Brain Research Bulletin 127 (2016) 47–55

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Postnatal changes in glutamatergic inputs of jaw-closing motoneuron dendrites

Shoko Nagata[a,b], Shiro Nakamura[a,*], Kiyomi Nakayama[a], Ayako Mochizuki[a], Matsuo Yamamoto[b], Tomio Inoue[a]

[a] Department of Oral Physiology, Showa University School of Dentistry, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo, 142-8555, Japan
[b] Department of Periodontology, Showa University School of Dentistry, 2-1-1 Kitasenzoku, Ohta-ku, Tokyo, 145-8515, Japan

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form 15 July 2016
Accepted 23 August 2016
Available online 24 August 2016

Keywords:
Dendrites
Active properties
Masseter motoneurons
Photostimulation
NMDA spikes/plateau potentials
Postnatal development

ABSTRACT

Dendrites of masseter (jaw-closing) motoneurons (MMNs) are well developed and ramify extensively throughout the trigeminal motor nucleus and often extend into the adjacent reticular formation. It is possible that the dendrites have active properties, which are altered with the development of the orofacial musculoskeletal system. Thus, we examined the changes in somatic voltage responses evoked by photostimulation of the MMN dendrites by laser photolysis of caged glutamate from postnatal day (P) 2–5 and 9–12 rats. We photostimulated 39 spots that were arranged around each recorded neuron in a concave shape and found that the dendritic stimulation induced somatic depolarization in the presence of tetrodotoxin in all MMNs. With increasing photostimulation intensity, the responses grew in amplitude up to a certain threshold, where a step-like increase in amplitude occurred. In 75% of P2–5 MMNs, the step-like increase in amplitude, which was blocked by 20 μM D(−)-2-amino-5-phosphonovaleric acid application, corresponded to the NMDA spikes/plateau potentials. In contrast, at P9–12 the responses became significantly smaller in amplitude and shorter in duration and only one neuron out of 12 MMNs showed NMDA spikes/plateau potentials. These results suggest that the glutamatergic responses evoked by photostimulation of the MMN dendrites change during the first two postnatal weeks, and these changes may be involved in the transition from suckling to chewing during postnatal development.

1. Introduction

Trigeminal motoneurons (TMNs), which send the final patterned motor commands to the jaw-closing and jaw-opening muscles, are one of the largest cells in the brain and spinal cord. The dendrites of adult cat TMNs are well developed, extend for ~2 mm in various directions, and often extend far beyond the boundaries of the trigeminal motor nucleus (MoV) and into the reticular formation (Shigenaga et al., 1988; Yoshida et al., 1987). Studies employing intracellular injections of horseradish peroxidase and postembedding immunogold labeling have demonstrated that excitatory and inhibitory synapses are densely distributed throughout the primary, intermediate, and distal dendrites of the jaw-closing and jaw-opening motoneurons in the cat (Bae et al., 1999; Shigenaga et al., 2005). These results suggest that the TMNs receive a number of inputs on the extensive surfaces of the dendrites.

The dendrites of some neurons such as neocortical neurons and motoneurons have active voltage-dependent conductances that are involved in the processing of synaptic inputs (Lee and Heckman, 1996; Schwindt and Crill, 1995). Cortical pyramidal neurons have been shown to have large dendrites that have multiple active conductances through Na^+, Ca^{2+}, and K^+ channels and *N*-methyl D-aspartate (NMDA) receptor channels (for reviews: Antic et al., 2010; Major et al., 2013). Dendritic NMDA spikes/plateau potentials were first described in the basal dendrites of cortical pyramidal neurons (Schiller et al., 2000), and were defined as step-like increases in depolarization with increasing glutamate concentration on the dendrites (Major et al., 2008). The NMDA spikes/plateau potentials have been suggested to play important roles in the supralinear synaptic integration along individual dendrites (Palmer et al., 2014; Schiller and Schiller, 2001) and shifts from burst to tonic mode in the thalamocortical neurons by lifting the membrane potentials (Augustinaite et al., 2014). The TMNs also have been shown to generate a strong persistent inward current that is largely mediated by persistent Na^+ (Hsiao et al., 1998; Lee and Heckman, 2001; Li

Abbreviations: APV, D(−)-2-amino-5-phosphonovaleric acid; DRL, dextran-tetramethylrhodamine-lysine; MMN, masseter motoneuron; MoV, trigeminal motor nucleus; NMDA, *N*-methyl D-aspartate; TMN, trigeminal motoneuron.

* Corresponding author.
E-mail address: snaka@dent.showa-u.ac.jp (S. Nakamura).

http://dx.doi.org/10.1016/j.brainresbull.2016.08.014
0361-9230/

and Bennett, 2003) and low-voltage-activated L-type Ca^{2+} channels (Hounsgaard and Kiehn, 1993; Hsiao et al., 1998). Moreover, NMDA receptor-mediated inward currents have been shown in the TMNs (Hsiao et al., 2002; Kim and Chandler, 1995); however, little is known about the generation of the NMDA spike/plateau potentials that can affect processing of synaptic inputs on the TMNs. Furthermore, because the feeding behavior in mammals changes dramatically from suckling to chewing and biting with development of the orofacial musculoskeletal system during the early postnatal period, it is also possible that the active dendritic properties of the TMNs are altered during the early postnatal period to enable this change.

In this study, we investigated the developmental changes of the glutamatergic responses elicited by local photostimulation of the dendrites using laser photolysis of caged glutamate combined with somatic whole-cell recordings in the masseter motoneurons (MMNs) obtained from postnatal day (P) 2–5 and 9–12 rats.

2. Materials and methods

Experimental protocols were approved by the International Animal Research Committee of Showa University, which operates in accordance with Japanese Government Law No. 105 for the care and use of laboratory animals.

2.1. Slice preparation

Coronal brainstem slices (400 μm thick) including the MoV were prepared from 22 P2–5 and 18 P9–12 Wistar rats as described previously (Nakamura et al., 2014; Nonaka et al., 2012). Briefly, the animals were anesthetized deeply with isoflurane until there was a loss of response to tail pinch and then the animals were decapitated. Each brainstem was rapidly removed and placed in ice-cold (4 °C), sucrose-based, modified artificial cerebrospinal fluid (ACSF) containing (in mM) 260 sucrose, 3 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose. Modified ACSF was continuously bubbled with a 95% O_2-5% CO_2 gas mixture to establish a pH of 7.4. Coronal sections were cut on a vibrating microtome (VT1200S, Leica Microsystems, Tokyo, Japan). The slices were allowed to recover in a holding chamber containing a 50:50 mixture of modified and normal ACSF at 34 °C for 30 min, followed by a 30 min incubation with normal ACSF at 34 °C. Normal ACSF contained (in mM) 130 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose. The slices were then maintained at room temperature (25–27 °C).

2.2. Retrograde labeling of jaw-closing motoneurons

One to three days before the preparation of slices, 22 P2–5 and 18 P9–12 animals were anesthetized with isoflurane as described above, and 2–5 μL of 5% dextran-tetramethylrhodamine-lysine (DRL, Life Science Technologies, Grand Island, NY, USA) (3000 or 10,000 MW) in distilled water was injected bilaterally into the masseter muscles with 10 μL Hamilton microsyringes (Hamilton, Reno, NV, USA) to retrogradely label the MMNs. After the animals recovered from anesthesia, they were returned to their mothers while the DRL was retrogradely diffused.

2.3. Electrophysiological recording

Brainstem slices were transferred to a recording chamber attached to the stage of an upright microscope (BX51WI; Olympus, Tokyo, Japan) and anchored with a nylon mesh. DRL-labeled neurons were identified under epifluorescence illumination with a rhodamine filter set. Whole-cell patch-clamp recordings in the current-clamp configuration were made with infrared videomicroscopy and a 40x water immersion objective (0.8 NA, LUMPlanFI, Olympus) with differential interference contrast. Patch electrodes were prepared from single-filament 1.5 mm diameter borosilicate capillary tubing (GD-1.5, Narishige, Tokyo, Japan) with a microelectrode puller (P-97, Sutter Instruments, Novato, CA, USA). Electrodes were filled with an internal solution of (in mM) 130 K-gluconate, 10 KCl, 10 HEPES, 0.4 EGTA, 2 $MgCl_2$, 2 Mg-ATP, and 0.3 Na_2-GTP (pH 7.3, 285–305 mOsm). Lucifer yellow (Sigma-Aldrich, St Louis, MO, USA) or biocytin (Sigma-Aldrich) was added to this internal solution at a concentration of 5 mg/mL to allow for post hoc morphological identification of the recorded cells. Pipette resistance was 2.5–5.0 MΩ when the electrodes were filled. The voltage of the responses evoked with laser photolysis of caged glutamate was recorded with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA). The data were filtered at 10 kHz, digitized at 20 kHz (Digidata 1440, Molecular Devices), and analyzed on a personal computer using pCLAMP 10.2 (Molecular Devices). The measured liquid junction potential of 10 mV was subtracted from all membrane potentials, and series resistance compensation was set to 60–80% in the whole-cell patch configuration. All of the experiments were performed at room temperature.

In all experiments, 1 μM tetrodotoxin (TTX; Tocris Cookson, Ellisville, MO, USA) was added to the bath to block the presynaptic action potentials in the premotor neurons projecting to the MMNs (see Ref. Nonaka et al., 2012). NMDA receptors were blocked with 20 μM D(−)-2-amino-5-phosphonovaleric acid (APV; Sigma-Aldrich).

2.4. Photostimulation

To systematically stimulate various sites in the slices using simultaneous whole-cell recordings from an MMN, laser photolysis of caged glutamate was performed using a 365 nm nitrogen-pulsed Micropoint laser system coupled with galvanometer-based steering lenses (Photonic Instruments, St. Charles, IL, USA). To position the laser beam, the steering lenses were controlled with MetaMorph software (Molecular Devices). After establishing the whole-cell configuration of MMNs using the 40x water immersion objective, the 40x objective was carefully switched to a 4x objective (0.28 NA, XLFluor, Olympus). Next, a concentration of 300 μM caged glutamate (4-methoxy-7-nitroindolinil-caged L-glutamate (MNI glutamate); Tocris Cookson) was added to the recirculating ACSF. All of the experiments began at least 10 min after the addition of MNI glutamate. Single pulses of laser beams (2–6 ns, 1.2–13.9 μJ) were delivered to the center of each location at 5 s intervals to trigger focal photolysis of MNI glutamate. The strength of the photostimulation was adjusted on the MetaMorph software. Each spot was stimulated 3–4 times.

To stimulate the dendrites of MMNs, we delivered photostimulation to 39 different spots for each neuron, which surrounded the recorded MMNs and were arranged in a concave shaped array with 100 μm spacing between adjacent rows and columns. The stimulation order was defined to maintain the distance between two consecutive stimulation points >300 μm. For statistical analysis, we selected direct dendritic responses evoked by photostimulation on or close to the labeled-dendrites (within a distance of 30 μm).

2.5. Histology

The neurons were filled by passive diffusion with Lucifer yellow ($n = 17$) or biocytin ($n = 23$) provided through the recording pipette during whole-cell recording (1–1.5 h) without current injection. These neurons were stained with Lucifer yellow and biocytin after the electrophysiological experiments were completed. At the end of the experiments, two sites in each slice were marked by passing a 10 s, 20 μA negative current through a Teflon-insulated tung-