**NICOTINE INDUCES [3H]-GABA RELEASE VIA NMDA RECEPTORS ACTIVATION IN THE DEVELOPING AVIAN RETINA**

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**Introduction:** Nicotine (Nic) is a classical agonist of cholinergic nicotinic receptors (nAChR). During retinal development, these receptors are usually expressed in the retinal layers, playing an important role in GABA circuitry, functioning, and organization. Our aim is to evaluate if an acute stimulation of nAChR is able to modulate [3H]-GABA transport in chicken embryos retinas staged E12.

**Methodology:** 96 retinas were used for [3H]-GABA uptake and release assays. They were conditioned on saline (Control) or exposed to Nic (Treated). Initially, we evaluated [3H]-GABA uptake without Na+, at 4ºC or after NO-711 (50μM) exposure 5’ before the assay. [3H]-GABA uptake at different times of incubation (5’, 10’, 15’ and 30’) with Nic 50µM was also evaluated. We performed a temporal (1’, 5’, 10’, 30’, and 60’) and Nic concentration (1, 10, and 50µM) curve. [3H]-GABA release was performed in both groups with or without MK-801 (50μM). One-way ANOVA followed by Bonferroni post-test were performed for results with 3 or more groups and unpaired t-test for results with 2 groups. Data were represented as mean ± SEM, % of the control group or in % of total. Statistical significance was achieved at p < 0.05. The project was approved in CEUA#IBCCF038/19.

**Results:** We observed that in the absence of Na+ ions, low temperature or in the presence of NO-711, [3H]-GABA uptake was completely blocked (Ctrl=99,83% ± 5,11, without Na+=12,76% ± 1,36, 4ºC=9,56% ± 2,91, NO-711=25,68% ± 1,77; % of control; p < 0.05, n=4). Further, we analyzed GABA uptake in the presence of Nic (1, 10 and 50 µM). We observed that Nic 50µM was able to inhibit 47% of [3H]-GABA uptake (Ctrl=99,83% ± 5,11, Nic 1µM=103,5% ± 7,83, Nic10µM=109% ± 4,03, Nic50 µM=53,83% ± 3,05; % of control; p < 0.05, n=4). In addition, we observed that Nic 50µM was able to reduce [3H]-GABA uptake after 10 minutes of incubation (Ctrl=99,83% ± 5,11, 5’=113,10 ± 6,62%, 10’=59,41 ± 8,64%, 15’=59,25 ± 9,74%, 30’=53,83 ± 3,05%; % of control; p < 0.05, n=3-6). The uptake of [3H]-GABA of the control group was approximately 209 fmol/mg/hour). We also noticed that after 30’ the temporal curve reached an equilibrium of [3H]-GABA uptake (1’=8±8, 5’=35±7, 10’=58±2, 30’=179±22, 60’=165±24, (fmol/mg/hour); p < 0.05, n=8). On the other hand, Nic 50µM enhanced in more than twofold the [3H]-GABA release (Ctrl=0,92 ± 0,10, Nic=2,01 ± 0,11; % of total; p < 0.05, n=4). Surprisingly, MK-801 was able to prevent the effect of Nic in [3H]-GABA release, turning it into similar control values (Ctrl=0,92 ± 0,10, MK-801=0,87 ± 0,10, Nic=2,01 ± 0,11, MK-801 + Nic=0,68 ± 0,10; % of total; p < 0.05, n=4).

**Conclusion:** We conclude that GABA uptake is mediated by GAT-1 and this transporter can be modulated by nicotinic receptors since Nic was able to regulate GABA uptake and release. Moreover, we also identified that Nic acute exposure was able to release [3H]-GABA via NMDA receptors activation.

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