Effects of presenilins and β-amyloid precursor protein on delayed rectifier potassium channels in cultured rat hippocampal neurons[1]

Wei ZHANG, Hong-wei JIN, Xiao-liang WANG[2]

Department of Pharmacology, Institute of Materia Medica,
Peking Union Medical College and Chinese Academy of Medical Science, Beijing 100050, China

KEY WORDS presenilin-1; presenilin-2; amyloid beta-protein precursor; potassium channels; transfection; patch-clamp techniques

ABSTRACT

AIM: To study the effects of presenilin-1 (PS-1), presenilin-2 (PS-2), and amyloid beta-protein precursor (APP695) on delayed rectifier potassium channels (I_K) in the cultured rat hippocampal neurons. **METHODS:** PS-1, PS-2, and APP695 were transfected into the cultured rat hippocampal neurons by transient transfection techniques. The I_K current was observed by the whole cell patch-clamp techniques. **RESULTS:** I_K was increased in cultured rat hippocampal neurons, after transient transfection of PS-1, PS-2, and APP695. I_K amplitudes and densities were significantly increased from (1689±412) pA, (48±18) pA/pF (mock cells, GFP alone, n=17) to (5565±1403) pA, (252±107) pA/pF (PS-1/GFP, n=22, P<0.01), (3804±1651) pA , (120±58) pA/pF(PS-2/GFP, n=16, P<0.01), and (4978±904) pA, (218±70) pA/pF (APP695, n=22, P<0.01). But PS-1, PS-2, and APP695 did not alter the activation curve of I_K (P>0.05). **CONCLUSION**: Overexpression of PS-1, PS-2, and APP695 increased I_K in the cultured rat hippocampal neurons. The upregulation of I_K may be related to neuronal apoptosis after PS-1, PS-2, and APP695 were transfected.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. The neuropathology of AD is characterized by extensive neuronal loss and deposition of senile plaques and neurofibrillary tangles in the cerebral cortex and hippocampus. Molecular insights into AD pathogenesis have arisen from genetic studies in families affected by inherited forms of AD (FAD). These account for only a small percentage of AD cases but have allowed the identification of mutations in three different genes that are responsible for triggering the disease. These genes are the *presenilins*-1 and -2 (PS-1 and PS-2) and the amyloid precursor protein (APP)[1].

Many experiments show that overexpression of some form of APP, as well as PS-1, PS-2 appear to induce apoptosis *in vivo* and *in vitro*, which is a fundamental mechanism of neurodegeneration in AD. Recently, Yu *et al* put forward that delayed rectifier K^+ current (I_K) plays a critical role in neuronal apoptosis[2,3].

[1] Project supported by the State Key Technologies R & D Programme (973), No 1998051106.
[2] Correspondence to Prof Xiao-liang WANG.
Phn 86-10-6316-5183. Fax 86-10-6301-7757.
E-mail wangxl@imm.ac.cn
Received 2003-01-10 Accepted 2003-07-03

In addition, some reports implicated that potassium current densities significantly increased when wild type PS-1 and PS-2 were transfected into HEK-293 cell and rat ventricular myocytesl[4]; whereas secreted APP also increased K^+ current in the hippocampal neurons[5]. Therefore, we speculated that I_K upregulation might also be involved in the neuronal apoptosis induced by overexpression of PS and APP. In order to further study the relationship between AD related genes and potassium channels, we transfected the PS-1, PS-2, and APP695 into the cultured rat hippocampal neurons using transient transfection techniques and observed the effects of PS-1, PS-2, and APP695 on I_K using the whole cell patch-clamp techniques.

MATERIALS AND METHODS

Cell culture Hippocampal neurons were prepared from 1-d-old fetal Wister rats according to Banker's *et al* methods (Animal Center of Chinese Academy of Medical Sciences)[6]. Briefly, dissected hippocampi were mechanically dissociated, spun down, and resuspended in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Berlin, Germany) supplemented with 10 % fetal bovine serum albumin (FBS Hyclone Laboratories, Logan, UT), 10 % horse serum (Hyclone Laboratories, Logan, UT), benzyl penicillin 100 kU/L, and streptomycin 100 mg/L. The cell suspension was seeded on poly-*D*-lysine-coated plastic dishes (Nunc, Wiesbaden, Germany) or coverslips at a density of $1\times10^5/cm^2$. After 3 d, cytosine arabinoside 5 μmol/L was added to inhibit the proliferation of non-neuronal cells. Cultures were kept at 37 ℃ in a humidified atmosphere of 95 % air 5 % CO_2. One-third of the culture medium was changed weekly.

Transient transfection Rat hippocampal neurons were cultured for 8 d to perform transient transfections[7]. Cells were transiently transfected with plasmids encoding pEGFP-N1 (1 μg), PS-1 (1 μg), PS-2 (1 μg) and APP695 (1 μg) using LipofectAMINE (Life Technologies, Inc). After 5 h of exposure, cells were added DMEM with 20 % FBS without removing the transfection mixture. Approximately 10-12 h after transfection, cells were washed once with normal growth medium and then incubated in normal growth medium under normal growth conditions before elctrophy-siological recordings. Parallel nontransfected cultures or cells transfected with GFP only (mock transfection) served as controls.

Electrophysiological recordings Whole cell patch-clamp recordings were applied for 10-14 d cultured neurons. The pipette solution contained (in mmol/L): KCl 140, $MgCl_2$ 1, egtazic acid 5, Na_2ATP 4, HEPES 10, pH adjusted to 7.2 with KOH. The external solution contained (in mmol/L): NaCl 140, KCl 5.4, $CaCl_2$ 2, $MgCl_2$ 1, 0 glucose 1, HEPES 10, pH adjusted to 7.4 with NaOH. The membrane currents were elicited by membrane depolarization from -50 to +40 mV for 250 ms for cultured neurons. The series resistance varied between 3-6 MΩ, and was always compensated by 60 %-80 % (HEKA Pusle 8.5). Data were acquired and stored on an IBM computer using an EPC-9 amplifier and the HEKA Pulse 8.5 software (HEKA Elektronik, Lambrecht, Germany). Pipettes were pulled from thin wall borosilicate glass capillaries on micropipette Puller (Narrishage, Japan). The pipette tip resistance was 3-5 MΩ, when the pipettes were filled with the pipette solution and placed into perfusion solution.

Plasmids resource and drugs PS-1 and PS-2 plasmid were provided by Prof Dennis J SELKOE (University of Harvard), pEGFP-N1 plasmid was provided by Prof Michael A KIEBLER (European Molecular Biology Laboratory), and APP695 plasmid was provided by Prof Christoph KAETHER (University of Heidelberg). Zeocin was kindly provided by Dr Huiliang LI (Department of Pathogen Biology, Chinese Academy of Medical Science and Peking Union Medical College). All the other chemicals were of AR grade.

Statistics All data were analyzed by HEKA Puslefit 8.5 and Ori5.0 software, and were expressed as mean±SD. Statistical significances were analyzed by ANOVA and *t*-test.

RESULTS

Effects of PS and APP695 on densities of I_K current in cultured hippocampal neuronal cells Transfected cells were identified by IX-70 fluorescence microscope (Olypmus, Japan) equipped for epifluorescence with a B2A filter assembly to observe the GFP fluorescence of transfected cells (Fig 1). Overexpression of wild type PS-1, PS-2, and APP695 significantly increased the amplitudes and density of I_K currents at +40 mV in GFP-positive neurons when compared with mock cells (GFP transfected alone) (Fig 2, 3, Tab 1) ($P<0.01$).

Effect of PS and APP695 on activation of I_K current in cultured hippocampal neuronal cells On

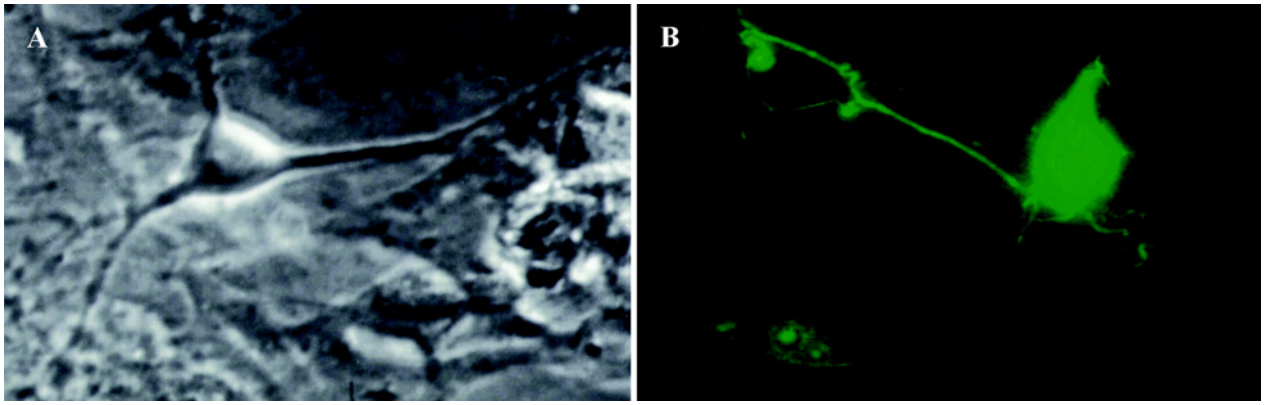

Fig 1. Appearance of transfected hippocampal neurons. (A) neurons are captured under phase-contrast microscope. (B) neurons transiently transfected with GFP, and captured under fluorescence microscope.

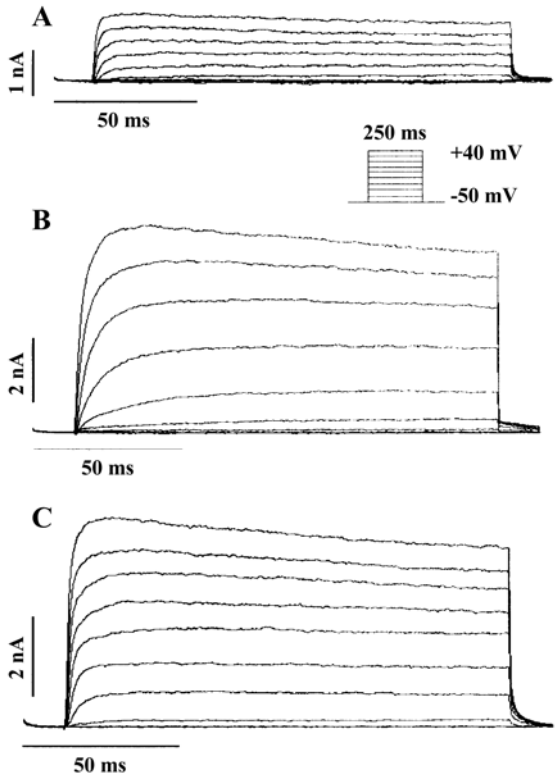

Fig 2. Effect of transfection of wild-type PS-1, wild type PS-2 on I_K in the cultured rat hippocampal neurons. (A) a mock (GFP alone)-transfected; (B) a GFP/PS-1 transfected; (C) a GFP/PS-2 transfected hippocampal.

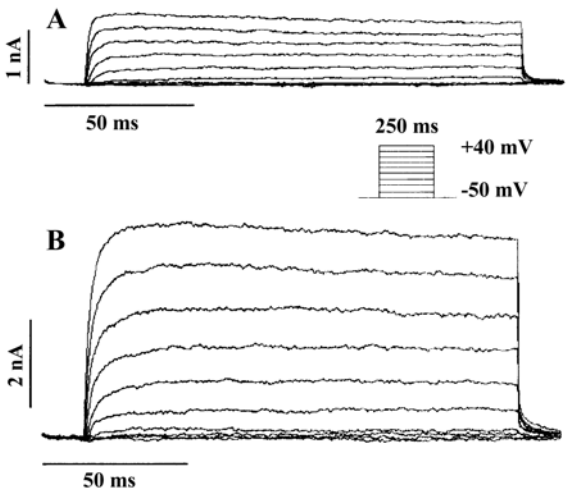

Fig 3. Effect of transfection of APP695 on I_K in the cultured rat hippocampal neurons transfected. (A) a mock (GFP alone)-transfected, (B) a APP695 transfected hippocampal.

the basis of data obtained from current voltage relationship, activation curves of I_K current in cultured hippocampal neuronal cells were determined before and after transient transfection of wild type PS-1, PS-2, and APP695. The activation curve was fitted by the Boltzmann equation $G/G_{max}=1/[1\text{-exp}\ (V\text{-}V_{1/2})/k]$, where G is the membrane conductance at potential V, $V_{1/2}$ is the half activation voltage, k is the slope factor. Overexpression of PS and APP695 had no effect on the steady-state activation curve of I_K current in the rat hippocampal neurons (Fig 4, Tab 1) (P>0.05).

DISCUSSION

The present work reveals that overexpression of PS and APP significantly increased I_K current in cultured rat hippocampal neurons. The upregulation of I_K is related to neuronal apoptosis, which was found in our study.

It is well known that K^+ channels play an important role in maintaining neuronal excitability. Accordingly, alternations of neuronal K^+ channels may lead to

Tab 1. Effects of the PS-1, PS-2, and APP on I_K current in the cultured rat hippocampal neurons. Mean±SD. $^bP<0.05$ *vs* control.

	Steady-state current/pA	I_K density /pA·pF^{-1}	$V_{1/2}$/mV	*k*/mV	*n*
Control	1568±289	39±16	5.5±2.4	12.6±1.3	20
Mock	1689±412	48±18	5.4±2.5	12.6±1.4	17
PS-1	5565±1403^b	252±107^b	4.6±2.9	10.1±2.9	22
PS-2	3804±1651^b	120±58^b	4.9±2.9	12.6±2.2	16
APP695	4978±904^b	218±70^b	3.9±2.1	12.2±3.6	22

$V_{1/2}$, potential for half-maximal activation; *k*, slope factor; *n*, number of cell.

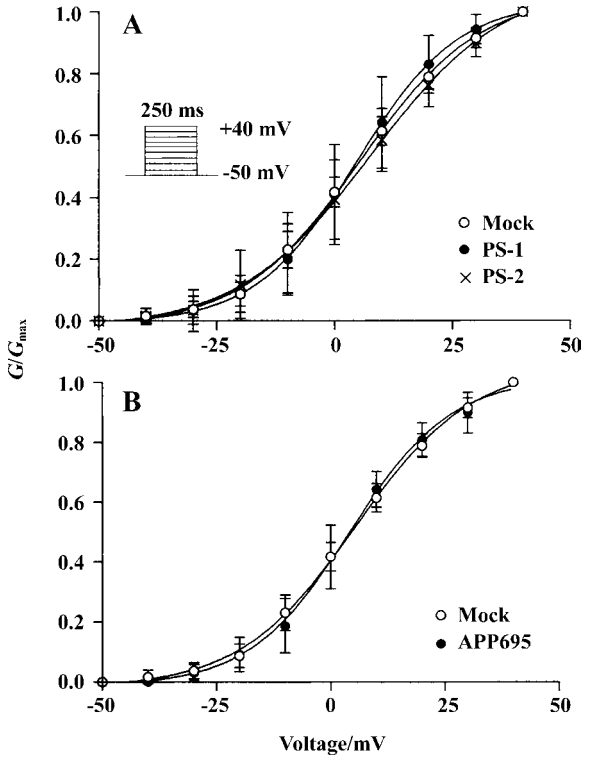

Fig 4. Effect of wild-type PS-1 (*n*=16), wild type PS-2 (*n*=22), and APP695 (*n*=19) on the activation curves of I_K currents in the cultured rat hippocampal neurons.

profound changes in excitability and subsequent cell apoptosis or death. Several years ago, Etcheberrigaray *et al* proposed there existed a link between AD and K^+ channels. They discovered that there was K^+ channel dysfunction in fibroblasts obtained from patients with AD[8], and this dysfunction could be mimicked in normal fibroblasts by the addition of β-amyloid[9]. In addition, some investigations suggested the delayed rectifier K^+ channel was upregulated in apoptosis mediated by β-amyloid in cortical neurons and cholinergic spetal cell line[3,10]. TEA (K^+ channel blocker) or raising extracellular K^+ could attenuate cell death induced by β-amyloid[3,10]. Therefore, increase of I_K current play an important role in triggering neuronal apoptosis.

The presenilins as polytopic membrane protein mutated in the majority of early onset of FAD. Both wild type and H115Y mutant form of PS-1 enhance Fas-mediated apoptosis in Jurkat cells[11]. PS-2, a close homologue of PS-1 also plays a pivotal role in cellular apoptosis. Overexpression of full length wild type PS-2 in neuronal cells increased apoptosis induced by trophic withdrawal and β-amyloid[12-14]. Recently, Malin *et al* reported that potassium current densities significantly increased when wild type PS-1 and PS-2 were transfected into HEK-293 cell and rat ventricular myocytes, whereas the expression of wild type PS-1 in COS-7 cells did not increase outward K^+ currents[4]. Based on the multiple membrane-spanning topology of presenilins, it was proposed that presenilins might function as parts of a channel, transporter or pore[15,16]. In our study we also discovered that overexpression of wild–type PS-1 and PS-2 in hippocampal neurons resulted in a significant increase of outward K^+ current densities. However, the mechanism of I_K upregulation after overexpression of PS is still unclear. The most possible interpretation of these results is that the presenilins do not actually form K^+ channels, but rather that these proteins upregulate functional K^+ channel expression either directly by associating with K^+ channel pore-forming subunits or indirectly by increasing the synthesis, assembly, and/or transportation of these subunits to the plasma membrane.

APP is the source of β-amyloid peptide, which is a major component of the amyloid deposits characterized in AD. The intracellular accumulation of wild type-APP in the rat hippocampus caused a specific type of neuronal degeneration *in vivo*, and the overexpression

of wild-type APP mediated by viral vector induced neurons apoptosis both *in vivo* and *in vitro*[17]. Recently, Furukawa *et al* found that the secreted form of APP (sAPP) was released from membrane-spanning APP by electrical stimulation of neural circuits in hippocampal slices, and sAPP has been shown to stabilize intracellular calcium concentrations in the cultured hippocampal neurons[5]. Additionally, sAPP also reduced the frequency of spontaneous postsynaptic current (SSC) in *Xenopus* spinal motoneurons. And the reduction of SSC frequency by sAPP may be mediated by activation of potassium channels through a cGMP-dependent pathway[18]. In this study, we also found that I_K current was significantly increased by over expression of wild type APP695 in hippocampal neurons. Further studies are required to clarify the precise mechanism of the overexpression of APP on I_K current.

Our present study shows that overexpression of APP and PS in neurons might impair cellular K^+ homeostasis through special I_K channels. And the K^+ unbalance disturbs normal metabolism of neurons and renders neurons vulnerable to apoptosis. But it still need further study which kind of I_K channel subunit is involved in such upregulation and what is the precise mechanism that APP and PS regulate I_K channel. However, the present study provides a new clue to investigate the pathogenesis of AD.

ACKNOWLEDGMENT We thank Prof Dennis J SELKOE, Prof Michael A KIEBLER, Prof Christoph KAETHER for kindly providing plasmids, Dr Hui-liang LI for kindly providing zeocin, Prof Michael A KIEBLER and Dr Paolo MACCHI for kindly suggestions and help on transfection experiment.

REFERENCES

1 Hardy J. New insights into the genetics of Alzheimer's disease. Ann Med 1996; 28: 255-8.
2 Yu SP, Yeh CH, Sensi SL, Gwag BJ, Canzoniero LM, Farhangrazi ZS, *et al*. Mediation of neuronal apoptosis by enhancement of outward potassium current. Science 1997; 278: 114-7.
3 Yu SP, Farhangrazi ZS, Ying HS, Yeh CH, Choi DW. Enhancement of outward potassium current may participate in beta-amyloid peptide-induced cortical neuronal death. Neurobiol Dis 1998; 5: 81-8.
4 Malin SA, Guo WX, Jafari G, Goate AM, Nerbonne JM. Presenilins upregulate functional K^+ channel currents in mammalian cells. Neurobiol Dis 1998; 4: 398-409.
5 Furukawa K, Barger SW, Blalock EM, Mattson MP. Activation of K^+ channels and suppression of neuronal activity by secreted beta-amyloid-precursor protein. Nature 1996; 379: 74-8.
6 Banker GA, Cowan WM. Rat hippocampal neurons in dispersed cell culture. Brain Res 1977; 126: 397-402.
7 Martin K, Wulf H, Indradeo H, Christoph K, Volkmar JL, Michael AK. Fast, convenient and effective method to transiently transfect primary hippocampal neurons. J Neurosci Res 1999; 58: 831-5.
8 Etcheberrigaray R, Ito E, Oka K, Tofel-Grehl B, Gibson GE, Alkon DL. Potassium channel dysfunction in fibroblasts identifies patients with Alzheimer disease. Proc Natl Acad Sci USA 1993; 90**:** 8209-13.
9 Etcheberrigaray R, Ito E, Kim CS, Alkon DL. Soluble beta-amyloid induction of Alzheimer's phenotype for human fibroblast K^+ channels. Science 1994; 264: 276-9.
10 Colom LV, Diaz ME, Beers DR, Neely A, Xie WJ, Appel SH. Role of potassium channels in amyloid-induced cell death. J Neurochem 1998; 70**:** 1925-34.
11 Wolozin B, Alexander P, Palacino J. Regulation of apoptosis by presenilin 1. Neurobiol Aging 1998; 19: S23-7.
12 Alves da Costa C, Paitel E, Mattson MP, Amson R, Telerman A, Ancolio K, *et al*. Wild-type and mutated presenilins 2 trigger p53-dependent apoptosis and down-regulate presenilin 1 expression in HEK293 human cells and in murine neurons. Proc Natl Acad Sci USA 2002; 99: 4043-8.
13 Araki W, Yuasa K, Takeda S, Takeda K, Shirotani K, Takahashi K, *et al*. Pro-apoptotic effect of presenilin 2 (PS2) overexpression is associated with down-regulation of Bcl-2 in cultured neurons. J Neurochem 2001; 79: 1161-8.
14 Araki W, Yuasa K, Takeda S, Shirotani K, Takahashi K, Tabira T. Overexpression of presenilin-2 enhances apoptotic death of cultured cortical neurons. Ann N Y Acad Sci 2000; 920: 241-4.
15 Clark RF, Hutton M, Fuldner RA, Froelich S, Karran E, Talbot C, *et al*. The structure of the presenilin 1 (S182) and identification of six novel mutations in early onset AD families. Nature Genet 1995;11: 219-22.
16 Li X, Greenwald I. Membrane topology of the C. elegans SEL-12 presenilin. Neuron 1996; 17:1015-21.
17 Nishimura I, Uetsuki T, Kuwako K, Hara T, Kawakami T, Aimoto S, *et al*. Cell death induced by a caspase-cleaved transmembrane fragment of the Alzheimer amyloid precursor protein. Cell Death Differ 2002; 9:199-208.
18 Morimoto T, Ohsawa I, Takamura C, Ishiguro M, Nakamura Y, Kohsaka S. Novel domain-specific actions of amyloid precursor protein on developing synapses. J Neurosci 1998; 18: 9386-93.