ACTIONS OF PAPAVERINE ON INTESTINAL SMOOTH MUSCLE AND ITS INHIBITION OF CYCLIC AMP AND CYCLIC GMP PHOSPHODIESTERASES

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Papaverine, a nonspecific smooth muscle relaxant, is well known to be a potent cyclic AMP phosphodiesterase inhibitor (1, 2). In recent papers (3–8) it has been reported that smooth muscle relaxing action of papaverine is likely to be mediated through an increase of cyclic AMP due to its phosphodiesterase inhibition. Recently attention has drawn to the role of cyclic GMP, which often presents physiological and pharmacological properties quite different from those of cyclic AMP (9). Lugnier and Stoclet (10) recently reported that papaverine inhibits both cyclic AMP and cyclic GMP phosphodiesterases. In the present experiment, we attempted to determine the inhibition constants (Ki values) of papaverine on cyclic AMP and cyclic GMP phosphodiesterases obtained from the guinea pig taenia, and to observe changes in the intracellular cyclic AMP and cyclic GMP levels after treatment of the guinea pig taenia with papaverine. The relationship between the action of papaverine on the intestinal smooth muscle and changes of the both cyclic nucleotides levels is also discussed.

Male guinea pigs (250–400 g) were sacrificed by a blow on the neck and the taenia was isolated from the caecum. The tissue was homogenized three times in 8 volumes of 25 mM Tris-HCl buffer with Polytron, with rheostat setting on 8 for 5 seconds. The homogenate was centrifuged at 1000 × g for 15 min at 0–4°C. The 1000 × g supernatant was then used as the crude enzyme preparation.

Cyclic GMP or cyclic AMP phosphodiesterase activity was estimated using radioactively labelled cyclic GMP (guanosine 8-labelled 3H-3',5'-cyclicphosphate, the Radiochemical Center) or labelled cyclic AMP (adenosine 8-labelled 3H-3',5'-cyclicphosphate, Daiichi Pure Chemicals. Ltd., Japan) as a substrate. An appropriately diluted enzyme preparation was incubated in 0.4 ml of 40 mM Tris-HCl buffer solution (pH 7.5) containing 5 mM MgCl₂,
3 mM 2-mercaptoethanol, $10^{-7}$ M $^3$H-cyclic GMP (or $^3$H-cyclic AMP). After a 10 min incubation at 37°C, the reaction was terminated by boiling for 3 min. Then 5'-nucleotidase (0.1 unit/ml) was added in the reaction mixture at 37°C for 10 min in order to change 5'-GMP (or 5'-AMP) into guanosine (or adenosine). The reaction products were separated by paper chromatography (a solvent: a mixture of ethanol 70 ml and 1 M ammonium acetate 30 ml) and radioactivities of unhydrolyzed cyclic nucleotides were counted in 10 ml of scintillation fluid. The rates of hydrolysis of cyclic nucleotides were adjusted to 20% to 60% in control samples.

In order to measure the intracellular levels of cyclic AMP and cyclic GMP, a taenia was divided into two pieces. One was used for measuring the control level of both cyclic nucleotides and the other for estimating changes in the concentrations of these cyclic nucleotides after treatment of the tissue with the drugs. As the level of cyclic GMP in this tissue is low, four pieces of taenia were used to form one preparation. In order to estimate changes in both cyclic nucleotides levels induced by papaverine, preparations were frozen in liquid nitrogen and used for measurements after the incubation of the tissue with papaverine for 3 min. This treatment with papaverine caused the maximum relaxation in taenia. Measurement of cyclic AMP was carried out by the method of Gilman (11). The cyclic GMP level was measured by the radioimmunoassay method of Steiner et al. (12) modified by Yasuda et al. (13). Protein was determined by the method of Lowry et al. (14).

Taenia strips of about 30 mm length were suspended in 10 ml organ bath to record changes in isometric tension produced by the drugs. These strips were arranged under 0.7 g initial tension for the isometric recordings of responses. All experiments were done at 37°C in Locke-Ringer solution which had the following composition (g/l): NaCl 9.0, KCl 0.4, CaCl₂ 0.2, MgCl₂ 0.2, NaHCO₃ 0.5 and glucose 0.5.

The cyclic GMP phosphodiesterase activity was measured with the substrate concentrations of $10^{-7}$-$10^{-5}$ M and an apparent Michaelis constant (Km value) was $1.5 \times 10^{-5}$ M and was obtained by the Lineweaver-Burk plots. The crude enzyme preparation was separated into mitochondrial and microsomal fractions. Cyclic GMP phosphodiesterase activity of these fractions was also assayed and the similar Km values were obtained (data not included). It has, however, been reported by Inatomi et al. (7) that cyclic AMP phosphodiesterase preparations obtained from the guinea pig taenia had low and high Km values (6.7 $\times 10^{-8}$ M and 9.1 $\times 10^{-5}$ M). In all the following experiments, the crude enzyme preparation was used. Papaverine ($2.5 \times 10^{-6}$ g/ml) inhibited cyclic GMP phosphodiesterase non-competitively. The inhibition constant (Ki value) of papaverine was $5.1 \times 10^{-5}$ M for cyclic GMP phosphodiesterase and $5.6 \times 10^{-5}$ M for low Km cyclic AMP phosphodiesterase, respectively. Aspaminol, $10^{-5}$ g/ml (1,1-diphenyl-3-piperidinobutanol hydrochloride, Kowa Co. Ltd., Japan), a synthetic antispasmodic, which previously showed no effects on cyclic AMP phosphodiesterase activity (7, 15), was also without any effects on cyclic GMP phosphodiesterase activity.

The 3 min treatment of the taenia with papaverine ($10^{-5}$ g/ml) significantly increased both cyclic nucleotide levels at 95% probability level, as shown in Table 1. Dibutryl
cyclic GMP caused contraction of taenia (16). A 10 min pretreatment with dibutyryl cyclic AMP (3 x 10^-4 - 1.2 x 10^-3 g/ml) depressed contractile responses of the taenia to dibutyryl cyclic GMP (3 x 10^-4 g/ml). The inhibitory effect of dibutyryl cyclic AMP was dose dependent and reversible as shown in Fig. 1.

It is known that cyclic GMP and its hydrolyzing enzyme, phosphodiesterase, are localized in various smooth muscles (17). Our present results show that guinea pig taenia also has cyclic GMP phosphodiesterase activity and its Km value is twice as large as the low Km value of cyclic AMP phosphodiesterase in the taenia as reported previously (7). Such a difference in the ratio of cyclic GMP to cyclic AMP hydrolysis was also shown in other tissue (17). Recent reports (10, 18) have stated that papaverine inhibits cyclic GMP phosphodiesterase from various tissues and its inhibition constant (Ki value) is similar to that for cyclic AMP phosphodiesterase. Our present data show a similar tendency. Recently the possible role of cyclic GMP in contractile responses of some smooth muscles to smooth muscle stimulants has been widely investigated (9, 19, 20). It is considered that in various smooth muscles, cyclic AMP plays an important role in relaxation induced by isoprenaline and papaverine and that cyclic GMP may be involved in contractions caused by smooth muscle stimulants (9, 19, 20). In order to explain various effects of drugs obtained as the result of their phosphodiesterase inhibition, it has been proposed that these effects are determined primarily by the relative selectivity of the compounds to either cyclic AMP phosphodiesterase or cyclic GMP phosphodiesterase (18). In the present work, papaverine

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<th>TABLE 1. Effect of papaverine on the intracellular levels of cyclic AMP and cyclic GMP of the guinea pig taenia</th>
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<tr>
<td>Cyclic GMP level (mean±S.E.) pmoles/mg protein</td>
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<tr>
<td>Cyclic AMP level (mean±S.E.) pmoles/mg protein</td>
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<td>No. of expts.</td>
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<td>Control (untreated)</td>
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<td>Papaverine (10^-5 g/ml)</td>
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* p<0.05

Fig. 1. Inhibitory action of dibutyryl cyclic AMP on the contraction of guinea pig taenia produced by dibutyryl cyclic GMP.
dib-c-AMP: dibutyryl cyclic AMP,  dib-c-GMP: dibutyryl cyclic GMP
increased the levels of the both cyclic nucleotides, however, dibutyryl cyclic AMP inhibited
the contraction produced by dibutyryl cyclic GMP, dose dependently. These results suggest
that cyclic AMP increased by an inhibition of cyclic AMP phosphodiesterase inhibits the
stimulating action of cyclic GMP produced by an inhibition of cyclic GMP phosphodies-
terase. We thus conclude that the nonspecific inhibitory action of papaverine on the
guinea pig taenia is mediated by an increase in cyclic AMP, as a result of inhibition of cyclic
AMP phosphodiesterase. On the other hand, the nonspecific antispasmodic action of
Aspaminol is not related with the intracellular levels of cyclic AMP and cyclic GMP.

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STUDIES ON 3,7-DIMETHYL-1-(5-OXO-HEXYL)-XANTHINE
(BL 191): THE INHIBITORY EFFECT OF BL 191
ON PDE IN VARIOUS TISSUES OF RATS

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Since Sutherland and Rall (1) reported that adenosine 3',5'-monophosphate (cyclic
AMP) was hydrolysed to 5'-AMP by tissue extracts from several sources, many investigations
have been made as regards the inhibitory effects of xanthine derivatives like caffeine and