Properties of membrane potential activities in *Onchidium* photoresponsive neurons

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Abstract

The intrinsically photoresponsive neurons lp-1 and lp-2 often display spontaneous action potential firing without any stimulus input. Their pattern of endogenous activities can be divided into 2 types of firing: bursting and beating. In this paper, I qualitatively describe the properties of these activities to further analyze the intracellular mechanism of information processing as potential multimodal sensors.

Introduction

In both vertebrate and invertebrate nervous systems, action potentials convey information from the peripheral organs to the central nervous system and vice versa. The mechanism of action potentials in the nervous system is similar to that of digital signal processing; they convey the information in an all-or-nothing manner, and changes in information are relayed by differences in the frequency of spikes. On the other hand, some neurons generate action potentials spontaneously without any synaptic inputs. There are 2 types of such neuronal activities. The first is bursting, which is composed of repetitive firing of action potentials separated by periods of silence. The second is beating, which is composed of tonic firing without long periods of silence. Bursting and beating are considered the basic and fundamental properties of rhythm formation in the nervous system.

The photoresponsive neurons, named lp-1 and lp-2, have been extensively studied as non-visual photoreceptors. Both of these neurons respond to light with hyperpolarizing receptor potentials, and they act as interneurons to convey the tactile stimulation (Review; Gotow and Nishi, 2008). At resting membrane potentials (approximately $-50 \text{ mV}$), both neurons often show endogenous repetitive burst activities (several minutes of firing and subsequent silent periods). It has been shown that an extremely weak light stimulus is effective for decreasing the spike frequency when the light stimulus is presented within the firing period (Shimotsu et al., 2010).

Recently, I have found that carbon dioxide (CO$_2$) induces them excitation in lp-1 and lp-2,
resulting in an increase in firing frequency (Nishi and Shimotsu, 2011). These findings indicate that Ip-1 and Ip-2 act as modulators of signaling; the frequency of spikes are accelerated by CO₂ stimulation and inhibited by light stimulation. It suggests that Ip-1 and Ip-2 may also function as multimodal sensors.

To date, there has been no description regarding the endogenous membrane activities of Ip-1 and Ip-2. Here, I categorize their membrane activities according to their firing patterns.

**Procedure**

The experimental specimens were prepared from *Onchidium verruculatum* within 3 months of collecting them from a habitat in Sakurajima, Kagoshima. The abdominal ganglion, including both Ip-1 and Ip-2 neurons, was isolated from the animals, and placed in a 2-ml volume recording chamber. The membrane potential activities of Ip-1 and Ip-2 were recorded intracellularly using a conventional electrophysiological apparatus, as previously described (Shimotsu et al., 2010). CO₂ gas was dissolved in artificial sea water (ASW), and its concentration was measured with the specific concentration meter for CO₂ gas (TOA DKK CGP-1). The solution was continuously perfused at a rate of 1 ml per minute by using gravity-driven perfusion system. Experiment animals are 10–15 grams, middle size in the natural condition. All recordings showing in the Figures 1 and 2 are obtained from different animals respectively. The Ip-1 and Ip-2 neurons could not be distinguished by their membrane properties; therefore, the results will be described using the term 'Ip-1/2.'

**Result and Discussion**

The membrane potential activities of Ip-1 and Ip-2 differed among each preparation and exhibited several different patterns of activity. Figures 1 and 2 illustrate typical examples of the firing patterns of Ip-1/2.

Figure 1 shows the bursting pattern of Ip-1/2. Panel A(i) shows the regular type of burst activity, where the duration of firing and cessation times are relatively constant. The repetitive firing lasts for longer than one minute and the subsequent silent period continues for a few minutes. This pattern of activity continues for more than one hour. The interval of each action potential is 1–2 seconds and depends on the membrane potentials, the more depolarized the membrane potential, the shorter the interval. This mode of firing is relatively simple. The frequencies of firing are rather "monotonic," though the frequency of the spikes decreases slightly during the later end of each burst period. The A(ii) and A(iii) panels show more complex bursting activity. Here, it appears as though there are several peaks of firing within one bursting period, or in other words, a few short-time firings make up 1 burst. However, the total durations of the burst and cessation periods are relatively constant.
Figure 1. Spontaneous membrane activities of Ip-1/2 and their responses to 7% concentration of CO₂. The experimental solution was changed from normal ASW to the CO₂-containing ASW, as shown by the bars in all figure panels. A(i), A(ii), and A(iii) show the relatively regular pattern of bursting; B(i) and B(ii) show the irregular pattern of bursting; and C(i) and C(ii) show the "silent-type" of activity. In panel A, the most hyperpolarized membrane potentials are -48 mV, and -50 mV in panel B. The membrane potentials at the beginning of panel C are -50 mV. The top parts of the spikes are cut.

It can be seen that the firing patterns completely changed in each of the preparations. When the solution included CO₂ gas, as CO₂ was added into the ASW, the burst periods lengthened and the subsequent silent periods shortened; it seemed as though 2 firing periods fused into 1 burst. The durations of the burst and cessation periods became irregular in A(i), A(ii), and A(iii).

In contrast to A, the durations of the bursts and subsequent silent periods are not constant, as shown in Fig. 1B. The membrane potentials fluctuated by 5 mV or less, and the duration of repetitive firing changed from about 1 minute to 5 minutes in the normal ASW for this preparation. Spikes were superimposed when the membrane potential reached the threshold of action potentials. Accompanying the depolarization induced by CO₂, several short-duration (within 1 minute) firings appeared in B(ii). When the solution was replaced with normal ASW, membrane activities returned to the former firing pattern within 15 minutes.

Unlike Fig. 1A and 1B, some preparations exhibited "silent" activities; there was no firing of action potentials, as shown in Fig. 1C(i) and C(ii). Here, the changes in membrane potential are below 1 mV. When CO₂ was added to the solution, membrane potentials gradually depolarized.
Once the threshold of action potentials was reached, long-lasting repetitive firings were introduced in this preparation. Firing activities are monotonous in C(i), but small changes in spike frequency during repetitive firing was observed in C(ii). These repetitive firings disappeared within one minute when the solution was replaced with the normal ASW.

Figure 2. Beating-type membrane potential activity of lp-1/2. Constant and repetitive firing without a long silent period is observed. When 7% CO₂ was added in the external solution (indicated by the arrow), the frequency of firing increased approximately 1.5 times. Lower panel shows the expanded time and voltage scales of upper panel. The resting potential was -45 mV, and the membrane potential was depolarized slightly (2 - 3 mV) in response to adding CO₂ in the solution. The top parts of the spikes are cut.

Figure 2 shows the beating type of activity in lp-1/2. In contrast to the bursting shown in Figure 1, a fairly constant repetitive firing occurs here. The intervals of firing last several seconds when the membrane potential is -40 mV, which is longer than those of the burst type. The neurons respond to CO₂ application in the ASW with an increase in firing, much like a digitized signal; the frequency of spikes increased 1.5 times in this preparation. The membrane potential increased by 2 - 3 mV with increasing application of CO₂, consistent with the case of the burst type shown in Figure 1 of this report.

As shown in this paper, the membrane activities of lp-1/2 exhibit different patterns among different preparations. As such, it is impossible to quantitatively describe their activities as of now. However, it is possible to say that a high proportion of the preparations (2 of the 3 preparations done) show burst activities.

There are many experimental and theoretical studies regarding the burst mechanism of the R15 neuron, which is found in the visceral ganglion of the related animal, the Aplysia. R15 is the one of
the model as a burst neuron in the nervous system of whole animal not restricted in invertebrates (Yu et al., 2004). However, the burst time scale of the *Onchidium Ip-1/2* is quite different from that of the R15. While the durations of the bursts and intervals are several minutes in the Ip-1/2, these only last for tens of seconds in R15, which is a difference of an order of magnitude. This indicates that the Ip-1/2 and R15 mechanisms of burst are quite different.

The duration of bursts (and subsequent intervals) is usually on the time scale of seconds in both vertebrate and invertebrate neurons. Instead, the Ip-1/2 bursting characteristics are reminiscent of the cells of the pancreas, which secrete insulin. It has been shown that an application of glucose extracellularly induces bursting in the cells. The regulation of insulin from the molecular level to the behavioral level of organisms has been extensively studied (Review; Bertram et al., 2010). Interestingly, an insulin-like hormone is found in a diverse range of species. This structurally related hormone, "*Aplysia insulin*," is involved in the regulation of glucose metabolism (as in vertebrates), and also regulates secretion of the egg-laying hormone (Floyd et al., 1999). Therefore, this insulin-like hormone is expected to be found in the *Onchidium Ip-1/2*. Future studies should investigate the involvement of insulin and glucose-related metabolism in Ip-1/2.

**References**


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