Does an homomeric α7 nicotinic receptor exist in bovine chromaffin cells?

Jonathan Rojo[a], Victoria Maneu[a], Laura Tapia[a], J.María González-Rubio[a], Ricardo de Pascual[a], José Mulet[b], L. Miguel Valor[b], Francisco Sala[b], Manuel Criado[b] and Luis Gandía[a]

a. Instituto Teófilo Hernando. Departamento. de Farmacología y Terapéutica. Facultad de Medicina. Universidad Autónoma de Madrid.

b. Instituto de Neurociencias. Universidad Miguel Hernández. Campus de San Juan. San Juan. Alicante, Spain.

Correspondence: Dr. Luis Gandía, Instituto Teófilo Hernando.
Dpto. de Farmacología y Terapéutica. Facultad de Medicina.
Universidad Autónoma de Madrid. Arzobispo Morcillo 4, 28029 Madrid, Spain.
Phone: 34-91-4975396; **Fax**: 34-91-4975380; **Email**: luis.gandia@uam.es

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The bovine adrenal chromaffin cell offers a unique model to explore the question of whether various subtypes of native and heterologously expressed neuronal nicotinic receptor subtypes share the same properties, as far as their ability to form a functional ion channel pore is concerned. This is so because, although from the molecular point of view various of the known subunits for the nAChR (α3, α5, α7, β4) seem to be present in bovine chromaffin cell[1-3], the subunit composition of the native receptor is still unknown, but it is assumed that it is formed by α3β4 and α7 independent receptors.

RESULTS AND DISCUSSION

In a previous work of our group[4], using short agonist applications (1s) and selective toxins, we see that the native nicotinic currents was generated by the simultaneous activation of a mixed pool of homomeric α7 and heteromeric α3β4 nicotinic acetylcholine receptors (nAChR); So the nicotinic native currents of the bovine chromaffin cells (BCC) should be similar to the nicotinic currents obtained when *Xenopus* oocytes are injected with the nAChRs present in the bovine chromaffin cells.

In this study, we intended to contribute to the knowledge of the neuronal nicotinic receptor composition in the BCC by comparing the effects of known nonselective nAChR agonist with those of selective α7 nAChR agonist. When we use different nonselective agonists (ACh, DMPP, Nicotine, and Epibatidine) we found that, ionic currents through nAChRs, measured with the whole-cell configuration of the patch-clamp technique in BCC, showed similar activation and inactivation kinetics with all nonselective agonist employed, and this kinetics are dependent on the agonist concentration employed. When we did the dose response curves with the different agonists and normalized the data with respect to the currents obtained in the same cell with 100 μM ACh, we observed a similar efficacy for Epibatidine, DMPP, and ACh, while nicotine showed 50% efficacy; This value was unexpected, and could not be explained because of receptor desensitization, because epibatidine is much more desensitizing than nicotine and appears to be as efficacious as ACh and DMPP. On the other hand the order of potencies for the different agonists was Epibatidine>DMPP>Nicotine>ACh. Surprisingly when we applied the

α7 selective agonists choline and 4-OH-GTS21, the response was less than 5% that induced by ACh 100μM; and when the current was evoked by cytisine, an α3β4 selective agonist, the current was only about 10% of the I_{ACh}.

On the other hand when the bovine nicotinic receptors α7 and α3β4 where expressed heterolousgy in *Xenopus* oocytes, neither of them showed a similar activation and inactivation kinetics that the present in CCB. Besides the selective agonists choline and 4-OH-GTS21 were check on bovine α3β4 and α7 nicotinic receptors heterolousgy expressed in *Xenopus* oocytes, and in this case the selective agonists activated only the α7 nicotinic receptors, producing an inward current comparable to that induced by ACh 100 μM, and not activated the α3β4 receptors. These experiments suggest that α7 subunits are not forming functional homomeric α7 receptor in bovine chromaffin cells and that they could be part of an heteromeric α3β4α7* nAChR.

Due to this surprised data and the absence of other pharmacological tools, we decided to further investigate the nature of the native nAChR to null the function the one of the other subtype of nAChR by incubing chronically the cells with selective antibodies against α3 subunits (mAb35) and against α7 subunits (H-302) (24-48h) of the nAChR. The results show a similar decline of the nicotinic response in both cases. In Addition, the catecholamine secretory response in cell population treated with specific antibodies against α3 or α7 subunits showed a similar decreased of ACh-evoked secretory responses, unrelated to the specific antibody used.

These results further support the presence of a heteromeric complex receptor formed by the combination of α3, α7, α4, and/or α5 subunits; this conclusion disagrees with the current hypothesis that assumes the existence of separated α 7 homomeric and α3β4 heteromeric receptors, based only in functional studies carried out in bovine Chromaffin cells.

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