Channels 5:2, 1-5; March/April 2011; © 2011 Landes Bioscience

Role of different voltage-gated Ca^{2+} channels in cortical spreading depression

Specific requirement of P/Q-type Ca^{2+} channels

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Gain-of-function mutations in $Ca_V2.1$ (P/Q-type) Ca^{2+} channels cause familial hemiplegic migraine type 1 (FHM1), a subtype of migraine with aura. Knockin (KI) mice carrying FHM1 mutations show increased neuronal P/Q-type current and facilitation of induction and propagation of cortical spreading depression (CSD), the phenomenon that underlies migraine aura and may activate migraine headache mechanisms. We recently studied cortical neurotransmission in neuronal microcultures and brain slices of FHM1 KI mice, and showed (1) gain-of-function of excitatory neurotransmission, due to increased action potential-evoked Ca^{2+} influx and increased probability of glutamate release at pyramidal cell synapses, but unaltered inhibitory neurotransmission at fast-spiking interneuron synapses and (2) a causative link between enhanced glutamate release and facilitation of CSD induced by brief pulses of high K^+ in cortical slices.[1] Here, we show that after blockade of either the P/Q-type Ca^{2+} channels or the NMDA receptors, CSD cannot be induced in wild-type mouse cortical slices. In contrast, blockade of N- or R-type Ca^{2+} channels has only a small inhibitory effect on CSD threshold and velocity of propagation. Our findings support a model in which Ca^{2+} influx through presynaptic P/Q-type Ca^{2+} channels with consequent release of glutamate from recurrent cortical pyramidal cell synapses and activation of NMDA receptors are required for initiation and propagation of the CSD involved in migraine.

Key words: calcium channel, synaptic transmission, migraine, spreading depression, NMDA receptor, cortex

Submitted: 11/08/10

Accepted: 11/08/10

Previously published online: www.landesbioscience.com/journals/channels/article/14149

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Addendum to: Tottene A, Conti R, Fabbro A, Vecchia D, Shapovalova M, Santello M, et al. Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in $Ca_V2.1$ knockin migraine mice. Neuron 2009; 61:762–73; PMID: 19285472; DOI: 10.1016/j.neuron.2009.01.027.

Introduction

Missense mutations in the gene that encodes the pore-forming α_1 subunit of voltage-gated $Ca_V2.1$ (P/Q-type) Ca^{2+} channels cause a rare autosomal dominant subtype of migraine with aura: familial hemiplegic migraine type 1 (FHM1).[2] $Ca_V2.1$ channels are located in somatodendritic membranes and presynaptic terminals throughout the brain, and play a dominant role in initiating action potential-evoked neurotransmitter release at central nervous system synapses.[3] FHM1 mutations produce gain-of-function of human recombinant $Ca_V2.1$ channels, mainly due to a shift to lower voltages of channel activation.[4,5] Knockin (KI) mice carrying FHM1 mutations show increased P/Q-type Ca^{2+} current density in central neurons including cortical pyramidal cells.[1,6-8] Interestingly, the FHM1 KI mice also show a reduced threshold for induction of cortical spreading depression (CSD) and an increased velocity of propagation of CSD.[1,6,8] CSD can be induced in animals by focal stimulation of the cerebral cortex and consists in a slowly propagating wave of cortical neuronal and glial depolarization, whose mechanisms remain unclear and controversial.[9,10] Neuroimaging studies in humans indicate that CSD underlies the migraine aura; animal studies indicate that CSD may also trigger the migraine headache mechanisms.[11-13]

To study the cortical mechanisms that produce facilitation of CSD in FHM1 mutant mice, we investigated cortical neurotransmission in neuronal microcultures

and in brain slices from FHM1 KI mice.[1] The results show increased strength of excitatory synaptic transmission due to increased action potential-evoked Ca^{2+} influx through presynaptic P/Q-type Ca^{2+} channels and increased probability of glutamate release at cortical pyramidal cell synapses of mutant mice. In striking contrast, inhibitory neurotransmission at connected pairs of fast-spiking interneurons and pyramidal cells was unaltered in FHM1 mice, despite being initiated by P/Q-type Ca^{2+} channels.[1] To test the hypothesis that the gain-of-function of glutamate release at synapses onto pyramidal cells may explain the facilitation of induction and propagation of experimental CSD in FHM1 KI mice, we measured the threshold for CSD induction and the velocity of CSD propagation in acute slices of somatosensory cortex of R192Q KI mice before and after perfusion with a concentration of ωAgaIVA that reduced glutamate release at KI pyramidal cell synapses to wild-type (WT) levels. Strikingly, restoration of glutamate release to WT levels completely rescued CSD facilitation, as both CSD triggering threshold and CSD propagation rate in mutant mice became similar to those in WT mice.[1] This finding provides direct evidence of a causative link between enhanced glutamate release at pyramidal cell synapses and facilitation of experimental CSD, thus giving insights into the controversial mechanisms of CSD initiation and propagation. The initiation of the positive feedback cycle that ignites CSD and almost zeroes the neuronal membrane potential depends on the local increase of the extracellular concentration of K^+ ions $[K^+]_o$ above a critical value and on the activation of a net inward current at the pyramidal cell dendrites;[9] the nature of the cationic channels mediating this inward current remains unclear and controversial, although there is strong pharmacological support for a key role of NMDA receptors[9,10] (cf also Discussion and references therein). The findings of Tottene et al.[1] support a model of CSD initiation in which activation of presynaptic P/Q-type Ca^{2+} channels with consequent release of glutamate from recurrent cortical pyramidal cell synapses and activation of NMDA receptors (and possibly postsynaptic P/Q-type Ca^{2+} channels) are key components of the positive feedback cycle that ignites CSD in normally metabolizing cortical tissue. Regarding CSD propagation, the findings are consistent with a model based on interstitial K^+ diffusion initiating in adjacent dendrites this positive feedback cycle.[1]

Since it is controversial whether activation of NMDA receptors and in particular Ca^{2+} influx and synaptic transmission play only a modulatory role or are required for CSD induction and propagation,[9,10] we further investigated the role of NMDA receptors and of the different voltage-gated Ca^{2+} channel by studying the effect of specific blockers of these channels on the threshold for CSD induction and the velocity of CSD propagation in acute slices of mouse cerebral cortex.

Results

To investigate the role of the NMDA receptors and the P/Q-, N-, R- and L-type Ca^{2+} channels in initiation and propagation of experimental CSD, we measured the threshold for CSD induction and the velocity of CSD propagation, induced by brief pulses of high K^+ in acute slices of mouse somatosensory cortex, before and after application of saturating concentrations of D-AP5 (50 uM), ωAgaIVA (300 or 400 nM), ωCgTxGVIA (1 μM), SNX-482 (300 or 500 nM) and nimodipine (10 μM), respectively, as described in Tottene et al.[1] Pressure pulses of increasing duration were applied to a 3 M KCl-containing pipette positioned on layer 2/3 until a CSD was observed. CSD was revealed by both the associated changes in light transmittance and the typical depolarization to almost zero mV recorded in a pyramidal cell located 600 μm apart from the pressure-ejection pipette tip (to ensure that a true propagating event was studied). The duration of the first pulse eliciting a CSD was taken as CSD threshold, and the rate of horizontal spread of the change in intrinsic optical signal as velocity of CSD propagation.[1]

CSD could not be induced after blocking either the NMDA receptors or the P/Q-type Ca^{2+} channels; in fact, in the presence of either D-AP5 or ωAgaIVA, neither a depolarization of the patch-clamped pyramidal cell nor a propagating change in intrinsic optic signal were measured even with K^+ pulses 30 times longer than the threshold pulses triggering a CSD in control (**Table 1**). After the inhibition of P/Q-type Ca^{2+} channels, K^+ pulses of 10 s duration were unable to elicit a CSD in slices in which the average threshold duration in control was 0.29 ± 0.01 s (n = 7). Similarly, after the inhibition of NMDA receptors with D-AP5, K+ pulses of 10 s duration were unable to elicit a CSD in slices in which the average threshold duration in control was 0.34 ± 0.03 s (n = 5). Moreover, CSD was also not elicited in 9 (in the presence of ωAgaIVA) and 9 (in the presence of D-AP5) additional slices in which the maximal duration of the K^+ pulse was 1 s (about 3 times larger than the threshold in control) (**Table 1**).

In contrast, inhibition of the other types of presynaptic voltage gated Ca^{2+} channels had only a relatively small effect on CSD initiation and propagation. In the presence of either ωCgTxGVIA (n = 13) or SNX-482 (n = 10), the CSD threshold was about 10% higher and the CSD rate of propagation 15% lower than in control (**Fig. 1 and Table 1**). Neither threshold nor velocity of propagation of CSD was significantly affected by inhibition of L-type Ca^{2+} channels with nimodipine (n = 5) (**Table 1**).

Discussion

Our pharmacological study supports the conclusions that (1) Ca^{2+} influx through P/Q-type Ca^{2+} channels and activation of NMDA receptors are required for CSD induction by K^+ pressure pulses in acute slices of mouse sensory cortex; (2) Ca^{2+} influx through N- and, probably, R-type Ca^{2+} channels may play a modulatory role on CSD threshold and velocity of propagation.

In agreement with our findings, most previous studies investigating the effect of NMDA receptor antagonists on CSD, induced by brief K^+ pulses in cortical slices, reported complete blockade of CSD recorded at ≥500 um from the local K^+ ejection,[14-17] (but reviewed in ref. 18 for an exception in hippocampal slices); moreover CSD could not be recorded after perfusing the slices in Ca^{2+}-free medium or after blocking the Ca^{2+} channels with

Table 1. Effect of inhibition of the different types of voltage-gated Ca^{2+} channels or the NMDA receptors on CSD threshold and CSD velocity

		Control		Channel inhibition	
	n	Threshold (ms)	Velocity (mm/min)	Threshold (ms)	Velocity (mm/min)
ωAgaIVA	7	293 ± 12	2.3 ± 0.2	No CSD (10 s)	
	9	292 ± 7	2.1 ± 0.1	No CSD (1 s)	
D-AP5	5	338 ± 27	1.8 ± 0.1	No CSD (10 s)	
	9	286 ± 10	2.4 ± 0.1	No CSD (1 s)	
ωCgTxGVIA	13	261 ± 9	2.1 ± 0.1	284 ± 7 (***)	1.8 ± 0.1 (**)
SNX-482	10	288 ± 9	2.4 ± 0.2	317 ± 10 (**)	2.0 ± 0.1 (*)
Nimodipine	5	310 ± 21	2.2 ± 0.1	322 ± 19	2.1 ± 0.1

The threshold for CSD induction and the velocity of CSD propagation were measured before (control) and after (channel inhibition) application of saturating concentrations of D-AP5, ωAgaIVA, ωCgTxVIA, SNX-482 and nimodipine to inhibit NMDA receptors and P/Q-, N-, R- and L-type Ca^{2+} channels, respectively. CSD was induced in acute cortical slices by high K^+ pressure pulses of increasing duration: the duration of the first pulse eliciting a CSD was taken as CSD threshold and the rate of horizontal spread of the change in intrinsic optical signal as velocity of CSD propagation. In the presence of ωAgaIVA or D-AP5, the CSD could not be induced even with very long pulses, about 3 (1 s) or 30 (10 s) times larger than the control CSD triggering threshold.

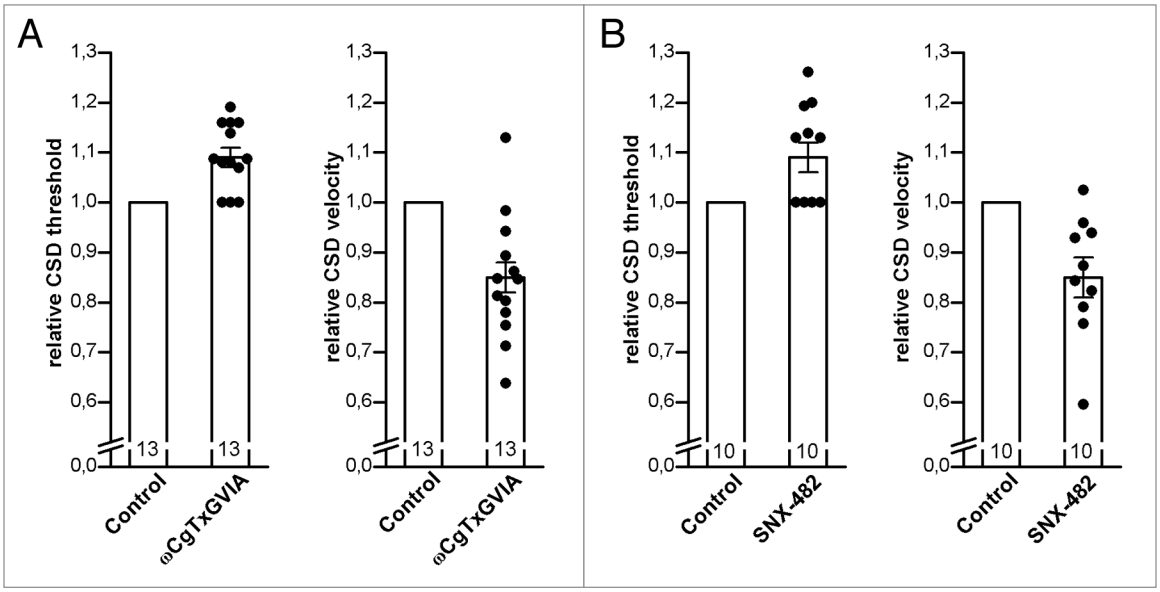

Figure 1. Relative values of CSD threshold and velocity before and after inhibition of N-type or R-type Ca^{2+} channels. The threshold for CSD induction and the velocity of CSD propagation were measured before (control) and after application of saturating concentrations of ωCgTxGVIA (A, n = 13) or SNX-482 (B, n = 10). The values obtained in the individual experiments in the presence of toxin relative to the corresponding control values are shown as dots and the average relative values as bar plots.

Cd^{2+},[14,16] or Ni^{2+} and Co^{2+}.[19] These findings are consistent with the conclusion that activation of NMDA receptors and Ca^{2+} influx through Ca_V channels are both required for CSD induction and/or propagation (although a possible caveat is the uncertainty regarding how much the K^+ stimulus exceeded the CSD threshold). Here, we have confirmed and extended the previous in vitro studies by showing that activation of P/Q-type Ca^{2+} channels is specifically required for induction of CSD by K^+ pulses, and that, after blockade of either the P/Q-type Ca^{2+} channels or the NMDA receptors, stimuli 30 times larger than the CSD threshold are unable to induce a CSD. Our findings in cortical slices are consistent with in vivo studies of CSD induced by electrical stimulation of the cortex, showing that, after i.p. injection of NMDA antagonists, even stimulation currents 10 times longer and 8 times larger than the CSD triggering threshold were unable to induce a CSD,[20] and spontaneous *cacna1a* mouse mutants, with mutations that produce partial loss-of-function of the P/Q-type Ca^{2+} channel, required an approximately 10 fold higher electrical stimulation intensity to evoke a CSD than wild-type mice.[21]

However, if one considers previous in vitro and in vivo pharmacological studies of CSD induced by perfusing the cortical slices or the cortex with a high K^+ solution (rather than with brief K^+ pulses or electrical stimulation) a completely different picture emerges: NMDA antagonists only slightly increase the CSD threshold without affecting its velocity;[17,22] similarly, blockade of the P/Q-type (or the N-type) Ca^{2+} channels hardly affects the CSD threshold obtained by perfusing cortical

slices with progressively increasing K^+ concentrations,[22] and removal of extracellular Ca^{2+} ions does not block CSD induced by perfusing the slices with a high K^+ solution for 80–90 s (but reduces to about half the rate of propagation).[23] Moreover, multiple CSDs induced in vivo by continuous K^+ microdialysis or topical application of a KCl cristal are strongly reduced in frequency but not completely suppressed by P/Q-type (or N-type) Ca^{2+} channel blockers[24] and by NMDA antagonists (that also reduce their amplitude and duration).[25,26] The Ca^{2+} channel blockers do not affect CSD induced by pinprick in vivo.[24,27]

Thus, even limiting our discussion to the methods that are used to elicit CSD in normally metabolizing cortical tissues (because the pharmacological profile of hypoxia and/or ouabain-induced CSD is again different), it is clear that different methods lead to different pharmacological profiles regarding the role of NMDA receptors and Ca^{2+} influx through P/Q-type Ca^{2+} channels in CSD induction and propagation. The different pharmacological profiles likely reflect the fact that CSD is a complex phenomenon and there may be sequential mechanisms with different pharmacology leading to the final common downstream CSD event;[10] some of the upstream mechanisms may be variably bypassed and/or occluded by the different CSD-inducing methods.

It becomes then crucial to understand which experimental CSD-inducing method is more relevant (and eventually most predictive of drug efficacy) for the CSDs that arise "spontaneously" in a given brain pathology.[12] In the case of migraine, insights into how "spontaneous" CSDs may arise have been provided by the interesting finding of enhanced excitatory neurotransmission at cortical pyramidal cell synapses but unaltered inhibitory neurotransmission at fast-spiking interneuron synapses in FHM1 KI mice.[1] A plausible working hypothesis is that the differential effect of FHM1 mutations on excitatory and inhibitory neurotransmission may, in certain conditions (cf migraine triggers), lead to disruption of the cortical excitation-inhibition balance due to excessive recurrent excitation, resulting in overexcitation and neuronal hyperactivity, that may increase $[K^+]_o$ above the critical value for CSD ignition.[28] Thus, to study mechanisms of experimental CSD that may be relevant to understand the mechanisms of the "spontaneous" CSD underlying migraine aura, electrical stimulation and/or brief applications of high K^+ appear as more appropriate CSD-inducing stimuli than prolonged applications of high K^+ and/or pinprick.

Our previous[1] and present findings support the idea that Ca^{2+} influx through presynaptic P/Q-type Ca^{2+} channels with consequent release of glutamate from recurrent cortical pyramidal cell synapses and activation of NMDA receptors are required for initiation and propagation of "spontaneous" CSDs in migraineurs. The specificity of the P/Q-type Ca^{2+} channel requirement compared to that of the other presynaptic N- and R-type Ca^{2+} channels may reflect the fact that excitatory synaptic transmission at pyramidal cell synapses in different areas of the cerebral cortex depends predominantly on P/Q-type Ca^{2+} channels (reviewed in ref. 28), and/or may point to a specific role of postsynaptic P/Q-type Ca^{2+} channels in CSD induction.

Acknowledgements

This work was supported by Telethon Italy (grant GGP06234), the Italian Ministry of University and Research (PRIN2007), the Fondazione CARIPARO (Calcium signalling in health and disease) and the University of Padova (Strategic project: Physiopathology of signalling in neuronal tissue).

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