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Research article

Cholinergic responses of satellite glial cells in the superior cervical ganglia

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ABSTRACT

Satellite glial cells (SGCs) surround the neurons in sympathetic ganglia and are believed to make important contributions to the function of the ganglia under normal and pathological conditions. It has been proposed that SGCs communicate chemically with the neurons, but little is known about their pharmacological properties and there is no information on whether they respond to acetylcholine (ACh), which is the major neurotransmitter in these ganglia. We used calcium imaging to examine responses of SGCs in the mouse superior cervical ganglion to ACh. The SGCs responded to ACh (0.01–2 mM) with an elevation of intracellular Ca^{2+}, which appeared to be due to direct action on these cells, as the response persisted in the presence of the nerve blocker tetrodotoxin (1 μM). The response was largely inhibited by atropine, indicating an action on muscarinic ACh receptors. In contrast to this, sensory ganglia (nodose and trigeminal) were not sensitive to ACh. Incubation of the ganglia in ACh (0.5 or 1 mM) increased the expression of glial fibrillay acidic protein, which is a marker for glial activation. Such incubation also increased the electrical coupling of SGCs, which is known to occur in sensory ganglia following injury. We conclude that SGCs in the superior cervical ganglia display muscarinic ACh receptors, which enable them to communicate chemically with the sympathetic neurons.

1. Introduction

It is now well established that glial cells are essential to the normal function of the nervous system and that they also play crucial roles in a variety of pathological states [1]. Most of the research on glial cells has been done on the central nervous system, and much is known about astrocytes, microglia and oligodendrocytes. In recent years there has been considerable amount of research on satellite glial cells (SGCs) in sensory ganglia, which form a tight envelope around the neuronal somata, and it was revealed that these cells play important roles in chronic pain [2–5]. In contrast, knowledge on SGCs in sympathetic ganglia is still scarce (for review see [6]).

The basic organization of SGCs in sensory ganglia and sympathetic ganglia is quite similar [6,7]. However, there is ground to believe that SGCs in these two ganglion types may be functionally different, largely because in sensory ganglia there are no synapses and no dendrites, whereas all the neurons in sympathetic ones receive synapses and extend dendrites [8,9]. Ultrastructural studies have shown that SGCs in these ganglia cover the axon terminals that make contact on, or near the neuronal somata [6,10,11], and it is therefore likely that the proximity of SGCs to the synapses allows these cells to influence synaptic transmission. As glial cells communicate with neighboring cells mainly by chemical messengers, it is of considerable interest to learn about the pharmacological properties of SGCs in sympathetic ganglia. It has been reported that these cells have P2 receptors [12,13], although there was some disagreement on the P2 subtypes (see [6] for discussion). We have shown recently that SGCs in the mouse superior cervical ganglion (Sup-CG) are highly sensitive to endothelin-1, which acts predominantly on ETb receptors [14]. There is evidence that SGCs in sensory ganglia display a high degree of plasticity following nerve damage [2,5]. Less is known about plasticity of SGCs in sympathetic ganglia, but the available information indicates that SGCs in these ganglia are altered by peripheral injury. For example, following cardiac ischemia the expression of P2X7 purinergic receptors is elevated in SGCs in rat Sup-CG [15], and sciatic nerve axotomy upregulated the glial activation marker glial fibrillary acidic protein (GFAP) in rat sympathetic ganglia [16]. Also, SGCs in sympathetic ganglia have a role in synaptic stripping following nerve injury [17,18]. As found for sensory ganglia, inflammation augments dye coupling between SGCs in sympathetic ganglia [19].

In the present work we studied responses of SGCs in mouse Sup-CG to ACh, using calcium imaging. We also investigated the effects of ACh on some properties of SGCs.

Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; GFAP, glial fibrillary acidic protein; SGC, satellite glial cell; Sup-CG, superior cervical ganglion

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2. Materials and methods

The procedures were approved by the Animal Care and Use Committee of the Hebrew University-Hadassah Medical School and conform to the National Institutes of Health standards for the care and use of laboratory animals. Balb/c-mice 3–5 months old of either sex (males:females about 1:1), weighing 19–23 g, were used (N = 72).

2.1. Ganglia incubation (in vitro) and immunohistochemistry

Mice were sacrificed by CO_2 inhalation, and the Sup-CG were removed and placed in a bath filled with Krebs solution; composition (in mM): NaCl 118, KCl 4.7, $NaHCO_3$ 14.4, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.5, pH 7.3, saturated with 95% O_2 and 5% CO_2. Incubations of the ganglia in drugs (ACh, tetrodotoxin, curare) were carried out at 37 °C in a CO_2 incubator. For the dye injection and calcium imaging, the capsule of the ganglia was removed. Control ganglia were incubated under the same conditions in Krebs solution. After the treatments, ganglia were studied by immunohistochemistry. Ganglia were fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.4) for 90 min at room temperature, then washed in 0.1 M PBS and incubated overnight at 4 °C in PBS with 20% sucrose before freezing in Tissue-Tek embedding medium (Sakura Fintek, Torrance, CA) . Sections were cut 10 μm thick using a cryostat (Jung CM3000, Leica Microsystems, Wetzlar, Germany) and thaw-mounted on glass slides. Sections were washed in PBS and incubated with 50 mM ammonium chloride for 30 min to reduce autofluorescence, then washed in PBS and incubated in a blocking solution containing 3% bovine serum albumin (BSA) in PBS with 0.3% Triton X-100 for 2 h. Primary antibody against GFAP (rabbit), was diluted 1:400 in PBS containing 1% BSA, and incubated overnight at 4 °C. Controls omitted the primary antibody. Sections were washed in PBS and incubated with secondary antibody; donkey anti-rabbit conjugated to Alexa Fluor 594 diluted 1:400 in PBS containing 1% BSA and 10 μM 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) to stain the nuclei for 2 h at room temperature. Finally sections were washed in PBS and visualized using an upright microscope (Axioskop FS2, Zeiss, Jena, Germany), equipped with fluorescent illumination and a digital camera (Penguin 600CL, Pixera, Los Gatos, USA), connected to a personal computer. Microscope fields (315 μm × 235 μm) were selected randomly. All the images were taken under identical conditions, and were analyzed in a blinded manner. Neuronal profiles that were surrounded by GFAP-positive SGCs by more than 50% of their circumference were counted and expressed a percentage of the total number of nucleated neuronal profiles present in the field analyzed. This criterion was used because the SGC sheath can be partly very thin and invisible under light microscopy. Only neurons with visible nuclei were counted. Four fields from different, non-adjacent sections were analyzed from each ganglion and then averaged.

2.2. Intracellular labeling

Freshly isolated ganglia were attached to the bottom of a silicon rubber-coated dish using fine pins. The dish was placed on the stage of an upright microscope, equipped with fluorescent illumination and a digital camera. The dish was superfused with Krebs solution, saturated with 95% O_2 and 5% CO_2 at 22–24 °C. Individual SGCs were injected with the fluorescent dye Lucifer yellow (LY), 3% in 0.5 M LiCl solution from sharp glass microelectrodes, connected to a preamplifier (NeuroData Instrument Corp., New York, NY, USA). The dye was passed by hyperpolarizing current pulses, 100 ms in duration; 0.5 nA in amplitude at 10 Hz for 3–5 min. Cells were injected blindly because individual cells are not visible in the intact ganglia; however, dye-injected cells were clearly visible, which enabled their identification during the injections, using a x40 water immersion objective. After the injection of each cell, we checked whether labeled SGCs (dye-coupled cells) were present around neighboring neurons as a result of dye passage from the injected cell. In some of the experiments drugs were added into the bathing medium 15 min before testing the effects of agonists. The coupling incidence was calculated as the ratio between the total number of injected cells to the number of dye-coupled ones. ACh was added to the bathing medium 15 min before starting to test its effects on coupling. When antagonists (TTX or atropine) were used, they were added 15 min before adding ACh.

2.3. Ca^{2+} imaging

Freshly isolated ganglia were fixed to the bottom of a silicon rubber-coated dish using fine pins. For Ca^{2+} microfluorimetry SGCs in intact ganglia were loaded with the Ca^{2+} indicator Fluo-3 AM (10 μM) in minimum essential medium-α for 70 min in an incubator at 37 °C [20,21]. Ganglia were mounted in a recording chamber on the stage of an upright microscope and superfused at 4 ml/min with Krebs solution saturated with 95% O_2 and 5% CO_2 at 32 °C. Test substances were applied by rapidly changing the bath solution. Antagonists were introduced to the bath 15 min before testing their effects. Images were acquired with cooled CCD camera (PCO, Kelheim, Germany), using Imaging Workbench 5 software (www.imagingworkbench.com). Fluorescence was excited at 450–490 nm, and emission above 520 nm was increased by elevated intracellular Ca^{2+} concentration ($[Ca^{2+}]_{in}$). Images were recorded at 0.3 Hz. The fluorescence ratio F/F_0, where F_0 is the baseline, was used to describe relative changes in $[Ca^{2+}]_{in}$.

2.4. Statistical analysis

In the Ca^{2+} imaging experiments, responses of all the cells in the field (responding and non-responding) were averaged. One-way ANOVA with Tukey's Multiple Comparison Test was used to analyze the immunohistochemical results. Data are from experiments on at least 6 ganglia for each of the data points. The number of cells for each of the data points was about 1300. Dye coupling data were pooled from multiple experiments. When an LY-injected cell was found to be dye-coupled it was marked as 100, and when it was not coupled, as 0. These data were analyzed using One-way ANOVA with Tukey's Multiple Comparison Test. Data are from experiments on 6 ganglia (3 mice) at least for each of the data points. The number of cells for each of the data points was between 38 and 70.

One-way ANOVA with Dunnett's Multiple Comparison Test was used in Ca^{2+} imaging experiments. Each of the data points is based on recordings of the responses from 4 to 7 ganglia (3–4 mice). A total of about 20–80 cells were recorded from each ganglion. $P < .05$ was considered as statistically significant. Values are expressed as mean ± SEM.

2.5. Chemicals

From Sigma-Aldrich, St. Louis, MO, USA: LY, DAPI, ACh, curare, atropine. From Alomone, Jerusalem, Israel: Tetrodotoxin (TTX). From Invitrogen, www.invitrogen.com: Fluo-3 AM. From Dako, Copenhagen, Denmark: Antibody against GFAP. From Abcam, www.abcam.com: Donkey anti-rabbit conjugated to Alexa Fluor 594

3. Results

We used Ca^{2+} imaging to learn about the presence of functional ACh receptors in SGCs in mouse Sup-CG. As described for sensory ganglia [20,21], at the concentrations used, AM- Ca^{2+} indicators dyes label only SGCs, and not neurons, which facilitated the recording from the SGCs (see Fig. 1A). ACh at concentrations over 0.01 mM produced clear elevation in intracellular Ca^{2+} in SGCs (Fig. 1). As can be seen in Fig. 1B not all the cells responded to ACh, and about 50% responded to ACh at 0.5 mM. For comparison we carried out the same experiments in mouse sensory ganglia (trigeminal and nodose) and observed negligible

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