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L-type calcium channels as drug targets in CNS disorders

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Abbreviations: Ca^{2+}, calcium; CCB, calcium channel blocker; DHP, dihydropyridine; FLIPR, fluorescence imaging plate reader; I_{Ca}, calcium inward current; LTCC, L-type calcium channel; PD, Parkinson disease; SNc, substantia nigra pars compacta; TS, Timothy syndrome; SNP, single nucleotide polymorphism; ASD, autism spectrum disorder; PYT, pyrimidine-2,4,6-trione; Ba^{2+}, barium; PCRD, proximal C-terminal regulatory domain; DCRD, distal C-terminal regulatory domain; CTM, C-terminal modulator; CaM, Calmodulin.

L-type calcium channels are present in most electrically excitable cells and are needed for proper brain, muscle, endocrine and sensory function. There is accumulating evidence for their involvement in brain diseases such as Parkinson disease, febrile seizures and neuropsychiatric disorders. Pharmacological inhibition of brain L-type channel isoforms, Cav1.2 and Cav1.3, may therefore be of therapeutic value. Organic calcium channels blockers are clinically used since decades for the treatment of hypertension, cardiac ischemia, and arrhythmias with a well-known and excellent safety profile. This pharmacological benefit is mainly mediated by the inhibition of Cav1.2 channels in the cardiovascular system. Despite their different biophysical properties and physiological functions, both brain channel isoforms are similarly inhibited by existing calcium channel blockers. In this review we will discuss evidence for altered L-type channel activity in human brain pathologies, new therapeutic implications of existing blockers and the rationale and current efforts to develop Cav1.3-selective compounds.

Almost 50 y after the discovery that drugs like nifedipine, verapamil and diltiazem exert their vasorelaxant and cardiodepressant effects by selectively inhibiting Ca^{2+} currents through voltage-gated Ca^{2+} channels in arterial smooth muscle and cardiac myocytes and after several decades of use of these L-type Ca^{2+} channel (LTCC) blockers (CCBs) as antihypertensives and antianginals, LTCCs again evolve as potential drug targets. This novel aspect stems from recent findings that different L-type channel isoforms have different biophysical properties and serve distinct physiological functions in tissues such as the brain, the cardiovascular system and in endocrine cells.

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From the 4 LTCC subtypes, those formed by Cav1.2 and Cav1.3 α1-subunits are the most widely expressed. They are often present in the same cell, such as in neurons,[1-3] sinoatrial node, atrial cardiomyocytes,[4] and adrenal chromaffin cells.[5] Despite high structural similarity of their pore-forming α1-subunit and sensitivity to CCBs, they show distinct gating behaviors, engage in different protein – protein interactions, and different mechanisms of fine-tuning by alternative splicing (**Fig. 1**).[6,7]

In this review we will first briefly summarize recent evidence for a potential pathogenic role of LTTCs in neurological and neuropsychiatric disease. This evidence suggests that block of LTCCs in the brain could provide a therapeutic benefit in different disorders. Selective inhibition of Cav1.3 channels, which do not contribute much to the peripheral blood pressure lowering and cardio-depressant effects of CCBs, is expected to cause less cardiovascular side effects and may thus allow more efficient dosing to target Cav1.3 channels in the brain. We will therefore also briefly discuss current efforts to develop Cav1.3-selective inhibitors.

Pharmacological Inhibition of LTCCs in the Brain

In contrast to Cav1.3, Cav1.2 is abundantly expressed in vascular smooth muscle and heart muscle.[8-10] It therefore accounts for most of the cardiovascular effects of organic LTCC blockers. In the brain, both isoforms are located postsynaptically at somatodendritic locations.[11] They regulate neuronal excitability and synaptic plasticity not only by carrying depolarizing Ca^{2+} inward currents but also by raising intracellular free Ca^{2+} which serves as an important second messenger for many Ca^{2+}-dependent signaling pathways, including modulation of gene-transcription.[12]

The long clinical experience with CCBs as widely used antihypertensives, in particular with brain-permeant dihydropyridines (DHPs) such as nifedipine, nimodipine, nitrendipine and isradipine (refs.[13,14]), raise the important question about their pharmacological actions in the brain. Nimodipine is also licensed for neuroprotection after subarachnoidal hemorrhage but this is believed to be due to the prevention of vasospasms rather than direct protection of neurons.[14] Patients treated with these DHPs do not experience adverse CNS effects. Nevertheless, there is

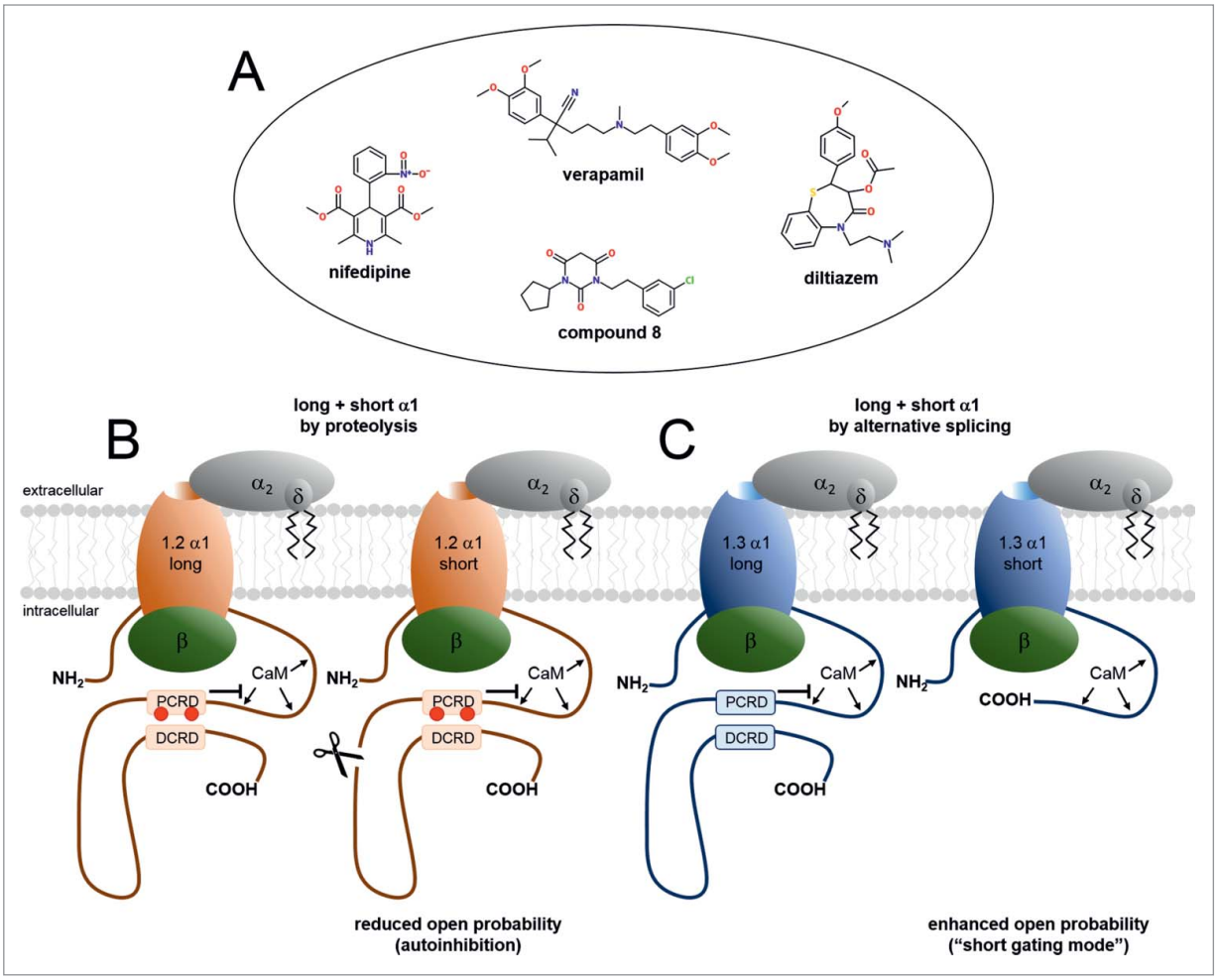

Figure 1. Long and short α1-subunit variants of Cav1.2 and Cav1.3 containing channels complexes. The intramolecular interaction of a proximal (PCRD) and distal C-terminal regulatory domain (DCRD) within the C-terminus forms a C-terminal modulator (CTM) that alleviates calmodulin (CaM)-mediated Ca^{2+}-dependent inactivation.[71] (**A**) Chemical structure of the pyrimidine-2,4,6-trione (PYT)-derivative compound 8 (ref. [60]) and representatives of the 3 major CCB classes: verapamil (phenylalkylamines), diltiazem (benzothiazepines), nifedipine (dihydropyridines). (**B**) Proteolytic cleavage of the Cav1.2 C-terminus releases a distal C-terminal fragment that can either translocate to the nucleus to regulate gene transcription[72, 73] or stays non-covalently attached to the proximal C-terminal part and mediates a potent autoinhibitory effect.[68] Inhibition can be released by PKA-mediated phosphorylation of Ser1700 and Thr1704 (indicated as red circles) within the PCRD, required for physiological β-adrenergic modulation of cardiac Cav1.2 channels during the fight-or-flight response.[74,75] (**C**) The C-terminus of Cav1.3 channels undergoes alternative splicing generating long and short splice variants. The lack of a functional CTM in short splice variants profoundly alters the gating by increasing the open probability and Ca^{2+}-dependent inactivation, and shifting the voltage-dependence of activation toward more hyperpolarized potentials.[7,71]

convincing evidence from a clinical study in healthy individuals demonstrating nimodipine-induced effects on long-term potentiation (LTP) and long-term depression (LTD) induced by non-invasive continuous theta burst stimulation.[15] This suggests that at least some populations of LTCCs are inhibited at therapeutic doses of DHP CCBs. It is known that the apparent sensitivity of LTCCs (i.e. the IC_{50} for current inhibition) depends on several factors. The most important are the LTCC pore-forming α1-subunit isoforms themselves, alternative splicing and a cell's electrical activity pattern (membrane resting potential, action potential width and firing rate). Cav1.2 channels appear about one order of magnitude more sensitive to DHP CCBs than Cav1.3.[16,17] In the case of Cav1.2, splice variants expressed in smooth muscle are more sensitive to DHPs than splice variants predominating in cardiac muscle.[18,19] DHPs are voltage-dependent blockers and bind with higher affinity to inactivated channel states (modulated receptor hypothesis).[20] Therefore the apparent sensitivity to DHPs for LTCCs increases in splice variants enabling more inactivated channel states at a given voltage (such as Cav1.2 α1-subunit variants in arterial smooth muscle) and during electrical activity patterns favoring channel inactivation. This is the case in cells that reside at more positive resting membrane potentials and undergo long depolarization. In contrast, DHPs appear less potent and show a slow onset of action in cells that fire brief action potentials from more negative membrane potentials, such as most neurons.[21] Taken together, therapeutic steady-state plasma concentrations (about 5–10 nM for isradipine;[22] www.drugs.com, Dynacirc^{R} Summary of Product

Characteristics) of clinically used DHPs efficiently inhibit Cav1.2 channels in vascular smooth muscle but are expected to cause much weaker block of Cav1.2, and especially of Cav1.3, in neurons. Most studies investigating DHP effects in rodents used inappropriately high doses of CCBs applied subcutaneously or intraperitoneally. This results in peak plasma concentrations several orders of magnitude higher than in humans and induce a strong aversive and fearful state[23,24] that results from inhibition of peripheral Cav1.2 channels (presumably excessive cardiovascular depression).[25] In such studies pharmacological effects on *in vivo* brain function assessed in behavioral experiments are therefore difficult to interpret.[23-25]

In conclusion, inhibition of LTCCs in the brain at therapeutic doses in humans cause measurable changes in neuronal plasticity[15] but no obvious side effects. We also know from work in Cav1.3-deficient mice that complete ablation of Cav1.3 causes antidepressant-like effects[26] and prevents development of psychostimulant-induced sensitized behaviors.[27] It is therefore likely that a global decrease of LTCC function in the brain does not induce disease-relevant functional changes. However, this raises the important question about the pathogenic role of the opposite, abnormally enhanced brain LTCCs activity. For the treatment of such conditions existing CCBs could be repurposed, or, alternatively, Cav1.3-selective compounds could be developed.

Evidence of Enhanced LTCC Activity in Brain Disorders

Data from studies in mutant mice and from human genetics strongly suggest a central role of LTCCs in both neurological as well as neuropsychiatric disease.

LTCCs in the pathophysiology of Parkinsons disease (PD): Cav1.2 and Cav1.3 LTCCs are expressed in substantia nigra pars compacta (SNc) neurons,[28] which degenerate in PD. They contribute to somatodendritic Ca^{2+} oscillations during autonomous pacemaking or bursting in these cells.[29] It is currently believed that this constant Ca^{2+} load contributes to the vulnerability of SNc neurons to degeneration in PD by enhancing mitochondrial oxidative stress[30] (for review see ref.) [31] and most likely also multiple systems atrophy (a PD related disorder).[32] Evidence from several epidemiological studies for a neuroprotective effect of brain permeable LTCC blockers in PD[33-36] support such a role and provide a rational basis for using LTCC blockers as disease modifiers in PD. Currently a phase 3 clinical trial (NCT02168842) is recruiting patients to study the neuroprotective potential of the DHP isradipine in early PD. Since some experimental evidence points to an involvement of Cav1.3 LTCCs,[28,37,38] Cav1.3-selective blockers appear especially suited for this indication. As predicted from Cav1.3-deficient mice, such drugs may also alleviate depression often associated with PD. Adverse effects in the cardiovascular system should be minimal and limited to a mild slowing of heart rate (Cav1.3 contributes to cardiac pacemaking).[4] Adverse effects on hearing cannot be excluded (Cav1.3 controls sound-induced neurotransmitter release from cochlear inner hair cells).[39] However, these appear unlikely because even high (toxic) doses of currently available CCBs were so far not reported to affect hearing.

LTCCs in the pathophysiology of febrile seizures: LTCCs, most likely Cav1.2, appear to contribute critically to the generation of febrile seizures. This has been shown using patch-clamp recordings from hippocampal pyramidal cells in acute rat pup brain slices.[40] Nimodipine could block hyperthermia-induced abnormal spontaneous activity of these neurons *in vitro* as well as in an *in vivo* model. This indicates a role of Cav1.2 in febrile seizures and, based on the known safety profile of CCBs, justifies clinical trials in humans.

LTCCs in neuropsychiatric diseases: Several independent lines of evidence imply enhanced LTCC activity as an important risk factor or cause for neuropsychiatric disease. First evidence came from patients with Timothy syndrome (TS), a rare autosomal dominant condition caused by *de novo* gain-of-function mutations in the pore forming α1-subunit of Cav1.2 (CACNA1C; OMIM # 601005). It is a multiorgan disease with cardiac and extracardiac symptoms. The underlying mutations reduce voltage-dependent inactivation of Cav1.2.[41,42] In the heart depolarizing Ca^{2+} current increases, causing delayed cardiomyocyte repolarization, prolongation of the QT-interval and enhances the risk for lethal ventricular arrhythmias. These are the primary cause of reduced average life expectancy (2.5 years) in these patients. Typical extracardiac features include dysmorphic facial features, syndactyly, and mental retardation.[43-45] Surviving patients often develop autism[44] suggesting enhanced neuronal Cav1.2 channel activity as a pathogenic factor. Genome-wide association studies found a strong association between intronic SNPs in CACNA1C and susceptibility for psychiatric disorders, including bipolar disease, schizophrenia, major depression and autism spectrum disorders (ASD). It is one of the most consistent associations reported in psychiatric genetics.[46-48] One SNP (rs1006737) leads to increased Cav1.2 activity in induced neurons[49] again pointing to a pathogenic role of enhanced Cav1.2 activity. This strongly motivates the re-evaluation of CCBs for the treatment of bipolar disease, schizophrenia and major depression. In contrast to earlier clinical studies[50,51] future trials could take advantage of these genetic findings and stratify patients according to risk alleles to define cohorts who may benefit most from the addition of CCBs to standard therapy.[52] Non-selective brain-permeable CCBs, such as isradipine, are expected to block both Cav1.2 and Cav1.3. Based on the preclinical findings discussed above the inhibition of Cav1.3 may contribute additional antidepressant effects.

Human genetics also strongly point to an important role of Cav1.3 LTCCs in the pathophysiology of neuropsychiatric disease, including ASD. Germline *de novo* mutations resulting in a gain-of-function of the Cav1.3 α1-subunit (CACNA1D) were described in 2 patients with a severe congenital syndrome presenting with primary aldosteronism (Cav1.3 provides Ca^{2+} for aldosterone production in zona glomerulosa cells)[53,54] but also with neurodevelopmental deficits and seizures at early age (PASNA, OMIM #615474;).[54] In addition, *de novo* CACNA1D mutations have also been reported as high risk mutations in 2

patients with sporadic autism and intellectual disability.[55-57] Both mutations induce a strong channel gain-of-function when expressed in tsA-201 cells[58] very similar to the biophysical changes observed for mutations in PASNA and, in the case of Cav1.2, TS. Given the important role of Cav1.3 for many brain functions (see above) and the causal role of Cav1.2 gain-of-function in autism associated with TS, these data do not prove but strongly suggest an even causative role of the 2 *de novo* mutations in the ASD patients. Further studies therefore need to address the important question if PASNA and ASD patients with CACNA1D mutations would benefit from therapy with LTCC blockers.

Cav1.3-Selective LTCC Blockers

Does the high structural similarity between Cav1.2 and Cav1.3 α1-subunits (approx. 75% overall sequence identity) and the high sequence conservation within their drug binding regions[59] allow the development of isoform-selective modulators? Several lines of evidence suggest that this should be possible. Although the affinity of DHP CCBs for Cav1.2 and Cav1.3 is similar in radioligand binding studies, their binding kinetics differ substantially, with much faster association and dissociation kinetics of [^{3}H]isradipine for Cav1.3 than for Cav1.2.[16] Moreover, as outlined above, isradipine and other DHPs block Cav1.2 channels with about one order of magnitude lower IC_{50} values than Cav1.3.[16,17] This clearly shows that the DHP binding pocket differs in these 2 channels and that voltage-dependent conformational changes induce further changes in apparent drug potency. Although not (yet) understood at the molecular level, these structural differences should allow development of novel scaffolds to selectively inhibit Cav1.3. Such a discovery would be important for several reasons. First, it would provide a proof-of-concept for the existence of such drugs; second, such compounds could serve as lead compounds for drug discovery and structure-activity studies to reveal the molecular features underlying selectivity and, third, basic scientist could use these drugs to further dissect the differential functional role of Cav1.2 and Cav1.3 without the need for gene knockout or knockdown strategies.

Recently, the discovery of first Cav1.3-selective compounds has been reported.[60,61] However, as outlined below, 2 subsequent studies[62,63] could not confirm the high selectivity found in the original report. Since all 3 studies were performed in experienced laboratories using comparable techniques the variable results are puzzling. These may be due to subtle differences in experimental conditions or in the properties of the recombinant channel constructs employed.

The original study[60] described the discovery of pyrimidine-2,4,6-trione (PYT) as a scaffold with Cav1.3-selective Ca^{2+} channel blocking activity (**Fig. 1**). Active compounds were identified by first screening a non-commercial chemical library using a fluorescence imaging plate reader (FLIPR)-based assay which identified the PYT scaffold as potential Cav1.3-selective inhibitors. Activity was further enhanced by structural modification. The most selective derivatives were Cp8 (1-(3-chlorophenethyl)-3-cyclopentylpyrimidine-2,4,6-(1H,3H,5H)-trione; also termed Cp6 or BPN-4689[61,64]), Cp1 and Cp3.[60] Cp8 was more than 600-fold selective for Cav1.3 over Cav1.2 in the FLIPR assay and 16- or 77-fold more selective than Cp3 or PYT, respectively. The higher selectivity of Cp8 was mainly due to its weaker inhibition of Cav1.2. The selectivity of Cp8 was confirmed in whole-cell patch-clamp studies which allow holding cells at defined membrane potentials. Cell lines stably expressing C-terminally long rat Cav1.3 (GenBank accession number: AF370010) or rabbit Cav1.2 (P15381) together with rat β3 (M88751) and rat α2δ1 (AF286488) were used with Ba^{2+} as the charge carrier. Holding the cell at – 70 mV, cells were depolarized for 100 ms to 0 mV with a frequency of 0.05 Hz and drugs were perfused with a flow rate of 2 ml/min. Five μM Cp8 reversibly inhibited 31% of Cav1.3 and 4.4% of Cav1.2 Ba^{2+} (10 mM) currents, with an IC_{50} of 24.3 μM for Cav1.3. The rat Cav1.3 α1-subunit used (AF370010) contains mutations that profoundly influence channel gating[65] and could thereby also alter its apparent drug sensitivity.

A second independent study[62] also found an inhibitory activity of Cp8, but could neither confirm high selectivity nor high potency of the PYT compounds. Recordings were made in transiently transfected HEK293 cells expressing C-terminally long ($Cav1.3_{42}$) and short ($Cav1.3_{42a}$) rat Cav1.3 α1-subunit splice variants or rat $Cav1.2_{B15}$[66] together with rat α2δ and different rat β subunit isoforms. Ca^{2+} currents (10 mM) were evoked by 100-ms or 1-s test pulses from – 70 mV to 10 mV at 0.05 Hz and compounds were applied with a flow rate of 1 ml/min. In general, 50 μM Cp8 inhibited between 23 – 44% of I_{Ca}, with only slight selectivity for $Cav1.3_{42}$ compared to $Cav1.2_{B15}$ and $Cav1.3_{42a}$ when expressed together with auxiliary β1, β3 or β4 subunits (e.g. β3: ~40%, 23.5% or 29.1% inhibition of I_{Ca} through $Cav1.3_{42}$, $Cav1.2_{B15}$ or $Cav1.3_{42a}$, respectively). Notably, with β1, β3 or β4 subunits, $Cav1.2_{B15}$ and the C-terminally short Cav1.3 splice variant, $Cav1.3_{42a}$, were inhibited to a similar extent. In contrast, when β2a, a palmitoylated β subunit that slows the inactivation of voltage-gated Ca^{2+} channels[67] was coexpressed, inhibition by 50 μM Cp8 was even $Cav1.2_{B15}$-selective (44.0% inhibition of I_{Ca} for $Cav1.2_{B15}$ vs 29.5% and 23.6% for $Cav1.3_{42}$ and $Cav1.3_{42a}$). Furthermore, Huang et al[62] also determined the blocking activity of Cp8 for the mutated Cav1.3 (termed $Cav1.3_{42\_UC}$ in their report) construct used in the original report. However, enhanced inhibition of $Cav1.3_{42\_UC}$ by Cp8 was not observed. Overall, blockage of $Cav1.3_{42}$ was less pronounced in this study (~40 % vs ~60 % in the original paper with 50 μM Cp8), while Cav1.2 inhibition was increased (23.5 % vs ~10 % inhibition by 50 μM Cp8 in the original report).

In contrast to the first 2 studies, our group found PYT compounds to exert pronounced gating changes.[63] TsA201 cells were transiently transfected with the long splice variants of rat ($rCav1.3_L$)[65] or human Cav1.3 ($hCav1.3_L$; EU363339), full-length rabbit Cav1.2 ($rbCav1.2_L$, X15539 identical to P15381 of the original study) or $rbCav1.2_S$ (truncated at amino acid position 1800 to account for proteolytically processed forms in heart and brain)[68] together with rat β3 (NM_012828) and rabbit α2δ-1 (NM_001082276). Ca^{2+} and Ba^{2+} currents (15 mM)

were recorded using 100-ms long depolarizations from – 80 mV to V_{max} with a frequency of 0.2 Hz. Additionally, a similar bath solution (10 mM Ba^{2+}) and pulse protocol (0.05 Hz) to reproduce the conditions of the original paper were used. Drugs were locally perfused with a flow rate of 0.6 ml/min. 50 μM Cp8 and Cp3 reproducibly altered Ca^{2+} and Ba^{2+} current kinetics of Cav1.2 as well as Cav1.3 channels using both protocols (0.2 and 0.05 Hz). This kinetic change was characterized by a slowing of the activation and inactivation time course as well as a more pronounced deactivation upon repolarization (**Fig. 2**; compare blue [Cp8] vs black [control] trace). These changes resembled the actions of the LTCC activator FPL64176.[69] However, this pharmacological modulation seemed to be complex. First, with Ba^{2+} as the charge carrier, a minority of cells also showed a weak and non-selective inhibition of $rbCav1.2_S$ and $rCav1.3_L$ by 50 μM Cp8 (21.7% and 9.1% inhibition depending on stimulation protocol without change in channel kinetics, similar to the findings in the other studies.[60,62] Inhibition of Cav1.2 in this subset of cells was comparable to the original report, while Cav1.3 exhibited a lower apparent sensitivity toward Cp8.[63] Second, in some experiments with Ca^{2+} as conducting ion, the typical slowing of the gating kinetics was preceded by a weak inhibition in which only the current amplitude was decreased but gating kinetics (slowing of activation, inactivation and deactivation) had not yet occurred (**Fig. 2**). This inhibition of peak inward I_{Ca} was found in about 50 % of the cells transfected with human or rat full-length Cav1.3, ranged between 9 and 11 % (7 out of 14 cells for $rCav1.3_L$, 6 out of 12 cells for $hCav1.3_L$; see also legend to **Fig. 2**) and developed within 3–4 sweeps (10–15 s). This inhibition of I_{Ca} was always followed by the typical strong changes in channel kinetics. It therefore appears that inhibitory effects of Cp8 occur first and are then surpassed by a Ca^{2+} channel activator-like effect.

The activating activity of Cp8 on Ca^{2+} inward current (I_{Ca}) was also seen using 5-s long or action potential (AP)-like depolarizations. It was also not an artifact of heterologous expression because it was further confirmed in native Cav1.2 and Cav1.3 LTCC currents (2 mM Ca^{2+}) recorded in mouse chromaffin cells. During action potential clamp 50 μM Cp8 increased overall cellular Ca^{2+} load. It also nearly doubled the spontaneous firing frequency of mouse chromaffin cells, accompanied by a reduction of the after-hyperpolarization.[63]

How can the diverging results in potency, selectivity and mode-of-action (inhibition vs activation of Ca^{2+} inward current) elicited by the same compound under similar experimental conditions be explained? One clue toward this question may come from the biphasic modulation of LTCC activity by 50 μM Cp8 described above. One theoretical possibility that could account for this biphasic action may be the presence of different binding sites for this compound that can be accessed with different time constants and cause opposite effects on channel function (simple inhibition vs complex gating changes). In line with that, it was demonstrated that voltage-gated potassium (Kv) channels possess 2 discrete drug-binding sites for Psora-4, a potent Kv1 channel blocker: one in the central pore below the selectivity filter that is highly conserved in Na^+, Ca^{2+} and Kv channels, and 4 less conserved side-pocket cavities formed by the backsides of S5 and S6.[70] Mutations in these side-pockets of the Psora-4 sensitive Kv1.5 channel diminished Psora-4 inhibition while introducing 4 crucial residues of these side pockets into Kv2.1 channels enhanced their sensitivity toward Psora-4. Moreover, both binding pockets change their conformation during channel activation and channel inhibition and seemed to depend on allosteric cooperativity between them. Since there is also evidence of non-pore facing residues in Ca^{2+} and Na^{2+} channels that are required for drug inhibition, it is possible that 2 drug binding sites for PYT compounds exist in LTCCs mediating different pharmacological effects. Thereby several factors like the applied drug concentration, relative affinities of the diverse binding pockets as well as the association rate and velocity of the

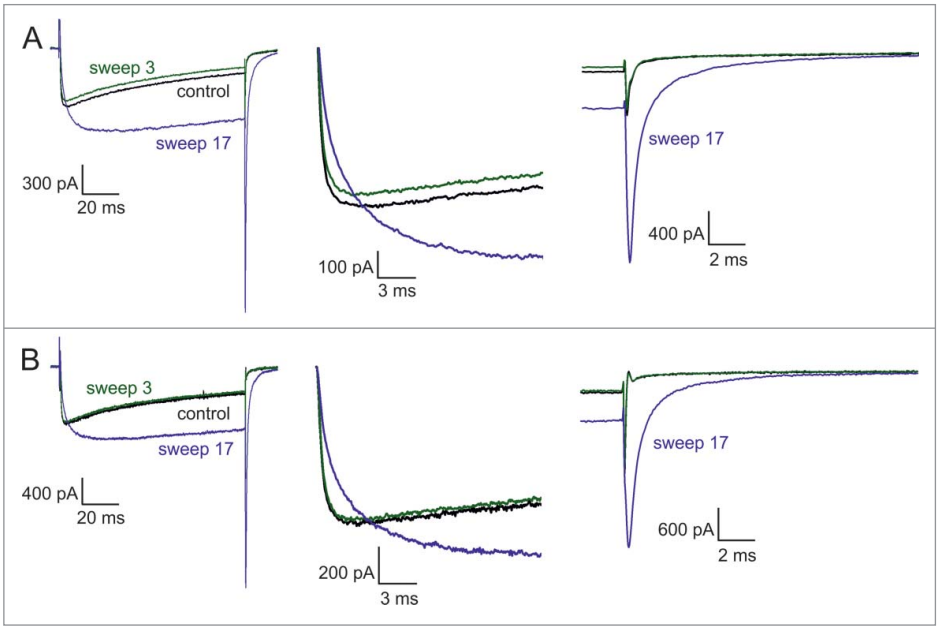

Figure 2. Modulation of full-length rat Cav1.3 ($rCav1.3_L$) I_{Ca} by 50 μM Cp8 with (**A**) or without (**B**) an initial inhibitory response. $rCav1.3_L$ α1-subunits were transiently expressed in tsA201-cells together with β3 and α2δ1 subunits. I_{Ca} (15 mM Ca^{2+} as charge carrier) was evoked by 100-ms long depolarizations from a holding potential of −80 mV to the voltage of maximal activation with a frequency of 0.2 Hz. An initial inhibitory response was defined as Cp8-induced reduction of peak I_{Ca} of >5% within 3–4 sweeps without kinetic changes (sweep 3 is shown in green; control sweep in black; sweep 17 with Cp8-induced kinetic change in blue). The left panel shows representative traces, the middle panel the first 20 ms of depolarization of the same recordings, and the right panel the tail currents after repolarization at higher time resolution. Representative traces for cells with (**A**, 7 out of 14 cells) and without (**B**, 7 out of 14 cells) initial inhibition are illustrated.

application of the drug may influence the occupation of these sites and account for varying effects.

In conclusion, isoform-selective modulators that would limit adverse side-effects by specifically targeting the disease-causing channel subtype, would be the preferential approach with respect to patient safety. In the absence of such compounds, existing non-selective CCBs may reveal their potential value for the treatment of human brain disorders in future clinical studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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