



**Karolinska
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Institutionen för Neurovetenskap

**THE GALANIN TYPE 2 RECEPTOR:
MOLECULAR BIOLOGICAL, HISTOCHEMICAL AND
ELECTROPHYSIOLOGICAL STUDIES**

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ABSTRACT

In the present thesis, the aim was to probe the distribution and functions of galanin type 2 receptor (GalR2) in the rodent brain. In order to overcome the lack of a reliable antibody to localize GalR2 by immunohistochemistry, we generated a construct which expresses GalR2 tagged with enhanced GFP (EGFP). We first studied the trafficking and function of this fusion protein in cell lines, including PC12 cells. The results showed that GalR2-EGFP is predominantly localized on the plasma membrane with some intracellular fluorescent structures (vesicles), mainly in the perinuclear region. After activation by galanin, the GalR2-EGFP is able to induce a concentration-dependent increase in intracellular Ca^{2+} level, suggesting that the conjugate is functional. The results also indicate that GalR2 undergoes constitutive endocytosis and recycling, using the clathrin-dependent endocytic recycling pathway.

Then we examined the localization of the GalR2-EGFP in a transgenic mouse brain expressing the same GalR2-EGFP construct under the GalR2 promoter, using a GFP antibody. The immunostaining was mainly found in cell bodies and to a limited extent also in presumable nerve terminals. GalR2-EGFP positive processes and cell bodies were located in many brain regions, including the olfactory bulb, certain limbic cortical areas, the basal forebrain, amygdala, subregions of the hippocampal formation, thalamus, hypothalamus, periaqueductal grey, locus coeruleus, and some further areas in the midbrain and medulla oblongata. Using double-staining we analyzed some cell groups giving rise to major ascending systems, and could demonstrate presence of the GalR2-EGFP construct in noradrenergic/galaninergic locus coeruleus neurons. The GalR2-EGFP was also detected in calcium-binding protein- and GAD-positive cell bodies in the basal forebrain, but not in cholinergic neurons in this area, or in the 5-hydroxytryptamine neurons in the dorsal raphe nucleus. Our results support the concept that GalR2 primarily is a presynaptic autoreceptor in noradrenergic locus coeruleus neurons. Galanin is presumably involved in a wide range of brain functions, which are partly executed through GalR2.

Plasticity is an important characteristic of galanin signaling. The expression levels of galanin and its receptors are highly dynamic in various circumstances. In vitro electrophysiological study was done with hippocampal slices obtained from mice overexpressing galanin under the promoter for the platelet-derived growth factor-B (GalOE mice). Slices from young adult wild-type (WT) animals showed significant paired-pulse facilitation (PPF) of the 2nd excitatory postsynaptic field potential (fEPSP) evoked with paired-pulse stimuli, while PPF was less strongly expressed in slices from young adult GalOE mice, as well as aged WT mice, but were not observed at all in slices from aged GalOE animals. The results indicate that galanin is involved in hippocampal synaptic plasticity, in particular in age-related reduction of synaptic plasticity in the lateral perforant path input to the dentate gyrus.

Hypertrophic galanin-containing fibers engulf cholinergic basal forebrain neurons in Alzheimer's disease, a phenomenon that has been interpreted as a region-specific innate neuroprotection. We have developed an en masse cell isolation strategy based on the p75NTR to establish 90-95% homogenous cholinergic neuron cultures from dissociated embryonic basal forebrains. We exploited this in vitro system to dissect the cellular mechanisms of how galanin impacts cholinergic neurons. Cultured cholinergic neurons express GalR2 that is located in the soma and distributed to the axonal growth cone. Stimulation of cultured cholinergic neurons with galanin or GalR2 agonist ARM1896 induces ERK phosphorylation. Stimulation of GalR2 by galanin and its synthetic analogue resulted in increased neurite outgrowth. Furthermore, we demonstrate that GalR2 stimulation can protect cholinergic neurons against β -amyloid ($\text{A}\beta$) toxicity in vitro. Therefore, we speculate that the hypertrophy of galaninergic nerve endings in the basal forebrain of Alzheimer's patients may represent a signalling arrangement poised to activate specific GalR2-dependent intracellular signaling cascades to induce neuroprotection in situ as proposed previously.

In summary, the study with PC12 cell line elucidated the subcellular distribution and trafficking pattern of GalR2; this gave us some idea how the GalR2 receptor exerts its function at the cellular level. The immunohistochemical study with GalR2-EGFP transgenic animal provided the information about the distribution pattern of GalR2 in the brain, including its relation to some ascending systems. The study about the effect of galanin signaling in the aged mice and GalOE mice showed plasticity of the galanin signaling system and the effect of overexpressed galanin on neurotransmission at the presynaptic site. With the newly developed target-specific-isolated cholinergic basal forebrain neuron culture, we provide further evidence for a trophic effect of GalR2 activity and the plasticity of GalR2 related to $\text{A}\beta$ toxicity. These studies may provide a basis for further discussion about the function of galanin signaling and potential therapeutic applications.

Keywords: Galanin, galanin receptor 2, PC12 cell, transgenic mouse, cholinergic basal forebrain neuron, target-specific-isolation, Alzheimer's disease

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