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A new role for T-type channels in fast "low-threshold" exocytosis

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Abstract

Evidence is accumulating on a key role of T-type channels in neurotransmitter release. Recent works have brought undisputable proofs that T-type channels are capable of controlling hormone and neurotransmitters release in association with exocytosis of large dense-core and synaptic vesicles. T-type channel-secretion coupling is not as ubiquitous as that shown for N- and P/Q-type channels in central neurons. In this case, the high-density of Ca_v2 channel types and co-localization to the release sites ensure high rates of vesicle release and synchronous synaptic responses. Nevertheless, when sufficiently expressed in distal dendrites and neurosecretory cells, T-type channels are able to drive the fast fusion of vesicles ready for release during "low-threshold" Ca^{2+}-entry. T-type channels appear effectively coupled to fast vesicle depletion and may possibly regulate other Ca^{2+}-dependent processes like vesicle recycling and vesicle mobilization from a reserve pool that are important mechanisms controlling synaptic activity during sustained stimulation. Here, we will briefly review the main findings that assign a specific task to T-type channels in fast exocytosis discussing their possible involvement in the control of the Ca^{2+}-dependent processes regulating synaptic activity and vesicular hormone release.

Keywords: LVA Ca^{2+} channels; Capacitance increases; Neurotransmitter release; Synaptic activity; Vesicle release

1. Introduction

T-type channels are transient low-voltage-activated (LVA) Ca^{2+} channels that control Ca^{2+} entry in excitable cells during small depolarizations around resting potential [1–8]. Studies in the past 20 years focused on the biophysical, physiological and molecular characterization of T-type channels in most tissues, leading to a clear-cut picture of their functional role in controlling: low-threshold spikes, oscillatory cell activity, muscle contraction, hormone release, cell growth and differentiation [9–13]. Molecular cloning has now provided evidence for the existence of three different pore forming α1 subunits (α1G, α1H, α1I) with biophysical profiles similar to the endogenous T-type channels expressed in most tissues [14,15]. This broadened the area of investigation, particularly for the structure–function relationships, which brought new insights into the mechanisms of ion channel selectivity and activation-inactivation gating [11] (see also [16]).

So far, little attention has been devoted to the role of T-type channels in transmitter release, which mainly involves channel types belonging to the high-voltage-activated (HVA) Ca^{2+} channel family. However, evidence is accumulating in favor of a unique participation of T-type channels in fast transmitter release. Clear data are now reported in reciprocal synapses of the retina and olfactory bulb [17–20], synaptic contacts between primary afferent and second order nociceptive neurons [21], rhythmic inhibitory interneurons of invertebrates [22,23] and clonal cell lines transfected with recombinant α1 channel subunits [24]. T-type channels also regulate the large dense-core vesicle (LCDV) release of neuroendocrine cells [25] where Ca^{2+}-dependence, rate of vesicle release and size of readily releasable pool appear comparable to those associated with HVA channels [26]. This suggests that when sufficiently expressed and properly located near the release zones, T-type channels can trigger low-threshold secretion.

In this review we will focus on the role that T-type channels play in the control of fast vesicular exocytosis. This aspect has been largely overlooked in the past and, given its importance, may turn of broad interest for future investigations.

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As the contribution of T-type channels to exocytosis derives mainly from channels located at distal dendrites and spines, the progress in this field will rely on whether optical techniques for recording Ca^{2+} signaling and electrophysiological detection of vesicle secretion will improve their sensitivity to reveal Ca^{2+} signals and exocytic events in narrow regions of neuronal terminals. T-type channels are known to play also a main role in the control of hormone release from cells lacking secretory vesicles and exocytotic mechanisms. This aspect, however, is discussed in other chapters of this special issue and in recent up-to-date reviews on the argument [27,28].

2. T-type channels in neuronal terminals

Pharmacological studies and immunocytochemical characterization of Ca^{2+} channel distribution suggest that N- and P/Q-type channels are the predominant species controlling synaptic transmission in central neurons [29]. The two Ca_v2 channel types bind to syntaxin and synaptotagmin through a "synprint" region [30] and are closely associated with the SNARE complex. They mediate vesicle docking, fusion and neurotransmitter release in highly specialized presynaptic regions (active zones). Co-localization of N- and P/Q-type channels to the active zones ensures high Ca^{2+} concentrations and fast synchronous synaptic responses during action potentials-evoked neurotransmitter release. In this way, probability of release associated with N- and P/Q-type channels is relatively large when $[Ca^{2+}]_i$ is >10 μM, ensuring high rates of vesicle release during rapid responses [31]. There are, however, exceptions to this general rule suggesting that also other voltage-gated Ca^{2+} channels (R-, L- and T-type) play a role in the control of presynaptic Ca^{2+} levels in a variety of neurons.

In cerebellar parallel fibers [32] and hippocampal CA3-CA1 synapses [33] a significant fraction of presynaptic $[Ca^{2+}]_i$ persists in the presence of ω-CgTx, ω-Aga-IVA and nifedipine, suggesting that either R- or T-type channels may contribute to Ca^{2+} elevations in these terminals during synaptic transmission. Evidence for a contribution of R-type channels to transmitter release is reported at the calyx of Held [34] and at the mouse neuromuscular junction in KO mice lacking P/Q-type channels [35]. α1F L-type channels are shown to control glutamate release in rod photoreceptor terminals and to co-localize at the hot spots of bipolar cell terminals [36,37]. L-type channels are expressed also in AII amacrine cells and localized at the distal dendrites where synaptic transmission takes place [38]. L-type channels regulate also transmitter release in single boutons of rat hippocampal neurons [39], are involved in presynaptic Ca^{2+} accumulation during high-frequency stimulation in GABAergic hippocampal neurons [40] and control short-term plasticity of GABA release in cerebral cortex during presynaptic firing rates in the gamma frequency range (40 Hz) [41]. This helps maintaining high rates of vesicle release during tetanic stimulation and to enhance transmitter release during post-tetanic periods. The action is likely mediated by second messengers, which may activate various kinases that control vesicle recycling and mobilization from a reserve pool.

To date, there is increasing evidence that also T-type channels mediate neurotransmitter release in a number of neurons. They control the response to graded depolarizations in rod bipolar cells [17,18], are involved in neurotransmitter release in dendrodendritic reciprocal synapses between granule and mitral cells of olfactory bulbs [19,20], regulate the frequency of spontaneous excitatory postsynaptic currents (mEPSCs) in nociceptive neurons [21] and control graded synaptic transmission between oscillatory hearth interneurons of leech [22]. Looking closely at these reports, it appears that T-type channels play a role in those neurons that use graded potentials for synaptic transmission and exhibit Ca^{2+} signals at distal dendrites and spines that are strongly dependent on holding potential. In all cases, the indication is unambiguous and uncovers a direct action of T-type channels on neurotransmitter release that will be described in the following paragraphs.

2.1. T-type channels in invertebrate inhibitory synapses

In the leech, the neuronal network generating rhythmic heartbeats includes two segmental bilateral pairs of reciprocal inhibitory interneurons. The bilateral neurons are active and alternate bursts, which inhibit one another via graded and spike-mediated transmission. T-type channels mediate the graded response, whereas L-type channels control the spike-mediated transmission [42]. Simultaneous recordings of presynaptic Ca^{2+} currents, presynaptic $[Ca^{2+}]$ and inhibitory postsynaptic currents (IPSCs) show that transient T-type currents at −35 mV produce sustained $[Ca^{2+}]$ signals at the fine neuritic branches near the region of synaptic contact. The presynaptic $[Ca^{2+}]$ increases are closely associated with the transient IPSCs [22]. There is nice correlation between presynaptic Ca^{2+} increases and rise of postsynaptic conductance and an even better correlation between the transient kinetics of T-type currents and transient IPSCs, proving an unequivocal association between LVA channels activation and graded postsynaptic response in these inhibitory interneurons. T-type channels also control the background $[Ca^{2+}]$ changes that modulate the strength and waveform of spike-mediated synaptic transmission [23] and are thus critical for setting the oscillatory rhythms of leech heartbeats.

2.2. T-type channels regulate GABA release in olfactory bulbs

T-type channels have a unique functional role also at the large spines of granule cells (GCs) that form dendrodendritic reciprocal synapses with mitral and tufted cells of olfactory bulbs. Distal spines of granule cells possess presynaptic and postsynaptic elements and express sufficient densities of T-type channels to modulate transmitter release [19]. The presence of T-type channels is evident by two-photon Ca^{2+} imaging showing that Ca^{2+} transients at the spines and adjacent

dendrites of GCs are strongly dependent on holding potential. Their size decreases with depolarization and increases with hyperpolarization. Both the voltage-dependence and time course of inactivation of Ca^{2+} transients are consistent with the known properties of T-type channels and Ca^{2+} signals appear markedly reduced by applications of Ni^{2+} (100 μM) and mibefradil (1–10 μM). Mibefradil reduces also the action potential-evoked synaptic transmission from granule to mitral cells. In this case, presynaptic action potentials are evoked by extracellular stimulation while inhibitory postsynaptic potentials (IPSPs) are recorded in whole-cell mode at the soma of mitral cells. Under these conditions, small IPSPs with short latency and slow decay time constant can be recorded in a number of mitral cells. The IPSPs are fully blocked by bicuculline and strongly attenuated by 10 μM mibefradil, suggesting that they are mediated by $GABA_A$ receptors and that mibefradil-sensitive Ca^{2+} channels contribute to part of the global inhibition of mitral cells induced by granule cell stimulation. Thus, T-type channels control the graded regulation of GC-mediated lateral inhibition in the olfactory bulb. A graded "low-threshold" control of GABAergic synaptic release mediated by T-type channels may represent a new way to gate the entry of olfactory information to the cortex [20].

2.3. T-type channels regulate the frequency of mEPSCs in nociceptive neurons

Increasing evidence indicates that T-type channels play a key role in the control of nociceptive signaling [43]. LVA channels are highly expressed in mammalian sensory neurons [1,3,44,45] and up-regulation of their current by endogenous L-cysteine induces thermal hyperalgesia in animal models of neuropathic pain [46]. T-type channels are also directly involved in the control of synaptic activity between primary afferents and second order nociceptive neurons at the dorsal horn of the spinal cord by controlling the frequency of mEPSCs [21]. T-type channel blockers like Ni^{2+} and mibefradil are very effective in reducing the frequency of mEPSCs without significantly affecting the latency and amplitude of action potential-evoked EPSCs. In contrast, HVA channel blockers like Cd^{2+} and ω-CgTx-GVIA can block the EPSCs without affecting the frequency of mEPSCs. Worth mentioning is the fact that small depolarizations induced by raising $[K]_o$ to 10 mM produce a marked increase of mEPSCs frequency, prevented by mibefradil. This indicates that in nociceptive neurons spontaneous glutamate release near resting conditions is effectively controlled by T-type channels. Since mEPSCs occur often enough to affect postsynaptic activity, it is evident that the availability of T-type channels alters the excitability level of nociceptive neurons. Excitatory transients occurring at a rate of 10–30/s, each lasting 10–30 ms, can easily overlap with the evoked activity of other terminals and summate to threshold levels for a postsynaptic afferent neuron, lowering the threshold of pain sensation.

2.4. T-type channels regulate glutamate release in retinal bipolar cells

In mammalian retina, T-type channels are co-expressed with L-type channels in rod bipolar cells [47] and contribute to transmitter release at the reciprocal synaptic contacts that bipolar cells form with A17 and AII amacrine cells [17,18]. Three main lines of evidence support a functional role and a tight coupling of T-type channels to glutamate release in these synapses. The first evidence concerns the high-density of T-type channels expressed in these cells compared to L-type channels [17,18]. The two channel types give rise to Ca^{2+} currents of comparable amplitude which implies rather similar densities if correcting for the different driving force, single channel conductance and open probability. The second evidence concerns the strong correspondence between the time course of presynaptic Ca^{2+} currents of bipolar cells and the fast glutamatergic responses in synaptically interconnected AII amacrine cells [18]. T-type channels recruited by strong hyperpolarization (−100 mV) give rise to transient presynaptic Ca^{2+} currents that markedly increase the amplitude of fast EPSCs with little changes to their transient time course [18]. EPSCs recorded in AII cells are already transient when L-types are the only Ca^{2+} channels contributing to the presynaptic current, but the activation of T-type currents almost doubles the amplitude of AMPA-mediated EPSCs. These findings suggest unequivocally that, when available, T-type channels contribute to neurotransmitter release with the same efficiency of HVA channels.

The third evidence concerns the strict correlation existing between LVA currents, Ca^{2+} influx and membrane capacitance increases (ΔC's) in isolated rod bipolar cells [17]. The latter is used as a measurement of the increased membrane surface area during presynaptic vesicle fusion [48]. ΔC's induced by L-type currents associated with 500 ms depolarizing pulses to −10 mV are in the order of 13 fF (corresponding to the release of 169 synaptic vesicles) and are fully abolished by 5 mM Co^{2+} or by replacing intracellular EGTA with BAPTA. ΔC's, however, occurs also at very low membrane voltages (−40 to −30 mV) where LVA channels are available by holding the axon terminals at −85 mV. These "low-threshold" capacitance changes are in the order of 7 fF and are fully blocked by 10 μM mibefradil. Nimodipine has no action on them but drastically reduces the ΔC's at −10 mV, associated with the L-type channels expressed in rod bipolar cells. These findings thus furnish the most direct evidence that T-type channels can support fast exocytosis in synaptic terminals subjected to graded depolarizations.

How rapid is the exocytotic response associated with T-type channels? Presently, there are no data available on capacitance measurements at intervals below 500 ms and thus it is impossible to make precise estimates of the rate of vesicle release and Ca^{2+}-dependence of exocytosis in retinal bipolar cells. However, intracellular Ca^{2+} fluxes at the presynaptic terminals of bipolar cells rise with time constants in the order of 100–200 ms [17] and it is thus possible that capacitance

changes will follow this speed. In addition, LVA currents lasting 50 ms can evoke reciprocal inhibitory currents in bipolar cells of retinal slices that form reciprocal synapses with AII amacrine cells. A transient fluctuating currents associated with the activity of $GABA_A$ receptors is already evident after 10 ms from the start of the current [17], indicating that LVA channels are capable of stimulating fast glutamate release in less than 10 ms. Thus, it is likely that T-type channels trigger neurotransmitter release in bipolar cell and contribute to the overall retinal information processing.

3. T-type channels and exocytosis in neuroendocrine cells

3.1. T-type channels mediate fast exocytosis in rat melanotropes

Rat melanotropes express all types of voltage-gated Ca^{2+} channels (HVA and LVA), which fully control the release of predocked large dense-core vesicles during action potential stimulation [25]. T-type channels in rat melanotropes contribute to a small fraction of the total current and produce a mean ΔC increase of about 7 fF during 40 ms step depolarization that is only about half the ΔC associated with HVA channels (L, N, P/Q, R). Normalizing the capacitance changes for the quantity of Ca^{2+} ions entering the cells it can be shown that T-type channels control secretion with the same efficacy of HVA channel types. The immediate significance of this result is that T-type channels are coupled to LDCVs with the same strength of HVA channels. It is interesting to notice, however, that in rat melanotropes as in chromaffin cells, voltage-gated Ca^{2+} channels are not co-localized with LDCVs. This implies some delay between Ca^{2+} entry and vesicle secretion, which may result in poor coupling between single action potentials and exocytosis if the action potential lasts few milliseconds and Ca^{2+} must diffuse considerable distances (~200 nm) to reach docked vesicles [49]. This however is not the case of rat melanotropes in which action potentials last very long (50–300 ms) and Ca^{2+} entry persists for the entire duration of the depolarization. Under these conditions, all voltage-gated Ca^{2+} channels can contribute to exocytosis by different degrees during the various phases of action potential generation. In the case of T-type channels, they activate around −60 mV and contribute to the slow initial phase of depolarization and to the "low-threshold" component of exocytosis with the same efficacy of HVA channels that participate to the late phases of action potential depolarization. This ensures that exocytosis takes place over the entire duration of the action potential with the same Ca^{2+} sensitivity regardless of the type of open Ca^{2+} channel.

3.2. Exocytosis in pheochromocytoma cell lines expressing recombinant α1G Ca^{2+} channels

T-type channels are effectively coupled to secretion also when they are overexpressed in pheochromocytoma cell lines that lack endogenous Ca^{2+} channels but contains secretory vesicles and protein components necessary for exocytosis [24]. MPC9/3L cells secrete dopamine and catecholamines when Ca^{2+} enters the cell through exogenously applied Ca^{2+} ionophores or when heterologously expressed Ca^{2+} channels open on step depolarizations. The interesting aspect of this secretory cell line is that newly expressed α1G T-type channels give rise to Ca^{2+} current densities (70 pA pF^{-1}) that are significantly smaller than those associated with α1B N-type channels (400 pA pF^{-1}) but produce capacitance increases with comparable efficiency (0.5 fF pC^{-1} for the N-type channel versus 0.6 fF pC^{-1} for the T-type channel). T-type channels are also capable of inducing cumulative capacitance increases during brief trains of 5–10 depolarizations of 10 Hz, suggesting that they can sustain marked exocytosis also when activated at frequencies comparable to sympathetic stimulation (2–20 Hz). The rationale for these observations is that in MPC9/3L cells T-type channels can functionally couple to release sites and control exocytosis despite they lack the "synprint region" [14] that is necessary for binding syntaxin and trigger vesicle fusion.

3.3. Fast exocytosis associated with α1H T-type channels in rat chromaffin cells (RCCs)

T-type channels are either absent or weakly expressed in bovine chromaffin cells (BCCs) and rat chromaffin cells [50–53] but can be expressed when RCCs are exposed to cAMP for several days [26,54] and after overnight incuba-

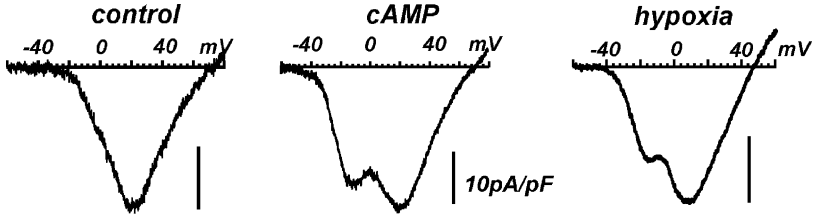

Fig. 1. Recruitment of α1H T-type currents after exposure to cAMP and chronic hypoxia. Ca^{2+} currents recorded during a ramp command from −60 to +60 mV from −80 mV holding potential in control (left), after 5 days treatment with 200 μM pCPT-cAMP (center) and overnight exposure to an hypoxic atmosphere (5% CO_2 and 3% O_2; right). Recordings were in whole-cell configuration (10 mM Ca^{2+}) for the center and left panel and in patch-perforated conditions (5 mM Ca^{2+}) for the right panel. Notice the second "low-threshold peak" associated with the newly recruited α1H T-type currents after pCPT-cAMP [54] and hypoxic treatment [55] (see also [56]).

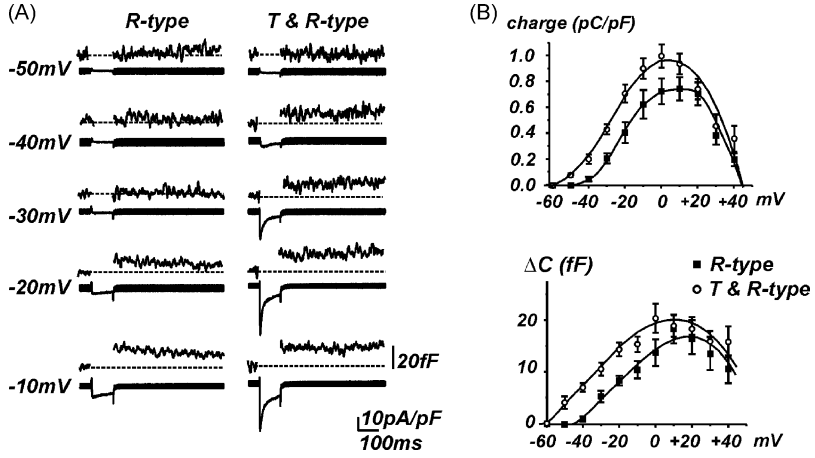

Fig. 2. "Low-threshold" capacitance changes associated with α1H T-type channels. (A) Secretory responses evoked by R- and T-type currents from a control RCC and after exposure to pCPT-cAMP recorded in patch-perforated mode. RCCs were incubated with ω-toxins and nifedipine was in the bath. (B) Quantity of Ca^{2+} charge and corresponding secretion measured as ΔC increases at the potentials indicated. The quantity of charge was calculated as the time integral of the current entering during the 100 ms depolarization (adapted from [26]).

tion in hypoxic conditions (3% O_2) [55] (Fig. 1) (see also [56]). Under these conditions, newly expressed α1H T-type channels appear effectively coupled to catecholamine secretion and produce rapid exocytic events that closely resemble those produced by HVA channels [57]. α1H T-type channels have, however, unique properties in controlling secretion that derive from their activation-inactivation gating. Secretion starts at significantly negative membrane potentials (−50, −40 mV) (Fig. 2(A)) and the voltage-dependence of ΔC's and quantity of charge (Q) do not show "double peaks"

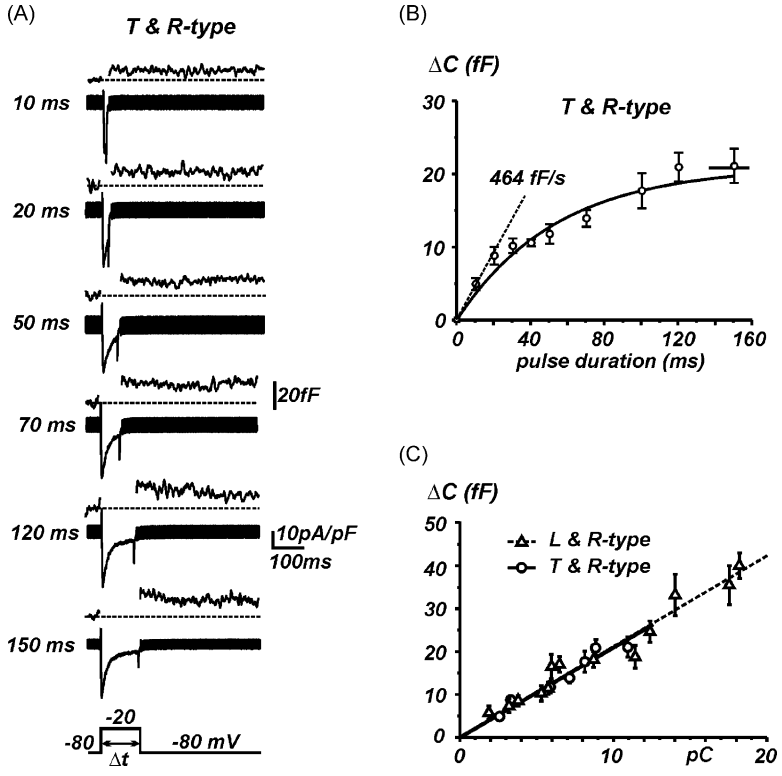

Fig. 3. Maximal rate of vesicle depletion mediated by α1H T-type channels in RCCs. (A) RCCs were pretreated with ω-toxins and nifedipine and depolarized with pulses of increasing length at −20 mV in order to progressively deplete the pool of vesicles ready for release. (B) Plot of ΔC increases vs. the corresponding pulse length. Data are averaged from 20 cAMP-treated cells as the one of panel (A). The solid curve is an exponential fit with $\tau = 54$ ms and maximal value indicated by the asymptote (21 fF). The dashed line drawn to fit the first two points at 10 and 20 ms has a slope of 464 fF/s and indicates the maximum rate of vesicle depletion (adapted from [25]). (C) ΔC's vs. the Q for cAMP-treated RCCs expressing T- and R-type channels and incubated in a medium containing ω-toxins and nifedipine (empty circles, solid line) compared to ΔC's induced by L- and R-type channels of control RCCs treated with ω-toxins (empty triangles, dashed line). The linear regressions have almost identical slope (adapted from [26] and [57]).

as for the *I/V* characteristics [1,2]. They rather exhibit a net broadening due to the constant contribution of T-type channels to both $\Delta C(V)$ and $Q(V)$ over a wide range of voltages (Fig. 2(B)). This derives from the weak voltage-dependence of the quantity of charges passing through transiently open T-type channels. The increased capacitance associated with T-type channels is effectively blocked by low concentrations of Ni^{2+} (50 μM), which also block α1H T-type currents [58]. Finally, long-term cAMP treatment and chronic hypoxia recruit T-type channels without altering the HVA channels density and their related secretion [26].

3.4. Rate of vesicle release, Ca^{2+}-dependence and pool size of immediately releasable vesicles coupled to α1H T-type channels

Despite α1H channels have unique gating properties they contribute to secretion with kinetics, Ca^{2+}-dependence and probability of release that are comparable to that of HVA channels normally expressed in RCCs. This is suggested by several observations. First, the time course of depolarization-evoked exocytosis is exponential, with an initial maximal rate of release of 464 fF/s (equivalent to ~464 vesicles/s) that decreases drastically with time (Fig. 3(A and B)). ΔC saturates with prolonged depolarizations because approaching complete mobilization of the immediately releasable pool (IRP) of vesicles and because of the fast inactivation of T-type currents that limits the quantity of charges for pulse >100 ms. Exponential time courses for the depolarization-evoked exocytosis are reported also for the HVA channels, with maximal rate of release of 580 fF/s [57] and 680 fF/s [59] for the same cell preparation. A second important observation is that the size of the IRP and probability of release (p) estimated by double-pulse protocols are comparable to those associated with HVA channels [26,57]. In particular, p is remarkably high (0.6 versus 0.68 for the HVA channels) indicating a high degree of coupling between T-type channels and release sites.

Finally, the Ca^{2+}-dependence of exocytosis evaluated by plotting ΔC's versus the corresponding quantity of Ca^{2+} charges (~2 pF pC^{-1}) is roughly linear and with identical slope to that estimated for the L-type channels (Fig. 3(C)). This indicates that T- and L-type channels couple with the same Ca^{2+} efficacy to exocytosis and that T-type channels contribute to the release of catecholamine at low voltages with the same efficacy that HVA channels contribute to the secretion at high voltages. Taken together, these findings indicate that T-type channels are effectively coupled to exocytosis in RCCs and that they contribute to secretion with the same efficacy of HVA channels normally expressed in these cells. Interestingly, T-type channels do not produce fast secretion coupling at all stages of chromaffin cell development. In embryonic RCCs, LVA channels are expressed in half of the cells but Ca^{2+} currents through these channels are unable to evoke sizable secretion [60].

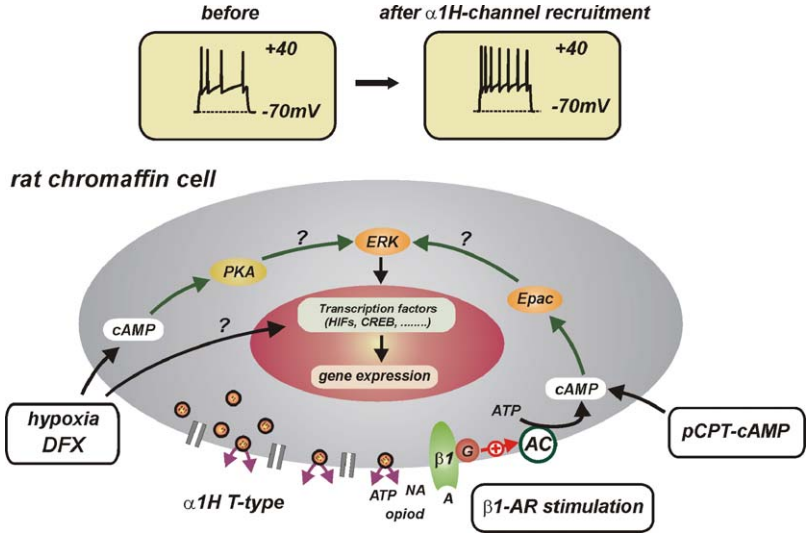

Fig. 4. Molecular pathways regulating α1H T-type channels recruitment in adrenal chromaffin cells during three different stress-mimicking conditions: exposure to pCPT-cAMP, β1-AR stimulation and chronic hypoxia. The model is based on experimental data supporting: (1) the existence of endogenous β1-AR in RCCs which rise the levels of cAMP when stimulated by isoprenaline [50]; (2) a marked recruitment of α1H T-type channels by cAMP and β1-AR stimulation via cAMP-receptor proteins (Epac) which lowers the threshold of RCCs excitability and increases spike frequency as illustrated (see [54]); (3) a recruitment of α1H T-type channels induced by chronic hypoxia and the hypoxia-mimicking agent deferoxamine (DFX) [55]; (4) an effective "low-threshold" secretion mediated by newly recruited α1H channels [26,55]; (5) the involvement of a cAMP/PKA pathway (Carabelli et al., unpublished results) and hypoxia-inducible transcription factors (HIFs) [56] in the hypoxia and DFX-induced up-regulation of α1H channels in RCCs and PC12 cells. The involvement of ERK in the activation of transcription factors (HIF, CREB, etc.) is hypothetical, as it is the pathway indicated by the arrow pointing directly to the nucleus. This alternative pathway is representative of the various hypoxia-induced signal transduction pathways that proceed regardless of cAMP activation [63].

4. Conclusions

This review aims at highlighting the increasing evidence for a role of T-type channels in the control of transmitter release in central neurons and neurosecretory cells. This new functional aspect becomes critical in pathological conditions in which overexpression of T-type channel enhances the ability of LVA channels to regulate Ca^{2+}-entry near resting conditions. In the case of chromaffin cells, expression of α1H T-type channels during exposure to cAMP [26], β1-adrenergic stimulation [54] and chronic hypoxia [55,56] represents a nice example of how T-type channels recruitment and coupling to exocytosis may generate a positive feedback that mimics the adrenal responses to stress stimuli (“fight-or-flight” response) [61,62].

As illustrated in Fig. 4, the marked recruitment of α1H T-type channels induced by exposures to cAMP, β1-adrenergic stimulation and hypoxia through either cAMP-dependent and cAMP-independent intracellular pathways [63] lowers the threshold of cell excitability and increases further the quantity of catecholamines (adrenaline and noradrenaline) secreted near resting potentials. In this way, the up-regulation of T-type channels effectively coupled to catecholamine release enhances the functional role of these channels in the positive feedback originating from the increased content of adrenaline and noradrenaline in single vesicles [64], the autocrine activation of β1-adrenoreceptors [50] and the increased levels of cAMP [54]. Cyclic AMP in turn activates transcription factors, gene expression and protein synthesis that are at the basis of the adaptive responses to stressful conditions [61].

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