Involvement of the Prokineticin system in in vivo models of Aβ-induced neurotoxicity

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Background: Prokineticin 2 (PK2) is a new chemokine that regulates multiple biological functions in the CNS including hyperalgesia, neurogenesis, neuronal survival or inflammation activating two GPCRs: prokineticin receptor 1 and 2 (PKR1 and PKR2), localized in neurons, astrocytes, granulocytes, macrophages and endothelial cells. PK2 is strongly up-regulated in neutrophils by inflammatory stimuli (Geannini et al., 2009) and in DRG's neurons and in spinal cord astrocytes by peripheral nerve injury (Maftei et al., 2014).

Recently, it was reported that PK2 is an insult-inducible endangering mediator for cerebral ischemic injury. Indeed, PK2 mRNA is up-regulated by several pathological stressors including hypoxia, reactive oxygen species (Cheng et al., 2012) and excitotoxic glutamate (Landucci et al., 2016).

We have recently demonstrated that, in vitro, in primary cortical cultures (CNs), amyloid beta (Aβ)1-42 increases the expression of PK2 and its receptors and that the PKRs antagonist, PC1, dose-dependently protects CNs against Aβ1-42-induced neurotoxicity, by reducing the Aβ1-42–induced PROK2 neuronal up-regulation (Severini et al., 2015).

Aim: we investigate the involvement of the prokineticin system in an in vivo model of Aβ-induced neurotoxicity and which are the effects of PC1 treatment.

Methods: Rats were intracerebroventricular (icv) infused with Aβ1-42 (5 mg) or its vehicle and treated s.c. with PC1 (150 μg/Kg, twice/day) or its vehicle for 14 days starting from the day of the surgery.The Morris water maze task was carried out 4 weeks after Aβ1-42 inoculation. Brain samples were collected at given time points: 1, 7, 14 and 35 days after Aβ infusion to evaluate PK2/PKRs mRNA levels by RT-PCR and at 45 days to evaluate PK2/PKRs protein levels and localization by Western Blot (WB) and immunofluorescence (IF). Moreover, 45 days after Aβ infusion we also evaluated the effects of PC1 on Aβ-induced: glial activation, neuronal death and hippocampal decrease of neurogenesis by WB and IF.

Results: the behavioral results showed that Aβ1–42-infused rats treated with PC1 were able to acquire the cognitive task more quickly than Aβ1–42-infused rats treated with saline.

RT-PCR analysis demonstrated that in pre-frontal cortex and hippocampus of Aβ1-42–infused rats PK2 and PKRs mRNA levels were significantly increased already 1 day after Aβ1-42 infusion and reached the maximum after 14 and 35 days. IF staining indicated a localization for PK2 and PKR1 in neurons and astrocytes of hippocampus, whereas PKR2 was localized only in neurons. Aβ1-42-infusion increased the PK2 and PKRs immunofluorescence signal mainly in hippocampal CA1, CA2 and dentate gyrus regions, whereas in prefrontal cortex increased only PK2 immunofluorescence signal.

Interestingly, chronic treatment with PC1 significantly reduced PK2 levels (both mRNA and protein) in hippocampus and prefrontal cortex, without affecting PKR1 or PKR2 levels.

Moreover, PC1 treatment reduced the astrocytic and microglial activation, exerted protective effects against Aβ1-42–induced neuronal death and restored neurogenesis in dentate gyrus.

To exclude that the activation of the PK system might be a consequence of the neuroinflammation produced by icv infusion of exogenous Aβ1-42, we also evaluated the mRNA levels of PK2 and PKRs in cortex of Tg2576 mice of 3, 6, and 20 month of age compared with age-matched wild-type as controls, and we found a significant increase of PK2 mRNA levels in brain cortex of mice at 6 and 20 months of age.

Conclusions: These results indicated that Aβ is involved in the activation of the prokineticin system in cortex and hippocampus, and blocking the PKRs with PC1 not only reduces the PK2 levels but also displays neuroprotective effects.

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