Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 373 (2005) 99–104
Neuroscience Letters
www.elsevier.com/locate/neulet

# Delayed rectifier potassium currents and Kv2.1 mRNA increase in hippocampal neurons of scopolamine-induced memory-deficient rats

Zhong Chong-Bo, Pan Ya-Ping, Tong Xiao-Yong, Xu Xiang-Hua, Wang Xiao-Liang*

*Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking Union Medical College, 1 Xian Nong Tan Street, Beijing 100050, China*

Received 22 June 2004; received in revised form 25 September 2004; accepted 28 September 2004

**Abstract**

To explore the ionic mechanisms of memory deficits induced by cholinergic lesion, whole-cell patch clamp recording techniques in combination with single-cell RT-PCR were used to characterize delayed rectifier potassium currents ($I_K$) in acutely isolated hippocampal pyramidal neurons of scopolamine-induced cognitive impairment rats. Scopolamine could induce deficits in spatial memory of rats. The peak amplitude and current density of $I_K$ measured in hippocampal pyramidal neurons were increased from $1.2 \pm 0.6$ nA and $38 \pm 19$ pA/pF of the control group ($n = 12$) to $1.8 \pm 0.5$ nA and $62 \pm 24$ pA/pF ($n = 48$, $P < 0.01$) of the scopolamine-treated group. The steady-state activation curve of $I_K$ was shifted about 8 mV ($P < 0.01$) in the direction of hyperpolarization in scopolamine-treated rats. The mRNA level of Kv2.1 was increased ($P < 0.01$) in the scopolamine-treated group, but there was no significant change of Kv1.5 mRNA level. The present study demonstrated for the first time that $I_K$ was enhanced significantly in hippocampal pyramidal neurons of scopolamine-induced cognitive impairment rats. The increase of Kv2.1 mRNA expression in hippocampal pyramidal cells might be responsible for the enhancement of $I_K$ and could be the ionic basis of the memory deficits induced by scopolamine.


*Keywords:* Potassium current; Scopolamine; Patch clamp; Cholinergic dysfunction; Kv2.1; Single-cell RT-PCR

Alzheimer's disease (AD), a progressively neurodegenerative disorder causing dementia in the elderly, is characterized by the deposition of beta-amyloid peptide (Aβ), neurofibrillary tangles, and the loss of neuronal synapses and pyramidal neurons in a variety of brain regions, particularly the content of acetylcholine (ACh) being reduced markedly [24]. It has been proposed that the degeneration of cholinergic neurons in the basal forebrain and associated loss of cholinergic neurotransmission in the neocortex and hippocampus may contribute significantly to the cognitive function deterioration observed in AD patients [8]. This hypothesis has been further supported in humans by recent clinic therapeutic trials with acetylcholinesterase inhibitor, such as tacrine and donepezil [21].

Because of these, a great deal of efforts has been focused on determining the cellular mechanisms involved in cholinergic modulation of hippocampal function. But little is known about the ionic mechanisms underlying the decreased excitability and memory loss during the cholinergic neurotransmission lesion. Potassium channels are known to be critical in regulating neuronal excitability and synaptic transmission. There has been a growing interest in the role of potassium channels in learning and memory processes. Several studies have demonstrated a key role of potassium channels in the mechanisms of learning and memory by applying different potassium channel modulators in various experimental models [11,18]. It has been reported by several, including our laboratories that an enhancement of outward $K^+$ current with delayed rectifier characteristics might play an important role in the pathogenesis of Aβ-induced neuronal death and related memory impairment [12,25]. Presenilins also could upregulate functional potassium channel currents in mammalian

* Corresponding author. Tel.: +86 10 6316 5193; fax: +86 10 6301 7757.
*E-mail address:* wangxl@imm.ac.cn (X.-L. Wang).

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.09.069

cells and result in profound changes in neuronal excitability and contribute to the cognitive decline commonly associated with AD [15]. All these reports indicate that potassium channel dysfunction might be related to clinical symptoms of cognitive decline in AD patients.

Scopolamine is one of classical antagonists at muscarinic ACh receptors (mAChRs). It could pass through the Brain–Blood Barrier and block muscarinic receptors throughout the brain including hippocampus and cerebral cortex. By blocking the conduction of cholinergic system, scopolamine could decrease neuronal excitability and induce memory impairment [7]. In the present study, scopolamine was used as a tool to mimic the downregulated cholinergic function. Under the condition, voltage-activated delayed rectifier $K^+$ currents ($I_K$) and mRNA expression of $K^+$ channel subunits in hippocampal pyramidal neurons of scopolamine-induced memory-deficient rats were analyzed by whole-cell patch clamp recording in combination with semi-quantitative single-cell reverse transcription (RT) followed by polymerase chain reaction (PCR) (single-cell RT-PCR).

The study was performed on male Wistar rats weighing 150–200 g, provided by Institute of Experimental Animal of Chinese Academy of Medical Science, according to the guidelines of the Chinese Academy of Medical Science for Institutional Animal Care, based on the National Institute of Health Guide for the care and use of laboratory animals. Rats were injected subcutaneously with saline ($n = 10$) or scopolamine (1 mg/kg, $n = 10$) per day for consecutive 7 days. From 4th day, the spatial memory test was done 30 min after the injection of saline or scopolamine. The procedure used for spatial memory test was a modification of that described by Morris [19]. For each training trial, the latency to escape onto the hidden platform was recorded. If the rat was unable to find the platform within 120 s, the training trial was terminated and a maximum score of 120 s was assigned. Each rat was given two trials per day for 4 days. The point of entry into the pool remained unchanged between trail 1 and trail 2, but was changed on each day.

After injected subcutaneously daily with saline or scopolamine for 7 days, hippocampal pyramidal neurons from the rats were dissociated acutely, using the modified methods of Kay and Wong [13]. In brief, the rats were decapitated, hippocampus were rapidly removed and submerged in ice-cold artificial cerebrospinal fluid (ACSF, in mM: NaCl 126, KCl 5, $NaH_2PO_4$ 1.25, $MgSO_4$ 2, $NaHCO_3$ 26, glucose 10, $CaCl_2$ 2, pH 7.2). Coronal slices (350–500 μm) were cut and incubated for 60 min in a chamber filled with oxygenated (95% $O_2$ + 5% $CO_2$) ACSF. Following the pre-incubation, the slices were placed in fresh oxygenated ACSF containing a low concentration of 0.05% trypsin for 30 min, followed by a 30-min incubation with 0.05% protease E at 32 °C. Slices were then washed in fresh oxygenated ACSF and maintained for up to 6 h. Immediately prior to recording, a slice of hippocampus was removed, and neurons were dissociated in ACSF by gentle triturating with fire polished Pasteur pipettes. Only neurons that exhibit the stereotypical morphology of pyramidal neurons and no visible evidence of injury will be selected for recording.

To measure $K^+$ outward currents of neurons, the patch clamp whole-cell recording technique was used with an EPC-9 patch clamp amplifier (HEKA, Germany) [10]. The extracellular solution consisted of (in mM): choline chloride 120, KCl 6, $MgCl_2$ 10, glucose 20, HEPES 10 (pH adjusted to 7.30 with KOH). Patch pipettes were pulled from borosilicate glass and had a tip resistance of 5–7 MΩ when filled with pipette solution containing (in mM): KCl 140, $MgCl_2$ 0.5, EGTA 10, HEPES 10, pH adjusted to 7.20 with KOH. All voltage protocols were applied to neurons via an IBM computer running Pulse + Pulsefit (version 8.5, HEKA, Germany) software. Series resistance compensation was routinely used to reduce the effective series resistance by about 70%. Outward currents were usually filtered at 3 kHz. Current recordings were not corrected for leak currents and capacitive components, which were very small, compared with the voltage-activated currents. All recordings were done at room temperature (22–24 °C).

All data were analyzed using Pulsefit software. Steady-state activation curves were fitted using the following Boltzmann equation: $G/G_{max} = [1 + \exp(V_{1/2} - V_{test})/k]^{-1}$ in which $G$ is the conductance value at each potential, $G_{max}$ is the maximal conductance. $G$ was calculated from the peak current with the equation: $G = I/(V - V_k)$, where $V$ is the step command voltage and $V_k$ is the estimated $K^+$ reversal potential, here it was taken to be –70 mV. $V_{1/2}$ is the membrane potential at which the current is half-activated. $V_{test}$ is the test potential, and $k$ is a factor describing the steepness of the activation curve.

Single-cell RT-PCR followed the strategy described by Seifert et al. [23] with modifications. After recording, the cell was harvested with a second pipette (diameter about 20 μm) under microscopic control. To maximize mRNA yields, we also aspirated some neurons without recording. Isolated neurons were patched in the cell-attached mode and lifted into a stream of control solution. Neurons then were aspirated into the electrode. Electrodes contained 8 μl of intracellular solution. The contents were then transferred into a 0.25-ml PCRP tube containing 2.5 μl of 5× RT buffer, 1 μl of 0.1 M dithiothreitor and 0.5 μl of RNasin (40,000 U/ml) by breaking the tip of the pipette and expelling about 5.5 μl solution under positive pressure. The tube was frozen and stored in liquid nitrogen until the RT reaction was carried out. To prevent amplification of genomic DNA, RT was preceded by a DNase step. Therefore, 0.5 μl DNase I was added to the tube, the mixture was incubated at 37 °C for 15 min to destroy any genomic DNA. The DNase was inactivated by heating the tube to 95 °C for 5 min. Single-strand cDNA was synthesized from the cellular mRNA by the addition of SuperScript II (0.5 μl, 200 U/μl), random primers (0.5 μl) and mixed dNTPs (1.5 μl, 2.5 mM of each), followed by incubation at 42 °C for 50 min. The reaction was terminated by heating the mixture at 70 °C for 15 min and then the samples were stored at −20 °C. The cDNA from RT of RNA in a single neuron was subjected