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Review

Measuring brain lipids ☆

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ABSTRACT

The rapid development of analytical technology has made lipidomics an exciting new area and this review will focus more on modern approaches to lipidomics than on earlier technology. Although not fully comprehensive for all possible brain lipids, the intent is to at least provide a reference for the analysis of classes of lipids found in brain and nervous tissue. We will discuss problems posed by the brain because of its structural and functional heterogeneity, the development changes it undergoes (myelination, aging, pathology etc.) and its cellular heterogeneity (neurons, glia etc.). Section 2 will discuss the various ways in which brain tissue can be extracted to yield lipids for analysis and section 3 will cover a wide range of techniques used to analyze brain lipids such as chromatography and mass-spectrometry. In Section 4 we will discuss ways of analyzing some of the specific biologically active brain lipids found in very small amounts except in pathological conditions and section 5 looks to the future of experimental lipidomic modification in the brain. This article is part of a Special Issue entitled Brain Lipids.[1]

1. Introduction

The brain contains an incredible mixture of lipids and has been a focus for lipid chemists since brain cholesterol became the first organic compound to be purified (crystallized) in 1834. It has continued to attract attention since both genetic diseases and common neurodegenerative diseases such as multiple sclerosis, leukodystrophies, Parkinson's, amyotrophic lateral sclerosis, glioblastomas and Alzheimer's have been associated with disturbances in brain lipid content. Thus a normal MRI reveals uniform lipid-rich structures and pathology whereas in multiple sclerosis MRI reveals Dawson fingers, a radiographic feature resulting from loss of lipids and demyelinating plaques through the corpus callosum (Fig. 1A, B). They are arranged at right angles along medullary veins (callososeptal location) and give some clues that lipids have a definite organization in the brain and that they are worthy of being measured in great detail.

A more accentuated demyelination is shown in Fig. 1 panel C. This is of a patient with a leukodystrophy of unknown etiology, which has destroyed many brain lipids. Many such changes may not be causative but may be associated with inflammation or other degenerative processes but in all cases, a lipid analysis can start the pathway to diagnosis and possible therapy.

The rapid development of analytical technology has made brain lipidomics an exciting new area and this review will focus more on modern approached to lipidomics than more outdated technology which will be referenced but not discussed in any detail. The brain poses unique sampling problems due to developmental changes (especially myelination) and aging, rapid turnover rates of some lipids and cellular heterogeneity. This is reflected in the changes in major lipids during the aging process. Thus in human brain (Fig. 2), phosphatidylcholine (PC) predominates at birth (50%) but then declines and sphingomyelin (SM) increases from 2 to 15% at 3 years, consistent with its role in the myelin sheath and mature membranes. Less attention has been paid to the problem of metabolic instability post-mortem, perhaps because many initial studies were done on human autopsy material. Methods now exist for rapidly freezing or denaturing mouse brain and with increasing interest in phosphorylated lipids, which tend to have a short half-life, we may see more attention to this area in the future. What has been a focus of attention since the very early days is that molecular oxygen is destructive to polyunsaturated fatty acids, generating reactive lipids such as 1-palmitoyl-2-(5″-oxovaleryl-sn-glycero-3-phosphorylcholine) from brain phosphatidylcholines. Thus most extraction procedures are careful to keep extracted lipids either in solvent or dried down and stored under nitrogen.

The cellular heterogeneity of brain is equally dramatically variable so sampling differences have complicated the quantitative analysis of brain lipids in the past. The individual cell types (neurons, astrocytes and oligodendrocytes etc.) all have their very distinctive lipid composition. Thus complex gangliosides are typical of neurons and galactosylceramides are uniquely expressed by oligodendrocytes and this has to be taken account of in any attempt to quantify "brain lipids". The problem of sampling different brain regions is a critical part of measuring brain lipids and we will discuss new technology (MALDI) that has been developed to

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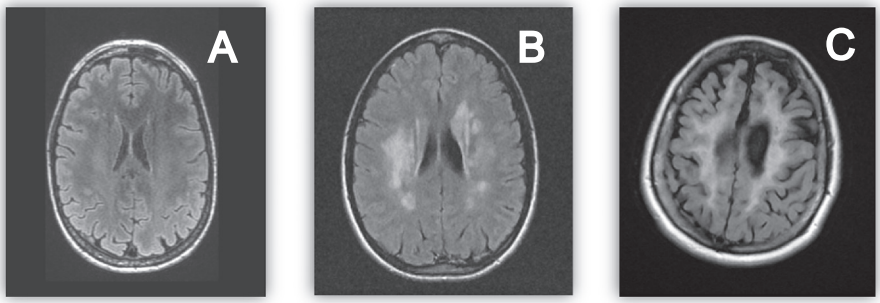

Fig. 1. A crude way of measuring lipids by magnetic resonance images of human brain. These show even lipid distribution in controls (A) and the abnormal lipid composition and shrinkage of the brain associated with neurodegeneration (B,C).

solve this problem. Some brain lipids are also highly metabolically dynamic, so that post-mortem changes can add to the complexities of interpretation, as well as lipid levels being affected by diet and general health-related issues such as malnutrition, metal toxicity etc.). The genes for the enzymes responsible for the synthesis and metabolism of most brain lipids are known and the use of knock-out mice in conjunction with HPLC/MS/MS has started to reveal some structure-functional insights as well as the complexity of the metabolic connections between lipids.

Most early lipid analyses were done on human autopsy tissue in an endeavor to provide diagnostic help to clinicians. A good example would be the increased brain sphingomyelin found in Niemann–Pick disease and the specific ganglioside accumulations in Tay–Sachs (GM2-gangliosidosis) and GM1-gangliosidosis, which was first observed more than 50 years ago. Fig. 3 shows the diagnosis of Tay–Sachs disease on the basis of the accumulation of GM2 ganglioside primarily in neurons [2,3].

In this review I will attempt to focus on the issues, solutions, techniques and future direction of 180 years of brain lipid measurements.

2. Pre-analytical work-up

2.1. Homogenization and extraction techniques

The exact extraction method may vary but after 50 years of research all extraction methods involve a mixture of polar and non-polar organic solvents, most commonly chloroform and methanol (the Folch procedure [4] or the Bligh and Dyer procedure [5]). Total lipids are typically extracted from half brains of mice or 0.1 g of other brains by homogenization in 3 ml of PBS, followed by protein standardization. Age-matched samples are essential since myelination increases with age and myelin is greatly enriched in lipids [7]. Lipids are extracted by adding methanol (5 ml) and incubating at room temperature for 1 h, followed by the addition of 1 drop of conc. HCL and chloroform (10 ml) to achieve the Folch partition in which most of the lipids are in the lower phase and the gangliosides in the upper phase (C-M-water 1:47–48), also known as "theoretical upper phase".

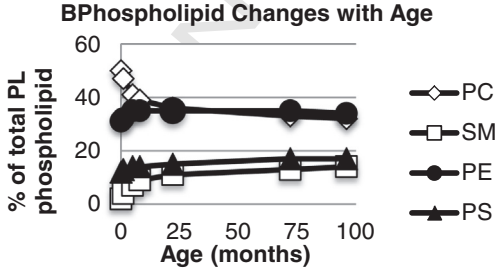

Fig. 2. The phospholipid content of control human brain with age. Phosphatidylcholines (PC) are 50% at birth, declining to 25% at age 8 whereas sphingomyelin (SM) (ceramidephosphorylcholine) increases from 2% to 16% over the same time period. In contrast, phosphatidylserine (PS) and phosphatidylethanolamines (PE) show modest increases over the same time-period. Extracted brain lipids were resolved by 2D HPTLC and qualified by lipid phosphorus analysis according to Rouser [1]. Such analyses do not take into account any regional differences in lipids, fatty acid chain length heterogeneity degree of unsaturation, plasmalogen content and other metabolic modifications.

The Bligh and Dyer initial approach [5] is to add $CHCl_3$ and CH_3OH (1:2 *v*/*v*) to a 1 ml sample, vortex and then add 1.25 ml of $CHCl_3$ and 1.25 ml of water, vortexing well. Most of the lipids will be in the lower phase, which is removed with a pipette. The phases can each be washed with a theoretical upper or lower phase and combined and concentrated before HPTLC (Fig. 3A).

For ganglioside isolation from the upper phase, prior to HPTLC it is necessary to get rid of salt, usually with a Sep-Pak or Lichoprep column and any traces of detergent. Typically, following extraction of fresh or frozen brain tissue with 20 volumes of chloroform-methanol–water (4:8:3 *v*/*v*), water is added to make a final ratio of 4:8:5.6 *v*/*v* [6]. The lower phase is partitioned twice with water and this is combined with the upper phase. The lower phase is used for total lipid analysis and the aqueous (upper phase) is desalted on a c18 reversed phase (eg: Lichoprep 0.3 g/g brain wet weight) and analyzed for more hydrophilic lipids such as gangliosides [7]. Gangliosides are eluted with methanol after extensive washing with water. The dried extract is treated with 0.6 N NaOH to destroy any trace phosphoglycerides and repurified on the column as indicated above. For gangliosides, further purification on DEAE-Sephadex can be done if necessary [7–9].

2.2. Isotope-labeling

Pre-1960s, the direct injection of isotopic precursor intracerebrally into live rodents was used to label lipids for quantification and for use as substrates for lysosomal hydrolase assays. For example, to make [3H]-sphingomyelin for use in sphingomyelinase assays for diagnosis of Niemann–Pick disease, the rat was made choline-deficient and just before death was injected with ^{3}H-choline. The [^{3}H]-SM was extracted from the brain as described above and cleaned up on a column. Other lipids can be labeled by general precursors such as [^{3}H]-palmitate [36] or specific precursors such as [^{3}H-inositol] for polyphosphoinositides [10] and these are now all obtainable from commercial sources. However, such studies led to the realization that minor species of lipids, such as the