α7 NICOTINIC ACETYLCHOLINE RECEPTORS PROMOTE RYANODINE-SENSITIVE Ca^{2+}-INDUCED Ca^{2+} RELEASE IN PC12 CELLS

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Neuronal nicotinic acetylcholine receptors (nAChR) are ligand-gated channels that can elicit changes in intracellular Ca^{2+} (Dajas-Bailador et al., 2002). In this study the sources of Ca^{2+} that give rise to α7 nAChR- and non-α7 nAChR-mediated increases in Ca^{2+} have been examined in PC12 cells.

PC12 cells were cultured in 96 well plates, pre-loaded with Fluo3 AM and stimulated with drugs in the presence or absence of blocking agents. Changes in fluorescence were measured (F538) for 20 s. Nicotine (100 μM) produced a rapid increase in fluorescence, which was decreased by 19.8 ± 2.2 % in the presence of the α7 nAChR antagonist α-bungarotoxin (αbgt 100 nM, n = 4). However, application of the selective α7 nAChR agonist compound A ((R)-N-(1-Azabicyclo[2.2.2]oct-3-yl)(5-(2-pyridyl)thiophene-2-carboxamide)), 0.1 nM – 10 μM, failed to elicit a response. Pre-incubation for 2 min with the α7 nAChR-selective positive allosteric modulator PNU 120595 (10 μM; Hurst et al., 2005) resulted in rapid increases in fluorescence upon application of compound A (10 nM). PNU 120596 did not increase fluorescence when added alone, nor did it significantly enhance responses evoked by KCl (60 mM, 97.4 ± 4.7 % control) or the non-α7 nAChR agonist 5-iodo-A-85380 (30 μM, 103.1 ± 21.7 % control) ($P > 0.05$; one-way ANOVA with post-hoc Dunnett's test, n = 5).

Compound A in the presence of PNU 120596 evoked increases in fluorescence that were equally sensitive to αbgt (100 nM) and the non-selective nAChR antagonist mecamylamine (mec, 20 μM); 86.0 ± 1.4 % and 78.5 ± 7.0 % decrease, respectively (n = 5, $P < 0.01$; one-way ANOVA, post-hoc Dunnett's test), consistent with stimulation of α7 nAChR. 5-iodo-A-85380-evoked increases were insensitive to αbgt, but significantly decreased by 78.2 ± 5.1% in the presence of mec (n = 5, $P < 0.01$; one-way ANOVA, post-hoc Dunnett's test).

α7 nAChR-mediated increases in fluorescence were unaltered in the presence of 50 μM Cd^{2+} or specific voltage operated Ca^{2+} channel (VOCC) inhibitors ω-conotoxin GVIA (1 μM; N-type), ω-conotoxin MVIIC (1 μM; N-,P-, Q-type) or verapamil (10 μM; L-type). In comparison non-α7 nAChR-mediated increases in fluorescence were insensitive to N-, P- and Q-type specific VOCC inhibitors but were significantly decreased in the presence of 50 μM Cd^{2+} (70.8 ± 12.7 % decrease, n=4, $P < 0.01$, one-way ANOVA with post-hoc Dunnett's test) and 10 μM verampamil (78.6 ± 6.0 % decrease, n = 5; $P < 0.01$, one-way ANOVA with post-hoc Dunnett's test). α7 nAChR-mediated increases in fluorescence were significantly decreased in the presence of ryanodine (30 μM, 66.6 ± 7.6 % decrease, n = 4, $P < 0.01$, one-way ANOVA with post-hoc Dunnett's test), which did not significantly alter non-α7 nAChR-mediated responses (16.8 ± 6.2 % decrease). These data suggest that activation of α7 nAChRs results in Ca^{2+} entry through its intrinsic ion channel that subsequently elicits Ca^{2+} induced Ca^{2+} release. This mechanism is distinct from that of non-α7 nAChR.

F. Dajas-Bailador *et al*., (2002) *J. Neurochem* **81**:606-614; Hurst *et al.,* (2005) J. *Neurosci.,* **25**(17):4396-405.

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