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MODULATION OF NEURONAL ACTIVITIES BY INTERACTIONS BETWEEN NEURONS AND GLIAL CELLS

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Abstract Action potentials are the fundamental signals for relaying information from one region to another in the nervous system. Action potentials are propagated along axons without decrease of their amplitudes and are conducted with constant velocity depending on axonal diameter and myelin. It is considered that the modulation of firing patterns of action potentials in the neural circuit influences the information processing in the brain. We investigated the modulatory effects of glial cells on the firing pattern and the axonal conduction of action potentials using rat hippocampal slice preparation. In our previous study, we focused on interneuron / perineuronal glial cell pairs in CA1 region and reported that perineuronal glial cells could be classified into two groups, one group belong astrocytes (perineuronal astrocytes) and the other group oligodendrocytes (perineuronal oligodendrocytes), based on their membrane properties and immunohistochemical study. Direct depolarization of perineuronal astrocytes modulated the directly induced firing pattern of the interneuron, with initial facilitation and subsequent suppression. We also studied the oligodendrocytes in the alveus and examined their modulatory effects on the conduction of action potentials along axons of CA1 pyramidal cells. Direct repetitive depolarization of oligodendrocytes shortened the latencies of action potentials evoked by antidromic stimulation. These results indicate that glial cells influence the firing pattern and axonal conduction of action potentials, and that their effects involve both facilitation and suppression.

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Key words: perineuronal glial cell; astrocyte; oligodendrocyte; conduction velocity; hippocampus

1. Introduction

Glial cells in the central nervous system play important roles in supporting neurons, such as providing nutrition, protection from circulating toxins by surrounding a blood-brain barrier, myelin formation for saltatory conduction of action potentials, and structural support, but have traditionally been considered as silent cellular elements in electrical activity and information processing since they show no action potentials. However, recent evidence has demonstrated that glial cells express a variety of ion channels and neurotransmitter receptors[1-4)] and, especially in astrocytes, have the ability to modulate neuronal activity by composing a functional synaptic unit called tripartite synapse[5,6)]. Moreover, a new concept called glia-neuron network which is more extended idea beyond the tripartite synapse and includes the roles of oligodendrocytes is also proposed[7)]. Therefore, the interaction between neurons and glial cells should be explored to obtain a better understanding of brain function.

Action potentials are the signals used in the nervous system to relay information. The changes of firing patterns of action potentials in the neural circuit influence the information processing in the brain. We investigated the modulatory effects of glial cells on the firing pattern and the axonal conduction of action potentials using acute hippocampal slices of rats.

In this review, we focus on the mutual direct

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interaction between a pair of the interneuron and the perineuronal astrocytes in the stratum radiatum of CA1 region. We also discuss the modulatory effects of oligodendrocytes in alveus on the conduction of action potentials along axons of pyramidal cells.

2. The modulatory effects of perineuronal astrocytes on interneuron firing

2.1. Identification of perineuronal astrocytes in the stratum radiatum of the CA1 region

Although glial cells contact tightly with many neurons, it is difficult to find the appropriate cell pair in which we can detect their interactions in physiological examinations. To investigate these interactions, the positional relation should be a matter for careful consideration[8]. A pair of glial soma and neuronal soma within a small area could be an ideal target for the investigation[9], thus, we chose a hippocampal perineuronal glial cell, the soma of which was just beside the neuronal soma.

Transverse 400 μm thick slices were prepared from male Sprague Dawley rats (23- to 30-days-old) using a rotary slicer, submerged, and continuously perfused at a rate of 2-3 ml/min with artificial cerebrospinal fluid at 28-30° C. Cells were visualized using a microscope equipped with a ×40 water immersion objective and infrared differential interference contrast (IR-DIC) optics. The soma of the perineuronal glial cell was visually identified as a round and relatively smaller cellular component beside the large soma of the interneuron (Fig. 1A). Whole-cell recordings using K-gluconate based pipette solution were obtained from the soma of an interneuron and a perineuronal glial cell. The distinction between neurons and glial cells was confirmed by the depolarizing current injection. All cells with larger soma responded with action potentials, while none of cells with paired smaller soma showed action potentials even by

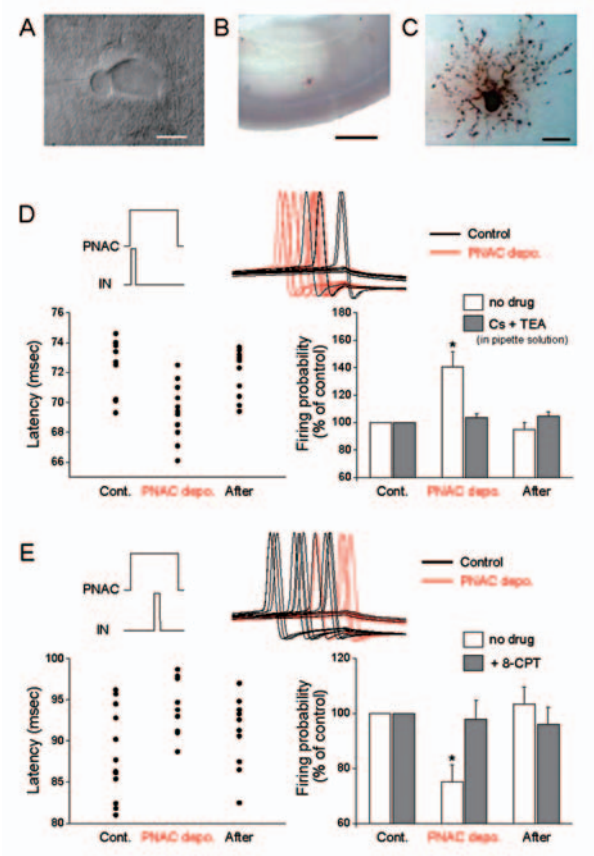

Figure 1 (A) IR-DIC image of an interneuron-perineuronal glial cell pair. Scale = 10 μm. (B, C) Light microscopy showing a biocytin-stained perineuronal astrocyte. The stained perineuronal astrocyte is positioned in stratum radiatum (B). The perineuronal astrocyte has many short, thin, radial processes. Scale bar = 100 μm and 10 μm in (B) and (C), respectively. (D, E) Time dependent change of the perineuronal astrocyte-induced modification on the interneuron firing. Adopted from Yamazaki et al., 2005, with permission. The effects of perineuronal astrocyte depolarization on interneuron firing using a short delay (20 ms) (D) or a long delay (400 ms) (E) between perineuronal astrocyte and interneuron depolarization. The timing of the depolarizing currents injected into both cells is shown in the inset. Upper panel: Superimposed action potentials (black, before perineuronal astrocyte depolarization; red, during perineuronal astrocyte depolarization) on an expanded time scale. Traces showing failure of generation of action potentials are also shown. Lower left panel: Typical changes in the latency before (Cont.), during (perineuronal astrocyte depo.), and after (After) perineuronal astrocyte depolarization. Lower right panel: Summary plot for the effects of intracellularly-applied potassium channel blockers (cesium; Cs, and tetraethylammonium; TEA) (D) and bath-applied 8-cyclopentyl-1,3-dimethylxanthine (8-CPT) (E) on the changes in the firing probability. PNAC and IN indicate perineuronal astrocyte and interneuron, respectively. $^{*}P < 0.05$.

large depolarizing current injection. We have previously reported that perineuronal glial cells could be classified into two groups, one group belong astrocytes (perineuronal astrocytes) and the other group oligodendrocytes (perineuronal oligodendrocytes), based on their membrane properties (i.e., resting membrane potential and input resistance) and immunohistochemical study[10]. To obtain input resistances of perineuronal glial cells, small hyperpolarizing voltage steps (10 or 20 mV, 600 ms duration) were applied in voltage-clamp mode, and then divided by the value of current responses at the plateau portion. The perineuronal astrocytes had deep resting membrane potentials (-81.3 ± 0.4 mV, n = 46) and relatively lower input resistances of 118.7 ± 8.1 MΩ, whereas the perineuronal oligodendrocytes showed shallow resting membrane potentials (-44.6 ± 0.8 mV, n = 56) and larger input resistances of 596.8 ± 33.9 MΩ. To examine the morphological properties of perineuronal astrocytes, biocytin (0.5%) was added to the pipette solution. The biocytin staining for perineuronal astrocytes showed small soma and a number of fine processes emitting radially from the soma (Fig. 1B, C). This classification was confirmed histologically on a sample of these cells by double staining with biocytin and anti-GFAP antibody, specific for astrocytes[10]. Dye coupling was not observed in the perineuronal astrocyte stainings with biocytin.

2.2. Depolarization of the perineuronal astrocytes modulates interneuron firing

To assess whether the perineuronal astrocytes had a direct effect on the interneuron, we performed simultaneous whole-cell recording from an interneuron-perineuronal astrocyte pair. The interneuron was injected with a depolarizing current to generate action potential. The magnitude and duration of the interneuron depolarization were set to induce single action potential with a probability of about 50%. After several action potentials were recorded in response to depolarization, the interneuron was depolarized with a delay from the start of perineuronal astrocyte depolarization. To investigate the time dependency of the perineuronal astrocyte depolarization, we employed two different paradigm of the delay (20 and 400 ms) between the start of perineuronal astrocyte depolarization and the start of interneuron depolarization (Fig. 1D, E, inset). When the delay was short (20 ms) and the time from the start of perineuronal astrocyte depolarization to the first spike less than 100 ms, the latency of the first action potential decreased and the firing probability increased (Fig. 1D), whereas, when the delay was 400 ms, the latency was increased and the firing probability decreased (Fig. 1E). Because the perineuronal astrocytes possess the 4-aminopyridine- and tetraethylammonium-sensitive currents induced by depolarizing voltage steps[10], the increase of extracellular potassium concentration should be the matter for consideration. When potassium channels of the perineuronal astrocytes were blocked by intracellular cesium and tetraethylammonium, the effects of the perineuronal astrocytes depolarization on the latency and firing probability of interneurons were inhibited in 20 ms delay (Fig. 1D). These results indicate that injection of a depolarizing current to the perineuronal astrocytes can induce a transient increase in the extracellular potassium concentration. This potassium increase will induce a small depolarization of the interneuron, leading to a shortening of the latency and an increase in the firing probability of the generation of action potentials. The short time window of this effect (< 100 ms) also suggests the contribution of transient currents. Alternatively, the bath application of 8-cyclopentyl-1,3-dimethylxanthine, an adenosine A_1 receptor antagonist, inhibited the perineuronal astrocytes-induced modulation in the case of 400 ms delay (Fig. 1E). From these results, it is

suggested the adenosine A_1 receptors is involved in the suppressive effects during the late phase of the perineuronal astrocytes depolarization. Recent studies indicated that ATP released from astrocytes could be the source of adenosine which modulates neuronal activities[11,12]. Therefore, the release of neuroactive substances, such as ATP and adenosine, from perineuronal astrocytes is the most likely mechanism for the suppressive effect on action potentials seen in the late phase. These results indicate that the perineuronal astrocyte directly modulates the firing activity of the interneuron, with facilitation in the early phase, which is mediated by the efflux of potassium from the depolarized perineuronal astrocytes, and suppression in the late phase of perineuronal astrocyte activation, which is mediated via adenosine A_1 receptors.

3. Modulatory effects of oligodendrocytes on the conduction velocity of action potentials in the alveus.

3.1. Identification and properties of oligodendrocytes in the alveus of the CA1 region

The well-known function of the oligodendrocytes is myelination around axon in the central nervous system. The myelin plays a crucial role in the saltatory conduction of action potentials along axons, an efficient manner of conduction. Like astrocytes, K^+ buffering along axons were also suggested[13,14]. Since oligodendrocyte processes form tight contacts with many axons, it is possible to modify the conduction property of action potentials along axons.

To obtain long alveus fibers in a hippocampal slice, 400 μm thick slices were prepared at a 45° plane to the septo-temporal axis. Under the IR-DIC microscope, glial cells were identified in the alveus of the CA1 region by their small round somata, with a diameter of less than 10 μm (Fig. 2A). These cells never showed action potentials on injection of a large depolarizing current. Under our experimental conditions, their resting membrane potential and input resistance were -75.2 ± 0.4 mV and 116.4 ± 10.7 MΩ (n = 64), respectively. The biocytin staining revealed the morphological feature of oligodendrocytes. Most of their processes were aligned in the same direction as the pyramidal cell axon, with some processes being vertical to the axon (Fig. 2B, C). The identification of the cells as oligodendrocytes was confirmed by double-staining with injected biocytin and anti-CNPase antibody, a specific antibody against oligodendrocytes[15].

3.2. Increase in the conduction velocity of action potentials by depolarization of the oligodendrocyte

The conduction velocity of action potentials depends mainly on the diameter of the axon and myelination and has been considered to be constant along a given axon. The oligodendrocytes in alveus form myelin sheathes for the axons of pyramidal cells. To examine whether oligodendrocytes modulated neuronal activities, we performed simultaneous whole-cell recording from pyramidal cells and oligodendrocytes in the alveus (Fig. 2D). We recorded action potentials induced by antidromic stimulation every 2 s in the presence of glutamatergic and GABAergic antagonists, and measured the latencies of the action potentials (Fig. 2D). After stable action potentials were recorded, oligodendrocytes were directly depolarized to between -30 and -20 mV with a train of current pulses (500 ms duration, 30 pulses at 1 Hz) through a recording electrode (Fig. 2D). In four out of 27 experiments, the latency of the action potential was influenced by oligodendrocyte depolarization. The latency of the action potentials was shortened to 89.7 ± 2.5% of control levels and returned to control levels about 20 s after the end of oligodendrocyte depolarization. In these four experiments, no effect was observed on the latencies of action potentials when the oligodendrocytes were not depolarized, (100.8 ± 0.4% of control levels). The

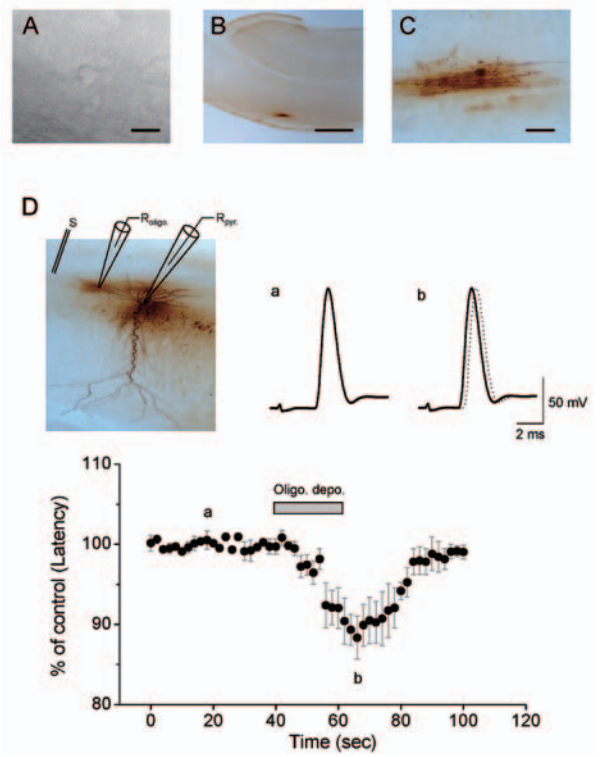

Figure 2 (A) IR-DIC image of an oligodendrocyte. Scale bar = 10 μm. (B, C) Light microscopy showing a biocytin-stained oligodendrocyte. The stained oligodendrocytes is positioned the border between stratum oriens and alveus (B). The oligodendrocyte has many processes aligned parallel to the axon and some vertical to the axon(C). Scale bar = 100 μm and 20 μm in (B) and (C), respectively. (D) Depolarization of oligodendrocytes shortens the latency of action potentials. Upper Left panel: Schematic diagram of dual whole-cell recording from a pyramidal cell ($R_{pyr.}$) and an oligodendrocyte ($R_{oligo.}$) with a stimulating electrode (S) in the alveus of the rat hippocampus. Upper right panel: Typical action potentials evoked antidromically without (a) or with (b) repetitive depolarization of the simultaneously recorded oligodendrocyte. The dotted line in (b) is identical to (a). The latency of the action potential is shortened by oligodendrocyte depolarization. Adopted from Yamazaki et al., 2007, with permission. Lower panel: Time-course of the changes in action potential latency induced by oligodendrocyte depolarization. The gray bar indicates the period of oligodendrocyte depolarization. Traces a and b in upper right panel were obtained at the times indicated in the lower panel.

amplitude and half-width of the action potentials were not significantly changed by oligodendrocyte depolarization.

After electrophysiological experiments, the recorded oligodendrocytes and pyramidal cells were stained with biocytin and examined the positional relationship between the axons of the recorded pyramidal cells and the oligodendrocytes. In the pairs in which we observed change of the latency caused by oligodendrocyte depolarization, the axon of the recorded pyramidal cell passed through the field of the recorded oligodendrocyte[15]. In contrast, in all cell pairs in which we did not detect latency changes, the pyramidal cells axons and oligodendrocyte processes were separate. These results indicate that depolarization of oligodendrocytes in the alveus directly increases the conduction velocity of action potentials.

The underlying mechanism that explains the influence of oligodendrocytes depolarization on action potential conduction is unknown. However, resulting from structural changes in the oligodendrocyte processes, a change in myelin insulation might be involved. Since a variety of ion channels, transporters and neurotransmitter receptors are expressed in the oligodendrocyte processes[13,16-21] and since the intracellular volume of the myelinating processes is small, the ion influx might cause a large increase in the ion concentration, leading to osmotic water influx, although excess ion influx can cause myelin disruption. At the physiological level, the volume increase in the myelinating processes caused by osmotic water influx might decrease extracellular conductance along axons which might enable more rapid conduction of action potentials[22].

The other suggested mechanism is the change of ion environment caused by oligodendrocyte depolarization. Depolarization of oligodendrocytes increases the extracellular K^+ concentration. Tetraethylammonium-sensitive K^+ channels are found on mature oligodendrocytes[20] and activation of these channels by depolarization induces K^+ efflux. Moreover, when the oligodendrocytes are depolarized, influx of K^+ from the extracellular space into the oligodendrocyte cytoplasm is reduced because the conductance of the inward rectifier K^+ channels is decreased, so

the extracellular K^+ concentration is increased. Because depolarization of oligodendrocytes induces a significant increase in the K^+ concentration due to the extracellular space being small[14], the axolemma around depolarized oligodendrocytes might be depolarized. Thus, action potentials might be easily evoked at the nodes and the conduction velocity might be increased by depolarization of the oligodendrocytes.

Because a single oligodendrocyte in the alveus forms the myelin for 10-30 axons of pyramidal cells, this modulation by oligodendrocytes might synchronize the output of a subset of pyramidal cells. Moreover, since the oligodendrocyte depolarization induced by electrical stimulation lasted for a long period[15], many action potentials could be affected over this time. Thus, hippocampal oligodendrocytes could influence the conduction of action potentials over a wide area and over a long period.

4. Conclusion

We demonstrate direct evidence for modulatory effects of glial cells on neuronal activities in different neuron-glial cell pairs. These evidences provide a new insight into the understanding of glial cell function in the brain in relation to the firing pattern or axonal conduction of action potentials. Further studies of interaction between neurons and glial cells relating to both physiological and pathophysiological conditions are needed to evaluate the exact functions of glial cells.

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