FTOX015

Evaluation of uptake glutamate in cultures of retina cells in the presence of cunaniol.

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Introduction: In 1968, Quilliam & Stables ictiotóxica showed activity in fish tank extracts derived from *Clibadium sylvestre*. Costa et al (2006) demonstrated the seizure activity in mice caused by ethanol extract of *Clibadium surinamese*, and that the substances causing majority of excitatory activity correspond to acetate and cunaniol cunaniol. However, the proconvulsant mechanisms remain unknown (Hamoy, 2002 and Costa et al, 2006). Thus we sought to evaluate the activity of cunaniol in vitro and determine possible changes in glutamate uptake in cultured retinal cells of chick embryo. **Methods**: Culture were made (C7) in the retina of the chick embryo, cells were incubated at 37 °C and washed with Hanks' solution (pH= 7.4) containing the following composition (120 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, $CaCl_2$ 2 mM glucose 12 mM and 20 mM HEPES), and then preincubated for 10 minutes with the following concentrations cunaniol 0,1, 0,2, and 0,4 mM. After pre-incubation the uptake assays were performed in Hanks' solution containing 0,5 µCi of [3H] - glutamate for 5 minutes. Uptake was blocked by washing with ice Hanks' solution, and then the cells were lysed with 300 µl of 5% trichloroacetic acid (TCA). After 30 minutes (TCA), the cells were removed and analyzed in a scintillator for determining the radioactivity, each experiment was done in triplicate. The experimental values of the samples were corrected for amounts of protein by the Lowry method (1951), using it to a standard curve made with bovine serum albumin (BSA) 0,1mg/ml, where increasing dilutions were made, reaching concentrations of 0mg, 0,005mg, 0,01 mg, 0,015 mg and 0,02 mg. **Results**: According to the results showed no was statistical difference between the uptake in control samples (0,058 ± 0,3004) and in cells treated with a concentration of 0.1 mM cunaniol (0,292 ± 0,041). The glutamate uptake in the presence of 0,2 mM showed a decrease in uptake (0,222 ± 0,021), making the sample statistically different from control. The samples submitted to the concentration of 0,4 mM cunaniol obtained statistically significant results in the control reaction, being the result of (0,069 ± 0,0081). Thus the concentration of cunaniol interfere with the uptake of glutamate by increasing the extracellular concentration. The percentage inhibition of glutamate uptake in a concentration of 0,2 mM cunaniol reduced by 26,10% compared to control, and the concentration of 0,4 mM, the reduction was 77,04%. In an attempt to find a possible action on the NMDA receptor, the cells were incubated with labeled glutamate, and Hanks' solution containing 0,4 mM cunaniol, 50 µM of MK-801, these results demonstrated a reduction in glutamate uptake (0,0535 ± 0,014), which was statistically different from control. **Conclusion**: The cunaniol was able to block the uptake of glutamate in neuronal cell cultures, confirming that this effect was concentration dependent. The use of NMDA antagonist with MK-801 was not effective in blocking the effects of cunaniol on glutamate uptake, suggesting that the molecular mechanisms of action of cunaniol is related to the operation of transporters present in glial cells.