Identification of a G Protein-coupled Receptor Specifically Responsive to β-Alanine*

Received for publication, December 29, 2003, and in revised form, March 22, 2004
Published, JBC Papers in Press, March 22, 2004, DOI 10.1074/jbc.M314240200

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We isolated a cDNA encoding an orphan G protein-coupled receptor, TGR7, which has been recently reported to correspond to MrgD. To search for ligands for TGR7, we screened a series of small molecule compounds by detecting the Ca\(^{2+}\) influx in Chinese hamster ovary cells expressing TGR7. Through this screening, we found that β-alanine at micromolar doses specifically evoked Ca\(^{2+}\) influx in cells expressing human, rat, or mouse TGR7. A structural analogue, γ-aminobutyric acid, weakly stimulated cells expressing human or rat TGR7, but another analogue, glycine, did not. In addition, β-alanine decreased forskolin-stimulated cAMP production in cells expressing TGR7, suggesting that TGR7 couples with G proteins Gq and Gi. In guanosine 5'-O-3-thiotriphosphate binding assays conducted using a membrane fraction of cells expressing TGR7, β-alanine specifically increased the binding of guanosine 5'-O-3-thiotriphosphate. When a fusion protein composed of TGR7 and green fluorescent protein was expressed in cells, it localized at the plasma membrane but internalized into the cytoplasm after treatment with β-alanine. In addition, we found that β-[\(^{3}H\)Jalanine more efficiently bound to TGR7-expressing cells than to control cells. From these results, we concluded that TGR7 functioned as a specific membrane receptor for β-alanine. Quantitative PCR analysis revealed that TGR7 mRNA was predominantly expressed in the dorsal root ganglia in rats. By in situ hybridization and immunostaining, we confirmed that TGR7 mRNA was co-expressed in the small diameter neurons with P2X3 and VR1, both in rat and monkey dorsal root ganglia. Our results suggest that TGR7 participates in the modulation of neuropathic pain.

G protein-coupled receptors (GPCR)* constitute a family with hundreds of members and have a variety of physiological functions. We have established previously (1, 2) a strategy widely applicable to identify orphan GPCR ligands. Our strategy involves searching for ligands by monitoring signal transductions, such as changes in cAMP or Ca\(^{2+}\) mobilization, in cells expressing orphan GPCRs (3). By applying this approach, we have succeeded in identifying various orphan GPCR ligands (4–10).

In the course of our search for orphan GPCRs, we isolated TGR7, which was recently found to correspond to MrgD (11). TGR7 has been classified as a member of the GPCR subfamily referred to as Mas-related genes (Mrgs), also known as sensory neuron-specific GPCRs. These GPCRs are known to be expressed in the sensory neurons of the dorsal root ganglia (DRG). To date, the following ligands have been identified for Mrgs: several neuropeptides, with an RFamide structure at their C termini, act as ligands for mouse MrgA1, MrgA4, MrgC11, and MAS1 (11, 12); proenkephalin A gene products, especially BAM22, that stimulate human MrgX1 (hSNSR4) and SNSR3 (13); adenine, which activates a member of the Mrg family found in rats (14); and a recent report indicates that cortistatin-14 is a potent agonist for human MrgX2 (15). However, no ligand has yet been identified for TGR7 (MrgD). Because TGR7 is co-expressed with major nociceptors, a purinergic receptor (P2X3) and a vanilloid receptor (VR1), in the sensory neurons of the DRG in rats, it has been thought to play some role in pain sensation or modulation (11, 16).

β-Alanine is a small amino acid structurally related to the major inhibitory neurotransmitters γ-aminobutyric acid (GABA) and glycine. Various studies (17, 18) indicate that β-alanine acts as a depressant in the central nervous system. It has been reported that β-alanine pharmacologically activates the glycine and GABA receptors with less efficacy than their native ligands (19, 20). It has also been reported that β-alanine decreases glutamatergic excitation by binding to the glycine co-agonist site on the N-methyl-D-aspartate receptor (21, 22). These dual effects of β-alanine to both decrease excitation and increase inhibition are very unique. The high affinity uptake of β-alanine has been detected in various types of the neurons (23–26). In addition, a sodium-dependent transporter for β-alanine has been identified in the mouse brain (27, 28). There is therefore the possibility that β-alanine acts as a neurotransmitter and/or neuromodulator. However, no receptor specific for β-alanine has yet been identified.

Here we report that an orphan GPCR, TGR7, which is highly expressed in the DRG, acts as a specific receptor for β-alanine. Our findings raise the possibility that β-alanine modulates pain sensation through TGR7.

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Cloning of TGR7 cDNAs—We designed primers (5'-GTCGACAT- 
GAACATCATTCTTATACCCCGACACCC-3' and 5'-ACTAGTTCA- 
GGACACTAGTACTGGGTTCG-3') to isolate an entire TGR7 cDNA by reverse transcriptase PCR. PCR was performed in a reaction mixture (25 μl in total) containing 0.2 μM of each primer, a template cDNA synthesized from rat cerebellum cDNA, 0.2 mM dNTPs, 1.25 units of Advantage 2 polymerase mix (Clontech), and 2.5 μl of a buffer provided by the manufacturer. The mixture was heated once at 95 °C for 30 s, 5 cycles at 94 °C for 5 s and at 70 °C for 5 min, 5 cycles at 94 °C for 5 s and at 68 °C for 5 min, 35 cycles at 94 °C for 5 s and at 65 °C for 5 min, and finally at 65 °C for 5 min for an extension reaction. By this means, we obtained an ~1-kb product containing a full coding region and then determined its nucleotide se- quence. In a similar manner, we isolated human, mouse, and cynomol- gus monkey TGR7 DNA fragments by PCR from human liver cDNA, mouse genomic DNA, and cyonmolgus monkey genomic DNA, respectively. To obtain these DNA fragments, we designed the following primer sets: 5'-GTCGACATGGAACACATCTTATACCCCGACACCC-3' and 5'-ACTAGTTCAACATCTTATACCCCGACACCC-3' for human TGR7; 5'-GGACACTGAGCAGCAGCTGACTGTTATACCCCGACACCC-3' and 5'-GGATGCTGACACCCCGACACCCCGACACCC-3' for mouse TGR7; and 5'-GGATGGCCACACCGGGTATACCCCGACACCC-3' and 5'-GGATGGCCACACCGGGTATACCCCGACACCC-3' for cynomolgus monkey TGR7.

Preparation of Chinese Hamster Ovary (CHO) Cells Expressing TGR7—The entire coding region of human, rat, or mouse TGR7 cDNA was inserted downstream of the SRα promoter in the expression vector pAKKO-111H (29). The resultant expression plasmids were transfected into CHO cells, and transformed cells (i.e. CHO cells expressing human, rat, or mouse TGR7 (CHO-hTGR7, CHO-rTGR7, and CHO-mTGR7 cells, respectively) were selected as described elsewhere (29).

Ca2+ Mobilization Assays—CHO cells expressing TGR7 and mock-transfected (i.e. the empty expression vector was transfected) CHO cells were seeded in black-walled clear-bottomed 96-well tissue culture plates (Costar) at 3 x 10^4 cells/well and cultured overnight. The cells were then incubated at 37 °C for 1 h in HEPES-buffered Hank's balanced salt solution (pH 7.4) containing 2.5 mM probenecid and 4 μM fluo-3AM (Dojindo). Next, the cells were washed four times with the solution without fluo-3AM. Intracellular Ca2+ concentrations ([Ca2+]i) were measured with a fluorometric imaging plate reader system (Molecular Devices) both before and after samples were added.

Guanosine 5'-O-3-Thiotriphosphate (GTP-S) Binding Assays—Mem- brane fractions prepared from CHO-hTGR7 and mock-transfected CHO cells as described elsewhere (29) were suspended at 500 μg/ml in a binding buffer (pH 7.4) containing 50 mM Tris, 150 mM NaCl, 5 mM MgCl2, 1 mM EDTA, 30 μM GDP, and 0.05% CHAPS. The membrane fractions were mixed with 200 μM [32P]GTP-S (Amersham Biosciences) (2 μl). After incubation at 25 °C for 60 min, the reaction mixtures were diluted with 2 ml of a chilled washing buffer, which was a modified binding buffer without GDP, and then filtered through GF/F filters (Whatman). The filters were washed with 2 ml of the washing buffer, dried, and subjected to a liquid scintil- lation counter to measure [32P]GTP-S bound to the membrane fractions.

cAMP Production-inhibitory Assays—The inhibitory activities of β-alanine on cAMP production in CHO-hTGR7 cells in the presence of forskolin were examined according to our method described previously (30). For pretreatment with pertussis toxin (PTX), the cells were incubated with 100 ng/ml of PTX (Sigma) overnight before performing the cAMP production-inhibitory assays.

Internalization of TGR7—An expression vector (pAKKO-hTGR7-GFP) with a fusion protein composed of human TGR7 and green fluo- rescent protein (hTGR7-GFP) was constructed using the enzyme of a fused DNA (human TGR7 and GFP-coding regions were connected in tandem) into pAKKO-111H. CHO cells stably expressing hTGR7-GFP were seeded onto chambered coverglasses (Nalgene) and cultured overnight. After treatment with 0.3 mM β-alanine for 30 min, the fusion protein in the cells was observed under a confocal fluorescence microscope.

Trinitiated β-Alanine Binding Assay—β-[3H]Alanine (60 Ci/mmol) was purchased from Muramachi (ART-205, Tokyo, Japan). CHO cells expressing TGR7 and mock-transfected CHO cells were harvested with phosphate-buffered saline (PBS) and suspended in HEPES buffer. β-[3H]Alanine binding was performed in a final volume of 200 μl containing 100 μl of cell suspension (0.6–10 x 10^6 cells/tube), 50 μl of 0.1 M NaCl (1.5–150 mM), and 50 μl of a Hanks’ balanced salt solution with or without β-alanine (a final concentration of 1 mM). After incubation at 37 °C for 30 min, the cells were collected by centrifugation and washed three times with 3 ml of the HEPES buffer chilled in ice. The cells were then suspended in a lysis buffer (Hanks’ balanced salt solution containing 1% SDS), and radioactivity retained in the cells was quantified with a liquid scintillation counter.

Quantitative Reverse Transcriptase PCR Analyses for TGR7 mRNA—Poly(A)+ RNA fractions were prepared from the tissues of 8–12-week-old Wistar rats, and cDNAs were synthesized from them as described previously (31). Total RNA fractions were prepared from the DRG and spinal cord of a 3-year-old female cynomolgus monkey, and cDNAs were synthesized using SuperScript II reverse transcriptase (Invitrogen). Rat and monkey TGR7 mRNA expressions were deter- mined with a Prism 7700 sequence detector (PE Biosystems) with the following primers and probes (5'-CTGTCGAGTTTCCACAGGTTCC-3' and 5'-TGACGAACTGACTGGGTTCG-3'), 5'-5-carboxyfluorescein-ATCCGACACCGAGCTGCTTGGTG-3'-carboxytetramethylrhodamine-3'-rat TGR7; 5'-TGGCTGGCTATGTCACCTG-3', 5'-GAGCTGGATCGTACTGGGTTCG-3', and 5'-5-carboxyfluorescein-TGGAAACCCCCGCTCTGTTGCTG-3'-5-carboxytetramethylrhodamine-3'-monkey TGR7).

In Situ Hybridization and Immunohistochemistry—We isolated DRG from 16-week-old male Wistar rats and an adult female cynomolgus monkey. The DRG were embedded in optimal cutting temperature (OCT) compound frozen in liquid nitrogen. Fresh frozen sections (6 μm) were attached to sialanized slides and fixed with 4% paraformaldehyde. In situ hybridization was carried out using digoxigenin-labeled ribo- probes prepared from full-length rat and monkey TGR7 cDNAs. To be visualized as purple, TGR7 mRNA was treated with alkaline phos- phatase-conjugated anti-digoxigenin antibody, using 4-nitrotetrazo- lium blue chloride and 5-bromo-4-chloro-3-indolyl phosphate (which changes to red under a confocal laser microscope). For double staining in immunohistochemistry, we used anti-neurofilament 200 kDa (Chemicon), anti-calcitonin gene-related peptide (Affiniti Research Products), anti-substance P (American Research Products), anti-soma- tostatin (Biogenesis), anti-P2X (Chemicon), and anti-VRI (Chemicon) primary antibodies, and Alexa 488-labeled secondary antibodies (Molecular Probes). For the labeling of Grifonia simplicifolia isoscillatin B4 (IB4), the sections were incubated with fluorescein isothiocyanate-con- jugated IB4 (Sigma). After processing, the sections were mounted and then examined by light and confocal laser microscopy.

RESULTS

Demonstration of β-Alanine as a Ligand for TGR7—We iso- lated TGR7 cDNAs from human, rat, mouse, and monkey. The proteins from these cDNAs encoded had amino acid lengths of 321, 319, 321, and 320, respectively, and showed 80% (human versus monkey), 58% (human versus rat), and 84% (rat versus mouse) amino acid identity. Although the TGR7 amino acid sequences were not highly conserved among the species, a phylogenetic analysis of all known GPCR showed that they were the closest counterparts (data not shown). To search for ligands of TGR7, we transiently expressed human TGR7 cDNA in CHO cells and then examined changes in [Ca2+]i, by adding various test samples. Through our screening of over 1500 com- pounds, we discovered that β-alanine induced the rapid mobilization of [Ca2+]i, in CHO cells expressing human TGR7 in a dose-dependent manner, whereas β-alanine did not induce an increase of [Ca2+]i in mock-transfected CHO cells (Fig. 1A). To confirm this receptor-ligand relationship in other animal species, we prepared CHO cells stably expressing human, rat, or mouse TGR7 (i.e. CHO-hTGR7, CHO-rTGR7, and CHO-mTGR7 cells, respectively) and examined changes in [Ca2+]i in response to β-alanine. The EC50 values of β-alanine in CHO-hTGR7, CHO-rTGR7, and CHO-mTGR7 cells were 15, 14, and 44 μM, respectively (Table I). Slight stimulatory activity was detected for GABA in CHO-hTGR7 and CHO-rTGR7 cells. 1-Carnosine was found to be active only in CHO-rTGR7 cells. Other compounds structurally related to β-alanine, that is, l-glutine, l-alanine, taurine, and anserine, did not induce an evident increase of [Ca2+]i, when examined in CHO-hTGR7 cells. We subsequently examined the effect of β-alanine on cAMP production in CHO cells expressing TGR7. As shown in Fig. 1B, β-alanine suppressed forskolin-induced cAMP produc-
tion in CHO-hTGR7 cells; however, this decrease did not occur after pretreatment with PTX. Similar suppressive effects of β-alanine on cAMP production in CHO-rTGR7 and CHO-mTGR7 cells were also observed (data not shown). These results suggest that human TGR7 couples to Gq and Gi in CHO cells. To confirm further that the interaction of β-alanine and TGR7 occurred at the plasma membrane, we prepared membrane fractions from CHO-hTGR7 cells and examined

![Figure 1](http://www.jbc.org/)

**Fig. 1. TGR7 as a specific cell surface receptor for β-alanine.** A, β-alanine-induced intracellular Ca²⁺ influx in CHO-hTGR7 cells. CHO-hTGR7 cells and mock-transfected CHO cells were cultured in 96-well plates overnight. The cells were then loaded with fluo-3AM, and fluorescent changes were measured by the fluorometric imaging plate-reader system. β-Alanine was added to the cells after 10 s. The concentrations of β-alanine used were 100 μM (●), 30 μM (▲), 10 μM (●), and 3 μM (◆) for CHO-hTGR7 cells, and 100 μM (○) for the mock-transfected CHO cells. Data are shown as representative traces. B, inhibition of cAMP production in CHO-hTGR7 cells. CHO-hTGR7 cells cultured in 96-well microplates were pre-incubated overnight with PTX at 100 ng/ml (PTX (+)) or without PTX (PTX (−)). The cells were then incubated for 30 min without β-alanine and forskolin (black columns), with 1 μM forskolin (white columns), or with forskolin and 300 μM β-alanine (gray columns). The amounts of intracellular cAMP were measured with enzyme immunoassay. Data are expressed as mean ± S.E. in triplicate assays. C, β-alanine-induced [35S]GTPγS binding to membrane fractions of CHO-hTGR7 cells. Binding of [35S]GTPγS to CHO-hTGR7 cell (●) and mock-transfected CHO cell (○) membrane fractions was determined in a binding buffer containing 100 μM GDP and the indicated concentrations of β-alanine. The increase in [35S]GTPγS binding is indicated as the percentage of specific binding to basal binding. Data represent the mean ± S.E. in three independent experiments done in triplicate assays. D, internalization of hTGR7-GFP induced by β-alanine. The left panel shows CHO cells expressing hTGR7-GFP without treatment. The right panel shows CHO cells expressing hTGR7-GFP treated with β-alanine (300 μM) at 37 °C for 30 min. E, specific binding of β-[3H]alanine to CHO-hTGR7 cells depending on increasing cell number. TGR7-specific binding (●) was calculated from binding of CHO-hTGR7 cells minus binding of mock-transfected CHO cells in the absence of unlabeled β-alanine. Nonspecific binding (○) was estimated from binding in the presence of 1 mM unlabeled β-alanine. β-[3H]Alanine at 50 nM (4.8 × 10⁵ cpm) was incubated with the cells for 30 min at 37 °C in this experiment. Data are expressed as mean ± S.E. in triplicate assays. F, specific binding of β-[3H]alanine to CHO-hTGR7 cells depending on increasing β-[3H]alanine concentrations. The cell number used in this experiment was 5.0 × 10⁵. TGR7-specific (●) and nonspecific binding (○) were determined as described in E. Data are expressed as mean ± S.E. in triplicate assays.

| Ligand     | Human EC₅₀ (μM) | Rat EC₅₀ (μM) | Mouse |
|------------|-----------------|--------------|-------|
| l-Glycine  | Inactive        | Inactive     | Inactive |
| β-Alanine  | 15 ± 1          | 14 ± 2       | 44 ± 6 |
| l-Alanine  | Inactive        | Inactive     | Inactive |
| GABA       | 191 ± 12        | 165 ± 2      | >300   |
| l-Carnosine| Inactive        | 34 ± 1       | >300   |
| Anserine   | Inactive        | >200         | Inactive |
| Taurine    | Inactive        | ND           | ND     |
[35S]GTPγS binding to these fractions (Fig. 1C). Significant levels of binding were detected with 3 μM of β-alanine, the EC50 value was at 25 μM, and maximum levels were reached with 100–300 μM. Such increases in [35S]GTPγS binding were not detected in the membrane fractions of mock-transfected CHO cells.

To confirm that TGR7 functions as a cell surface receptor, we stably expressed a fusion protein (hTGR7-GFP) in CHO cells and then examined its subcellular localization. In the absence of a ligand, hTGR7-GFP was localized typically at the plasma membrane (Fig. 1D, left panel). However, in the presence of β-alanine, hTGR7-GFP was internalized into the cytoplasm (Fig. 1D, right panel). Treatment with l-alanine or l-glycine had no effect on hTGR7-GFP localization (data not shown). We examined the binding of [3H]alanine to intact CHO-hTGR7 cells. As shown in Fig. 1 (E and F), β-[3H]alanine more effectively bound to CHO-hTGR7 cells than to mock-transfected CHO cells in a manner dependent on the cell number and β-[3H]alanine concentration, indicating that β-alanine specifically binds to CHO-hTGR7 cells.

Tissue Distribution of TGR7 mRNA in Rat and Monkey—We first analyzed the tissue distribution of TGR7 mRNA in rats. TGR7 mRNA was primarily expressed in the DRG (Fig. 2A). Moderate levels of expression were detected in the testes, urinary bladder, arteries, and uterus (Fig. 2A). To examine whether TGR7 mRNA was highly expressed not only in rodents but also in primates, we analyzed the expression of TGR7 and GABA<sub>µ</sub>1a mRNAs in monkey DRG and the spinal cord. A high level of TGR7 mRNA was detected in the DRG, whereas only a low amount was found in the spinal cord (Fig. 2B, left panel). In contrast, a high level of GABA<sub>µ</sub>1a mRNA was detected in both the DRG and spinal cord. These results suggest that the high level of TGR7 mRNA expression in the DRG is conserved between rodents and primates.

Expression of TGR7 mRNA in DRG—To compare the detailed expression of TGR7 mRNA in the neurons of the DRG between rodents and primates, we performed in situ hybridization using digoxigenin-labeled riboprobes. TGR7 mRNA signals were detected in small diameter DRG neurons in both the rat and monkey (Fig. 3), suggesting that TGR7 is expressed in C-fibers mediating pain or nociceptive responses in both rodents and primates.

To determine whether TGR7 co-localized with neuronal markers, we conducted double-staining in situ hybridization and immunostaining for various markers in rat and monkey DRG neurons. We employed seven kinds of neuronal markers (i.e., neurofilament 200 kDa, calcitonin gene-related peptide, substance P, IB4, somatostatin, P2X<sub>3</sub>, and VR1) to distinguish the subgroups of rat DRG neurons. Anti-neurofilament 200 kDa antibody recognizes high molecular weight neurofilaments and therefore is one of the markers for myelinated large diameter neurons. TGR7 mRNA was not detected in neurofilament 200 kDa immunoreactive neurons in the rat DRG (Fig. 4, A–C). TGR7 mRNA was also not expressed in calcitonin gene-related peptide-, somatostatin-, and substance P-immunoreactive neurons (images not shown). Most of the TGR7 mRNA-positive neurons were labeled with IB4, a marker of non-myelinated small diameter neurons in rat DRG (Fig. 4, D–F). These results suggest that TGR7 mRNA expresses in non-peptidergic nociceptive neurons. Likewise, most of the TGR7 mRNA-positive

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**Fig. 2.** Tissue distribution of TGR7 mRNA in the rat and monkey. Poly (A)⁺ RNA from rat tissues and total RNA from monkey tissues were subjected to quantitative reverse transcriptase PCR analyses using an ABI Prism 7700 sequence detector. A, rat; B, monkey. Each column represents a mean value in duplicate determinations.
neurons co-localized P2X3-immunoreactive and VR1-immunoreactive neurons in the rat DRG (Fig. 4, G–L). These results are highly consistent with those reported by other groups (11, 16).

To compare with the localization of TGR7 mRNA in rat DRG neurons, we also performed the double-staining hybridization of TGR7 and neuronal markers with monkey DRG neurons. We found that most TGR7 mRNA-positive neurons were also positive for IB4 (Fig. 4, M–O). P2X3 (Fig. 4, P–R), and VR1 (Fig. 4, S–U) indicating that the expression pattern of TGR7 is similar between rats and monkeys. Because P2X3 and VR1 are important molecules for mediating pain (32), these data suggest that TGR7 plays a role in pain modulation both in rodents and primates.

FIG. 3. Expression of TGR7 in DRG neurons in the rat and monkey. In situ hybridization for TGR7 using digoxygenin-labeled cRNA probe showed that TGR7 was expressed in DRG small diameter neurons in a rodent and primate. A, rat TGR7 antisense probe; B, rat TGR7 sense probe; C, cynomolgus monkey TGR7 antisense probe; D, cynomolgus monkey TGR7 sense probe. Scale bar = 50 μm.

DISCUSSION

In this paper, we have demonstrated that an orphan GPCR, TGR7, is specifically responsive to β-alanine. We found that β-alanine induced intracellular Ca2+ influx and suppressed cAMP production in CHO cells expressing TGR7. The responsiveness of TGR7 to β-alanine was detected in all species examined (i.e. human, rat, and mouse) even though the amino acid sequence of TGR7 among these species was not always highly conserved. We showed through [35S]GTPγS binding assays that β-alanine induced the replacement of GDP with GTPγS through the activation of G proteins in membrane fractions of CHO cells expressing TGR7. In addition, by utilizing a fusion protein combining TGR7 and GFP, we demonstrated that in CHO cells β-alanine caused the internalization of TGR7 from the plasma membrane into the cytoplasm. These results suggest strongly that TGR7 functions as a specific membrane receptor for β-alanine. However, we failed to detect specific binding of radiolabeled β-alanine to membrane fractions of CHO cells expressing TGR7 (data not shown). We therefore conducted the binding experiments using intact CHO-hTGR7 cells instead of the membrane fractions, because we have experienced previously that the procedure to prepare membrane fractions damaged the binding functions of some receptors including thyrotropin-releasing hormone receptor (29). In the experiments using intact live cells, we found that β-3H]alanine more efficiently bound to CHO-hTGR7 cells than to mock-transfected CHO cells suggesting that β-alanine binds to TGR7. However, to analyze the interaction between β-alanine and TGR7 more precisely, it will be necessary in future studies to establish a binding assay using a synthetic compound with higher affinity to TGR7. Tissue concentrations of β-alanine are reportedly ~50 μM in rat sciatic nerve and 60 μM in cat brain (34, 35). Because the EC50 values of β-alanine were 14–44 μM in our Ca2+-mobilization assays using CHO cells expressing TGR7, we believe that TGR7 can respond to the physiological concentrations of β-alanine. β-Alanine is closely related to l-glycine, l-alanine, GABA, taurine, and anserine in structure. However, TGR7 selectively responded only to β-alanine. In addition, other amino acids including l-serine and l-aspartic acid did not act as ligands for TGR7 (data not shown), suggesting that for TGR7 to recognize a ligand, it must meet very strict specifications. l-Carnosine (β-alanyl-l-histidine) is a precursor of β-alanine. l-Carnosine acted on rat TGR7 at doses similar to β-alanine. However, because the human and mouse TGR7 did not respond to l-carnosine, it should be considered that it may be a surrogate ligand.

Nociceptive primary sensory neurons in the DRG are classified into subclasses, which can be distinguished on the basis of their functions, dependence on neutrophins, or expression of molecular markers (36, 37). In rat and mouse DRG, TGR7 is predominantly expressed in nociceptors that express the glial cell-line-derived neurotrophic factor receptor, c-Ret, and binds...
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with IB4 (11, 16). It has been reported (11, 16) that TGR7 co-localized with P2X3 in rat and mouse DRG. In addition, TGR7 reportedly co-localized with VR1 in rat DRG but not in mouse DRG (11, 16). Both P2X3 and VR1 act as cation channels that function as nociceptors and play important roles in detecting noxious stimuli (38, 39). We have shown here that TGR7 co-localized with P2X3 and VR1 in monkey DRG. In addition, we found that TGR7 mRNA expression levels were changed in the DRG of chronic constrictive injury-model rats, which are widely known as models of neuropathic pain (data not shown). These results suggest that TGR7 plays a role in the modulation of pain.

Our results indicate that TGR7 couples with the G proteins Gq and Gi in CHO cells. The functional significance of the activation of Gq and Gi in the DRG neurons has been reported previously (40, 41). The activation of bradykinin and P2Y1 receptors, which couple with Gq, potentiates the sensitivity of VR1 in the DRG (40, 41), whereas the activation of opiate and cannabinoid receptors, which couple with Gi, causes analgesia (37). Thus, there is the possibility that TGR7 regulates pain sensitivity through the activation of the Gq and/or Gi signaling pathways in the DRG, although further studies are necessary to confirm whether TGR7 modulates pain in a similar manner.

Electrophysiological and biochemical studies have suggested that β-alanine participates in synaptic transmission as a neurotransmitter and/or neuromodulator. Hosli and Hosli (42) have reported that [3H]β-alanine uptake is observed in both neurons and glial cells in the spinal cord and brain stem. In rat DRG, TGR7 mRNA was reported to be mainly expressed in small diameter neurons, whereas the glycine receptor β-subunit and the GABA_A receptor γ-subunit mRNAs were mainly expressed in large diameter neurons, implying that TGR7 might have functions distinct from glycine and GABA in pain modulation (43). β-Alanine might function as a neurotransmitter and/or neuromodulator through the activation of TGR7, although future studies are necessary to clarify this point.

In this study, we have demonstrated that a specific receptor for β-alanine exists in DRG. We hope that our findings here will contribute to the understanding of pain regulation and provide an opportunity to develop anti-nociceptive drugs.

Acknowledgments—We thank Drs. M. Fujino, T. Soda, Y. Fujisawa, K. Konogmi, M. Kobayashi, T. Doi, F. Itoh, K. Fukatsu, T. Iwama, S. Sato, T. Ito, and Y. Shintani for their helpful discussions and collaboration.

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J. Biol. Chem. 2004, 279:23559-23564.
doi: 10.1074/jbc.M314240200 originally published online March 22, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M314240200

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