_2$ increased with a rise in deoxycholate concentration up to 0.3 mM and decreased above 0.3 mM. An addition of deoxycholate exceeding 0.7 mM reduced the rate of Ca^{2+}-ATPase activity to less than control value. The 44 per cent inhibition induced by 2 mM deoxycholate was near the maximum value. However, in the presence of 2.5 mM Ca^{2+}, no concentration of deoxycholate stimulated Ca^{2+}-ATPase activity. The rate of Ca^{2+}-ATPase activity decreased with an increase in deoxycholate concentration up to 5 mM. The deoxycholate concentration for 50 per cent inhibition of Ca^{2+}-ATPase was approximately 0.640 mM in the presence of 2.5 mM Ca^{2+}. The 82 per cent inhibition caused by 5 mM deoxycholate was near its maximum value.

The turbidity of brush border membrane ATPase was reduced with an increase in deoxycholate concentration up to 5 mM, as shown in Fig. 2. The 50 per cent decrease in turbidity was observed at a concentration of 2.2 mM deoxycholate. Deoxycholate concentrations exceeding 5 mM were necessary for maximal decrease in turbidity.

The rate of Ca^{2+}-ATPase activity was raised with an increase in ATP concentration up to 1 mM. As illustrated in Fig. 3, Lineweaver–Burk plots of Ca^{2+}-ATPase activity vs ATP concentration yield a linear regression, from which the value of V_{max} was calculated to be 0.629 μmole P_i/mg of protein · min with a K_m value of 0.110 mM. In the presence of 0.8 mM deoxycholate, Lineweaver–Burk plots of Ca^{2+}-ATPase activity presented two linear regressions at ATP concentrations up to 1 mM (Fig. 3). From these lines, the value of V_{max} was calculated to be 0.175 and 0.356 μmole P_i/mg of protein · min with a K_m value of 0.0176 and 0.131 mM respectively.

The rate of Ca^{2+}-ATPase activity increased with a rise in Ca^{2+} concentration, as shown in Fig. 4. Deoxycholate (0.8 mM) depressed the Ca^{2+}-induced activation of ATPase. The optimal Ca^{2+}

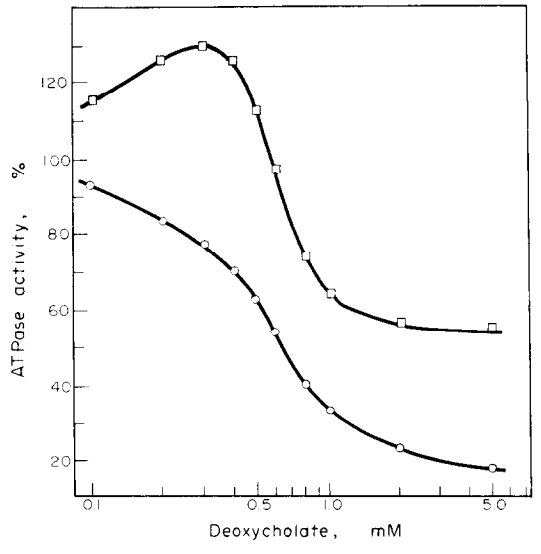

Fig. 1. Effect of deoxycholate on Ca^{2+}-ATPase activity in intestinal brush border membranes in the presence of different Ca^{2+} concentrations. A 1-ml volume of reaction mixture (pH 7.4) containing about 15 μg protein of ATPase, 0.1 (□) or 2.5 (○) mM $CaCl_2$ and various concentrations of sodium deoxycholate was preincubated at 37° for 5 min. Thereafter, the 3-min reaction of Ca^{2+}-ATPase was started by addition of ATP. The final concentration of ATP-2Na was 1 mM.

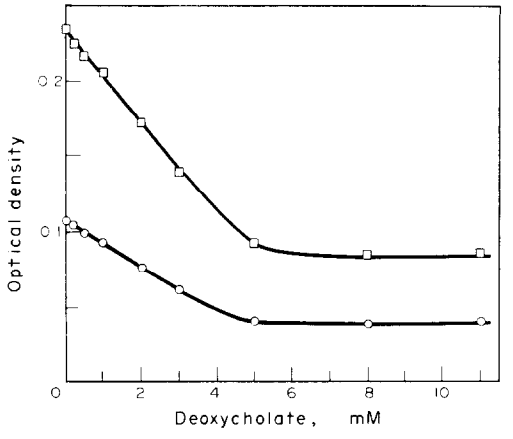

Fig. 2. Reduction in turbidity of brush border membrane ATPase seen with increasing sodium deoxycholate. A fraction of brush border membrane ATPase (700 μg, protein) was suspended in 5 ml of 50 mM Tris–Cl buffer (pH 7.4). The optical density of this suspension was determined at 350 nm (□) and 480 nm (○) about 10 min after addition of the indicated amount of sodium deoxycholate.

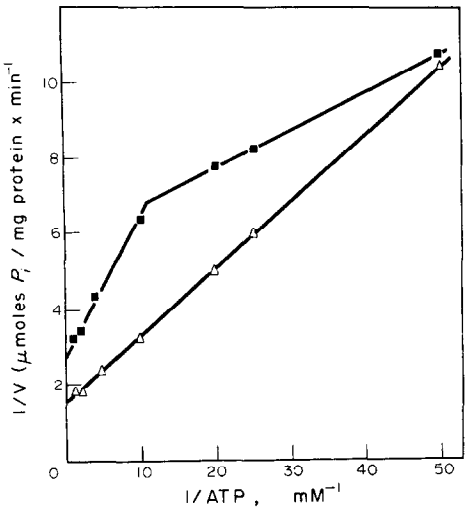

Fig. 3. Double-reciprocal plots of Ca^{2+}-ATPase activity vs ATP concentration in the presence or absence of deoxycholate. The 2-min reaction of Ca^{2+}-ATPase was started by addition of ATP to 50 mM Tris–Cl buffer (pH 7.4) containing 2.5 mM $CaCl_2$ and about 15 μg protein of ATPase in the presence (■) or absence (△) of 0.8 mM deoxycholate.

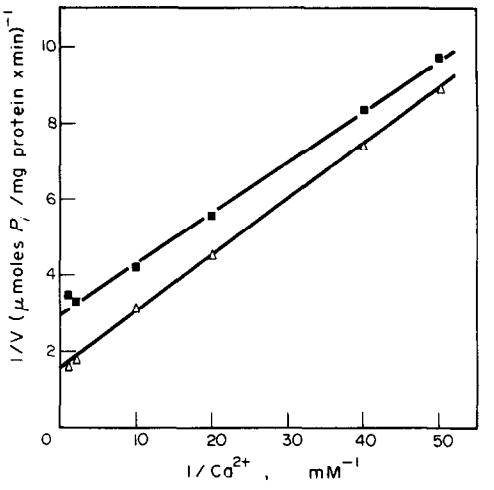

Fig. 5. Interaction of deoxycholate and Ca^{2+} on Ca^{2+}-ATPase of brush border membrane. Double-reciprocal plots of the values are given in Fig. 4. Key: (△) no deoxycholate; (■) 0.8 mM deoxycholate.

concentration for ATPase activation was about 2.5 mM, but 0.8 mM deoxycholate changed it to 0.5 mM. Lineweaver–Burk plots of Ca^{2+}-ATPase activity vs Ca^{2+} concentration are illustrated in Fig. 5. The K_m value of ATPase for Ca^{2+} was estimated to be 0.0925 mM in the absence of deoxycholate and 0.0450 mM in the presence of 0.8 mM deoxycholate.

In the presence of 0.8 mM deoxycholate, sodium and potassium ions inhibited Ca^{2+}-ATPase, as described in Table 1. The comparatively high dose of potassium or sodium was necessary for significant effectiveness on the inhibition by deoxycholate of Ca^{2+}-ATPase.

DISCUSSION

Intestinal brush borders are composed mainly of plasmic membranes related to membrane transport [42–45] and fibrillar constituents (terminal web and filament core) related to brush border motility [46, 47]. Thus, the brush border plasmic membranes must be separated from the fibrillar constituents to study brush border membrane transport. If the brush borders are disrupted by incubation in distilled water [38] or a 1 per cent Triton X-100 solution [46, 47], fibrillar constituents are collected as a high density fraction. Even when the separated fibrillar fraction was frozen, thawed and sonicated in 2.5 mM EDTA-NaOH (pH 7.4), the fibrillar constituents were collected as a higher density fraction on a ficoll density gradient than brush border membranes. In these experiments therefore, the brush border membrane fractions I

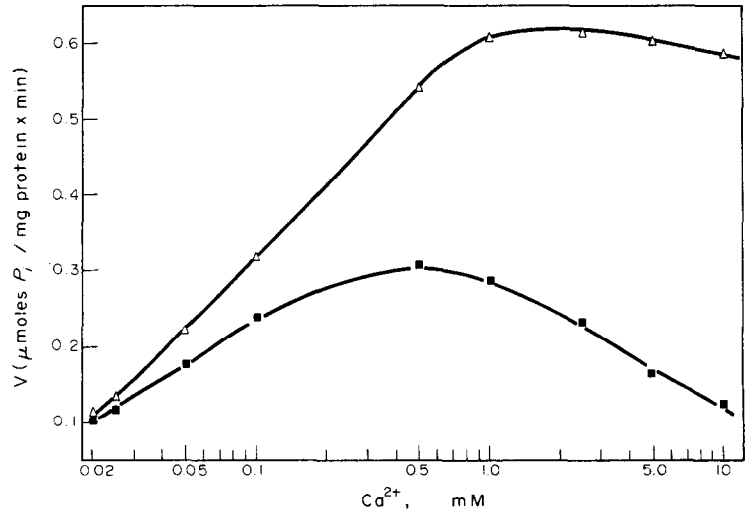

Fig. 4. Effect of deoxycholate on the activation by Ca^{2+} of ATPase in brush border membranes. A 1-ml volume of reaction mixture (pH 7.4) containing about 15 μg protein of ATPase, 50 mM Tris–Cl, 1 mM ATP-2Na and various concentrations of $CaCl_2$ was incubated at 37° for 2 min in the presence (■) or absence (△) of 0.8 mM sodium deoxycholate.

Table 1. Effect of sodium and potassium on Ca^{2+}-ATPase activity in brush border membranes in the presence of deoxycholate*

Cation	Concn. (mM)	Ca^{2+}-ATPase activity (%)
Sodium	20	99.2 ± 3.5
	40	95.1 ± 3.0†
	180	79.7 ± 4.3‡
	200	75.5 ± 3.1‡
Potassium	5	99.1 ± 2.9
	20	95.3 ± 3.1†
	40	91.0 ± 3.5§
	80	84.2 ± 3.8§
	140	78.5 ± 3.9‡

* Experimental details are the same as in Fig. 1. A 1-ml volume of reaction mixture (pH 7.4) containing approximately 15 μg protein of ATPase, 0.8 mM deoxycholic acid, 2.5 mM $CaCl_2$, 1 mM ATP–Tris, 50 mM Tris–Cl and various concentrations of potassium or sodium was incubated at 37° for 3 min. Each value indicates the mean and standard deviation of five percentages relative to control determinations.

† $P < 0.05$.

‡ $P < 0.001$.

§ $P < 0.01$.

and II, prepared as a fraction of low density by the method of Forstner *et al.* [38], were further purified by sonication and ficoll density gradient centrifugation to remove the contaminating fibrillar constituents and membrane peripheral proteins from the brush border membranes.

It has been shown that deoxycholate causes perturbation of membrane structure in liver cells [22] but Sladen and Harries [6] have found that deoxycholate produces a 50 per cent reduction of water absorption at lower concentrations than cause histological alterations of mucosal cells. In the experiments in Figs. 1 and 2, deoxycholate influenced the activity of brush border membrane Ca^{2+}-ATPase at lower concentrations than affected the turbidity of enzyme suspensions. It is probable that deoxycholate affects the activity of Ca^{2+}-ATPase by changing lipid–ATPase interactions at lower concentrations than by solubilizing ATPase. Simons *et al.* [48] have also suggested that, below the critical micellar concentration (less than 3 mM), deoxycholate interacts with lipid–protein complexes without delipidation taking place. Deoxycholate, even at 0.1 mM, affected the activity of brush border membrane Ca^{2+}-ATPase (Fig. 1). Thus, this compound may change the activity of Ca^{2+}-ATPase by interacting with lipid–ATPase complexes rather than by delipidating ATPase. Simons *et al.* [48] have also suggested that deoxycholate solubilizes membrane proteins by binding to the hydrophobic sites of these proteins. Deoxycholate increased the activity of Ca^{2+}-ATPase at a low concentration of 0.1 mM Ca^{2+} (Fig. 1) and decreased the K_m value of Ca^{2+}-ATPase for Ca^{2+} (Fig. 5). These results may be interpreted as an improved accessibility of Ca^{2+} to ATPase molecules by changing the hydrophobic interaction of lipids and ATPase when the lipid environment is modified by deoxycholate. A change in the hydrophobic interaction may be relevant to the interpretation of the results that an affinity component of Ca^{2+}-ATPase for ATP was separated into lower and higher affinity components by deoxycholate.

Deoxycholate has been shown to be effective in removing lipid from Ca^{2+}-ATPase of sarcoplasmic reticulum and inactivating the enzyme by delipidation [49]. The liberation of proteins from membranes begins at 1.5 mM deoxycholate and the proteins released are free from phospholipid above 2 mM deoxycholate [32]. A lipid–ATPase interaction is accepted as an important factor in the regulation of membrane-bound ATPase [34–37]. Thus, the high concentration of deoxycholate may inhibit Ca^{2+}-ATPase by delipidation.

The above results of the effect of deoxycholate on brush border membrane Ca^{2+}-ATPase are similar to the results of Jorgensen and Skou [50] that 1.5 mM deoxycholic acid produces a stimulation of Na^+–K^+-ATPase but higher concentrations inactivate the enzyme. However, some investigators [25–27, 30, 31] have reported different results. These different effects of deoxycholate may be attributed to differences in ionic species and concentration of the medium or ATPase preparation and its tissue concentration, because calcium, sodium and potassium change the effect of deoxycholate on brush border membrane Ca^{2+}-ATPase (Figs. 1 and 4, Table 1) and deoxycholate produces different effects with the increase in concentration (Figs. 1 and 2).

Figure 5 shows an apparent uncompetitive interaction of Ca^{2+} and deoxycholate on a Ca^{2+}-affinity site of Ca^{2+}-ATPase within the range of low Ca^{2+} concentrations. The marked inhibition of Ca^{2+}-ATPase by deoxycholate at high Ca^{2+} concentrations yielding the departure of Lineweaver–Burk plots from linearity suggests that deoxycholate is a much more effective inhibitor of the Ca^{2+}–enzyme complex on different Ca^{2+}-affinity sites than the free enzyme. Thus, the effect of Ca^{2+} is different from that of sodium and potassium, which may have only a more nonspecific effect of ionic strength on deoxycholate action (Table 1).

Guiraldes *et al.* [31] have suggested that deoxycholate inhibits sodium-coupled glucose transport by inhibition of Na^+–K^+-ATPase at the lateral and basal membranes of epithelial cells rather than at the brush border membranes. However, these results of inhibition of brush border membrane Ca^{2+}-ATPase suggest that deoxycholate has an effect on solute transport in the brush border membranes. Recently, it has been suggested that brush border membrane Ca^{2+}-ATPase may be related to the calcium pumping mechanism [51]. It is probable that deoxycholate interferes with the supply of energy to the Ca^{2+} pump that is concerned with pumping Ca^{2+} out of the brush border membrane in epithelial cells. An increase in calcium absorption by bile acids [10, 11] may be due to the decrease of Ca^{2+} efflux with the inhibition in Ca^{2+}-ATPase and also the increase of passive Ca^{2+} influx with the perturbation in lipid–protein interaction.

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