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

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Serine protease thrombin, a key factor of blood coagulation, participates in many neuronal processes important for normal brain functioning and during pathological conditions involving abnormal neuronal synchronization, neurodegeneration and inflammation. Our previous study on CA3 pyramidal neurons showed that application of thrombin through the activation of specific protease-activated receptor 1 (PAR1) produces a significant hyperpolarizing shift of the activation of the TTX-sensitive persistent voltage-gated Na^+ current (I_{NaP}) thereby affecting membrane potential and seizure threshold at the network level. It was shown that PAR1 is also expressed in CA1 area of hippocampus and can be implicated in neuronal damage in this area after status epilepticus. The aim of the present study was to evaluate the effect of thrombin on I_{NaP} in CA1 pyramidal neurons from adult and young rats. Using whole cell patch-clamp technique we demonstrate that thrombin application results in the hyperpolarization shift of I_{NaP} activation as well as increase in the I_{NaP} amplitude in both age groups. We have found that I_{NaP} in pyramidal neurons of hippocampal CA1 region is more vulnerable to the thrombin action than I_{NaP} in pyramidal neurons of hippocampal CA3 region. We have also found that the immature hippocampus is more sensitive to thrombin action which emphasizes the contribution of thrombin-dependent pathway to the regulation of neuronal activity in immature brain.

Key words: hippocampus, persistent sodium current, thrombin.

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 epilptiform 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_2PO_4 – 1, Na_2HPO_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_2PO_4 – 1, Na_2HPO_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

[20]. The subtraction of sodium current traces evoked by ramp-protocol before and after 1 μM TTX reveals I_{NaP}. Voltage dependence of I_{NaP} conductance (G) was calculated from

$$G = \frac{I}{(V - E_{Na})},$$

where I is the amplitude of I_{NaP} induced by potential V, and E_{Na} – 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_{NaP} in isolated pyramidal CA1 neurons. Fig.1.A and Fig.2.A show representative current traces of I_{NaP} registration in rats two weeks and two months old, respectively. In young rats (Fig.1.B) thrombin induced the hyperpolarization shift of I_{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_{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_{NaP} activation between age groups was not significant (F (1, 12) = 0.97, $P = 0.35$). Up-regulation of I_{NaP} amplitude after thrombin exposure was more pronounced in the group of young rats (Fig.3). I_{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_{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_{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_{NaP}.

Our previous findings in hippocampal slices revealed that thrombin induces hyperpolarization shift of I_{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_{NaP} peak amplitude up-regulation. Such ef-

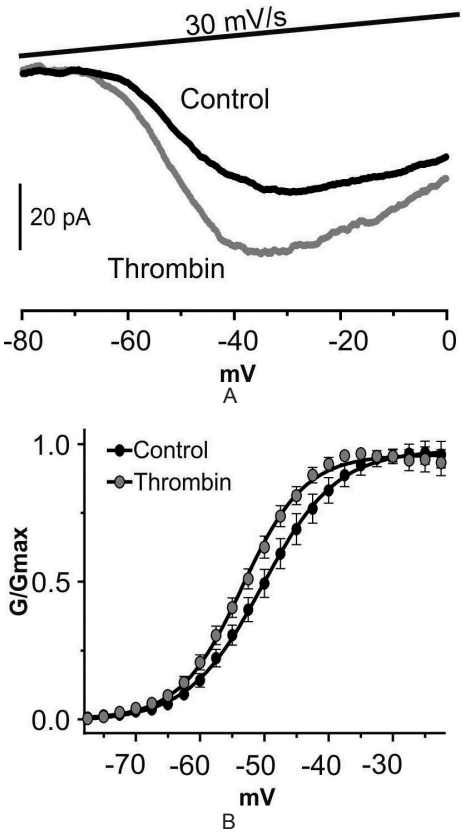

Fig.1. Effect of thrombin application on I_{NaP} in CA1 pyramidal neurons isolated from young rats. (A) Representative smoothed sodium current traces obtained using slow depolarizing ramp protocol shown in the upper panel. Ramp stimulation was elicited from holding potential -80 mV to 0 mV. Thrombin application results in the increase of I_{NaP} peak amplitude. (B) Boltzmann fit of summarized curve of normalized activation voltage dependences before and after thrombin application. Thrombin induces hyperpolarization shift of I_{NaP} activation

fect could be a basis for neuronal hyperexcitation and capability for long-lasting generating action potentials. For example, it was shown in sensorimotor 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.

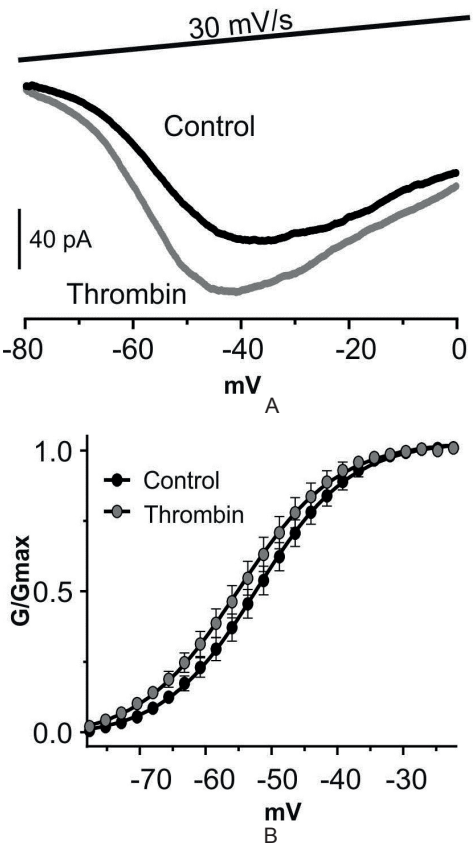

Fig. 2. Effect of thrombin application on I_{NaP} in CA1 pyramidal neurons of adult rats. (A) The traces demonstrate I_{NaP} registration in adult rats in control conditions and in the presence of thrombin. (B) Effect of thrombin on I_{NaP} activation curve. Straight curves represent a Boltzmann fits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

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ВЛИЯНИЕ ТРОМБИНА НА ПОСТОЯННЫЙ НАТРИЕВЫЙ ТОК В ПИРАМИДАЛЬНЫХ НЕЙРОНАХ СА1 ЗОНЫ ГИППОКАМПА МОЛОДЫХ И ВЗРОСЛЫХ КРЫС

Сериновая протеаза - тромбин, ключевой фактор свертывания крови, участвует во многих нейронных процессах, важных для нормального функционирования мозга и при патологических состояниях, сопровождающихся аномальной нейрональной синхронизацией, нейродегенерацией и воспалительными процессами. Наши предыдущие исследования на пирамидальных нейронах зоны СА3 показали, что аппликация тромбина приводит к увеличению постоянного натриевого тока (I_{NaP}) через активацию

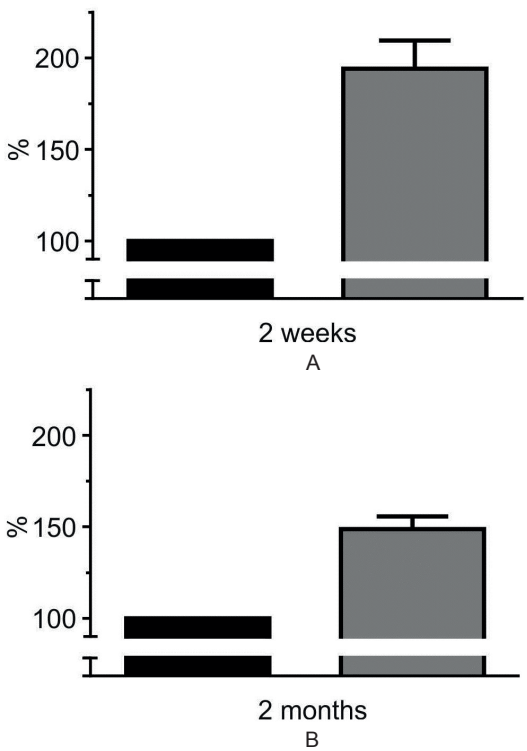

Fig. 3. Comparison of thrombin effect on I_{NaP} peak amplitude in 2-week-old (A) and 2-months-old rats (B)

протеазаактивированого рецептора 1 (ПАР1) и, таким образом, влияет на мембранный потенциал, а также порог эпилептиформной активности на уровне нейрональной сети. Было показано, что ПАР1 также экспрессируется в СА1-зоне гиппокампа и вовлечен в процесс повреждения нейронов после эпилептического статуса. Цель данной работы состоит в установлении эффекта тромбина на I_{NaP} в пирамидальных нейронах СА1-зоны гиппокампа взрослых и молодых крыс. С использованием методики patch-clamp в конфигурации «целая клетка» мы показали, что аппликация тромбина вызывает гиперполяризационный сдвиг активации и увеличение амплитуды I_{NaP} в обеих возрастных группах. Наши результаты также свидетельствуют о том, что I_{NaP} в пирамидальных нейронах СА1-зоны гиппокампа является более уязвимым к действию тромбина, чем в нейронах СА3-зоны. Также нами было установлено, что гиппокамп молодых крыс более чувствителен к действию тромбина, что в свою очередь приводит к усилению вклада тромбинзависимого пути в регуляцию нейрональной активности в незрелом головном мозгу.

Ключевые слова: гиппокамп; постоянный натриевый ток; тромбин.

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ВПЛИВ ТРОМБІНУ НА ПОСТІЙНИЙ НАТРІЄВИЙ СТРУМ В ПІРАМІДАЛЬНИХ НЕЙРОНАХ СА1 ЗОНИ ГІПОКАМПА МОЛОДИХ І ДОРОСЛИХ ЩУРІВ

Серинова протеаза - тромбін, ключовий фактор згортання крові, бере участь у багатьох нейронних процесах, важливих для нормального функціонування мозку і в патологічних станах, що супроводжуються аномальною нейрональною синхронізацією, нейродегенерацією і запальними процесами. Наші попередні дослідження на пірамідальних нейронах СА3-зони показали, що аплікація тромбіну призводить до збільшення постійного натрієвого струму (I_{NaP}) через активацію протеазаактивованого рецептора 1 (ПАР1) і, таким чином, впливає на мембранний потенціал, а також на поріг епілептиформної активності на рівні нервової мережі. Було показано, що ПАР1 також експресується в СА1-зоні гіпокампа і залучений до процесу пошкодження нейронів після епілептичного статусу. Мета нашої роботи полягає у встановленні ефекту тромбіну на I_{NaP} в пірамідальних нейронах СА1-зони гіпокампа дорослих та молодих щурів. З використанням методики patch-clamp у конфігурації «ціла клітина» ми показали, що аплікація тромбіну викликає гіперполяризаційний зсув активації та збільшення амплітуди I_{NaP} в обох вікових групах. Наші результати також свідчать про те, що I_{NaP} в пірамідальних нейронах СА1-зони гіпокампа є більш уразливим до дії тромбіну, ніж в нейронах СА3-зони. Також нами було встановлено, що гіпокамп незрілих щурів чутливіший до дії тромбіну, що у свою чергу призводить до збільшення внеску тромбінзалежного шляху в регуляцію нервової активності в незрілому головному мозку.

Ключові слова: гіпокамп; постійний натрієвий струм; тромбін.

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