Choline as a tool to evaluate nicotinic receptor function in chromaffin cells.

Juana M[a] González-Rubio[a], Jonathan Rojo[a], Laura Tapia[a], Victoria Maneu[d], José Mulet[c], Luis M. Valor[c], Manuel Criado[c], Francisco Sala[c], Antonio G. García[a,b] and Luis Gandía[a]

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

b. Servicio de Farmacología-Unidad Clínica and Instituto de Gerontología, Hospital de la Princesa, Madrid

c. Instituto de Neurociencias, Universidad Miguel Hernández-CSIC, Sant Joan d'Alacant,

d. Departamento Interuniversitario de Óptica. Universidad de Alicante. San Vicente del Raspeig, 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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Choline is present in the synaptic cleft as a result of the rapid degradation of acetylcholine (ACh) by acetylcholinesterase (AChE). The hypotesis that choline will exert some modulatory function of synaptic neurotransmission is supported by few studies; for instance, choline has muscarinic effects on central neurons[1], evokes catecholamine release by acting as a partial agonist at neuronal nicotinic receptors for ACh (nAChRs), and blocks partially the release of catecholamines evoked by ACh, in cultures of bovine adrenal medullary chromaffin cells[2]. Choline has been also shown as a selective agonist of α7 nAChRs in brain neurons[3]. As far as we know, a detailed study of the effects of choline on the kinetic properties of inward currentes through α7 and α3β4 nAChRs is not available. Hence, we decided to express homomeric α7 and heteromeric α3β4 bovine nicotinic receptors in *Xenopus* oocytes and study the effects of choline on these receptors. We report here the results of such study that might be relevant in the frame of the growing number of physiological and physiopathological roles that are being adscribed to brain nAChRs, i.e. in neuroprotection, analgesia, addiction to nicotine, dementia or behaviour[4,5].

RESULTS AND DISCUSION

Application of brief (1-5 s) pulses of **c**holine (0.3-10 mM) elicited inward current in oocytes expressing α7 nAChRs, acting as a full agonist of these receptors (Figure 1) with an EC_{50} of about 0.6 mM. In contrast, in oocytes expressing α3β4 nAChRs, choline did not elicit any inward current by itself. However, choline blocked the inward current through these receptors, induced by 5-s pulses of 0.1 mM ACh (I_{ACh}) (figure 2), with an IC_{50} of about 0.38 mM. This blockade was quickly reversible after choline washout. The nature of I_{ACh} blockade by choline was further tested by challenging the oocytes with increasing concentrations of ACh (from 10 μM to 3 mM). In these experiment, α3β4 oocytes were sequentially stimulated with 5-s pulses of increasing concentrations of ACh, given first in the absence and subsequently in the presence of 1 mM choline. The blockade induced by choline was around 65-70% in all cases, suggesting a non-competitive nature of the blockade, as the case is for hexamethonium[6].

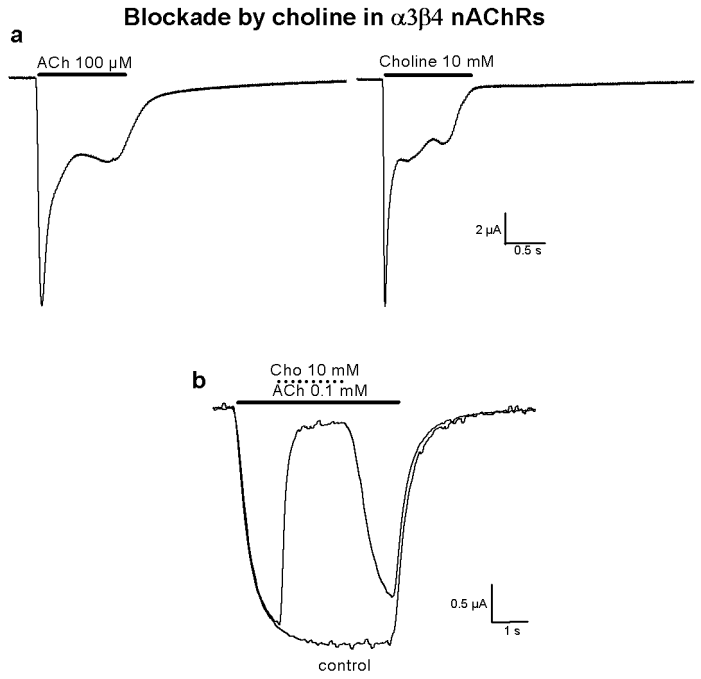

Figure 1. Effects of choline α7 and α3β4 nAChRs expressed in xenopus oocytes. a) Application of choline (10 mM) elicited an inward current in α7 nAChRs, acting as a full agonist of this receptor. In the same oocyte, ACh 0.1 mM elicited similar inward current. **b)** In an oocyte expressing α3β4 nAChRs, ACh 0.1 mM is capable to elicit a response (control) that was fully blocked by choline 10 mM. The blockade of the current was partially recovered when choline was removed from the perfusion solution.

We also wanted to study if the blockade induced by choline of I_{ACh} exhibited voltage-dependence, as has been decribe for different blockers of nAChRs[7-9]. For it, a voltage ramp protocol (-100 mV to +60 mV) was applied to α3β4 oocytes voltage-clamped at –80 mV and pulses of ACh (0.1 mM, 5-s) were applied either in the absence or in the presence of increasing concentrations of choline. The I-V curves for the traces are plotted, and calculations were made to estimate the degree of current blockade achieved by each concentration of choline at different voltages. That revealed that I_{ACh} blockade by choline was more pronounced at more hyperpolarised

potentials, as compared to depolarised potentials, in all of the three concentrations of choline tested.

Ours results reported here shown that, with a similar concentration range, choline exerted dual opposite effects in the two main receptor subtypes of nAChRs expressed by bovine chromaffin cells, α7 and α3β4[10-12].

The blockade of I_{ACh} through α3β4 receptors was voltage-dependent and was of a non-competitive nature, suggesting that choline had a binding site different to that for the agonist ACh, i.e. choline may bind to the ACh locus on the α7 receptor to cause its activation and to a site different to that of ACh on the α3β4 receptor to cause its blockade. The similar concentration at which choline exerts its dual action on α7 receptor activation and α3β4 receptor blockade, reinforces the hypothesis that choline has a role beyond that of being the simple precursor of ACh.

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