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Internalization of scavenger receptor ligands by cortical neurons

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Abstract

Scavenger receptors (SRs) are a family of receptors displaying affinity for a wide variety of ligands including modified lipoproteins. SRs may play a range of physiological functions including intracellular transport, lipid transport and pathogen clearance. The role of SRs has been documented in pathologies such as atherosclerosis and Alzheimer's disease. Although most studies on SRs have focused on macrophages, they are also present in other cells like endothelium, smooth muscles and brain tissue. Within brain, due to its functional similarity, SRs have been studied mostly in microglia. However, in situ images from Allen's brain atlas suggest SRs are abundant in neurons. In this study we have used two fluorophore labeled well characterized SR ligand, maleylated-BSA (MBSA) and polyguanylic acid (polyG) to probe acute cortical slices. Our data indicate that within cortex, neurons avidly endocytose both ligands. Thus in cerebral cortex neurons may have higher number of functional SRs on the surface than other cell-types.

Introduction

SRs were identified in the laboratory of Goldstein and Brown due to their ability to endocytose modified low density lipoproteins, modified proteins like maleylated albumin and nucleotides (polyguanylic and polyinosinic acid) (1, 2). At present at least 10 different classes of SRs are known (3). SRs are versatile receptor and play numerous physiological and pathological roles and have also been successfully used for drug targeting specifically in macrophages (4, 5). Most study till date has focused on immunological (6) and cardiovascular role of SRs. SRs also play a crucial role in lipid transport. Although SRs are known to express in brain tissue, limited number of studies have focused on its function in brain. Microglia, astrocytes and brain macrophages expresses SRs (7). It has been demonstrated that SRs are involved in uptake of amyloid β and thus play a role in Alzheimer's disease (8). Some forms of SRs are also known to be expressed in neurons (3), however functional studies of SRs in neuron has not been extensively performed. In situ images of different SRs from Allen's brain atlas surprisingly suggest that neurons may express many types of SRs. In this study we probed acute rat cortical slices with two independent fluorophore labeled ligands to examine their neuron-specific uptake.

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Objective

The objective of this study was to investigate which brain cell type exhibited highest affinity for scavenger receptor ligands in cerebral cortex.

Results and discussion

MBSA has been a widely used ligand for SRs. We generated Texas-red labeled MBSA and tested it on a macrophage cell line RAW264.7 which is known to express scavenger receptors. Our data indicate that MBSA-Texas red was rapidly internalized by RAW264.7 cells. Internalization of BSA was negligible during the same time. To ensure that the internalized MBSA was indeed transported to endo-lysosomal compartment, we did a pulse chase experiment on RAW264.7 cells. Within 90 minutes MBSA-Texas-red co-localized with lysotracker indicating that indeed MBSA is transported to endo-lysosomal compartment. We next examined uptake of MBSA-Texas red on acute cortical slices generated from adult rats. MBSA was rapidly taken up by cells of cortex which stained positive for NeuN indicating the cells which maximally endocytosed MBSA were neurons. In order to confirm our observations we repeated our experiments with another well characterized SR ligand, polyG. We examined the uptake of polyG labeled with FAM on acute cortical slices. Our data show that NeuN positive cells rapidly internalize polyG but not polyA (polyadenylic acid) indicating the neurons in cortex have large number of SRs on the surface. Our imaging results indicate most cells in the cortex that internalized detectable quantities of SR ligands are NeuN positive. Thus contrary to expectations, neurons rather than microglia or astrocytes have higher amounts of SRs in cortex. Other lipoprotein receptors like ApoER2 and VLDLR are also present on neurons and play valuable function (9). Large quantities of lipoproteins are synthesized by glial cells (10). In periphery SRs play a critical role in lipid transport (11), it is therefore likely that SRs may play physiological role in lipid homeostasis of the brain also. SRs are also known to bind and internalize A β , neurons are known to internalize A β which are neurotoxic (12). It may be possible that SRs present on neurons contribute to this uptake. Finally, our data indicate that much like in macrophages SR ligand may be useful to target drugs and bioactive molecules to neurons.

Conclusion

Our study indicates that cortical neurons express large amounts of functional scavenger receptors based on the internalization studies. Surprisingly we detected very few non-neuronal cells within the cortex that exhibited similar levels of internalization.

Limitations

One big limitation of this study is that it does not specify which types of SRs are active in neuronal cortex. Furthermore levels of SR expression may vary with age as well as pathology. It is likely that during inflammatory and degenerative states cortical macrophages may express more scavenger receptors. In future immunohistochemistry and internalization studies in pathological conditions will be required.

Materials and method

Cell culture and internalization assay

Raw 264.7 cells were kind gift from Prof. Liwu Li, they were maintained in DMEM containing 10% FBS at 37°C and 5% CO₂. For internalization assay, RAW 264.7 cells transduced with lentivirus expressing GFP were plated on poly-lysine coated coverslips. 24 hours later, cells were exposed to MBSA-Texas red for 4 minutes at 37°C. Cells were washed and fixed. Cells were imaged using an upright Zeiss laser scanning microscope (LSM 710) with a 63X oil immersion lens. For pulse-chase experiments RAW 264.7 cells were plated on polylysine coated coverslips. 24 hours later cells were pulsed with MBSA-Texas red for 4 minutes washed and fresh media added. Cells were incubated at 37°C for 65 minutes, lysotracker was added to the cells and they were returned to the incubator for additional 25 minutes. Live cells were imaged using 63X oil immersion lens in an inverted Zeiss laser scanning microscope.

Texas-red maleyl BSA generation

BSA (Sihma) was incubated with NHS-Texas red (ThermoFisher) at pH 8.5 in a 1:3 ratio for half an hour at 40°C. Conjugation was stopped using Tris and the protein was dialyzed against PBS exhaustively. The ratio of Texas red to BSA was found to be ~1:2.2. BSA-Texas red was then maleylated using maleic anhydride following a protocol described earlier (5); briefly maleic anhydride was added to BSA-Texas red with constant stirring, the pH was maintained above 8.5 using 5N NaOH. This was followed by dialysis against PBS overnight.

Acute cortical slice experiment

Adult .....

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure. Cortical neurons internalize SR ligands

(A) Internalization of MBSA-Texas red by Raw264.7 cells; (B) Internalization of BSA-Texas red by Raw264.7 cells; (C) MBSA-Texas red colocalizes with lysotracker within 90 minutes; and (D) Internalization of MBSA-Texas red by NeuN positive cells in rat cortex. (E) Internalization of PolyG-FAM by NeuN positive cells in rat cortex; (F) PolyAFAM is not internalized by cortical cells.