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T-type channel-mediated neurotransmitter release

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Abstract

Besides controlling a wide variety of cell functions, T-type channels have been shown to regulate neurotransmitter release in peripheral and central synapses and neuroendocrine cells. Growing evidence over the last ten years suggests a key role of Cav3.2 and Cav3.1 channels in controlling basal neurosecretion near resting conditions and sustained release during mild stimulations. In some case, the contribution of low-voltage activated (LVA) channels is not directly evident but requires either the activation of coupled presynaptic receptors, block of ion channels or chelation of metal ions. Concerning the coupling to the secretory machinery, T-type channels appear loosely coupled to neurotransmitter and hormone release. In neurons, Cav3.2 and Cav3.1 channels mainly control the asynchronous appearance of “minis” (mIPSCs and mEPSCs). The same loose coupling is evident from membrane capacity and amperometric recordings in chromaffin cells and melanotropes where the low-threshold driven exocytosis possesses the same linear Ca^{2+} -dependence of the other voltage-gated Ca^{2+} channels (Cav1 and Cav2) that is strongly attenuated by slow calcium buffers. The intriguing issue is that, despite not expressing a consensus “synprint” site, Cav3.2 channels do interact with syntaxin-1A and SNAP-25 and, thus, may form nanodomains with secretory vesicles that can be regulated at low voltages. In this review, we discuss all the past and recent issues related to T-type channel-secretion coupling in neurons and neuroendocrine cells.

T-types and other non-canonical presynaptic Ca^{2+} channels regulate neurotransmitter release

T-type Ca^{2+} channels are transient, low-voltage activated Ca^{2+} channels that control Ca^{2+} entry during depolarizations near resting potential. Due to their biophysical properties and widespread expression, T-type channels control key functions like: low-threshold spikes, oscillatory cell activity, muscle contraction, hormone and neurotransmitter release, cell growth, differentiation and proliferation (see [21, 28, 61, 75, 78, 94] for a review). Given this, they are now proposed as therapeutic targets for a variety of diseases like: hypertension, angina pectoris, heart failure, atrial fibrillation, neuropathic pain, epilepsy, sleep disorders, obesity and cancer [39].

T-type channels possess unique gating properties that allow their electrophysiological identification: *i*) activation is voltage-dependent and channels open at very low voltages [19], *ii*) inactivation is voltage-dependent and complete within tens of milliseconds above 0 mV, *iii*) deactivation is slow at potentials near rest [18, 20], *iv*) Ca^{2+} and Ba^{2+} are carried equally well and single channel conductance is 3 to 4-fold lower than Cav1 and Cav2 channels [74], and *v*) activation and steady-state inactivation overlap at potentials near resting, generating a sizeable ‘window Ca^{2+} current’ [61].

Molecular cloning of T-type channels has provided evidence for the existence of three different pore forming $\alpha 1$ subunits (Cav3.1, Cav3.2, Cav3.3) with only 25% amino acid homology but similar pore structure organization to Cav1 (L-type) and Cav2 (N, P/Q, R-type) channels [78]. Cav3 channels differ from Cav1 and Cav2 channels for two other important singularities: *i*) they are not surrounded by β , $\alpha_2\delta$ and γ auxiliary subunits [62] and, *ii*) they lack the consensus “synprint site” (synaptic protein interaction site) [78] which allows Cav2.1 and Cav2.2 channels to bind SNARE proteins (syntaxin-1A/B, SNAP-25) and non-SNARE proteins (synaptotagmin) (see [22] for a review). This binding is crucial to give rise to Ca^{2+} nanodomains with docked presynaptic vesicles, ensuring high rates of vesicle release during rapid synaptic activity [73, 99].

Despite Cav2.1 (P/Q-type) and Cav2.2 (N-type) are the dominant Ca^{2+} channel species controlling presynaptic vesicle release in most neurons, there is increasing evidence that also other Ca^{2+} channel isoforms lacking the consensus synprint site (Cav2.3, Cav1 and Cav3) contribute to

presynaptic Ca^{2+} elevations and vesicle release. There are now abundant proofs that Cav3.2 (R-type) channels are localized at the presynaptic terminals [29, 51] and trigger neurotransmitter release by enhancing presynaptic Ca^{2+} levels [10, 31, 37, 55, 97, 104]. The same is true for Cav1 (L-type) channels, which regulate glutamate release in rod photoreceptor terminals [103] and co-localize at the hot spots of bipolar cell terminals [6]. L-type channels also regulate transmitter release in isolated hippocampal single buttons [71], cultured GABAergic hippocampal neurons [53] and cerebral cortical neurons [54] during high-frequency presynaptic activity. To date there is also overwhelming evidence that Cav3 channels are also directly involved in neurotransmitter release in an increasing number of neuronal preparations and neuroendocrine cells, making undisputable their key role in neurotransmitter release [17, 21, 102]. Here we summarize earlier and recent findings that support a direct role of T-type channels in the control of Ca^{2+} -dependent transmitter release and discuss recently suggested molecular mechanisms of T-type channel coupling to synaptic vesicles that do not occur through the consensus synprint site [100].

T-type channels and neurotransmitter release: earlier evidence (1997-2005)

The first report describing a role of T-type channels on neurotransmitter release was on the activity of the oscillatory heart GABAergic interneurons of the leech, where fast inactivating presynaptic T-type currents were shown to regulate the graded response of heart interneurons [3, 50]. At nearly the same time, Bao et al., 1998 [5] showed that, T-type channels highly expressed at the soma of afferent nociceptive neurons [94], are also involved in the control of synaptic activity between primary afferents and second-order nociceptive neurons in the dorsal horn of the spinal cord. Presynaptic T-type channels control the frequency of spontaneous glutamatergic EPSCs mediated by AMPA/kainate receptors while the latency and amplitude of AP-evoked EPSCs is controlled by HVA channels. Interestingly, the frequency of mEPSCs increases greatly with mild KCl depolarizations and is prevented by mibefradil, suggesting a critical role of T-type channels in basal nociception activity near rest. A role for T-type channels in the control of mEPSCs is relevant since the frequency of events can markedly increase during hyperalgesia to reach the threshold of nociception responses (see below).

Clear evidence of the involvement of T-type channels in fast neurotransmitter release came also from early studies at the reciprocal synapses that retinal bipolar cells form with A17 and AII amacrine cells [77, 88]. Bipolar cells express both L-type (Cav1.4) and T-type channels, which give rise to Ca^{2+} currents of comparable amplitude: at -40 mV for T-type and -10 mV for L-type channels. Correcting for the driving force, single channel conductance and open probability this implies comparable expression densities of the two channels. Evidence for the involvement of T-type channel-mediated exocytosis comes from membrane capacitance measurements (ΔC) [see [72] for details] in which glutamate release is directly measured as an increase of membrane surface area during presynaptic vesicle fusion. Pan et al. [77] estimated a ΔC of 7 fF attributed to T-type channels, corresponding to the fusion of about 90 vesicles of 50 nm diameter during pulses of 500 ms at -40 mV. T-type channels could be recruited only if the cells were previously hyperpolarized to -80 mV and the ΔC was fully blocked by mibefradil and spared by nimodipine.

T-type channels play a unique functional role also at the large spines of axonless granule cells (GCs) that form dendrodendritic reciprocal synapses with the mitral and tufted cells (M/TCs) of olfactory bulbs. These terminals express sufficient high densities of T-type channels to regulate fast GABAergic responses [32]. Small inhibitory post-synaptic potentials (IPSPs) with short latency and slow decay time constant can be recorded in whole-cell clamped mitral cells. The IPSCs are fully blocked by bicuculline and strongly attenuated by mibefradil, suggesting direct involvement of T-type channels on the inhibitory GABA_A-mediated control of mitral cells by GCs stimulation. GABA release from GC dendrites can be induced also by Ca^{2+} entry through NMDA receptors activation and Ca^{2+} release from intracellular stores [33]. The co-participation of T-type channels to GABA release from GC spines thus strengthens the GC-mediated lateral inhibition of M/TCs. It is

also worth noticing that, high-densities of T-type channels at distal and proximal dendrites ensure the possibility of evoking sub-threshold Ca^{2+} transients during back-propagation of low-threshold spikes from the soma to the spines. Under physiological conditions this warrants a better graded control of olfactory bulb function.

Early observations on the involvement of T-type channels in neurosecretion include also the original report by Harkins et al. [44] in a mouse pheochromocytoma (MPC) cell line. In this work newly expressed Cav3.2 channels produce Ca^{2+} current densities that generate membrane capacitance changes associated to vesicle secretion with comparable Ca^{2+} -efficiency to Cav2.2 (N-type) and Cav1.2 (L-type) channels. Since MPC cells express most proteins involved in Ca^{2+} -dependent neurotransmitter release (SNAP-25 and syntaxin), the rationale of these findings is that in MPC cell lines all voltage-gated Ca^{2+} channels can functionally couple to catecholamines release sites and control fast exocytosis, regardless of the availability of the “synprint site” that is present only in N and P/Q-type channels. Similar conclusions are drawn in rat melanotropes where T-type currents can drive membrane capacitance changes associated with vesicle secretion, with the same Ca^{2+} -dependence of HVA channels (L-, N-, P/Q- and R-type). This, together with the findings on chromaffin cells discussed in the following sections, allows to conclude that in neurosecretory cells there is no preferential Ca^{2+} channel coupling to secretory vesicles. The presence of T-type channels in neuroendocrine cells is thus mainly to ensure functional secretion over a broader range of membrane voltages: from near resting conditions where a T-type “window current” warrants sufficient basal hormone release to very positive potentials where HVA channels are maximally activated.

T-type channels-mediated transmitter release in neurons: recent findings (2006-2014)

Following the above early reports, there has been an increasing interest on the role of T-type channels in neurotransmitter release in the last eight years. Here we illustrate the main findings on presynaptic T-type channels controlling GABAergic and glutamatergic synapses of central neurons and T-type channels-mediated hormone secretion in neuroendocrine cells.

Cav3.2-mediated control of excitatory synapses in nociceptive dorsal-horn neurons

T-type channels are widely expressed both at the soma of small diameter nociceptive afferent neurons and at the presynaptic terminals of nociceptive dorsal horn neurons in lamina I-II, where they control the activity of mEPSCs [5] (see [94] for a review). T-type channels are also expressed at the soma of the lamina I neurons that are responsible for forwarding nociceptive signals to different regions of the brain as part of the ascending pain pathway [49]. In this way, Cav3 channels have a wide control of pain-processing pathways both at the pre- and postsynaptic level of nociceptive projecting neurons. Until recently the identity of the presynaptic Cav3 channel type involved in such pain responses was unknown. This has been solved recently, by showing that: *i*) Cav3.2 channels are expressed at the presynaptic terminals of nociceptive projecting neurons [52], *ii*) the frequency of mEPSCs is strongly attenuated by TTA-P2 and Ni^{2+} and *iii*) TTA-P2 have no action on the mEPSCs of Cav3.2^{-/-} dorsal horn neurons. Interestingly, T-type channels control specifically the nociceptive neurons of superficial laminae I-II but not the excitatory transmission of non-nociceptive neurons of deeper laminae (III-V), which are regulated by Cav2.1 and Cav2.2 channels. The Cav3.2-mediated regulation of mEPSCs is also remarkably more robust in superficial dorsal horn neurons of animal models of painful diabetic neuropathy as compared to healthy age-matched animals. The frequency of the mEPSCs increases drastically in diabetic animals and can thus critically trigger the transmission of hyperalgesic signals through the ascending pain pathway.

Cav3.2-mediated ATP release in dorsal root ganglion sandwich synapses

Subpopulations of dorsal root ganglion (DRG) neurons can communicate with their immediate neighbors via neuron–glial cell–neuron "sandwich synapses" [81]. Stimulation of one neuron leads to transglial activation of its partner by a purinergic synaptic contact between the stimulated neuron and the glial cell and a glutamatergic synaptic contact between the glial cell and the receiving neuron [83]. Release of ATP from the stimulated neuron is attributed to a classical mechanism involving Ca^{2+} entry via Cav3.2 channels, while the release of glutamate from the glia is mediated by a Gq/PLC pathway causing intracellular Ca^{2+} increase [82]. In the case of ATP, the Cav3.2-mediated release is blocked by moderate depolarization (-50 mV) or low-concentration of Ni^{2+} and is insensitive to specific blockers and toxins of Cav1 and Cav2 channels [84]. Synaptic transmission is particularly sensitive to the holding potential as expected from the presence of T-type channels at the somatic presynaptic terminal. The presence of Cav3.2 to gate the transmission at the DRG "sandwich synapses" thus broadens the possibility of modulating nociceptive sensory signals via sub-threshold voltage oscillations in addition to AP evoked responses. Taken together, the Cav3.2-driven release pathway at the DRG neuron–glial cell–neuron may play a critical role in the transmission of abnormal pain sensation.

Cav3.1-mediated GABA release at hippocampal perisomatic interneurons

T-type channels have also a key role in the release of GABA from hippocampal perisomatic-targeting interneurons [93]. More specifically, immune-histochemically identified Cav3.1 channels are co-localized with $\alpha_3\beta_4$ nicotinic receptors (nAChR) at the synaptic regions of parvalbumin expressing GABAergic interneurons. Local applications of ACh activate Cav3.1 currents, which in turn trigger bursts of mIPSCs that can transiently reduce pyramidal cell excitability. The inhibitory response is only partially attenuated by mibefradil, low doses of Ni^{2+} and TTA-P2, suggesting that T-type channels contribute only to a fraction of the overall mIPSCs bursting response. The remaining part is associated to a Ca^{2+} -induced release from presynaptic Ca^{2+} stores. In addition, bath application of EGTA-AM strongly reduces the nAChR-induced inhibitory response, suggesting loose coupling between T-type channels and GABA release. This is different from the HVA-driven pathway in which few Cav2.1 (P/Q-type) channels are sufficient to elicit synaptic activity from parvalbumin basket cells [11]. The Cav3.1-driven transmission occurs regardless of HVA channel Ca^{2+} influx and it may thus operate in parallel with the normal AP-driven HVA channel-dependent mechanism. This ensures a broader control of inhibitory interneurons on pyramidal cell excitability in hippocampus.

Cav3.2-driven glutamate release in cortical neurons

Presynaptic Cav3.2 channels are also expressed in glutamatergic cortical synaptic terminals contacting entorhinal cortical layer III pyramidal neurons [48]. These terminals, express high densities of the hyperpolarization-activated cyclic-nucleotide gated channel type 1 (HCN1) that partially depolarize the terminal by passing a net inward current. A high resting potential (-50 mV) inactivates functionally available T-type channels, reduces basal presynaptic Ca^{2+} levels and attenuates the frequency of spontaneous mEPSCs. Block of HCN1 channels by ZD-7288 or genetic deletion of the channel causes a net increase in the frequency of spontaneous mEPSCs that is effectively blocked by mibefradil (10 μM), TTA-A2 (1 μM) and TTA-P2 (1 μM) [87, 96]. Applications of ω -agatoxin IVA, ω -conotoxin GVIA and SNX-482 have no effect on the ZD-7288-induced increase of mEPSCs frequency, suggesting a dominant presynaptic control of Cav3 channels on the increased mEPSCs activity following HCN1 block. Given that changes in synaptic strength are pivotal to the induction and maintenance of synaptic plasticity and that entorhinal cortical layer III neurons are involved in memory formation and spatial navigation [80], these findings indicate that a HCN1/Cav3.2-driven excitatory transmission may critically contribute to such physiological processes.

T-type channel-mediated GABA release of fast spiking interneurons in dentate granule cells

In line with the above findings T-type channels are also known to play a role at the mono- and di-synaptic GABAergic inputs of dentate granule cells [41]. Zinc conditions the functioning of these synapses, but its action is widely unexplored. Zinc inhibits ionotropic receptors commonly found at central synapses, as well as a number of voltage-gated ion channels including Cav3 channels [91, 95]. In fast spiking GABAergic interneurons Zn blocks somatic T-type Ca^{2+} currents. Thus, zinc chelation enhances these currents, decreases spike threshold and broadens AP half-width. Zinc also reversibly depresses GABAergic transmission while zinc chelation enhances the inhibitory response. Since zinc chelation is prevented only by T-type and not by HVA channel blockers, a reasonable assumption is that T-type channels controls GABA release of fast spiking interneurons. Although lacking a convincing immunoreactivity proof of presynaptically expressed Cav3 channels, the reported electrophysiological findings together with the evidence of a dense localization of Cav3 channel gene transcripts in the cell granule layer [92] and parvalbumin-immunoreactive interneurons [7, 86], strongly support a role of T-type channels in GABA transmission.

T-type channel-driven dopamine release from the somata of dopaminergic neurons

T-type channels control also dopamine release from the soma of dopaminergic neurons in the substantia nigra *pars compacta*, as shown by amperometric recordings with carbon fiber microelectrodes [57]. Prolonged depolarizations induced by high KCl solutions cause massive release of dopamine in the form of bursts of amperometric events that are associated to the quantal dopamine release. Mibefradil (10 μM), reduces the frequency of amperometric spikes thus suggesting a direct role of T-type channels in dopamine release in substantia nigra *pars compacta* neurons.

T-type channel-driven synaptic exocytosis in immature auditory hair cells

A role of T-type channels on vesicle exocytosis has been reported also in chick auditory hair cells during development [63]. Immature chick hair cells (embryonic *days 10–12*) possess a low-voltage-activating, rapidly inactivating T-type current and a high-voltage-activating, non-inactivating L-type current to control vesicle exocytosis. Synaptic activity evoked by T-type channels and measured through membrane capacitance changes (ΔC) displays a fast release component but lacks the slow sustained release component. This suggests an efficient recruitment of nearby synaptic vesicles and a less efficient action on more distant vesicles by T-type channel Ca^{2+} currents in immature hair cells. During hair cell development, the participation of T-type currents progressively decreases, whereas that of L-type increases. A property observed also in other cell types, including chromaffin cells (see below [64]). Of relevance is the observation that with maturation (from E12 to P2) the Ca^{2+} -efficiency of the T-type channel increases markedly, although less steeply than L-type channels. These latter dominate during development.

T-type channels-driven hormone release in neuroendocrine cells

In line with the early reports in MPC cells [44] and melanotropes [67] described above and those on chromaffin cells described below [15, 16, 38], two recent reports confirm that T-type channels can effectively drive hormone secretion in neuroendocrine cells. Using markers of prostate secretion and FM1-43 fluorescence imaging of membrane trafficking, Gackiere et al [35] have shown unequivocally that prostate cell differentiation is associated with an increase in Ca^{2+} -dependent secretion that critically relies on Cav3.2 channel activity. Cav3.2 channels up-regulation may account for the alteration of secretion during prostate cancer development. T-type channels promote the secretion of potential mitogenic factors and participate to the progression of the disease toward an androgen-independent stage. In line with this, Zanatta et al. [106] show that exocytosis in immature rat Sertoli cells is modulated by the reverse T3 thyroid hormone (rT3) through the activation of T-type channels. Cav3 channels are expressed in Sertoli cells [60] and are up-regulated by rT3 through an integrin-mediated action and consequent PKC and MEK activation.

T-type channels secretion coupling in adrenal chromaffin cells

Despite widely distributed in most nervous, muscular and epithelial tissues, T-type channels are weakly expressed or absent in adult chromaffin cells. In bovine chromaffin cells of adult animals, the mRNA encoding for Cav3.1 and Cav3.2 is clearly expressed [36], but functional T-type channels are mainly absent [1, 4, 13, 23, 24]. T-type channels are usually expressed and carry sufficient Ca^{2+} currents in embryonic and neonatal RCCs [9, 64, 90]. They are also available in a small percentage of adult RCCs [47]. T-type channels have been reported to be absent in adult mouse chromaffin cells (MCCs) [45, 69, 70, 98]. Preliminary data from our lab, however, clearly show that the I/V characteristics of adult MCCs display the typical “low-threshold shoulder” associated with T-type channels during ramp commands (see Fig. 4 in [40]). It is thus very likely that availability of T-type channels may critically depend on chromaffin cell isolation, culture cell conditions or exogenous stimulations, as in the case of applied chronic stressors which up-regulate Cav3.2 channels in RCCs [16, 38, 76, 90] and MCCs [46, 89].

When expressed, T-type channels appear effectively coupled to chromaffin secretory granules and control a sizeable amount of “low-threshold” catecholamine release [15, 16, 38, 59, 68]. The Ca^{2+} -dependence of T-type channel driven secretion is linear and strikingly similar to that of Cav1 channels [15], which is comparable to that of Cav2.1 and Cav2.2 channels [14]. Independently of the low voltage range of activation, T-type channels are thus closely distributed near the docked secretory vesicles, just like Cav1 and Cav2 channels and control catecholamine secretion (see Fig. 1 for a T-type channel vs. vesicles arrangement derived from [15, 16, 38]). Thus, by lowering the threshold of AP generation T-type channels broaden the interval of voltage control of catecholamine release. This extended Ca^{2+} channel secretion coupling may be relevant when chromaffin cells depolarize steadily or fire at very high frequencies during stressful conditions.

T-type channels and low-threshold exocytosis: an adaptive response to stress

As for the nicotinic receptors and gap-junction channels [42, 43] also T-type channels undergo functional remodeling during stress [17, 21, 66]. This is particularly evident in chromaffin cells of adult animals as an adaptive response to stress conditions like chronic and intermittent hypoxia [16, 90], cAMP/PKA, β -adrenoreceptor and high-frequency sympathetic stimulation [46, 59, 76, 89]. T-type channel remodeling occurs also during the transition from intra- to extra-uterine life [64], when newborns experience episodic hypoxia conditions and catecholamine secretion switches from a non-neurogenic to a neurogenic control mode [85].

A common molecular mechanism in response to stressors is a marked recruitment of functioning Cav3.2 channels. Up-regulation of Cav3.2 channels lowers the threshold of AP firing [76], increases the amount of catecholamines secreted at low-voltages [16, 38] and mobilizes a RRP equivalent to that of L-type channels with the same Ca^{2+} -dependence (see Fig. 1). An increased density of T-type channels is also expected to decrease the resting potential and increase the firing frequency, as it occurs in spontaneously firing rat chromaffin cells in the adrenal-gland slices from stressed rats [25]. Indeed, an increased density of functioning T-type channels is a remodeling mechanism that many cells and tissues develop in response to stressors. Beside hypoxia, β -AR and high-frequency sympathetic stimulation other stressors (glucocorticoids, aldosterone, ACTH, 17- β estradiol) and stress-induced conditions (hypertension, cardiomyopathies, diabetic and chronic visceral pain) lead to the up-regulation of Cav3.1 and Cav3.2 channels in neuronal, muscular and endocrine cells (see Table 1 in [65]). This reinforces the view that T-type are “stress-activated channels” [17, 21, 27, 89].

As listed above, there are multiple ways by which Cav3.2 channels are either activated or up-regulated by stressors in chromaffin cells. They act either acutely within minutes (sustained sympathetic stimulation, PACAP release) or slowly, requiring hours or days (chronic and

intermittent hypoxia, β_1 -AR stimulation, VIP, aldosterone, ACTH). The rapidly developing mechanism (minutes) is most evident in mouse innervated adrenal gland slices [46]. This pathway is activated by PACAP, released during high-frequency splanchnic stimulation, and mediated by cAMP-activated exchange proteins (Epac) that stimulate PLC and PKC through a non-canonical cAMP-dependent pathway. The PKC target is the phosphorylation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger [59] and a fast recruitment of Cav3.2 channels [46] (Fig. 2a). The former induces a small depolarization near resting potential (ΔV_m) sufficient to activate a robust “low-threshold” Cav3.2 current that triggers exocytosis.

The second pathway, slowly developing, is a long lasting mechanism that requires hours or days to progress. This is the case for chronic/intermittent hypoxia and cell exposure to β_1 -AR agonists (adrenaline, isoprenaline), which both increase the levels of CACNA1H mRNA (Cav3.2 gene). Their action is prevented by protein synthesis inhibitors [15, 16, 38, 76, 90], suggesting net recruitment of newly synthesized Cav3.2 channels (Fig. 2b). For the case of β_1 -AR stimulation, Cav3.2 recruitment is mediated by a cAMP-dependent (PKA-independent) Epac pathway which leads to the activation of transcription factors [76], most likely through a Rap-mediated pathway and the phosphorylation of ERK [8]. This cAMP-dependent mechanism is completely different from the fast cAMP-dependent up-regulation of Cav1.2 and Cav1.3 channels observed in MCCs [66].

Concerning chronic and intermittent hypoxia, recent findings indicate the involvement of hypoxia-inducible factors. HIF-2 α is involved in the up-regulation of Cav3.2 in PC12 cells exposed to chronic hypoxia [30]. In line with this, overnight incubations with the unspecific HIF activator desferrioxamine mimics the effects of hypoxia in PC12 cells [30] and RCCs [15]. In addition to HIFs, also NADHP-oxidases (NOX2, NOX4) and reactive-oxygen species [90] appear to play an important role in the recruitment of Cav3.2 in neonatal RCCs subjected to intermittent hypoxia [61]. A hypothetical scheme in which NOX, ROS and HIF are linked together is shown in Fig. 2c. The scheme is derived from [105] in which ROS is shown to activate PLC, DAG and PKC, favoring the stabilization and translocation of HIF-1 α to the nucleus to start transcription and gene expression.

T-type channel-driven catecholamine release and O₂ sensing during development

Chromaffin cells possess an inborn chemosensitivity to O₂ levels during fetal life, when the control of hormone release from the adrenal medulla is mostly non-neurogenic [85] and effectively regulated by the gap-junction coupling existing between neighboring cells [25]. During this developmental phase, chromaffin cells respond to acute hypoxia with a robust catecholamine release, mostly controlled by Cav3.2 channels [64]. High densities of T-type channels and enhanced electrical coupling facilitate action potential generation and propagation, as well as the synchronous release of catecholamines from cell populations of the adrenal medulla. This increases the amount of released adrenaline that helps sustaining the cardiovascular response to hypoxia [42, 43]. Of great significance, however, is the observation that cholinergic innervation of the adrenal gland, following postnatal development [85], causes a loss of functional Cav3.2 channels [64] paralleled by an increased density of α_7 -built nAChRs [12]. But even more interesting is the observation that O₂-sensitivity and Cav3.2 channel expression is reestablished in chromaffin cells after adrenal gland denervation, suggesting an opposing correlation between nicotinic AChRs expression and Cav3.2 channels down-regulation or vice versa [64].

This apparent coupling between AChRs and Cav3.2 is worth being investigated since T-type channels co-localize with a number of ion channels. In central neurons, Cav3.2 channels are co-localized to voltage-gated (Kv4) [2], Ca²⁺-gated K⁺ channels (KCa1.1, KCa3.1) [34, 79] and HCN1 channels [48] to widen the voltage range where K⁺, Ca²⁺ and HCN1 channels can control the shape and frequency of AP trains. Cav3.2 channels are also effectively inhibited by closely associated TRPV1 channels in small diameter DRG neurons, which would predict a TRPV1-mediated

analgesic action via an increased threshold of nociceptive signals [26]. Indeed, a TRPV1-mediated inhibition of Cav3.2 channels has been recently proposed to regulate the analgesic effect of paracetamol in supraspinal DRG neurons [56]. All these findings widen the range of operation of T-type channels in the regulation of cell excitability and underline once more the amazing functional importance of these voltage-gated Ca^{2+} channels in the control of vital cell functions.

Cav3.2 channel binding to syntaxin-1A and SNAP-25: a likely mechanism for fast neurosecretion?

It is surprising that despite the overwhelming evidence in favor of a role of T-type channels in neurotransmitter release there are still few data available on the molecular mechanism that triggers these events. Most data in presynaptic terminals, neuronal somata and neuroendocrine cells suggest loose coupling between T-type channels and secretory vesicles. This contradicts the idea of the formation of “nanodomains” between T-type channels, SNARE proteins and vesicles. However, recent reports clearly show that Cav3.2 channels biochemically interact with syntaxin-1A and SNAP-25 [100] at the carboxy-terminal domain. Electrophysiological recordings show that syntaxin-1A binding to Cav3.2 channels causes marked changes to channel gating, resembling those regarding N- and P/Q-type channels (see [22, 101] for a review). Most relevant is the observation that the Cav3.2-syntaxin-SNAP-25 interaction somehow affects a reconstituted T-type-driven exocytosis in MPC cells. Expression of Cav3.2 in MPC9/3L cells, which possess native SNARE proteins [44], produces a marked “low-threshold” membrane capacitance (ΔC) increase that is fully prevented by uncoupling T-type channels from SNARE proteins. Taken together these findings suggest that Cav3.2 channels co-localize with SNARE proteins and may thus potentially form membrane nanodomains with secretory vesicles able to trigger vesicle fusion and neurotransmitter release.

Concluding remarks

There is clearly no doubt that T-type channels contribute to neurotransmitter release in neurons and neuroendocrine cells. The growing evidence over the last ten years suggests a key role of these channels in controlling basal neurosecretion near resting conditions and sustained release during mild stimulations. In some cases, the contribution of T-type channels is not direct but requires either the activation of coupled presynaptic receptors [93], the block of ion channels [48] or the chelation of metal ions [41]. The effect is nevertheless remarkable and in all cases of physio-pathological significance. Concerning the coupling to the secretory apparatus, T-type channels appear loosely coupled to neurotransmitter and hormone release. In central neurons, T-type channels mainly control the asynchronous appearance of mIPSCs and mEPSCs while AP-evoked IPSCs and EPSCs are dominated by P/Q and N-type channels. The same loose coupling is evident from the membrane capacity changes in chromaffin cells and melanotropes where the low-threshold-driven exocytosis possesses the same Ca^{2+} dependence of the other HVA calcium channels that is strongly attenuated by slow calcium buffers [58]. The intriguing issue is that, despite not expressing a consensus synprint site, T-type channels do interact with syntaxin-1A and SNAP-25 and, thus, may form nanodomains with secretory vesicles that can be regulated at low voltages. Future studies will clarify this issue, may be by showing that a fraction of T-type channels couple directly to the vesicle via SNARE-proteins and the remaining does not and that the picture may be different in presynaptic terminal and neurosecretory cells.

Figure Legends

Figure 1 – Loose coupling of T- and L-type channels to exocytosis in chromaffin cells.

Left) Schematic drawing illustrating the loose coupling of T- and L-type channels to secretory granules in adrenal chromaffin cells. The two voltage-gated channels are coupled to secretion with the same efficacy for Ca^{2+} . This implies that on average they are at an equal distance from fused vesicles ready for release. For more details see ref [15]. *Top-right*) Membrane capacity changes (ΔC) versus the corresponding quantity of calcium charge (pC) for cAMP-treated RCCs expressing T- and R-type channels and incubated in a medium containing ω -toxins and nifedipine (black circles, solid line) compared to the ΔC increases induced by L- and R-type channels expressed in control RCCs treated with ω -toxins (red squares, dashed line). The linear regressions have nearly equal slope (adapted from [15] and [38]). *Bottom-right*) ΔC recordings driven by T-type currents passing during step depolarization of 150 ms to -20 mV (black traces) and L-type currents during pulse of 200 ms to +10 mV (red traces) (adapted from [15] and [38]).

Figure 2 - Cell signaling pathways leading to fast or slow recruitment of Cav3.2 channel-mediated secretion in chromaffin cells.

a) PACAP-stimulated secretion in mouse chromaffin cells through the acute phosphoregulation of Cav3.2 channels [46, 59, 89]. PACAP activation of the PAC1 receptor elevates cAMP that activates Epac within minutes (not shown). The subsequent activation of PLC and PKC leads to the phosphorylation of a sodium-calcium exchanger (NCX) that causes a small cell depolarization (ΔV_m), the opening of T-type channels and release of catecholamines. T-type channels may also be directly up-regulated by PKC activation. *b*) cAMP and β_1 -AR mediated up-regulation of Cav3.2 channels through an Epac-mediated pathway that activates transcription factors and CACNA1H gene expression in rat chromaffin cells [76]. Cav3.2 channel recruitment leads to a well resolved “low-threshold” exocytosis [38]. The involvement of Rap and ERK in the modulatory pathway is only hypothetical (?). *c*) Cell signaling pathway leading to CACNA1H gene expression and Cav3.2 channels recruitment during hypoxia in rat chromaffin cells [16]. The final result of chronic/intermittent hypoxia is the activation of transcription factors (HIF, CREB, etc...) and CACNA1H gene expression that leads to Cav3.2 channels recruitment and associated exocytosis. The schematic pathway induced by hypoxia (NOX, ROS, PLC, PKG, HIF) is adapted from [65, 105].

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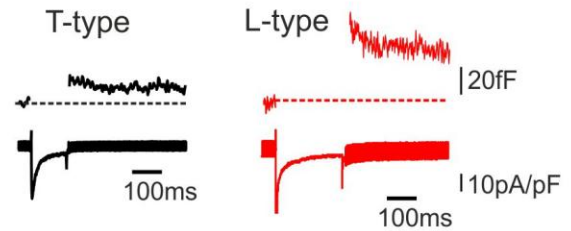
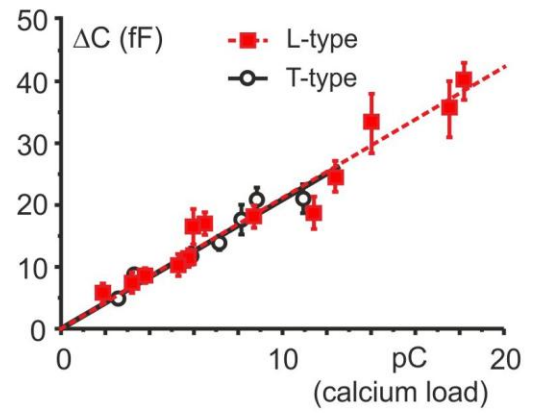
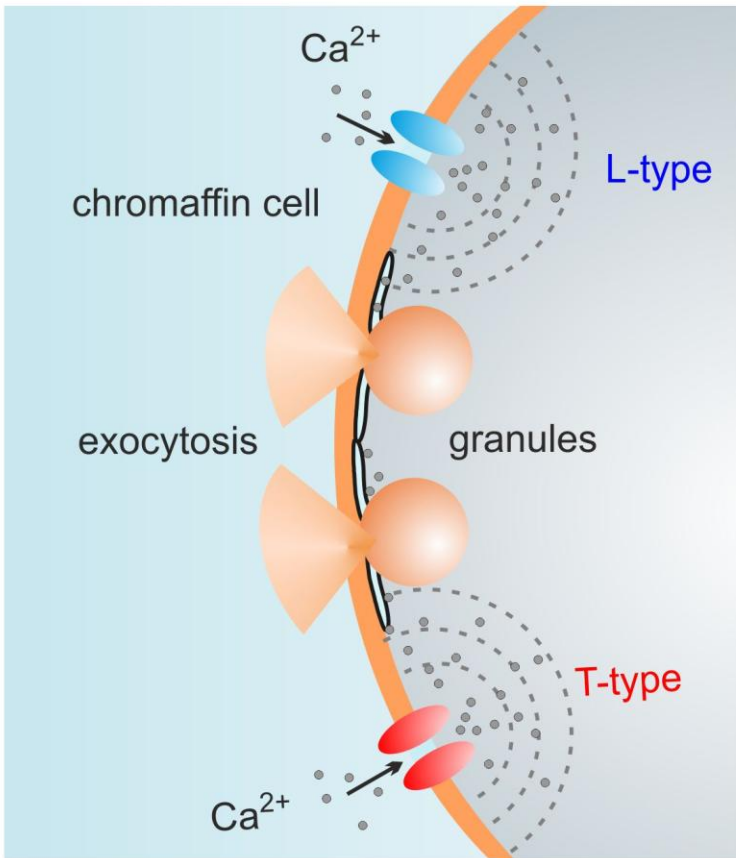


Fig. 1

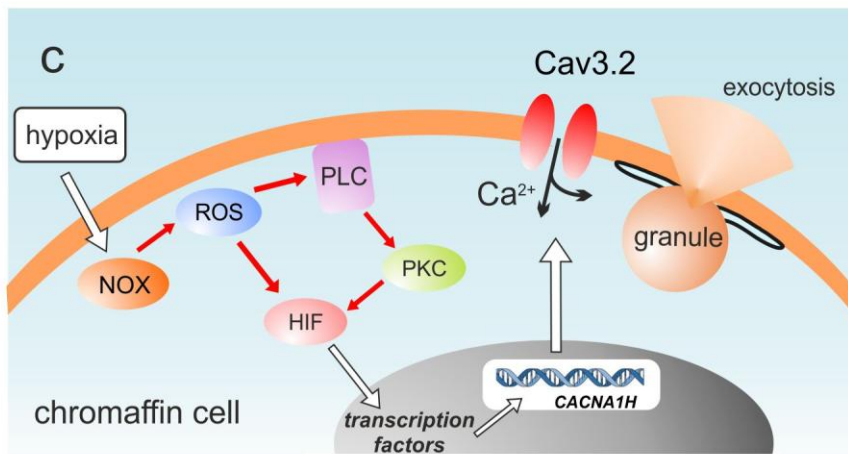
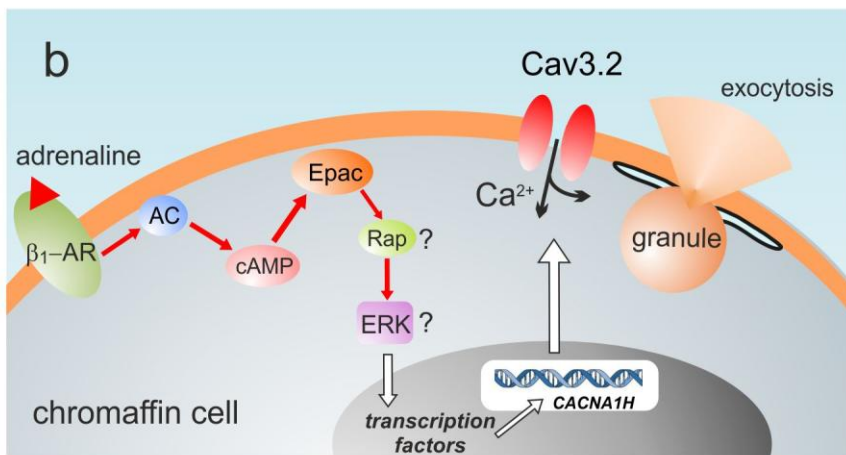
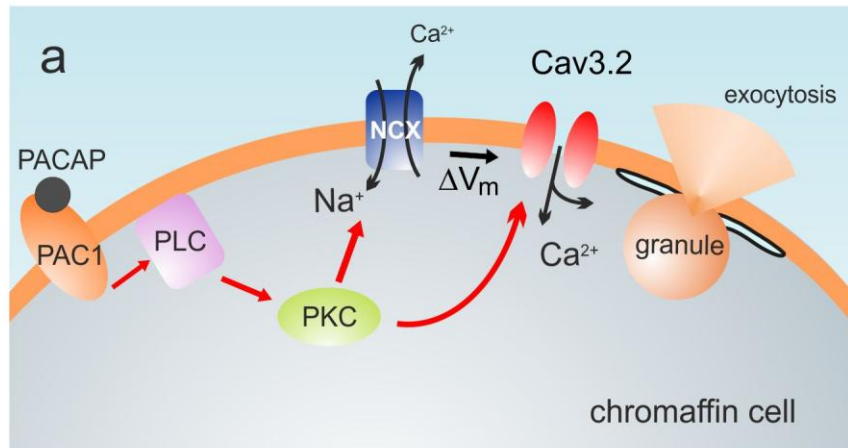


Fig. 2