Design, Synthesis and Characterization of ‘Responsive’ MR Contrast Agents Sensitive to Glutamate

Reporting

Project Information

GLURESPROBES

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Final Report Summary - GLURESPROBES (Design, Synthesis and Characterization of ‘Responsive’ MR Contrast Agents Sensitive to Glutamate)

Glutamate (Glu) is the major mediator of excitatory signals in the mammalian central nervous system,

involved in most aspects of normal brain function including cognition, memory and learning. The interaction of this neurotransmitter with its receptors was selected as the model system in this project. Glutamate is abundantly distributed in the brain and plays a central role in brain metabolism.
Most of the neurons in the mammalian brain release Glu as a neurotransmitter. When the presynaptic neuron is stimulated, synaptic vesicles containing Glu merge with the neuron's plasma membrane and release their contents outside the cell. Glu diffuses to the postsynaptic neuron and binds to receptors there, activating the postsynaptic cell (Figure 1[A, B]). The released Glu is then cleared away by the glutamate transporters (present on astroglial and support cells) and to a later extent by transporters on postsynaptic neurons. In astroglial cells, Glu is enzymatically converted to glutamine, later it is supplied to presynaptic neurons through the neutral amino-acid-transporters and finally is recovered as glutamate to repackage into vesicles (Figure 1C).
Glutamate mediates its effect through both the G-protein coupled metabotropic receptors (mGluRs) and the ligand-gated ionotropic receptors (iGluRs). Only the mGluR subtype-5 (mGluR5) are found to be actively involved in transducing excitatory signals between neurons through Glu. These receptors are widely localized on the postsynaptic membrane and are distributed in various brain regions, including the spinal cord, thalamic nuclei and the hippocampus. Next to neurons, mGluR5 is also expressed on astrocytes where it is thought to be part of Glu transmission by astrocytes. In addition, a role in the Glu-mediated astrocyte-to-neuron signalling has also been discussed.
Hypothesis. We hypothesized that in the brain resting stage, a selective glutamate responsive contrast agent (GluRCA) would bind to mGluR5 and provide a high contrast in magnetic resonance images. At the same time, the GluRCA will bind to mGluR5 expressed on astrocytes, which are located nearby the synaptic cleft. Therefore, using mGluR5 as the target for our GluRCA was considered to give an advantage of increasing the number of available binding sites, leading to a larger signal change.
Design, synthesis and characterisation of GluRCA. We designed, synthesised and evaluated eight GluRCAs containing various selective mGluR5 binding moieties linked to 'DOTA' derived gadolinium (Gd3+) complexes, exploring their potential application as responsive MR imaging probes. These binding moieties have been as established specific mGluR5 antagonists (alkynes and dipyridyl/heterobiaryl amides derivative) and have been integrated into these structures in a modular fashion, involving linkage to a macrocyclic ligand core (i.e. 'DOTA') to allow the targeting of mGluR5 receptors (Figure 2A).
Probe-receptor binding studies. Primary rat astrocytes were chosen as the cellular model, as these cells are known to express mGluR5 efficiently. However, we did not use additional differentiation of the cells with a G5-supplement, as sufficient expression of the receptor in our model was revealed by immunofluorescence staining studies.
The cytotoxicity of the gadolinium complexes [Gd.L1-8] was examined with a proliferation assay (XTT: mitochondrial redox perturbation) in combination with a cell number assay (Hoechst 33342: stains DNA in cells). Apart from [Gd.L3] none of the complexes exhibited significant effects in each assay over the range of 50 to 200μM after 24 h.

Related documents

final1-mc-report.doc

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