conferenceseries.com

Clin Exp Pharmacol 2016, 6:4(Suppl)
http://dx.doi.org/10.4172/2161-1459.C1.014

The allosteric regulation of neuropeptide class B G-protein coupled receptor PAC1-R by doxycycline and minocycline

Rongjie Yu
Biomedical Institute of Jinan University, P.R. China

Doxycycline has significant neuroprotective effect with anti-inflammatory and anti-apoptotic activity, but it was not clear which molecular target mediates its positive effects in nervous system. We found that doxycycline promoted the internalization of neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) preferring class B G protein-coupled receptor (GPCR), PACAP receptor 1 (PAC1) tagged with yellow fluorescent protein (YFP) in Chinese hamster ovary (CHO) cells significantly, and the protectvie effect of doxycyline on Neuro2a cells was inhibited by PAC1 specific shRNA indicating that doxycyline inteacts with PAC1. The homology modeling of PAC1-R and molecular docking of doxycycline with PAC1 showed the theoretical binding of doxycycline to PAC1 at the site where PACAP(30-37) recognized. The following competition binding assay and PAC1 site-specific mutation of Asp116, which formed two hydrogen bonds with Dox, confirmed the binding of doxycycline to PAC1 imitating PACAP(30-37). Doxycycline (100 ng/mL) significantly promoted the proliferative activities of vasoactive intestinal polypeptide (VIP) and oligopeptide HSDGIF responsible for the activation of PAC1 in PAC1-CHO cells, indicating that doxycycline facilitated the binding and the activation of PAC1 imitating PACAP(28-38). In Neuro2a cells with endogenous expression of PAC1 and its ligands, doxycycline not only promoted the proliferation of Neuro2a cells but also protected the cells from scopolamine induced apoptosis, which was inhibited by cAMP-PKA signal pathway inhibitor H-89, PAC1 shRNA or PACAP antagonist PACAP(6-38). The *in vivo* study showed long-term treatment with doxycycline (100ug/kg) had significant effect against scopolamine induced amnesia, and the synergetic anti-apoptotic, anti-oxidative and neuroprotective effect of doxycycline with VIP was more efficient than doxycycline alone or VIP alone, indicating doxycycline enhanced the activation of PAC1 *in vivo* effectively. Furthermore, doxycycline analogue minocycline also had similar theoretically binding site on PAC1 to doxycycline and displayed corresponding similar activity on PAC1 to doxycycline. All these results confirmed for the first time that doxycycline specially targeted PAC1 imitating PACAP(30-37) and acted as an enhancer by facilitating the subsequent ligand binding and the activation of PAC1. The confirmation of PAC1 as a novel molecular target of doxycycline and the novel mechanism by which doxycycline enhances the activity of PAC1 will help further clinical development of doxycycline as novel therapy for nervous system diseases such as neurodegenerative diseases targeting PAC1.

rongjie_yu1123@163.com