Possible Mechanisms of Spasmolytic Action of Bile Salts on the Isolated Guinea-Pig Gallbladder

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Abstract

Sunagane N., T. Kobori, T. Uruno and K. Kubota. Possible mechanisms of spasmolytic action of bile salts on the isolated guinea-pig gallbladder. The spasmolytic action of bile salts on gallbladder smooth muscle could explain the alleged relief of biliary colic seen during bile acid therapy. The mechanisms of spasmolytic action of bile salts, ursodeoxycholate and deoxycholate were studied in the isolated gallbladder of guinea-pigs. The bile salts accelerated the 45Ca-efflux from the gallbladder with synchronous relaxation and inhibited the cellular 45Ca-uptake by the depolarized muscle preparation. Further, they sensitively inhibited CaCl2-induced contraction of the depolarized muscle. The tissue cyclic AMP content of the gallbladder was significantly elevated by the bile salts. Dibutyryl cyclic AMP mimicked the effects of bile salts on the Ca-efflux and the muscle relaxation, but showed no effect on the cellular Ca-uptake. From these results, it is suggested that the bile salts produce the relaxant action through accelerating Ca-efflux, which is probably coupled with the elevation of the cellular cyclic AMP level, and through suppressing the Ca-influx across the cell membrane.

Introduction

Bile salts such as ursodeoxycholate and chenodeoxycholate have been clinically used for patients with gallstones in order to dissolve the stones. Clinical studies have suggested that the frequency and severity of biliary pain can be reduced during the bile salt therapy (Maton et al., 1977; Frigerio, 1980; Iwamura, 1980; Polli et al. 1980). Such mitigation in the pain occurred before any significant reduction in the size of gallstones due to dissolution of the stones. Altered gallbladder motility might explain the relief of biliary colic ( Forgacs et al., 1984). Our previous study demonstrated that bile salts non-specifically relaxed the guinea-pig gallbladders contracted by several different types of agonists (Sunagane et al., 1984). Therefore, the non-specific relaxant action of bile salts may be responsible for alteration of gallbladder motility. However, precise mechanisms of their spasmolytic action have remained obscure.

Investigations on the mechanisms of action of spasmyotics have focused on their effects on the calcium movements of various types of smooth muscle since the contraction-relaxation cycle of muscle cells is controlled by cytoplasmic calcium ions. However, little is known about the effects of bile salts on the calcium movements in the gallbladder.
It is well established that cyclic AMP is a second messenger of smooth muscle relaxation for some spasmyotics. Uruno et al. (1974) showed that bile salts could inhibit cyclic AMP phosphodiesterase (PDE) activity, resulting in the accumulation of cytoplasmic cyclic AMP. Good correlation between the increment of cyclic AMP level and the relaxant effects of bile salts was also found in the rat uterus (Uruno et al., 1975). Francavilla et al. (1981) demonstrated the inhibition by bile salts of the cyclic AMP phosphodiesterase isolated from the human gallbladder. Thus, bile salts may cause relaxation of the gallbladder through cyclic AMP-mediated mechanisms.

The present study, therefore, was undertaken to elucidate the mechanisms of relaxant action of bile salts in the guinea-pig gallbladder through comparing with that of a dibutyryl derivative of cyclic AMP, from the point of view of their effects on the calcium movements. Some of these studies have been reported in abstract form (Kobori et al., 1986).

**Materials and Methods**

The gallbladders were dissected from male guinea pigs weighing 350-500 g and helical strips were prepared from the gallbladders. The mucosae of the tissues were removed by rubbing with sharp-edged glass. Each muscle strip was then cut into two segments about 4 mm wide and 10 mm long for paired tests; one was used as a control and the other as a test tissue. They were mounted in a tissue bath filled with Locke-Ringer solution which was kept at 32°C and bubbled with air. The Locke-Ringer solution had the following composition (mM) NaCl 154, KCl 5.6, CaCl₂ 2.2, MgCl₂ 2.1, NaHCO₃ 5.9 and glucose 2.2 (pH 7.8). Isometric tension was recorded by an isometric transducer (ME commercial Co. Ltd., Tokyo). After 1 hr equilibration under 1 g resting tension, the muscle preparations were repeatedly challenged with excess 60 mM KCl at intervals of 30 min until the contractile responses of the muscles attained a constant level. Thereafter the following experiments were carried out.

Concentration-response relationships for deoxycholate, ursodeoxycholate and dibutyryl cyclic AMP were obtained in the muscle partly depolarized by excess 20 mM KCl. The drugs were applied cumulatively to the muscles after the KCl-induced contractions had been reached a plateau level. The degree of relaxation induced by each drug was expressed as relaxation percentage in which the complete relaxation of the KCl-induced contraction was taken as 100%. In order to compare effects of deoxycholate and ursodeoxycholate on the different types of agonists, the cumulative concentration-contraction curves for CaCl₂ and acetylcholine were established in the presence or absence of the bile salts. CaCl₂ was added to the muscle which had been immersed in a Ca-free medium and then depolarized by 60 mM KCl. The concentration of each bile salt was selected nearly to produce 50% inhibition of 20 mM KCl-induced contraction. These antagonists were added to the bath 5 min before addition of agonists.

Effects of drugs on the Ca-movements were assessed by determining their effects of ⁴⁵Ca-efflux and the cellular ⁴⁵Ca-uptake of the depolarized gallbladder. ⁴⁵Ca-efflux from the muscle was measured according to the method described previously (Sunagane et al., 1986). In this experiment, a specially designed apparatus was set up and the recording of the mechanical response and quick collection of washout solution were made simultaneously. The ⁴⁵Ca-efflux
was expressed as the rate constant of $^{45}\text{Ca}$-loss from the tissue. For determination of the cellular $^{45}\text{Ca}$-uptake, the tissues were first rinsed for 10 min in a Ca-free depolarizing Locke-Ringer solution, which was prepared by adding 20 mM KCl and by removing CaCl$_2$ to and from the normal solution, and then immersed in a depolarizing solution containing 1 mM CaCl$_2$ and $^{45}\text{Ca}$ (37kBq/ml) in the presence or absence of the drugs. The tissues were washed with a ice-cold Ca-free Tris-buffered solution containing 10 mM LaCl$_3$ for 10 min, blotted with ashless filter paper (Whatman No. 42) and quickly weighed. In this experiment, the $^{45}\text{Ca}$-uptake was estimated as the tissue $^{45}\text{Ca}$-space non-displaceable by 10 mM lanthanum ion. The radioactivity of the washout solutions or in the tissues which were digested with a tissue solubilizer, Soluene-350 (Packard Instrument Co.), was counted in a liquid scintillator, Univergel II (Nakarai Chemicals, Kyoto) with liquid scintillation counter (Aloka, model 673).

For the determination of tissue cyclic AMP content, the gallbladders depolarized in Locke-Ringer solution containing 20 mM KCl were treated with or without drugs for 10 min and then immediately frozen by immersing them in liquid nitrogen. The frozen tissues were homogenized with ice-cold trichloroacetic acid (6%) and the supernatant of the homogenate was collected as assay samples. Tissue concentration of cyclic AMP was determined by the method of Honma et al. (1977) using radioimmunoassay kit for cyclic AMP (Yamasa Shoyu Co. Ltd., Chiba). Radioactivity of $^{125}\text{I}$ was counted with gamma counter (Aloka, model ARC 300).

The data were analyzed by Student’s $t$-test and statistical significance was set at $p<0.05$ or better.

The drugs and chemicals used were as follows; sodium deoxycholate (Sigma Chemicals Co. Ltd., Missouri), sodium ursodeoxycholate (Tokyo–Tanabe Seiyaku, Tokyo), dibutyryl cyclic AMP (Sigma Chemicals Co., Missouri) and $^{45}\text{CaCl}_2$ (New England Nuclear, Massachusetts).

Results

Deoxycholate and ursodeoxycholate caused a concentration-dependent relaxation in the gallbladder precontracted by 20 mM KCl. Higher concentrations of both bile salts not only relaxed the KCl-induced contraction but lowered the muscle tone below the resting level. Relaxant action of the bile salts was reversible at concentrations range tested in the present experiments. Dibutyryl cyclic AMP mimicked the relaxation of the preparation, its action being quantitatively similar to that of bile salts. The concentration–response curves for these drugs are shown in Fig. 1. Complete relaxation of the KCl-induced contraction was obtained with deoxycholate, ursodeoxycholate and dibutryl cyclic AMP at 0.3 mM, 1 mM and 3 mM, respectively. Thus, the concentrations of these drugs used in the following experiments were selected to cause complete relaxation of the 20 mM KCl-induced contraction.

The effects of deoxycholate and ursodeoxycholate on the Ca-efflux were tested in the gallbladder depolarized by 20 mM KCl and were compared with that of dibutyryl cyclic AMP. Fig. 2 shows effects of the drugs on $^{45}\text{Ca}$-efflux from the tissues and typical mechanical responses recorded during the Ca-efflux measurement. When deoxycholate (0.3 mM) and ursodeoxycholate (1 mM) were added to the washing solution in a period between 30th and 50th min of efflux, marked acceleration of $^{45}\text{Ca}$-efflux and synchronous full relaxation of the muscle
Fig. 1. Concentration–relaxation curves for deoxycholate (●), ursodeoxycholate (▲) and dibutyryl cyclic AMP (■) in the guinea-pig gallbladder. Each drug was cumulatively applied to the muscle preparation precontracted by 20 mM KCl. The relaxation in response to a test drug was expressed as percentage of the complete relaxation of 20 mM KCl-induced contraction. Each point represents the mean S.E. of 6 experiments.

Fig. 2. Effects of deoxycholate (DC), ursodeoxycholate (UDC) and dibutyryl cyclic AMP (db-cAMP) on 45Ca-efflux and mechanical responses of the guinea-pig gallbladder. Upper panel: typical response of the muscle to each drug, which was simultaneously recorded during Ca-efflux measurement. Horizontal bar indicates the duration of drug application. Lower panel: Ca-efflux in the presence (●) or absence (○) of a test drug. The Ca-efflux was expressed as the rate of loss of 45Ca from the muscle. Early phase of rate of 45Ca-loss is not shown in each figure. Data were obtained from 6 experiments.
Table 1. Effects of bile salts and dibutyryl cyclic AMP on the cellular $^{45}\text{Ca}$-uptake of the depolarized guinea-pig gallbladder

<table>
<thead>
<tr>
<th>Bile Salts</th>
<th>Inhibition %</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>Deoxycholate</td>
<td>0.3 mM</td>
<td>17.2±5.2</td>
</tr>
<tr>
<td>Ursodeoxycholate</td>
<td>1 mM</td>
<td>18.0±2.9</td>
</tr>
<tr>
<td>Dibutyryl c-AMP</td>
<td>3 mM</td>
<td>9.4±9.5</td>
</tr>
</tbody>
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Each value represents mean S.E. of 4 experiments. The $^{45}\text{Ca}$-uptake was determined after 10 min incubation in a $^{45}\text{Ca}$ containing solution and was estimated as $^{45}\text{Ca}$-space. The $^{45}\text{Ca}$-space of the control tissues was 0.60±0.03 m mol/kg wet weight of tissues.

did not take place. Dibutyryl cyclic AMP (3 mM) similarly accelerated the $^{45}\text{Ca}$-efflux with the synchronous muscle relaxation. No significant difference was observed in the extents of acceleration of $^{45}\text{Ca}$-efflux by two bile salts and dibutyryl cyclic AMP.

Table 2 represents the effects of bile salts and dibutyryl cyclic AMP on the cellular $^{45}\text{Ca}$-uptake by the depolarized gallbladder determined after 10 min incubation in the radioactive solution. Incubation time was selected to allow the strips to respond maximally to the drugs. Deoxycholate (0.3 mM) and ursodeoxycholate (1 mM) significantly inhibited the cellular $^{45}\text{Ca}$-uptake of gallbladder by similar extents. By contrast, dibutyryl cyclic AMP failed to inhibit the cellular $^{45}\text{Ca}$-uptake.

The effects of bile salts on the tissue cyclic AMP levels were also tested in the partly depolarized tissues. After the treatment with deoxycholate (0.3 mM) or ursodeoxycholate (1 mM) for 10 min, the cytoplasmic levels of the nucleotide were markedly elevated. No significant difference between two bile salts with regard to the elevation of cyclic AMP contents was observed. The results were summarized in Table 2.

Effects of the bile salts on the contractions induced by two different types of agonists, CaCl$_2$ and acetylcholine were also compared. The concentration–response curves for the agonists were established in the presence or absence of the bile salts. In the control tissues, CaCl$_2$ and acetylcholine produced almost the same maximum contraction. As shown in Fig. 3, deoxycholate and ursodeoxycholate at 6×10$^{-5}$ M and 3×10$^{-4}$ M, respectively, caused a rightward and downward shift of the concentration–response curves for CaCl$_2$ and acetylcholine. The degree of the suppression of the maximum response was marked in CaCl$_2$ contraction.

**Discussion**

The present results showed that in the guinea-pig gallbladder deoxycholate and ursodeoxycholate relaxed the contraction induced by a non-specific stimulant, high potassium, and inhibited non-competitively the CaCl$_2$- and acetylcholine–induced contractions (Figs. 1 and 3). These findings were coincident with the view of our previous study that bile salts induce non-specific relaxation of the gallbladder smooth muscle (Sunagane et al., 1984). Such a non-specific spasmolytic action of bile salts have also been demonstrated in the intestine (Kimura et al., 1967; Kubota et al., 1970; Sunagane et al., 1986) and the uterus (Uruno et al., 1974, 1975).
Deoxycholate and ursodeoxycholate accelerated the $^{45}$Ca-efflux from the gallbladder partly depolarized by potassium when the drugs were applied to the muscle during the later efflux phase where intracellularly bound calcium ions were effluxed (Fig. 2). The acceleration of Ca-efflux should lead to the muscle relaxation because calcium ions available for the contraction will be decreased. The present results further demonstrated good correlation between the time courses of the acceleration of Ca-efflux and the development of muscle relaxation. Thus, the acceleration of Ca-efflux is likely to be one of the mechanisms responsible for the muscle relaxation of the gallbladder in response to the bile salts.

In addition, the bile salts used inhibited the cellular $^{45}$Ca-uptake by the depolarized muscle preparation (Table 1). This finding indicates that the bile salts inhibit the Ca-influx across the cell membrane. Since calcium ions activating the contractions of visceral smooth muscles are mainly brought from the extracellular pools (Hurwitz et al., 1967), the inhibition of Ca-influx should suppress the supply of calcium ion to the contractile machinery, thus leading to the muscle relaxation. It is, therefore, likely that the inhibition of Ca-influx may also contribute...
to the bile salts–induced relaxation of the gallbladder. The present results further showed that the bile salts suppressed more sensitively the contraction induced by CaCl₂ than that by acetylcholine (Fig. 3). Existence of two different calcium mobilizing pathways in the membrane, voltage–dependent and receptor–operated channels is known. Since calcium ion utilized for the contraction induced by CaCl₂ in the depolarized muscle is considered to be mobilized mainly via the voltage–dependent channel, it is indicative of the possibility that deoxycholate and ursodeoxycholate prominently block the voltage–dependent channel.

It is clear from the present results that deoxycholate and ursodeoxycholate increase the tissue cyclic AMP content of the gallbladder (Table 2). The increase of the tissue cyclic AMP level may be due to the inhibition of the cyclic AMP phosphodiesterase (Francavilla et al., 1981). The present results further showed that dibutyryl cyclic AMP induced a concentration–dependent relaxation of the muscle preparation. These results suggest that the elevation of tissue cyclic AMP levels mediates the bile salts–induced relaxation of the gallbladder. This view is supported by the observation of Andersson et al. (1972) that agents increasing the tissue cyclic AMP contents induce muscle relaxation of the gallbladder.

With regard to the mechanisms of cyclic AMP–mediated smooth muscle relaxation, it is proposed that the increased cyclic AMP activates the protein kinase which results in the phosphorylation of myosin light chain kinase (MLCK), resulting in the inactivation of MLCK and the inhibition of muscle contraction. We have no available data at present to discuss the role of phosphorylation of MLCK in the relaxation of the gallbladder in response to the bile salts. The acceleration of ⁴⁶Ca–efflux from the smooth muscle cells has been suggested to be related to the elevation of cellular cyclic AMP levels (Tomiyama et al., 1973; Shiba et al., 1981; Sunagane et al., 1986). In the present results, dibutyryl cyclic AMP was found to accelerate the Ca–efflux from the gallbladder (Fig. 2). Therefore, the acceleration of Ca–efflux may be able to explain the cyclic AMP–mediated mechanisms of the muscle relaxation even in the gallbladder. Meanwhile, dibutyryl cyclic AMP failed to inhibit the cellular ⁴⁶Ca–uptake of the depolarized muscle preparation. Thus, the inhibition of Ca–influx seems not to be mediated through the cyclic AMP system. In this context, the bile salts may induce the relaxation of the gallbladder not only through the mechanisms due to the elevation of cyclic AMP levels, but also through those independent of the cyclic AMP system.

In conclusion, bile salts have been shown to produce their spasmolytic actions in the gallbladder through accelerating Ca–efflux, which is probably coupled with the elevation of cyclic AMP level, and through suppressing the Ca–influx across the cell membrane.

References


(Received May 1 1990)