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Short Communication

Differential Distribution of 5-Hydroxytryptamine$_3$ Receptor in the Colon between Human and Guinea Pig

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Abstract

Localization of 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor in the human colon was examined by in vitro receptor autoradiography using [^{125}I](S)iodozacopride, and compared with that in the guinea pig colon. [^{125}I](S)iodozacopride binding sites were found with high densities around the myenteric plexus, but with low ones in the muscle layer and mucosa of the human colon, and the binding was abolished by granisetron, a specific 5-HT$_3$ receptor antagonist. While in the guinea pig colon, specific [^{125}I](S) iodozacopride binding was not detected in either the myenteric plexus or the muscle layers. Thus, the 5-HT$_3$ receptors are present in the human colon, especially densely located in the myenteric plexus, but not in the guinea pig colon, and those may participate in the colonic motility. The results of functional studies of 5-HT$_3$ receptor obtained from experiments using guinea pig are not always applying to the human.

Key Words: [^{125}I](S)iodozacopride binding, human colon, guinea pig colon, *in vitro* receptor autoradiography

Introduction

Effects of 5-hydroxytryptamine (5-HT) on the gastrointestinal contractility are complex and these events may depend on differential functions and localizations of 5-HT receptor subtypes as targets of 5-HT. The multiple 5-HT receptor subtypes cloned to date are the largest of all known neurotransmitter receptor families. The 5-HT$_1$, 5-HT$_2$ and 5-HT$_4$ - 5-HT$_7$ receptor families are members of the superfamily of G-protein coupled receptors and the 5-HT$_3$ receptor, on the other hand, is a ligand-gated ion channel. According to the current classification, four main subtypes of functional 5-HT receptors, termed 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ can be distinguished (3), and these 5-HT receptors are recognized in the gastrointestinal tract. It is generally accepted that the 5-HT$_1$ receptor is relaxant and the 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors are contractile in the gastrointestinal tract. The 5-HT$_3$ receptor located on the nerve terminals of parasympathetic afferent neurons in the stomach is known to participate in the emetic response. Furthermore, activation of 5-HT$_3$ receptor causes contractions of intestinal tissues (1, 11). Electrophysiological studies have shown the 5-HT$_3$ receptor-mediated fast-activating depolarizing response of the myenteric neurons in the guinea pig stomach (7, 9). Stimulation of the 5-HT$_3$ receptor facilitates the release of acetylcholine (ACh) from enteric neurons of the guinea pig ileum (11) and the release of ACh and tachykinin-like neurotransmitter from enteric neurons of guinea pig colon (1). Thus, the 5-HT$_3$ receptor appears to be located on the neurons.

There are some literatures on the functional studies of 5-HT$_3$ receptor using laboratory animals, while the localization of 5-HT receptors in the

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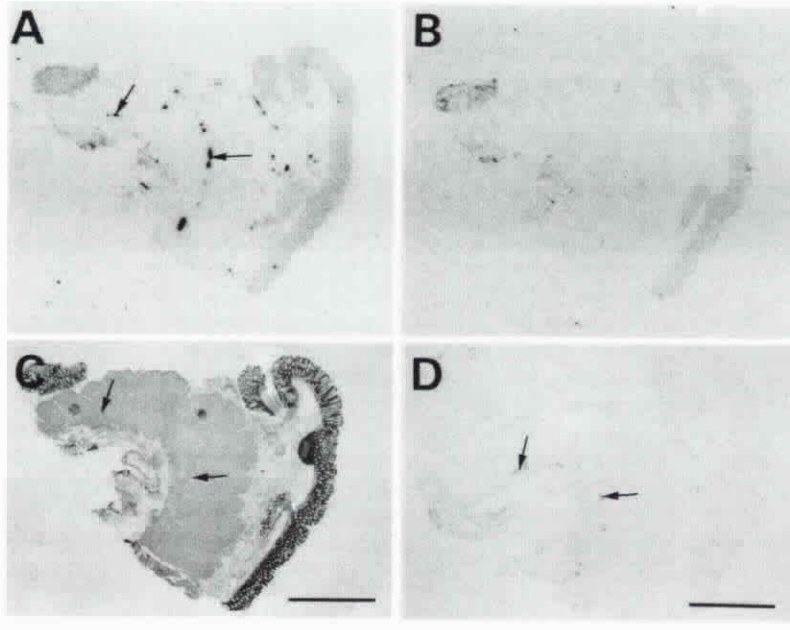

Fig. 1. Receptor autoradiographic evidence of [^{125}I](S)iodozacopride binding sites and microscopic evidence in human colon.
Tissue sections from the human colon were labelled with 100 pM of [^{125}I](S)iodozacopride (A and B) in the absence (total binding, A) or presence of 10 μM granisetron (B). Consecutive sections were stained by hematoxylin-eosin (C) or modified Karnovsky's choline esterase-staining method (D). Scale bars in C and D=2.5 mm.

gastrointestinal tract has been obscure, especially in the human tissues. Mechanism underlying the effects of prokinetics which act at the 5-HT receptors, such as benzamide derivatives, on motility of the gastrointestinal tract has been evaluated in laboratory animals as substitution of the human. In order to determine whether or not the function of 5-HT_3 receptor evaluated in laboratory animals can be applied to human, the present study was attempted to examine the localization of the receptor in the colon of human and guinea pig, using *in vitro* receptor autoradiography.

Materials and Methods

Human specimens of descending colon were obtained from patients undergoing surgical resection for colonic cancer (3 patients). Normal adjacent tissues to the pathological ones were cut and used. Use of specimens for this study was approved by The Ethical Committee of Nagasaki University School of Medicine, and written informed consent from the patients was obtained. Adult guinea-pigs of either sex, weighing between 250 and 400 g, were killed by cervical dislocation, and the descending colon were immediately excised.

The excised specimens were immediately immersed in isopentane at -30 °C. The frozen tissues were cut into 20-μm-thick sections on a cryostat, thaw-mounted onto gelatin-coated glass slides, then stored overnight under vacuum at 4 °C. For binding experiments of [^{125}I](S)iodozacopride ([^{125}I]zacopride) (2), tissue sections were incubated with 100 pM of [^{125}I]zacopride in the 25 mM Tris-HCl buffer (pH 7.4) which included 157 mM NaCl and 0.56 mM ascorbic acid for 60 min at 23 °C following preincubation in the same buffer for 30 min (14). Non specific binding was determined by incubating consecutive sections in the presence of 10 μM granisetron, a specific 5-HT_3 receptor antagonist (12). The labeled sections were then washed three times (for 1 min each) at 4 °C in 50 mM Tris-HCl buffer (pH 7.2), tapped in ice-cold distilled water and dried under a stream of cold air. The labeled sections were washed and dried using the same method as for the case of [^{125}I]zacopride binding. To obtain autoradiograms of a higher resolution, the dry-labeled sections were apposed against Hyperfilm-[^{3}H] (Amersham, U.K.) and the films were developed with a D19 developer (Eastman Kodak, USA) for 7 min at 4 °C. For histochemical staining of choline esterase, we used modified Karnovsky's method (4). [^{125}I](S)iodozacopride (74 TBq/mmol) was purchased from Amersham, U.K. Granisetron were generously provided by Smith Kline Beecham, U.K.

Results

Figures 1 and 2 show typical receptor autoradiograms of [^{125}I]zacopride binding in the human (Fig. 1A and B) and guinea pig colon removed mucosa

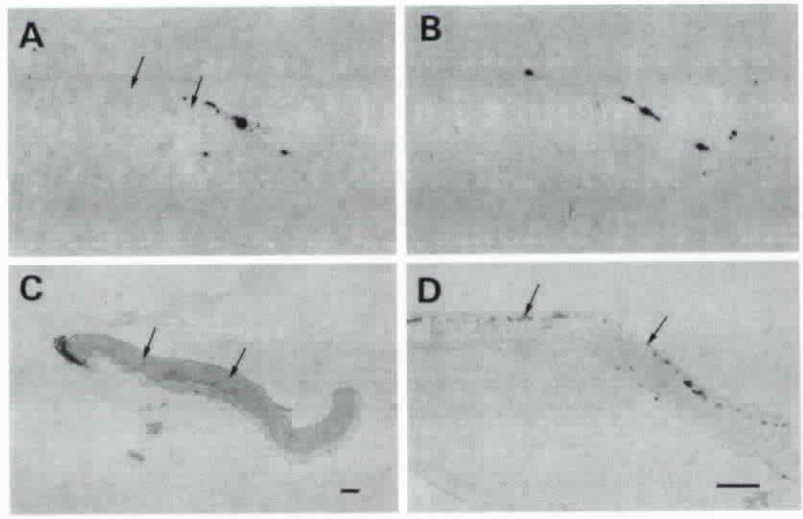

Fig. 2. Receptor autoradiographic evidence of [^{125}I](S)iodozacopride binding sites in guinea pig colon.
Tissue sections from the guinea pig colon removed mucosa were labelled with 100 pM of [^{125}I](S)iodozacopride (A and B) in the absence (total binding, A) or presence of 10 μM granisetron (B). Consecutive sections were stained by hematoxylin-eosin (C) or modified Karnovsky's choline esterase-staining method (D). Scale bars in C and D=2.5 mm.

(Fig. 2A and B). In the human colon, [^{125}I]zacopride binding sites were found with high densities around the myenteric plexus, but with low ones in the muscle layer and mucosa (Fig. 1A), and the binding was abolished by granisetron, a specific 5-HT_3 receptor antagonist (Fig. 1B). The precise localization of [^{125}I] zacopride binding sites was examined by hematoxylin-eosin staining and choline esterase staining to visualize myenteric neurons containing acetylcholine esterase, in the consecutive tissue sections (Fig. 1C and D). These staining show the high densities of [^{125}I] zacopride binding in the human colon corresponding to the myenteric plexus (indicated by arrows in Fig. 1A, C and D). In the guinea pig colon removed mucosa, specific [^{125}I]zacopride binding was not detected in neither the myenteric plexus indicated by arrows in Fig. 2A, C and D, nor muscle layers. When the concentration of [^{125}I]zacopride was increased up to 1 nM, the binding was almost same either in the absence or presence of granisetron and the specific binding was less than 10 % in the guinea pig.

Discussion

The present study demonstrated that 5-HT_3 receptors were densely located in the myenteric plexus, but so slightly in the muscle layer of human colon, while was not detected in either the myenteric plexus or the muscle layer of guinea pig colon.

[^{3}H]Zacopride has been reported to associate with the 5-HT_3 receptor in membrane preparations from rabbit ileum muscularis (6), and iodinated zacopride, [^{125}I](S)iodozacopride ([^{125}I]zacopride) is also a high affinity radioligand for the 5-HT_3 receptor (2), while zacopride has affinity for not only the 5-HT_3 receptor but also the 5-HT_4 receptor (8). The specific binding of [^{125}I]zacopride to the 5-HT_3 receptor was evaluated by granisetron, a potent and specific 5-HT_3 receptor antagonist. As the dense [^{125}I]zacopride binding in the myenteric plexus of human colon was abolished by granisetron, the 5-HT_3 receptor is present in the myenteric plexus of human colon. When we used [^{125}I]zacopride as a radioligand to the 5-HT_3 receptor, the specific [^{125}I]zacopride binding sites were not detected in the guinea pig colon until the ligand concentration up to 1 nM. It cannot be elucidated whether the 5-HT_3 receptor is not present in the guinea pig colon or the affinity of zacopride to the 5-HT_3 receptors in the guinea pig colon is very low. The cloned 5-HT_3 receptor from the guinea pig intestine has been shown to be different from that from mouse and human in sensitivity to several agonists and antagonists for 5-HT_3 receptor (5).

The 5-HT_3 receptor has been reported to participate in stimulation of the motility of guinea pig gastrointestinal tract. Stimulation of 5-HT_3 receptors has been shown to induce depolarization of the myenteric neurons in the stomach (7, 9), and facilitation of ACh release from enteric neurons of the ileum (11), and the release of both ACh and tachykinin-like neurotransmitter from enteric neurons of guinea pig colon (1). Similarly, the 5-HT_3 receptors present in the myenteric plexus of human colon may be located on the enteric excitatory neurons, such as cholinergic and/or non-cholinergic neurons, and stimulation of the receptor may enhance the colonic motility. The 5-HT_3 receptor blockade has been

demonstrated to slow colonic transit (10) and cause constipation as the main adverse effect in healthy man [12], and some patients with diarrhoea-predominant irritable bowel syndrome have accelerated small bowel or colon transit (13), therefore the 5-HT_3 receptor antagonists may be available for the treatment of diarrhoea-predominant diseases.

In the human colon, 5-HT_3 receptors were located on the myenteric plexus, being different from the guinea pig colon, in which the receptor was not detected. These results indicate that the results of functional studies of receptors obtained from experiments using guinea pig are not always applying to the human.

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