Characterization of GABA_B Receptor in the Human Colon

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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(ε) 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 μl using random primers and a reverse transcriptase supplied with the RT-PCR kit. PCR was done in a final volume of 50 μl containing 1 μM 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 in levels of nucleotide sequences.

RT-PCR revealed the expression of both GABA_B1 and
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GABA$B_2$ in the human colon (Fig. 1A). mRNAs for 2 isoforms of GABA$B_{1\alpha}$, GABA$B_{1\alpha(e)}$ and GABA$B_{1\beta}$, and GABA$B_{2(b)}$ were detected in the human colon, as well as dog small intestine and human brain. The GABA$B_2$ receptors have been shown to be heterodimers of GABA$B_{1\alpha}$ and GABA$B_{2(b)}$ 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$B_2$ agonist has not been detected in the ileal preparations from GABA$B_{1\beta(e)}$-receptor subunit knock-out mice (6). Thus, the functional GABA$B_2$ receptors in the human colon possibly form heterodimers consisting GABA$B_{1\alpha}$ and GABA$B_{2(b)}$, and the coupling of GABA$B_{2(b)}$ with GABA$B_{1\alpha}$ may express a fully functional GABA$B_2$ receptor in the human colon. It has been reported that the GABA$B_{2(b)}$ 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$B_{2(b)}$ subunit designed on the basis of the cloned human GABA$B_{2(b)}$ subunit. There may be very little GABA$B_{2(b)}$ mRNA, relative to GABA$B_{1\alpha}$ mRNA, as noted in the hypothalamus and caudate-putamen (8). mRNAs for 4 isoforms other than GABA$B_{1\beta(a)}$ and GABA$B_{1\beta(b)}$ of GABA$B_{1\alpha}$, such as GABA$B_{1\beta(c)}$, GABA$B_{1\beta(d)}$, GABA$B_{1\beta(e)}$, and GABA$B_{1\beta(f)}$, were detected in the intestine of human and dog, but GABA$B_{1\beta(d)}$ mRNA was not detected, while the mRNAs for GABA$B_{1\beta(e)}$ and GABA$B_{1\beta(f)}$ were not detected in the human brain (Fig. 1B). The GABA$B_{1\beta(e)}$ isoform has been shown to be the primary isofrom detected in the peripheral tissues and a minor component in the central nervous system (9).

There are 7 isoforms of splice variants of GABA$B_{1\alpha}$ (3, 4); therefore, the localization and function of GABA$B_2$ receptor may vary with the different isoforms of GABA$B_{1\alpha}$. The affinity of the GABA$B_2$ receptor for agonist and the distribution of GABA$B_2$ receptor may be dependent on the GABA$B_{1\alpha}$ isoform. There are some reports on the different distributions of GABA$B_{1\beta(a)}$ and GABA$B_{1\beta(b)}$ isoforms in the brain. In the cerebellum and spinal cord, the GABA$B_{1\beta(a)}$ isoform is associated with presynaptic receptors, and the GABA$B_{1\beta(b)}$ isoform is located predominantly at postsynaptic sites (10). On the other hand, in other areas of the brain, GABA$B_{1\beta(a)}$ and GABA$B_{1\beta(b)}$ isoforms are located at the postsynaptic site and presynaptic terminals, respectively (11).

The human colon was found to possess the same mRNAs for GABA$B_{1\alpha}$ isoforms and GABA$B_{2(b)}$ as the dog small intestine. Our study demonstrated that the GABA$B_2$ receptor, relative to the GABA$A_{\beta_2}$ receptor, operated predominantly in the physiological motility of dog small intestine (5). The GABA$B_2$ receptor was proposed to be localized on the enteric cholinergic neurons of dog small intestine, based on the findings that stimulation of the GABA$B_2$ 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$B_2$ receptor is located at enteric cholinergic neurons as a heterodimer with the subunits of GABA$B_{1\alpha}$ and GABA$B_{2(b)}$ in the human colon, as noted in the dog intestine. What isofrom 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.

![Image](image.png)
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References