current and fluorescence signals, which corresponded to specific VSD rearrangements during channel activation. We found that the G406R mutation dramatically altered the operation of VSDs I and III compared to wild-type channels, by inducing a hyperpolarizing shift in their activation voltage dependence of ~80mV and ~50mV, respectively. These shifts were associated with a significant reduction in the effective valences of VSD I and III by ~50% and ~42%, respectively. Moreover, the sign of the fluorescence signals detected from TS channels was opposite to that observed in wild-type CaV1.2 channels. Taken together, these results suggest that the TS-causing mutation causes an overall voltage perturbation, manifest as a change in both the fluorophore quenching process reported from VSD I and VSD III and their altered voltage-dependence. Funded by: NIH, AHA, FONDECYT, ACT.

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Three Splice Variants of the Calcium Channel Beta4 Subunit Display Differential Targeting and Gene Regulation in Neurons
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The β subunits of voltage-gated calcium channels regulate surface expression and gating properties of CaV-1 and CaV-2 α subunits. All four CaV-β genes are expressed in the brain, but only mutation/lack of βα causes a neurological phenotype (epilepsy, ataxia) in humans and mice. The βα isoform is also targeted into the nucleus. There it directly interacts with the epigenetic machinery, suggesting a calcium channel-independent role of βα in transcriptional regulation. CaVβ subunits are subject to abundant alternative splicing. However, little is known about the specific functions of individual β splice variants in excitable cells. Here we identified a newly alternatively spliced ββ transcript, ββ mRNA and protein of this splice are highly expressed in mouse cerebellum and cultured cerebellar granule cells (CCG). Overexpression of ββαα modulates P/Q-type calcium currents in tSA cells and promotes surface expression of native synaptic CaV-2.1 channels in hippocampal neurons. Compared to the other two known full-length βα variants (ββαα, βαιααβαιαα) βαιααβαιαα is most abundantly expressed in the distal axon. Consistent with the described role of N-terminal sequences in nuclear import, βαιααβαιαα, which lacks these sequences, does not show nuclear targeting. The importance of nuclear targeting for the putative role of βα in transcriptional regulation was examined by whole genome expression profiling of CCG's from βαιαα-null mice individually reconstituted with βαιαα, βαιαα, or βαιαα. Strikingly, the capacity of βαιαα splice variants to regulate neuronal genes depended on their nuclear targeting with a rank order βαιαα > βαιαα > βαιαα. Together these findings indicate that in neurons the three βαιαα splice variants serve distinct functions. Whereas βαιαα plays a dual role in channel modulation and gene regulation, the newly detected βαιαα variant functions primarily as calcium channel subunit. Support: FWF P23479, P24079, W1101, F4406.

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Identification of a Determinant of High Affinity Calcium Binding in the Selectivity Filter of a Mammalian Calcium Channel
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Voltage-gated calcium channels (CaVs) provide the primary source of calcium influx in excitable cells and couple electrical signals to chemical signaling cascades. Due to CaV size and the difficulty of expressing CaVs at quantities sufficient for high-resolution determination, detailed structural information is limited to isolated cytoplasmic domains. However, CaVs are homologous to voltage-gated sodium channels (NaVs) and NaV structure can provide a template for CaV structure. We determined the structure of the closed conformation of NaVα1p, a pore-only bacterial NaV derived from NaVα1, an Alkalimimiccola ehrlichei bacterial NaV. This structure reveals the site of a putative calcium ion at the extracellular mouth of the selectivity filter liganded by four serines. At the equivalent site in mammalian calcium channel selectivity filters, there is a conserved aspartate in one of the calcium channel domains. Our functional studies show that this aspartate is a previously unidentified location for cis-gating and calcium binding in a mammalian calcium channel CaV1.2. These findings show the extent of similarities between bacterial sodium channels and eukaryotic voltage gated channels and shed new light on the selectivity filter in mammalian calcium channels.

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Functional Clustering of L-Type CaV1.3 Channels
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Pancreatic β-cells, the major cellular component of the island of Langerhans, are responsible for the synthesis of insulin and its secretion in response to elevated blood glucose. High voltage-gated calcium channels (HVCC) are intimately involved in excitation-secretion coupling in pancreatic islet cells and calcium entering through HVCC is an important regulator of insulin synthesis. HVCC are multi-subunit protein complexes comprised of the main pore-forming β1 subunit and auxiliary extracellular β2 and intracellular β subunits. Here we show that genetic ablation of the β2β1 subunit (the main pancreatic β2β1 isoform) results in the postnatal development of diabetes. Homozygous β2-/-KO mice show highly elevated urine production and develop ~9-fold higher blood glucose levels compared to WT littermates. Morphological analysis of the pancreas shows a reduction in the number of islets and their size due to a dramatic decrease in β-cell mass in an age-dependent manner. The reduced β-cell mass is not caused by an islet-specific autoimmune reaction, but might result from prolonged hyperglycaemia toxicity. Voltage-clamp recording in dissociated pancreatic β-cells shows a more than two-fold decrease in calcium current amplitude. Glucose stimulated calcium oscillations in whole isolated pancreatic islets shows a strong decrease in amplitude of both the first and second phase of insulin release, and an increased oscillation frequency of the second phase. On-going pharmacological experiments will identify which pore-forming β2 subunits are primarily affected by β2β1 deletion and how this effects insulin secretion. These findings indicate that β2β1 is an important determinant of normal β-cell physiology, critical for insulin release. Support: FWF W1101, P23479, LFU-P7400-027-011.

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Calcium Channel α2δ-1 Subunit Knockout Causes Diabetes Due to Impaired Insulin Release
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Pancreatic β-cells, the major cellular component of the island of Langerhans, are responsible for the synthesis of insulin and its secretion in response to elevated blood glucose. High voltage-gated calcium channels (HVCC) are intimately involved in excitation-secretion coupling in pancreatic islet cells and calcium entering through HVCC is an important regulator of insulin synthesis. HVCC are multi-subunit protein complexes comprised of the main pore-forming β1 subunit and auxiliary extracellular β2 and intracellular β subunits. Here we show that genetic ablation of the α2δ-1 subunit (the main pancreatic α2δ-1 isoform) results in the postnatal development of diabetes. Homozygous α2δ-1KO mice show highly elevated urine production and develop ~9-fold higher blood glucose levels compared to WT littermates. Morphological analysis of the pancreas shows a reduction in the number of islets and their size due to a dramatic decrease in β-cell mass in an age-dependent manner. The reduced β-cell mass is not caused by an islet-specific autoimmune reaction, but might result from prolonged hyperglycaemia toxicity. Voltage-clamp recording in dissociated pancreatic β-cells shows a more than two-fold decrease in calcium current amplitude. Glucose stimulated calcium oscillations in whole isolated pancreatic islets shows a strong decrease in amplitude of both the first and second phase of insulin release, and an increased oscillation frequency of the second phase. On-going pharmacological experiments will identify which pore-forming β1 subunits are primarily affected by β2β1 deletion and how this effects insulin secretion. These findings indicate that β2β1 is an important determinant of normal β-cell physiology, critical for insulin release. Support: FWF W1101, P23479, LFU-P7400-027-011.