The secretory responses to choline and acetylcholine show different patterns and calcium dependence in chromaffin cells.

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Acetylcholine (ACh) is the physiological neurotransmitter at central and peripheral cholinergic synapses[1], and choline its catabolic metabolite; However, the role of choline in these synapses was changing in the time, and this change is due to the discovery of various activities of choline i.e. muscarinic effects on brain neurons[2], secretion of catecholamines from bovine adrenal medullary chromaffin cells[3], inward currents in neurons[4], or the increase of the cytosolic concentration of calcium ($[Ca^{2+}]_c$) in sympathetic neurons[5]. In oocytes expressing different subtypes of neuronal nicotinic receptors for ACh (nAChR)[6], choline does not activate $\alpha4\beta2$ nor $\alpha3\beta2$ receptors; it is, however, a partial agonist of several heteromeric receptors, including the $\alpha_3\beta_4$ subtype, and behaves as a full agonist at homomeric $\alpha7$ receptors[7].

In the study presented here, we used choline effects in chromaffin cells, a cholinergic neuronal model; using choline as a selective agonist for $\alpha7$ receptors and ACh as a non-selective agonist for $\alpha7$ and $\alpha3\beta4$ receptors, as well as high $[Ca^{2+}]_e$ solutions in order to further characterize the functional role of nicotinic receptors in bovine chromaffin cell. We compared the secretory responses, the confocal $[Ca^{2+}]_c$ signals and the changes in membrane potential elicited by choline and ACh in bovine chromaffin cells.

RESULTS

Ca^{2+}-depencence catecholamine release induced by choline. The catecholamine secretory response was studied in cell populations of bovine chromaffin cells. Choline, ACh and K^+ (as a functional control) were applied during 5-s, at 1 min intervals. Choline secretory responses exhibited a strong dependence on the extracellular concentration of Ca^{2+} ($[Ca^{2+}]_e$), those of ACh showed less dependence, and those of K^+ exhibited little Ca^{2+} dependence (fig.1A: 1 mM Ca^{2+}; fig 1B: 20 mM Ca^{2+}). The summary of pooled data is shown in the figure 1C (range 1-20 mM Ca^{2+}).

Intracellular Ca^{2+} increments elicited by choline and ACh, measured by confocal imaging in chromaffin cells. Cytosolic ($[Ca^{2+}]_c$) and nuclear ($[Ca^{2+}]_N$) confocal images of increments in the Ca^{2+} levels associated to patch clamp inward currents produced by

choline and ACh pulses given in high $[Ca^{2+}]_e$ solutions were measured. Under these conditions, changes in intracellular calcium levels are due to calcium entry through nicotinic receptors, especially α7 subtype that shows a high permeability to this cation.

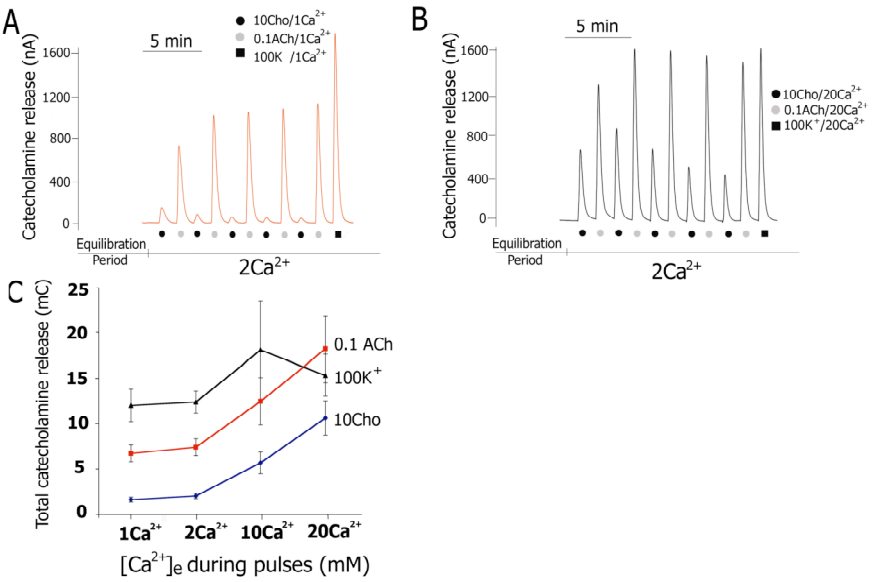

Figure 1: Cells were challenged at 1-min intervals with alternate 5-s pulses of a Krebs-Hepes solution containing 1 mM Ca^{2+} (panel A, 10Cho/1Ca^{2+}) or 20 mM Ca^{2+} (panel **B**, 10Cho/20Ca^{2+}). After applying 5 pulses of choline alternating with 5 pulses of ACh, a final pulse was given with a Krebs-Hepes solution containing 100 mM K^+ (with isoosmotic reduction of Na^+). Panel **C** shows pooled results on total secretion elicited by the three secretagogues, in the presence of four $[Ca^{2+}]_e$ tested. The secretion areas were calculated in each individual experiment for each stimulus and expressed in nA.s (μC). Data are means±SE, n=30 performed with at least 6 different batches of cells.

The holding potential was fixed at -80 mV. Figure 2A shows original inward current traces elicited by the application of 1-s pulses of ACh (0.1 mM) and Cho (10 mM). Panel B shows the changes in the fluorescence taken from an equatorial plane of the cell, before and after the applications of the agonist pulses. Panel C shows the differences on increments in $[Ca^{2+}]_c$ and $[Ca^{2+}]_N$ generated by choline and ACh pulses in different areas of the cytosol and the nucleus. Panel

D shows the pooled data of inward current generated by choline referred to the inward current elicited by 0.1 mM ACh (as %). Panel E shows the pooled data on cytosolic and nuclear calcium levels. Choline increased the $[Ca^{2+}]_c$ as well as the $[Ca^{2+}]_N$; the $[Ca^{2+}]_c$ induced by choline was about 20% of that induced by ACh and the $[Ca^{2+}]_N$ induced by choline about 70% of that of ACh.

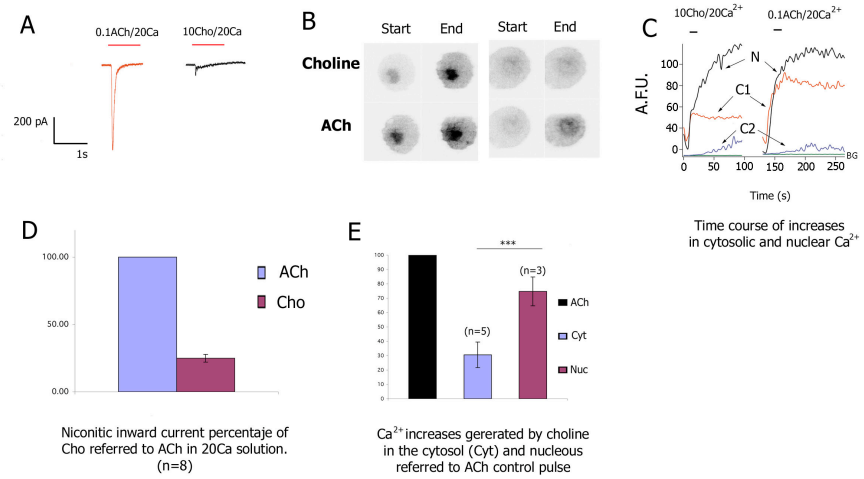

Figure 2: Choline increased the $[Ca^{2+}]_c$ as well as the $[Ca^{2+}]_N$. Cytosolic and nuclear confocal images of increments of the $[Ca^{2+}]_c$ associated to patch clamp inward currents produced by choline and ACh pulses given in high $[Ca^{2+}]_e$ solutions. Were recorded by using Fluo3 (5 µM), and acquired every 350 ms. Panel **A** shows original inward current traces elicited by application of 1-s pulses of 0.1ACh/20Ca^{2+} and 10Cho/20Ca^{2+} solutions. Panel **B** shows the changes in the fluorescence before and after the application of the agonist pulses. Panel **C** shows the differences on increments in $[Ca^{2+}]_c$ and $[Ca^{2+}]_N$ generated by choline and ACh pulses in different areas of the cytosol and the nucleus. Panel **D** show the pooled data of inward current generated by choline referred to ACh (as %). Panel **E** shown the pooled data of cytosolic and nuclear calcium increases of all cell (n=5 for cytosol and n=3 for nucleus).

Effects of choline and ACh on the membrane potential of chromaffin cells. Under the current clamp configuration of the whole cell patch clamp technique, we observed that in contrast to ACh, 10 mM choline (in low Ca^{2+} solutions) produced little or no depolarization, while ACh induce a large depolarization of membrane potential (fig. 3C-D). Those results change drastically when we

increase the extracellular Ca^{2+}; in these conditions, choline induce the same first phase of little or no depolarization followed by a second phase of strong hyperpolarization, while ACh only induced moderated hyperpolarizing phase (fig3. A-B).

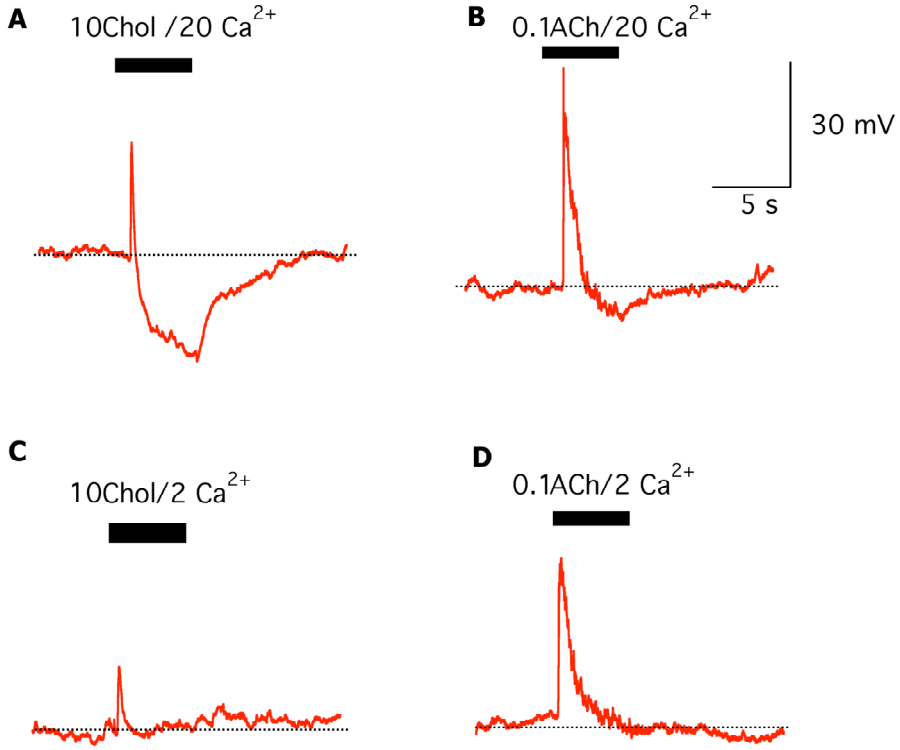

Figure 3: Original recordings of changes in membrane potential induced by nicotinic agonist applied in high-Ca^{2+}-solution (panels A-B) or low-Ca^{2+}-solution (panels C-D), choline elicited an hyperpolarizing response while ACh evoked a depolarizing followed a hyperpolarizing response. The high permeability of α_7 nicotinic receptors to Ca^{2+} ions could explain the pronounced hyperpolarization observed when 10Chol/20 Ca^{2+} was applied by means of the activation of co-localized Ca^{2+}-dependent K^+ channels.

In conclusion, choline acting as full agonist of $\alpha 7$ nAChR, shows striking differences respect to ACh, concerning their ability to generate depolarizing or hyperporalizing changes of the membrane

potential, inward currents, Ca^{2+} signals and exocytosis in bovine chromaffin cells. While the responses of choline were strongly dependent upon the $[Ca^{2+}]_e$, those of ACh were much less dependent on the changes of this cation. These differences are interpreted on the basis of a preferential selective activation of the highly Ca^{2+}-permeable α7 nicotinic receptor subtype, elicited by choline; this leads to activation of Ca^{2+}-dependent K^+ channels of the SK subtype, and to the choline hyperpolarizing response. These findings illustrates a novel mechanism of action of choline, that may be highly relevant to substantiate the proposal that it may have a physiological role in synaptic controlling neurotransmission

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