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Short-Range Functional Interaction Between Connexin35 and Neighboring Chemical Synapses

A. PEREDA¹, J. O'BRIEN², J. I. NAGY³, M. SMITH¹, F. BUKAUSKAS¹, K. G. V. DAVIDSON⁴, N. KAMASAWA⁴, T. YASUMURA⁴, and J. E. RASH⁴

¹ Department of Neuroscience, Albert Einstein College of Medicine, NY, USA

² Department of Ophthalmology and Visual Science, University of Texas at Houston-Health Science Center, Houston, TX, USA

³ Department of Physiology, University of Manitoba, Winnipeg, Canada

⁴ Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

Abstract

Auditory afferents terminating as mixed, electrical, and chemical, synapses on the goldfish Mauthner cells constitute an ideal experimental model to study the properties of gap junctions in the nervous system as well as to explore possible functional interactions with the other major form of interneuronal communication—chemically mediated synapses. By combining confocal microscopy and freeze-fracture replica immunogold labeling (FRIL), we found that gap junctions at these synapses contain connexin35 (Cx35), the fish ortholog of the neuron-specific human and mouse connexin36 (Cx36). Conductance of gap junction channels at these endings is known to be dynamically modulated by the activity of their co-localized chemically mediated glutamatergic synapses. By using simultaneous pre- and postsynaptic recordings at these single terminals, we demonstrate that such functional interaction takes place in the same ending, within a few micrometers. Accordingly, we also found evidence by confocal and FRIL double-immunogold labeling that the NR1 subunit of the NMDA glutamate receptor, proposed to be a key regulatory element, is present at postsynaptic densities closely associated with gap junction plaques containing Cx35. Given the widespread distribution of Cx35- and Cx36-mediated electrical synapses and glutamatergic synapses, our data suggest that the local functional interactions observed at these identifiable junctions may also apply to other electrical synapses, including those in mammalian brain.

Keywords

Auditory; connexin36; electrical coupling; electrical synapse; gap junction; NMDA; synaptic plasticity

INTRODUCTION

Because of the ability to combine anatomical and physiological analysis, identifiable auditory afferents terminating as Large Myelinated Club endings (Club endings) on the lateral dendrite of the goldfish Mauthner (M-) cell historically have constituted a powerful system to study the nature and properties of electrical transmission between neurons in vertebrates (1,2). Both gap junctional and glutamatergic synaptic transmission occur at these terminals (2–4), thus providing an ideal model to study interactions between these modalities of synaptic

transmission (Figure 1A). Experimental evidence has shown that gap junctional conductance at Club endings is enhanced by sustained cellular activity (5–7). Because this enhancement requires NMDA receptor activation (5), it has been suggested that the observed activity-dependent modification of electrical synapses depends on functional interaction with their co-localized glutamatergic synapses (6,7).

Given the widespread distribution of both glutamate receptors and gap junctions in vertebrate CNS, such functional interaction may constitute a common property of electrical synapses. As an initial step toward investigating this possibility we attempted to: a) identify which member of the multigene family of gap junction forming proteins (connexins) that is responsible for electrical transmission at these endings and b), directly determine the existence and spatial extent of such a relation between chemical and electrical synapses. We review here recent anatomical and physiological evidence (8,9) indicating that gap junctions at these terminals contain Cx35 (10,11), and that chemical synapses modulate the conductance of gap junctions that are within a few micrometers.

MATERIAL AND METHODS

Electrophysiology

For these experiments, goldfish (*Carassius auratus*) were used. Surgical and recording techniques were similar to those described previously (4). Detailed illustration and interpretation of electrophysiological recordings described here can be found in a previous report (9).

Immunolabeling

Two anti-connexin antibodies (against Cx35 and Cx36) and two anti-NR1 subunit of the NMDA glutamate receptor were used in this study. Methods for FRIL and confocal microscopy, as well as

Dye Coupling

For dye coupling evaluation, the M-cells were intracellularly injected with the tracer Neurobiotin (cation MW: 286; Vector; for a detailed description see reference [9]).

RESULTS AND DISCUSSION

Electrical Synapses at Large Myelinated Club Endings Contain Cx35

Confocal immunofluorescence microscopy revealed abundant Cx35 in M-Cell/Club Ending synapses (Figure 1B1). These synapses were identified by their uniquely large size and distinctive subcellular location on lateral dendrites, as well by confocal grid-mapping of M-cell lateral dendrites that had been injected with Lucifer Yellow during intracellular recordings. Immunofluorescence mapping followed by FRIL revealed abundant Cx35 in gap junction plaques at these Club Ending synapses (Figure 1C).

The intensity of labeling for Cx35 observed by confocal microscopy and the presence of this connexin in virtually every gap junction plaque as demonstrated by FRIL indicates that Cx35 is a major component of these junctions and thus primarily responsible for electrical transmission at Club Endings. Moreover, our identification of Cx35 by FRIL on both the M-cell side and Club ending side of gap junctions between these structures suggests that electrical transmission at Club ending synapses may occur through homotypic gap junction channels. Nevertheless, we do not exclude that additional connexins may be present at these terminals.

Postsynaptic Densities at Large Myelinated Club Endings Contain NMDA Receptors

Activity-dependent modification of the strength of electrical transmission seems to depend on functional interactions with neighboring, co-localized glutamatergic synapses. Thus, it has been proposed that NMDA receptors (5) acting postsynaptically via local calcium signaling cause activation of calcium/calmodulin-dependent kinase II, which is essential for induction of the modifications (6,7). Confocal microscopy demonstrated the presence of glutamate receptor NR1-immunogold labeling at Club Endings (Figure 1B2). Consistent with ultrastructural data describing the predominance of postsynaptic densities (PSDs) in the periphery of these terminals (12), the labeling was largely localized to the periphery of the contacts. Accordingly, FRIL double labeling revealed the presence of Cx35 in gap junctions and NR1 glutamate receptors in nearby E-face PSDs in identified M-cells, including at identified Club Ending synapses and in other synapses that because of their size, location, and complement of abundant gap junctions, likely correspond to M-cell Club Ending synapses (Figure 1C).

Activity-Dependent Modulation of Electrical Coupling Requires Activity of Nearby Chemical Synapses

Consistent with the presence of gap junctions and chemical synapses, extracellular stimulation of a population of these afferents in the posterior branch of the eighth nerve (where these afferents run) evokes a mixed excitatory postsynaptic potential in the dendrite. This synaptic potential is composed of a fast electrical component, followed by a chemical component mediated by glutamate (2,4,13). High frequency stimulation of the eighth nerve induces potentiation not only of the glutamatergic response but also of the electrical potential, and this potentiation usually persists for the remainder of an experiment (5,6). Activity-dependent depression of both electrical and chemical components has also been reported (14). As previously mentioned, it has been suggested that the observed activity-dependent modification of the electrical component depends on interaction with the chemically transmitting region(s) of the same synapse (5–7).

To directly determine the existence and spatial extent of such a relation between chemical and electrical synapses, we obtained multiple paired recordings between single afferent fibers and the same M-cell dendrite. We were able to explore the distribution of synaptic efficacy and of activity-induced changes across the population of single terminals. These recordings provided evidence for the existence of local interactions between chemical and electrical transmission sites in single synaptic contacts at Club Endings (for a detailed description of these experiments see reference [9]).

Activity-dependent modulation of electrical coupling required activity of nearby chemically receptive zone(s) in the same synapse and, although we cannot rule out a contribution from neighboring terminals, the data suggest that there is a critical distance for such regulatory control. Anatomical data suggest that this “critical distance” is at least a few nanometers (distance between a given PSD and the closest gap junction at a Club Ending; [12]) but necessarily less than ~5 μm , the average distance between the closest neighboring terminals on the surface of the M-cell’s lateral dendrite.

Such localized, Club ending-specific, functional interactions have important consequences. Because auditory afferents are differentially activated by sound and therefore likely subject to different levels of activation, electrical synapses between these terminals might be expected to exhibit different junctional conductances due to differences in potentiation/depotentiation. Such expectation was supported by the facts that: a) unitary electrical synaptic potentials dramatically vary in amplitude (Figure 2A), as demonstrated by multiple simultaneous pre- and postsynaptic recordings obtained sequentially from individual afferents and the same M-

cell lateral dendrite, and b) tracer coupling to the afferents after injection of the M-cell with Neurobiotin varied markedly between neighboring Club Endings; unlabeled afferents, and afferents showing different degrees of staining were observed in the same dendritic area (Figure 2B). Thus, localized functional interactions between chemical and electrical synapses regulate the degree of coupling, making it possible for the M-cell to independently modify coupling at different electrical synapses with auditory afferents.

CONCLUSIONS

Gap junctions at mixed synapses known as Large Myelinated Club endings contain Cx35, the fish ortholog of the mammalian Cx36 (15). These gap junctions interact with their co-localized chemical synapses by short range cytoplasmic signaling. The PSD-mediated signaling discussed here (see also [7]) can be relevant not only to M-cell and, potentially, mammalian mixed synapses (16), but also to situations in which PSDs belonging to chemical synapses are situated close to dendrodendritic or axodendritic gap junctions formed with other cells.

Recent freeze-fracture studies on different mammalian CNS neurons (17) revealed that PSDs are located at distances from gap junction plaques comparable to those in the Club Endings (12). Such an arrangement suggests that this novel form of gap junction modulation could constitute a widespread property of electrical synapses, relevant to structures where glutamatergic transmission and gap junctions co-exist.

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Figure 1.

Cx35 mediates electrical transmission at Large Myelinated Club endings. (A) Auditory afferents (Club endings) terminate on the ipsilateral M-cell lateral dendrite as mixed, electrical and chemical, synaptic contacts known as Large Myelinated Club endings (*, Mixed synapse). (B) Confocal microscopy reveals the presence of Cx35 and NMDA receptors in Club endings. (B1) Laser scanning confocal immunofluorescence showing Cx35 at a Club Ending using monoclonal anti-Cx35 antibody. These terminals, identified by their large size and delineated by immunofluorescence, exhibit multiple sites of punctate labeling for Cx35. Image derived from 3 confocal z sections (2 μ m) through the surface of the distal portion of a M-cell lateral dendrite. (B2) Laser scanning immunofluorescent image of a Club Endings labeled with anti-

NR1 antibody. The image also represents a stack of 3 z sections (2 μm) through the surface of the M-cell lateral dendrite. Calibration bars: 5 μm . (C) Freeze fracture immunogold labeling (FRIL) confirms the presence of Cx35 and NMDA receptors. FRIL double labeling of Cx35 in gap junction plaques within the presynaptic membrane and of NR1 subunit of NMDA glutamate receptors in nearby IMPs in postsynaptic particle clusters (designated PSDs) in the postsynaptic membrane of a Club ending. Image shows five gap junctions, delineated by the gray areas, labeled for Cx35 (10 nm gold beads, Ab298 antibody) in a small portion of a Club Ending. A nearby small PSD is labeled for NR1 (18-nm gold bead, monoclonal NR1 antibody). Calibration bar: 0.1 μm . Interpretations of these images, are described in a previous report (9).

Figure 2.

Electrical synapses belonging to neighboring Club endings co-exist at different degrees of conductance. (A) Electrical component of mixed synaptic potentials (electrical) evoked by stimulation of different afferent fibers differ dramatically in amplitude. Mixed unitary excitatory synaptic potentials (EPSPs, a–d) evoked by stimulation of four afferents obtained sequentially while recording in the same fish at the same dendritic site. The electrical component of the unitary synaptic potentials show much greater variability in amplitude than the presynaptic action potentials (not shown, see reference [9]). Only one of these four unitary synaptic potentials exhibited a clear chemical component (chemical, trace a). (B) Transfer of Neu-robiotin at neighboring Club Endings differs dramatically, indicating that they differ in permeability. View of the bifurcation of the lateral dendrite (dark branches) obtained with Nomarski optics and revealing differences of labeling between two neighboring Club Endings. Labeled (bottom) and unlabeled (top) fibers terminate in the same area of lateral dendrite. Only a small number of Club Endings were detected by transfer following injection of the tracer Neurobiotin into the M-cells (5–25% of the estimated population) suggesting that gap junction

permeability at Club Endings is generally low and that most fibers did not accumulate enough Neurobiotin to produce a visible reaction product. Calibration bar: 20 μm .