cAMP and β-adrenergic stimulation regulate L-type channel gating and exocytosis in rat chromaffin cells.

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In rat chromaffin cells (RCCs), L-channels carry ~50% of the total Ca^{2+} current and are effectively inhibited by the neurotransmitters released by their own secretory granules. This inhibition is membrane-delimited and mediated by G protein-coupled receptors co-localized with L-channels in membrane microdomains. The inhibition is reversed when intracellular cAMP is raised or after exposure to isoprenaline (β-AR-stimulation), suggesting the existence of parallel and opposite effects on L-channel gating by distinctly activated membrane autoreceptors. Rising intracellular cAMP by either applying the membrane permeable analogue pCPT-cAMP or through β-AR stimulation induces multiple effects on RCCs: *i)* a 20% increase of L-currents, *ii)* a 100% potentiation of the depolarization-evoked secretion occurring downstream of Ca^{2+}-entry and *iii)* a slow recruitment of T-type channels following long-term exposures of permeable cAMP or isoprenaline. This multiform action of pCPT-cAMP appears to nicely counterbalance the inhibitory effect on L-channels and argues in favor of an effective increase of catecholamine secretion and cell excitability under conditions of sustained cell stimulation with minor increases of intracellular Ca^{2+}.

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Cell Biology of the Chromaffin Cell
R. Borges & L. Gandía Eds.
Instituto Teófilo Hernando, Spain, 2004

Modulation of Ca^{2+}-entry through voltage-gated L-channels may occur in various ways: by down regulating or recruiting newly available channels or by inhibiting or facilitating the channel gating[1-3]. L-channel gating modulation has received great attention in the last two decades and appears an effective system for controlling Ca^{2+} ions entering the cell. Among the many modulatory pathways, two appear of particular interest because of their autocrine nature: the G protein-dependent inhibition and the cAMP/PKA-mediated potentiation[1-3]. In chromaffin cells, both pathways are activated by autoreleased neurotransmitter molecules and produce opposing effects of comparable entity[4]. The inhibition is complete within few seconds and is mediated by PTX-sensitive G proteins coupled to P_{2y}-purinergic, δ/κ-opioidergic, α_2- and β_2-adrenergic receptors[5-10]. In contrast, the potentiation is selectively triggered by β_1-ARs and occurs slowly through the activation of a cAMP/PKA pathway, which may act at distant sites from receptors[11].

An interesting issue is whether Ca^{2+} changes associated to L-channel modulation play a critical role in the control of exocytosis. Recent observations suggest that down- or up-modulation of L-currents do not always produce proportional effects on exocytosis indicating that intracellular [Ca^{2+}] increases following Ca^{2+} channels activation are preliminary to secretion but may be either amplified[12] or depressed[13] by a downstream action on the secretory machinery. Here, besides discussing the molecular features of cAMP-mediated potentiation and G protein–induced inhibition of L-channels via β_1-ARs and β_2-ARs stimulation, we will focus on some recent findings concerning L-channel modulation and its coupling to secretion.

The β1- and β_2-ARs modulation of L-channels: an example of direct and remote signaling pathways in chromaffin cells. As recently shown, the $G_{i,o}$ protein-dependent inhibition of L-channels in RCCs is not limited to opioidergic, purinergic and α_2-adrenergic autoreceptors[3]. There is in fact evidence also for the involvement of β-ARs[9]. Thus, the question is whether there is a rationale for the presence of β-ARs, which can up- or down-modulate the major Ca^{2+} current component controlling neurotransmitter release in RCCs. The answer comes from recent findings in which RCCs are shown to

express two distinct β_1- and β_2-AR activated signaling pathways[4]. The β_1-AR cascade acts by selectively up-regulating the L-channel through a PKA-mediated pathway and develops slowly due to its diffusive characteristics. On the contrary, the β_2-AR signaling is fast and primarily coupled to PTX-sensitive G proteins. Fig.1A shows an example in which a RCC responds to ISO stimulation with rapid inhibition of Ca^{2+} currents followed by a slow potentiation. The final balance is a slight increase of Ca^{2+} currents, which allows the cell to elevate cAMP concentration, with little increases of Ca^{2+} fluxes. This is different from the β-AR stimulation of cardiac cells in which the cAMP/PKA stimulation is accompanied by a marked amplification of Ca^{2+} fluxes required for increasing the strength of cardiac contraction during sympathetic discharges[14].

An interesting aspect of the β-ARs modulation in RCCs is the peculiar role of β_2-ARs, which are directly coupled to an inhibitory PTX-sensitive G protein pathway and are unable to produce L-currents potentiation through the activation of adenylate cyclase, as in cardiac myocytes[15]. Fig.1B shows that zinterol (a β_2-AR selective agonist) does not produce the slow stimulatory effect of isoprenaline, but causes fast inhibition of Ca^{2+} currents. Sequential application of zinterol and isoprenaline nicely mimics the effects of isoprenaline alone; indicating that complete activation of β_2-ARs produces an inhibition (direct action) followed by a slow potentiation mediated by β_1-ARs (remote action) (see Fig.1C).

L-channel modulation and exocytosis. An interesting issue concerning L-channel modulation is how these mechanisms interfere with the exocytotic cell activity. Excitation-secretion coupling in chromaffin cells is triggered by elevations of cytosolic [Ca^{2+}] mainly associated to Ca^{2+} influx through voltage-gated Ca^{2+} channels. Thus, modulation of L-channel gating by neurotransmitters may represent an effective mechanism for regulating secretory responses. This is particularly critical in rat, mouse and human chromaffin cells, which express high densities of L-channels[16]. However, modulation by neurotransmitters may occur also directly on the secretory machinery (downstream of Ca^{2+}-entry), thus introducing a further degree of variability to the phenomenon.

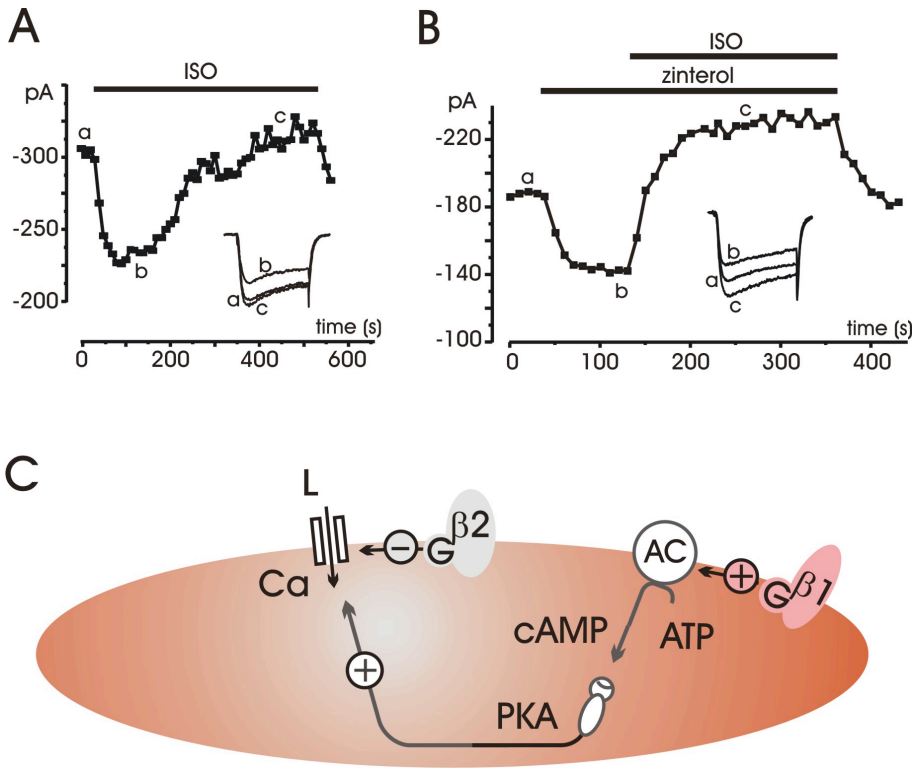

Fig. 1. Sequential inhibition and potentiation of L-type Ca^{2+} currents during β_2- and β_1-ARs stimulation in RCCs. **A)** Isoprenaline alone (ISO, 1 μM) causes a rapid inhibition and a slow recovery of L-current amplitude. **B)** Addition of isoprenaline following the fast inhibition induced by zinterol (selective β_2-ARs agonist; 1 μM) induces a marked potentiation of L-currents. The symbols are peak current amplitudes measured during a 25 ms step depolarization to +10 mV repeated every 10 sec (holding potential –40 mV). The insets show the original recordings taken at the time indicated. Modified from ref [4]. **C)** Schematic drawing of the signaling pathways associated to β_1- and β_2-ARs stimulation on a RCC.

The effects of neurotransmitters on Ca^{2+} currents and secretion have been studied in bovine chromaffin cells (BCCs) and RCCs by combining membrane capacitance measurements and whole-cell current recordings[13,17,18]. Most of the works point to a marked inhibition of ATP on L- N- and P/Q-type currents and a proportional inhibition of exocytosis. In BCCs, ATP neither alters the Ca^{2+}-

dependent fusion of vesicles to the plasma membrane nor the vesicle supply to release sites, thus confirming that the ATP-induced inhibition of exocytosis is primarily associated to its action on Ca^{2+} channels[17,18]. The action of ATP appears more complex in RCCs[13]. ATP inhibits exocytosis by either depressing Ca^{2+} currents (L, N and P/Q) or by directly acting on the secretory machinery through a Ca^{2+}-independent pathway. The latter occurs independently of Ca^{2+} channels and accounts for most of the inhibitory effect on exocytosis induced by ATP.

The cAMP/PKA-mediated potentiation of secretion and L-channel gating. The effects of cAMP on secretion in chromaffin cells are quite heterogeneous. Some reports point to a marked increase of basal and stimulus-evoked secretion from adrenal chromaffin cells together with an increased Ca^{2+}-entry through L-channels induced by cAMP, PACAP and forskolin[19-22]. On the contrary, other data support the existence of an inhibitory action of cAMP on nicotine-induced release and Ca^{2+} currents in BCCs[23,24]. In some works, L-channels and membrane voltage are shown to play an exclusive role in increasing the stimulus-induced secretion by cAMP[25], while in others the role of these components appears more limited or unnecessary[20,26]. Among this complex pattern of responses, the contribution of Ca^{2+} channels and their modulation by cAMP in the control of exocytosis has been recently investigated in RCCs[12]. The cAMP permeant analog pCPT-cAMP is found to potentiate both the L-currents and the depolarization-evoked secretion, but the current increase accounts for only 20% of the total secretory response. cAMP doubles the size of the readily-releasable pool (RRP) of vesicles by almost doubling the mean size of unitary exocytic events (from 1.1 to 2.1 fF), without affecting the probability of release. cAMP potentiates the secretion independently of the activated Ca^{2+} channel type and the same effects are induced by β_1-AR stimulation through a PKA-mediated pathway.

CONCLUSIONS

Given the critical role of L-type Ca^{2+} channels in controlling cell excitability and neurotransmitter release, it is not surprising that this class of channels is extensively regulated by a number of signaling

pathways. The work of the last five years has shown that neuroendocrine L-channels undergo a marked autocrine modulation induced by the material released during cell activity, causing either inhibition or potentiation of the Ca^{2+} current controlling exocytosis. The most original aspect of this action is the existence of a direct G protein-mediated inhibition of L-channels, which co-exists with the classical up regulation mediated by the cAMP/PKA-signaling cascade. In chromaffin cells, these opposing modulations of L-channels have the advantage of allowing the increase of intracellular cAMP by means of β-ARs stimulation, with consequent increased exocytosis and recruitment of newly synthesized T-type channels[27], without significantly altering the intracellular Ca^{2+} levels. The overall effect of L-channels auto-regulation by released neurotransmitters (adrenaline, noradrenaline, ATP and opioids) is therefore a remodeling of cell excitability and an enhancement of neurotransmitter release with little changes to intracellular [Ca^{2+}], which may result deleterious for cell survival during maximal sympathetic stimulation and sustained catecholamine release.

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