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The p75 neurotrophin receptor is localized to primary cilia in adult murine hippocampal dentate gyrus granule cells

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ABSTRACT

The densely ciliated granule cell layer of the adult murine hippocampal dentate gyrus is one of two sites of adult neurogenesis. The granule cells have already been proven to localize their SSTR3 (somatostatin receptor 3) receptors to their so-called primary cilia. Here we show for the first time that 70–90% of these cells in 7–18 months-old wild-type and 3×Tg-AD (Alzheimer disease transgenic) mice also load $p75^{NTR}$ receptors into the structures containing SSTR3, i.e., their primary cilia. On the other hand, $p75^{NTR}$'s TrkA co-receptors were not localized to cilia but conventionally distributed throughout the cell surface. Significantly fewer cells (20–40%) in the hippocampal CA1 and CA3 regions and cerebral cortex have $p75^{NTR}$ containing cilia. While we don't know what the impact of the cilial localization of $p75^{NTR}$ on dentate gyral adult neurogenesis and memory encoding might be, the cilia's amyloid β-activatable $p75^{NTR}$ receptors could be damaging or lethal to the hippocampal functioning of amyloid β–accumulating Alzheimer brain.

1. Introduction

Dahl [1] was the first to report that all rat hippocampal dentate granule neurons have a primary immobile cilium and this has since proven to be true for mouse granule cells [2]. This small immobile primary cilium is an ideal "remote" sensor because of its protrusion from the cell surface into the extracellular milieu where it can contact neighboring cells and their synapses, measure local fluid flow and respond to various growth and other factors [3–5]. Because of the cilium's limited volume and gated access, the cell can sharpen its focus on, and responsiveness to, an extracellular target(s) by clustering a set of receptors onto the cilial surface and squeezing their signal-transducing machineries into the cilial interior for close contacts and rapid interactions. Armed with an array of receptors, the cilium detects important extracellular factors with its compact signal-transduction machinery and promptly sends this information to the cell center. Receptors so far proven to be on the cilia of different cell types are 5-HT_6 (serotonin receptor-6), MCHR1 (melanin-concentrating hormone 1), PDGFRα (platelet-derived growth factor α), sonic hedgehog, SSTR3 (somatostatin receptor 3), and VPR2 (vasopressin receptor 2) [6–12].

Here we have discovered that nearly all hippocampal dentate granule cells, but significantly fewer hippocampal CA1 and CA3 cells and cerebral cortical cells, of adult wild-type and transgenic AD (Alzheimer's disease)-model mice localize the p75 neurotrophin receptor ($p75^{NTR}$) along with the expected SSTR3 [10,12] to their primary cilia. $p75^{NTR}$ is a member of the TNF receptor superfamily, that complexes with neurotrophin tyrosine kinase receptors such as Trk A, that are activated by neurotrophins such as NGF(nerve growth factor) [13]. Interestingly, the dentate granule cells do not appear to co-localize TrkA to their cilia. As indicated by the involvement of the cilium-localized SSTR3 in novelty detection in mice reported by Einstein et al. [14], cilium-localized $p75^{NTR}$ in the dentate granule cells is more than likely involved in memory formation because the dentate gyrus is the site of the neurotrophin-driven adult neurogenesis that is needed for memory encoding [15–20].

2. Materials and methods

2.1. Reagents

SSTR3 polyclonal antibody against somatostatin receptor, type SST3, was obtained from Gramsch Laboratories (Schwabhausen, Germany). $p75^{NTR}$ and $p75^{NTR}$ – FITC rabbit polyclonal antibodies, directed against the receptor's extracellular domain (amino acids 188–203), was obtained from Alomone Labs Ltd. (Jerusalem Israel).

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TrkA antibody was from Novus Biologicals, LLC (Littleton, CO). Alexa Fluor 488 and 680 goat anti-rabbit IgG were from Invitrogen Canada (Burlington, ON).

2.2. Wild-type and AD-transgenic mice

Wild-type sv129/C57BL6 mice and AD-triple transgenic sv129/C57BL6 mice (3×Tg-AD), harbouring $PS1_{M146V}$, APP_{Swe} and tau_{P301L} transgenes were developed in the Department of Neurobiology and Behaviour of the University of California, Irvine, CA and the animals used in this study were bred and maintained in-house at NRC. All animal studies were approved by NRC Institute's Animal Care Committee.

2.3. Immunohistological methods

Anesthetized female wild-type and transgenic AD mice (age-matched 7–8 and 14–18 months-old, three animals in each group) were decapitated, their brains rapidly removed and split into two equal halves (hemi-brains), snap frozen on dry-ice and stored at −80 °C until needed.

Frozen hemi-brain was embedded in OCT and 10 μm sections were prepared using a Jung CM 3000 cryostat and stored at −80 °C until use. Tissue sections were fixed in acetone and then permeabilized in 0.1% TritonX-100, 2% normal goat serum, 0.02% Na azide, 10 mg/ml BSA. After blocking with a solution of 5% normal goat serum, 0.02% Na azide, 10 mg/ml BSA for 1 h, sections were incubated overnight at 4 °C with various primary antibodies diluted in 2% normal goat serum, 0.02% Na azide, 10 mg/ml BSA at indicated dilutions: 1:700 dilution for SSTR3, 1:100 dilution for $p75^{NTR}$, FITC-$p75^{NTR}$ and TrkA antibodies. After incubation with primary antibodies, sections were washed in PBS and incubated with Alexa Fluor 488 goat anti-mouse IgG at 1:400 dilution for 1 h at room temperature. Please note that sections incubated with FITC-$p75^{NTR}$ were not incubated with secondary antibody.

All sections were then washed and cover-slipped with Dako fluorescent mounting medium containing the DAPI (4′,6-diamidino-2-phenylindole) DNA-binding nuclear stain, examined and cilial lengths measured using an Olympus fluorescent microscope.

2.4. Immunoblot analysis

In some experiments hippocampi from wild type (Wt) and transgenic (Tg) mice were homogenized in ice-cold Tris–HCl buffer (20 mM, pH 7.4, 5 μg/ml AEBSF, 0.8 μg/ml aprotonin, pepstatin, and leupeptin) with 10 up-and-down strokes in Glas-Col tissue homogenizer at maximum speed (4000 rpm). Hippocampal homogenates were centrifuged at 600×*g* for 5 min at 4 °C and the supernatants (tissue homogenate) were subjected to Western blot analysis as previously described by Chakravarthy et al. [21]. Briefly, proteins in tissue homogenate were separated on 10% polyacrylamide gels and transferred to PVDF membranes as previously described [21]. The immunoblots were then blocked with 5% non-fat milk in TBST (10 mM Tris pH 8.0, 150 mM NaCl, 0.05% Tween 20) for 1 h and incubated with primary TrkA antibody (1:2000 dilution) overnight at 4 °C. After three washes in TBST, immunoblots were incubated with peroxidase-conjugated secondary antibody (1:5000 dilution) in TBST containing 1% non-fat milk for 30 min at room temperature. Western blots were visualized using Western Lightning Chemiluminescence Reagent Plus kit.

2.5. Statistical analysis

Data were analyzed with ANOVA and Bonferroni's post hoc test. Values of $p < .05$ were considered statistically significant.

3. Results

Stanić et al. [12] and Einstein et al. [14] have reported that rodent dentate granule cells localize SSTR3 receptors to their primary cilia. Consistent with this, more than 90% of the dentate gyral granule cells in the hippocampi of the wild-type sv129/C57BL6 mice also localized SSTR3 receptors to their single primary cilia (Fig. 1). The lengths of the SSTR3-containing cilia were (means ± SEMs): 7–8 months, 4.08 ± 0.29 μm; 14–18 months, 4.33 ± 0.07 μm).

We had recently reported that the level of the hippocampal p75 neurotrophin receptor ($p75^{NTR}$) significantly increased in 3×Tg-AD (Alzheimer's disease) transgenic mice accumulating $Aß_{1-42}$ [21]. Therefore it was of interest to determine the $p75^{NTR}$ distribution in cells of the various murine hippocampal subfields. We did this via immunohistochemical analysis using $p75^{NTR}$ –selective antibodies (normal as well as FITC-tagged antibodies). Surprisingly, both normal and FITC-tagged $p75^{NTR}$ antibodies only stained single 4-μm structures identical to the granule cells' SSTR3-bearing cilia (Fig. 1).

Since most adult mouse dentate granule cells have only one SSTR3-bearing primary cilium [10,12,14] (Fig. 1), and since the granule cells' cilia are loaded with SSTR3, it was reasonable to expect co-localization of SSTR3 and $p75^{NTR}$. This expectation was tested by double-labeling of hippocampal sections with SSTR3 and $p75^{NTR}$ antibodies. Indeed, the red fluorescence from the SSTR3 antibodies and the green fluorescence from the $p75^{NTR}$ antibodies merged to produce yellow-fluorescing dentate gyral cell cilia (Fig. 2). Therefore the dentate granule cells loaded both $p75^{NTR}$ and SSTR3 into their primary cilia.

We then analysed immunohistochemical staining in differ hippocampal regions and cortex to determine the ratio of $p75^{NTR}$-loaded cilia to nuclei. As was the case with SSTR3, a vast majority (70–90%) of gentate gyral cells had $p75^{NTR}$-loaded cilia and relatively fewer hippocampal CA1 and CA3 cells and cortical cells had $p75^{NTR}$ containing cilia in 7–8 and 14–18 months-old wild-type and 3×Tg mice (Fig. 2A and B). Interestingly, the $p75^{NTR}$-loaded cilia to nuclei ratio in 14–18-months-old 3×Tg mice tended to be higher (though not significantly so) than in wild-type mice in CA1 and CA3 hippocampal regions, and in the dentate gyrus the increase (>25%) was very highly significant ($p < 0.001$) (Fig. 2B).

We then asked whether cilial $p75^{NTR}$ was associated with its TrkA co-receptors. This was a very important question when we found $p75^{NTR}$ localized to the granular cilia because signals from $p75^{NTR}$-TrkA or uncomplexed $p75^{NTR}$ are involved in driving brain development or neuronal apoptosis respectively (reviewed in [20]). Therefore, we also determined TrkA localization in the dentate gyral cells of the normal wild-type mice. TrkA antibody detected TrkA receptor in the hippocampal homogenate as determined with immunoblot analysis (Fig. 3A). TrkA was not localized to the cilium (Fig. 3B). Instead it was seen as puncta distributed throughout the cell. The striking difference between the distribution of TrkA and $p75^{NTR}$ receptors in the dentate gyral granule cells can be seen in Fig. 3B.

4. Discussion

The dentate cell's SSTR3-bearing cilium shown in Fig. 1 was the first receptor proven to be localized to the primary cilia of the granule cells of rodent dentate gyri and proven to be involved in memory encoding [9,10,12,14]. We have now shown that the dentate granule cells also put $p75^{NTR}$ into their primary cilia. We have shown that 70–85% of dentate granule cells, but significantly fewer CA1 (50–60%), CA3 (20–35%) and cortical neurons (30–45%), in normal adult wild-type sv129/C57BL6 mice put $p75^{NTR}$ into their