MECHANISMS OF SPASMOLYTIC ACTION OF BILE SALTS IN DEPOLARIZED GUINEA-PIG TAENIA COLI

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The effects of bile salts on the calcium movements and the electrical activity of the guinea-pig taenia coli were investigated and compared with those of papaverine in order to explore the mechanisms of their spasmolytic action. Four bile salts, deoxycholate, chenodeoxycholate, ursodeoxycholate and cholate, as well as papaverine, dose-dependently relaxed the depolarized taenia coli. The bile salts and papaverine caused the acceleration of $^{45}Ca$-efflux with the synchronous muscle relaxation and inhibited the cellular $^{45}Ca$-uptake by the depolarized muscle preparation. The bile salts also inhibited the increased spike frequency and the developed tension in the depolarized taenia coli. Furthermore, the dose-relaxation curves for bile salts were shifted to the right as the external calcium ion was increased. These findings suggest that the bile salts, like papaverine, may exert their spasmolytic action through accelerating the Ca-efflux and inhibiting the Ca-influx of the smooth muscle cells.

Keywords — bile salt; deoxycholate; ursodeoxycholate; spasmolytic action; calcium movement; calcium-influx; calcium-efflux; guinea-pig taenia coli

It has been reported by several investigators that bile salts such as ursodeoxycholate relax smooth muscles through their non-specific inhibitory actions.1–5) Kubota et al. mentioned that the spasmolytic action of bile salts is not attributable to their surface active properties.1) Uruno et al. demonstrated the inhibitory action of bile salts on the adenosine 3',5'-cyclic monophosphate (cyclic AMP) phosphodiesterase (PDE) activity and suggested that their spasmolytic actions may be mediated through an increase of the intracellular cyclic AMP content.2) The inconsistency in the relationship between the increment of cyclic AMP level and the spasmolytic effects of bile salts was also reported.3) Thus, the mechanisms of spasmolytic action of bile salts remain controversial.

In order to explore the mechanisms of action of spasmolytics, investigations have been focused on the effects of the drugs on the calcium movements of smooth muscle cells since it is generally accepted that the contraction-relaxation cycle in the muscle cells is controlled by cytoplasmic free calcium ion. However, little is known about the involvement of bile salts in the calcium movements in the smooth muscle. The present study was undertaken therefore to elucidate the mechanisms of spasmolytic action of bile salts mainly by analyzing their effects on the calcium movements in guinea-pig taenia coli. A comparison of the effects of the bile salts and papaverine on the calcium movements was also made because papaverine is known as one of the prototypes of spasmolytic agents.

MATERIALS AND METHODS

Strips of taenia coli, approximately 1.5 cm long, were dissected from male guinea pigs weighing 300 to 500 g and suspendend in Locke-Ringer solution which was composed of (in mM): NaCl 154, KCl 5.6, CaCl$_2$ 2.2, MgCl$_2$ 1.2, NaHCO$_3$ and glucose 2.8. The solution was kept at 32 °C and bubbled with air. The mechanical response of the muscle was recorded by means of an isotonic transducer (ME Commercial Co., Ltd). The muscle preparation was equilibrated for 30 min under 1 g resting tension and then repeatedly applied with hypertonic 20 mM KCl at intervals of 15 min until the contractile response of the muscle attained a constant level. Thereafter, the following experiments were carried out.

The dose-relaxation curves for four bile salts, cholate, deoxycholate, ursodeoxycholate and chenodeoxycholate, and papaverine were obtained in the muscle precontracted by 20 mM KCl. The drugs were applied cumulatively to the muscle when the KCl-induced contracture
reached a plateau level, approximately 10 min after the application of KCl. The relaxation induced by each drug was expressed as the percentage of the complete relaxation of the KCl-induced contracture.

To examine the effects of spasmytic drugs on the Ca-efflux, ⁴⁶Ca-efflux from the taenia coli was determined. Determination of the ⁴⁶Ca-efflux was carried out according to the methods described previously.⁷,⁸ In these experiments, a specially designed apparatus was set up and the recording of the mechanical response and rapid collection of the washout solution were simultaneously made. The ⁴⁶Ca-efflux was expressed as the rate constant of ⁴⁶Ca-loss from the muscle. In some experiments, the cellular ⁴⁶Ca-uptake by the taenia coli was determined in order to examine the effects of the drugs on the Ca-influx. For determining the cellular ⁴⁶Ca-uptake, the muscle, preincubated in Ca-free depolarizing (20 mM KCl) Locke-Ringer solution, was soaked in a depolarizing solution containing 1 mM CaCl₂ and ⁴⁶Ca (1 μCi/ml) for 10 min and then washed with ice-cold Ca-free Tris-buffered solution containing 10 mM LaCl₃ for 10 min. After removing the muscle from the solution, it was blotted with ashless filter paper and quickly weighed. The radioactivity of the washout solution or in the muscle which was digested with a tissue solubilizer, Soluene-350 (Packard Instrument Co.) was counted in a liquid scintillator, Univergel II (Nakarai Chemicals).

In examining the effects of the bile salts on electrical activity, the membrane potential changes and the developed isometric tension were measured with a single sucrose-gap method. The organ chamber of the apparatus was continuously circulated with the bathing solution which was kept at 32 °C and bubbled with 100% O₂. A test drug was added to the circulating solution. The exchange of the circulating solution in the chamber was completed within 1 min.

Sodium salts of ursodeoxycholic acid, chenodeoxycholic acid, deoxycholic acid and cholic acid were offered from Tokyo Tanabe Co., Ltd. Paraverine hydrochloride and ⁴⁶CaCl₂ were purchased from Sigma Chemical Company and New England Nuclear, respectively.

RESULTS
1. Dose-Response Relationships of Bile Salts and Papaverine

The relaxant effects of four bile salts, cholate, deoxycholate, ursodeoxycholate and chenodeoxycholate, and papaverine were determined in the taenia coli depolarized by hypertonic 20 mM KCl. All of these drugs caused the dose-

![Graph showing dose-response curves for bile salts and papaverine.](image)

**FIG. 1. Dose-Relaxation Curves for Bile Salts and Papaverine on the Taenia Coli**

Deoxycholate (▲), chenodeoxycholate (■), ursodeoxycholate (○), cholate (●) and papaverine (▲) were applied to the muscle precontracted by 20 mM KCl. The relaxation ratio was expressed as the percentage for the complete relaxation of the KCl-induced contraction. Each point represents means ± S.E. of 6 experiments.

| **TABLE I. ID₅₀ Values for Bile Salts and Papaverine to the 20 KCl-Induced Contraction of Taenia Coli** |
|---------------------------------|---------------------------------|
| Deoxycholate                    | 1.14±0.17 (× 10⁻⁵ M)            |
| Chenodeoxycholate               | 1.71±0.09 (× 10⁻⁵ M)            |
| Ursodeoxycholate                | 4.24±0.73 (× 10⁻⁴ M)            |
| Cholate                         | 9.56±1.16 (× 10⁻³ M)            |
| Papaverine                      | 0.72±0.10 (× 10⁻⁷ M)            |

ID₅₀ values were calculated from the data in Fig. 1.
dependent relaxation of the taenia coli, as shown in Fig. 1. The ID$_{50}$ values of these drugs for KCl-induced response are also listed in Table I. These data show that the potency of spasmyloytic action of each drug is in the order of papaverine > deoxycholate > chenodeoxycholate > ursodeoxycholate > cholate. This order was coincident with those in other smooth muscles, which were previously reported.$^{1,2,5}$ In our present experiments, the most potent bile salt, deoxycholate, and the medium potent one, ursodeoxycholate, were chiefly used.

2. Effects of Bile Salts and Papaverine on Ca-Efflux

Effects of deoxycholate and ursodeoxycholate on the Ca-efflux were investigated and compared with that of papaverine. Figure 2 shows the effects of the drugs on the $^{45}$Ca-efflux from the depolarized taenia coli and typical mechanical responses of the muscle, which were simultaneously recorded during the Ca-efflux measurement. Deoxycholate ($3 \times 10^{-4}$ M), ursodeoxycholate ($10^{-3}$ M), and papaverine ($3 \times 10^{-5}$ M) which caused the complete relaxation of the depolarized muscle were added to the washout solution after 30th min of efflux for a duration of 20 min. These drugs induced marked acceleration of $^{45}$Ca-efflux and synchronous relaxation of the muscle. The extents of $^{45}$Ca-efflux induced by these drugs were almost similar to one another.

3. Effects of Bile Salts and Papaverine on Cellular $^{45}$Ca-Uptake

Effects of bile salts and papaverine on the cel-

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**FIG. 2. Effects of Deoxycholate (DC), Ursodeoxycholate (UDC) and Papaverine (Papa) on the $^{45}$Ca-Efflux and Mechanical Activities of Taenia Coli**

Upper panel: typical response of the muscle to each drug which was simultaneously recorded during $^{45}$Ca-efflux measurement. Lower panel: effect of each drug on the $^{45}$Ca-efflux from the depolarized taenia coli. ●, in the presence of; ○, in the absence of the drugs. Horizontal bars indicate the duration of drug application. Data were obtained from 6 experiments.

**TABLE II. Inhibitory Effects of Bile Salts and Papaverine on the Cellular $^{45}$Ca-Uptake by the Depolarized Taenia Coli**

<table>
<thead>
<tr>
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<th>Inhibition% (mean ± S.E.)</th>
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<tbody>
<tr>
<td>Deoxycholate</td>
<td>$3 \times 10^{-4}$ M</td>
</tr>
<tr>
<td>Ursodeoxycholate</td>
<td>$10^{-3}$ M</td>
</tr>
<tr>
<td>Papaverine</td>
<td>$3 \times 10^{-5}$ M</td>
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The $^{45}$Ca-uptake by the control tissues determined after 10 min incubation in the 20 mM KCl containing solution was 0.33 ± 0.03 mmol/kg wet tissue. Data were obtained from 6 experiments. Significant difference from control, a) : $p < 0.01$, b) $p < 0.05$. 
lular 45Ca-uptake were examined by using the depolarized taenia coli. In these experiments, doses of these drugs were selected to cause full relaxation of the taenia coli. As shown in Table II, deoxycholate (3 × 10^{-4} M) and ursodeoxycholate (10^{-3} M) markedly inhibited the cellular 45Ca-uptake determined after 10 min incubation. The significant inhibition of cellular 45Ca-uptake was also observed with 3 × 10^{-5} M papaverine, but the extent of inhibition was smaller than that of bile salts.

4. Effect of Bile Salt on Electrical Activity of Taenia Coli

Fig. 3 represents changes in the electrical and mechanical activities of the taenia coli depolarized with 20 mM KCl. Application of KCl to the muscle produced a depolarization accompanied by an increase in spike frequency and a sustained contracture. When deoxycholate (3 × 10^{-4} M) was applied to the muscle approximately 10 min after the application of KCl, the spike discharge ceased but the membrane potential was not changed, and the developed tension was synchronously decreased. These effects of deoxycholate were reversible. Effects of ursodeoxycholate on the electrical and mechanical activities of taenia were qualitatively similar to those of deoxycholate (data not shown).

5. Effect of Increasing External Calcium Ion Concentration on Spasmyotic Action of Bile Salts

The dose-relaxation curves for deoxycholate and ursodeoxycholate were obtained in the bathing solution in which the calcium ion concentration was increased from normal level (2.2 mM to 5 mM in order to examine the influence of external calcium ion on the spasmyotic action of the bile salts. CaCl_2 was added to the bathing solution to increase calcium ion concentration and 5 min later the bile salts were cumulatively applied to the depolarized muscle. The spasmyotic action of the bile salts were attenuated by increasing the external calcium ion concentration since the dose-relaxation curves for deoxycholate and ursodeoxycholate were shifted to the right in the 5 mM calcium ion containing solution (Fig. 4).

DISCUSSION

It has been well documented that bile salts inhibit the contraction of smooth muscle induced by various agonists in a non-competitive manner.1,4,5 The present results also showed that deoxycholate and ursodeoxycholate, as well
as papaverine, relaxed the KCl-induced contraction of taenia coli. Thus, bile salts may exert their inhibitory effect on the smooth muscle contractility through a so-called papaverine-like action. The mechanisms of the relaxant effect of papaverine itself have been extensively studied, and it is now considered that papaverine exerts its inhibitory actions by changing the cellular calcium movements of smooth muscle, including an acceleration of Ca-efflux and an inhibition of Ca-influx. Therefore, if bile salts act in a way similar to papaverine, their effects would be assumed to be caused through changes in Ca-efflux and Ca-influx across the cell membrane action.

The present results demonstrated that deoxycholate and ursodeoxycholate, like papaverine, accelerated the 45Ca-efflux from the depolarized taenia coli and caused synchronous muscle relaxation. The acceleration of Ca-efflux is likely to be one of the mechanisms for the bile salt-induced smooth muscle relaxation because the availability to calcium by the contractile machinery would be decreased. It has been demonstrated that the papaverine-induced acceleration of the Ca-efflux is coupled with the elevation of cellular cyclic AMP level, due to the inhibition of phosphodiesterase. The bile salts have also been shown to inhibit the phosphodiesterase activity. Thus, the action of bile salts on the Ca-efflux can be considered to be also related to the elevation of cellular cyclic AMP content.

Deoxycholate and ursodeoxycholate exerted a potent inhibitory effect on the cellular 45Ca-uptake, suggesting that they suppressed the Ca-influx. In addition, our preliminary study where the change in calcium ion concentration in the bathing fluid was monitored by means of Ca-selective electrode demonstrated that the bile salts had no chelating action (unpublished observation). Thus, bile salts are suggested to act as calcium antagonists and to produce muscle relaxation. This suggestion is consistent with the results of the present electrophysiological study that bile salts inhibited the increased spike discharge and the developed tension in the depolarized taenia coli. Since it is generally accepted that the spike generation in smooth muscle is due to the inward movement of calcium ion, the inhibitory action of bile salts on the Ca-influx is considered to be due to the inhibition of inward movement of calcium ion.

The fact that the spasmylytic action of bile salts was antagonized by the increase in calcium ion concentration in the bathing medium was also obtained in the present study. Since the increase of external calcium ion concentration should counteract the actions of bile salts on the Ca-efflux and Ca-influx, this finding may support the view that bile salts induce their spasmylytic action by affecting the cellular calcium movements of smooth muscle.

In summary, bile salts have been shown to exert spasmylytic effect through accelerating Ca-efflux which is probably coupled with an increase in cyclic AMP content and suppressing Ca-influx across the cell membrane of taenia coli. The actions of bile salts seem to be like those of papaverine. However, considering our previous finding that in the absence of external sodium ion papaverine could induce muscle relaxation while deoxycholate was ineffective, the precise mechanism of spasmylytic action for bile salts appears to be different from that of papaverine.

REFERENCES


