Brief Communication

Cooperative Glutamatergic and Cholinergic Mechanisms Generate Short-Term Modifications of Synaptic Effectiveness in Prepositus Hypoglossi Neurons

Juan de Dios Navarro-López, José M. Delgado-García, and Javier Yajeya

1Centro Regional de Investigaciones Biomédicas, Universidad de Castilla-La Mancha, 02071 Albacete, Spain, 2División de Neurociencias, Universidad Pablo de Olavide, 41013 Sevilla, Spain, and 3Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, 37007 Salamanca, Spain

To maintain horizontal eye position on a visual target after a saccade, extraocular motoneurons need a persistent (tonic) neural activity, called “eye-position signal,” generated by prepositus hypoglossi (PH) neurons. We have shown previously in vitro and in vivo that this neural activity depends, among others mechanisms, on the interplay of glutamatergic transmission and cholinergic synaptically triggered depolarization. Here, we used rat sagittal brainstem slices, including PH nucleus and paramedian pontine reticular formation (PPRF). We made intracellular recordings of PH neurons and studied their synaptic activation from PPRF neurons. Train stimulation of the PPRF area evoked a cholinergic-sustained depolarization of PH neurons that outlasted the stimulus. EPSPs evoked in PH neurons by single pulses applied to the PPRF presented a short-term potentiation (STP) after train stimulation. APV (an NMDA-receptor blocker) or chelerythrine (a protein kinase-C inhibitor) had no effect on the sustained depolarization, but they did block the evoked STP, whereas single pulses applied to the PPRF presented a short-term potentiation (STP) after train stimulation. APV (an NMDA-receptor blocker) or chelerythrine (a protein kinase-C inhibitor) had no effect on the sustained depolarization, but they did block the evoked STP, whereas pirenzepine (an M 1 muscarinic antagonist) blocked both the sustained depolarization and the STP of PH neurons. Thus, electrical stimulation of the PPRF area activates both glutamatergic and cholinergic axons terminating in the PH nucleus, the latter producing a sustained depolarization probably involved in the genesis of the persistent neural activity required for eye fixation. M 1-receptor activation seems to evoke a STP of PH neurons via NMDA receptors. Such STP could be needed for the stabilization of the neural network involved in the generation of position signals necessary for eye fixation after a saccade.

Key words: acetylcholine; glutamate; short-term potentiation; prepositus hypoglossi; oculomotor system; rats

Introduction

It has been shown in both cats and monkeys that neurons located in the prepositus hypoglossi (PH) nucleus encode pure eye position and functionally related position–velocity and velocity–position signals (Delgado-García et al., 1989; Fukushima et al., 1992; McFarland and Fuchs, 1992; Moschovakis, 1997). Indeed, transient pharmacological inactivations or permanent electrolytic lesions of the PH nucleus indicate that prepositus neural circuits are necessary for the generation of eye position signals subsequent to on- and off-directed saccades (Cheron and Godaux, 1987; Arnold et al., 1999; Moreno-López et al., 2002). PH neurons receive eye velocity signals from excitatory burst neurons (EBN) located in the paramedian pontine reticular formation (PPRF) (Igusa et al., 1980), and they project monosynaptically on extraocular motoneurons and other brainstem and cerebellar structures related to eye movements (McCrea and Baker, 1985). As a result, it has been suggested that PH neurons perform the neural integration of eye velocity signals into eye position motor commands in the horizontal plane (Robinson, 1981; Delgado-García et al., 1989; Moschovakis, 1997).

It has been shown recently that the sustained activity present in PH neurons during eye fixations is the result, at least partially, of synaptic events evoked by EBN, located in the PPRF, and cholinergic neurons, located either in the pontomesencephalic region and/or in the PH nucleus (Navarro-López et al., 2004). These synaptic effects were mediated by AMPA-kainate and M 1 muscarinic receptors located in PH neurons. Here, we show that train stimulation of the PPRF (mimicking eye velocity signals) evokes a short-term potentiation (STP) that follows the sustained activity of PH neurons. In this case, the activation of NMDA receptors (NMDAR) and M 1 muscarinic receptors is apparently responsible for the short-lasting potentiation of PH neurons. We also provide evidence that the interplay between NMDA and M 1 receptors seems to be mediated intracellularly through intermediate steps including the activation of protein kinase C (PKC) (Salter and Kalia, 2004). Thus, more than one synaptic mechanism seems to be involved in the generation of eye position signals characterizing the firing of PH neurons.

Materials and Methods

Animals. Experiments were performed in 25–35 rats (50–75 g) raised in the Salamanca University Animal House. Experiments were performed according to the European Union directive (609/86/EU) for the use of laboratory animals in acute experiments.
Preparation of slices. Animals were anesthetized deeply with halothane gas and decapitated. The brain was excised and immersed rapidly in oxygenated ice-cold (4–6°C) artificial CSF (ACSF) with sucrose (234 mM) replacing the NaCl (117 mM) to maintain osmolarity. Brainstem sagittal slices (350 μm thick) were cut in cold oxygenated Ringer’s solution using a Vibratome-S1000 (Technical Products International, O’Fallon, MO) and placed in an incubation chamber, where they were maintained for ~2 h at room temperature. Additional details of this in vitro preparation have been described previously (Yajeya et al., 2000).

In vitro recordings. For recordings, a single slice containing PH nucleus and rostral PPRF was transferred to an interface recording chamber (BSC-HT and BSC-BU; Harvard Apparatus, Holliston, MA) and perfused continuously with ACSF comprising the following (in mM): 117 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 25 NaHCO₃, 1.2 NaH₂PO₄, and 11 glucose. The ACSF was bubbled with carbogen gas (95%O₂–5%CO₂) and maintained at 30 ± 2°C.

Intracellular records from PH neurons were obtained with borosilicate glass microelectrodes (140–180 MΩ; World Precision Instruments, Sarasota, FL) filled with a potassium acetate solution (3 M) and connected to the head stage of an intracellular recording amplifier (VF180; Biologic, Claix, France). Micropipette tips were directed to the rostral third of the PH nucleus, where neurons carrying eye position signals are located (Delgado-Garcia et al., 1989; Navarro-Lopez et al., 2004). Only data from neurons that had stable resting potential with absolute values greater than ~60 mV in the absence of DC holding currents and that presented overshooting action potentials were collected for analysis. Spike amplitude and afterhyperpolarizing potentials were measured relative to the threshold.

Synaptic potentials were elicited orthodromically by stimulating the ipsilateral PPRF using a monopolar stainless-steel electrode (2 MΩ, World Precision Instruments, Sarasota, FL) representing the number of averaged responses. Postsynaptic potentials were characterized according to their amplitude (as a function of the resting potential) and latency. When needed, the same stimulating pulses were also presented in trains (100 ms) at 50–200 Hz.

Identification of stimulating and recording sites. Recorded neurons were identified following procedures described previously (Navarro-Lopez et al., 2004). Briefly, selected neurons were stained by the intracellular injection of biocytin diluted in a 2 M potassium acetate solution (McDonald, 1992) using positive current pulses of 0.2 nA for 6 min. Slices were fixed and cut in sections (45 μm) using a freezing microtome (HM400R; Microm, Heidelberg, Germany). Sections were incubated with avidin-biotin–peroxidase complex (Vector Laboratories, Burlingame, CA). 3,3’-Diaminobenzidine was used as chromogen for visualization of the biocytin complex. Alternate sections were counterstained with cresyl violet. Neurons were reconstructed from serial sections using a camera lucida (Nikon Labophot; Nikon, Kawasaki, Japan).

Drugs. All chemicals used in this study were applied by superfusion in the ACSF. The chemicals used were atropine sulfate (a nonspecific antagonist of cholinergic receptors), 6-cyano-nitroquinoline-2,3-dione (CNQX; a potent, competitive AMPA-kainate receptor antagonist), and 2-amino-5-phosphonovlate (APV; a specific blocker of NMDAR) from Sigma (St. Louis, MO), pirenzepine (a selective blocker of M₁ muscarinic receptors) from Biogen Científica (Madrid, Spain), and chelerythrine chloride (specific and potent, cell-permeable inhibitor of PKC) from Alomone Labs (Jerusalem, Israel).

Data storage and statistical analysis. Data were acquired and stored, as an analog signal, on a videocassette, using a modified video recorder (Physiologic-3; Cibertec, Madrid, Spain). Off-line data acquisition and analysis were performed with the help of a Cambridge Electronic Design (Cambridge, UK) 1401 interface between the tape recorder and a computer, using the Mini Analysis program, version 5.2.1 (Synaptosoft, Decatur, GA). Unless otherwise indicated, the electrophysiological data are all expressed as mean ± SEM, and n represents the number of averaged neurons. Synaptic potentials were averaged (≥5) before quantitative analysis. Statistical analysis of collected data was performed using a paired Student’s t test and, when necessary, one-way ANOVA. Statistical significance was determined at a level of p ≤ 0.05.

Results

Characterization of PH neurons and their response to PPRF stimulation

This study comprises 42 intracellular recordings from PH, selected because of their resting potential (less than or equal to ~60 mV) and monosynaptic activation from the PPRF. PH neurons were identified electrophysiologically by the absence of spontaneous firing at resting membrane potential and the presence of a biphasic afterhyperpolarization in their action potential when depolarized (Navarro-López et al., 2004). The location of selected PH neurons (n = 12) filled with biocytin is illustrated in Figure 1B. The morphology and initial axonal trajectories of labeled PH neurons suggest that they could be considered “principal cells” (McCrea and Baker, 1985) (i.e., a type of PH cell projecting to many brainstem sites related to the oculomotor system, including the abducens nucleus).

Single subthreshold stimulations of the PPRF evoked monosynaptic EPSPs in PH neurons in all cases (n = 42). The EPSPs presented a mean latency of 2.57 ± 0.30 ms, a rise time of 6.5 ± 2.9 ms, a decay time of 20.8 ± 8.7 ms, and a duration of 71.6 ± 23.8 ms (Fig. 1C, top). EPSPs evoked in PH neurons by PPRF stimulation were not modified in amplitude or latency by slice superfusion with atropine sulfate (1–3 μM) or APV (50 μM), but were removed completely by the application of CNQX (10–12 μM) (Fig. 1C). These results indicate that the EPSP evoked in PH neurons by PPRF stimulation was mediated by glutamate acting exclusively on AMPA-kainate receptors, at least when stimulus rate was set at ≤0.2 Hz.

![Figure 1](https://example.com/figure1.png)
Differential effects of train stimulation of PPRF on PH neurons in presence of glutamatergic and/or cholinergic drugs

Train stimulation (200 Hz, 100 ms) of the PPRF evoked a sustained depolarization of PH neurons that exceeded the end of the train by up to 400–500 ms (Fig. 2). This persisting depolarization was large enough to evoke a burst of action potentials that reached peak frequencies of 150–200 spikes/s. The frequency of the burst of action potentials evoked by PPRF train stimulation decayed with time, with a time constant of 140 ± 15 ms (range, 70–210; n = 11; p < 0.01) (Fig. 3A) [i.e., a value similar to the time constants of cat abducens motoneurons (90–197 ms) (Delgado-García et al., 1986) and “position-velocity” PH neurons recorded in behaving cats (46–371 ms) (Delgado-García et al., 1989)].

The sustained depolarization was the probable result of M1 muscarinic-receptor activation, because the effect was removed by atropine sulfate (1 µM; n = 7) (Fig. 2A) and by pirenzepine (0.5 µM; n = 6) (Fig. 2B). However, superfusion with APV (50 µM; n = 8) or chelerythrine (2.5 µM; n = 5) superfused to the bathing solution.

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Persistent activity and STP are both present in PH neurons

As proposed recently (Navarro-López et al., 2004) and confirmed here, train stimulation of the PPRF, with stimulus parameters similar to those evoked in PPRF neurons during normal saccades in behaving mammals, is able to produce a sustained depolarization of PH neurons lasting the train by up to 500 ms. This sustained depolarization is dependent both on the activation of AMPA-kainate receptors by EBN (Igusa et al., 1980) and on cholinergic axons terminating on the same PH neurons and acting on M1 muscarinic receptors. These results, collected from brainstem slices, have been confirmed in behaving cats by the local (at the PH nucleus) injection of different cholinergic drugs (Navarro-López et al., 2004). Here, we also report an STP (lasting for ~120 s) evoked in PH neurons by train stimulation of the PPRF. In this case, the potentiation seems to be mediated by NMDAR, because it was easily blocked by superfusion with APV, indicating a postsynaptic origin. Additional proof that this STP has a postsynaptic origin is that both pirenzepine and chelerythrine were able to block it when applied to the bathing solution.

Although the sustained depolarization triggered in PH neurons by PPRF stimulation (i.e., by EBN) (Igusa et al., 1980) carrying eye velocity signals can be described as a synaptic mechanism involving the generation of the neural persisting activity (Aksay et al., 2003; Major and Tank, 2004; Navarro-López et al., 2004) necessary for eye positions of fixation, the role of the STP described here is of a difficult interpretation within oculomotor system performance. The involvement of NMDAR of the PH in the generation of eye position signals has been demonstrated previously in the alert cat (Cheron et al., 1992; Mettens et al., 1994). On the basis of in vivo and in vitro experiments, the NMDA-related STP mechanism seems to be crucial for persistent activity in the PH. This is somewhat paradoxical, because STP, mainly in the form of posttetanic potentiation, was first described in motor networks, although, in recent years, it has been related more with cortical network functioning (Nadim and Manor, 2000). Nevertheless, the reported STP could act as a mechanism for high-pass filtering in oculomotor function, facilitating the synchronous activation of (postsynaptic) PH neurons, increasing signal-to-noise ratio (Lisman, 1997), and canceling out the disturbing effects of low rate firing of EBN. STP could still be useful in facilitating the action of error signals addressed to correct aimed versus achieved eye positions (Schultz and Dickinson, 2000). In this regard, Shen (1989) proposed a model of oculomotor integration based on potentiation phenomena at the synaptic level. If we assume an opposite action (i.e., short-term depression) evoked by contralateral inhibitory burst neurons (Hikosaka et al., 1980), the in vivo interplay of short-term synaptic facilitation and depression would help network stability and maintenance (Nadim and Manor, 2000). In contrast, the sustained activity that follows train stimulation of the PPRF seems to be related more with the generation of eye position signals. Indeed, the amplitude and duration of the evoked EPSPs are related linearly to train frequency (Navarro-López et al., 2004), and, as shown here, the time constant of the decaying firing (Fig. 3A) is similar to the time constant of PH and abducens neurons and of the oculomotor plant (Robinson, 1981; Delgado-García et al., 1989; Fukushima et al., 1992; Moschovakis, 1997).

References


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