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Short communication

Localization of the 5-HT_4 receptor in the human and the guinea pig colon

Yasuko Sakurai-Yamashita [a,*], Kimihiro Yamashita [b], Takashi Kanematsu [c], Kohtaro Taniyama [a]

[a] *Department of Pharmacology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*

[b] *Section of Pharmacology, Department of Environmental Conservation, Faculty of Environmental Studies, Nagasaki University, Nagasaki 852-8521, Japan*

[c] *Department of Surgery II, Nagasaki University School of Medicine, Nagasaki 852-8523, Japan*

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Abstract

The functions of the 5-HT_4 receptor in the gastrointestinal tract are complex, depending on the species and anatomical regions, and localization of the receptor was not clear. The present study attempted to examine the localization of the 5-HT_4 receptor in the colon of human for comparison with that in guinea pig colon. Human specimens of sigmoid colon and the distal colon of guinea pig were used for in vitro receptor autoradiography using [^{125}I]SB207710, (1-*n*-butyl-4-piperidinyl) methyl-8-amino-7-iodo-1,4-benzodioxane-5-carboxylate, as a ligand. [^{125}I]SB207710 binding sites were distributed over the muscle layer of human colon, in the myenteric plexus and in the muscle. In the guinea pig colon, a much higher density was detected in the myenteric plexus than in the muscle. Therefore, in the human and guinea pig colon, the 5-HT_4 receptor was located both in the myenteric plexus and in the muscle, and in the guinea pig colon, the receptor was located more predominantly in the myenteric plexus of the muscle than it is in the human colon.

Keywords: 5-HT_4 receptor; Colon, human; Colon, guinea pig; Receptor autoradiography, in vitro

1. Introduction

The 5-HT_4 receptor participates in the modulation of gastrointestinal motility, in either an excitatory or an inhibitory manner, depending on the species and anatomical region (Ford and Clarke, 1993). Stimulation of the 5-HT_4 receptor has been noted to evoke the release of acetylcholine from guinea pig enteric nerve terminals (Kilbinger and Wolf, 1992; Matsuyama et al., 1996; Takada et al., 1999). In the stomach, the 5-HT_4 receptor mediates the contractile functions in isolated preparations from both human (Schuurkes et al., 1991) and guinea pig (Buchheit and Buhl, 1994; Matsuyama et al., 1996). Our previous results demonstrated the dense localization of the 5-HT_4 receptor in the myenteric plexus of the corpus and antrum of guinea pig stomach and its stimulation potentiated the release of acetylcholine (Takada et al., 1999). In the colon, the 5-HT_4 receptor-mediated response appears to be different in the human and in the guinea pig, so that there is a relaxing response in the circular muscle of human colon (Tam et al., 1994; McLean and Coupar, 1996) and a contractile response in the guinea pig colon (Elswood et al., 1991; Gale et al., 1994; Kojima and Shimo, 1995; Briejer and Schuurkes, 1996). The 5-HT_4 receptors which mediate relaxing effects appear to be mainly located on the smooth muscle cells (McLean and Coupar, 1996), while those which mediate contractile effects appear to be mainly located on the excitatory neurons, cholinergic neurons and non-cholinergic neurons (Gale et al., 1994; Kojima and Shimo, 1995; Briejer and Schuurkes, 1996). Thus, there

* Corresponding author. Tel.: +81-958-49-7047; fax: +81-958-49-7048.

E-mail address: yasukosy@net.nagasaki-u.ac.jp (Y. Sakurai-Yamashita)

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are many reports of functional studies using laboratory animals, while the localization of the 5-HT_4 receptor in the gastrointestinal tract, especially in the human tissues, has remained obscure. The present study attempted to examine the localization of the 5-HT_4 receptor in the colon of human and guinea pig, using in vitro receptor autoradiography.

2. Materials and methods

Specimens of human sigmoid colon were obtained from three patients (53–70 years old) who underwent surgical resection for cancer. The normal tissues adjacent to the pathological ones were cut away and used. Use of specimens for this study was approved by *The Ethical Committee of Nagasaki University School of Medicine*, and written informed consent was obtained from the patients. Adult guinea pigs of either sex, weighing between 250 and 400 g, were killed by cervical dislocation, and the distal colon was rapidly excised and the mucosa was removed.

2.1. Receptor autoradiography

The tissues were immediately immersed in isopentane at −30°C. The frozen tissues were cut into 20-μm thick

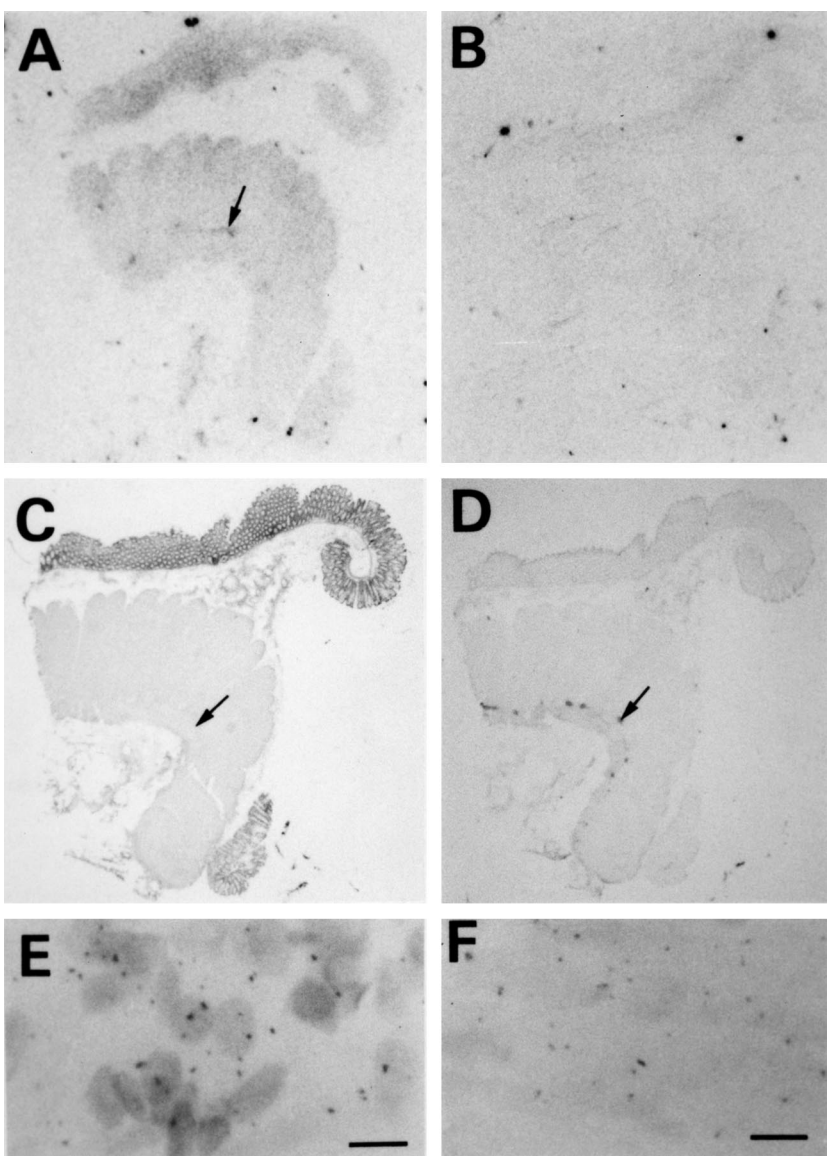

Fig. 1. Receptor autoradiographic evidence of [^{125}I]SB207710 binding sites in human colon. Tissue sections from human sigmoid colon were labelled with 25 pM of [^{125}I]SB207710 (A and B) in the absence (total binding, A) or presence of SB204070 (non-specific binding, B). Consecutive sections were stained with hematoxylin–eosin (C) or modified Karnovsky's cholinesterase staining method (D). Emulsion autoradiograms of [^{125}I]SB207710 binding in the myenteric plexus (E) and in the muscle (F). Scale bar = 10 μm.

sections on a cryostat, thaw-mounted onto gelatin-coated glass slides, then stored overnight under vacuum at 4°C. Tissue sections were incubated with 25 pM of [^{125}I]SB207710 under the same conditions as described previously (Sakurai-Yamashita et al., 1999; Takada et al., 1999). Non-specific binding was determined in the presence of 1 μM SB204070, a specific 5-HT$_4$ receptor antagonist (Wardle et al., 1994). Quantitation of the radioligand binding sites was made using the computerized radioluminographic imaging-plate system (Bio-imaging analyzer BAS 5000, Fuji Photo Film) with calibrated [^{125}I]standards ([^{125}I]microscales, Amersham, UK). The results were expressed as the means ± S.E.M. in attomoles per milligram. To obtain autoradiograms with a higher resolution, the dry-labeled sections were apposed against Hyperfilm-[^{3}H] (Amersham, UK) for 1 week. To confirm the presence of the binding sites in the myenteric plexus, the labeled sections were coated with NTB-3 liquid emulsion (Eastman Kodak, USA), developed with a D19 developer after exposure and counterstained as described previously (Yoshimura et al., 1996). For histochemical staining of cholinesterase, we used a modification of Karnovsky's method (Karnovsky and Roots, 1964).

2.2. Chemicals

[^{125}I]SB207710, (1-*n*-butyl-4-piperidinyl) methyl-8-amino-7-iodo-1,4-benzodioxane-5-carboxylate (74 TBq/mmol), was purchased from Amersham, UK. SB204070, (1-*n*-butyl-4-piperidinyl) methyl-8-amino-7-chloro-1,4-benzodioxane-5-carboxylate, was generously provided by Smith Kline Beecham, UK.

3. Results

Fig. 1A shows a typical receptor autoradiogram of [^{125}I]SB207710 binding in the human colon. Moderate levels of [^{125}I]SB207710 binding sites were observed over the muscle layers, and the binding was abolished by the addition of SB204070, a specific 5-HT$_4$ receptor antagonist (B). Histochemical staining with hematoxylin–eosin (C) and for cholinesterase (D) showed that there were also moderate levels (indicated by an arrow in A, C and D) of binding sites in the myenteric plexus. The concentration of specific [^{125}I]SB207710 binding sites at 25 pM in the muscle of the human colon was 42.8 ± 4.6 amol/mg (in triplicates of three patients). The concentration in the myenteric plexus was not calculated because the sites were not distinguishable from those in the muscle with our analyzing system; however, emulsion autoradiography further demonstrated that there were clearly [^{125}I]SB207710 binding grains in the myenteric plexus (E) and the muscle (F).

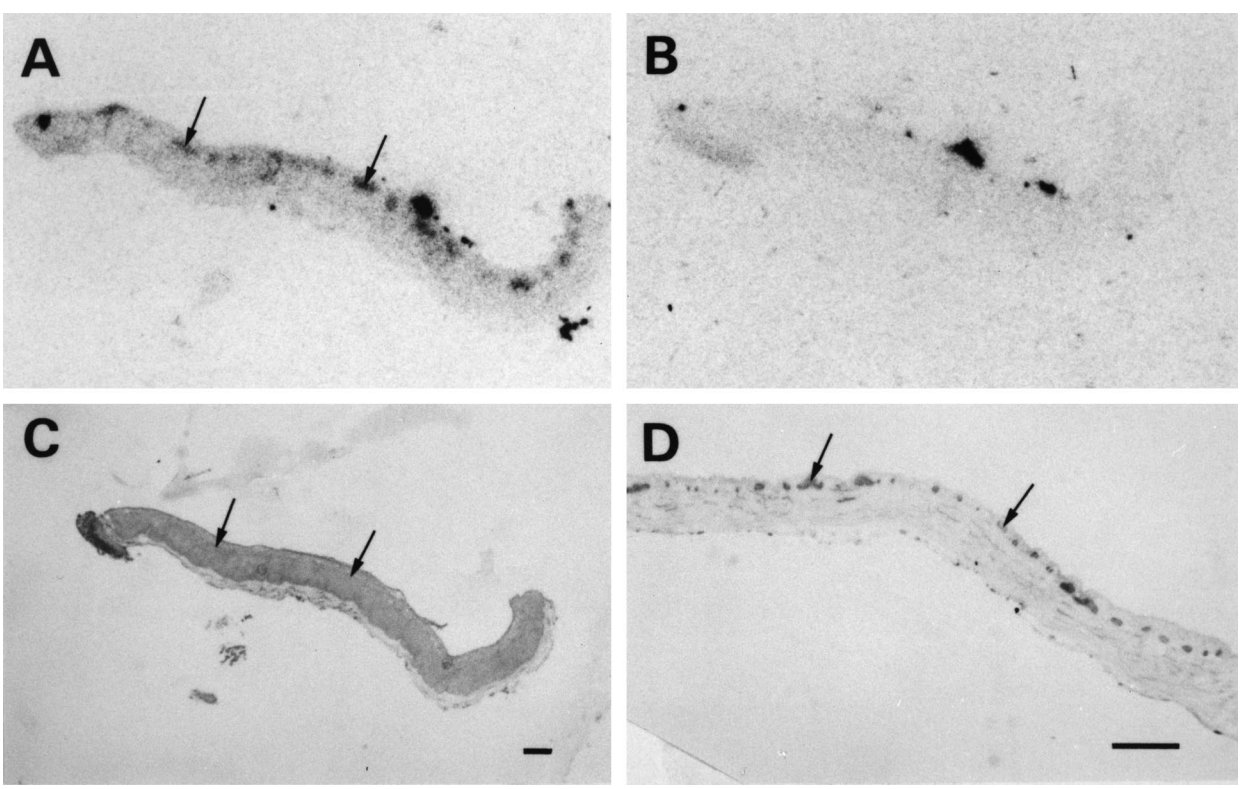

Fig. 2. Receptor autoradiographic evidence of [^{125}I]SB207710 binding sites in guinea pig colon. Tissue sections from guinea pig colon with the mucosa removed were labelled with 25 pM of [^{125}I]SB207710 (A and B) in the absence (total binding, A) or presence of SB204070 (non-specific binding, B). Consecutive sections were stained with hematoxylin–eosin (C) or a modified Karnovsky's cholinesterase staining method (D). Scale bar = 50 μm.

In the guinea pig colon, sites with both dense and moderate binding of [^{125}I]SB207710 were detected in the muscle layers (Fig. 2A), and SB204070, a specific 5-HT$_4$ receptor antagonist, abolished the binding (Fig. 2B). The precise localization of the dense [^{125}I]SB207710 binding sites was examined by hematoxylin–eosin and cholinesterase staining to visualize the myenteric neurons containing acetylcholine esterase, in consecutive tissue sections (Fig. 2C and D). This staining showed that the dense [^{125}I]SB207710 binding sites indicated by an arrow in Fig. 2A corresponded to the myenteric plexus (indicated by arrows in Fig. 2C and D). The concentration of specific [^{125}I]SB207710 binding sites at 25 pM in the guinea pig colon was 204.8 ± 38.4 amol/mg in the myenteric plexus, and 110.6 ± 20.2 amol/mg ($n = 4$) in the muscle.

4. Discussion

The present study demonstrated that the 5-HT$_4$ receptor was present in the myenteric plexus and in the muscle of both human and guinea pig colon. The difference in receptor densities between the myenteric plexus and the muscle was clearly greater in the guinea pig colon than in the human colon. In the guinea pig colon, the densities in the myenteric plexus were about twice those in the muscle.

Stimulation of the 5-HT$_4$ receptor modulates the motility of the gastrointestinal tract, in either an excitatory or an inhibitory manner, depending on the species and anatomical region (Ford and Clarke, 1993). The 5-HT$_4$ receptor detected in the muscle of the colon may be located on the smooth muscle cells. The concept is consistent with the findings of functional studies in which 5-HT caused relaxation of circular muscle preparations from human colon via the 5-HT$_4$ receptor (Tam et al., 1994; McLean and Coupar, 1996). On the other hand, the 5-HT$_4$ receptor was also detected in the myenteric plexus of the human colon, although the density was not compared with that in the guinea pig colon. The 5-HT$_4$ receptor detected in the myenteric plexus may be located on the excitatory neurons, and participate in the stimulation of motility, as in the case of the guinea pig stomach and ileum (Taniyama et al., 1991; Kilbinger and Wolf, 1992). The 5-HT$_4$ receptor-mediated contraction of guinea pig colon appears to be mediated by stimulation of cholinergic neurons and tachykinin-containing neurons (Gale et al., 1994; Kojima and Shimo, 1995; Briejer and Schuurkes, 1996).

In the human colon, the 5-HT$_4$ receptor was located both in the myenteric plexus and in the muscle. In the guinea pig colon, the receptor was much more predominantly located in the myenteric plexus than in the muscle. These results indicate that the predominance of the location of the 5-HT$_4$ receptor in the myenteric plexus to the muscle might contribute to the appearance of the contractile function of the receptor in the guinea pig colon.

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