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Antibiotic Supplements Affect Electrophysiological Properties and Excitability of Rat Hippocampal Pyramidal Neurons in Primary Culture

Farideh Bahrami[1] and Mahyar Janahmadi[*2]

[1]*Neuroscience Research Center and Dept. of Physiology, Baqiyatallah University of Medical Sciences, Tehran;*
[2]*Neuroscience Research Centre and Dept. of Physiology, Medical School, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

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ABSTRACT

Introduction: Antibiotic supplements are regularly used in neuronal culture media to control contamination; however, they can interfere with the neuronal excitability and affect electrophysiological properties. Therefore, in this study, the effect of penicillin/streptomycin supplements on the spontaneous electrophysiological activity of hippocampal pyramidal neurons was examined. **Methods:** Electrophysiological whole-cell patch-clamp recordings from rat hippocampal pyramidal cells in primary culture were performed to investigate the effects of antibiotic supplements on the intrinsic excitability of cultured cells. **Results:** The present findings indicated that presence of antibiotic supplements (penicillin/streptomycin) in the culture medium altered the intrinsic electrical activity of hippocampal pyramidal neurons in primary culture. These alterations included: 1) depolarized resting membrane potential; 2) a significant enhancement in the after-hyperpolarization amplitude; 3) a significant increase in the area under the action potential and in the decay and rise time of the action potential; 4) a significant broadening of action potential and 5) a significant reduction in the firing frequency. **Conclusion:** These findings suggest that addition of antibiotic supplements to culture media influences the neuronal excitability and alters the electrophysiological properties of cultured neurons, possibly through changing the ionic conductance underlying neuronal excitability. *Iran. Biomed. J. 17 (2): 101-106, 2013*

Keywords: Primary cell culture, Patch-clamp techniques, Hippocampus

INTRODUCTION

During recent decades, there have been many studies that indicate the influence of antibiotics on biological organisms and cell membranes [1, 2]. These influences include their translocation across the target membrane [3], inhibition of cell wall biosynthesis [4] and change in permeability of cell membrane [5]. In addition, ion channels are the targets for the action of antibiotics [6].

One of the important properties of antibiotics which need to be considered by investigators is their ability to disrupt the ion flow through cell membranes. This property becomes propounded when ionophore antibiotics are used. The concentration of ions in the extracellular and intracellular media is imbalance, which is necessary for normal cell function [7]. However, the ion imbalance across the membrane can be disrupted by application of ionophore antibiotics that form either ion channels or ion-ionophore complexes in biological membranes [8].

Several studies have indicated that aminoglycosides, that are common antibiotics with potent bacterial activities [9, 10], bind to ion channel proteins and induce neurotoxicity by altering channel behavior. For example, gentamicin and neomycin reduce the current through nicotinic acetylcholine channels [10, 11], whereas apramycin enhances the conductance through N-methyl-D-aspartate receptor channel [12]. In addition, it has been reported that aminoglycosides act on voltage-gated ion channels, including Ca^{2+}-activated K^+ channels [13], L-type Ca^{2+} channels [14], N-type Ca^{2+} channels [15] and P/Q-type Ca^{2+} channels [16] as pore blockers [17].

There is also consistent evidence of strong interference of antibiotics with cell membrane excitability [18]. It has been reported that penicillin induces epileptiform activity and seizures both in

*Corresponding Author; Tel & Fax: (98-21) 22439971; E-mail: mjanahmadi@yahoo.com or Janahmadi@sbmu.ac.ir

clinical situations [19] and following intracortical injection in rat [2, 20]. The proposed mechanism of its action was reported to be either synaptic or non-synaptic actions. Penicillin produces convulsion through inhibition of GABA receptor chloride channels [21-22]. It has been also shown that administration of penicillin into cortex activates voltage-dependent calcium channels, which can be suppressed by Ca^{2+} channel blocker, nimodipine [23]. There is also evidence showing that penicillin induces paroxysmal depolarization shift, followed by prolonged after hyperpolarizations (AHP) in hippocampal pyramidal neurons, which is caused, in part, by a Ca^{2+}-dependent K^+ current (K_{Ca}^{2+}) [24].

Based on the above backgrounds, in the present study, the question of whether application of antibiotic supplements (penicillin/ streptomycin) affects the electrophysiological properties of rat hippocampal pyramidal neurons in culture was investigated.

Since it has been shown that antibiotics affect neuronal physiological properties [25], there have been attempts to culture neurons in the absence of antibiotics [26].

MATERIALS AND METHODS

Cell culture. Hippocampal neurons were isolated from 36 brains of neonatal Wistar rats (1-4 days). To do this, hippocampi were transferred to dissociation buffer containing calcium and magnesium free Hank's balanced salt solution (0.976%), sodium bicarbonate (0.035%, Sigma, UK) and pyruvate (1 mM), HEPES (10 mM), pH 7.4. Then, the cells were dissociated by triturating (15-18 times) through a fire-polished Pasteur pipette. The dispersed cells were centrifuged at 360 ×g for 1 min. Next, the cell pellet was re-suspended in a fresh dissociation buffer. An aliquot was removed and mixed with an equal volume of 0.4% Trypan blue and counted for dye-excluding cells in a hemocytometer. Neurons were then plated on poly-L-lysine-coated coverslips (15 mm dia-meter culture dishes) in a B27/neurobasal medium containing B27 (2%), neurobasal (96.75%) and L-glutamine (200 mM, Sigma, UK) with or without penicillin-streptomycin (100 µg/ml) at a density of 1×10^6 cell/ml. Cells were incubated in 5% CO_2 at 37°C and fed twice weekly with B27/neurobasal medium. Penicillin-streptomycin mixture is effective against both Gram-negative and -positive bacteria. The morphological changes and growth of the neurons were observed under an inverted phase contrast microscope. Neurons were used for whole-cell patch-clamp recordings 14-21 days after plating on coverslip.

Whole-cell patch-clamp electrophysiological recordings. A coverslip with cultured pyramidal neurons was placed in a recording chamber, perfused at 1-2 ml/min with HEPES-based artificial cerebrospinal fluid containing (in mM) 140 NaCl, 2 $CaCl_2$, 1.4 KCl, 10 HEPES, 10 glucose, pH 7.3 (NaOH) and osmolarity 295-297 mOsm. Cultured hippocampal pyramidal neurons were visualized with an Olympus IX71 inverted microscope equipped with an Olympus DP12 camera. Cells were identified based on their pyramidal-shaped soma.

Whole-cell patch-clamp technique was used to record action potentials from spontaneously active cells in current clamp condition with zero current injection. A gap-free acquisition mode at room temperature (22-25°C) was used with a Multiclamp700 B amplifier (Axon Instruments, Foster City, CA) equipped with Digidata 1440 Data Acquisition System and pCLAMP 10 software (Axon Instruments, Foster City, CA). Electrophysiological recordings were filtered at 5 kHz, digitized at 10 kHz and stored on a personal computer for offline analysis.

Patch electrodes were pulled from thick-walled borosilicate glass capillary (1.5 mm O.D; Clark Instrument, UK) with a tip resistance of 3-6 MΩ using a PC10 two-stage vertical puller (Narishige, Japan). Pipettes were filled with a solution, containing (in mM) 145 KCl, 4 NaCl, 10 HEPES, 0.4 Na_2GTP and 2 Na_2ATP, pH 7.3 (with KOH) and osmolarity 290 ± 10 mOsm. Under an inverted microscope, the patch pipette was lowered onto the cultured cell surface, and a gentle suction was applied to establish a high resistance seal. When seal resistance was > 1 ΩM, a brief strong suction was applied to rupture the membrane for making whole-cell recording. Recordings were discarded if changes in series resistance were greater than 20%.

The following electrophysiological parameters were measured under current clamp condition: resting membrane potential (RMP), action potential duration at half-width after AHP, action potential number, action potential amplitude, rise time and decay time of action potential. The AHP amplitude was measured from the RMP before stimulation to the peak of the hyperpolarization. Action potential half-width was measured as the duration at the half of the peak amplitude.

Statistical analyses. In each group 14 cells were recorded. Significance was assessed at $P<0.05$ with two tailed Student's *t*-test. Data are presented as mean ± SEM.

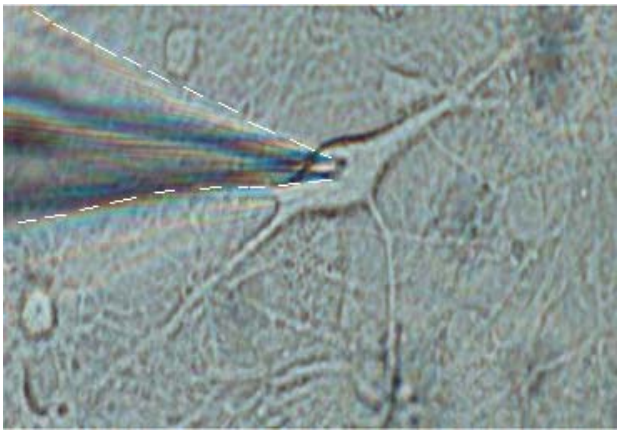

Fig. 1. Phase contrast image of a cultured hippocampal neuron with a patch pipette attached to the membrane of neuron ready for recording.

RESULTS

Cultured pyramidal neurons were identified based on their triangular-shaped soma with several neurites and smooth membranes (Fig. 1). Culturing the neurons in the presence of a medium containing antibiotics (penicillin/streptomycin) caused firing of action potentials, which were followed by after depolarization potential (compare Fig. 2A and 2B).

Cultured hippocampal pyramidal neurons significantly displayed depolarized membrane potential when compared with those cells cultured in the absence of antibiotics (Fig. 3A, $P<0.05$). Furthermore, the amplitude of AHP following action potential was significantly greater when cells were cultured in the presence of antibiotics (Fig. 3B, $P<0.01$). Addition of antibiotic supplements to the culture medium affected the area under the action potential (Fig. 3C), the rise time constant (Fig. 3D) and also the decay time constant (Fig. 3E) of action potential, so that these parameters were significantly ($P<0.001$) increased compared to the cells cultured without antibiotic application.

Furthermore, the use of antibiotics in culturing media was associated with lengthening action potential duration and lower neuronal excitability as compared to the cells cultured without antibiotics (Fig. 3F and 3G, $P<0.001$).

DISCUSSION

Cultured pyramidal neurons even in the control condition had more depolarized membrane potential, which it could be explained by depolarizing GABA actions in immature neurons. In neonatal (P6-10) rabbit hippocampal CA1 pyramidal neurons, membrane potential was reported to be -53 mV [27]. It was suggested that GABA through GABAA receptor depolarizes the immature cells. This depolarizing action of GABA has been also reported by other researchers in neurons from neonatal animals [28].

Several studies have indicated that antibiotics can change the membrane properties of excitable cells and could exert toxic effects on cultured neural network [29, 30].

Findings of the present study indicated that presence of antibiotics in a culture medium was associated with a marked depolarization in the membrane potential. Application of antibiotics was also caused a significant increase in the AHP amplitude, area under the action potential, rise and decay times of action potential and action potential half-duration, but it was led to a

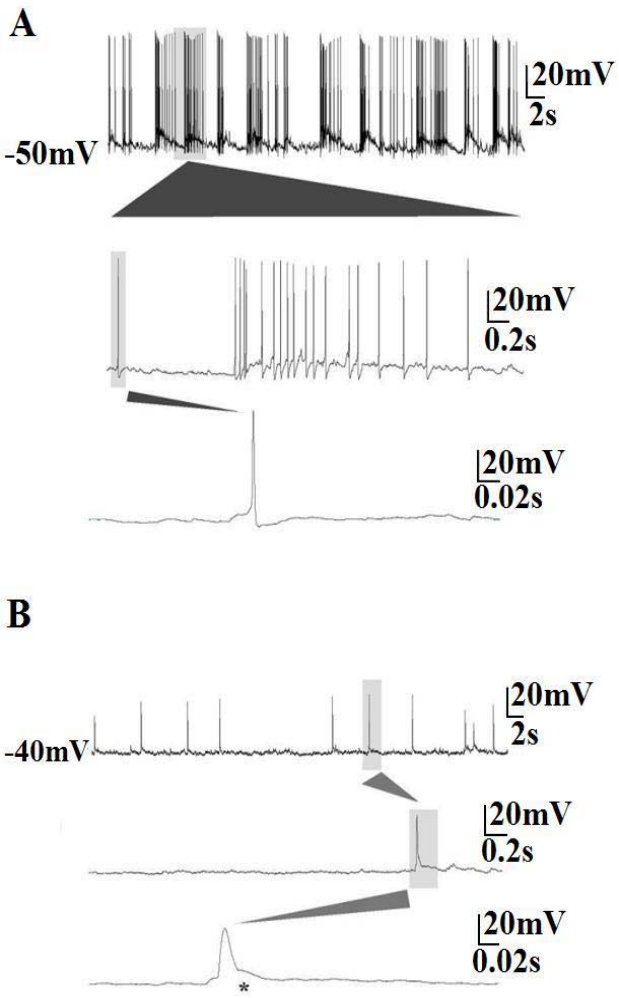

Fig. 2. Effect of antibiotic supplements on spontaneous electrical activity of cultured pyramidal neurons. Representative traces showing spontaneous activity of hippocampal pyramidal neurons in primary culture without **(A)** and with antibiotic supplements in the culture media **(B)**. Asterisk in C shows after depolarization potential.

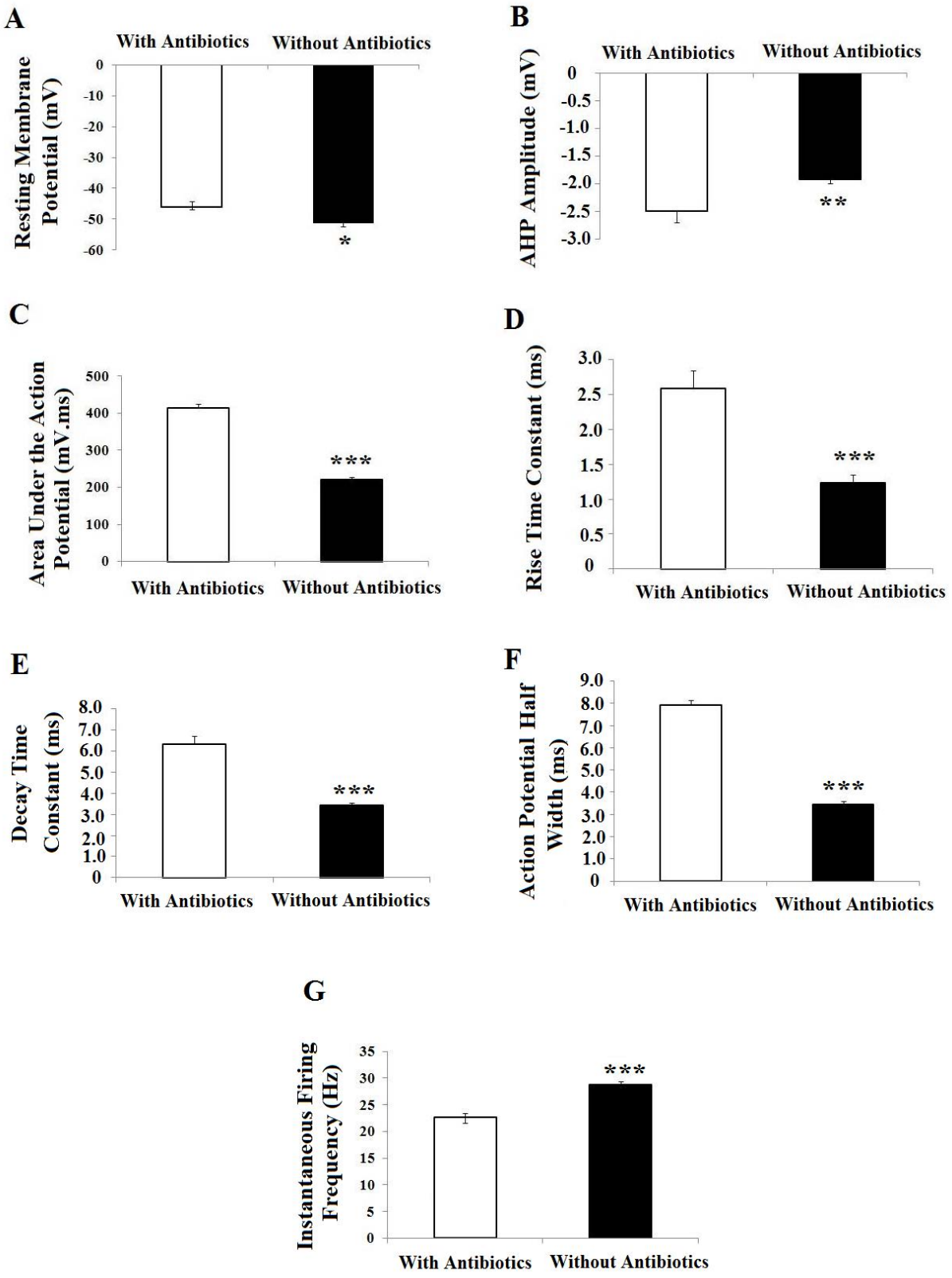

Fig. 3. Effect of antibiotics on the electrophysiological properties of hippocampal pyramidal cells in culture condition. Histograms illustrate the effect of antibiotic application in culture medium on **(A)** resting membrane potential, **(B)** AHP amplitude, **(C)** area under the action potential, **(D)** rise time, **(E)** decay time, **(F)** action potential half-width, **(G)** instantaneous firing frequency. Data represent mean ± SEM; Asterisks represent significant differences from the group with antibiotics : *P<0.05, **P<0.01 and ***P<0.001.

significant decrease in the firing frequency. Consistent with the previous reports [1, 31], here we demonstrated that antibiotics (penicillin/streptomycin) depolarized the cultured RMP and markedly prolonged the action potential of hippocampal pyramidal neurons in primary culture. Penicillin, which is a group of β-lactam antibiotics, is used to prevent bacterial infection (Gram-positive bacteria) and has been shown to alter the RMP and amplitude the spontaneous action potential [31]. Voltage clamp study also indicated that penicillin decreases sodium channel current [31] that might be the reason for increasing the time of the rising phase and decreasing the firing rate of action potential recorded in the present work. It has been shown that penicillin antagonizes GABA-mediated inhibition [32] and thereby induces neuronal hyperexcitability [1], which is in contrast to the present finding. One explanation for this contrast is that membrane depolarization induced by penicillin/streptomycin may lead to suppression of Na^+ channels [31], and thereby decreases the neuronal firing frequency.

In addition, we demonstrated that presence of penicillin/streptomycin in the culture medium was associated with a significant increase in the AHP amplitude and action potential duration. The increase in the AHP amplitude and prolongation of action potential duration could be due to an increase in the voltage-dependent calcium and/or calcium-activated potassium conductance. However, the action potential broadening which, in turn, increases the area under the action potential also might be due to the blockage of K^+ current, including sodium-activated potassium channel current. These findings suggest the effect of penicillin/ streptomycin on active properties including action potential parameters.

In many neurons including hippocampal pyramidal neurons, a rise in intracellular concentration of Ca^{2+} contributes to generation of AHP following action potential [32], which, in turn, plays an important role in neuronal excitability and in shaping the firing pattern [33]. In addition, activation of K_{Ca}^{2+} particularly big conductance K_{Ca}^{2+} results in spike broadening. Besides big conductance K_{Ca}^{2+} channel, K_{V1} channels also may contribute to the duration of action potential. These channels also contribute to action potential repolarization and thereby affect duration, shape and frequency of action potential [34].

Based on the above discussion, it can be concluded that antibiotic supplements influence hippocampal pyramidal neuronal excitability, possibly through modulation of ion channels; however, this needs to be further investigated using voltage clamp technique. Therefore, electrophysiological data obtained from cultured neurons in the presence of antibiotics must be interpreted with a certain caution.

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