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Glutamate release from satellite glial cells of the murine trigeminal ganglion

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HIGHLIGHTS

- Extracellular glutamate was recorded microfluorimetrically.
- Satellite glial cells released glutamate in response to internal Ca^{2+} increase.
- Glutamate was released via vesicular exocytosis and via hemichannels.

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ABSTRACT

It has been proposed that glutamate serves as a mediator between neurons and satellite glial cells (SGCs) in sensory ganglia and that SGCs release glutamate. Using a novel method, we studied glutamate release from SGCs from murine trigeminal ganglia. Sensory neurons with adhering SGCs were enzymatically isolated from wild type and transgenic mice in which vesicular exocytosis was suppressed in glial cells. Extracellular glutamate was detected by microfluorimetry. After loading the cells with a photolabile Ca^{2+} chelator, the intracellular Ca^{2+} concentration was raised in SGCs by a UV pulse, which resulted in glutamate release. The amount of released glutamate was decreased in cells with suppressed exocytosis and after pharmacological block of hemichannels. The data demonstrate that SGCs of the trigeminal ganglion release glutamate in a Ca^{2+}-dependent manner.

1. Introduction

Recent research on glial cells demonstrated that these cells are not only the supporting cells of the nervous tissue, but may be involved in signal processing by participating in bidirectional communication with neurons [1]. It has been shown that glial cells of the central nervous system are able to release chemical signals, so-called gliotransmitters, via various mechanisms, such as Ca^{2+}-dependent exocytosis [2] or hemichannels [3]. Release of gliotransmitters may modulate neuronal activity [4], synaptic transmission [5] and other processes. Typical gliotransmitters observed in astrocytic signaling are glutamate, D-serine, and ATP [6]. In transgenic mice in which the exocytotic machinery was eliminated specifically in glial cells, transmitter release from these cells in hippocampal slices was impaired, indicating that vesicular release occurs normally in astrocytes [7]. Recently, we established a fluorimetric method to record glutamate release from retinal Müller glial cells [8].

In comparison with the existing data on astrocytes of the central nervous system, much less is known about glial cells and neuron–glia-interaction in the peripheral nervous system. Sensory ganglia contain the somata of sensory neurons, which are surrounded by satellite glial cells (SGCs) [9]. SGCs are similar in many aspects to other glial cells; however, they are unique as they form a complete glial sheath around sensory neurons with a 20-nm wide gap between both cell types [9]. This has to be taken into account when investigating chemical communication within the ganglion. It is very likely that non-synaptic chemical communication not only occurs between neurons but also between SGCs and neurons [10]. For example, expression of P2 receptors on SGCs of the trigeminal ganglion has been described [11]. Bidirectional calcium signaling between SGCs and neurons involving nucleotide P2 receptors has

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been observed in cultured mouse trigeminal ganglia [12]. Moreover, it has been demonstrated that SGCs from dorsal root ganglia (DRG) release ATP via activation of P2X7 receptors [13]. To investigate the involvement of SGCs in glutamatergic signaling within sensory ganglia, we studied Ca^{2+}-dependent glutamate release from SGCs from the murine trigeminal ganglion.

2. Materials and methods

2.1. Animals and preparation of ganglia

The experiments were done in accordance with the European Communities Council Directive 86/609/EEC, and were approved by the local authorities. Adult (2–6 months old) dnSNARE mice [7] and wild type mice (C57Bl6J) of either sex were used. dnSNARE mice express the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) domain of synaptobrevin 2 and EGFP (enhanced green fluorescent protein) in GFAP (glial fibrillary acidic protein)-positive cells under the control of the "tet-Off" tetracycline transactivator. Transgene expression was suppressed during the first 3 postnatal weeks by application of doxycycline (25 mg/l) into the drinking water. Animals were euthanized by CO_2, the skull was opened and the brain removed, exposing the two trigeminal ganglia. To obtain isolated neurons, the ganglia were cut in two halves and incubated in Ca^{2+}, Mg^{2+}-free solution (140 mM NaCl, 3 mM KCl, 10 mM HEPES, and 11 mM glucose; pH 7.4) containing 1.5 mg/ml collagenase (type IA; Sigma–Aldrich, Taufkirchen, Germany) for 60 min at 37 °C in the dark. The Ca^{2+}, Mg^{2+}-free extracellular solution was supplemented with glutamine (0.25 mM), glutamate (0.5 mM), and a photolabile calcium chelator (O-nitrophenyl ethylene glycol tetraacetic acid acetoxymethyl, NP-EGTA, 10 μM; Invitrogen, Eugene, OR, USA). The incubation was performed on a laboratory shaker at 650 rpm. After several washes with normal extracellular solution (136 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, 11 mM glucose, pH 7.4), the ganglia were treated with DNase I (200 U/ml; Sigma–Aldrich) and washed again. Trituration of the ganglia using a wide-bore pipette resulted in isolated neurons that were still covered by SGCs (Fig. 1A). The components of the Amplex® Red Glutamic Acid kit (Invitrogen; 100 μM Amplex® Red reagent, 0.5 U/ml horseradish peroxidase, 0.16 U/ml L-glutamate oxidase, 1.0 U/ml L-glutamate-pyruvate transaminase, 400 μM L-alanine), NP-EGTA (10 μM), and D,L-*threo*-β-benzyloxyaspartate (200 μM) to block glial glutamate uptake were added before the final step of trituration.

2.2. Microfluorimetry

A fluorimetric enzyme assay based on the Amplex® Red Glutamic Acid kit was used to visualize glutamate release from SGCs [8]. The cell suspension obtained as described above was mixed with 1% agarose and incubated for 30 min in custom-made recording chambers. In some cases, NP-EGTA or the enzymes of the kit were removed for control experiments, and blocking substances were added for pharmacological experiments. Glutamate released from cells into the extracellular space is used in a cascade of enzymatic reactions resulting in the formation of the fluorescent product resorufin. Resorufin fluorescence was imaged by confocal laser microscopy above the soma of a SGC (LSM 510 Meta, 100×/0.9 Achroplan oil immersion objective, Zeiss, Oberkochen, Germany; 543 nm helium-neon laser, 585 nm long pass filter, pinhole maximally open).

Calcium transients that should evoke glutamate release were induced by four repetitive UV pulses (351 nm/364 nm Enterprise UV-Laser, 500 ms at maximal intensity) to release Ca^{2+} ions from NP-EGTA. To confirm that the cells showed UV-induced Ca^{2+} transients, cells were loaded with the Ca^{2+}-sensitive dye, fluo-4/AM (11 μM), in normal extracellular solution with 2% Pluronic® F-127 for 30 min at room temperature. SGCs preferentially took up the dye whereas neurons were filled to a much lesser extent. To fill neurons more efficiently, incubation time was increased to 150 min. After loading the cells with fluo-4 and NP-EGTA and subsequent washing, the cell suspension with 1% agarose was placed in chambers. Fluo-4 fluorescence was recorded every 1.5 s with the LSM 510 Meta (argon laser 488 nm, 505–550 nm band pass filter). Peak amplitudes were calculated as difference between mean fluorescence values (four time points) before and after the UV pulse. The changes in Ca^{2+} concentrations are given in relative units.

Significance was determined with the non-parametric Mann–Whitney *U* test.

3. Results

3.1. Ca^{2+} microfluorimetry

We first tested whether the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) could be increased in SGCs after loading them with NP-EGTA. UV pulses onto NP-EGTA-loaded cells resulted in transient increases of the $[Ca^{2+}]_i$ that could not be recorded in the absence of NP-EGTA (Fig. 1B). Because SGCs are still adhered to neurons, it could be argued that the recorded $[Ca^{2+}]_i$ increases occur in the neuron or are of neuronal origin and only secondarily evoked in SGCs by intercellular signaling. To clarify this point, we recorded responses in the somata of SGCs and in the neurons. We observed responses of significantly higher amplitudes when recording in SGCs as compared with neuronal responses (Fig. 1C). As we have shown previously [11], SGCs take up Ca^{2+}-sensitive dyes more efficiently than neurons (Fig. 1C and D). Therefore, it is possible that $[Ca^{2+}]_i$ increases are evoked in neurons but cannot be recorded because of low neuronal dye loading during 30 min of incubation. We therefore incubated the cell suspension for 150 min in fluo-4/AM, which resulted in clearly increased dye loading of neurons (Fig. 1D). However, even under this condition we recorded significantly higher responses in SGCs than in neurons (Fig. 1D). Moreover, glial responses did apparently not show delay after the neuronal ones. Therefore, we conclude that (i) UV pulses evoke Ca^{2+} transients directly in SGCs independently from neurons, and (ii) UV pulse-evoked Ca^{2+} transients in neurons are low, probably because of small amount of NP-EGTA uptake.

3.2. Ca^{2+}-dependent glutamate release

Next, we investigated whether UV pulse-evoked Ca^{2+} transients in SGCs elicited glutamate release. Using a fluorimetric assay that was established on isolated retinal glial cells [8], we studied the release of glutamate from SGCs. UV stimulation of SGCs from wild type mice resulted in distinct increases of the resorufin fluorescence when recorded directly above the glial cell somata (Fig. 2). Signals recorded above neurons at a distance from SGCs somata were markedly smaller. For control, fluorescence was recorded from an area distant from any cell, and no increase could be detected (Fig. 2A). The specificity of the assay was verified by omitting NP-EGTA or the glutamate-specific enzymes of the assay. Under both conditions the recorded fluorescence was significantly reduced (Fig. 2B).

We used transgenic dnSNARE mice that express the SNARE domain of synaptobrevin 2 in GFAP-positive cells, thus preventing exocytosis of vesicles from the respective cells. The cells also express EGFP under control of the GFAP promoter, which enables their identification. Resting SGCs express only low amounts of GFAP, and expression is upregulated after injury [9]. However, we