Effects of Various Selective Phosphodiesterase Inhibitors on Carbachol-induced Contraction and Cyclic Nucleotide Contents in Guinea Pig Taenia Coli

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ABSTRACT. Effects of various selective phosphodiesterase (PDE) inhibitors on muscle contractility and cyclic nucleotide contents in guinea pig taenia coli were investigated. Forskolin and sodium nitroprusside inhibited carbachol (CCh)-induced contraction in a concentration-dependent manner. Various selective PDE inhibitors, vinpocetine (type1), erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA, type 2), milrinone (type 3), Ro20–1724(type 4) and zaprinast (type 5) inhibited CCh-induced contraction in a concentration-dependent manner, but the inhibition of milrinone was noticeably smaller than that of the other PDE inhibitors. The rank order of potency was zaprinast > vinpocetine > EHNA > Ro20–1724 > milrinone. In the presence of CCh (0.3 µM), vinpocetine and Ro20–1724 both increased cAMP content, but not cGMP. By contrast, EHNA and zaprinast both increased cGMP content, but not cAMP. Pretreatment with ODQ (30 µM), a soluble guanylyl cyclase inhibitor, decreased the inhibition of CCh-induced contraction by EHNA or zaprinast. Pretreatment with SQ22536 (100 µM), an adenylyl cyclase inhibitor, decreased the inhibition of CCh-induced contraction by vinpocetine or Ro20–1724. In conclusion, it was indicated that vinpocetine- or Ro20–1724-induced relaxation was correlated with cAMP but EHNA- or zaprinast-induced relaxation was correlated with cGMP.

KEY WORDS: cAMP, cGMP, PDE inhibitor, taenia coli.

Cyclic nucleotides are important second messengers and have been associated with smooth muscle relaxation [5]. Cyclic nucleotides are synthesized by adenylyl cyclase or guanylyl cyclase and degraded by phosphodiesterase (PDE). Currently, PDEs are classified into 11 families [3, 6, 20], and selective PDE inhibitors have been found in some families of PDEs [4]. It has been reported that the relaxation induced by these selective PDE (type1-type5) inhibitors is involved in the increases in cAMP and/or cGMP contents in vascular [13], tracheal [15], urinary [14, 19] and ileal smooth muscle [9]. It has been reported that vinpocetine (type1 inhibitor) induces relaxation in rabbit aorta through the increase in cGMP content [1, 7] but in rat urinary bladder through the increase in cAMP content [14]. There have been a few reports indicating that relaxation induced by EHNA (type2 inhibitor) is associated with increases in cyclic nucleotide contents in smooth muscle [8, 12].

Additionally, the relationship of relaxation induced by these selective inhibitors and cyclic nucleotides is less clear in intestinal smooth muscle. Barnette et al. [2] showed that PDE3, 4 and 5 inhibitors caused relaxation in canine colonic smooth muscle, and that PDE3 and 4 inhibitors decreased the hydrolysis of cAMP, and PDE5 inhibitor decreased the hydrolysis of cGMP in smooth muscle. Tomkinson & Raeburn [18] investigated the inhibitory potency of PDE1,3,4 or 5 inhibitors on methacholine-induced contraction in guinea pig and rat ileal smooth muscle and showed the difference in the inhibitory potency of these agents. They did not clarify the relationship of the relaxations and the changes in cyclic nucleotide contents induced by selective PDE inhibitors in intestinal smooth muscle. In a previous paper, we reported that zaprinast-induced relaxation was greater than milrinone- or Ro20–1724-induced relaxation in guinea pig ileum and that zaprinast increased cGMP and milrinone or Ro20–1724 increased cAMP [9].

In the present study, we investigated the relationship between relaxation and cyclic nucleotide contents in response to the selective PDE (types 1–5) inhibitors in guinea pig taenia coli precontracted with carbachol.

METHODS

Muscle preparations and tension measurement: Male guinea pigs (Hartley, 300–400 g; Funabashi Farm, Funabashi) were bled after stunning, and then the taenia coli was quickly removed. The muscle strips were about 1–2 mm in width and 7 mm in length. The muscle strips were incubated with physiological salt solution (PSS) containing (in mM) 136.8 NaCl, 5.4 KCl, 2.5 CaCl₂, 1.0 MgCl₂, 11.9 NaHCO₃ and 5.5 glucose. PSS was aerated with 95% O₂ and 5% CO₂ to adjust pH to 7.2 at 37°C. This study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Nippon Veterinary and Animal Science University.

Muscle tension was recorded isometrically. One end of each strip was bound to a glass holder and the other end was connected by a silk thread to a strain-gauge transducer (TB-611T, Nihon Kohden, Tokyo) in an organ bath containing PSS with a resting tension of 1g. The muscle strips were equilibrated for 30 min to obtain a stable contractility induced by hyperosmotically added 65 mM KCl.

Assay of cGMP and cAMP contents: The cGMP or cAMP content in the muscle strips was measured by enzyme immunoassay. The weight of muscle strips was measured...
before starting the experiments and strips of approximately 15 mg wet weight were used for the experiments. After incubation of the strips with vehicle (dimethyl sulfoxide or distilled water), vinpocetine, EHNAA, milrinone, Ro20–1724 or zaprinast for 10 min in the presence of carbachol (0.3 µM), the strips were rapidly frozen in liquid nitrogen and stored at −80°C until homogenized in 6% trichloroacetic acid (0.4 ml). The homogenate was centrifuged at 3,000 × g for 15 min and the supernatant was washed with 1.5 ml of water-saturated diethyl ether four times. The cGMP or cAMP content of the strips was assayed with an enzyme immunoassay kit (Amersham Pharmacia Biotech, UK). The range of assay for cAMP or cGMP was 12.5–3200 or 50–12800 fmol/well, respectively. The cGMP or cAMP content was expressed as picomole per gram wet weight.

Chemicals: Chemicals used were forskolin, sodium nitroprusside, milrinone, zaprinast, carbachol (Sigma, U.S.A.), vinpocetine, erythro-9-(2-hydroxy-3-nonyl)-adenine·HCl (EHNA), SQ22536, ODQ (BIOMOL Research Laboratories, U.S.A.) and Ro20–1724 (LC Laboratories, U.S.A.). Vinpocetine crystallizes if the concentration of vinpocetine exceeds 30 µM in PSS. Therefore, we used vinpocetine below 30 µM in the present experiment.

Statistics: Values are expressed as the mean ± S.E.M. and statistical analyses were performed by Student’s t-test.

RESULTS

Effects of forskolin and sodium nitroprusside on carbachol-induced contraction: When a contractile response induced by 0.3 µM carbachol (CCh) reached a steady level about 15–20 min after addition, forskolin, an adenylyl cyclase activator, or sodium nitroprusside (SNP), a soluble guanylyl cyclase activator, was added cumulatively. Forskolin inhibited the CCh-induced contraction in a concentration-dependent manner (Figs. 1A, 2). The threshold concentration of forskolin was 0.1 µM. SNP also inhibited the CCh-induced contraction in a concentration-dependent manner (Fig. 2). The concentrations of forskolin and SNP producing 50% inhibition (IC50) of the CCh-induced contraction were 2.8 (2.2–3.4) and 2.5 (1.9–3.1) µM, respectively.

Effects of various selective PDE inhibitors on CCh-induced contraction: When a contractile response induced by 0.3 µM CCh reached a steady level about 15–20 min after addition, each PDE inhibitor was added cumulatively. Vinpocetine (type 1 PDE inhibitor) (Fig. 1B), EHNA (type 2), milrinone (type 3), Ro20–1724 (type 4) and zaprinast (type 5) inhibited the CCh-induced contraction in a concentration-dependent manner (Fig. 3). The IC50 and percentages of maximal inhibition of CCh-induced contraction in response to those agents are shown in Table 1. The inhibitory effect of milrinone was weaker than that of other PDE inhibitors. The order of potency for the relaxant effect was zaprinast > vinpocetine > EHNAA > Ro20–1724 > milrinone.

Effects of various PDE inhibitors on cGMP and cAMP contents: Vinpocetine and Ro20–1724 caused concentran.
tion-dependent increases in the cAMP content of muscle strips and high concentrations were required to cause a significant increase in cAMP content (Fig. 4A, D). But EHNA, milrinone and zaprinast, even at maximum concentration (100 µM), did not significantly increase the cAMP content (Fig. 4B, C and E).

EHNA caused concentration-dependent increases in cGMP content of the muscle strips in the presence of CCh and high concentrations were required to increase cGMP content (Fig. 4B). Zaprinast also caused a concentration-dependent increase in cGMP content and was effective at a low concentration of 1 µM (Fig. 4E), but vinpocetine, milrinone and Ro20–1724 did not significantly increase the cGMP content (Fig. 4A, C and D).

There was a positive correlation between the inhibition of CCh-induced contraction and the increase in cAMP content elicited by vinpocetine or Ro20–1724 (Fig. 5A), and between the inhibition of CCh-induced contraction and the increase in cGMP content elicited by EHNA or zaprinast (Fig. 5B).

**Effects of guanylyl cyclase and adenylyl cyclase inhibitor on the inhibition of CCh-induced contraction by various PDE inhibitors:** Furthermore, we used ODQ, a soluble guanylyl cyclase inhibitor, and SQ22536, an adenylyl cyclase inhibitor to clarify the relationship between the inhibition of CCh-induced contraction and cyclic nucleotides. SQ22536 or ODQ was added to the bath solution 30 min before application of CCh. SQ22536 and ODQ did not affect resting tension or CCh-induced contraction (data not shown). EHNA- and zaprinast-induced inhibition of CCh-evoked contraction were attenuated significantly by pre-incubation with 30 µM ODQ, but not 100 µM SQ22536 (Fig. 6B, D).

Vinpocetine- and Ro20–1724- induced inhibition of CCh-evoked contraction were decreased significantly by pre-incubation with 100 µM SQ22536, but not with 30 µM ODQ (Fig. 6A, C).

**DISCUSSION**

cAMP and cGMP are important second messengers that control many physiological processes, including smooth muscle contractility [5]. Adenylyl cyclase and guanylyl cyclase synthesize cAMP and cGMP, respectively and PDE degrades them. It has been reported that isoproterenol induces relaxation by increasing cAMP content [11] and SNP induced relaxation by increasing cGMP [10] in guinea pig taenia coli. In the present study, forskolin and SNP inhibited the CCh-induced contraction in a concentration-dependent manner (Fig. 2). These results suggested that the cAMP/adenylyl cyclase pathway and cGMP/guanylyl cyclase pathway were both involved with relaxation in guinea pig taenia coli.

PDEs metabolizing intracellular cAMP and cGMP are classified into 11 families [3, 6, 20]. There are selective inhibitors for PDE 1–5 families. It has been showed that isoproterenol induces relaxation by increasing cAMP content [11] and SNP induced relaxation by increasing cGMP [10] in guinea pig taenia coli. In the present study, forskolin and SNP inhibited the CCh-induced contraction in a concentration-dependent manner (Fig. 2). These results suggested that the cAMP/adenylyl cyclase pathway and cGMP/guanylyl cyclase pathway were both involved with relaxation in guinea pig taenia coli.

It has been reported that vinpocetine (type 1 inhibitor) induces relaxation by increasing cGMP content in rabbit smooth muscle [9].

### Table 1. IC50 and maximal inhibition (%) of various selective PDE inhibitors for contraction induced by 0.3 µM CCh

<table>
<thead>
<tr>
<th>Agents</th>
<th>IC50 (µM)</th>
<th>Maximal inhibition (%)</th>
<th>n</th>
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<tbody>
<tr>
<td>Vinpocetine</td>
<td>7.7 (7.4–8.0)</td>
<td>72.6 ± 2.9</td>
<td>6</td>
</tr>
<tr>
<td>EHNA</td>
<td>42.9 (38.0–45.8)</td>
<td>72.5 ± 3.6</td>
<td>6</td>
</tr>
<tr>
<td>Milrinone</td>
<td>&gt;100</td>
<td>12.4 ± 3.7</td>
<td>4</td>
</tr>
<tr>
<td>Ro20–1724</td>
<td>&gt;100</td>
<td>42.6 ± 3.8</td>
<td>6</td>
</tr>
<tr>
<td>Zaprinast</td>
<td>5.6 (3.6–7.6)</td>
<td>86.4 ± 3.2</td>
<td>5</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate 95% confidence limits. The inhibition induced by PDE inhibitors which fully recover to the basal tension before the application of CCh was considered to be 100% inhibition.
Fig. 4. Effects of vinpocetine (A), EHNA (B), milrinone (C), Ro20–1724 (D) and zaprinast (E) on cAMP and cGMP precontents of guinea pig taenia coli. Preparations were precontracted with 0.3 µM CCh and were treated with these agents for 10 min. Control was treated with vehicle instead of these PDE inhibitors. Each point represents the mean of 4 experiments. Vertical bars indicate the S.E.M. * and **: Significant difference from each respective control with P<0.05 and P<0.01, respectively.
aorta [1, 7] and by increasing cAMP content in rat urinary bladder [14]. In the present study, there was a positive correlation between the inhibition of CCh-induced contraction and the increase in cAMP content by vinpocetine in guinea pig taenia coli. Moreover, the inhibition of CCh-induced contraction by vinpocetine was attenuated significantly by pre-incubation with adenylyl cyclase inhibitor, SQ22536, but not soluble guanylyl cyclase inhibitor, ODQ. These results suggested that the relaxation induced by vinpocetine, PDE type 1 inhibitor, was involved with the increase in cAMP content in guinea pig taenia coli. PDE1 families are identified as PDE1A, 1B and 1C. PDE1A and 1B have high affinity for cGMP and PDE1C has similar affinity for cAMP and cGMP [3]. It is known that PDE1 hydrolyzes cGMP in some smooth muscle cells like PDE5 [16], but vinpocetine might inhibit PDE1C but not PDE1A and B in the guinea pig taenia coli.

It has been suggested that EHNA (type 2 inhibitor) relaxes rat pulmonary artery tone by increasing cAMP and cGMP [8]. Additionally, it was reported that the relaxation induced by EHNA might be involved in the increase in cAMP content in urinary bladder [14]. In this paper, we showed that there was a positive correlation between the inhibition of CCh-induced contraction and the increase in cAMP content caused by EHNA, and the inhibition of CCh-induced contraction caused by EHNA was attenuated significantly by pre-incubation with ODQ, but not by SQ22536 in taenia coli. These results suggested that the relaxation induced by EHNA, a PDE type 2 inhibitor, was involved with the increase in cGMP content in guinea pig taenia coli. The present data showed that the relationship between the relaxation and the increase in cyclic nucleotides in guinea pig taenia coli in response to type 1 or 2 PDE inhibitor was similar to that found in rat urinary bladder [14].

Milrinone (type 3 inhibitor) increased cAMP content and inhibited phenylephrine-induced contraction in a concentration-dependent manner in guinea pig aorta [17]. In the present study, the relaxation induced by milrinone was
smaller than that induced by the other PDE inhibitors and milrinone did not significantly increase either cAMP or cGMP in guinea pig taenia coli. These results suggest that PDE3 does not function in intestinal smooth muscle. It is well known that PDE4 is specific for the hydrolysis of cAMP and PDE5 is specific for that of cGMP [3]. The results of the present study suggest that the Ro20–1724-induced relaxation is related to cAMP and the zaprinast-induced relaxation to cGMP. Furthermore, these data are similar to our previous results observed in guinea pig ileum [9].

In the present study, selective PDE inhibitors inhibited CCh-induced contraction in guinea pig taenia coli and the rank order of inhibitory potency was zaprinast (type 5) > vinpocetine (type 1) > EHNA (type 2) > Ro20–1724 (type 4) > milrinone (type 3) (Table 1). The rank order of inhibitory potency of PDE inhibitors for histamine-induced contraction was rolipram (type 4) > milrinone (type 3) > zaprinast (type 5) > vinpocetine (type 1) in guinea pig tracheal smooth muscle [15], that for CCh-induced contraction was vinpocetine (type 1), sildenafil (type 5) > EHNA (type 2) > Ro20–1724 (type 4) > milrinone (type 3) in rat urinary bladder [14] and that for CCh or high K+-induced contraction was zaprinast (type 5) > Ro20–1724 (type 4) > milrinone (type 3) in guinea pig ileal smooth muscle [9]. These results suggest that the activity of PDE families is different according to the organ.

In conclusion, selective PDE inhibitors inhibited CCh-induced contraction in guinea pig taenia coli and the rank order of inhibitory potency was zaprinast (type 5) > vinpocetine (type 1) > EHNA (type 2) > Ro20–1724 (type 4) > milrinone (type 3). Moreover, it is suggested that vinpocetine or Ro20–1724-induced relaxation is correlated with cAMP, and that EHNA- or zaprinast-induced relaxation is correlated with cGMP in taenia coli.

REFERENCES