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Gender difference in BK channel expression in amygdala complex of rat brain

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ABSTRACT

The expression of large-conductance Ca^{2+}-activated K^+ (BK) channel protein in amygdala complex was higher in adult (8–10 weeks old) male rats than in female. Castration at 4–6 weeks old significantly reduced BK channel expression in amygdala to the level similar to that in female. Immunocytochemical analyses of pyramidal-like neurons isolated from amygdala revealed that somas with relatively large size were highly immunoreactive to both anti-androgen receptor (AR) and anti-BK channel antibodies, while those with smaller size were not. The double-immunopositive neurons were dominant (60%) among pyramidal-like neurons isolated from amygdala of male rats but rare among those from female. The membrane current sensitive to penitrem A, a BK channel blocker, was the major K^+ current component in large neurons and showed higher current-density than that in smaller ones. These results suggest the gender-dependent cell population expressing BK channels in amygdala complex and its up-regulation by AR stimulation.

Large-conductance Ca^{2+}-activated K^+ channel (BK channel) is a common regulator of membrane excitability in various types of cells [1]. BK channel is composed of tetrametric complex of the pore-forming α subunit (BKα) and also of the auxiliary β subunit (BKβ). The deficiency of BKα in mice causes abnormality in smooth muscle tissues such as hypertension, bladder overactivity, erectile dysfunction, and also dysfunction of pacemaker output from suprachiasmatic nucleus and circadian rhythm [2–4].

Amygdala is a complex of some nuclear groups, the basolateral nuclear group, the centromedial group, and other smaller groups. The basolateral amygdala is part of the limbic system that plays a key role in assigning emotional significance to sensory input, and has been shown to be particularly important for emotional learning, such as fear conditioning [5]. BK channel is functionally expressed in pyramidal-like neurons in rat basolateral amygdaloid complex and substantially contributes to outward K^+ currents, modulation of action potential duration and the Ca^{2+}-dependent regulation of electrical behavior in the neuron [6,7]. Medial amygdala plays a key role in the process transforming sensory cues into the particular motor patterns and the neuroendocrine events characteristic of sex-specific social behaviors and shows steroid-dependent plasticity [8]. The involvement of BK channels in these neuronal activities in amygdala still remains to be determined.

The activity and/or expression of BK channel can be modulated by steroid hormones as both long-term genomic and acute non-genomic effects. Female sex hormones, progesterone and estrogen regulates BK channel expression, the alternative splicing of BKα, so called STREX variants, and the membrane surface clustering of BK channel in uterine smooth muscles, particularly during pregnancy [3,9]. 17β-Estradiol enhances BK channel activity as acute effects and also up-regulates BKα mRNA expression in aorta but not in brain [10]. This study was undertaken to examine whether circulating testosterone in adult rats regulates functional expression of BKα in amygdara, one of steroid hormone sensitive regions of brain. We found that the protein expression of BKα in amygdala is gender-dependent in adult rats and higher in male rats than female.

Materials and methods

Animal and tissue preparation. Intact and castrated male and female adult rats (8–10 weeks old, Wistar-ST) were decapitated. Castration, when applicable, was performed 4 weeks prior to use. The brain were removed rapidly from the skull and placed in ice-cold oxygenated saline containing (in mM) KCl 2.4, $MgSO_4$ 10, $CaCl_2$ 0.5, piperazine-N,N′-bis(ethanesulfonic acid) (PIPES) 20, glucose 10, and sucrose 195, pH 7.35. Coronal slices (500 μm in thickness) were cut from a block of tissue containing the amygdala on a microslicer (DTK-1000, DOSAKA EM, Kyoto, Japan). The amygdaloid complex was dissected under a stereo microscope. Pituitary were obtained from this slice. Pyramidal cells were enzymatically isolated from amygdala complex. Tissue pieces of amygdala complex were incubated in a stirring chamber at 33 °C in oxygenated medium of the following composition (mM): NaCl 132.5, KCl 5, $MgCl_2$ 3, $CaCl_2$ 0.25, PIPES 20, dextrose 25, pH adjusted to 7.35 with NaOH. After incubation with enzyme (1 mg/ml trypsin, Sigma type

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XI, and 0.5 mg/ml bovine serum albumin), tissue was rinsed several times with enzyme-free solution and kept at 23 °C until further use. Neurons were mechanically dissociated by trituration of a single slice with fire-polished Pasteur pipettes and transferred into a recording chamber.

Real-time PCR. Real-time quantitative PCR was performed with the use of Syber Green chemistry on an ABI 7000 sequence detector (Applied Biosystems). Unknown quantities relative to the standard curve for a particular set of primers were calculated, yielding transcriptional quantitation of gene products relative to the endogenous standard (β-actin). The following PCR primers were used for real-time PCR analysis: BKα (GenBank Accession No. NM_031828, 408–509, 102 bp) , BKβ1 (NM_019273, 592–694, 103 bp) , BKβ2 (NM_176861, 547–655, 109 bp) , BKβ3 (NM_001066117, 370–471, 102 bp) , BKβ4 (NM_023960, 439–546, 108 bp) , AR (NM_012502, 2548–2669, 122 bp) , β-actin (NM_031144, 338–438, 101 bp).

Western blotting. Proteins (50 μg/lane) of the rat amygdala, pituitary, and hippocampus were subjected to SDS–PAGE and were then transferred to PVDF membrane. The blots were immunoblotted with the primary antibodies specific for AR (Perseus Proteomix), BKα, BKβ1 (Alomone Labs) and β-actin (Sigma). Resulting images were analyzed by a LAS-1000 (FUJIFILM, Tokyo, Japan) and the digitized signals were quantified with Image Gauge software (Ver. 3.3, FUJIFILM).

Immunocytochemistry. After fix and permeabilization, single pyramidal cells of rat amygdala complex were exposed to AR (1:50 dilution, Perseus Proteomix), anti-BKα, anti-BKβ1, anti-BKβ2, anti-BKβ4 (1:50 dilution, Alomone Labs) or anti-BKβ3 (1:50 dilution, Abnova) antibodies and then the cells were labeled with Alexa Fluor 488 anti-rabbit IgG and Alexa Fluor 633 anti-mouse IgG (1:200 dilution, Molecular Probes). After removing excess secondary antibody, digital images were analyzed on a laser scanning confocal microscope (LSM510, Carl Zeiss) [11,12].

Electrophysiology. Whole-cell voltage clamp was applied to single cells with patch pipettes using a CEZ-2300 amplifier (Nihon Kohden). Normal HEPES-buffered solution having the following composition was used as the external solution (mM), NaCl 137, KCl 5.9, $CaCl_2$ 2.2, $MgCl_2$ 1.2, glucose 14, and HEPES 10 (pH 7.4). $CdCl_2$ (100 μM) was added in the external solution. The pipette solution contained (mM), KCl 140, $MgCl_2$ 2.8, ATP-2Na 5, EGTA 0.05, and HEPES 10. Adding $CaCl_2$ and KOH adjusted the pCa and pH of the solution to 6.0 and 7.2. All experiments were done at room temperature (23 ± 1 °C). Data acquisition and analysis were carried out using the software (AQ and cell soft) developed in Dr. Giles laboratory [12].

Results

Regional expression of AR and BK channel in rat brain

The mRNA expression of AR and BKα in amygdala complex, pituitary, and hippocampus in male rat brain was examined by quantitative real-time PCR analyses (Fig. 1A). The expression of AR and BKα mRNAs was detected in these regions and BKα mRNA expression was apparently higher than that of AR. The mRNA expression analyses of BK channel β subunit isoforms (BKβ1, β2, β3, and β4) revealed that BKβ4 was predominant isoform in amygdala complex of male rats (Fig. 1B and C).

The expression of BKα and AR proteins was examined in cells freshly isolated from amygdala complex by immunocytochemical methods, using a confocal fluorescent microscope. Co-expression of BKα and AR proteins was observed in most of cells, which had pyramidal shape and relatively large size (>10 μm in diameter) (Fig. 1D, see also Fig. 3). In AR-positive cells, the protein expression of BKβ1 in addition to BKβ4 was also observed but neither BKβ2 nor BKβ3 was detected (Fig. 1E).

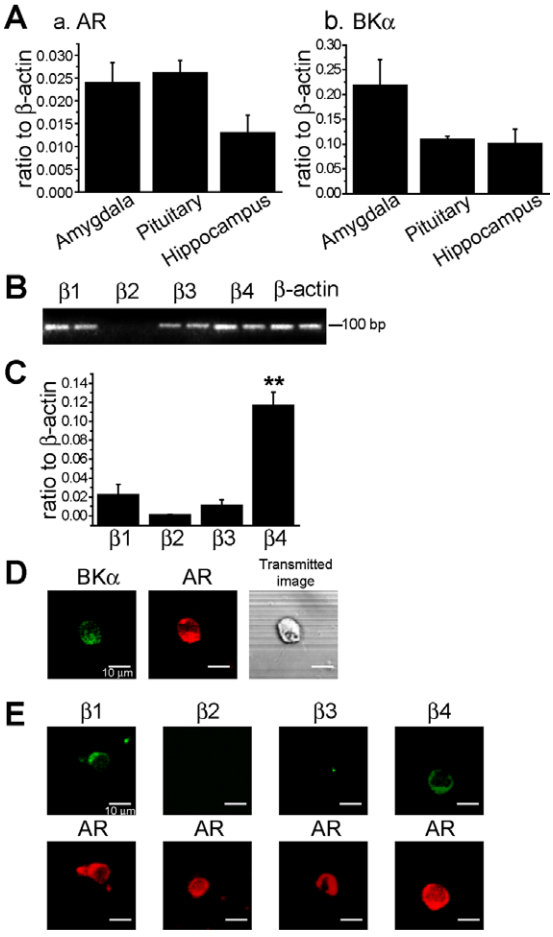

Fig. 1. The Expression of BKα, BKβ1∼4 subunits and AR in three separate regions of rat brain. (A) Real-time PCR analysis of AR, BKα in three separate regions in brain (amygdala, pituitary, hippocampus) from male rats ($n = 4$ for each). (B) The transcriptional expression of BKβ1∼4-subunits and β-actin was examined by RT-PCR in amygdala from male rats. (C) Relative expression of the BKβ1∼4-subunits by real-time PCR in rat amygdala as means ± SE. $^{**}P < 0.01$. (D) Confocal images of a pyramidal-like cell isolated from amygdala of male rat indicated specific immunoreactivity to both BKα (green) and AR (red). The bars represent 10 μm. (E) Images of immunoreactivity to BKβ1∼4 (green) and AR (red) from pyramidal-like cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Gender difference of and castration-induced effects on BK channel expression in amygdala

Next, the gender difference of BK channel expression in amygdala was examined by Western blotting (Fig. 2A). The expression of BKα protein was significantly higher in amygdala of male rats than that in female ($P < 0.05$) (Fig. 2A,b). Moreover, effects of castration on BKα in amygdala were examined in male rats (Fig. 2B). Castration was performed in male rats and the amygdala was dissected for Western blotting analysis 4 weeks after the castration. BKα protein expression in amygdala of castrated rats was significantly reduced in comparison with that in sham-operated rats ($P < 0.05$) (Fig. 2B,b). Castration-induced decrease in BKα protein expression was also observed in pituitary ($P < 0.01$ vs. SHAM) but not in hippocampus ($P > 0.05$ vs. SHAM) (Fig. 2C).

Cell population expressing BK channel and AR among those isolated from amygdala

The population of amygdala cells expressing BK channel and AR was compared between male and female rats. The immunoreactivity to anti-BKα and anti-AR antibodies was examined (Fig. 3A).