Contractile Responses of Longitudinal Muscle Strip to 5-HT and Influences of Divalent Cations in the Guinea-Pig Isolated Colon

Mitsuo Ishizawa

Laboratory of Physiology, School of Health Sciences, Sapporo Medical University, Sapporo, 060, Japan

Abstract

The contractile effects of 5-hydroxytryptamine (5-HT) and influences of several kinds of divalent cations were investigated on longitudinal muscle strips of the guinea-pig isolated distal colon. 5-HT (10 nM-10 μM) produced phasic contractions which were partially inhibited by atropine (1 μM) and markedly inhibited by tetrodotoxin (1 μM), indicating that 5-HT acts mainly on the myenteric plexus and releases transmitters to cause contraction of the longitudinal muscle.

The contractile response to 5-HT (3 μM) was almost completely inhibited by spantide (10 μM), a substance P antagonist, in the presence of atropine (1 μM), while spantide alone did not block 5-HT-induced contraction.

Of several divalent cations including Cd²⁺, Co²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Sr²⁺ and Zn²⁺, Cd²⁺ ions (10 μ-100 μM), which block L- and N-type Ca²⁺ channels, were most effective inhibitor of the 5-HT-induced contractions. While Sr²⁺ and Co²⁺ at a concentration of 100 μM did not have a significant effect. The order effectiveness of inhibition was Cd²⁺ ≫ Mn²⁺ > Mg²⁺ = Ni²⁺ = Zn²⁺.

Bay K 8644 (1 μM), a L-type Ca²⁺ channel activator, did not influence the contractile response of the longitudinal muscle strip to 5-HT (3 μM).

The present results suggest that 5-HT may mainly act on N-type Ca²⁺ channels in the myenteric neurones and cause the release of at least acetylcholine and substance P to induce contractions of the longitudinal muscle in the guinea-pig distal colon.

Key words: 5-hydroxytryptamine, divalent cations, myenteric plexus, longitudinal muscle strip, guinea-pig colon.

Introduction

The presence of 5-hydroxytryptamine (5-HT) in the enteric nervous system and the release of 5-HT from the myenteric plexus in the intestinal tract suggest that 5-HT is an enteric neurotransmitter (Costa et al., 1982; Holzer and Skofitsch, 1984). The released 5-HT is thought to act on the interneurones which release transmitters and cause contractions of the intestinal muscles (Gaddum and Picarelli, 1957; Brownlee and Johnson, 1963).

It is also accepted that at nerve terminals voltage-sensitive Ca²⁺ channels are essential for
the initiation of transmitter release (Katz and Miledi, 1967) and several kinds of divalent cations compete with Ca\(^{2+}\) uptake for the channel binding sites (Godfraind et al., 1986).

However, the relationship between Ca\(^{2+}\) channels and 5-HT-induced release of transmitters in myenteric neurones of the intestine is not well understood.

The present study was designed to define 1) which transmitters are released by 5-HT, 2) which types of Ca\(^{2+}\) channels are affected by 5-HT using several kinds of divalent cations, in the longitudinal muscle strip of the guinea-pig isolated distal colon.

**Methods**

Male guinea-pigs weighing 300-500 g were stunned and bled to death. The distal colon (ca 3 cm long) was excised at about 5 cm from the anus. The longitudinal muscle strip (10 mm long, 1 mm wide) was cut parallel to the longitudinal muscle layer. The muscle strip was separated from the mucosal layer and immersed in 10 ml of the bath solution containing NaCl 125.0, KCl 5.0, CaCl\(_2\) 2.0, glucose 11.0, and tris-maleate buffer 5.0 (mM). The bath solution was kept at 37\(^\circ\)C and gassed with O\(_2\) (pH : 7.2).

The contractile activity of the muscle strip was recorded isometrically with a force-displacement transducer and an ink-writing pen-recorder. The initial load on the muscle strip was 0.3 g.

Bay K8644 was dissolved in methanol (1 mg/ml) and diluted with saline. The maximum concentration of methanol in the bath solution was 0.1%. This did not influence tissue responsiveness. Other drugs and divalent cations (chloride compound) were dissolved in distilled water.

A small volume (10 \(\mu l\) - 100 \(\mu l\)) of the solution dissolved drugs or divalent cations was added to the bath solution and the concentrations refer to the final bath concentration. Divalent cations were added to the bath solution and stabilization was allowed at least 10 min before addition of 5-HT.

The following drugs were used: 5-hydroxytryptamine hydrochloride (Sigma), atropine sulphate (Sigma), tetrodotoxin (Sankyo), spantide (Sigma), Bay K8644 (Reserch Biochemicals) and carbachol (Sigma).

The statistical significance was analyzed by Student's \(t\)-test for paired data compared with control.

**Results**

1. Effects of atropine and tetrodotoxin on the contractile responses to 5-HT

5-HT (10 nM-10 \(\mu M\)), added to the bath solution, dose-dependently produced phasic contractions of the longitudinal muscle strip. These contractions were partially inhibited by atropine (1 \(\mu M\)) and markedly inhibited by tetrodotoxin (Fig. 1).

2. 5-HT-induced transmitter release from myenteric plexus

5-HT (3 \(\mu M\))-induced contraction of the longitudinal muscle strip was almost completely inhibited by spantide (10 \(\mu M\)), a substance P antagonist, in the presence of atropine (1 \(\mu M\)),
5-HT-induced contraction and divalent cations

Fig. 1. Effects of atropine and tetrodotoxin (TTX) on the contractile response of the longitudinal muscle strip to 5-HT in the guinea-pig isolated distal colon. Peak tension induced by 5-HT (10 μM) in control was taken as 100%.

●: control, ■: in the presence of atropine (1 μM, n=9) ▲: in the presence of TTX (1 μM, n=6).
Each point represents the mean±SEM.

Fig. 2. Effects of spantide and atropine on 5-HT-induced contraction of the longitudinal muscle strip in the guinea-pig isolated distal colon. The contractile response to 5-HT (3 μM) was inhibited in the presence of spantide (10 μM) with atropine (1 μM).
Time interval between traces was 30 min.

while spantide alone did not block 5-HT-induced contraction (Fig. 2).

3. Effects of several kinds of divalent cations on the contractile response to 5-HT

The effects of divalent cations (Cd²⁺, Co²⁺, Mn²⁺, Mg²⁺, Ni²⁺, Sr²⁺ and Zn²⁺) at a concentration of 100 μM on the contractile response to 5-HT (3 μM, ED₅₀) were examined in the longitudinal muscle strip (Fig. 3). 5-HT-induced contraction was completely inhibited by Cd²⁺ and slightly by Mn²⁺, Mg²⁺, Ni²⁺ and Zn²⁺. However, Sr²⁺ and Co²⁺ did not show significant inhibition (Table 1). The order of inhibitory effect was Cd²⁺≫Mn²⁺>Mg²⁺=Ni²⁺=Zn²⁺.

The inhibitory effects of Cd²⁺ (10, 20 and 100 μM) on dose-response curve for 5-HT in the longitudinal muscle strip are shown in Fig. 4. Dose-response curve for 5-HT parallelly shifted
Fig. 3. Effects of several kinds of divalent cations on the contractile response of the isolated distal colon to 5-HT.

In the presence of divalent cation (Cd²⁺, Mn²⁺, Mg²⁺, Zn²⁺, Ni²⁺, Sr²⁺ or Co²⁺) at a concentration of 100 μM, the contractile response induced by 5-HT (3 μM) was compared with the control response. Each trace was obtained from different preparations.

to the right by Cd²⁺ at concentrations of 10 μM and 20 μM, respectively. Cd²⁺ gave IC₅₀ value of 19.2 μM against 5-HT.

Additionally, carbachol (0.3 μM) showed almost same contractile response of the longitudinal muscle strip as 5-HT (3 μM). The carbachol (0.3 μM)-induced contraction, which is
Table 1. Effects of divalent cations on the contractile response of the longitudinal muscle strip to 5-HT in the guinea-pig isolated distal colon.

<table>
<thead>
<tr>
<th>Divalent Cation 100 μM</th>
<th>Contractile response to 5-HT (3 μM)</th>
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<tbody>
<tr>
<td>Cadmium (Cd)</td>
<td>0.0% n=10 **</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>96.0±3.9% n=8 ns</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>83.7±11.5% n=6 *</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>71.6±11.2% n=10 **</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>86.6±4.6% n=7 *</td>
</tr>
<tr>
<td>Strontium (Sr)</td>
<td>97.9±3.2% n=6 ns</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>78.7±4.9% n=8 *</td>
</tr>
</tbody>
</table>

Peak tension induced by 5-HT (10 μM) in control was taken as 100%.
Each response to 5-HT (3 μM) in the presence of divalent cation (100 μM) represents the mean±SEM.
**: p<0.05, *: p<0.1, ns: p>0.1.

Fig. 4. Effects of Cd²⁺ on the contractile response of the guinea-pig isolated distal colon to 5-HT.
Peak tension induced by 5-HT (10 μM) in control was taken as 100%.
○: control, ■: in the presence of Cd²⁺ (10 μM, n=10), ▲: in the presence of Cd²⁺ (20 μM, n=10), ×: in the presence of Cd²⁺ (100 μM, n=5).
Each point represents the mean±SEM.

5-HT-Mediated Contractile Response

Fig. 4. Effects of Cd²⁺ on the contractile response of the guinea-pig isolated distal colon to 5-HT.
Peak tension induced by 5-HT (10 μM) in control was taken as 100%.

- blocked by atropine (1 μM) but not by tetrodotoxin (1 μM), was not inhibited by Cd²⁺ (20 μM).
- However, the contractile response to carbachol was markedly inhibited by verapamil (1 μM).

4. Effect of Bay K8644 on the contractile response of the longitudinal muscle strip to 5-HT

Although Bay K8644 (0.1-1 μM) dose-dependently elevated the muscle tone which is inhibited by Cd²⁺ (100 μM), the contractile response of the longitudinal muscle strip to 5-HT (3 μM) at peak tension was not influenced in the presence of Bay K8644 (0.1 μM and 1 μM) compared with the control response (Fig. 5).
Fig. 5. Effect of Bay K 8644 on 5-HT-induced contraction of the longitudinal muscle strip in the guinea-pig isolated distal colon. 
In the presence of Bay K 8644 (0.1 and 1 μM), the contractile response of the guinea-pig distal colon to 5-HT (3 μM) at peak tension was almost equal to the control response. Time interval between traces was 30 min.

Discussion

In the present study, 5-HT dose-dependently produced phasic contractions of the longitudinal muscle strip, which were partially inhibited by atropine and markedly inhibited by tetrodotoxin. These results indicate that 5-HT acts mainly on the myenteric plexus and releases transmitters that cause contraction of the longitudinal muscle in the guinea-pig distal colon.

Fishlock and Parks (1966) have reported that contractions to 5-HT on the human ileum and colon were not antagonized by atropine. However, Chahl (1983) has reported that low doses of 5-HT (0.01–0.25 μM) produced contractions which were abolished by atropine in the guinea-pig ileum, and this atropine-sensitive responses were also inhibited following desensitization of preparation to substance P and the substance P antagonist. Buchheit et al. (1985) have also concluded that 5-HT at doses below 0.3 μM causes the release of substance P which subsequently releases acetylcholine, and 5-HT at doses higher than 0.3 μM releases substance P which then directly stimulates longitudinal muscle cells of the guinea-pig ileum. In the present study, 5-HT-induced contraction was inhibited by spantide, a substance P antagonist, in the presence of atropine, suggesting that 5-HT may release at least acetylcholine and substance P from the myenteric neurones of the longitudinal muscle strip in the guinea-pig distal colon as well as in the guinea-pig ileum.

It is generally accepted that the activation of voltage-sensitive Ca^{2+} channels increases cytosolic Ca^{2+} and causes the release of neurotransmitters at nerve terminals (Godfraind et al., 1986). However, the precise Ca^{2+} channel(s) that is involved in 5-HT-induced release of transmitters in the myenteric plexus is not known. There are some reports for an inhibition of transmitter release by divalent cations in the frog sartorius muscles (Weakly, 1973), in the rat brain (Drapeau and Nachshen, 1984), in the mouse spinal neurones in culture (Mayer and Westbrook, 1985) and in the mouse hippocampal neurones (Westbrook and Mayer, 1987). These reports showed that evoked transmitter release was inhibited by some divalent cations (e.g. Cd^{2+}, Co^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}). However, it is not accepted that all divalent cations act as a selective Ca^{2+} channel blocker (Godfraind et al., 1986). In sensory neurones of the chick...
dorsal root ganglion, Nowycky et al. (1985) have shown that the coexistence of three voltage-sensitive Ca\(^{2+}\) channel types (L-, N- and T-types). They also reported that Cd\(^{2+}\) blocks L- and N-type Ca\(^{2+}\) channels and Bay K8644, a dihydropyridine Ca\(^{2+}\) channel activator, strongly increases the opening probability of L-type Ca\(^{2+}\) channels. It is also reported that organic Ca antagonists, L-type Ca\(^{2+}\) channel blocker, did not inhibit substance P release evoked by action potentials in the chick dorsal root ganglion neurones (Rane, et al., 1987).

In the present study, Cd\(^{2+}\) ions, L- and N-type Ca\(^{2+}\) channel blocker, were most effective inhibitor of 5-HT-induced contractions of the longitudinal muscle strip, although it was unable to differentiate which type of Ca\(^{2+}\) channels (L- or N-type) is activated by 5-HT. However, Bay K8644, a L-type Ca\(^{2+}\) channel activator, did not influence the contractile response of the longitudinal muscle strip to 5-HT in the guinea-pig isolated colon. This evidence suggests that L-type Ca\(^{2+}\) channels may not be involved in the 5-HT-induced contraction of the longitudinal muscle strip.

Incidentally, Cd\(^{2+}\) ions may directly act on the longitudinal muscle cells. In this study, however, Cd\(^{2+}\) at a concentration of 20 \(\mu\)M did not affect carbachol (0.3 \(\mu\)M)-induced contraction which is almost equipotent to 5-HT (3 \(\mu\)M), although the contractile response to carbachol (0.3 \(\mu\)M) was strongly inhibited by verapamil (a L-type Ca\(^{2+}\) channel blocker). This finding indicates that Cd\(^{2+}\) at concentrations below 20 \(\mu\)M scarcely affects L-type of Ca\(^{2+}\) channels on the longitudinal muscle cells in the guinea-pig distal colon.

Frelin et al. (1986) have reported that Cd\(^{2+}\) and Zn\(^{2+}\) possess a blocking action of Na\(^{+}\) channels in different cell types of neuronal, cardiac or skeletal muscle origin. However, they indicated that high concentrations of Cd\(^{2+}\) (IC\(_{50}\) = 5 mM) and of Zn\(^{2+}\) (IC\(_{50}\) = 2 mM) are necessary to inhibit Na\(^{+}\) channels. Therefore, Cd\(^{2+}\) ions at low concentrations of Cd\(^{2+}\) (10-20 \(\mu\)M) in this experiments seem unlikely to inhibit Na\(^{+}\) channels of the interneurones in the myenteric plexus.

From the present experiments, although the results obtained here were not definitive evidences, it is suggested that 5-HT may act on the N-type Ca\(^{2+}\) channels, not on the T- and L-types, of interneurones in the myenteric plexus and cause the release of at least acetylcholine and substance P that induce contractions of the logitudinal muscle in the guinea-pig distal colon.

References


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