Thrombin modulates persistent sodium current in CA1 pyramidal neurons of young and adult rat hippocampus

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INTRODUCTION

The role of blood coagulation factor thrombin is well characterized in the processes of hemostasis, proliferation and inflammation [1]. In addition to these functions serine protease thrombin also acts as a hormone-like neuromediator and together with serpins regulates the protease-activated receptors (PAR) in the CNS [2]. PARs belong to the superfamily of 7-domain transmembrane G-protein coupled receptors which include four subtypes (PAR1-4). They are involved in the regulation of nerve cells morphology [3], and play an important role in learning and memory [4]. Through PAR serine proteases also regulate synaptic plasticity [5] and modulate neuronal activity [6]. Among four types of PAR family thrombin preferentially activates PAR1, which is also a major thrombin receptor in the brain [7]. The prominent expression of PAR1 mRNA is found in the hippocampus, as well as in the cerebellum, thalamic nuclei and cerebral cortex [8].

It was shown that in low concentrations thrombin exerts neuroprotective properties, while at high concentrations PAR1 activation leads to the damage of neurons and astrocytes [9]. Elevation of thrombin activity is marked after ischemic, hemorrhagic and traumatic brain injury (TBI) [9]. However, the molecular mechanism of thrombin-induced neuropathology is still remains unclear. Recent finding showed that thrombin activation of PAR1 leads to the epileptiform activity in CA3 area of hippocampal slices [10]. Our group revealed that one of the mechanisms that could contribute to the increased excitability produced by application of thrombin in CA3 pyramidal neurons is the
hyperpolarization shift of persistent sodium current ($I_{NaP}$) activation induced by PAR1-dependent pathway [11]. $I_{NaP}$ activates near resting membrane potentials and slowly inactivates during prolonged depolarizations. This TTX-sensitive sodium current have been shown to modulate subthreshold oscillations and is responsible for repetitive action potential generation [12]. $I_{NaP}$ regulates excitability of neurons in different brain areas and modulates synaptic plasticity contributing intrinsic pacemaking activity [13]. Up-regulation of $I_{NaP}$ in temporal lobe structures such as entorhinal cortex and hippocampus is known to be one of molecular mechanisms underlying epilepsy condition. For example, an increase of $I_{NaP}$ after status epilepticus (SE) contributes to prevalence of intrinsically bursting phenotypes among CA1 pyramidal neurons [14]. Our recent paper have also shown that using the potent PAR1 antagonist SCH79797 in adult rats after lithium-pilocarpine SE have neuroprotective and antiepileptogenic effect [15]. These findings suggest that thrombin through PAR1 signaling plays an important role in hyperexcitation and neuronal damage in CA1 area. We hypothesize that $I_{NaP}$ modulation could be possible mechanism of thrombin pathogenesis. Because studies of PAR1 participation in neuropathology of CA1 region were performed on adult rats and the study of the effect of thrombin on $I_{NaP}$ was focused on CA3 pyramidal neurons of young rats [16] we aimed to evaluate the effect of thrombin on $I_{NaP}$ obtained from isolated hippocampal CA1 pyramidal neurons of both ages.

METHODS

Experiments were carried out using Wistar rats P12-16 and P60-75. All experimental procedures were conducted in accordance with guidelines set by National Institutes of Health for the humane treatment of animals and approved by the Animal Care Committee of Bogomoletz Institute of Physiology. The experimental method was described previously in our paper [17]. Briefly, rats were anaesthetised with sevoflurane and after decapitation the brain was quickly transferred to ice-cold (0-4°C) solution contained (in millimoles per 1 L): NaCl – 130, KCl – 5, CaCl$_2$ – 0.1, MgCl$_2$ – 5, NaH$_2$PO$_4$ – 1, Na$_2$HPO$_4$ – 1, NaHCO$_3$ – 26, glucose – 10 (pH 7.35). Hippocampal slices (400 µm thick) were obtained using vibratome (Campden Instruments, Loughborough, UK) and were left for one hour (temperature 20-22°C) in the solution contained (in millimoles per 1 L): NaCl – 130, KCl – 5, CaCl$_2$ – 2, MgCl$_2$ – 2, NaH$_2$PO$_4$ – 1, Na$_2$HPO$_4$ – 1, NaHCO$_3$ – 26, glucose – 10 (pH 7.35). During these procedures solutions were bubbled with carbogen gas mixture (95% O$_2$/5% CO$_2$).

Enzymatic treatment of hippocampal slices was done during 15 min (32°C) with pronase E (2 mg/ml) in sucrose-based solution which contained (in millimoles per 1 L): sucrose – 290, KCl – 5, CaCl$_2$ – 0.5, MgCl$_2$ – 2, HEPES – 10, glucose – 15 (pH 7.35). Previously we have shown that enzymes used for cell dissociation do not alter $I_{NaP}$ kinetics [18]. After enzymatic treatment slices were washed in basic solution and vibrodissociated [19] in artificial cerebrospinal fluid (ACSF) with following content (in millimoles per 1 L): NaCl – 140, KCl – 5, CaCl$_2$ – 2, MgCl$_2$ – 2, HEPES – 20 (pH 7.35).

Electrophysiological experiments were done using patch-clamp technique in whole cell configuration using patch-clamp amplifier (A-M Systems, Calrsborg, WA). The resistance of pipettes was 3-4 MΩ. Pipettes were filled by intracellular solution (in millimoles per 1 L): CsF – 120, NaCl – 5, TEA-Cl – 30, EGTA – 10, TRIS – 10 (pH 7.2-7.3).

$I_{NaP}$ activation was induced by slow depolarizing voltage ramp (30 mV/s) from holding potential of -80mV to 0 mV. The effect of thrombin on $I_{NaP}$ was evaluated after 40 s of thrombin application. After registration recordings were analyzed using Origin 8.0 (“Origin Labs”, USA). Corrections of liquid junction potential errors were made as described.
The subtraction of sodium current traces evoked by ramp-protocol before and after 1 µM TTX reveals $I_{\text{NaP}}$. Voltage dependence of $I_{\text{NaP}}$ conductance ($G$) was calculated from

$$G = \frac{I}{V - E_N},$$

where $I$ is the amplitude of $I_{\text{NaP}}$ induced by potential $V$, and $E_N$ – calculated Nernst potential for Na⁺. Normalized activation curves were fitted by Boltzmann function. Statistical analysis was performed using Prism 5 (GraphPad, La Jolla, CA). All data are presented as mean ± standard error. Unpaired Student’s $t$ test and two-way ANOVA test were used for statistical analysis. Differences in intergroup comparisons were acknowledged statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

We estimated the effect of thrombin application (10 U/ml) on $I_{\text{NaP}}$ in isolated pyramidal CA1 neurons. Fig.1.A and Fig.2.A show representative current traces of $I_{\text{NaP}}$ registration in rats two weeks and two months old, respectively. In young rats (Fig.1.B) thrombin induced the hyperpolarization shift of $I_{\text{NaP}}$ activation ($V_{1/2}$ shifted from -49.6 ± 1.4 mV to -52.9 ± 0.8 mV, $n = 7$, $P < 0.005$). The same effect of thrombin was revealed in the group of adult rats ($V_{1/2}$ of $I_{\text{NaP}}$ activation shifted from -51.4 ± 1.0 mV to -54.1 ± 1.3 mV, $n = 7$, $P < 0.001$, Fig.2.B). The difference of the effect of thrombin on $I_{\text{NaP}}$ activation between age groups was not significant ($F(1, 12) = 0.97$, $P = 0.35$). Up-regulation of $I_{\text{NaP}}$ amplitude after thrombin exposure was more pronounced in the group of young rats (Fig.3). $I_{\text{NaP}}$ amplitude was increased from 30.7 ± 5.6 pA to 56.1 ± 7.6 pA (194 ± 16%, $P < 0.0005$, $n=7$) in young rats and from 68.7 ± 12.7 pA to 99.1 ± 14.9 pA (149 ± 7 %, $P < 0.0005$, $n=7$) in adult ones. The effect of age on up-regulation of $I_{\text{NaP}}$ amplitude, induced by thrombin, was significant ($F(1, 12) = 7.12$, $P < 0.05$).

In the current study we show that the effect of thrombin on $I_{\text{NaP}}$ is age-dependent. Interesting that expression of PAR1 decreases with age with the most marked decrease during a period between the second and third postnatal week [8, 21]. This finding could explain the age-related difference in the thrombin effect on $I_{\text{NaP}}$.

Our previous findings in hippocampal slices revealed that thrombin induces hyperpolarization shift of $I_{\text{NaP}}$ activation in CA3 pyramidal neurons. This effect is mediated by PAR1 and requires PKC activation. In the current study we use isolated neurons to reduce space clamp errors. We show that thrombin produces not only a change in activation kinetics but also a significant $I_{\text{NaP}}$ peak amplitude up-regulation. Such ef-
fect could be a basis for neuronal hyperexcitation and capability for long-lasting generating action potentials. For example, it was shown in sensory-motor cortex that depolarization shift of voltage dependency of inactivation and higher current density of $I_{NaP}$ in layer V compared to layer II/III was associated with generation of long depolarization plateau in response to depolarization impulse [22]. Increased $I_{NaP}$ amplitude is also determined in rats with temporal lobe epilepsy after lithium-pilocarpine injection [23]. In CA1 region of hippocampus status epilepticus (SE)-induced modulation of $I_{NaP}$ is associated with increase of fraction of bursting neurons [14].

In conclusion, this and previous findings [11] show that thrombin action on neuronal excitability at least in part is mediated by $I_{NaP}$ regulation in both CA3 and CA1 hippocampal areas. Taking together with studies on the synaptic transmission [24] these findings elucidate diverse molecular mechanisms of thrombin action on CNS, which may contribute to pathological conditions associated with BBB dysfunction.

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Fig. 2. Effect of thrombin application on $I_{NaP}$ in CA1 pyramidal neurons of adult rats. (A) The traces demonstrate $I_{NaP}$ registration in adult rats in control conditions and in the presence of thrombin. (B) Effect of thrombin on $I_{NaP}$ activation curve. Straight curves represent a Boltzmann fits of normalized voltage dependent conductance

Fig. 3. Comparison of thrombin effect on $I_{NaP}$ peak amplitude in 2-week-old (A) and 2-months-old rats (B)
ВПЛИВ ТРОМБИНИ НА ПОСТІЙНИЙ НАТРІЄВИЙ СТРУМ В ПІРАМІДАЛЬНИХ НЕЙРОНАХ СА1 ЗОНИ ГІПОКАМПА МОЛОДИХ І ДОРОСЛИХ ЩУРІВ

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Серинова протеаза - тромбін, ключовий фактор згортання крові, бере участь у багатьох нейронних процесах, важливих для нормального функціонування мозку і в патологічних станах. Спробуватимемося показати, що аплікація тромбіну призводить до збільшення постійного натрієвого струму, що у свою чергу призводить до збільшення внеску тромбіна в регуляцію нервової активності в незрілому головному мозку.

Ключові слова: гіпокамп; постійний натрієвий струм; тромбін.

REFERENCES


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