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Expression and distribution of GABAergic system in rat knee joint synovial membrane

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Summary. The GABAergic system, found in the adult mammalian brain and composed of γ-aminobutyric acid (GABA), GABA synthesizing enzyme glutamate decarboxylase (GAD) and GABA receptors, is also located in many peripheral nonneuronal tissues. Studies suggest that synovial membranes possess GABA, and that GABA participates in the control of the inflammatory response in rheumatoid arthritis (RA). However, no studies on the GABAergic system in synovial membranes have been done so far. Therefore, expression and distribution of the GABAergic system in the synovial membrane of the normal rat knee joint were investigated by reverse transcription-polymerase chain reaction (RT-PCR) analyses and immunohistochemistry. Results of RT-PCR analysis showed that mRNA encoding the GAD65 and $GABA_B$ receptor subunits necessary for the assembly of functional receptors, R1 and R2, are expressed in the synovial membrane. GAD and $GABA_B$ receptor subunits were localized in macrophage-like A cells of the synovial membrane. Macrophage-like A cells of the synovial membrane have a GABA production system and $GABA_B$ receptors, and GABA seems to play functional roles in the synovial membrane.

Key words: GABA, $GABA_B$ receptor, Macrophage-like A cell, Synovial membrane

Introduction

The principal inhibitory neurotransmitter in the adult mammalian brain, γ-aminobutyric acid (GABA), is also detected in many peripheral non-neuronal tissues (Watanabe et al., 2002, 2006). GABA is synthesized primarily from glutamic acid by a decarboxylation reaction catalyzed by glutamate decarboxylase (GAD). Mammals express two isoforms of GAD, GAD65 and GAD67, which are encoded by two distinct genes (Watanabe et al., 2002). In the brain, GAD67 is responsible for the synthesis of more than 90% of GABA and is a soluble cytosolic protein, whereas GAD65 is preferentially localized near neuronal synaptic vesicles (Soghomonian and Martin, 1998). In peripheral neuronal and non-neuronal tissues, the type of GAD expressed depends on the cells (Watanabe et al., 2002).

The effects of GABA are exerted through ionotropic $GABA_A$ and $GABA_C$ receptors, as well as metabotropic $GABA_B$ receptors (Watanabe et al., 2002). $GABA_A$ receptors are ligand-gated chloride channels, and are pentameric assemblies derived from a combination of at least 16 subunits, α1-6, ß1-3, γ1-3, δ, π, ε and θ. $GABA_C$ receptors are also pentameric assemblies derived from a combination of 3 ρ subunits and linked to chloride channels. $GABA_B$ receptors are coupled to G proteins, and active functional $GABA_B$ receptors are heterodimers that are composed of two subunits, R1 and R2. $GABA_A$ and $GABA_B$ receptors are present in many peripheral non-neuronal tissues, indicating that GABA exerts physiologic effects other than neurotransmitter effects in these tissues. In fact, GABA has been shown to be involved in the development of osteoblasts (Fujimori et al., 2002) and chondrocytes (Tamayama et al., 2005). Nakano et al. (1988) studied GABA content in the rat synovial membrane and suggested that GABA is present in nerve fibers. They further studied GABA with arthritis model rat knee joints, and claimed that GABA presents in non-neuronal cells of synovial membrane, in addition to neuronal elements, and suggested that GABA participates in the control of the inflammatory response (Nakano, 1988). Gabapentin, which is a GABA analog and widely used anticonvulsant and antinociceptive drug, is used for the prevention and treatment of inflammatory diseases, including arthritis

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(Schrier et al., 2001). These data imply that the GABAergic system, which is composed of GABA, GADs, and GABA receptors, may play a certain physiologic role in the synovial membrane. However, no studies on the GABAergic system in synovial membrane have been done so far. In the present study, we therefore investigated the expression and distribution of the GABAergic system in the synovial membrane of the normal rat knee joint.

The synovial membrane is a unique tissue, and has features distinguishable from the epithelial linings in other body cavities. The synovial membrane has neither a continuous cellular lining nor a basement membrane (Kamimoto et al., 2003). The synovial membrane is composed of two layers: the surface intimal lining cell layer (intima layer) and the deeper subintima layer. The latter layer consists of connective tissue containing blood vessels and nerve fibers. The intimal lining cell layer consists of two predominant cells, known as synoviocytes: macrophage-like A cells and fibroblast-like B cells (Barland et al., 1962; Iwanaga et al., 2000; Nozawa-Inoue et al., 2003). Macrophage-like A cells function in the homeostasis of the joint cavity by phagocytosing waste and cell debris in the synovial fluid (Graabaek, 1985; Senda et al., 1999). Fibroblast-like B cells secrete intercellular matrix collagen (Visnapuu et al., 2000) and a component of synovial fluid, hyaluronic acid (Roy and Ghadially, 1967; Okada et al., 1981).

Materials and methods

Animals

All animal experiments were reviewed by the Ethics Review Committee for Animal Experimentation of Osaka Medical College. Five-week-old male Lewis rats were obtained from Clea Japan Inc., Osaka, Japan. All animals were housed in a temperature-controlled room (23°C) with free access to water and regular food (CE-2; Clea Japan) under a standard dark/light schedule of 12/12 h before use in the experiments.

RNA preparation and reverse transcription-polymerase chain reaction (RT-PCR)

The rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and perfused transcardially with Ringer's solution via the left ventricle. The articular capsule (synovial membrane + fibrous capsule) was dissected from the knee joint (n=6). RT-PCR of GAD65, GAD67, and $GABA_A$, $GABA_B$ and $GABA_C$ receptor subunits was done as described before (Tamayama et al., 2005) with the primers shown in Table 1. The PCR products were separated on 1.5% agarose gels, stained with 0.1 mg/mL ethidium bromide, visualized by UV transillumination, and documented on black and white instant film.

Immunohistochemistry of GAD, GABA, heat shock protein 25 (HSP25), CD68, $GABA_A$, $GABA_C$ and $GABA_B$ receptor subunits in rat synovial membrane

The rats (n=15) were anesthetized with sodium pentobarbital and perfused transcardially with 100 mL of Ringer's solution, and then fixed with 50 mL of 4% paraformaldehyde (and 0.1% glutaraldehyde for GABA) in 0.1M phosphate-buffered saline (PBS, pH 7.4). The articular capsules of knee joints were dissected and postfixed for 4-5 h in the same fixative at 4°C. The specimens were rinsed with PBS and then immersed in 30% sucrose at 4°C for cryoprotection. Specimens embedded in a medium for frozen tissue specimens (Tissue-Tek® O.C.T compound, Miles, Elkhart, IN, USA) were frozen with liquid N_2, and then 12 µm thick frozen sections were cut on a freezing microtome (Leica CM3050, Nussloch, Germany). Sections for GAD, $GABA_A$ receptor subunit and $GABA_B$ receptor subunit immunostaining were incubated with Block Ace (Dainippon Pharmaceutical, Osaka, Japan) containing 0.1% Triton X for 1 h at room temperature to control nonspecific reactions, and then incubated at 4°C overnight with a rabbit-anti GAD65/67 polyclonal antibody (1:1000, Sigma, Saint Louis, MO, USA), a guinea pig anti-$GABA_B$ receptor polyclonal antibody that recognizes both the R1a and R1b subunits (1:2000, Chemicon International, Temecula, CA, USA), or a rabbit anti-$GABA_B$ receptor R2 polyclonal antibody (1:500, Alomone Labs Ltd. Jerusalem, Israel). For $GABA_A$ receptor subunit immunostaining, sections were incubated with a goat polyclonal antibody specific for each $GABA_A$ receptor subunit (α1, α2, α3, α4, ß2 ß3, δ) (1:150; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) (Hayasaki et al., 2006). Some sections were incubated with anti-CD16/32 antibody (BD Biosciences-Pharmingen, San Diego, CA, USA) for 10 min at 4°C prior to incubation with primary antibody, to reduce Fc receptor-mediated binding by antibodies (Maemura et al., 2006). For $GABA_C$ receptor subunit immunostaining, sections were incubated with a goat polyclonal antibody specific for $GABA_C$ receptor subunit ρ1 and ρ2 (1:250. Santa Cruz).

To identify the cell types of the synovial membrane, double immunostaining with CD68 or HSP25 was performed. CD68 is a marker of macrophage-like A cells (Hamann et al., 1999) and HSP25 is a marker of fibroblast-like B cells (Andoh et al., 2001; Ikeda et al., 2004) in the synovial membrane. The antibodies were mouse anti-CD68 monoclonal antibody (1:200, BD Biosciences Pharmingen, CA, USA) and mouse anti-HSP25 monoclonal antibody (1:100, Chemicon). For GABA immunostaining, sections were preincubated with 1% sodium borohydride in PBS for 30 min at room temperature, washed in PBS, and then incubated with Block Ace followed by rabbit anti-GABA polyclonal antibody (1:2000, Chemicon) overnight at 4°C. After incubation with a primary antibody, all sections were

rinsed in PBS and incubated with secondary antibodies for 1 h at room temperature. The secondary antibodies used were Alexa 488™-conjugated goat anti-rabbit IgG, Alexa 488™-conjugated goat anti-guinea pig IgG, Alexa 488™-conjugated donkey anti-goat IgG, Alexa 546™-conjugated goat anti-rabbit IgG, Alexa 546™-conjugated goat anti-guinea pig IgG, and Alexa 546™-conjugated goat anti-mouse IgG. These secondary antibodies were purchased from Molecular Probes, Eugene, OR, USA, and diluted 1: 300. Some sections were stained with propidium iodide (Molecular Probes). Expression of GAD65/67 and GABA was verified in positive control tissues (cerebellum of wild-type ICR mouse). Negative immunostaining controls comprised sections incubated with non-immune serum instead of a primary antibody. All controls generated the predicted positive or negative results.

Fluorescence was observed and photographed using a fluorescence microscope (Nikon Eclipse E600, Tokyo, Japan) equipped with a digital camera (VB7000/7010, Keyence Co., Osaka, Japan) or with a confocal laser microscope (LSM 510, Carl Zeiss Co., Ltd., Oberkochen, Germany).

Results

Analyses of GAD, $GABA_A$, $GABA_B$ and $GABA_C$ receptor subunit mRNA in the rat articular capsule

RT-PCR analyses revealed that GAD65 was expressed in the articular capsule of the rat knee joint. However, GAD67 mRNA was not detected in the articular capsule (Fig. 1). We detected $GABA_A$ receptor subunit α1-4, ß1-2 and δ mRNAs (Fig. 2) and $GABA_B$ receptor subunit R1a, R1b and R2 mRNA in the articular capsule of the rat knee joint (Fig. 3). In this experiment,

Table 1. Primer sequences used for RT-PCR of rat GAD65 and GAD67, and $GABA_A$, $GABA_B$ and $GABA_C$ receptor subunits.

Primers	Sequences	Product size	References or GenBank accession No.
GAD65	F 5'- GTCATCTCAAACCCTGCA-3' R 5'- ACTGGACTCTACTGTGAC-3'	209	M72422
GAD67	F 5'- GTGCAATGAAAGGGGGAA-3' R 5'- CGCTCCATAAACAGTCGTG-3'	121	M76177
α1	F 5'- AGCTATACCCCTAACTTAGCCAGG -3' R 5'- AGAAAGCGATTCTCAGTGCAGAGG -3'	305	Akinci and Schofield, 1999
α2	F 5'- ACAAGAAGCCAGAGAACAAGCCAG -3' R 5'- GAGGTCTACTGGTAAGCTCTACCA -3'	334	Akinci and Schofield, 1999
α3	F 5'- CAACATAGTGGGAACCACCTATCC -3' R 5'- GGGGTTTGGGATTTTGGATGCTTC -3'	352	Akinci and Schofield, 1999
α4	F 5'- AAATGCAGCGAGACTATCTCTGC -3' R 5'- AGACAGTCTGTATTTCCATCACGC -3'	395	Akinci and Schofield, 1999
α5	F 5'- CAAGAAGGCCTTGGAAGCAGCTAA -3' R 5'- GGTTTCCTGTCTTACTTTGGAGAG -3'	339	Akinci and Schofield, 1999
α6	F 5'- ATCGTTCCATCTTCTGAGCCA -3' R 5'- CTGAAATGAGACCATTTATAC -3'	322	Luddens et al., 1990
ß1	F 5'- CCTGGAAATCAGGAATGAGACCAG -3' R 5'- GGAGTCTAAACCGAACCATGAGAC -3'	342	Akinci and Schofield, 1999
ß2	F 5'- TGAGATGGCCACATCAGAAGC -3' R 5-' TCATGGGAGGCTGGAGTTTAGTTC -3'	318	Akinci and Schofield, 1999
ß3	F 5-' GAAATGAATGAGGTTGCAGGCAGC -3' R 5'- GCAGGGTAATATTTCACTCAG -3'	356	Akinci and Schofield, 1999
γ1	F 5'- CAGAGACAGGAAGCTGAAAAGCAA -3' R 5'- CGAAGTGATTATATTGGACTAAGC -3'	361	Akinci and Schofield, 1999
γ2	F 5'- TGTGAGCAACCGGAAACCAAGCAA -3' R 5'- CGTGTGATTCAGCGAATAAGACCC -3'	375	Akinci and Schofield, 1999
γ3	F 5'- CATCCAGATTCAACAAGATG -3' R 5'- AGCTCAGAGACGTCAATG -3'	256	Akinci and Schofield, 1999
δ	F 5'- TGAGGAACGCCATTGTCCTCTTCT -3' R 5'- ACCACCGCACGTGGTACATGTAAA C-3'	360	Akinci and Schofield, 1999
R1a	F 5'- TGCTGCTGCTGGTGCCTCTCTT -3' R 5'- GTCACACTTGCTGTCGTGGT -3'	653	Castelli et al., 1999
R1b	F 5'- CGCTGCCTCTTCTGCTGGTG -3' R 5'- GTCACACTTGCTGTCGTGGT -3'	275	Castelli et al., 1999
R2	F 5'- CGGAGGTGAGCGTGCGTCTG -3' R 5'- GGGGCGAGAGAAACTCCTCA -3'	360	Castelli et al., 1999
ρ1	F 5'- ACAGACCTGCTCTCTGGAAATC -3' R 5'- GGGTTTCTCTCCGTTCTCAGGC -3'	633	NM_008075
ρ2	F 5'- AGCAGCACTGGCTGGTACAACC -3' R 5'- AGAATGTGGCTTGTTGGGTAGCC -3'	452	NM_008076

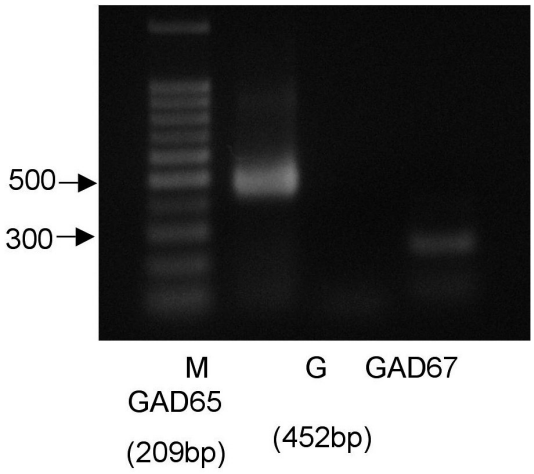


Fig. 1. Reverse transcription-polymerase chain reaction analysis of glutamate decarboxylase (GAD) mRNAs of rat synovial membrane. G: GAPDH, M: 100 bp DNA ladder.

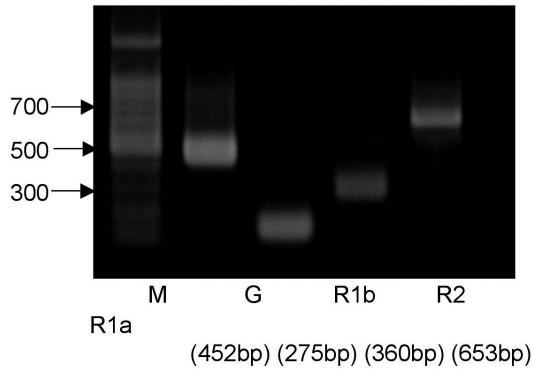


Fig. 3. Reverse transcription-polymerase chain reaction analysis of $GABA_B$ receptor subunit mRNAs of rat synovial membrane. G: GAPDH, M: 100 bp DNA ladder.

mRNA of the $GABA_C$ receptor subunits was not detected (Fig. 4). GAPDH (resultant PCR product was 452 bp) was used as a housekeeping gene (Figs. 1, 2 and 3).

Immunohistochemical analyses of GAD, GABA, and $GABA_A$, $GABA_B$ and $GABA_C$ receptor subunits in rat synovial membrane

GAD65/67 immunoreactivity was detected in the

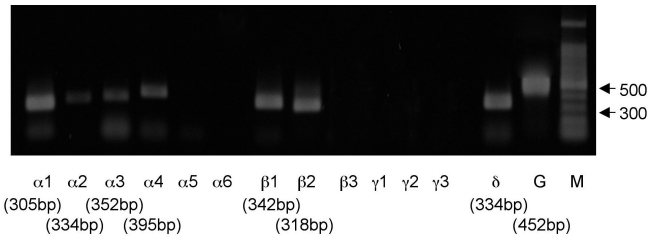


Fig. 2. Reverse transcription-polymerase chain reaction analysis of $GABA_A$ receptor subunit mRNAs of rat synovial membrane. G: GAPDH, M: 100 bp DNA ladder.

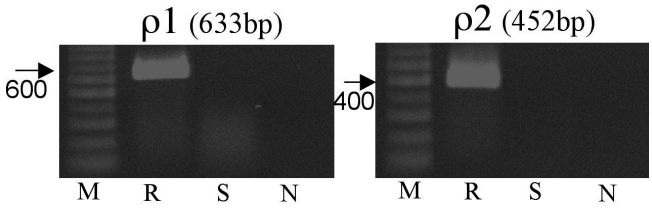
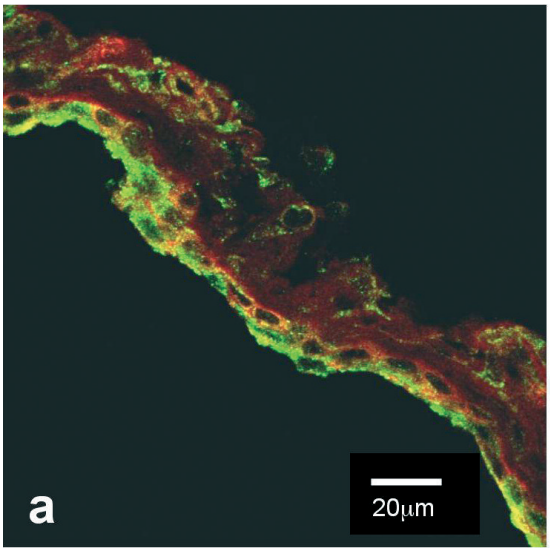


Fig. 4. Reverse transcription-polymerase chain reaction analysis of $GABA_C$ receptor subunit mRNAs of rat synovial membrane. M: DNA ladder, R: positive control (retina), S: synovial membrane, N: negative control (water).



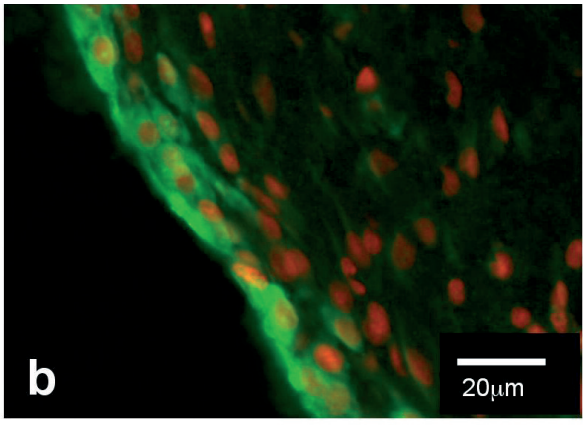


Fig. 5. Confocal image of GAD65/67 immunostaining with HSP25 in rat synovial membrane **(a)**. GAD65/67 immunopositive cells (green) were not immunopositive for HSP25 (red) which is a marker of fibroblast-like B cells. Fluorescent micrograph of GABA immunostaining (green) in rat synovial membrane **(b)**. Section was stained with propidium iodide for nuclei (red). GABA immunopositive cells are observed in the intimal cell layer.

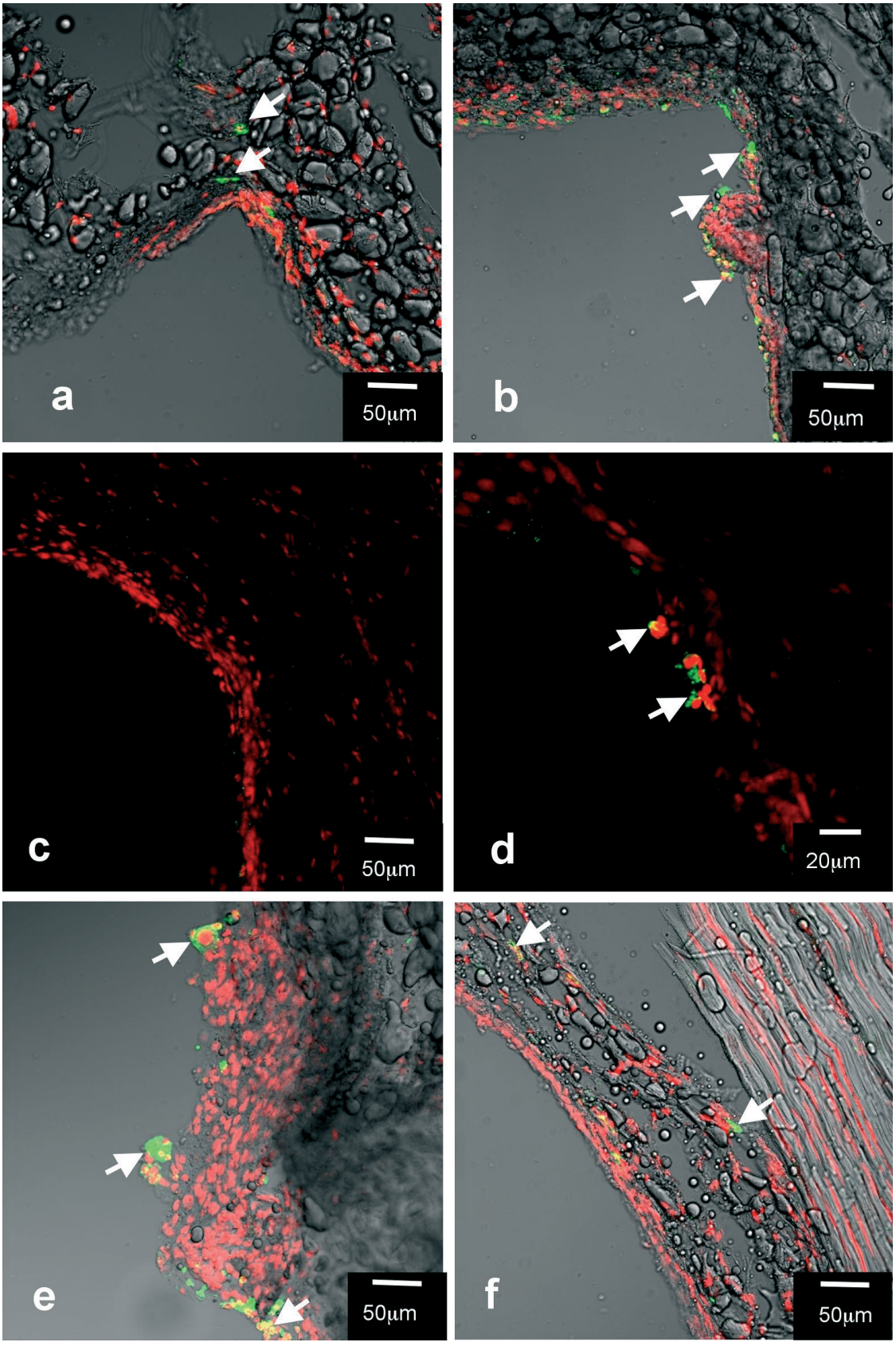


Fig. 6. Confocal images of rat synovial membrane stained for $GABA_A$ α1 (**a**, green), α2 (**b**, green), α3 (**c**, green), α4 (**d**, green), ß1 (**e**, green), and ß2 subunits (**f**, green) immunostaining. Sections are stained with propidium iodide for nuclei (red). Images of **a, b, e and d** are merged images of fluorescence and differential interference contrast images. These images show no expression of $GABA_A$ receptor subunits in the intimal lining cells of the synovial membrane. However, certain immunopositive cells are found in the subintima layer of synovial membrane (arrows in a and f) or in the synovial cavity (arrows in b, d and e).

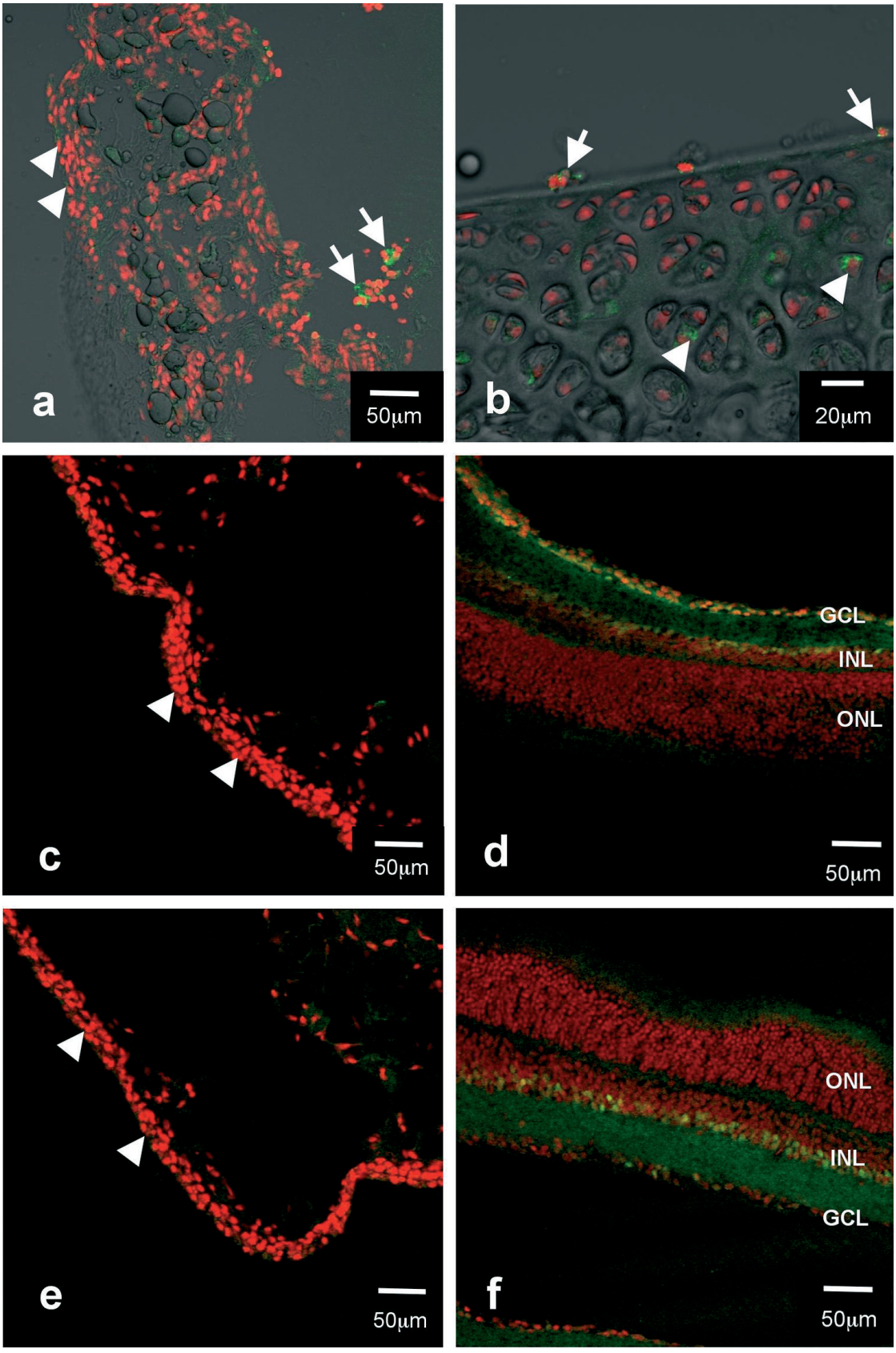


Fig. 7. Confocal images of $GABA_A$ receptor δ subunit (**a and b**, green), immunostaining in the rat knee joint, and confocal images of $GABA_C$ receptor ρ1 and ρ2 subunit of rat synovial membrane (**c and e**, respectively) and rat retina (**d and f**, respectively) as a positive control. Images of a and b are merged images of fluorescence and differential interference contrast images. $GABA_A$ δ subunit immunopositive cells (long arrows in **a and b**) are found in the rat knee joint apart from the synovial membrane (short arrows in **a**) or surface of the articular cartilage (**b**). Chondrocytes in the articular cartilage are immunopositive for $GABA_A$ δ subunit (short arrows in b). $GABA_C$ ρ1 and ρ2 subunits are immunopositive in the inner nuclear layer (INC in **d and f**) and ganglion cell layer (GCL in **d and f**) of rat retina, but no immunoreactivity is observed in the synovial membrane (short arrows in **c and e**).

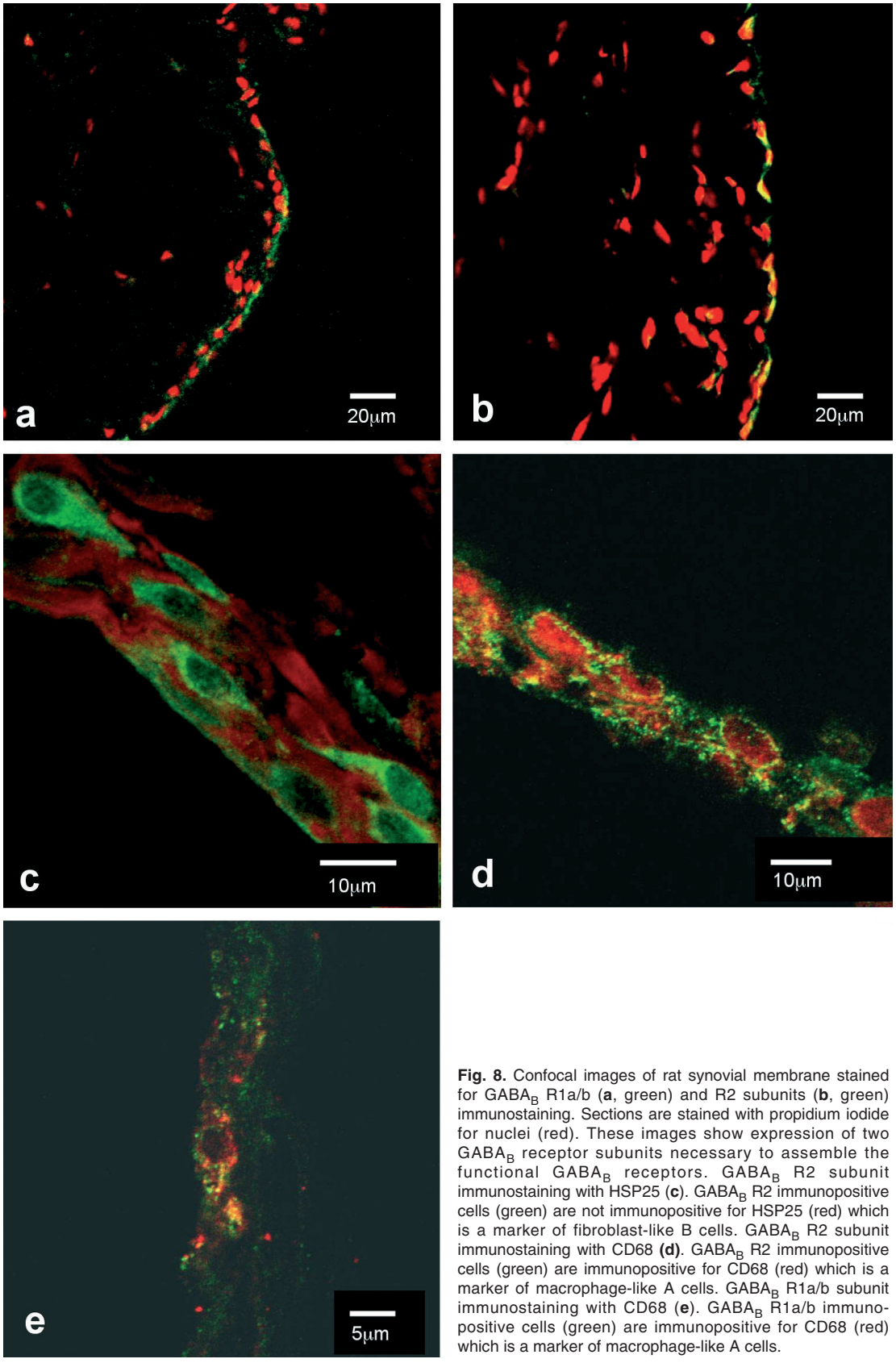


Fig. 8. Confocal images of rat synovial membrane stained for $GABA_B$ R1a/b (**a**, green) and R2 subunits (**b**, green) immunostaining. Sections are stained with propidium iodide for nuclei (red). These images show expression of two $GABA_B$ receptor subunits necessary to assemble the functional $GABA_B$ receptors. $GABA_B$ R2 subunit immunostaining with HSP25 (**c**). $GABA_B$ R2 immunopositive cells (green) are not immunopositive for HSP25 (red) which is a marker of fibroblast-like B cells. $GABA_B$ R2 subunit immunostaining with CD68 **(d)**. $GABA_B$ R2 immunopositive cells (green) are immunopositive for CD68 (red) which is a marker of macrophage-like A cells. $GABA_B$ R1a/b subunit immunostaining with CD68 (**e**). $GABA_B$ R1a/b immunopositive cells (green) are immunopositive for CD68 (red) which is a marker of macrophage-like A cells.

intimal lining cells, and GAD65/67 immunopositive cells were immunonegative for HSP25, which is a marker of fibroblast-like B cells (Fig. 5a). Some cells in the intimal cell layer of the synovial membrane were immunopositive for GABA (Fig. 5b).

Immunohistochemistry for $GABA_A$ receptor subunits α1-4, ß1-2 and δ, did not show any specific immunoreation in the intimal lining cells of synovial membrane (Figs. 6, 7a,b), though chondrocytes of articular cartilage showed specific immunostaining for all $GABA_A$ receptor subunits studied with the different staining intensity. In addition to the chondrocytes, certain leukocytes in the synovial fluid were immunopositive for α2 (Fig. 6b), α4 (Fig. 6d), ß1 (Fig. 6e), and δ (Fig. 7a,b) subunits. Furthermore, $GABA_A$ receptor α1 (Fig. 6a), and ß2 (Fig. 6f) immunopositive cells were observed in the subintima layer of the synovial membrane. These immunoreactivites did not change by preincubation with anti-CD16/32 antibody. Immunohistochemistry for $GABA_C$ receptor subunits ρ1 and ρ2 did not show any specific immunoreaction in the knee joint region, including synovial membrane and articular cartilage (Fig. 7c-f).

However, immunoreactivity for $GABA_B$ receptor subunits R1a/b and R2 was detected in intimal lining cells (Fig. 8a,b). Double immunostaining of $GABA_B$ subunit R2 with HSP25 revealed that $GABA_B$ receptor was expressed on the macrophage-like A cells, but not on the fibroblast-like B cells (Fig. 8c). This was further confirmed by double immunostaining of $GABA_B$ subunit R2 with macrophage-like A cell marker CD68 (Fig. 8d). Double immunostaining with CD68 revealed that $GABA_B$ subunits R1a/b also localized on macrophage-like A cells (Fig 8e).

Discussion

The intimal lining cells of synovial membrane consist of two predominant cells, macrophage-like A cells and fibroblast-like B cells. Type A cells can be identified immunohistochemically by using antibodies against CD68 (Hamann et al., 1999) and type B cells can be identified with HSP25 as the cell marker (Andoh et al., 2001; Ikeda et al., 2004). The present study indicated that the intimal lining cells of the synovial membrane expressed the enzyme GAD, which synthesizes GABA. Since GAD65/67 immunopositive cells in the synovial membrane were not immunopositive for HSP25, GABA production cells were macrophage-like A cells.

The effects of GABA are mediated through ionotropic $GABA_A$ and $GABA_C$ or metabotropic $GABA_B$ receptors (Watanabe et al., 2002). As to the $GABA_A$ receptors, most studies of heterologous expression systems have shown that the functional $GABA_A$ receptor contains at least one α subunit, one ß subunit, and one γ or δ subunit. In the present study, RT-PCR analyses showed expression of certain mRNAs of $GABA_A$ receptor subunits, but no substantial immunoreactivity for any $GABA_A$ receptor subunits was observed in the synovial membrane. Therefore, functional $GABA_A$ receptors might not be present in the synovial membrane of the rat knee joint. However, interestingly, certain cells in the synovial cavity or in the subintima layer of synovial membrane showed specific immunoreactivity for $GABA_A$ subunits studied, except for α3 subunit. These cells are not macrophage-like A cells or fibroblast-like B cells. These cells seem to be leukocytes including T cells and macrophages from the morphological characteristics. These are unexpected findings, and therefore further well-designed experiments are needed before discuss the significance of these findings. However, reports that $GABA_A$ α1, α2, ß1, ß2 and δ subunit present in native mouse T-cell (Tian et al., 1999, 2004) and GABA present in macrophage (Stuckey et al., 2005), lead us to assume that the GABAergic system has certain roles for inflammatory disease.

As our RT-PCR analyses showed that mRNAs of $GABA_C$ receptor subunits were not detected, and immunohistochemistry for the ρ1 and ρ2 subunits did not show any specific immunoreactivity, functional $GABA_C$ receptors also might not be present in the rat synovial membrane. $GABA_C$ receptors were first found exclusively in the retina (Qian and Dowling, 1993), and demonstrated that ρ1 or ρ2 subunits were largely localized in bipolar cells in the inner nuclear layer (Enz et al., 1995). Recent studies show that these receptors are expressed in many brain regions (Liu et al., 2004; Alakuijala et al., 2006; Harvey et al., 2006), while in non-neuronal tissues functional $GABA_C$ receptors have not been found so far.

In the synovial membrane, however, we confirmed here the expression of $GABA_B$ receptors. $GABA_B$ receptors are coupled to G proteins, and active functional $GABA_B$ receptors appear to be heterodimers of the R1 and R2 subunits. In the R1 subunit, two splice variants (R1a and R1b) have been identified (Kaupmann et al., 1997). In the present study, we detected mRNAs of the R1a, R1b and R2 subunits of $GABA_B$ receptors. Furthermore, present immunohistochemical analyses revealed that all these $GABA_B$ receptor subunits were expressed in macrophage-like A cells. This means that the GABA produced in macrophage-like A cells acts on itself in autocrine and/or paracrine fashion. Both splice variants of the R1 subunit, R1a and R1b, are expressed in macrophage-like A cells, though these variants exhibit different anatomical distribution and functions in the central nervous system (Kaupmann et al., 1998; Benke et al., 1999).

The function of the GABAergic system, composed of the GABA production system and $GABA_B$ receptors in the synovial membrane, remains unclear. The $GABA_B$ receptor has been detected in a variety of peripheral tissues, including the heart (Lorente et al., 2000), testis (Kanbara et al., 2005), epididymis (Abe et al., 2005), chondrocytes (Tamayama et al., 2005), and lung (Yabumoto et al., 2008) and would support the possibility that the receptor may be functional outside

the brain. It is known that activation of $GABA_B$ receptors provokes diverse cellular and biochemical responses through modulation of adenylyl cyclase activity or Ca^{2+} or K^+ channels (Cunningham and Enna, 1996; Couve et al., 2000; Onali et al., 2003). Since the R1a subunit is transiently expressed at high levels in the developing brain (Benke et al., 1999), it has been considered that $GABA_B$ receptors play a role in the developmental process (Behar et al., 2001; Mukherjee et al., 2006). Recent findings regarding the GABAergic system in non-neuronal peripheral cells suggest that GABA contributes to cell proliferation via $GABA_B$ receptors in osteoblasts (Fujimori et al., 2002) and in murine embryonal carcinoma-derived ATDC5 cells (Tamayama et al., 2005). In rat testis, GABA may contribute to spermatogenesis via $GABA_B$ receptors (Kanbara et al., 2005). The proliferation modalities of the two types of synovial lining cells are not well understood. In humans, fibroblast-like B cells proliferate locally in synovial tissue (Edwards, 1982), while macrophage-like A cells originate in bone marrow, and are constantly replaced via circulation (Cutolo et al., 1993). In this context, it is difficult to relate the $GABA_B$ receptor expression in macrophage-like A cells to cell proliferation in normal synovial membrane. In a diseased state, such as rheumatoid arthritis, the situation of the synovial lining cells should be changed substantially. In rheumatoid arthritis, the synovial lining thickens. The increase in cell number is considered to be due to a combination of cellular infiltration by chronic inflammatory cells and hyperplasia of resident cells (Cutolo et al., 1993). Evidence suggests that the rate of cell division of fibroblast-like B cells does not increase in rheumatoid synovial lining compared to that of normal lining (Mohr et al., 1975). On the contrary, 80 to 100% of rheumatoid synovial membrane is composed of macrophage-like A cells (Kouri et al., 1984; Cutolo et al., 1993; Van Lent et al., 1995). Nakano (1988) reported that the rat synovial membrane of adjuvant-methylated bovine serum albumin-induced arthritis contains a significantly higher amount of GABA when compared with that of the normal rat. Nakano also measured the GABA content in synovial membranes obtained from patients with rheumatoid arthritis or osteoarthritis, and showed that synovial membrane from rheumatoid arthritis patients contained a significantly higher amount of GABA than that from osteoarthritis patients. These facts may suggest that one of the possible functions of the GABAergic system in the synovial membrane is macrophage-like A cell growth.

On the other hand, Schrier et al. (2001) states that Gabapentin and Pregabalin (structural analogs of GABA) are useful to treat inflammatory diseases, including arthritis. The mechanism of these GABA analogs in inflammatory disease is obscure at present, but it has been shown that although Gabapentin does not bind with high affinity to $GABA_A$ receptors (Taylor, 1994; Taylor et al., 1998), it acts on $GABA_B$ receptors (Ng et al., 2001; Bettrand et al., 2003; Parker et al., 2004). Recently, a hypothesis was advanced to the effect that GABA may play a role in downregulating mechanisms that lead to the production of proinflammatory agents, such as interleukins and matrix metalloproteinase, via p38 MAP kinase pathway inhibition (Kelley et al., 2008). The signaling mechanisms from the activation of the $GABA_B$ receptor to regulation of MAP kinase were proposed by Ortega (2003) and Kelley et al. (2008). As to the metalloproteinase, we showed that GABA increased the invasive ability of prostate cancer cell line C4-2 (Azuma et al. 2003). The mechanism is thought to be that the expression of matrix metalloproteinases is substantially elevated by stimulation of $GABA_B$ receptors in the cancer cells by the GABA produced in cancer cells themselves. The same result was obtained in renal carcinoma cell line Caki-2, and its underlying mechanism involves activation of MAP kinase, including ERK1/2, JNK, and p38 (Inamoto et al., 2007). It is known that fibroblast-like B cells, which have no $GABA_B$ receptors, are responsible for the production of MMPs (Murphy et al., 2002; Davis, 2003; Pakozdi et al., 2006), but a recent report suggests that MMP-12 (a macrophage elastase is a possible candidate) in the pathogenesis of rheumatoid arthritis (Chen, 2004).

In summary, we demonstrated that the GABA production system and $GABA_B$ receptors are both present in the synovial membrane of the normal rat knee joint. These data provide a foundation for further studies to elucidate the precise nature of the function of the GABAergic system in synovial membrane.

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