mGluR1/TRPC3-mediated Synaptic Transmission and Calcium Signaling in Mammalian Central Neurons

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Metabotropic glutamate receptors type 1 (mGluR1s) are required for a normal function of the mammalian brain. They are particularly important for synaptic signaling and plasticity in the cerebellum. Unlike ionotropic glutamate receptors that mediate rapid synaptic transmission, mGluR1s produce in cerebellar Purkinje cells a complex postsynaptic response consisting of two distinct signal components, namely a local dendritic calcium signal and a slow excitatory postsynaptic potential. The basic mechanisms underlying these synaptic responses were clarified in recent years. First, the work of several groups established that the dendritic calcium signal results from IP_3 receptor-mediated calcium release from internal stores. Second, it was recently found that mGluR1-mediated slow excitatory postsynaptic potentials are mediated by the transient receptor potential channel TRPC3. This surprising finding established TRPC3 as a novel postsynaptic channel for glutamatergic synaptic transmission.

Glutamate is the predominant neurotransmitter used by excitatory synapses in the mammalian brain (Hayashi 1952; Curtis et al. 1959). At postsynaptic sites, glutamate binds to two different classes of receptors, namely the ionotropic glutamate receptors (iGluRs) and the metabotropic glutamate receptors (mGluRs) (Sladeczek et al. 1985; Nicoletti et al. 1986; Sugiyama et al. 1987). The iGluRs represent ligand-gated nonselective cation channels that underlie excitatory postsynaptic currents (EPSCs). Based on their subunit composition, gating, and permeability properties, they are subdivided into three groups named after specific agonists: AMPA- (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), NMDA receptors (*N*-methyl *D*-aspartate receptors) and kainate receptors (Alexander et al. 2009). The other class of glutamate receptors, the mGluRs, consists of receptors that are coupled to G proteins and act through distinct downstream signaling cascades. They are structurally different from iGluRs and characterized by the presence of seven transmembrane domains (Houamed et al. 1991; Masu et al. 1991). The mGluRs exist as homodimers that do not by themselves form an ion-permeable pore in the membrane (Ozawa et al. 1998). To date, eight different genes (and more

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splice variants) encoding mGluRs have been identified and form the mGluR1 through mGluR8 subtypes (Alexander et al. 2009). Based on the amino acid sequence homology, downstream signal transduction pathways, and pharmacological properties, each of the subtypes was assigned to one of three groups. Group I receptors consist of mGluR1 and mGluR5 that positively couple to the phospholipase C (PLC). The receptors mGluR2 and mGluR3 constitute group II, whereas the remaining mGluRs, namely mGluR4, mGluR6, mGluR7, and mGluR8, belong to group III. Both groups II and III inhibit the adenylyl cyclase and thereby reduce the concentration of cAMP in the cytosol.

Of all different subtypes, mGluR1 is the most abundantly expressed mGluR in the mammalian central nervous system. In the brain, mGluR1 is highly expressed in the olfactory bulb, dentate gyrus, and cerebellum (Lein et al. 2007). The highest expression level of mGluR1 in the brain is found in Purkinje cells, the principal neurons of the cerebellar cortex (Shigemoto et al. 1992; Lein et al. 2007). Together with the AMPA receptors, mGluR1s are part of the excitatory synapses formed between parallel fibers and Purkinje cells (Fig. 1A). Each Purkinje cell is innervated by 100,000–200,000 parallel fibers (Ito 2006) that are axons of the cerebellar granule cells, the most abundant type of neuron in the brain. A second type of excitatory input to Purkinje cells is represented by the climbing fibers that originate in the inferior olive in the brain stem (Ito 2006). The two excitatory synaptic inputs to Purkinje cells are important determinants for the main functions of the cerebellum, including the real-time control of movement precision, error-correction, and control of posture as well as the procedural learning of complex movement sequences and conditioned responses.

It is expected that mGluR1 is involved in many of these cerebellar functions. This view is supported by the observation that mGluR1-deficient knockout mice show severe impairments in motor coordination. In particular, the gait of these mice is strongly affected as well as their ability for motor learning and general coordination (Aiba et al. 1994). The phenotype of the general mGluR1-knockout mice is rescued by the insertion of the gene encoding mGluR1 exclusively into cerebellar Purkinje cells (Ichise et al. 2000) and blockade of mGluR1 expression only in Purkinje cells of adult mice leads to impaired motor coordination (Nakao et al. 2007). These findings established mGluR1 in Purkinje cell as synaptic receptors that are indispensable for a normal cerebellar function.

Synaptic transmission involving mGluR1s is found at both parallel fiber-Purkinje cell synapses (Batchelor and Garthwaite 1993; Batchelor et al. 1994) as well as at climbing fiber-Purkinje cell synapses (Dzubay and Otis 2002). Most of our knowledge on the mGluR1 was gained from the analysis of the parallel fiber synapses. The parallel fiber synapse is quite unique in the central nervous system regarding its endowment with neurotransmitter receptors. In contrast to most other glutamatergic synapses in the mammalian brain, it lacks functional NMDA receptors (Shin and Linden 2005). The entire synaptic transmission at these synapses relies on AMPA receptors and on mGluR1 (Takechi et al. 1998). Although AMPA receptors are effectively activated even with single shock stimuli (Konnerth et al. 1990; Llano et al. 1991b), activation of mGluRs requires repetitive stimulation (Batchelor and Garthwaite 1993; Batchelor et al. 1994; Batchelor and Garthwaite 1997; Takechi et al. 1998). A possible explanation for the need of repetitive stimulation may relate to the observation that mGluR1s are found mostly at the periphery of the subsynaptic region (Nusser et al. 1994). At these sites outside the synaptic cleft, glutamate levels that are sufficiently high for receptor activation may be reached only with repetitive stimulation.

At parallel fiber-Purkinje cell synapses, repetitive stimulation produces an initial AMPA receptor postsynaptic signal component, followed by a more prolonged mGluR1 component (Fig. 1). Figure 1B shows a current clamp recording of this response consisting of an early burst of action potentials, followed by a prolonged depolarization known as a “slow excitatory postsynaptic potential” (slow EPSP)

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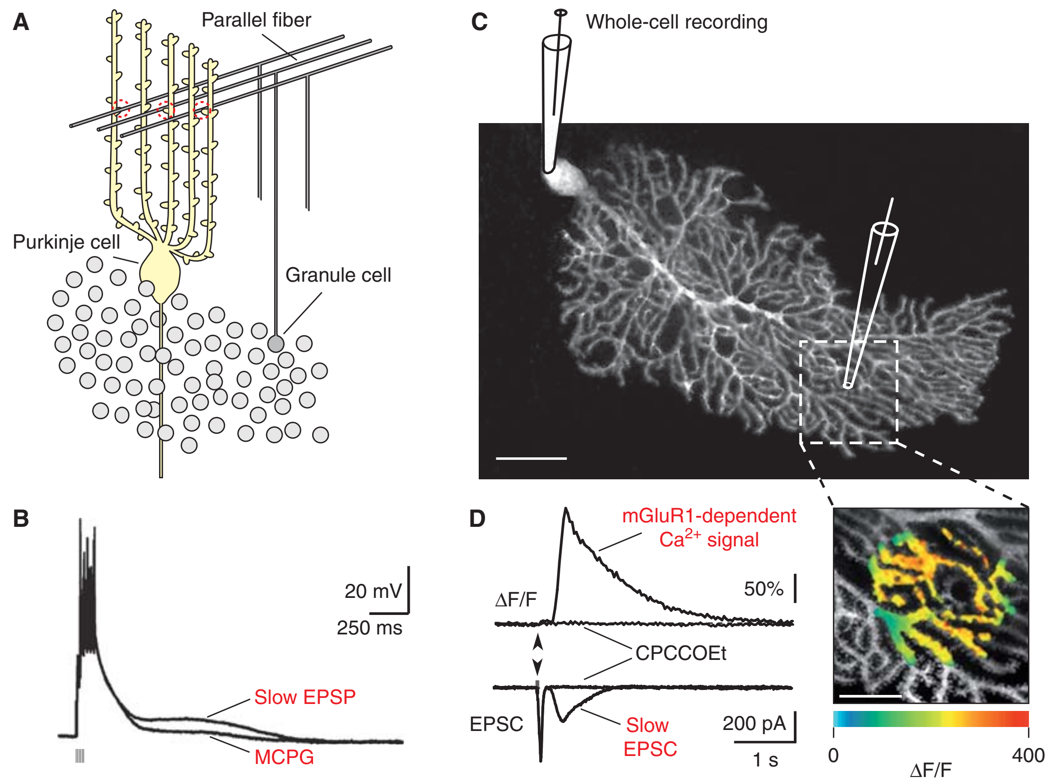

Figure 1. Parallel fiber-evoked mGluR1-dependent signals. (*A*) Diagram showing the parallel fiber synaptic input to Purkinje cell dendrites. (*B*) Microelectrode recording of glutamatergic postsynaptic potentials from a Purkinje cell in an acute slice of adult rat cerebellum. Short trains of stimuli to the parallel fibers (5–6 at 50 Hz) caused summation of the early AMPA receptor-dependent EPSPs (leading to spike firing) and a slow, delayed, depolarizing potential (slow EPSP), which was reversibly inhibited by antagonist of mGluRs (+)-MCPG (1mM). (*C*) Confocal image of a patch-clamped Purkinje cell in a cerebellar slice of an adult mouse. The patch-clamp pipette and the glass capillary used for electrical stimulation of parallel fibers are depicted schematically. The site of stimulation is shown at higher magnification in *D*. (*D*) *Left*: Parallel fiber-evoked (five pulses at 200 Hz, in 10 mM CNQX) synaptic responses consisting of a dendritic mGluR1-dependent Ca^{2+} transient ($\Delta F/F$, *top*) and an early rapid and a slow excitatory postsynaptic current (EPSC, *bottom*). Block of the mGluR1-dependent components by the group I-specific mGluR-antagonist CPCCOEt (200 µM) is shown as indicated. *Right*: Pseudocolor image of the synaptic Ca^{2+} signal. (*B*, Reprinted with modifications, with permission, from Batchelor and Gaithwaite 1997 [Nature Publishing Group].)

(Batchelor and Garthwaite 1993; Batchelor et al. 1994; Batchelor and Garthwaite 1997). Voltage-clamp recordings allow a clear separation of the initial rapid, AMPA receptor mediated excitatory postsynaptic current (EPSC) and the mGluR1-mediated slow EPSC (Fig. 1D) (Takechi et al. 1998; Hartmann et al. 2008). In addition of inducing the slow EPSPs, mGluR1s mediate a large and highly localized dendritic calcium transient in cerebellar Purkinje cells (Fig. 1D) (Llano et al. 1991a; Finch and Augustine 1998; Takechi et al. 1998).

mGluR1-DEPENDENT POSTSYNAPTIC Ca^{2+} RELEASE FROM INTERNAL STORES

The mGluR1-mediated synaptic Ca^{2+} transients constitute a distinct class of postsynaptic response because they may occur independently from changes in membrane potential (Takechi

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et al. 1998). The generation of these Ca^{2+} transients is the result of a cascade of signaling events. During the initial step, binding of glutamate to the mGluR1 activates the phospholipase C (PLC). This is followed by inositoltrisphosphate (IP_3) production and accumulation in the cytosol. IP_3 binds to its own receptor channels that are located in the endoplasmic reticulum (ER) membrane and are permeable for Ca^{2+} (Verkhratsky 2005). At parallel fiber-Purkinje cell synapses, the release of Ca^{2+} from ER stores forms a characteristic local signaling response (Finch and Augustine 1998; Takechi et al. 1998). An important feature of the mGluR1-dependent Ca^{2+} signal is its spatial restriction ranging from dendritic regions (Fig. 1C) to small dendritic terminal branchlets or even to single spines (Fig. 2). Focal synaptic stimulation involving the activation of just a few neighboring parallel fibers causes mGluR1-dependendent Ca^{2+} signals that occur at tiny spino-dendritic regions (Fig. 2, spine 2-dendrite 2) or in individual spines (Fig. 2, spine 1) (Fig. 2B) (Takechi et al. 1998). However, there is evidence that nominally similar parallel fiber synaptic inputs may cause variable postsynaptic Ca^{2+} transients, depending apparently on the dendritic location (Hartmann et al. 2004). Possibly, this variability may relate to a heterogeneity of the organization of the smooth ER network in the Purkinje cell dendrites and spines (Harris and Stevens 1988; Martone et al. 1993; Terasaki et al. 1994). Alternatively, there may be an uneven distribution of IP_3 receptors, ryanodine receptors (RyRs), Ca^{2+}-ATPases and luminal Ca^{2+}-binding proteins, like calsequestrin, calnexin and calreticulin, in the dendritic tree of Purkinje cells (Villa et al.

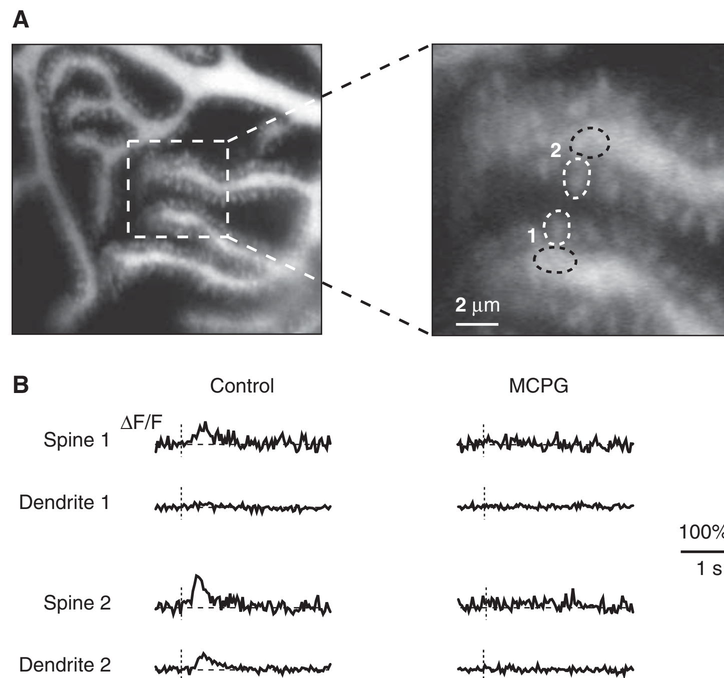

Figure 2. Identification of synaptic Ca^{2+}-release signals in spines and dendritic microdomains (Takechi et al. 1998). (*A*) Images of spiny dendrites of a Purkinje cell, indicating the sites of fluorescence measurements. (*B*) Fluorescence measurements taken from the dendrites shown in A. In spine 1, the synaptic Ca^{2+} transient was restricted to an individual spine. Note the complete absence of any Ca^{2+} signal in the immediate dendritic vicinity. A large synaptic Ca^{2+} transient was detected in spine 2 and a smaller signal occurred in the subspine region. MCPG (1 mM) completely blocked all synaptic Ca^{2+} transients.

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1991; Takei et al. 1992; Villa et al. 1992; Nori et al. 1993).

A more detailed dissection of the intracellular signaling cascade shows that mGluR1s couple to their downstream effectors through members of the G_q protein subclass of heterotrimeric G proteins (Masu et al. 1991). Only two members of this G protein family, $G\alpha_q$ and $G\alpha_{11}$, are expressed in the brain (Nakamura et al. 1991; Wilkie et al. 1991). Both are present also in Purkinje cells (Tanaka et al. 2000) and it has been shown that the $G\alpha_q/G\alpha_{11}$ immunoreactivity colocalizes with mGluR1 in Purkinje cell spines (Tanaka et al. 2000). However, measurements of postsynaptic Ca^{2+} signals in Purkinje cells in mice deficient for either $G\alpha_q$ or $G\alpha_{11}$ revealed that only $G\alpha_q$, but not $G\alpha_{11}$, is required for mGluR-mediated Ca^{2+} transients (Hartmann et al. 2004).

G_q proteins activate the PLCβ of which four subtypes (PLCβ1-4) have been identified (Blank et al. 1991; Berstein et al. 1992; Rebecchi and Pentyala 2000). Purkinje cells express all subtypes with the exception of the PLCβ2 (Tanaka and Kondo 1994; Roustan et al. 1995; Watanabe et al. 1998; Lein et al. 2007). PLCβ1 has the lowest and PLCβ4 the highest expression level in Purkinje cells (Lein et al. 2007). PLCβ3 prevails in the caudal cerebellum (Hirono et al. 2001), whereas PLCβ4 is reciprocally expressed in the rostral lobuli of the cerebellum (Kano et al. 1998). PLCβ1 is present primarily in the somata of Purkinje cells and is, therefore, unlikely to play a major role in synaptic transmission at parallel fiber synapses that are all located in the spiny dendrites (Kano et al. 1998).

Of all three IP_3 receptor subtypes, IP_3R1 is expressed in Purkinje cells with extraordinary high density (Sharp et al. 1999; Lein et al. 2007). IP_3R2 is present in low amounts, whereas IP_3R3 is not found in Purkinje cells (Lein et al. 2007). Most remarkably, the signaling properties of the IP_3R1 in its native environment in the Purkinje cell cytosol differ largely from those detected in isolated preparations or other cell types (Khodakhah and Ogden 1993; Fujiwara et al. 2001). With an EC50 of 25.8 μM (Fujiwara et al. 2001) its sensitivity for IP_3 is exceptionally low, indicating that 10- to 20-fold higher concentrations of IP_3 are required to evoke Ca^{2+}-release from stores as compared with astrocytes, hepatocytes, exocrine cells and vascular endothelium (Khodakhah and Ogden 1993). Intriguingly, IP_3Rs isolated from Purkinje cells have a sensitivity for IP_3 that is similar to that found in other cell types (Fujiwara et al. 2001). In general, the IP_3R forms a "macro" signal complex in which it operates as an integrator and regulator for signaling cascades. The most important regulators of the IP_3R are Ca^{2+} ions, calmodulin, the immunophilin FKBP12, ATP, and protein kinases (reviewed in Patterson et al. [2004]; Mikoshiba [2007]). Notably, calmodulin specifically inhibits IP_3-binding to IP_3R1 receptors and IP_3-evoked Ca^{2+} mobilization in a Ca^{2+}-independent manner (Patel et al. 1997; Cardy and Taylor 1998). Because of its high expression in Purkinje cells, calmodulin may be responsible for the low affinity of the IP_3R1 for IP_3 in the Purkinje cell cytosol. A Ca^{2+}-independent binding to the IP_3R1 has been reported also for CaBP1, a member of the neuronal Ca^{2+} sensor family of Ca^{2+} binding proteins. The interaction of CaBP1 with the amino terminus of the IP_3R1 results in a weakened IP_3 binding to the receptor, similarly to the effect of calmodulin (Kasri et al. 2004). However, because of its very low expression in Purkinje cells (Lein et al. 2007), CaBP1 does not seem to be a major cause for the specific features of IP_3R-dependent Ca^{2+}-signaling in this cell type. Instead, it is assumed that, in addition to calmodulin, in Purkinje cells the IP_3R1 is specifically regulated by proteins like the IP_3 inhibitor IRI (Watras et al. 2000) or the carbonic anhydrase-related protein CARP (Nogradi et al. 1997). Interestingly, the expression of CARP is restricted to Purkinje cells (Nogradi et al. 1997) in which it reduces the affinity of IP_3R1 for IP_3 by binding to the modulatory domain of IP_3R1 (Hirota et al. 2003). On the behavioral level, the relevance of the carbonic anhydrase activity for IP_3R-dependent signaling in Purkinje cells is emphasized by the fact that a mutation in the carbonic anhydrase related protein 8 (Car8) is associated with a pronounced ataxic gait (Jiao et al. 2005), whereas on the ultrastructural level,

Purkinje cells show various synaptic anomalies (Hirasawa et al. 2007). Even though the affinity of the IP_3R1 for its agonist in Purkinje cells is low, the magnitude of Ca^{2+} release signal from the ER Ca^{2+} store is much larger than in other cell types (Khodakhah and Ogden 1993; Ogden and Capiod 1997). Furthermore, in comparison to cells in other tissues, in Purkinje cells the kinetics of activation and Ca^{2+}-dependent inactivation (Bezprozvanny et al. 1991) of the IP_3R1 are particularly fast (Khodakhah and Ogden 1995; Ogden and Capiod 1997). For a cytosolic IP_3 concentration of 38 µM following photorelease of "caged" IP_3, the efflux of Ca^{2+} ions from the ER was estimated to be ≈1 µM/ms in Purkinje cells and only ≈0.03 µM/ms in cells from peripheral tissues (Khodakhah and Ogden 1993). Together, these observations suggest that IP_3-dependent Ca^{2+}-release in Purkinje cells is particularly fine-tuned for signaling on the time scale of synaptic transmission in the millisecond range, which takes place in a localized space adjacent to the site of synaptic inputs (Takechi et al. 1998).

An increasing amount of evidence indicates that the mGluR1-mediated synaptic Ca^{2+} release signal from stores plays an important role in activity dependent synaptic plasticity. Thus, parallel fiber synapse-mediated IP_3R1-dependent Ca^{2+} transients are required for the induction of long-term depression (LTD) at parallel fiber-Purkinje cell synapses (Kasono and Hirano 1995; Khodakhah and Armstrong 1997; Finch and Augustine 1998; Inoue et al. 1998; Daniel et al. 1999). LTD, the presumed cellular basis of motor learning in the cerebellum (Ito 2000) requires the conjunctive stimulation of parallel and climbing fiber inputs (Gao et al. 2003). During LTD induction, parallel fiber firing activates mGluR1-dependent pathways that include IP_3R1-mediated release of Ca^{2+} ions from ER Ca^{2+} stores, whereas climbing fiber activity strongly depolarizes the Purkinje cells and induces influx of Ca^{2+} ions through voltage-gated Ca^{2+} channels (Knöpfel et al. 1991). The increased cytosolic concentrations of Ca^{2+} and IP_3 are, thus, the result of the concerted parallel and climbing fiber activity. Because of their sensitivity to both IP_3 and Ca^{2+} (Bezprozvanny et al. 1991), IP_3R1s act as coincidence detectors for the two excitatory inputs onto Purkinje cells and mediate an amplification of the postsynaptic Ca^{2+} release signal. This facilitates the induction of LTD (Wang et al. 2000; Doi et al. 2005). Experimental evidence indicates that IP_3R1-mediated Ca^{2+} release in the spines themselves is of outstanding importance for the induction of LTD. Thus, LTD was absent in a myosin-Va mutant mouse line, in which the ER does not extend into dendritic spines (Miyata et al. 2000). Furthermore, IP_3R-dependent Ca^{2+} release in single spines elicited by sparse parallel fiber stimulation is able to induce LTD specifically at the activated input (Wang et al. 2000).

MECHANISMS UNDERLYING mGluR1-DEPENDENT SYNAPTIC DEPOLARIZATION

As mentioned above, repetitive stimulation of parallel fiber inputs evokes a slow EPSC that is sensitive to mGluR-specific antagonists (Batchelor and Garthwaite 1993; Batchelor et al. 1994). Remarkably, the frequencies that are most effective in brain slices (Batchelor and Garthwaite 1997) resemble those that are encountered with the stimulation of parallel (Isope et al. 2004) or mossy fibers in vivo (Chadderton et al. 2004). The slow EPSC is characterized by a characteristic time course: it starts with a latency of 100–200 ms after the stimulation of afferent parallel fibers and lasts for about 1 second (Fig. 1D)(Batchelor et al. 1994). The long search for the mechanisms underlying the slow EPSC involved pharmacological experiments that indicated that the mGluR1-mediated slow EPSC is not mediated by hyperpolarization-activated cation channels, purinergic receptors (Canepari et al. 2001), Na^{+}/Ca^{2+}-exchangers (Hirono et al. 1998) or voltage-gated Ca^{2+} channels (Tempia et al. 2001). Instead, the ionic properties of the slow EPSCs are reminiscent of currents that permeate through the canonical transient receptor potential (TRPC) channels. Indeed, a few years ago (Kim et al. 2003) reported experiments that

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suggested that the mGluR1-dependent slow EPSC is mediated by TRPC1.

Later studies investigated in more detail TRPC channels in Purkinje cells (Huang et al. 2007; Lein et al. 2007; Hartmann et al. 2008). Although the general notion of an involvement of TRPCs was confirmed, the new experiments did not provide evidence for a specific role of TRPC1. Instead, several lines of evidence pointed to TRPC3 as the postsynaptic channel that mediates the slow EPSC. Thus, the TRPC3 protein was found to be abundant in the somatodendritic compartment of Purkinje cells in the adult mouse brain (Fig. 3A,B) (Hartmann and Konnerth 2008). The number of mRNA copies for TRPC3 is 8–10 times higher in single Purkinje cells than that of TRPC1 whereas mRNA of the other subunits is present in low amounts. Furthermore, of all TRPC subunits the expression of TRPC3 is most tightly coupled to a developmental time window in which the outgrowth of the dendritic tree of Purkinje cells (Hendelman and Aggerwal 1980), the formation of parallel fiber synapses and the elimination of supernumerary climbing fibers (Scelfo and Strata 2005) take place (Fig. 3C) (Huang et al. 2007). The outstanding role of TRPC3 channels for mGluR1-dependent synaptic transmission in Purkinje cells was unambiguously shown by a recent study that analyzed mGluR1-mediated transmission in specifically designed TRPC-deficient mice (Hartmann et al. 2008) (Figs. 4A–F). The importance of TRPC3 and the mGluR1-dependent slow EPSC for cerebellar function was further emphasized by the behavioral impairments that were observed in TRPC3 null mutant mice. These mice show a movement deficit of their hindpaws that leads to an ataxic wide-based gait and poor performance when they walk on a horizontal ladder or an elevated beam (Hartmann et al. 2008).

The role of TRPC3 for mGluR1-mediated signaling and sensorimotor integration was further corroborated in a study that used mice with a gain-of-function mutation in the *Trpc3* gene. A single amino acid exchange in the TRPC3 protein (T635A) results in loss of a phosphorylation site of TRPC3 and altered gating of the channel. Therefore, mGluR1-dependent inward currents are increased and motor coordination is heavily impaired. These so-called *moonwalker* (*Mwk*) mice display massive symptoms of ataxia. Remarkably, and in distinct contrast to the TRPC3 knockout mice (Hartmann et al. 2008), increased TRPC3-mediated signaling in *Mwk* mice is accompanied by a reduced outgrowth of the dendritic arborization of Purkinje cells and even Purkinje cell loss after 4 months of age (Becker et al. 2009). It is remarkable that the absence of TRPC3 is less deleterious for Purkinje cell development than the excess of TRPC3-mediated signaling. The high impact of TRPC3 overexpression may relate to the fact that the developmental increase in TRPC3 expression in Purkinje cells during the first two postnatal weeks (Huang et al. 2007) coincides with the most intensive phase of dendritic growth in these neurons (Hendelman and Aggerwal 1980).

The signaling cascade that links mGluR1 and TRPC3 is not yet fully elucidated. There is evidence that both mGluR1-evoked signal components, the Ca^{2+} release signal and the slow EPSC, are mediated by G_q proteins, but subsequently follow divergent pathways (Fig. 5). It has been shown that both $G\alpha_q$ and $G\alpha_{11}$ contribute to the generation of the slow EPSC (Hartmann et al. 2004). However, pharmacological manipulations that block the mGluR1-dependent Ca^{2+} release from internal stores do not affect the mGluR1-dependent depolarization (Finch and Augustine 1998; Takechi et al. 1998). Importantly, the two signal components of mGluR1-mediated transmission, the Ca^{2+} signal and the slow EPSC, may occur independently of each other (Hirono et al. 1998; Takechi et al. 1998; Tempia et al. 1998; Hartmann et al. 2008), as expected from processes that involve distinct intracellular signaling pathways.

The role of PLCβ in the generation of the mGluR1-dependent slow EPSC is controversial. An involvement of PLCβ was indicated by the observation that slow EPSC were absent in PLCβ4 deficient mutant mice (Sugiyama et al. 1999). However, slow EPSCs seem to be insensitive to the PLCβ antagonist U73122 (Hirono et al. 1998; Canepari et al. 2001; Glitsch 2010). It has been reported that U73122 abolished

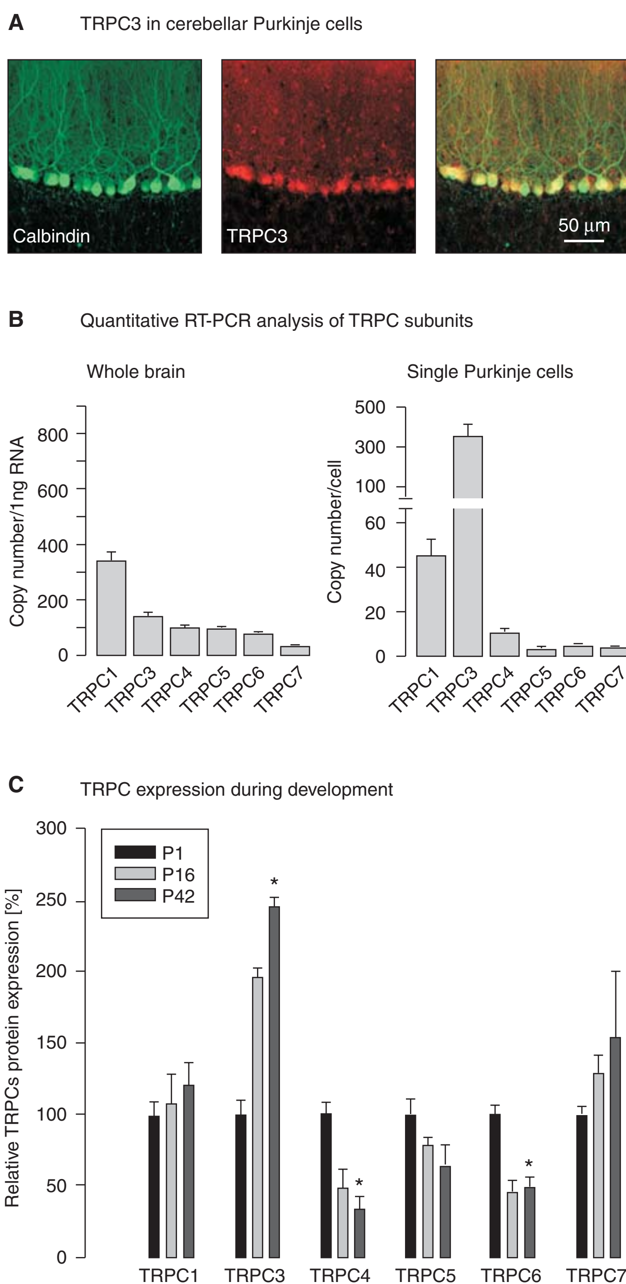

Figure 3. Expression of TRPC channel subunits in the murine brain and single Purkinje cells. (*A*) A dual-channel confocal scan of an immunohistochemical staining in an acute cerebellar slice. Calbindin D28k immunoreactivity is shown in green (*left*) and that for TRPC3 in red (*middle*). *Right*: Merged images (Hartmann et al. 2008). (*B*) Copy numbers of TRPC subunit mRNA detected in 1ng total RNA of mouse whole brain (*left*) and in single Purkinje cells (*right*). (*C*) Relative TRPC expression at postnatal days 1 (P1), 16 (P16) and 42 (P42), normalized to average expression value obtained at P1 for a given TRPC subunit. For the quantification, relative Western blot band intensities were analyzed. Data represent averages from three to four independent Western blots. There is a significant increase in TRPC3 expression and a significant decrease in both TRPC4 and TRPC6 expression (unpaired t-tests). (Reprinted with modifications, with permission, from Huang et al. 2007 [Elsevier].)

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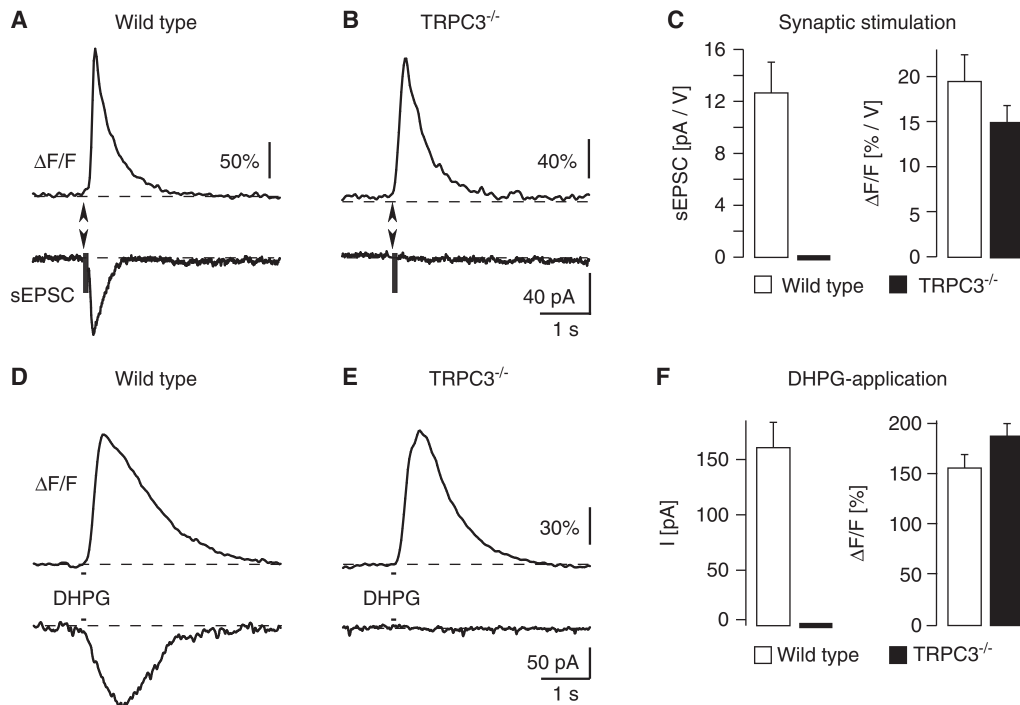

Figure 4. Analysis of TRPC3-deficient mice (Hartmann et al. 2008). (*A*) Slow EPSC in a wild-type mouse (*lower trace*) and the corresponding local dendritic Ca^{2+} response (*upper trace*). (*B*) Similar recording in a $TRPC3^{-/-}$ mouse. (*C*) Summary graphs for normalized (to stimulation strength) slow EPSCs and Ca^{2+} transients. (*D*) DHPG evoked a slow inward current (*lower trace*) and a local Ca^{2+} transient (*upper trace*) in a wild-type mouse. (*E*) Similar recording in a $TRPC3^{-/-}$ mouse. (*F*) Summary of DHPG-evoked current (*right*) and Ca^{2+} (*left*) responses.

the mGluR1-dependent Ca^{2+} release signal in rat cerebellar slices (Takechi et al. 1998). A possible explanation for these discrepancies may be that the genetic removal of PLCβ disrupted the tight assembly of the molecular components of the mGluR1-dependent signaling cascade at the postsynaptic density (Nakamura et al. 2004) and that PLCβ activity is generally not needed for the signal transduction between mGluR1 and TRPC3. A recent study indicated that the activation of mGluR1 initiates two independent signaling pathways downstream of the G_q proteins: one dependent on the PLCβ and another one dependent on phospholipase D1 (Fig. 5) (Glitsch 2010). It was shown in that paper that activation of mGluR1 is followed by the translocation of the phospholipase D to the plasma membrane and that the TRPC3-dependent slow EPSCs in Purkinje cell require the activity of the phospholipase D (PLD). However, PLD most likely does not gate the TRPC3 directly, as suggested by experiments that were performed in a cell line transfected with human TRPC3 (Glitsch 2010). In Purkinje cells, DHPG-evoked inward currents are sensitive to antagonists of the small G proteins of the rho family. From other studies it is known that PLD1 is one of the effectors of rho GTPases (Jenkins and Frohman 2005; Weernink et al. 2007). Thus, it is possible that the activation of TRPC3 through mGluR1 involves a rho GTPase-dependent pathway that depends on PLD1 (Glitsch 2010).

Another subtype of the PLC, namely PLCγ, which is also found in Purkinje cells (Lein et al. 2007) has been shown to activate TRPC3 in other cell types (van Rossum et al. 2005; Lockwich et al. 2008; Tong et al. 2008) including

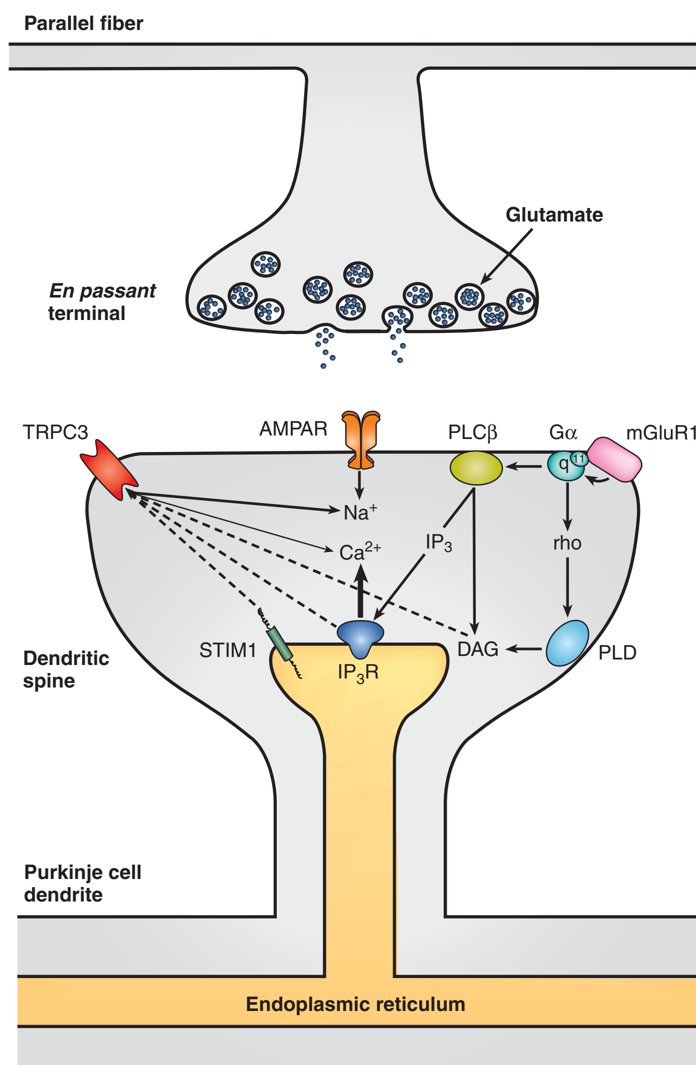

Figure 5. Model of glutamatergic synaptic signaling at parallel fiber-Purkinje cell synapses. Glutamate released from presynaptic terminals binds to AMPA receptors and the mGluR1. Influx of sodium ions through AMPA receptor channels leads to a fast postsynaptic depolarization. The mGluR1 couples predominantly to $G\alpha_q$ but also to $G\alpha_{11}$. This is followed by the activation of the phospholipase Cβ that cleaves phosphatidyl-4,5-bisphosphate (PIP_2) into inositoltrisphosphate (IP_3) and diacylglycerol (DAG). By binding to its receptor (IP_3R) in the endoplasmic reticulum membrane, IP_3 releases Ca^{2+}-ions from this intracellular Ca^{2+}-store. TRPC3 channels in Purkinje cells can be activated following stimulation of the PLD and require activity of a small G protein of the Rho family. Possible other candidates for TRPC3 gating are DAG, the stromal interaction molecule 1 (STIM1), the amino terminus of the IP_3R1 a.o.

neurons (Li et al. 1999). However, the internal effector molecule that activates the TRPC3 remains to be determined. It is well established that, when activated, the PLCs, in addition to the soluble factor IP_3, produce the lipophilic compound diacylglycerol (DAG) (Weernink et al. 2006). In heterologous expression systems, TRPC3 was shown to be directly regulated by diacylglycerol (DAG) (Fig. 5) (Hofmann et al. 1999; Lemonnier et al. 2008) and by the

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substrate of PLCs, namely phosphatidylinositol-4,5-bisphosphate (Lemonnier et al. 2008). In transfected HEK293 cells, the opening of TRPC3 channels is induced by their binding to the amino-terminal domain of the activated IP_3 receptor (Kiselyov et al. 1998; Kiselyov et al. 1999). In the same cell type, there is also an obligatory role of tyrosine kinase Src (Vazquez et al. 2004). In pancreatic acinar cells, the scaffolding protein Homer 1b/c is critical for the regulation of the activity of TRPC3 by the IP_3 receptor (Kim et al. 2006). Finally, another interesting component involved in TRPC3 signaling is the stromal interacting molecule I (STIM1) that has an essential role for the Ca^{2+} homeostasis in the ER in many cell types (Roos et al. 2005; Zhang et al. 2005). STIM1 is a Ca^{2+} sensor that, following depletion of ER Ca^{2+} stores, activates a Ca^{2+} influx through Orai channels in the plasma membrane of nonneuronal cells (Zhang et al. 2005). This so-called store-operated Ca^{2+} entry (SOCE) ensures the replenishment of ER Ca^{2+} stores. Because of their opening downstream of the PLC and their permeability for Ca^{2+} ions, TRPC channels were proposed to function as store-operated channels (Zhu et al. 1996; Zitt et al. 1997). In HEK293 cells, it has been shown that STIM1 exerts a gating action on TRPC3 by intermolecular electrostatic interaction (Zeng et al. 2008). TRPC3 opens when two positively charged lysine-residues in the carboxyl terminus of STIM1 (684 and 685) get in contact with two negatively charged residues of aspartate (697 and 698) in the intracellular carboxy-terminal domain of TRPC3. Purkinje cells express all the factors mentioned above (Lein et al. 2007) and it is important to note that TRPC3 is part of a multi-protein signaling complex that is located postsynaptically and, in addition to the mGluR1, contains also the IP_3R1, PLCβ4 (Nakamura et al. 2004), Homer proteins (Tu et al. 1999; Yuan et al. 2003; Kim et al. 2006) and STIM1 and other TRPC subunits (Yuan et al. 2007). However, whether SOCE requires TRPC channels and whether they are part of the STIM1-Orai-complex is controversially discussed (Liao et al. 2007; Liao et al. 2008; DeHaven et al. 2009).

As most other TRP channels, TRPC3 channels are permeable not only for Ca^{2+} but also for Na^+ ions (Zhu et al. 1996; Alexander et al. 2009). It is, therefore, not surprising that TRPC3-mediated slow EPSCs are associated with dendritic Na^+ transients in Purkinje cells (Knöpfel et al. 2000). These synaptic Na^+ signals are restricted to dendritic regions that are innervated by the afferent parallel fibers. In addition to Na^+ transients, mGluR1-dependent slow EPSCs are also associated with dendritic Ca^{2+} transients that may result in part from Ca^{2+} entry through TRPC3 channels (Tempia et al. 2001). However, it remains unclear how Ca^{2+} entry through TRPC3 channels compares to the Ca^{2+} release signal from internal stores. The answer to this question is important for a better understanding of the induction of LTD, a process known to require a transient elevation in intradendritic Ca^{2+} concentration (Konnerth et al. 1992).

Another open question concerns the possible molecular partners of TRPC3 channels in Purkinje cells. In view of the fact that Purkinje cells express various TRPCs (Fig. 3), TRPC3 may form heteromers with one or several of those channels (Zagranichnaya et al. 2005). The specific arrangement may determine the relative composition of the ion fluxes (Nilius et al. 2007). Furthermore, the existence of heteromeric complexes of TRPC- and Orai-channels, which may be regulated by STIM1, has been suggested and is controversially discussed at present (Liao et al. 2007; Ong et al. 2007; Cheng et al. 2008; Liao et al. 2008; Zeng et al. 2008; DeHaven et al. 2009). Because all three subtypes of Orai-proteins are expressed in Purkinje cells (Hartmann et al. 2010), such complexes represent possible candidates for the ion channels underlying the mGluR1-dependent slow EPSC.

CONCLUSIONS

In the mammalian central nervous system, mGluR1 is involved in a variety of physiological functions including memory, cognition, the sensation of pain and fear (Gravius et al. 2010). An extensively studied function of the

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mGluR1 is its role in cerebellar sensorimotor integration. The absence of mGluR1 activity in the cerebellar cortex has deleterious consequences for synaptic plasticity (for review, see Rose and Konnerth [2001]) as well as for motor coordination (Aiba et al. 1994; Ichise et al. 2000; Coesmans et al. 2003; Nakao et al. 2007). Even a mild interference with mGluR1-dependent signaling cascades invariably leads to ataxia (Hartmann et al. 2004; Hartmann et al. 2008). At parallel fiber-to Purkinje cell synapses, important aspects of mGluR1-dependent signaling became clear in recent years (Fig. 5). Thus, it was found that mGluR1 induces via PLCβ the release of Ca^{2+} ions from internal Ca^{2+} stores in dendrites and spines (Takechi et al. 1998). This process was shown to be important for synaptic plasticity and for motor learning (Miyata et al. 2000). In addition to mediating the release of Ca^{2+} ions from internal stores, mGluR1 was shown to activate the cation channels TRPC3 (Hartmann et al. 2008). This surprising observation established TRPC3 as a novel postsynaptic channel for glutamatergic synaptic transmission. The gating of TRPC3 downstream of the synaptic activation of mGluR1 has not been entirely clarified, but seems to involve various signaling events, one of which is the rho GTPase-dependent activation of the PLD1 (Glitsch 2010). Other candidate signaling molecules include the IP_3R itself (Kiselyov et al. 1999), diacylglycerol (Hofmann et al. 1999) and STIM1 (Zeng et al. 2008). A promising experimental strategy for the elucidation of Purkinje cell-specific intracellular signaling events downstream of mGluR1 may involve the functional analysis of cell type-selective mutant mice in vitro and in vivo.

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