ACTIVATED P2X7 RECEPTORS ALLOW CALCIUM ENTRY AND GLUTAMATE EXIT: INVESTIGATION ON TRANSFECTED HEK 293 CELLS

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The $P2X_7$ receptor allows passage of small cations on immediate activation by agonist, while long-lasting activation leads to formation of a non selective large pore and membrane blebbing. We have shown the ability of $P2X_7$ receptor activation to stimulate glutamate efflux in Ca^{2+}-independent manner (not involving glutamate transporters or anion channels) from isolated rat cerebral cortex nerve endings. The aim of the present study was to investigate on the mode of exit of glutamate following activation of rat $P2X_7$ receptors stably transfected on HEK293 cells. **Experimental procedures:** HEK293 cells stably expressing rat GFP-tagged $P2X_7$ (HEK293r$P2X_7$) and native HEK293 were incubated with [^{3}H]D-aspartate in HEPES medium; [^{3}H]D-aspartate efflux induced by BzATP from cells was evaluated in superfusion system; the effects of 0.01mM Mg^{2+}medium, oATP, DL-TBOA and niflumic acid were evaluated. Ca^{2+} accumulation was measured by microfluorimetric tecniques in cells loaded with FURA2-AM (5µM). **Results:** Both native cells and HEK293r$P2X_7$ were proven to take up [^{3}H]D-aspartate through glutamate transporters: [^{3}H]D-aspartate uptake was almost totally blocked in Na^+-free medium or by the non transportable glutamate transporter blocker DL-TBOA (10µM). BzATP, ineffective in control native HEK293 cells, increased [^{3}H]D-aspartate efflux in a concentration-dependent, extracellular Mg^{2+}-sensitive manner in HEK293r$P2X_7$. Preincubation with oxATP (300µM), a selective $P2X_7$ receptor irreversible antagonist, prevented the BzATP-evoked [^{3}H]D-aspartate efflux. BzATP-evoked [^{3}H]D-aspartate efflux was unaffected in conditions where any possible exit of the glutamate analogue through the glutamate transporter or anion channels was prevented by DL-TBOA or 300µM niflumic acid, respectively. Moreover, BzATP increased Ca^{2+}-accumulation only in HEK293 cells stably expressing the $P2X_7$ receptor, being ineffective in native cells. **Conclusions:** We assessed the ability of [^{3}H]D-aspartate to exit the cells following $P2X_7$ receptor activation, when other possible ways of exit, such as glutamate transporters or anion channels, were blocked. [^{3}H]D-aspartate efflux in stably transfected HEK293 cells, consistent with in-out [^{3}H]D-aspartate transport through the activated $P2X_7$ receptor, suggests that $P2X_7$-evoked Ca^{2+}-independent glutamate efflux from nerve terminals could occur through the receptor itself. We propose that the characteristics of [^{3}H]D-aspartate movement(s) through the $P2X_7$ channel/pore could be suitably investigated in the new and simple model of stably transfected HEK293 cells.