UDC 577.359

DIMINAZENE ACTION ON ACID-SENSING ION CHANNELS (ASICS) IN RAT AMYGDALA NEURONS

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Abstract

Different properties of neuronal cells connection named gap junction, especially its bidirectional nature, can be evaluated from experiments with dual, whole-cell voltage-clamp recording of activity from electrically coupled neuronal cells. However, the process of recording becomes very complicated because of inability to receive online analyzed data during the experiment. In our work we view the method of synchronization of software, which would perform needed analysis, and test it within dual, whole-cell voltage clamp recording experiments.

Keywords: gap junction, stimulus-evoked current, dual current clamp

Introduction

Interneuronal communication can occur in form of indirect or direct transmission. Indirect transmission acts by neurotransmitter release in extracellular space to bind to postsynaptic membrane. Direct communications are performed by electrical synapses. Main group of electrical synapses are gap junctions, which provide a high conductance pathway for ionic current between two cells. Gap junction channels are formed by apposing of two contacting hexahedral connexin hemichannels (connexons). Connexons may have only one type of connexins and gap junction can be formed by identical or different connexons. Gap junctions allow bidirectional intercellular passage for molecules, thereby mediating ionic and metabolic communications between directly connected cells. This isotropic property can be displayed in the protocol of current responses for stimulus in dual, whole-cell voltage clamp recording of electrically coupled cells (Fig. 1).

For each sweep, the pipette current and pipette voltage for both cell A (I_a, V_a) and cell B (I_a, V_b) are recordered. When the pipette potential V_a of cell A is changed from V_{aa} toV_{ab} (with the pipette potential of cell B held at V_{bb}) current responses I_{ab} in cell A and I_{bb} in cell B are evoked. When the pipette potential V_b of cell B is changed from V_{bb} to V_{ba} (with the pipette potential of cell A held at V_{aa}), current responses I_{ba} in cell B and I_{aa} in cell A are evoked. Stimulus-evoked amplitudes for current and voltage were measured by averaging across the periods marked by light rectangles. Thef series o experimental data, acquired by PatchMaster (HEKA Elektronik, Lambrecht/Pfalz, Germany) are opened in FitMaster (HEKA Elektronik, Lambrecht/Pfalz, Germany), which carry series analysis. The advantage of such configuration is that FM cannot perform types of analysis at series level that cannot be done in PM. Nonetheless FM can not display results on the Group level, so they are exported to be displayed in IgorPro.

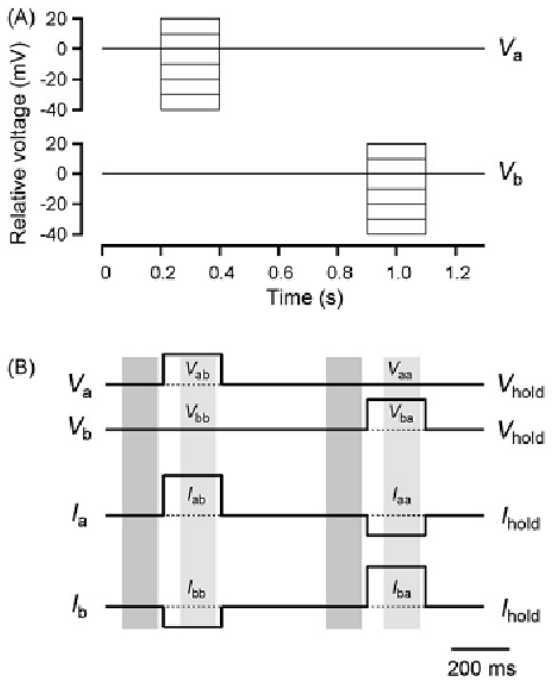

Fig. 1. (A) Schematic stimulus protocol for dual whole-cell voltage clamp recording. V_a, V_b – voltages of the recording electrodes of cell A and cell B respectively. This protocol illustrates seven sweeps with 10mV increment for each. (B) Schematic overview of one sweep of the voltage stimulus protocol with accompanying current responses.

1. Methods

For applying our method we construct model cell, with electrical circuits that mimic recording from two electrically coupled neuronal cells. Each cell (A,B) was represented by a resistor (R_{m1}, R_{m2}) in parallel with a

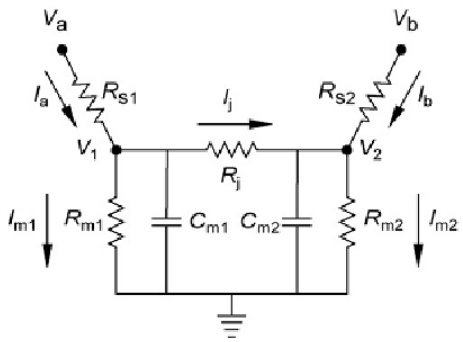

Fig. 2. Electrical cirquit of model cell used to mimic dual, whole-cell voltage clamp recording.

capacitor (C_{m1}, C_{m2}).Only symmetrical cell pairs (i.e., $R_{m1} = R_{m2}$) were examined. The pipette series resistances (R_{s1}, R_{s2}) and junctional resistance (R_j) were represented by corresponding resistors (fig. 2). To explore the influence of each component of the model cell on the experimental errors obtained with the voltage- and current-clamp measurements, the values of the different resistors could be varied to represent realistic physiological situations. The exact value of a resistor was measured by connecting the resistor to ground and recording the voltage evoked by a current pulse. The value of the resistor was then calculated directly from Ohm's law.

The resistors R_{m1} and R_{m2} of the model cell circuit were connected directly to the headstages of two amplifiers (see below) via the resistors R_{s1} and R_{s2}, respectively. The model cell did not incorporate an explicit pipette capacitance and the effective pipette capacitance corresponded to stray capacitances surrounding the pipette resistor. Whole-cell, single-electrode voltage-clamp and current-clamp recordings were performed with EP with an EPC10-triple patch-clamp amplifier controlled by PatchMaster software. Data is acquired by the PatchMaster, running in Master mode, controlling its second instance in addition to the instance of FitMaster (HEKA Elektronik, Lambrecht/Pfalz, Germany). After completing acquisition of series PatchMaster sends a batch command to FitMaster that starts series analysis. It's results are opened by IgorPro (HEKA Elektronik, Lambrecht/Pfalz, Germany) and are appended to the previous results, to be displayed at the group level.

2. Results

For our model cell we perform dual whole-cell voltage-clamp simulated recording and get the following protocol (fig. 3)

As we can see, this protocol matches the schematic stimulus protocol for whole-cell voltage-clamp (Fig. 1.). The magnitude of received voltage response equals the magnitude of voltage pulse in voltage-clamp protocol. This tells us, that selected model and method of acquiring online analyzed data work properly.

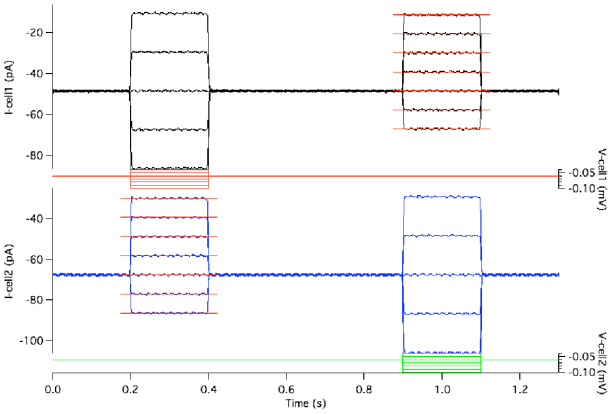

Fig. 3. Recording from Model Cells for simulating electrical coupling – gap junction – between neuronal cells.

So,

- we can represent coupled neuronal cells by the equivalent electrical cirquit from fig.2;
- our model and method of acquiring online analyzed data for simulated recording of gap junction between coupled neuronal cells work properly;
- we can perform online data analysis over recording the coupled via gap junction neuronal cells activity in dual, voltage-clamp whole-cell experiments.
- in our work we perform such online data analysis by synchronization of protocols in PatchMaster, FitMaster and IgorPro suit of programs (HEKA Elektronik, Lambrecht/Pfalz, Germany) . This configuration is highly effective because it allows to execute acquiring, analyzing and displaying results at the series level and enables real-time investigation of gap junction

Discussion

The listed suit of programs was shown to perform accurate real-time investigation of gap junction. This was tested in dual, whole-cell voltage clamp recording experiment. The further investigation is required to apply this method for researching the passive membrane properties of electrically coupled cells, such as AII amacrine cells in rat retina which should be the object of future series of experiments.

References

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