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BRAIN RESEARCH

Research Report

Characterization of voltage-activated ionic currents in the GnRH-containing terminalis nerve in transgenic zebrafish

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ABSTRACT

The terminalis nerve (TN) is in a class of cranial nerves that plays important roles in animal development, physiology and behavior. Here, we report a study on the characterization of voltage-activated ionic currents in GnRH-containing TN cells in zebrafish. The experiments were performed using acutely dissociated TN cells from the transgenic zebrafish Tg (GnRH-3::GFP). In the transgenic zebrafish, the TN cells express GFP under the transcriptional control of the zebrafish GnRH-3 promoter. In all of the GnRH-containing TN cells examined, we recorded both low-voltage-activated (LVA) and high-voltage-activated (HVA) calcium current (I_{Ca}). The characteristics of the I_{Ca} were similar to those described in other zebrafish cell types. However, the distribution patterns of the currents in the GnRH-containing TN cells were different in comparison to the distribution of the currents in other cell types. In addition, we characterized TTX-sensitive sodium current (I_{Na}) and 4AP-sensitive and TEA-resistant potassium current (I_K). The characteristics of voltage-activated I_{Na} and I_K in the GnRH-containing TN cells were similar to those described in other zebrafish cell types. Together, the data from this study revealed the electrophysiological properties of the GnRH-containing TN cells, thereby providing insight on the regulatory mechanisms of TN-signaling in animal physiology.

1. Introduction

The terminalis nerve (TN) is in a class of cranial nerves that has been described in all vertebrate species (Arey, 1916; Münz et al., 1982; Demski and Northcutt, 1983). Previous studies have demonstrated that the TN plays an important role in animal development, behavior and physiology (for reviews, see Wirsig-Wiechmann and Oka, 2002; Whitlock, 2004). In fish, the TN cells are located in the olfactory bulbs and/or its nerve tracts (Stell et al., 1984; Chiba et al., 1996). The TN cells project axons into many brain areas, which include the neural retinas (Stell et al., 1984; 1987; Zucker and Dowling, 1987). Recent studies have suggested that TN-signaling may play an important role in sensory integration between the olfactory and visual systems. For example, in response to olfactory stimulation zebrafish visual sensitivity (measured by behavioral assays or by electrophysiological recordings) increased by nearly half a log unit (Maaswinkel and Li, 2003; Huang et al., 2005). The integration of olfactoretinal signals is likely mediated by the olfactoretinal centrifugal pathway, which originates from TN cells in the olfactory bulb and terminates in the inner plexiform layer in the retina (Stell et al., 1984; Behren and Wagner, 2004). In zebrafish, for example, after olfactory bulbectomy or drug inhibition of the TN synaptic input to retinal dopaminergic cells, stimulation of olfactory neurons produced no effect on visual sensitivity (Maaswinkel and Li, 2003).

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Recently, we generated a transgenic zebrafish line [Tg (GnRH-3::GFP)], which provides a tool for easy identification of the GnRH-containing TN cells in live embryos and in cell culture (Wang et al., 2010). In the transgenic fish, the TN cells express GFP, which is under the transcriptional control of the zebrafish GnRH-3 promoter. Using the transgenic fish, Wang et al. (2010) characterized the GnRH-containing TN cell development and axonal projection. They also characterized, to some extent, the physiological properties of the GFP-tagged GnRH-containing TN cells, including the firing patterns of spontaneous and evoked action potentials. They found that the GnRH-containing TN cells express ionotropic glutamate receptors, suggesting that the activity of TN cells may be influenced by signals from the olfactory bulbs.

Whereas the developmental course of TN cells and the potential roles of TN-signaling in zebrafish visual system functions have been documented (Torgersen et al., 2002; Abraham et al., 2008; Wang et al., 2010), the physiological properties of GnRH-containing TN cell membranes (e.g., the expression of voltage-activated ionic currents) remain to be further characterized. In this study, we characterized voltage-activated calcium current (I_{Ca}), sodium current (I_{Na}) and potassium current (I_K) using acutely dissociated GFP-tagged GnRH-containing TN cells from Tg (GnRH-3::GFP) fish. This study provides data on the electrophysiological properties of GnRH-containing TN cells, thereby shedding insight on the mechanisms of TN-signaling in animal physiology.

2. Results

2.1. *Voltage-activated calcium currents (I_{Ca}) in the GnRH-containing TN cells*

In this study, we examined voltage-activated ionic currents from freshly dissociated GFP-tagged TN cells from the olfactory bulbs of adult transgenic animals (between 2 and 3 months of age). In the transgenic zebrafish [Tg (GnRH-3::GFP)], the TN cells can be readily identified in live embryos and in cell culture (Fig. 1).

We recorded voltage-activated calcium current (I_{Ca}) in dissociated GFP-tagged GnRH-containing TN cells. During the recordings, the cells were perfused with medium that contained TTX and TEA in order to block voltage-activated sodium currents and potassium currents, respectively. All the examined GnRH-containing TN cells ($n=12$) express low-voltage-activated (LVA) I_{Ca} and high-voltage-activated (HVA) I_{Ca} (Figs. 2A and B). Depolarizing steps from a holding potential of −60 mV elicited a mixture of transient and sustained inward currents, which showed the characteristics of LVA and HVA I_{Ca}. With the increase of membrane depolarization, the amplitude of the current increased, until it reached the peak value at about −30 and +10 mV (transient and sustained currents, respectively). We measured the activation and steady-state inactivation voltages of LVA and HVA I_{Ca} (Figs. 2C and D).

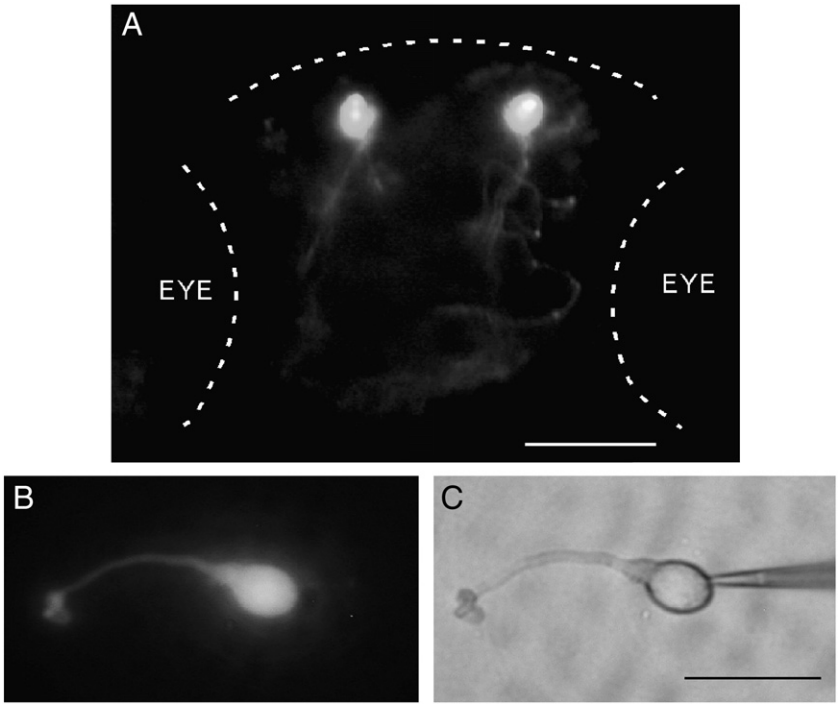

Fig. 1 – Images of a transgenic [Tg(GnRH-3::GFP)] zebrafish embryo and an isolated GFP-tagged GnRH-containing TN cell. A. A fluorescent image of a 4-day-old transgenic embryo that shows the TN cells in the olfactory bulb (dorsal view). Dashed lines outline the head and eyes (anterior, top). Scale bar: 30 μm. B and C. Fluorescent and bright-field images of an acutely dissociated GFP-tagged GnRH-containing TN cell. Note the recording electrode placed on the right side of the cell. Scale bar: 15 μm.