DRUG-INDUCED SMOOTH MUSCLE CONTRACTION WITH NO CHANGE IN THE LEVEL OF CYCLIC GMP

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Beta-adrenergic action is postulated as being related to the ability of beta-adrenergic stimulants to increase the intracellular level of cyclic AMP. Evidence has also been presented that relaxation of intestinal smooth muscle produced by papaverine is mediated by an increase in the intracellular levels of cyclic AMP as a result of inhibition of cyclic AMP phosphodiesterase. Recently, there is evidence that cyclic GMP has physiological and pharmacological properties quite different from those of cyclic AMP (1, 2). It has also been suggested that the smooth muscle contraction mediated through cholinergic and other receptors may be associated with increase in the intracellular level of cyclic GMP (2, 3). In the present study, we have obtained data, which refutes a correlation between cyclic GMP level and contraction of the longitudinal muscle of rat duodenum as caused by drugs.

Male Wistar rats of 250 to 350 g were sacrificed by a blow on the neck and the duodenum was isolated. The longitudinal muscle was dissected free from the circular muscle and mucosa.

To determine the pharmacological actions of 5-hydroxytryptamine creatinine sulfate (5-HT) and butyltrimethylammonium bromide (BuTMA), a piece (3 to 4 cm) of the longitudinal muscle was suspended in an organ bath filled with Locke Ringer solution (NaCl 9.0 g, KCl 0.4 g, CaCl₂ 0.2 g, NaHCO₃ 0.5 g, MgCl₂ 0.2 g and glucose 0.5 g in a litre), kept at 37°C and bubbled with air. Responses of the longitudinal muscle preparation to the drugs were isotonically recorded.

Cyclic GMP levels were measured by the radioimmunoassay of Steiner et al. (4) as modified by Yasuda et al. (5). The measurable minimum amount of cyclic GMP technique was found to be 0.1 pmoles/tube. Two pieces of the longitudinal muscle were obtained from one duodenum. One was used for measuring the control level of cyclic GMP and the other for estimation of a change after exposure to a drug. After response of the longitudinal muscle to a drug was confirmed, the preparation was immediately frozen in liquid nitrogen and used for measurement of cyclic GMP (6, 7). The muscle thus treated was homogenized with a glass homogenizer in 2 ml of cold 6% trichloroacetic acid. The homogenate was centrifuged at 1000 x g at 0°C for 30 min and the supernatant was acidified by 1 N HCl, thereafter, trichloroacetic acid was extracted 4 times with 3 volumes of ether. The lyophilized sample was dissolved with distilled water and was applied to Sephadex G-25 (0.9 x 2.4 cm)
column which was equilibrated with 1.2 ml of water. Cyclic GMP was then eluted with a further 2.5 ml of water. The elutes were lyophylized to dryness, redissolved with 200 µl of this solution was used for estimation of cyclic GMP content. Reactions were initiated by addition of 50 µl of antiserum of cyclic GMP in the mixture, which consisted of 100 µl cyclic GMP sample and 50 µl of 3H-cyclic GMP (1.33 p moles). These reactions were allowed to proceed for 90 min at 0°C. The mixture was passed through a Millipore filter (HAWP-02500) and the filter was washed with 6 ml of cold sodium acetate buffer (pH 6.2) and placed in a counting vial with 1 ml of methyl cellosolve. Nine ml of toluene scintillator (4.8 g of DPO, 300 ml of methyl cellosolve and 600 ml of toluene) was then added and the radioactivity was counted using a Packard Tri-carb liquid scintillation counter (model 3203). The trichloroacetic acid precipitate was solubilized with 1 N NaOH and assayed for protein by the method of Lowry et al. (8) using bovine serum albumin as the standard.

BuTMA (1.5 × 10⁻⁴ M) and 5-HT (2.5 × 10⁻⁶ M) were applied for 20 sec and 2 min. These concentrations used were sufficient to induce a maximal contraction. The smooth muscle contractions reached 70 to 80 percent of the maximal magnitude 20 sec after application and reached the maximum at 2 min (Fig. 1). Papaverine hydrochloride was used as the smooth muscle relaxant. When this agent (10⁻⁴ M) was applied for 3 min, the relaxation was observed to the extent of the maximal magnitude. These smooth muscle preparations were used for the estimation of cyclic GMP contents. A mean (± standard error) content of cyclic GMP in the untreated preparations was 0.18 (±0.04) p moles/mg protein (no. of experiments : 6). The level of cyclic GMP after exposure of the preparation to a drug is expressed as a percentage of the level in the untreated one, as shown in Table 1. There was no significant change in the cyclic GMP level with application of BuTMA (1.5 × 10⁻⁴ M) and 5-HT (2.5 × 10⁻⁶ M) for 20 sec and 2 min (Table 1). On the other hand, with a 3 min application of papaverine hydrochloride (10⁻⁴ M) the cyclic GMP level in the longitudinal muscle was significantly increased. (Table 1).

BuTMA is a cholinergic stimulant not hydrolyzed by acetylcholinesterase. Since the response to BuTMA was completely abolished by atropine and was unaffected by hexamethonium and tetrodotoxin, the site of action of BuTMA was estimated to be the acetylcholine receptor in the longitudinal muscle of the rat duodenum (9). 5-HT acts directly on the D-receptor of 5-HT (10) of the smooth muscle cells of the duodenum, since the contractile response to 5-HT was unaffected by tetrodotoxin and atropine (11).

![Fig. 1. Registrogram of contraction of the longitudinal muscle of the rat duodenum induced by butyltrimethylammonium (BuTMA) and 5-hydroxytryptamine (5-HT).](image-url)
TABLE 1. Cyclic GMP levels in the presence of 5-hydroxytryptamine, butyltrimethylammonium and papaverine in the longitudinal muscle of rat duodenum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after application of drug</th>
<th>20 sec</th>
<th>2 min</th>
<th>3 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyltrimethylammonium (1.5 x 10^{-4} M)</td>
<td></td>
<td>81.2±21.8[6]</td>
<td>115.9±47.4[6]</td>
<td></td>
</tr>
<tr>
<td>5-Hydroxytryptamine (2.5 x 10^{-4} M)</td>
<td></td>
<td>109.7±20.0[6]</td>
<td>107.7±31.2[6]</td>
<td></td>
</tr>
<tr>
<td>Papaverine (10^{-4} M)</td>
<td></td>
<td>246.3±42.0*[5]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cyclic GMP level (mean±S.E.) was indicated as a percentage of cyclic GMP level in the untreated longitudinal muscle. [ ]: no. of experiments. * P-value was determined by comparing the cyclic GMP level of the treated longitudinal muscle of rat duodenum with that of control (P<0.02).

It has been recently observed that contraction of smooth muscles from some species induced by drugs is associated with an increase of the intracellular cyclic GMP level (1, 2, 3, 12, 13) and it was suggested that cyclic GMP may mediate contraction following stimulation of cholinergic and other receptors. We also observed that the level of cyclic GMP in the tracheal smooth muscle of the guinea pig is increased by acetylcholine and histamine (6). In the present study, increase in the level of cyclic GMP in the longitudinal smooth muscle of the rat duodenum after BuTMA and 5-HT was not observed.

On the other hand, it has been reported that papaverine inhibits cyclic GMP phosphodiesterase as well as cyclic AMP phosphodiesterase (14, 15). More recently, we reported that papaverine increases the level of cyclic GMP in the guinea pig taenia caecum by the inhibition of cyclic GMP phosphodiesterase and a relationship between increase in the cyclic GMP level and relaxation of smooth muscle produced by papaverine has been reported by our group (7). In the present study, papaverine also significantly increased the level of cyclic GMP (Table 1). In our previous work (6) increase in the cyclic GMP level in the tracheal smooth muscle of guinea pig by acetylcholine and histamine was observed using the same procedure as described herein. The present data with papaverine together with our previous results (6, 7) indicate that the evidence against a correlation between cyclic GMP level and contractions of the longitudinal muscle of the rat duodenum induced by BuTMA and 5-HT cannot be due to a lack of sensitivity in the assay method employed. Therefore, contractions of the longitudinal muscle of rat duodenum induced by BuTMA a cholinergic stimulant and 5-HT are apparently not associated with the level of cyclic GMP.

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REFERENCES
COMPARISON OF NEWLY SYNTHESIZED β-ADRENERGIC BLOCKERS, OPC 1085 AND SQ 11725, WITH PINDOLOL AND PROPRANOLOL IN THE BLOOD-PERFUSED CANINE SA NODE AND PAPILLARY MUSCLE PREPARATIONS

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In previous studies (1, 2, 3), we assessed the β-adrenergic blocking activity of 11 compounds against the positive chronotropic and inotropic effect of norepinephrine in the isolated and blood-perfused canine sino-atrial (SA) node and papillary muscle preparations with donor animals. The blocking potencies of these compounds on the β-adrenergic effects on the cardiac functions were roughly divided in those of propranolol and of pindolol, and also in two groups on the difference of presence or absence of sympathomimetic effect. In this paper, two newly synthesized compounds were compared with the potencies of pindolol and propranolol.

The isolated SA node and papillary muscle preparations were perfused at 100 mm Hg with the arterial blood conducted from the carotid artery of a heparinized donor dog by the aid of a peristaltic pump. The experimental setup of these preparations have been described in detail in previous papers (4, 5). Drugs used were as follows: 1-norepinephrine, dl-propranolol hydrochloride, dl-pindolol, dl-5(3-tert-butylamino-2-hydroxy) propoxy-3,4 dihydrocarbostyril hydrochloride (OPC 1085, Otšuka) (6) and dl-2,3-cis-1,2,3,4-tetrahydro-