Purinergic signaling modulates adult neurogenesis in the subventricular zone: role of parenchymal astrocytes

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The subventricular zone (SVZ) of the lateral ventricles is one of the two neurogenic regions persisting in the adult brain. Here, $GFAP^+$ precursors (Type B cells) give raise to an intermediate population of transit-amplifying $Mash1^+$ Type C cells, which eventually further differentiate to $doublecortin^+$ neuroblasts (Type A cells). Evidence is accumulating that neurogenesis in the SVZ is boosted following trauma or ischemia, also through the interaction with surrounding parenchyma or niche cells, although very few newborn neuroblasts survive and integrate after damage. Astrocytes are key components of the neurogenic niche, and play a vital role in regulating neural stem cells (NSC) proliferation and differentiation. However, the exact molecular mechanisms by which astrocytes modulate NSC functions have not been identified. Besides significantly contributing to reactive astrogliosis, increasing evidence suggests that extracellular nucleotides play a role in controlling adult neurogenesis; these functions become prominent especially under pathological conditions where nucleotides concentrations raise several folds. From the few data published so far, a primary role for the $P2Y_1$ G protein-coupled receptor subtype is clearly emerging in controlling the proliferation and differentiative potential of SVZ cells. Therefore we examined the role of ADPβS, a $P2Y_{1,12,13}$ receptor agonist, in modulating NSC properties in the mouse adult SVZ both *in vivo* and *in vitro*, with a particular focus on the possible modulatory effects exerted by reactive astrocytes.

A 7-day long i.c.v. administration of 100 mM ADPβS stimulated reactive astrogliosis in the brain parenchima surrounding the SVZ, and induced a massive reaction of GFAP-expressing precursors and astrocytes in the SVZ, which became hypertrophic. Moreover, ADPβS promoted BrdU incorporation, indicating a proliferative effect, which was paralleled by a significant expansion of the population of $Mash1^+$ transit-amplifying cells and of $doublecortin^+$ neuroblasts. By taking advantage of a conditional GLAST::CreERT2 Rosa YFP mouse model, we also demonstrated that ADPβS promoted the proliferation of GLAST-expressing progenitors in the neurogenic niche, and sustained their progression towards the generation of rapidly dividing transit-amplifying cells. To confirm our *in vivo* data, we also performed *in vitro* experiments by the neurosphere (NS) assay. When cells derived from the dissociation of SVZ were plated in the presence of ADPβS, an increased number of NS was generated. Moreover, ADPβS stimulated the differentiation of undissociated NS towards GFAP+ astrocytes, and $\beta\text{-IIITub}^+$ neurons, fully confirming our *in vivo* data showing an increased generation of more mature cells by the nucleotide agonist. To test whether ADPβS was acting only directly on NSCs or whether reactive astrocytes are involved, we grew NS in the conditioned media derived from Control astrocytic cultures or from astrocytes cultured in presence of ADPβS. Both astrocyte-conditioned medium reduced the number and size of primary NS with respect to control neurosphere medium. Interestingly, an increase in the number and size of secondary NS was instead observed when cells obtained from the dissociation of primary NS grown in medium from ADPβS-treated astrocytes were replated in control medium,. This suggests that the purine analogue led to the release of a yet-to-be identified astrocytic mediator that directly inhibited NS generations, but primed cells to boost their stem cell potential when removed from the culture medium. Our preliminary results from an ELISA assay suggest that IL-10 could likely play a role in this effect. Taken together, our data suggest that nucleotides can be used to increase the pool of NSCs and their differentiation towards neuroblasts, either directly or through the activation of parenchymal astrocytes.