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Two independent forms of activity-dependent potentiation regulate electrical transmission at mixed synapses on the Mauthner cell

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Abstract

Mixed (electrical and chemical) synaptic contacts on the Mauthner cells, known as Club endings, constitute a valuable model for the study of vertebrate electrical transmission. While electrical synapses are still perceived by many as passive intercellular channels that lack modifiability, a wealth of experimental evidence shows that gap junctions at Club endings are subject to dynamic regulatory control by two independent activity-dependent mechanisms that lead to potentiation of electrical transmission. One of those mechanisms relies on activation of NMDA receptors and postsynaptic CaMKII. A second mechanism relies on mGluR activation and endocannabinoid production and is indirectly mediated via the release of dopamine from nearby varicosities, which in turn leads to potentiation of the synaptic response via a PKA-mediated postsynaptic mechanism. We review here these two forms of potentiation and their signaling mechanisms, which include the activation of two kinases with well-established roles as regulators of synaptic strength, as well as the functional implications of these two forms of potentiation.

Keywords

Gap junction; Connexin 36; Endocannabinoids; Long-term potentiation; NMDA; Dopamine; PKA; CaMKII; Auditory

1. Introduction

In contrast with mammalian electrical synapses that generally have limited experimental access, the wide distribution and sometimes particular sub-cellular localization of some electrical synapses in some species have provided with advantageous experimental models in which basic properties of electrical transmission can be more easily studied (Bennett, 1977). This is the case of a special class of auditory afferents terminating on the lateral dendrite of the teleost Mauthner cells known as Large Myelinated Club Endings (Bartelmez, 1915; Bartelmez and Hoerr, 1933; Bodian, 1937; Robertson, 1963; Furshpan, 1964). These endings are identifiable “mixed” (electrical and chemical) synaptic terminals that offer the unique opportunity to correlate physiological properties with biochemical composition and

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specific ultrastructural features of individual synapses (Pereda et al. 2004). A wealth of evidence shows that electrical transmission at these afferents is very dynamic and is mediated by teleost homologs of connexin36 (Cx36) (O'Brien et al., 1996, 1998), the main neuronal connexin (Condorelli et al., 1998), which is responsible for electrical coupling at neocortical inhibitory interneurons and inferior olivary cells, among many cell types (Connors and Long, 2004). The widespread distribution of these neuron-specific connexins and of some of the proposed regulatory mechanisms raises the possibility that the observed properties could also pertain to most electrical synapses.

This article does neither attempt to be a comprehensive review of the plasticity of electrical synapses in general nor a detailed description of the physiology and anatomy of the Club endings. We concentrate here on an essential aspect of the physiology of these terminals: the activity-dependent potentiation of electrical (and chemical) transmission observed at these mixed synapses and the proposed underlying mechanisms.

2. The Club endings

The Club endings are single terminations of saccular afferents (~85 in number) (Bodian, 1937) on the distal portion of the lateral dendrite of the Mauthner cells (Fig. 1A). The Mauthner cells are a pair of unusually large reticulospinal neurons involved in tail-flip escape responses in fish, which are triggered by diverse sensory stimuli, particularly auditory (Eaton et al., 2001). These uncommonly large cells are anatomically and physiologically identifiable and constitute a valuable preparation for the study of the cellular correlates of behavior (Eaton et al., 2001; Korn and Faber, 2005; Faber and Pereda, 2011; Pereda and Faber, 2011). Auditory afferents that terminate as Club endings on the Mauthner cell provide essential auditory information to this decision-making neuron for the initiation of an escape response (Korn and Faber, 2005; Curti and Pereda, 2009; Faber and Pereda, 2011; Pereda and Faber, 2011).

A wealth of anatomical and electrophysiological data shows that Club endings support both chemical and electrical modalities of transmission (Lin and Faber, 1988; for review see Pereda et al., 2004). In fact, these contacts provided one of the first demonstrations of gap junction plaques (Robertson, 1963) and electrical transmission (Furshpan, 1964) in the vertebrate central nervous system. Detailed electron microscopic studies, including freeze-fracture techniques (Tuttle et al., 1986), have shown that while the specializations corresponding to chemical transmission predominate in the periphery, gap junction plaques are located on most of the surface area of the terminal. The plaques range in number from 63 to 243 gap junctions, and the total area they occupy is about 20% of the synaptic area (Tuttle et al., 1986). These gap junctions are formed by Cx35 (Pereda et al., 2003), the fish ortholog of the widely expressed Cx36. Recent data indicates that a second homolog of Cx36, Cx34.7, is also present at these terminals (Rash et al., 2010). From the electrophysiological point of view, a presynaptic impulse generates a “mixed” excitatory response. That is, stimulation of the posterior branch of the VIIIth nerve, where these fibers run, evokes an electrical potential followed by a chemically mediated excitatory postsynaptic potential (Fig. 1B). Due to the fast time constant of the Mauthner cell (~400 μ s) both components can be easily distinguished (Fukami et al., 1965).

While electrical transmission is supported by the existence of an unusually large number of gap junction plaques, chemical transmission is mediated by the release of glutamate from ~15 release sites (Lin and Faber, 1988b). The pharmacological characteristics and voltage dependence of the synaptic currents are consistent with glutamate being the transmitter at these contacts, that activates both non-NMDA and NMDA receptors (Wolszon et al., 1997). Glutamatergic synapses at Club endings characteristically exhibit frequency-dependent facilitation (Pereda and Faber, 1996; Wolszon et al., 1997). Despite that mixed synapses are ubiquitous in lower vertebrates, the combination of these two forms of transmission is particularly beneficial for the function of Club endings (Curti and Pereda, 2009). Electrical transmission provides speed and reliability of transmission and the presence of chemical transmission, with relatively longer duration, allows temporal summation during repetitive responses in a cell in which the membrane time constant is unusually brief. These properties are advantageous for an escape reflex network. In addition, facilitation of transmitter release is an essential property for the induction of one type of activity-dependent potentiation (see below) (Pereda and Faber, 1996).

3. Activity-dependent plasticity

The advantage and contribution of combining electrical and chemical synapses are emphasized by the existence of important functional interactions between these two modalities of transmission. The most remarkable of these interactions is the induction by glutamatergic synapses of long-term activity-dependent changes in the efficacy of both chemical and electrical synaptic transmission. There exist two forms of activity-dependent potentiation in Club endings, each involving the activation of different glutamate receptors, NMDA or mGluR, whose activation follow independent intracellular pathways.

3.1 Potentiation by activation of nearby NMDA receptors

Both components of the mixed synaptic response undergo activity-dependent potentiation of their synaptic strength in response to discontinuous high frequency stimulation of the VIIIth nerve. The induction of activity-dependent long-term potentiation (Fig. 2A) requires discontinuous high frequency stimulation of the VIIIth nerve (brief trains of 2 to 8 stimuli at 500 Hz applied once every two secs; “500 Hz Bursts” protocol) (Yang et al., 1990; Pereda and Faber, 1996), and is specific to the tetanized pathway. Physiological and pharmacological evidence showed that this discontinuous stimulating pattern is essential to evoke potentiation; continuous high frequency stimulation, which rapidly depresses the chemical component, does not cause potentiation (Pereda and Faber, 1996). Thus, while continuous stimulation at 500 Hz quickly depresses transmission, bursting facilitates release of glutamate and allows temporal summation of the synaptic responses for the postsynaptic depolarization presumably necessary to relieve Mg⁺ block of the NMDA receptors (Pereda and Faber, 1996), whose activation is necessary for potentiation (Yang et al., 1990; Pereda and Faber, 1996). It has been shown that the activation of postsynaptic NMDA receptors leads to a localized increase in the intracellular concentration of Ca⁺⁺, which in turns activates the kinase Ca⁺⁺-calmodulin-dependent kinase II (CaMKII) (Pereda et al., 1998), whose activity is necessary for the potentiations (Fig. 2B). Accordingly, confocal and freeze fracture immunolabeling of these terminals showed that the NR1 subunit of the NMDA

glutamate receptor, proposed to be the key regulatory element in this phenomenon, is present at PSDs closely associated with gap junction plaques containing Cx35 (Pereda et al., 2003). Therefore, evidence suggests that potentiation of electrical transmission in Club endings takes place as result of interactions between glutamatergic and electrical synapses.

Interestingly, interactions of gap junctions with glutamatergic synapses are short-range (Smith and Pereda, 2003). Simultaneous pre- and postsynaptic recordings at these single terminals, demonstrated that local, Club ending-specific, rather than widespread interactions between chemical and electrical components at different endings, are responsible for modifications of junctional conductance. Terminal-specific modification of electrical coupling was directly demonstrated by showing that the activity of many terminals did not induce changes in a neighboring inactive terminal (Smith and Pereda, 2003). Given the spacing of Club endings on the dendrite, it would appear that the maximum distance of spread is 5-10 microns (Bartelmez, 1915; Pereda et al., 2003) and possibly much less, the distance between NMDA receptors and gap junctions at single endings (Pereda et al., 2003). The Club ending-specific modulation of chemical and electrical transmission may be important in generating diversity between terminals. Because auditory afferents are differentially activated by sound and therefore likely subject to different levels of activation (Fay and Ream, 1986), electrical synapses between these terminals might be expected to exhibit different junctional conductances due to differences in potentiation (and de-potentiation) (Smith and Pereda, 2003; Pereda et al., 2004). Accordingly, electrical components markedly vary in amplitude among Club endings on the same Mauthner cell and the differences in tracer coupling to the afferents after injection of a Mauthner cell with neurobiotin (Smith and Pereda, 2003). Similar differences may occur in single fiber chemical components in population responses. Thus, localized interactions between NMDA receptors and gap junctions permit independent modification of electrical transmission at different Club endings on the Mauthner cell, generating a wide heterogeneity of electrical coupling within the population of Club endings (Smith and Pereda, 2003; Flores et al., 2010).

3.2 Activity-dependent release of endocannabinoids mediate potentiation

A second form of activity-dependent potentiation, which follows a different induction protocol and signaling mechanisms, was more recently reported at Club endings (Cachope et al., 2007). In this case (described in detail below), brief (1 sec) continuous stimulation of these afferents at 100 Hz (100 Hz protocol) (Fig. 3A), leads to release of endocannabinoids subsequently promoting release of dopamine, which triggers a long-term enhancement of both components of the synaptic response (Cachope et al., 2007).

Dopamine has been identified as a modulator of gap junction-based intercellular communication (Teranishi et al., 1983; Piccolino et al., 1984; Lasater and Dowling, 1985; Cepeda et al., 1989; Hampson et al., 1992). Immunochemical studies (using both anti-dopamine and anti-TH labeling) revealed the existence of a profuse dopaminergic innervation along the lateral dendrite of the Mauthner cell (Pereda et al., 1992). This finding was supported by immunolabeling combined with transmission electron microscopy which demonstrated the presence of dopaminergic varicosities in the Mauthner cell's lateral

dendrite in close proximity to Club endings (Pereda et al., 1992). However, no synaptic contacts were identified between those varicosities and other cellular structures, suggesting that dopamine actions follow a volumetric mode of neurotransmission, rather than direct terminal-to-neuron specific contacts (Pereda et al., 1992). Local application of dopamine in the vicinity of the Mauthner cell lateral dendrite with a help of a second (puffing electrode) was shown to be sufficient to trigger a long-term enhancement of both the electrical and chemical components of the mixed synaptic response (Pereda et al., 1992, 1994). This potentiation was prevented by superfusing the brain with aCSF containing the D1/D5 dopamine receptor blocker SCH-23390. Intradendritic injections of cAMP and cAMP-dependent kinase (PKA) activation were sufficient to trigger a potentiation of the synaptic response. Also supporting the involvement of a PKA-dependent postsynaptic mechanism, dopamine-evoked potentiation was blocked by intradendritic injection of the PKA inhibitor PKI₅₋₂₄ (Pereda et al., 1994). Further supporting a postsynaptic mechanism, dopamine potentiation did not alter paired-pulse facilitation of the glutamatergic component (Pereda et al., 1994). Altogether, the data supports that the notion that gap junction-mediated electrical and chemical synaptic transmission between the Club endings and the Mauthner cell are a target of modulation by dopamine, which by acting on putative postsynaptic D1/D5 receptors leads to activation of a postsynaptic PKA-dependent cascade.

More recent findings demonstrated that release of dopamine from these varicosities is under the regulatory control of the activity of Club endings. Activation of the cannabinoid 1 receptor (CB1) by application of its agonist WIN55,212-2 (WIN) led to long-term potentiation of the electrical and chemical components of the mixed synaptic potential (Cachope et al., 2007). Since activation of CB1 most commonly results in decreased neurotransmission (Chevalleyre et al., 2006), this unexpected finding directed the attention to the existence of an intermediary transmitter, such as dopamine. The WIN-evoked potentiation was blocked by D1/D5 antagonist and intradendritic application of PKA blockers, suggesting that locally-produced endocannabinoids mediate their action via dopamine. Activation of the metabotropic glutamate receptor type 1 (mGluR1), combined with postsynaptic depolarization led to a potentiation that was very similar to that triggered by pharmacological activation of CB1 receptors and it was blocked by SR141716 and AM251, antagonists of this receptor indicating that endocannabinoids can be released from the lateral dendrite of the Mauthner cell (Cachope et al., 2007). Both, mGluR activation and depolarization had been reported to trigger release of endocannabinoids (Chevalleyre et al., 2006). Given the properties of their mixed synaptic responses, the combined requirement of mGluR activation and dendritic depolarization for endocannabinoid release suggested, that patterns of activity of Club endings could be responsible for the release of endocannabinoids under natural conditions. More specifically: 1) Club endings represent the overwhelming majority of the excitatory inputs in this portion of the M-cell lateral dendrite (Bartelmez, 1915; Furshpan, 1964; Nakajima, 1974), and 2) the electrical component of their mixed synaptic potential evokes a large dendritic depolarization that is immediately followed by a glutamatergic response (the depolarization produced by the electrical component is generally 4-5 times bigger than that of the glutamatergic response; see Fig. 1B). Electrical stimulation of the VIIIth nerve in the form of long trains (1 sec each) of high frequency (100 Hz) evoked long-lasting potentiation of both components of the synaptic response. This

pattern of stimulation was proposed to enhance activation of mGluR receptors, as it sustained and does not depress transmitter release. The potentiation was prevented by pretreatment with aCSF containing the CB1 receptor antagonists (Cachope et al., 2007) and intradendritic injection of tetrahydrolipstatin (THL), blocker of the 2-AG-synthesizing enzyme diacylglycerol lipase [2-AG is an endocannabinoid which has been shown mediate actions of this system in the CNS (Chevalleyre et al., 2006), indicating the dependence of this phenomenon on endocannabinoid signaling (Cachope et al., 2007). Finally, indicating that endocannabinoids led to potentiation via local dopamine release, this activity-dependent potentiation was blocked by D1/D5 dopamine receptor antagonists (Cachope et al., 2007).

Supporting this conclusion, CB1 receptors were shown to be associated to dopaminergic varicosities (Cachope et al., 2007). While endocannabinoids have been shown to depress transmitter release (Chevalleyre et al., 2006) they usually lead to enhanced dopamine release by producing “dis-inhibition”, that is, reducing transmitter release from inhibitory terminals and indirectly enhancing the excitability of dopaminergic neurons (inhibition of the inhibition) (Szabo et al., 2002; Wallmichrath and Szabo, 2002). This was shown not to be the case in Club endings, as activity-dependent potentiation can be induced in the presence of GABA receptors antagonists (Cachope et al., 2007). Taken together, the evidence suggest that the most likely explanation is that CB1 receptor activation leads to dopamine release following alternative, non-conventional, intracellular pathways such as those reported to be involved in glutamate release from astrocytes (Navarrete and Araque, 2008). Thus, dopamine availability in the vicinity of Club endings is regulated by the activity of glutamatergic synapses at these contacts. In contrast to the Club ending-specific effects of the 500 Hz bursts protocol (Smith and Pereda, 2003) and based on the volumetric modality of dopamine transmission, this mechanism is likely to affect all the Club endings located in the vicinity of the targeted dopaminergic varicosities.

3.3 PKA and CaMKII converge for the regulation of synaptic strength

A wealth of evidence indicates that two important kinases known to regulate synaptic transmission of chemical and electrical synapses in other systems converge for the regulation of electrical synapses and chemical synapses in Club endings: CaMKII and PKA.

Multifunctional CaMKII has been implicated in mechanisms of activity-dependent plasticity in chemical synapses (Lisman, 1994; Pettit et al., 1994; Barria et al., 1997). This kinase is an essential and abundant component of glutamatergic postsynaptic densities (PSDs) (Kennedy, 2000) in which it associates with other proteins, such as NMDA receptors (Yang and Schulman, 1999; Strack et al., 2000). Consistent with its involvement in activity-dependent potentiation in Club endings, recent data indicate that this kinase can molecularly interact with Cx36 (Alev et al., 2008). That is, CaMKII was shown to bind to two cytoplasmic domains of Cx36 (Alev et al., 2008). These domains reveal striking similarities with segments of the regulatory subunit of CaMKII, such as the pseudosubstrate and pseudotarget sites of the kinase (Alev et al., 2008). Moreover, in similarity to the NR2B subunit of the NMDA receptor, both Cx36 binding sites exhibit phosphorylation-dependent interaction and autonomous activation of CaMKII (Alev et al., 2008). This study also revealed the existence of multiple phosphorylation sites for α CaMKII in Cx36 (Alev et al., 2008). In a second

study, *in silico* analysis showed that Cx36 shares most of the identified residues with its fish homologs Cx35 and Cx34.7 (Flores et al., 2010). This analysis also showed that residues S315 / S298 / S300 in Cx36, Cx35 and Cx34.7, respectively, constitute exclusive phosphorylation sites for CaMKII, which are not shared with other kinases. On the other hand, residues S110 and S293 in Cx36, S110 and S276 in Cx35, and S110 and S277 in Cx34.7, are shared with PKA (O'Brien et al., 1998; Mitropoulou and Bruzzone, 2003; Ouyang et al., 2005; Urschel et al., 2006).

Interestingly, a recent study suggests that although CaMKII directly associates with Cx36 and its homologs (Alev et al., 2008; Flores et al., 2010), it might not constitute an obligatory component of electrical synapses in Club endings (Flores et al., 2010). As shown in the inferior olive (Alev et al., 2008), CaMKII also associates with Cx35 at Club endings (Flores et al., 2010) (Figs. 4 A-C). Interesting, unlike other proteins, both CaMKII labeling and distribution were highly variable between contiguous Club endings (Fig. 4A), and it was not restricted to the periphery of the terminals, where glutamatergic synapses are located, but was also present at the center where gap junctions predominate. Strikingly, the variability of colocalization was found in immediately adjacent Cx35-labeled puncta, within the same region of a single Club ending (Fig. 4B). Since each punctum is thought to represent an individual gap junction plaque, such differential labeling of adjacent plaques indicates that the colocalization of Cx35 and CaMKII might not be obligatory in nature and reflective of their functional interaction. Because CaMKII characteristically undergoes activity-dependent translocation, the observed variability of labeling likely reflects physiological differences between electrical synapses of contiguous Club endings, which remarkably co-exist with differing degrees of conductance (Flores et al., 2010). That is, its presence is highly variable, even between gap junction plaques within the same contact, and likely related to differences in the degree of potentiation between individual terminals (see above). In comparison with ZO-1, which would play a more permanent or structural role (Flores et al., 2008, 2010), the presence of CaMKII seems to be non-obligatory and possibly regulated by neural activity. This conclusion is consistent with the dynamic translocation properties of CaMKII and whose presence is thought to represent the history of synaptic activation at chemical (Asrican et al., 2007) and electrical synapses (Flores et al., 2010).

Dopamine and PKA on the other hand are traditionally known to play an important regulatory role on electrical transmission. The regulatory role of dopamine on electrical synapses was shown to be widespread and observed at both vertebrate (Teranishi et al., 1983; Piccolino et al., 1984; Lasater and Dowling, 1985; Cepeda et al., 1989; Hampson et al., 1992; Pereda et al., 1992; O'Donnell and Grace, 1993; Urschel et al., 2006; Kothmann et al., 2007) and invertebrate (Johnson et al., 1993a, 1993b) nervous systems. Dopamine was shown to promote reduction of tracer and electrical coupling between retinal horizontal cells (Teranishi et al., 1983; Piccolino et al., 1984; Lasater and Dowling, 1985; Cepeda et al., 1989). The effects of dopamine in retina are not restricted to this cell type, and this neurotransmitter modulator was also shown to regulate electrical coupling in AII amacrine cells (Hampson et al., 1992; Urschel et al., 2006; Kothmann et al., 2007). Dopamine's action is mediated via activation of D1/D5 receptors, which leads to an elevation of cAMP activate cAMP-dependent protein kinase A (PKA) (Teranishi et al., 1983; Piccolino et al., 1984;

Lasater and Dowling, 1985; Cepeda et al., 1989; Hampson et al., 1992; O'Donnell and Grace, 1993). In contrast to horizontal cells, at which electrical coupling is mediated by Cx57 (Cx55.5 in teleost), coupling between amacrine type II cells is primarily mediated by Cx36 (for review of modulation of electrical coupling in retina see (Bloomfield and Völgyi, 2009). Two PKA phosphorylation sites were identified in Cx36 and its fish homolog: Ser110 and Ser293 (Ser276 in teleost) (Ouyang et al., 2005a; Kothmann et al., 2007), which are essential for PKA-mediated regulation of coupling. These two sites were shown to undergo dynamic changes in the phosphorylation state in retina (Kothmann et al., 2007; Li et al., 2009) and to underlie important functional roles in both AII amacrine cells (Kothmann et al., 2009) and photoreceptors (Li et al., 2009). Further, the regulatory effects of PKA on electrical conductance were also observed to occur following the activation of noradrenergic receptors, which dynamically regulate the degree of coupling between inhibitory interneurons in hippocampus (Zsiros and Maccaferri, 2008). Two aspects of the action of dopamine on these terminals are unusual: 1) in contrast to most examples, at which depression was reported (Teranishi et al., 1983; Piccolino et al., 1984; Lasater and Dowling, 1985; Cepeda et al., 1989; Hampson et al., 1992; O'Donnell and Grace, 1993), dopamine produces enhancement of gap junctional conductance in Club endings, and 2) rather than a modulator, dopamine acts as a trigger initiating a long-term modification of electrical transmission (Pereda et al., 1992, 1994; Cachope et al., 2007). This last property is emphasized by the fact that the sole intradendritic injection of the catalytic sub-unit of PKA was sufficient to trigger long-term enhancement of electrical and chemical transmission at these terminals (Pereda et al., 1994). Recent evidence indicates that dopamine (acting via D1/5 receptors), rather than promoting PKA-mediated phosphorylation of Cx36 at regulatory sites, decouples AII amacrine cells by PKA-activation of protein phosphatase 2A and subsequent dephosphorylation of Cx36 (Kothmann et al., 2009). This study also demonstrates a direct relationship between Cx36 phosphorylation and AII amacrine cell coupling strength, suggesting that PKA-mediated dopamine regulation relies in a balance between phosphorylation and de-phosphorylation of Cx36, which might result in both increase or decrease of the coupling strength. In agreement with this possibility (Kothmann et al., 2009), dopamine-mediated PKA activation in Club endings would favor its action on Cx36 teleost homologs promoting increase in coupling. Although according to this model D1/5 receptor activation would favor the direct action of PKA on Cx36 teleost homologs promoting increase in coupling, the mechanisms described by Kothman et al. (Kothmann et al., 2009) could underlie bidirectional control of coupling at Club endings. Ionotropic glutamate receptors were also shown to be the target of PKA modulatory action in a variety of experimental preparations (Swope et al., 1999). Thus, dopamine potentiates both forms of transmission via postsynaptic activation of PKA.

As mentioned above, Cx36 and its teleost homologs have been shown to be targets of PKA phosphorylation (Mitropoulou and Bruzzone, 2003; Ouyang et al., 2005; Urschel et al., 2006). Consistent with these reports, a recent study indicates that Cx35 is phosphorylated at regulatory PKA phosphorylation sites in Club endings. That is, western blots obtained from small areas of the goldfish hindbrain containing the Mauthner cells with a polyclonal antibody raised against a PKA phosphorylation site at serine 110 of Cx35 (anti-Cx35/P110) (Kothmann et al., 2007) labeled Cx35. This labeling was lost upon alkaline phosphatase

digestion, suggesting that this connexin is also the target of PKA phosphorylation in neurons of this region that includes the Mauthner cells (Fig. 4C) (Cachope et al., 2007). Consistent with this finding, immunohistochemical studies using the anti-Cx35/P110 antibody revealed abundant punctate labeling at Club endings, which were unambiguously identified by the presence of labeling with a monoclonal anti-Cx35 antibody (Fig. 4D) (Cachope et al., 2007), indicating that dopamine is likely to potentiate electrical transmission via a direct action on this connexin.

4. Conclusions

The evidence discussed here indicates that activity-dependent potentiation of electrical (and chemical) transmission involving different intracellular signaling molecules can be triggered by substantially different patterns of synaptic activity in mixed synapses on the Mauthner cell. Interestingly, the signaling pathways involve PKA and CaMKII, two kinases that have a well-established role in regulating electrical transmission (Kothmann et al., 2007; Alev et al., 2008). These two signaling pathways are independent; PKI5-24 does not block 500Hz Bursts potentiation (Kumar and Faber, 1999) and the dopamine-evoked (Kumar and Faber, 1999) and cannabinoid-evoked (Cachope et al., 2007) potentiations do not occlude 500Hz Bursts potentiation.

The synaptic plastic properties observed in Club endings are unusual for synapses of a primary auditory afferent and they are likely to represent a form of sensory-motor processing (Yang et al., 1990; reviewed in Curti and Pereda, 2009). They probably represent a cellular specialization of these contacts whose function is to provide a decision-making neuron with essential sensory information (Faber and Pereda, 2011). As mentioned above, the ability to trigger these synaptic changes in one case relies on stimulation with brief trains ("500 Hz Bursts protocol"). Interestingly, the initial selection of this pattern was inspired in the natural bursting properties of these afferents (Yang et al., 1990), which characteristically respond with multiple action potentials to brief or low frequency acoustic stimulation (Furukawa and Ishii, 1967). Brief trains at 500 Hz optimize NMDA receptor activation by promoting temporal summation and facilitating transmitter release (Pereda and Faber, 1996), whereas long trains at 500 Hz depressed chemical synaptic transmission at these synapses (Pereda and Faber, 1996; Curti and Pereda, 2009). On the other hand, the induction of endocannabinoid/dopamine-dependent potentiation required a different pattern of afferent activity in the form of longer trains of 100 Hz ("100 Hz protocol") (Cachope et al., 2007), which is more adequate for mGluR activation and endocannabinoid release. Thus, the synaptic properties of Club endings are adapted to the firing characteristics of the afferents and the requirements of the Mauthner cell system, where the increased synaptic gain of these auditory nerve synapses will sensitize a vital escape response, lowering its threshold to acoustic stimuli (Korn and Faber, 2005; Faber and Pereda, 2011).

Endocannabinoid signaling has recently received great attention due to the widespread distribution of their receptors and its well-established role in modulating chemical synaptic plasticity (Alger, 2002; Freund et al., 2003; Chevalleyre et al., 2006). The work discussed here (Cachope et al., 2007) indicates that their modulatory role is not restricted to a specific modality of synaptic transmission. Cannabinoids were also reported to modify gap

junctional communication in peripheral tissue (Boger et al., 1999; Chaytor et al., 1999; Brandes et al., 2002; Upham et al., 2003). In the nervous system, anandamide, an endogenous cannabinoid, was reported to decrease junctional conductance between cultured astrocytes, although this effect was not mediated via activation of CB₁ receptors (Venance et al., 1995). Thus, the work on Club endings demonstrated for the first time that electrical synapses are also the target of endocannabinoid control (Cachope et al., 2007).

A multiplicity of molecules has been reported to be involved in regulating the strength of synaptic transmission. Amongst them, three have been recurrently shown to lead to enhancement of synaptic transmission: the neurotransmitter modulator dopamine and the enzymes PKA and CaMKII. Dopamine has been shown to trigger enhancement of synaptic transmission in a wide variety of brain structures and species (Centonze et al., 2001; Jay, 2003). PKA and CaMKII are considered memory molecules and its involvement in hippocampal LTP has been documented (Lisman et al., 2002; Shobe, 2002; Waltereit and Weller, 2003). The effects of these molecules has been shown not to be restricted to chemical synapses but also modify the strength of electrical synapses (Pereda et al., 1998; Mitropoulou and Bruzzone, 2003; Ouyang et al., 2005; Urschel et al., 2006; Alev et al., 2008), suggesting a general role of these molecules in regulating interneuronal communication. The convergence of these molecules for the regulation of electrical transmission in Club endings indicates that electrical synapses are simultaneously targeted by a multiplicity of regulatory mechanisms.

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Figure 1. Club endings exhibit mixed synaptic transmission

(A) VIIIth afferents (Club endings) terminate as single mixed synaptic contact (Mixed synapse) on the lateral dendrite of the Mauthner cell (B) VIIIth nerve stimulation evokes a mixed electrical and chemical synaptic responses (trace represents the average of 20 individual responses).

Figure 2. Activity-dependent modulation of electrical coupling

(A) Discontinuous high-frequency stimulation (500 Hz Bursts protocol; indicated by HFS) of the VIIIth nerve can evoke persistent potentiation of both components of the mixed synaptic response. Plots here and in subsequent figures illustrate the amplitudes of the electrical (open circles) and chemical (filled circles) components versus time (each point represents the average of 20 traces) for one experiment. (B) Schematic representation of the proposed potentiating pathway. Ca⁺⁺ entering through NMDA receptors activates CaMKII which phosphorylates either glutamate receptors and connexins or regulatory molecules. Modified from Pereda et al., 1998.

Figure 3. Endocannabinoid mediated activity-dependent modulation of electrical coupling
(A) Repetitive stimulation of the posterior VIIIth nerve (100 Hz protocol; indicated by HFS) evoked robust potentiation of both components of the mixed synaptic potential ($n = 5$). (B) Model for endocannabinoid-mediated potentiation of electrical and chemical synaptic transmission at Club endings. Synaptic activity leads to mGluR activation paired with postsynaptic membrane depolarization, triggering endocannabinoid (eCB) release from the postsynaptic Mauthner cell dendrite, which activates CB1Rs on dopaminergic fibers. CB1R activation leads to dopamine release that, by activating postsynaptic $D_{1/5}$ receptors, increases PKA activity responsible for simultaneous potentiation of electrical (Cx35) and glutamatergic (GluR) synaptic transmission. Modified from Cachope et al., 2007.

Figure 4. Immunocytochemical evidence for the involvement of CaMKII and PKA in Club endings
 Connexin35 and α CaMKII colocalize at Club endings and associate in goldfish brain. (A), Confocal z-section of a single terminal showing colocalization (yellow) of Cx35 (green) and α CaMKII (red). Labeling of Cx35 and α CaMKII colocalized at the periphery and the center of the synaptic contact. (B), Higher magnification of the labeled boxed region in A. α CaMKII and Cx35 colocalize at some Cx35 puncta (arrowheads) but is absent at others (asterisks). (C), Immunoblot detection of Cx35 in two different samples (lanes 2 and 3) with monoclonal Cx35/Cx36 antibody after IP of α CaMKII from goldfish hindbrain with G301 antibody. The Cx35 immunoblot exhibits three identifiable bands. Modified from Flores et al., 2010. (C) Polyclonal anti-Cx35/P110 antibody recognizes Cx35 as a pair of bands at 32–33 kDa (arrow, left lane, AP-) in membranes from goldfish hindbrain. Labeling is lost in membranes digested with alkaline phosphatase (right lane, AP+). The high molecular weight phospho-proteins labeled nonspecifically by the antibody are not associated with Cx35 (Kothmann et al., 2007). 50 mg/lane crude membrane protein. (D) Laser scanning confocal immunofluorescence of Club endings with double labeling by polyclonal anti-Cx35/P110 (green, Alexa Fluor 488) and monoclonal anti-Cx35/36 (mCx35; red, Alexa Fluor 594)

antibodies. The image shows extensive colocalization at individual Club endings in a section of the Mauthner cell lateral dendrite. A DIC image of this region is superimposed. Modified from Cachope et al., 2007.

Convergence of two mechanisms of activity-dependent potentiation in Club endings

Figure 5. Convergence of two mechanisms of activity-dependent potentiation in Club endings
Two different patterns of activity lead to potentiation of electrical (and chemical) transmission in Club endings. Discontinuous 500 Hz burst-like activity leads to activation of CaMKII via the involvement of NMDA receptors (“500 Hz Bursts”, red pathway). Sustained 100 Hz synaptic activation leads instead to activation of mGluR receptors and the release of endocannabinoids, which in turn lead to the local release of dopamine and the activation of postsynaptic PKA (“100 Hz”, black pathway). The involvement of PKA and CaMKII in regulating electrical transmission, which are also known to regulate the strength of chemical synapses, suggests a general role for these molecules in regulating interneuronal communication in general.