ACTIONS OF PAPAVERINE, ASPAMINOL AND BILE SALTS AND INTRACELLULAR CYCLIC AMP LEVEL

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Abstract—Antioxytocin activities of bile salts and their effects on phosphodiesterase (PDE) activities and the intracellular level of cyclic AMP in the rat uterus were investigated, and the mode of action of the bile salts was compared with that of papaverine or Aspaminol. The bile salts, chenodesoxycholate, desoxycholate, ursodesoxycholate and cholate significantly decreased PDE activities and a good relationship between the PDE activities and the antioxytocin activities of the bile salts was observed. Chenodesoxycholate and desoxycholate significantly increased the intracellular level of cyclic AMP in the rat uterus. The exogenously applied CaCl₂ potentiated the inhibitory responses of the rat uterus to papaverine, aminophylline responses of the rat uterus to papaverine, aminophylline and bile salts, while the inhibitory response to Aspaminol was greatly reduced by excess CaCl₂. These results indicate that the antioxytocin action of the bile salts may be exerted through an increase in the intracellular level of cyclic AMP. Further, the results support the theory that there are two mechanisms for "papaverine-like" antispasmodics.

Recently it has been demonstrated that the relaxing actions of papaverine on smooth muscle may be exerted through phosphodiesterase (PDE) inhibition and consecutive accumulation of cyclic 3', 5'-AMP (cyclic AMP) (1, 2). Further, some workers (3, 4, 5, 6, 7, 8) have reported that papaverine and isoprenaline increased the intracellular level of cyclic AMP in the intestinal and uterine smooth muscle.

"Papaverine-like" actions of various spasmolytics are generally thought to be direct actions on smooth muscle. Takagi, Takayanagi and Fujie (9) and also Takagi et al. (10) divided "papaverine-like" antispasmodics into two groups according to their mechanisms of action; strong basic substances or most synthetic antispasmodics such as 1, 1-diphenyl-3-piperidinobutanol hydrochloride (Aspaminol) belong to the first group and weak bases such as papaverine and neutral substances as isoamylesters belong to the second group. This theory has been supported by Takayanagi et al. (11), Uruno et al. (8), Uruno et al. (12) and Inatomi et al. (3).

On the other hand, it has been reported that bile salts show anticholinergic activities, which are based on their "papaverine-like" actions on the intestinal smooth muscle (13, 14, 15). In this paper, the antioxytocin activities of bile salts and their effects on the PDE activity and the intracellular level of cyclic AMP in the rat uterus were investigated, and the mode of action of the bile salts was compared with those of papaverine and Aspaminol.
MATERIALS AND METHODS

Virgin female Wister rats weighing 150 to 250 g were ovariectomized and allowed to recover for more than 5 days before the experiment.

Oxytocin-induced contractions

These rats were sacrificed by a blow on the neck and the uterine horns were removed. One was suspended in a 10 ml organ bath. The bath was filled with Locke-Ringer solution, kept at 32°C, and gassed with a mixture of 95°o O2 and 5°o CO2. The composition of Locke-Ringer solution in gram per liter was NaCl 9.0, KCl 0.4, CaCl2 0.2, MgCl2 0.2, NaHCO3 0.5 and glucose 0.5. Uterine contractions were isotonically recorded by means of a lever loaded with 0.5 g on a smoked drum. Antispasmodics were allowed to act for 3 min and in their presence oxytocin was cumulatively added. Spasmolytic activities of the bile salts (sodium salts) were presented as the PD2°' values (16, 17). The PD2°' value of each spasmolytic was calculated from the value graphically determined from the concentration-action curves of oxytocin, which were obtained by application of the antispasmodic in two doses, one inhibited the oxytocin-induced maximum response by more than 50°", and the other by less than 50°", (17). The responses are expressed as the percentage of the maximum contraction and each curve represents the mean of at least six experiments.

Anti-phosphodiesterase activity of the test drugs

The uteri isolated from five ovariectomized rats were homogenized with 10-fold of a buffer-Mg2°-solution (Tris-HCl 16 · 10-2 M, Mg-acetate 5 · 10-2 M; pH 7.5) at 0°C according to the method of Puch (18). The homogenate was centrifuged at 2,000 g for 15 min at 0°C and the supernatant was used as enzyme preparation. PDE activity was assayed according to essentially the same method as that used by Puch (18).

Intracellular level of cyclic AMP

An ovariectomized rat (200 to 250 g in body wt.) was stunned and bled, and the uterine horns were removed and suspended in two similar organ baths of 10 ml. The baths were filled with Locke-Ringer solution, kept at 32°C, and bubbled with air. Responses of the uterus were isotonically recorded on a smoked drum. One horn always served as a control (treated only with oxytocin). The other horn was frozen by submerging into liquid nitrogen as fast as possible, after the isolated uterus had been exposed to a bile salt for 3 min and almost complete inhibition (90 to 100°") of the oxytocin-induced contraction by the drug had been confirmed, while dehydrocholate (3 · 10-4 g/ml) showed little inhibition on the contraction. The frozen tissue was quickly weighed, transferred without thawing to a glass homogenizer and homogenated at 0°C under addition of 3 ml of 6°, trichloroacetic acid. The homogenate was centrifuged at 2,000 g for 15 min at 0°C and the 2,000 g supernatant was extracted three times with 5 ml of ether after addition of 0.3 ml 1N HCl. The aqueous residue was freeze dried and the residue was dissolved in 0.5 ml acetate buffer (50 mM, pH 4). Twenty μl of this aliquot was used for measurement of the amount of cyclic AMP in the tissue. The measurement was carried out according to the method originally described by Gilman (19).
Drugs used

Papaverine hydrochloride (Tokyo Kasei Kogyo). 1,1-Diphenyl-3-piperidinobutanol hydrochloride (Aspinol, Kowa Co. Ltd.). Aminophylline ((Theophylline)$_2$. ethylenediamine, Sigma). Oxytocin (Atonin-S, Teikoku Zoki). Cyclic 3',5'-AMP (Cyclic adenosine-3', 5'-monophosphate, Daiichi Pure Chemicals Co. Ltd.). $^3$H-cyclic 3', 5'-AMP (adenosine-8-labeled, specific activity; 7.7 Ci/m mole, Daiichi Pure Chemicals Co. Ltd.). Desoxycholic acid (sodium salt, Sigma). Ursodesoxycholic acid (Tokyo Tanabe Co. Ltd.). Chenodesoxycholic acid (Tokyo Tanabe Co. Ltd.). Cholic acid (sodium salt, Sigma). Dehydrocholic acid (Tokyo Kasei Kogyo). Saponin (E. Merk).

RESULTS

Table 1 shows the PD$_{2}'$ values of papaverine and 4 bile salts, chenodesoxycholate, desoxycholate, ursodesoxycholate and cholate, and the mean values of percent inhibition of PDE activity by papaverine, the bile salts and saponin. The PD$_{2}'$ values show that the antioxytocin action of each bile salt was strong in order of desoxycholate > chenodesoxycholate > ursodesoxycholate > cholate > dehydrocholate. The antioxytocin activities of desoxycholate, chenodesoxycholate and ursodesoxycholate were approximately the same. The antioxytocin action of dehydrocholate was very weak and its PD$_{2}'$ value was below 2.3.

The PDE activity was determined by incubating the substrate (10$^{-4}$ M) in the presence of 1 mM 5'-AMP with a 25-fold crude PDE at 37°C, pH 7.5, for 30 min. The test drugs did not change the pH of the reaction mixture. The bile salts other than dehydrocholate in the concentrations of 10$^{-4}$ and 10$^{-3}$ g/ml significantly decreased PDE activity and there was a dose-dependent inhibition. Saponin had little influence on the PDE activity even in a concentration of 10$^{-3}$ g/ml. Moreover, a good relationship between the PDE activities and the antioxytocin activities of the bile salts was observed.

| TABLE 1. PD$_{2}'$ values of papaverine and bile salts, and percent inhibition of PDE activity by papaverine, bile salts and saponin. |
|---|---|---|---|
| **Concentration (g/ml)** | **Percent PDE-inhibition (mean ± S.E.)** | **Antispasmodic activity (PD$_{2}'$)** |
| **Compound** | **10$^{-4}$** | **10$^{-3}$** | **Antispasmodic activity (PD$_{2}'$)** |
| Chenodesoxycholate | 39 ± 4.6 (a) | 76 ± 1.7 (f) | 4.1 ± 0.11 (k) |
| Desoxycholate | 18 ± 5.3 (b) | 59 ± 3.3 (g) | 4.2 ± 0.09 (l) |
| Ursodesoxycholate | 35 ± 7.1 (c) | 56 ± 2.6 (h) | 3.9 ± 0.04 (m) |
| Cholate | 29 ± 7.6 (d) | 41 ± 1.5 (i) | 3.6 ± 0.03 (n) |
| Dehydrocholate | --2 ± 1.9 (e) | 8 ± 5.4 (j) | <2.3 |
| Saponin | 1 ± 1.6 | 2 ± 1.3 | -- |
| Papaverine | 94 ± 1.92* | -- | 5.3 ± 0.04 |

The amount of substrate hydrolysed in the absence of the test drug was 2.5 ± 1.0 nmol/12 mg wet wt. (3 estimations).

Significance of difference: p<0.05 (a, f), (b, g), (c, h), (f, g), (h, i), (i, j) and (m, n), p>0.05 (c, j), (d, l), (g, h), (k, l) and (l, m).

* The mean value of percent PDE-inhibition by papaverine was cited from reference 12.)
FIG. 1. Effects of exogenously applied CaCl₂ on inhibitory responses of the isolated rat uterus to papaverine (A), aminophylline (B), desoxycholate (C) and Aspaminol (D).

The effects of excess exogenous Ca²⁺ on inhibitory responses of the rat uterus to papaverine, Aspaminol, aminophylline and the bile salts are shown in Fig. 1. The antispasmodic and CaCl₂ were applied simultaneously, incubated for 3 min and in their presence oxytocin was cumulatively added. As a bile salt desoxycholate was used. The inhibitory actions of papaverine (10⁻⁶ g/ml), aminophylline (10⁻⁴ g/ml) and desoxycholate (2 x 10⁻⁵ g/ml) were potentiated by CaCl₂ (2.2 x 10⁻³ g/ml, 20 mM), while the inhibitory action of Aspaminol (10⁻⁵ g/ml) was greatly reduced by CaCl₂ (2.2 x 10⁻³ g/ml).

Table 2 shows the effects of chenodesoxycholate, desoxycholate and dehydrocholate on the intracellular cyclic AMP level in the rat uterus treated with oxytocin (10⁻² units/ml). The intracellular level of cyclic AMP after exposure of the uterus to the test drug is expressed as a percentage of the amount of intracellular cyclic AMP in the oxytocin-treated uterus. Chenodesoxycholate (5 x 10⁻⁴ g/ml) and desoxycholate (3 x 10⁻⁴ g/ml) significantly increased the intracellular level of cyclic AMP, while dehydrocholate did not affect the intracellular cyclic AMP level and had extremely weak antioxytocin action without inhibiting PDE.
**DISCUSSION**

It has been reported that papaverine, a potent inhibitor of PDE (2), elevates the intracellular content of cyclic AMP in rat uterus (5, 6). In our previous papers (8, 12), we have shown that papaverine strongly inhibited PDE from the rat uterus and increased the intracellular level of cyclic AMP, while Aspaminol, a synthetic antispasmodic, influenced neither the PDE activity nor the intracellular level of cyclic AMP. In the present study, the bile salts inhibited PDE activity. The order of potency for this action in the rat uterus was chenodesoxycholate > desoxycholate > ursodesoxycholate > cholate > dehydrocholate in the concentration of $10^{-3}$ g/ml and approximately the same as that for relaxation. Moreover, there was a dose-response relationship between the inhibition of PDE activity and the concentration of each bile salt. Saponin, surface-active agent, did not exert an antioxytocin action on the isolated rat uterus (unpublished observation) and little influenced the PDE activity in the concentration of $3 \times 10^{-4}$ g/ml. Therefore, it can be concluded that the antioxytocin actions of the bile salts are not concerned with their surface-active actions.

The exogenously applied CaCl$_2$ ($2.2 \times 10^{-3}$ g/ml, 20 mM) potentiated the inhibitory responses of the rat uterus to papaverine, aminophylline and the bile salts, while the inhibitory response to Aspaminol was remarkably reduced by the excess Ca$^{2+}$ ions. In the presence of the excess Ca$^{2+}$ ions, the inhibitory response to Aspaminol apparently differed from that to papaverine and those to the bile salts. The potentiation of the inhibitory responses to papaverine and the bile salts by CaCl$_2$ is probably the consequence of membrane "stabilization" (21).

Finally, we examined the changes of the intracellular level of cyclic AMP after exposure of the isolated rat uterus to the bile salts to determine whether or not the non-competitive antioxytocin action was related to the tissue cyclic AMP level. The results showed that the bile salts, chenodesoxycholate and desoxycholate, significantly increased the intracellular level of cyclic AMP. Dehydrocholate, which had weak antioxytocin action and little influenced PDE activity, did not increase the intracellular level of cyclic AMP. From these results, it appears that the non-competitive antioxytocin action of the bile salts...
tested may be exerted through inhibition of PDE and consecutive accumulation of cyclic AMP in the isolated rat uterus. Further, the fact that the inhibitory responses to papaverine, aminophylline and the bile salts, which inhibited PDE from the rat uterus, were not potentiated by the exogenously applied Ca²⁺ ions, and the inhibitory response to Aspaminol, which little influenced PDE activity, was greatly reduced, indicates that there are at least two different mechanisms for the so-called papaverine-like antispasmodics. These results support the theory proposed by Takagi, Takayanagi and Fujite (9) and Takagi et al. (10).

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