*Rapid Communication***Characterization of GABA_B Receptor in the Human Colon**Yasuhito Uezono¹, Muneshige Kaibara¹, Hideki Hayashi¹, Shunsuke Kawakami², Akihito Enjoji², Takashi Kanematsu², and Kohtaro Taniyama^{1,*}*Divisions of ¹Pharmacology and ²Surgery, Department of Translational Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8523, Japan**Received December 19, 2003; Accepted January 14, 2004*

Abstract. Characterization of the GABA_B receptor in the human colon was performed by the reverse transcription-polymerase chain reaction (RT-PCR). mRNAs for both subunits of the GABA_B receptor, GABA_{B1} and GABA_{B2}, were detected in the human colon. The GABA_{B1(e)} isoform was detected in the human colon, but not in the brain, and the other isoforms, except GABA_{B1(d)}, were detected in both tissues. Thus, the GABA_B receptor may be present as a heterodimer with subunits of GABA_{B1} and GABA_{B2} in the human colon.

Keywords: GABA_{B1} subunit, GABA_{B2} subunit, RT-PCR

Several reviews have described the presence of GABAergic neurons and two types of GABA receptors, GABA_A and GABA_B receptors, in the mammalian intestine (1). In vivo studies indicate that GABA produces more predominantly the GABA_B receptor-mediated response than the GABA_A receptor-mediated response in intestinal motility (2). GABA_B receptors, the first G protein-coupled receptor discovered to form heterodimers, consist of 2 different proteins, GABA_{B1} and GABA_{B2} (3). The GABA_{B1} subunit is necessary for agonist binding and the GABA_{B2} subunit activates the G protein-coupled signaling system (3). There are 7 known isoforms of splice variants of GABA_{B1}, from GABA_{B1(a)} to GABA_{B1(g)} (3, 4), and therefore the localization and function of the GABA_B receptor may vary with the different isoforms of GABA_{B1}. Thus, we attempted to elucidate the characteristics of the GABA_B receptor present in the human colon using the reverse transcription-polymerase chain reaction (RT-PCR) and compared them with those of the receptor in the dog small intestine and human brain.

Total RNA was extracted from the human colon and dog small intestine using a Qiagen Midi Kit (Qiagen GmbH, Hilden, Germany), and the Poly(A)RNA of human brain was purchased from Ambion (Austin, TX, USA). RT-PCR was done using a thermal cycler (Perkin-Elmer, Norwalk, CT, USA) and an RT-PCR

kit (Toyobo, Osaka). Specimens of colon were obtained from patients undergoing surgical resection for colonic cancer. Use of the specimens for the present study was approved by The Ethical Committee of Nagasaki University School of Medicine, based on the informed consent of patients. Seven independent forward and reverse primers specific for GABA_{B1} subunits and a pair of primers for GABA_{B2} subunit were designed on the basis of cloned human GABA_{B1} and GABA_{B2} subunits based on the data in GenBank and appeared in Ref. 5. Reverse transcription was done in a final volume of 20 μ l using random primers and a reverse transcriptase supplied with the RT-PCR kit. PCR was done in a final volume of 50 μ l containing 1 μ M primers, 1 mM each deoxynucleoside triphosphate (dNTP), 2.5 U of recombinant KOD dash DNA polymerase, and the RT-PCR buffer supplied with the kit. PCR was done under the following conditions: 30 cycles of 94°C 30 s, 60°C 30 s, and 72°C 90 s. At the end of PCR, samples were kept at 72°C for 10 min for final extension and then stored at 4°C. The amplification products were separated by electrophoresis (2.5% agarose gel) and visualized by SYBR Green nucleic acid gel stain (Molecular Probe, Eugene, OR, USA) with FMBIO-II luminescent system (Fuji, Tokyo). The bands for GABA_{B1} and GABA_{B2} receptors were sequenced and found to be 100% identical to the reported human GABA_B receptors (1a, 1b, 1c, 1e, 2) and rat GABA_B receptors (1f and 1g) in levels of nucleotide sequences.

RT-PCR revealed the expression of both GABA_{B1} and

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Fig. 1. RT-PCR analysis of GABA_B receptor mRNAs in the dog small intestine, human colon, and human brain and comparison with individual human cloned subunits. A: RT-PCR analysis of the GABA_{B1(a/e)}, GABA_{B1(b)}, and the GABA_{B2} receptor. B: RT-PCR analysis of isoforms of GABA_{B1} subunits. Amplification products were electrophoresed on a 2.5% agarose gel and visualized by SYBR Green staining.

GABA_{B2} in the human colon (Fig. 1A). mRNAs for 2 isoforms of GABA_{B1}, GABA_{B1(a)} and GABA_{B1(b)}, and GABA_{B2} were detected in the human colon, as well as dog small intestine and human brain. The GABA_B receptors have been shown to be heterodimers of GABA_{B1} and GABA_{B2} subunits, with both subunits critical for receptor function in the expression systems, such as *Xenopus* oocytes and HEK-293 cells (3). The effect of GABA_B agonist has not been detected in the ileal preparations from GABA_{B1}-receptor subunit knock-out mice (6). Thus, the functional GABA_B receptors in the human colon possibly form heterodimers consisting GABA_{B1} and GABA_{B2}, and the coupling of GABA_{B2}

with GABA_{B1} may express a fully functional GABA_B receptor in the human colon. It has been reported that the GABA_{B2} subunit is not detected in the small intestine of rats and humans (7), being different from the present findings in the human colon. In the present study, we used the primers for the GABA_{B2} subunit designed on the basis of the cloned human GABA_{B2} subunit. There may be very little GABA_{B2} mRNA, relative to GABA_{B1} mRNA, as noted in the hypothalamus and caudate-putamen (8). mRNAs for 4 isoforms other than GABA_{B1(a)} and GABA_{B1(b)} of GABA_{B1}, such as GABA_{B1(c)}, GABA_{B1(e)}, GABA_{B1(f)}, and GABA_{B1(g)}, were detected in the intestine of human and dog, but GABA_{B1(d)} mRNA was not detected, while the mRNAs for GABA_{B1(e)} and GABA_{B1(d)} were not detected in the human brain (Fig. 1B). The GABA_{B1(e)} isoform has been shown to be the primary isoform detected in the peripheral tissues and a minor component in the central nervous system (9).

There are 7 isoforms of splice variants of GABA_{B1} (3, 4); therefore, the localization and function of GABA_B receptor may vary with the different isoforms of GABA_{B1}. The affinity of the GABA_B receptor for agonist and the distribution of GABA_B receptor may be dependent on the GABA_{B1} isoform. There are some reports on the different distributions of GABA_{B1(a)} and GABA_{B1(b)} isoforms in the brain. In the cerebellum and spinal cord, the GABA_{B1(a)} isoform is associated with presynaptic receptors, and the GABA_{B1(b)} isoform is located predominantly at postsynaptic sites (10). On the other hand, in other areas of the brain, GABA_{B1(a)} and GABA_{B1(b)} isoforms are located at the postsynaptic site and presynaptic terminals, respectively (11).

The human colon was found to possess the same mRNAs for GABA_{B1} isoforms and GABA_{B2} as the dog small intestine. Our study demonstrated that the GABA_B receptor, relative to the GABA_A receptor, operated predominantly in the physiological motility of dog small intestine (5). The GABA_B receptor was proposed to be localized on the enteric cholinergic neurons of dog small intestine, based on the findings that stimulation of the GABA_B receptor inhibited the intestinal motility and decreased the release of acetylcholine in the dog small intestine (5). Thus, there is a possibility that the GABA_B receptor is located at enteric cholinergic neurons as a heterodimer with the subunits of GABA_{B1} and GABA_{B2} in the human colon, as noted in the dog intestine. What isoform is mainly involved in cholinergic nerve activity and whether the 6 isoforms are predominantly localized at the different cells remain the subject of ongoing studies.

Acknowledgment

This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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