Development of a medium-throughput method to screen the effect of test articles on mouse brain activity using 14C-2-deoxyglucose (14C-2DG) 3D autoradiography

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Results (continued)

Relative changes in 14C-2DG signal in brain subregions are detectable with 3D autoradiography

By collecting every 30-µm-thick section, we were able to build 3D brain volumes (Fig 2) and analyze the autoradiography data in 3D using conventional in vivo image processing methods. The pattern of 14C-2DG distribution agreed with published results for similar 14C-2DG or 18F-FDG studies [1-2]. Statistical maps demonstrated significant group-level effects (Fig 3). The group that received haloperidol (1 mg/kg) showed significantly higher normalized 14C-2DG signal in the dorsal striatum and lateral habenula and significantly lower normalized 14C-2DG signal in the caudate-putamen as well as in the ventral tegmental area (Fig 6A, B). Another group described by a significant lower signal in the haloperidol group control could be observed in the ventral tegmental area. Unique patterns of response in voxel-level results Patterns of response in voxel-level statistical maps varied by test article (Fig 3, 4, and 7). Atomoxetine (10 mg/kg) produced a significant bilateral decrease in 14C-2DG signal in the somatomotor areas. Both guanfacine (1 mg/kg) and clonidine (0.03 mg/kg) produced significant bilateral decreases in normalized signal in the sensory-motor cortex related area of the thalamus. Clonidine also produced significant bilateral increases in the basolateral and lateral amygdalar nuclei (LAB, LA, LAM), as well as in the dorsal and lateral habenula. The similarities in 14C-2DG uptake between guanfacine and clonidine may be explained by the underlying pharmacology of the drugs, which are both a2 agonists. However, while guanfacine is selective for a1, clonidine has activity at all three a neuroreceptor subtypes. Atomoxetine is a selective nonopiate norepinephrine uptake inhibitor.

Materials & Methods

C57BL/6J mice were separated into groups of nine animals. After an overnight fast, each mouse was administered one of 12 test articles while awake via intraperitoneal injection. Fifteen minutes after test article administration, each mouse was administered 14C-2DG intravenously (100 uCi/kg). Mice were euthanized 2-5 minutes post-injection of 14C-2DG, and their brains were harvested. Each brain was resected whole and frozen. Frozen brains were embedded in optimal cutting temperature (OCT) compound blocks, 54 mm thick (Fig 1). In grid-like configurations for autoradiography. Each block contained radioactive and white light visible fiducial markers. Transverse, 30-µm thick, brain sections were obtained using a cryomicrotome. High resolution optical (white light) images were acquired prior to each section being taken from the block. Sections were exposed to phosphor imaging plates with radioactive standards to measure radioactivity in the tissue and to produce autoradiograms.

Discussion/Conclusions

A medium-throughput 3D autoradiography method was developed and applied to measure the effect of test articles on 14C-2DG radioactivity in the mouse brain. As the resolution of quantitative autoradiography is on the order of 100 µm, this method provides a higher-resolution complement to precision in vivo PET imaging.

Reproducible results were produced with this method, as demonstrated by the consistent haloperidol response between studies. Unique patterns of response were seen in both the voxel-level and region-level results for different compounds. Similar response was seen for compounds with similar mechanism of action (guanfacine and clonidine). Together with the high resolution and replicability of this approach, this suggests that 14C-2DG autoradiography may be a useful assay to measure drug-induced modifications of brain activity.

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References