

Under The Action Of β -Amyloid Peptide₁₋₄₀ Hippocampal CA1 Pyramidal Neurons Membrane Ion Conductivity and its Current Dynamic Model

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Abstract—In this paper the Acutely isolated hippocampal CA1 pyramidal neurons is researched. under the mode of whole cell recording signals, Aging process of β -amyloid peptide₁₋₄₀ for Na^+ Current the effects, Aging process of β -amyloid peptide₁₋₄₀ the concentration of as needed $0.01 \mu\text{mol.L}^{-1}$. We clamp down on -60 mV , depolarization to -10 mV , 0 mV , 10 mV , 20 mV , compared before and after the use of $0.01 \mu\text{mol.L}^{-1}$ β -amyloid peptide₁₋₄₀ hippocampal neurons cell membrane conductivity changing with time. sodium channels in the process of activation is to escalate rapidly open process, the deactivation is to intensify in the process of closing the slow process of sodium channels. two different before and after the main current of $0.01 \mu\text{mol.L}^{-1}$ β -amyloid peptide₁₋₄₀ (sodium ion current) established the dynamic model of the current.

Index Terms—hippocampal CA1 pyramidal neurons; β -amyloid peptide₁₋₄₀; dynamic model

I. INTRODUCTION

Alzheimer Disease (AD) is the most common form of dementia and the most frequent degenerative brain disorder that humans encounter in old ages, The risk of developing AD substantially increases after age of 65[1] AD is rapidly becoming one of the major universal healthcare problems. While the cause of this disease remains unknown, there is evidence for substantial genetic influence[1]-[5]. With the unclear pathogenesis, several hypotheses about the pathogenesis of AD has been proposed, such as the abnormal protein hypothesis, cholinergic hypothesis, oxidative stress theory and the estrogen hypothesis, etc. were proposed. In particular, amyloid β -protein has been linked to the neurotoxic principle causing neuronal death in the disease, although the mechanism has remained elusive[6]-[9]. It has been shown that when amyloid β -protein was incorporated into lipid bilayer membranes, the amyloid formed cation-selective channels[10]-[12]

Mutations in three genes, a myloid precursor prote in

(APP), presenilin 1 and 2(PSEN1 , PSEN2), result in an early onset, autosomal dominant form of the disease beginning in the third or fourth decade. The $\epsilon 4$ allele of apolipoprotein E (APOE) increases the risk of both sporadic and familial AD occurring later in life around the sixth decade. Each of these genes is involved in the production or processing of the amyloid β peptide, which is deposited in the brain as dense plaques that are characteristic of the disease [13]. Today, however, there are neither precise diagnostic approaches nor effective therapeutic agents available for Alzheimer disease.

To understand the underlying mechanisms that cause amyloid β -protein induced ion flux, In particular, amyloid β -protein affected Voltage-dependent Calcium Current and potassium ion channel current. The main purpose of this paper is to research action Mechanism and kinetic character of β -amyloid₁₋₄₀ on Voltage-dependent Calcium Current and potassium single channel Current, from these data we analyze that free amyloid β -protein action, In order to discover the ion channel gating kinetics character. Thus the research has technically significance.

This paper is divided into four parts: the first part is the introduction; in the second part we briefly review materials and method; the third and four part are the results and discussion.

II. MATERIALS AND METHODS

A. Acutely isolated hippocampal CA1 pyramidal neurons

Age 7 ~ 10 d Wistar rats, male and female. Beheaded in brain, rapidly in low temperature ($0 \sim 4^\circ\text{C}$) oxygen saturation of extracellular fluid isolated from hippocampus, cut into $500 \mu\text{m}$ thick slices. Put slices in 32°C of extracellular fluid in continuous oxygen incubation. 1 h after moving the brain slice containing $610 \sim 710 \text{ U/ml}$ Pronase E (Sigma) in extracellular fluid, 32°C after enzymolysis 25 min wash slices with extracellular fluid above 6 times, and then move into the extracellular fluid back-up incubation at room temperature. In the process of the whole continuous oxygen. In brain slices to isolate CA1 area in extracellular fluid of oxygen saturation of the centrifugal tube, blowing gently with the dropper, let stand, centrifuge tube on the vertical drain upper cell suspension, to join in the perfusion slot of extracellular fluid. About 30 min after the cell wall, can make whole cell patch clamp experiment.

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B. Solutions and electrophysiology

Place the fresh separation of hippocampal neurons in the perfusion slot on the inverted microscope. The whole-cell patch clamp recordings was carried out at room temperature (21 ~ 22 °C) using an EPC-9 patch clamp amplifier (Germany). Recording Na⁺ Current bath solution contained (in mM): NaCl 150, KCl 5, MgCl₂ 11, CaCl₂ 2.5, HEPES 10, D-glucose 10, EGTA, 10 pH was adjusted to 7.4 with NaOH. The pipettes had resistances of 2-4 MΩ after backfilling with an internal solution. Recording Voltage-dependent Calcium Current The patch-pipette (internal) solution contained (in mM): NaCl 140, MgCl₂ 11, CsCl, 1 CdCl₂ 2, HEPES 10, EGTA 11, pH was adjusted to 7.2 with CsOH.

C. whole cell recording

Experimental instrument to record the EPC - 9 (Germany), under the mode of whole cell recording signals, current sampling interval is 0.5-5 ms. Signal by quadrupole low-pass filter filter, frequency for 1-3 KHZ. Acutely isolated hippocampal CA1 pyramidal neurons cell membrane potential is about 60 mV. When such potential V_c < -60mV membrane is superlarization. When V_c > -60 mV membrane depolarization [14]-[15]

D. Chemicals

Glass microelectrode using two step control, polishing, electrode resistance 2-5 MΩ. Drug delivery system of drug solution through the fused quartz tube by gravity (i.d. = 200 μm) and ordinary liquid flows into the load cells in a petri dish. By moving the electromagnetic switch, can quickly change the drug dosing system. We purchase $\hat{\alpha}$ -amyloid peptide₁₋₄₀, H-89, HEPES, TEA-Cl, Pronase E from Sigma Inc. and TTX from Boehringer Mannheim Inc. Aβ₁₋₄₀ Match with three steamed water into 100 μmol·L⁻¹ 20 °C storage, place it before using the incubation in the temperature of 37 °C box 7 d aging process to form the state of aggregation, and then the concentration of as needed.

E. Data analysis

Collect data by Igor Pro4.03 (WaveMetrics Inc) software for processing, four different concentrations measured in beta amyloid peptide1-40, under the action of the channel current amplitude, calculate the conductance, observation after dosing current change over time.

III. RESULTS

A. The change of drug under the action of ionic conductivity

Hodgkin and Huxley [16] membrane current only original research is divided into sodium and potassium current, they start to look for next membrane ion permeability changes of measuring method. In order to find out under the condition of the permeability constant, ion current and the membrane potential is a linear relationship, whether follow ohm's law. Hodgkin and Huxley, first of all make the axon to escalate to improve its permeability, and then step voltage by the switch to a level to another level, no changes in membrane permeability, measured between 10 to 30 milliseconds to voltage of electric current. In sodium ion permeability and

potassium ion permeability increases measurement, the two experimental results show that the approximation of the relationship between current and voltage follow ohm's law, and a linear relationship between changes. Therefore Hodgkin and Huxley ion conductance with formula is expressed as (1):

$$g_{Na} = \frac{I_{Na}}{E - E_{Na}} \quad g_K = \frac{I_K}{E - E_K} \quad (1)$$

Aging process of $\hat{\alpha}$ -amyloid peptide₁₋₄₀ for Na⁺ Current the effects, Aging process of $\hat{\alpha}$ -amyloid peptide1-40 the concentration of as needed 0.01 μmol·L⁻¹. We clamp down on -60 mV, depolarization to -10 mV, 0 mV, 10 mV, 20 mV, compared before and after the use of 0.01 μmol·L⁻¹ $\hat{\alpha}$ -amyloid peptide₁₋₄₀ hippocampal neurons cell membrane conductivity changing with time. Fig 1 shows the activation to the conductivity changing with time under different voltage. The experimental results show that activation to conductance maximum is different when different voltages, among them: in -10 mV, 0 mV, 10 mV, a maximum of 20 mV conductance when respectively: 15.9283, 15.5496, 14.61224, 11.9078. After adding 0.01 μmol·L⁻¹ $\hat{\alpha}$ -amyloid peptide1-40, when different voltages, among them: in -10 mV, 0 mV, 10 mV, a maximum of 20 mV conductance when respectively, and the maximum conductivity also change accordingly, in this is: 21.9761, 19.7953, 17.6042, 15.1316.

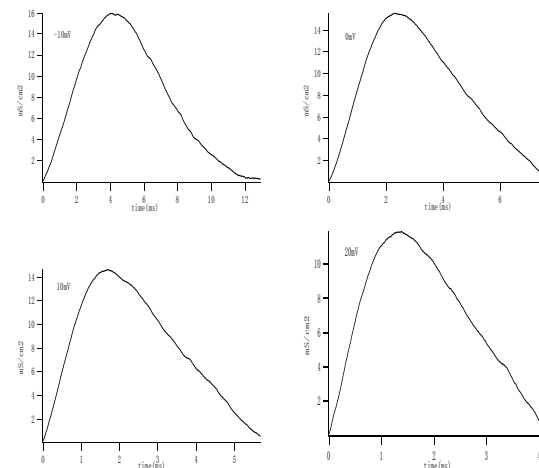


Fig 1 shows the activation to the conductivity changing with time under different voltage

Experimental results illustrate the change caused not only a conductance to escalate, and with the augmentation of the intensified maximum conductivity time shorten, accordingly when -10 mV is 4 ms, 0 mV is 2.28 ms, 10 mV is 1.66 ms, 20 mV is 1.38 ms. After using 0.01 μmol·L⁻¹ $\hat{\alpha}$ -amyloid peptide₁₋₄₀, respectively to escalate to -10 mV, 0 mV, 10 mV and 20 mV, maximum transconductance time was increased, respectively is: 4.72 ms and 3.1 ms and 2.06 ms and 1.52 ms. 0.01 μmol·L⁻¹ $\hat{\alpha}$ -amyloid peptide₁₋₄₀ further demonstrated by the changes in cell membrane of $\hat{\alpha}$ -amyloid peptide1-40 conductance, meaning that it changes the internal structure of the protein. Fig 2 shows the join 0.01 μmol·L⁻¹ $\hat{\alpha}$ -amyloid peptide₁₋₄₀ before and after the effect of $\hat{\alpha}$ -amyloid peptide₁₋₄₀ to intensify voltage and maximum Na conductivity.

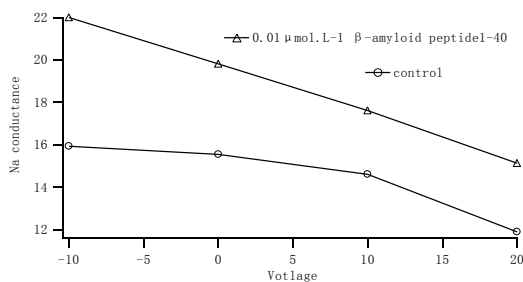


Fig 2. shows the join $0.01\mu\text{mol.L}^{-1}$ β -amyloid peptide₁₋₄₀ before and after the effect of intensify voltage and maximum Na^+ conductivity.

Hodgkin and Huxley research by huge squid nerve axons ion conductance peak is 20 to 50 mS/cm^2 , This conclusion shown conductance peak a maximum of not more than 25 mS/cm^2 , an important finding is $0.01\mu\text{mol.L}^{-1}$ β -amyloid peptide₁₋₄₀ improved the sodium ion conductance.

B. under drug the action of dynamic model of ion current changes

Hodgkin conductance with sodium, Huxley thought control two kinds of dynamics, the experiment showed that sodium conductance activation before the deactivation, and sodium channels in the process of activation is to escalate rapidly open process, the deactivation is to intensify in the process of closing the slow process of sodium channels. In the previous paper has discussed the sodium conductance peak depends on the corresponding pulse voltage. At the same time also depends on the drug. Due to the activation and inactivation process can be expressed as some form of exponential function, therefore, H - H model description of sodium ion permeability change equation as follows(2)

$$I_{Na} = -701.856(1.41026 - 0.887174\exp(-\frac{t}{1.66}))^3(-0.444739 + 1.74652\exp(-\frac{t}{4.02})) \quad (2)$$

Three of the microsomal control activation process, a process microsomal control deactivation. So in allows the possibility of ion appear a position for them. Under the influence of H - H model, we have to escalate to 10 mV with two different before and after the main current of $0.01\mu\text{mol.L}^{-1}$ β -amyloid peptide₁₋₄₀ (sodium ion current) established the dynamic model of the current. Fig 3 shows the membrane potential of 60 mV to escalate to 10 mV, the dotted line as the sign of the current change over time, the results of the solid line for curve fitting.

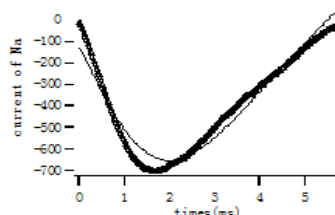


Fig3 shows the the results of curve fitting.

Fig4 shows the join $0.01\mu\text{mol.L}^{-1}$ β -amyloid peptide₁₋₄₀, membrane potential for 60 mV to escalate to 10 mV, main current Na^+ current changing with time curve (dotted line), the fitting curve for the solid line. Fitting curve equation is(3).

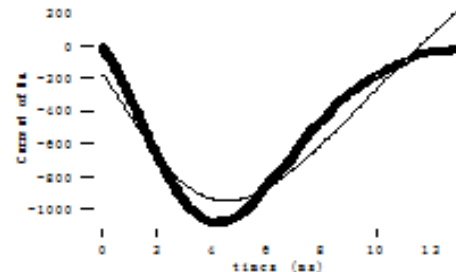


Fig4 shows the join $0.01\mu\text{mol.L}^{-1}$ β -amyloid peptide₁₋₄₀, Na^+ current changing with time curve

$$I_{Na} = -1083*(1.4704 - 0.9718\exp(-\frac{t}{4}))^3(-0.47428 + 1.7198*\exp(-\frac{t}{8.92})) \quad (3)$$

IV. CONCLUSION

Because of the influence of H - H model, to establish the dynamic model of the sodium ion current, although the fitting effect may be related to the actual electrophysiological signals have a certain degree of difference, but generally using quantitative analysis method to analyze the kinetics of ion channels to explain its physiological mechanism is very necessary. The results of the analysis on the one hand, through quantitative analysis means to analyze the cell membrane conductance, reveals the change of cell membrane conductivity before and after medication, show the changes of protein structure; On the other hand, through the establishment of corresponding dynamics model can explain the physiological phenomena and predict the evolution of the ion. This is further research content in the future.

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