Electrochimica Acta 282 (2018) 89–96

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Generating change in membrane potential by external electric stimulation and propagating the change by using nerve model cell systems

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ARTICLE INFO

Article history:
Received 18 April 2018
Received in revised form
21 May 2018
Accepted 4 June 2018
Available online 6 June 2018

Keywords:
External electric stimulation
Action potential
Nerve conduction
Nerve model cell system

ABSTRACT

A change in the membrane potential in nerve cells is thought to be generated and propagated mainly by a function of K^+ and Na^+ channels. The concurrent monitoring of multipoints on the axon has been generally conducted on the basis of the voltage-clamp or current-clamp method. Given that the respective membrane potentials have been evaluated by considering the applied potential, local current, and conductance, the propagation of the change in the membrane potential was measured. By using a nerve model system composed of some liquid membrane cells, we directly measured the actual membrane potentials and the local currents of the respective cells. We demonstrated that the local membrane current caused by an external voltage induced a change in the membrane potential and that the change was propagated by connecting the liquid membrane cells and mimicking voltage-gated Na^+ channels. It has been proved that hyperpolarization hardly occurs on the occasion of existence of the flux of K^+ and Na^+ only in the present model system and that the change in the membrane potential corresponding to the action potential is directionally propagated.

1. Introduction

A change in the membrane potential in nerve cells is generated and propagated mainly by the function of K^+ and Na^+ channels [1–4]. The usual membrane potential is determined by the ratio of the concentration of K^+ inside the nerve cell to that outside the cell; this ratio is called the resting potential. When neurotransmitters combine with channel-type receptors at the synapse by an external stimulus, Na^+ mainly flows from the outside of the cell into the inside of the cell around the receptors. Thus, the membrane potential shifts from resting potential to action potential, which is mainly determined by the ratio of Na^+ concentration inside the cell to that outside the cell at the synapse or at a partial area of the synapse. The change in the membrane potential is immediately propagated from the synapse to the axon. Considering that voltage-gated Na^+ channels that have short open lifetimes (approximately 1 m s) serve on the axon surface, the part indicating the action potential runs toward the axon terminal. Subsequently, the membrane potential on the axon goes back to the resting potential because of the function of delayed-K^+ channels after the hyperpolarization condition in which the membrane potential is more negative than the resting potential. Thus, it has been considered that a change in the membrane potential is propagated directionally along the axon toward the synaptic terminal.

The concurrent monitoring of the membrane potential at the multipoints on the axon has been performed on the basis of the voltage-clamp method [1,2,5]. Thus, the direct membrane potential has not been measured at the respective points because it is estimated by taking into account both the constant membrane potential and the potential difference converted from both the local current and membrane conductance. Several studies on nerve conduction using voltage-sensitive dyes have been conducted; however, the hyperpolarization has not been clearly observed yet because of the low time resolution of CCD cameras [6,7].

The authors elucidated the propagation of the change in membrane potential by using organic liquid membrane (LM) cells as a model of the nerve system. Ueya et al. [8] constructed an LM cell first to investigate the ways of propagating change in the membrane potential. Kushida et al. [9] made cells to mimic the resting potential and action potential by introducing a switch system. Furthermore, Takano et al. [10] developed an LM system with a

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https://doi.org/10.1016/j.electacta.2018.06.023
0013-4686/

function of K^+ and Na^+ channels to mimic actual nerve cells. Takano et al. [11] improved the cells to mimic voltage-gated Na^+ channels by using relay switches in the electric circuit and succeeded in reproducing the directional propagation of the change in the membrane potential. On the basis of these results, Shirai et al. [12] explained a new conduction mechanism based on the propagation of the change in the membrane potential using the liquid-membrane model system.

Several studies on nerve conduction have been performed by applying extracellular electric stimulus to nerve cells [1,2,5]. Action potential is locally generated between two extracellular points on the axon surface and is propagated to the nerve terminal along the axon under the condition clamping of the membrane potential. However, details on the mechanism responding to the extracellular electric stimulus have not been elucidated.

In this study, a model system mimicking the nerve cell was constructed to elucidate a way of responding to the external electric stimulus. The change in the membrane potential was generated by applying the potential gradient between two outside points, and the mechanism of change in the membrane potential was discussed by considering the effect of resistance within the circuit that was elucidated.

2. Experiments

2.1. Chemicals

Sodium (Na) tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB) (i.e., NaTFPB) was prepared in a similar manner as a previous study [13]. The $TFPB^-$ salt of potassium (KTFPB) and that of tetrabutylammonium (TBATFPB) were obtained as follows. The methanol solution of KTFPB and that of TBATFPB were prepared by mixing the methanol solution of KCl (Wako Pure Chemical Co.) and that of TBABr (Tokyo Kasei Chemical Co.) with that of NaTFPB. After adding ultrapure water to the methanol solutions of KTFPB and TBATFPB and after cooling them, the precipitations of KTFPB and TBATFPB were formed. KTFPB and TBATFPB were purified by repeating this recrystallization method. All other chemicals were of analytical reagent grade and were used without further purification.

2.2. Electrochemical cells

An LM cell made of glass was composed of two aqueous phases (W1 and W2) and a nitrobenzene phase (NB), as illustrated in

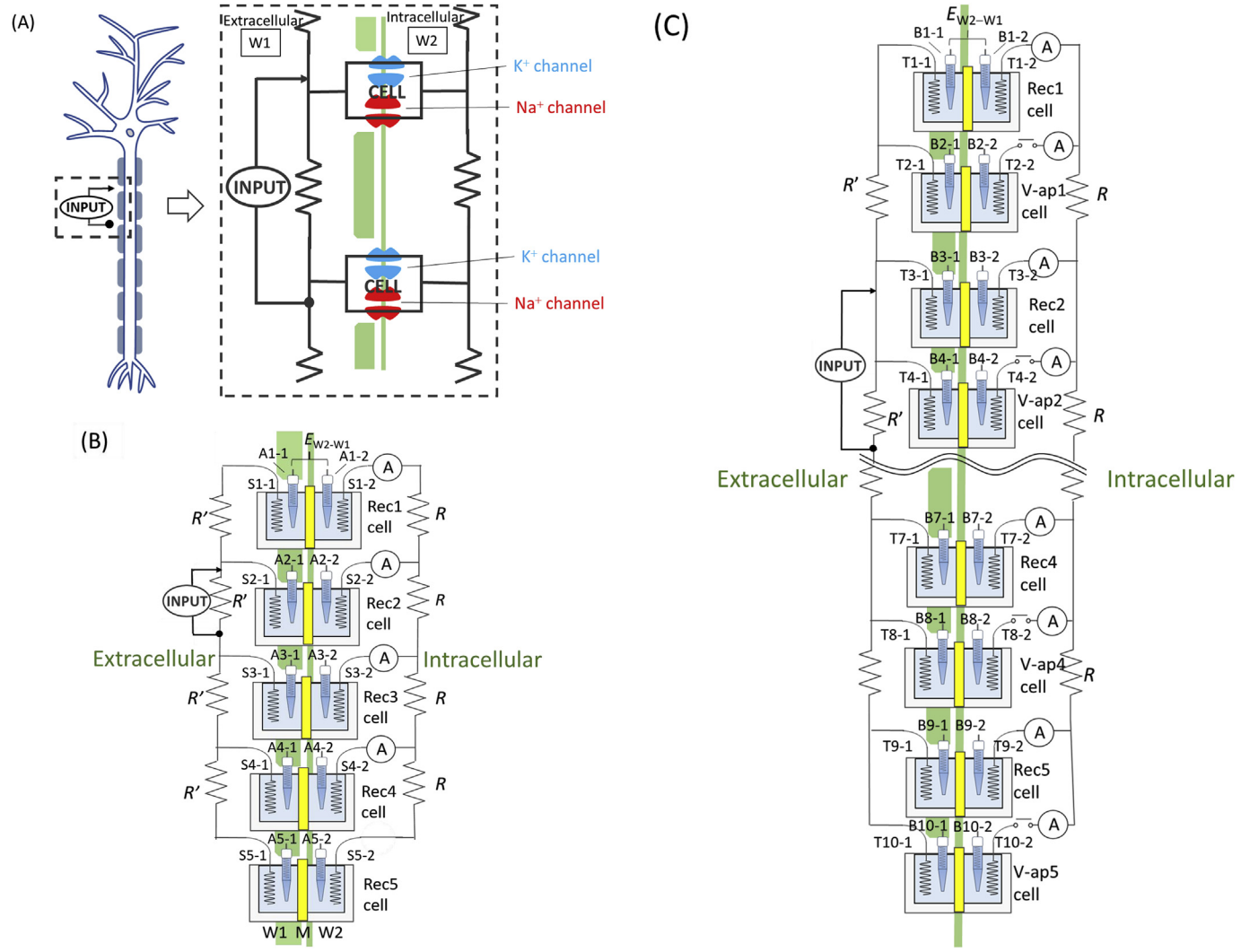

Fig. 1. Electrochemical cells. (A) Overview of a neuron and a model system. (B) The LM model system used to explain the mechanism responding to the external electric stimulation. (C) The LM model system including V-ap cells mimicking the voltage-gated channels.