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CELLULAR AND SUBCELLULAR DISTRIBUTIONS OF β1- AND β2-ADRENOCEPTORS IN THE CA1 AND CA3 REGIONS OF THE RAT HIPPOCAMPUS

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Abstract—β-Adrenoceptors (ARs) in the hippocampus play an important role in regulating synaptic plasticity and memory consolidation. However, little is known about the distributions of β-ARs in the hippocampus, especially in the cornu ammonis (CA)1 and CA3 regions of Sprague-Dawley rats. Here, we report that β1- and β2-ARs in the CA1 and CA3 regions have differential subcellular distributions. Using double immunofluorescence labeling and confocal laser scanning microscopy, we found that almost all of the neuronal nuclei positive cells express β1- and β2-ARs, while few glial fibrillary acidic protein positive cells express them. Interestingly, β1-ARs are predominantly distributed in the cell membrane and cytoplasm, whereas β2-ARs are predominantly distributed not only in the membrane and cytoplasm, but also in the nucleus. The differential subcellular distribution of β1- and β2-ARs may have functional significance. **© 2007 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: β-adrenoceptor, CA1, CA3, double immunofluorescence labeling, confocal laser scanning.

It is known that most norepinephrine (NE)-containing neurons arise from the locus coeruleus (LC) and project multiple branched axons throughout the CNS (Moore and Bloom, 1979). Because of the rich and widespread projections of adrenergic axons, it is thought that NE plays a modulatory role in global aspects of brain functions, including regulation of the sleep–wake cycle (Berridge and Waterhouse, 2003), electroencephalographic graphic activity (Foote et al., 1983), vigilance (Aston-Jones and Bloom, 1981), body temperature (Philipson, 2002), and learning and memory (Kobayashi and Kobayashi, 2001).

NE and the endogenous hormone epinephrine act through a family of G-protein-coupled receptors, termed adrenergic receptors or adrenoreceptors (ARs), to produce physiological effects. Pharmacological and molecular-cloning studies have revealed the existence of three β-AR subtypes (β1, β2, and β3), based upon sequence homologies and affinity for subtype-selective AR antagonists. Although all β-AR subtypes are positively coupled to adenyl cyclase via activation of Gs, each subtype has its own unique pharmacological characteristics, particularly for AR agonists (Pupo and Minneman, 2001). All the three β-AR subtypes are found in the brain, but β3-AR subtype seems to have a very limited expression (Pupo and Minneman, 2001).

The hippocampus is structurally well-defined, consisting of a highly stratified pyramidal cell layer surrounded above and below by large numbers of widely-dispersed glia cells. The hippocampus receives a dense noradrenergic innervation from the LC (Loy, 1980). Immunolocalization studies have revealed NE transporter labeling in many regions of the hippocampus (Schroeter et al., 2000). It is documented that β-AR in the hippocampus are involved in long-term depression (LTD), long-term potentiation (LTP) and memory consolidation (Thomas et al., 1996a; Katsuki et al., 1997; Ji et al., 2003a; Scheiderer et al., 2004).

The existence of β-ARs in the hippocampus (Booze et al., 1993; Milner et al., 2000) and the importance of β-ARs in regulating synaptic plasticity and learning/memory function are well documented. Milner et al. (2000) investigated the cellular and subcellular locations of β-AR in the dentate gyrus (DG) of the hippocampus using light and electron microscopy. They found that β-AR-immunoreactivity is localized principally in postsynaptic sites on DG granule cells and interneurons as well as in glial processes. However, little is known about the cellular and subcellular distributions of β-AR, especially the β1- and β2-AR subtypes, in the cornu ammonis (CA)1 and CA3 regions of the hippocampus. In the present study, we employed immunofluorescence staining and confocal laser scanning microscopy to determine the cellular and subcellular distributions of β1- and β2-AR subtypes in the CA1 and CA3 regions of the adult hippocampus. Immunohistochemical markers specific for mature neuron, astrocyte and karyon were used to localize the β-AR expressing cells.

EXPERIMENTAL PROCEDURES

All experimental protocols involving the use of the rats in the present study were in compliance with the *Guide for the Care and Use of Laboratory Animals* issued by National Institutes of Health, and were approved and monitored by the Ethical Committee of Animal Experiments at the Fudan University Institute of Neurobi-

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Abbreviations: AC, adenylyl cyclase; AR, adrenergic receptor or adrenoceptor; BSA, bovine serum albumin; CA1, CA3, cornu ammonis 1, 3; DAPI, 4′-6-diamidino-2-phenylindole; DG, dentate gyrus; FISH, florescence *in situ* hybridization; GFAP, glial fibrillary acidic protein; HEK, human embryonal kidney; LC, locus coeruleus; LTP, long-term potentiation; NE, norepinephrine; NeuN, neuronal nuclei; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PKA, protein kinase A; TBST, Tris-buffered saline with Tween.

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ology (Shanghai, China). All efforts were made to minimize the number of animals used and their suffering.

The primary antibodies against β1- and β2-ARs were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). In order to confirm the specificity of the antibodies, we conducted both positive and negative control experiments. For positive controls, we transfected and expressed β1- and β2-AR cDNAs in human embryonal kidney (HEK)293 cells. We also conducted antigen-competition experiments using specific blocking peptides. For negative control, we performed immunofluorescence staining by omitting the primary antibodies.

cDNA clones for β1- and β2-ARs

To isolate RNA, the hippocampus was dissected and lysed in Trizol (Invitrogen, Carlsbad, CA, USA), and then separated by addition of chloroform and centrifugation at 12,000$\times g$ for 15 min (4 °C). The aqueous phase was isolated, and RNA was then precipitated with isopropanol and pelleted by centrifugation for 15 min at 12,000$\times g$ (4 °C). The RNA pellet was washed with 75% ethanol and dissolved in diethylpyrocarbonate (DEPC)-treated water. Four micrograms of RNA was subjected to reverse transcription (RT) with oligo dt (Promega, Madison, WI, USA) as primer and expanded with M-MLV (Invitrogen). The rat β1- and β2-AR cDNAs were amplified by PCR using pyrobest (Takara Bio, Japan) with the following primers: β1-AR, sp ATGGGCGC-GGGGGCGCT, as CTACACCTTGGACTCGGAGGAGAAGCC; β2-AR, sp ATGGAGCCACACGGGAATG, as CTACAGTGGC-GAGTCGTTTGTG. The polymerase chain reaction (PCR) fragment, flanked by *Eco*RI and *Xho*I (Takara Bio) which were provided by the PCR primers, was cloned in frame into *Eco*RI and *Xho*I of 5′Flag-pcDNA3. The sequencing analysis revealed that β1-AR cDNA had two mutations (i.e. 162 leucine to serine and 267 threonine to serine) and β2-AR cDNA was completely correct.

Transfection and expression

HEK293 cells were seeded at a density of 2.0$\times$10^5 cells per well in 24 well plates. Transfection was performed 24 h later with Lipofectamine (Invitrogen). Briefly, 0.5 μg DNA and 1 μl lipofectamine, each diluted in 50 μl opti-mem (Invitrogen), were mixed and incubated for 20 min. The mixed solution was added into the plates after replacement of the medium with 0.3 ml opti-mem. After incubation at 37 °C with 5% CO_2 for 5 h, the medium was changed to Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS). The cells were harvested 24 h post-transfection and subjected to Western blotting analysis.

Western blot analysis

Western blotting was performed on total cellular extract from the transfected HEK293 cells, to detect the specificity of antigen-antibody interaction. Samples (20 μg protein per lane), after being quantified, were loaded, subjected to 12% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred electrophoretically onto polyvinylidene fluoride (PVDF) membrane (Roche Diagnostics Corporation, Indianapolis, IN, USA) using an electrophoresis system (Bio-Rad, Hercules, CA, USA) and a mini trans-blot electrophoretic transfer system (Bio-Rad). The membranes were blocked for 1 h at room temperature with 5% non-fat dry milk in Tris-buffered saline with Tween (TBST) buffer (20 mM Tris–HCl, pH 7.4, 137 mM NaCl, and 0.1% Tween-20) and then incubated overnight at 4 °C with the primary antibody against β1-AR (dilution 1:200; Santa Cruz Biotechnology, Inc.) or β2-AR (dilution 1:500; Santa Cruz Biotechnology, Inc.). The blots were washed three times for 10 min each in TBST and incubated for 1 h at room temperature with horseradish peroxidase–conjugated donkey anti-goat IgG (1:2000 dilution, Pierce Biotechnology, Inc., Rockford, IL, USA). Immunostaining was revealed by enhanced chemiluminescent substrate (Pierce Biotechnology, Inc.). The mouse anti-Flag (M2) monoclonal antibody (1:2000 dilution, Sigma Chemical Company, St. Louis, MO, USA) was used to identify the expression of β1- and β2-ARs. Expression of β-tubulin was assessed by using its specific monoclonal antibody (Sigma Chemical Company). The specificity of β1- and β2-AR immunoreactive bands was confirmed by using the specific antigenic peptides (sc-568P for β1-AR and sc-569P for β2-AR, Santa Cruz Biotechnology, Inc.), against which the β1- and β2-AR antibodies had been raised. To estimate the molecular weight of aimed proteins, a pre-stained marker (Bio-Rad) was used.

Hippocampal slice preparation

Hippocampal slices were prepared from adult male albino rats (Sprague–Dawley rats) weighing 220~250 g. Briefly, animals, maintained under 12-h light/dark cycle for at least 3 days, were anesthetized with sodium pentobarbital (40 mg/ml, i.p.) and transcardially perfused with 0.9% NaCl solution pre-heated at 37 °C, followed by fixation with 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (PB) at 4 °C. Brains were removed from the skull and stored at 4 °C in 30% sucrose solution until they sank. Brains were frozen sectioned using a sliding microtome (Leica, Nussloch, Germany) into 20-μm coronal sections and collected in 0.01 M PBS.

Double immunofluorescence staining

For labeling of β1- and β2-ARs, the specific rabbit polyclonal antibodies (Santa Cruz Biotechnology, Inc.) were used as the primary antibodies (at working dilutions of 1:500 for β1-AR; 1:50 for β2-AR). The specificity of the primary antibodies was assessed by using their specific antigenic peptides (sc-568P for β1-AR and sc-569P for β2-AR; Santa Cruz Biotechnology, Inc.), against which the antibodies had been raised. Mouse anti-neuronal nuclei (NeuN) monoclonal antibody (1:100 dilution, Chemicon, Temecula, CA, USA) and mouse anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (1:1000 dilution, Sigma Chemical Company) were used for labeling neurons and astrocytes, respectively. Free-floating sections were washed in 0.01 M phosphate-buffered saline (PBS, pH 7.4) for 10 min and blocked in 6% donkey serum (v/v) in PBS plus 1% bovine serum albumin (BSA) (w/v) and 0.2% Triton X-100 overnight at 4 °C. Single labeling experiments were performed by incubating these sections with the primary antibodies for 3 days at 4 °C in a medium containing 5% normal donkey serum (v/v), 0.2% Triton X-100 and 1% BSA (w/v). After washing three times with PBS (10 min each), binding sites of the primary antibodies were revealed by incubating with the secondary antibodies for 2 h at 4 °C in the same medium as used in incubation with the primary antibodies. For double immunofluorescence labeling, the sections were incubated sequentially in a mixture of the two primary and secondary antibodies. Texas Red dye-conjugated affinipure donkey anti-rabbit IgG (1:100 dilution, Jackson Immunoresearch Laboratories, West Grove, PA, USA) was used to immunolabel β-AR, whereas donkey anti-mouse IgG tagged with fluorescein isothiocyanate (FITC) (1:100 dilution, Jackson Immunoresearch Laboratories) to immunostain neurons and astrocytes. Control experiments were performed by omitting the primary antibodies during the incubation. Before mounted on gelatin-coated glass slides, brain sections were rinsed in 4′-6-diamidino-2-phenylindole (DAPI), a nuclei marker of natural cells, for 1~2 min in order for fluorescent complexes with double-stranded DNA to form.

Fluorescently-labeled sections were visualized with a confocal laser scanning microscope (Leica SP2, Mannheim, Germany) using a 40$\times$ and 63$\times$ oil-immersion objective lens. Single optical

sections were taken and recorded digitally. To avoid any possible reconstruction stacking artifact, double labeling was precisely evaluated by sequential scanning on single-layer optical sections.

Identification of β1- and β2-ARs in cytoplasm and nucleus

Hippocampi were dissected and stored frozen at −80 °C. To isolate cytoplasmic component from nuclear one, hippocampal tissue was mechanically homogenized, treated with a nuclear protein extraction kit (Beyotime Biotechnology, Wuhan, China) and centrifuged at 3400 r.p.m. for 10 min at 4 °C. The cytoplasmic and nuclear components were then subjected to Western blotting. Expression of β-tubulin and CREB, as markers for cytoplasm and nucleus, was assessed by using their specific monoclonal antibodies (Sigma Chemical Company). The specificity of β1- and β2-AR immunoreactive bands was confirmed by using the specific antigenic peptides (sc-568P for β1-AR and sc-569P for β2-AR, Santa Cruz Biotechnology, Inc.), against which the β1- and β2-AR antibodies had been raised.

RESULTS

Determination of specificity of β1- and β2-AR antibodies

To assess antibody specificity, we transfected HEK293 cells with the pcDNA3 vector bearing 5′ flag-tagged β1- or β2-AR cDNA, and with pcDNA3 vector as a control. Western blotting was performed on total cellular extract from the transfected cells, to detect the specificity of antigen-antibody interaction.

Fig. 1 (*left panel*) shows the immunoblots of the constructed β1- and β2-AR clones expressed in the HEK293 cells. We detected the expression of the flag ligated to β1- or β2-AR to clarity the expression of the β1- and β2-AR clones. The β1- and β2-AR clones were then identified by the antibodies against β1- and β2-ARs. As shown, the same bands were recognized by the two pair of antibodies (anti-flag and anti-β1-AR antibodies, and anti-flag and anti-β2-AR antibodies). The β1-AR antibody could not recognize the exogenously-expressed β2-AR, and the β2-AR antibody could not recognize the exogenously-expressed β1-AR., demonstrating the specificity of the β1- and β2-AR antibodies.

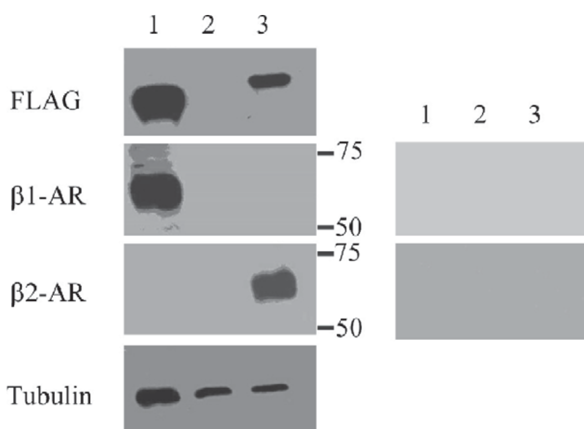

Fig. 1. Immunoblots of β1- and β2-AR clones expressed in the HEK293 cells. pcDNA3 vectors bearing 5′ flag tagged β1- or β2-AR cDNA were transfected into HEK293 cells. *Lane 1*: clone expressing flag-tagged β1-AR (65 kDa); *lane 2*: pcDNA3 vector control. *Lane 3*: clone expressing flag-tagged β2-AR (68 kDa). As shown, the same bands were recognized by the two pair of antibodies (antibodies for flag and β1-AR, and antibodies for flag and β2-AR). The β1-AR antibody did not recognize β2-AR and the β2-AR antibody did not recognize β21-AR (*left*). The immunoreactivity for β1- or β2-AR was blocked by the pre-incubated specific antigenic peptide (*right*).

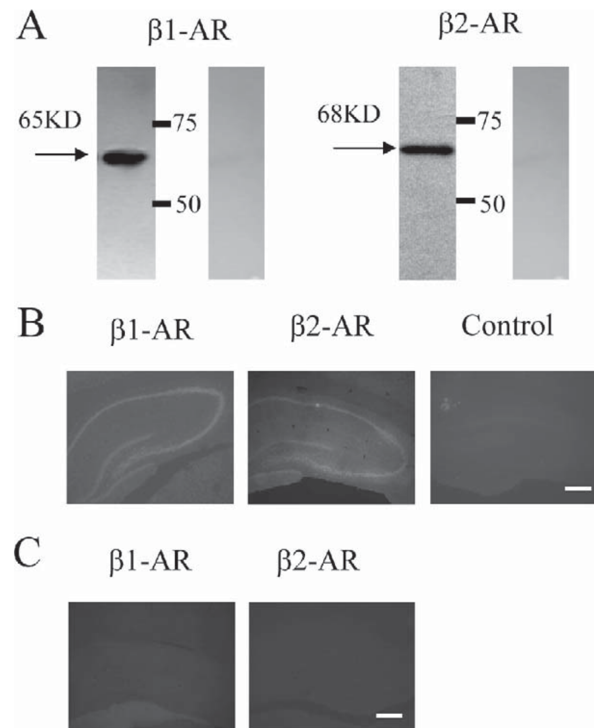

Fig. 2. Immunolabeling of the hippocampus with β1- and β2-ARs. (A) Western blot analysis of hippocampal extracts using the β1- and β2-AR antibodies. The immunoreactivity for β1-AR (65 kDa) or β2-AR (68 kDa) was blocked by the pre-incubated specific antigenic peptide. (B) Low-power images for β1- and β2-AR staining. Dominant staining was seen in the CA1 and CA3 regions, as well as in the DG region. The *right* panel is a control section treated with the secondary antibody but not with the primary antibody for β1- or β2-AR. (C) Low-power images for β1- and β2-AR staining under antigen-competition condition. Scale bar=400 μm.

To verify the specificity of the Western-blot bands for β1- and β2-ARs, we also performed antigen-competition experiments. The antibodies for β1- and β2-ARs were pre-incubated with their corresponding antigenic peptides, respectively. As shown in Fig. 1 (*right panel*), the specific antigenic peptides for β1- and β2-AR antibodies completely blocked the bands recognized by the antibodies.

Immunolabeling of β1- and β2-ARs in the hippocampus

Fig. 2A shows the Western blots for β1- and β2-ARs performed on total extracts from the hippocampus. As shown, the bands were recognized by the β1- and β2-AR antibodies, respectively (*left*). The antigen-competition experiment showed that the Western-blot bands recognized

by the β1- and β2-AR antibodies were completely blocked by the pre-incubated specific antigenic peptides (*right*).

Fig. 2B shows the florescence *in situ* hybridization (FISH) staining for β1- and β2-ARs in the hippocampal sections. The left and middle panels are the low-power fluorescent images for β1- and β2-AR staining. Dominant staining was seen in the pyramidal cell layer of the CA1 and CA3 regions and in the granular cell layer of the DG region. The right panel is a control section treated with the secondary antibody, but not with the primary antibodies. The absence of fluorescence in this control section indicates that the staining for β1- and β2-ARs was not due to the non-specific reactivity of the secondary antibody.

Fig. 2C shows the FISH staining for β1- and β2-ARs in the hippocampal sections under antigen-competition experimental condition. As shown, the specific blocking peptides completely blocked the immunofluorescent staining by the β1- and β2-AR antibodies.

Cellular distributions of β1- and β2-ARs in the CA1 region

The expressions of β1- and β2-ARs in CA1 neurons were investigated by double immunofluorescence labeling. To avoid stacking artifact by reconstruction, single layer optical sectioning was performed. Fig. 3A shows the representative staining of β1-AR and NeuN and Fig. 3B the staining of β2-AR and NeuN. As shown in the computer-merged images, almost all of the β1- or β2-AR positive cells are stained positively for NeuN, indicating that β1- and β2-ARs are expressed in CA1 neurons.

We also preformed immunofluorescence staining for β1- or β2-AR and GFAP. Fig. 3C shows the staining of

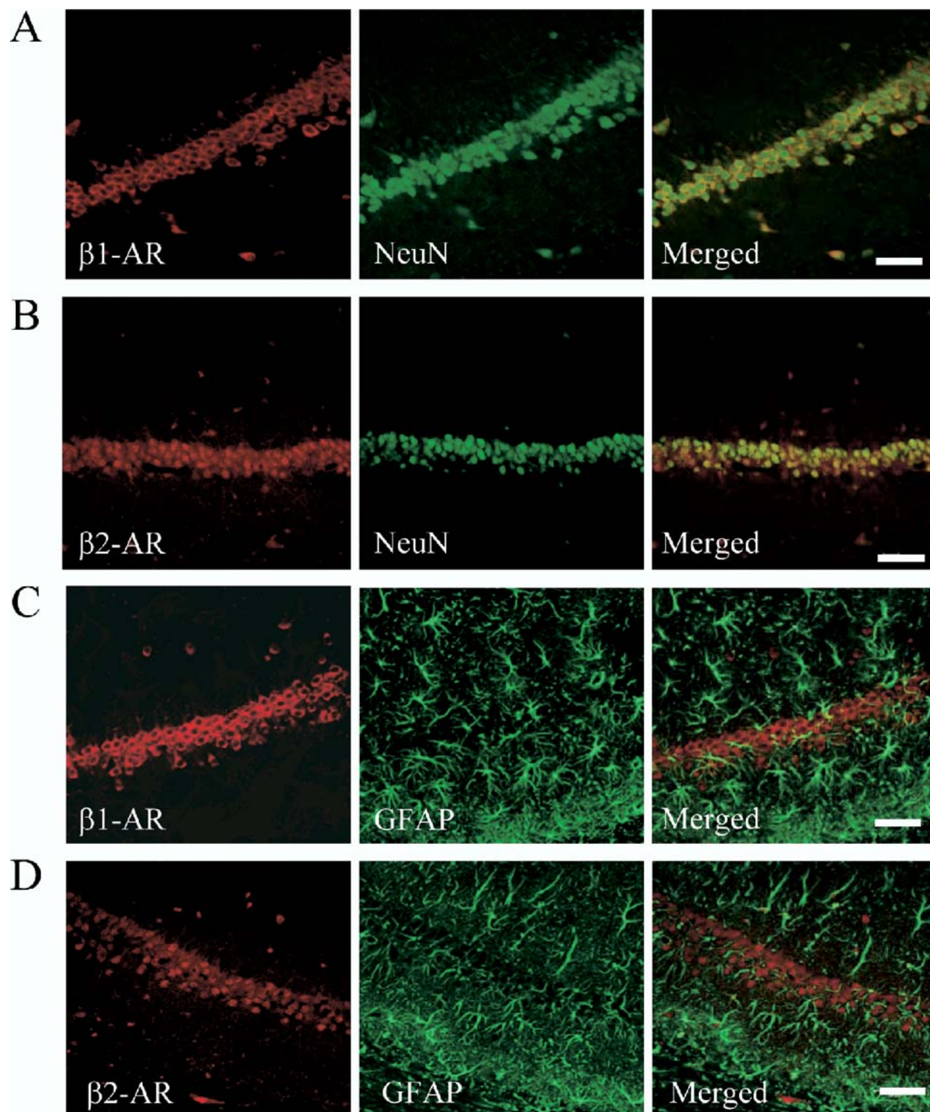

Fig. 3. Immunoreactivity of β1- and β2-ARs in the CA1 region. (A) Co-staining of β1-AR and NeuN. (B) Co-staining of β2-AR and NeuN. (C) Co-staining of β1-AR and GFAP. (D) Co-staining of β2-AR and GFAP. Double-stained cells appear in yellow in the merged images. Scale bar=50 μm.

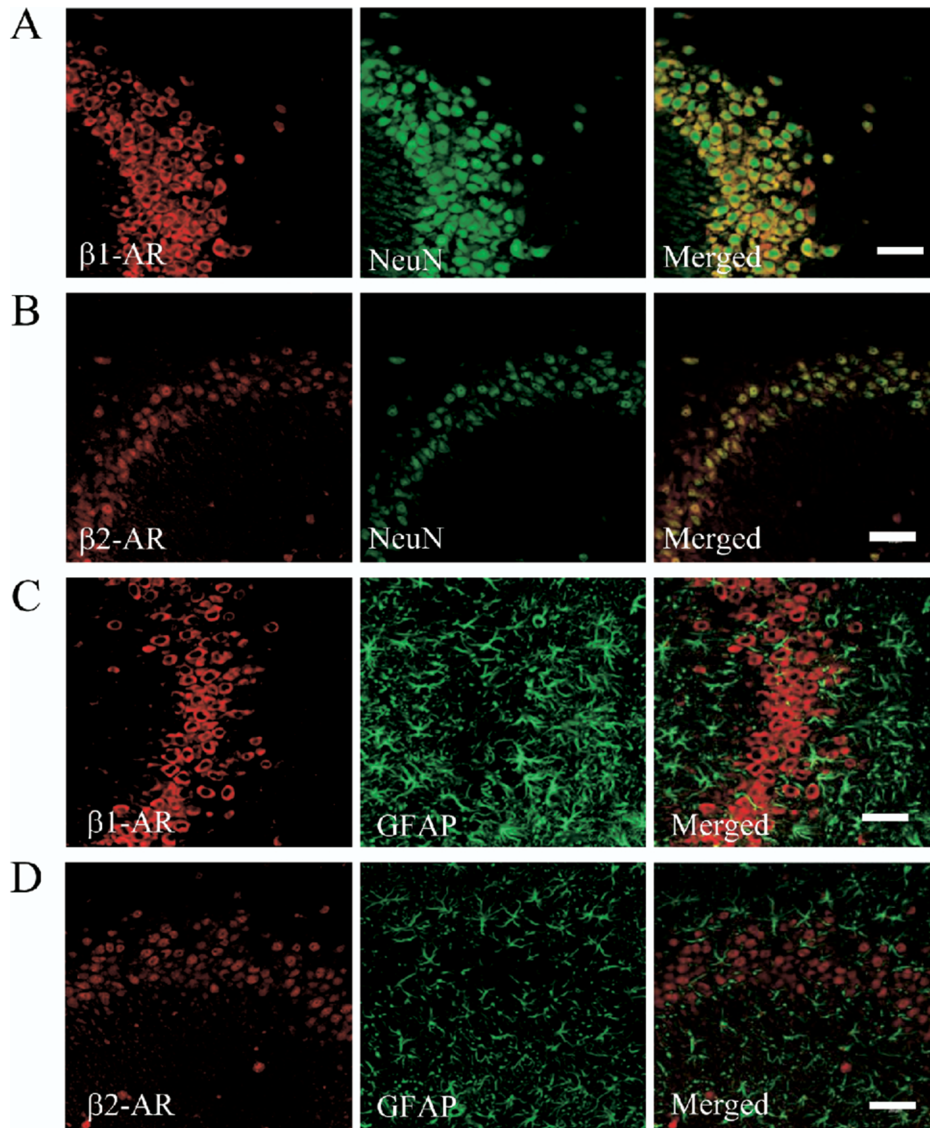

Fig. 4. Immunoreactivity of β1- and β2-ARs in the CA3 region. (A) Co-staining of β1-AR and NeuN. (B) Co-staining of β2-AR and NeuN. (C) Co-staining of β1-AR and GFAP. (D) Co-staining of β2-AR and GFAP. Double-stained cells appear in yellow in the merged images. Scale bar=50 μm.

β1-AR and GFAP, and Fig. 3D the staining of β2-AR and GFAP. As indicated by the computer-merged images, few β1- or β2-AR positive cells were stained positively for GFAP, indicating that β1- and β2-ARs are not expressed in CA1 astrocytes.

Cellular distributions of β1- and β2-ARs in the CA3 region

The distributions of β1- and β2-ARs in area CA3 were also investigated by double immunofluorescence labeling. The staining of β1-AR and NeuN was displayed in Fig. 4A, and that of β2-AR and NeuN in Fig. 4B. As shown by the merged images, almost all of the β1- or β2-AR positive cells were stained positively for NeuN, as in the CA1 region. This indicates that β1- and β2-ARs are expressed in CA3 neurons.

Fig. 4C shows the staining of β1-AR and GFAP, and Fig. 4D the staining of β2-AR and GFAP. As seen in the merged images, few β1- or β2-AR positive cells were stained positively for GFAP, as in the CA1 region. These results indicate that β1- and β2-ARs are not expressed in CA3 astrocytes.

Subcellular distributions of β1- and β2-ARs in CA1 and CA3

We examined the cytoplasmic and nuclear distributions of β1- and β2-ARs in both CA1 and CA3 regions. DAPI was used as a marker for karyon. Fig. 5A shows the representative staining of β1-AR and DAPI and Fig. 5B the staining of β2-AR and DAPI in the CA1 region. Fig. 5C shows the representative staining of β1-AR and DAPI and Fig. 5D the staining of β2-AR and DAPI in the CA3 region. As shown

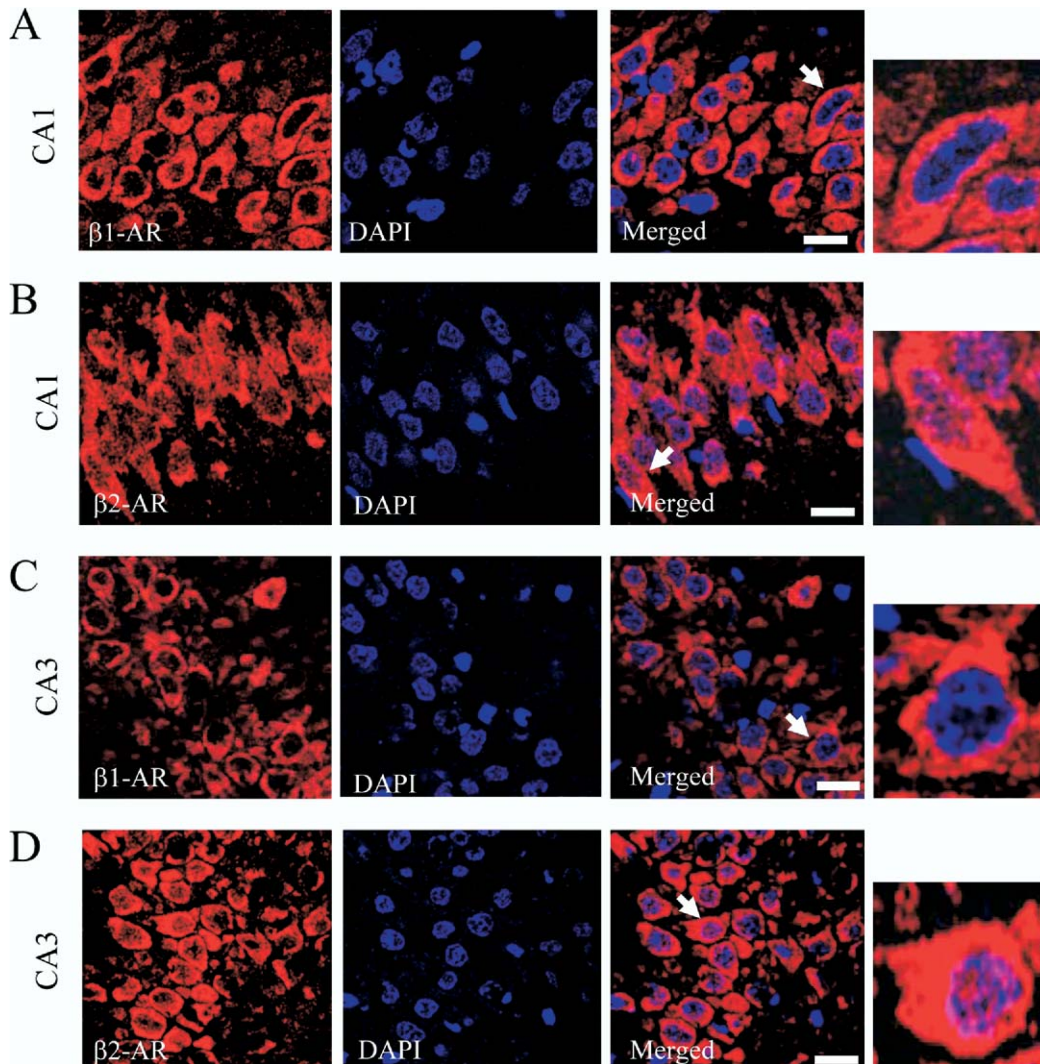

Fig. 5. Immunoreactivity of β1-AR, β2-AR and DAPI in the CA1 and CA3 regions. (A) Co-staining of CA1 for β1-AR and DAPI. (B) Co-staining of CA1 for β2-AR and DAPI. (C) Co-staining of CA3 for β1-AR and DAPI. (D) Co-staining of CA3 for β2-AR and DAPI. The *right-most* panels are the digitally-enlarged images for neurons stained with β1- and β2-ARs. Double-stained cells appear in purple. Arrows in the merged images indicate neurons digitally enlarged in the right-most images. Scale bar=20 μm.

in the merged images, β1-AR is always expressed in the membrane and cytoplasm, with no positive staining in the nucleus. Interestingly, β2-AR is localized not only in the membrane and cytoplasm, but also in the nucleus. Presented on right-most panel are the digitally-enlarged images for neurons stained with β1- and β2-ARs in the CA1 and CA3 regions.

The differential subcellular distributions of β1- and β2-ARs were further confirmed by Western blot analysis. Fig. 6 shows that the cytoplasmic and nuclear components extracted from the hippocampus express proteins stained by the antibodies for β1-AR (65 kDa), β2-AR (68 kDa), tubulin and CREB. As shown, β1-AR was expressed in the cytoplasm but not in the nucleus, and β2-AR not only in the cytoplasm but also in the nucleus. Tubulin was used as a cytoplasmic marker and CREB as a nuclear marker. The Western-blot bands recognized by the β1- and β2-AR antibodies were totally blocked by pre-incubation with the specific blocking peptides (Fig. 6, *right*).

DISCUSSION

The present study shows that 1) β1- and β2-ARs are expressed in neurons but not in astrocytes in the CA1 and CA3 regions, and 2) β1-AR is distributed in the cell membrane and cytoplasm, and β2-AR not only in the membrane and cytoplasm but also in the nucleus.

The antibodies for β1- and β2-ARs used in the present study are affinity-purified rabbit polyclonal antibodies raised against peptides mapping at the C-terminus with 45% homology for β1- and β2-ARs. These antibodies have been previously used by other authors to detect the expression of β1- and β2-ARs (Naren et al., 2003; Liang and Fishman, 2004; Rouget et al., 2006). In the present study, we performed both positive and negative control experi-

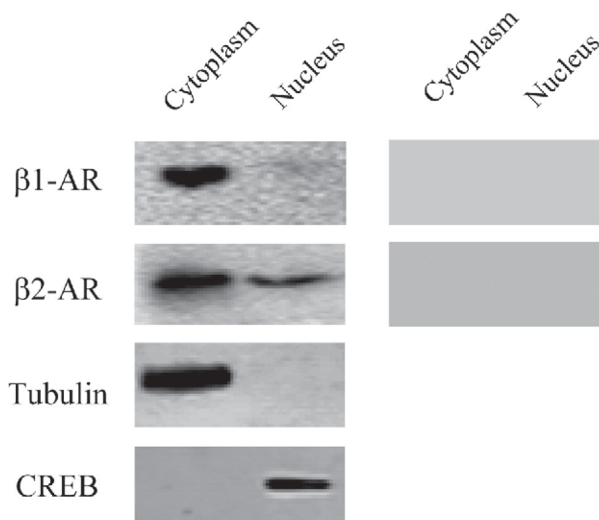

Fig. 6. Western blot analysis of cytoplasmic and nuclear components for β1- and β2-ARs. Tubulin and CREB were used as specific markers for the cytoplasm and nuclear components, respectively. β1-AR could be detected from the cytoplasmic component (65 kDa), and β2-AR from both the cytoplasmic and nuclear components (68 kDa; *left*). The immunoreactivity for β1- or β2-AR was blocked by the pre-incubated specific antigenic peptide (*right*).

ments and demonstrated the specificity of the β1- and β2-AR antibodies. The cDNA clone and exogenous-expression experiment in HEK293 cells showed that β1-AR was not recognized by the β2-AR antibody and β2-AR not by the β1-AR antibody. The antibody specificity was further confirmed by antigen-competition experiment using the specific blocking peptides. Furthermore, the negative control experiment with omission of the primary antibodies for β1- and β2-ARs indicated that the staining for β1- and β2-ARs was not due to the non-specific reactivity of the secondary antibody.

For the cDNA clone and exogenous-expression experiment, the constructed β1-AR plasmid had two mutations: 162 leucine to serine and 267 threonine to serine. The linear structures of expressed β1-AR included about 470 amino acids, as revealed by Western-blotting. The two mutations were not located at the C-terminal of β1-AR and produced no impact on the antigen-antibody reactivity in the C-tail.

Nicholas et al. (1993) reported that both β1- and β2-ARs reside in the rat hippocampus, with β1-AR mRNA a light labeling and β2-AR mRNA a moderate labeling in the CA1 stratum pyramidale. Duncan et al. (1991) reported that the ventral subiculum and entorhinal cortex in the rat have the highest density of β-AR. Jurgens et al. (2005) reported that β1-AR labeling was present on the pyramidal cells in the hippocampus and the heaviest labeling was observed along the plasma membrane and within the cytoplasm of cells in the CA3 region. Zhu and Kimelberg (2004) found that β-AR is expressed in astrocytes freshly isolated from the CA1 region of postnatal 9–10 day rats. In the present study, we did not find any positive staining of β1- or β2-AR in astrocytes in the CA1 and CA3 regions of adult rats. It is possible that acute isolation performance in the previous study by Zhu and Kimelberg (2004) altered gene expression pattern of astrocyte, inducing β-AR expression. Indeed, evidence shows that manipulation with brain may induce change in expression pattern for some proteins (Cremin and Smith, 2002).

β-AR (β_1, β_2, β_3) is G protein-coupled receptors (GPCRs) that mediate physiological responses to NE. β-AR is positively coupled to adenylyl cyclase (AC) through Gs proteins (Daly et al., 1981). When activated, AC triggers production of cAMP from ATP. cAMP activates protein kinase A (PKA). Activated PKA phosphorylates many pre- and post-synaptic protein substrates, as well as intracellular proteins. It is known that the β-AR–cAMP–PKA signaling pathway in the hippocampus plays an important role in regulating synaptic transmission, synaptic plasticity and memory formation (McGaugh, 2000; Sara, 2000; Kandel, 2001). For example, stimulation of β-AR in the hippocampus lowers the threshold for induction of E-LTP (Thomas et al., 1996a,b; Katsuki et al., 1997) and elicits a type of L-LTP that requires ERK activation and protein synthesis (Gelinas and Nguyen, 2005). Blockade of β-AR in the CA1 region impairs consolidation of contextual fear memory and water-maze spatial memory (Ji et al., 2003a,b).

Importantly, the present study revealed for the first time the differential distributions of β1- and β2-ARs in subcellular structures of hippocampal CA1 and CA3 neurons. β1-AR is predominantly distributed in the cell membrane and cytoplasm, and β2-AR not only in the membrane and cytoplasm but also in the nucleus. The existence of β2-AR in NeuN strongly suggests a different and unique functional significance of this receptor subtype. To fully understand this, it is necessary to answer where the nuclear β2-ARs come from. Here, we propose two possibilities that remain to be demonstrated. First, some β2-ARs synthesized in the cytoplasm are trafficked onto the membrane to mediate adrenergic response, whereas other β2-ARs are translocated into the nucleus to regulate gene expression. Second, β2-ARs in the cell membrane are internalized into the cytoplasm after binding with its ligands such as NE and epinephrine and then translocated to the nucleus, which might be a novel signaling pathway for transduction of extra-cellular physiological signals to the nucleus.

CONCLUSION

In summary, the present study provides immunohistochemical evidence showing that hippocampal CA1 and CA3 neurons but not astrocytes express β1- and β2-ARs, with β1-AR distributed in the cell membrane and cytoplasm, and β2-AR not only in the membrane and cytoplasm but also in the nucleus.

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