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Accumulation of N-Acylphosphatidylserines and N-Acylserines in the Frontal Cortex in Schizophrenia

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Abstract

Background—While schizophrenia is generally considered a neurodevelopment disorder, our basic understanding of the biochemical processes involved in disease etiology and/or progression is limited. One class of biochemical mediators that has been suggested to play a role in the development of schizophrenia is N-acyl ethanolamine metabolites of N-acylphosphatidylethanolamines. However, no investigations of N-acylphosphatidylserines or their N-acylserine metabolites have been published.

Methods—We undertook a targeted *postmortem* lipidomics analysis of N-acylphosphatidylserines (NAPS) and N-acylserines (NAS) in gray matter of the frontal cortex of schizophrenia subjects.

Results—Our data are the first to demonstrate that NAPS and NAS are present in human brain. Furthermore, NAPS and their bioactive metabolites, N-acylserines (NAS), were found to be significantly elevated in the frontal cortex of schizophrenia subjects.

Conclusions—Elevated levels of NAPS lipid pools in schizophrenia may result in complex alterations in the structural function of neuronal membranes while increases in NAS may alter signal transduction pathways.

Keywords

schizophrenia; N-acylserines; N-acylphosphatidylserines; frontal cortex; lipidomics

Introduction

N-Acylphosphatidylethanolamines (NAPE) function as structural components of membranes, with acylation of the polar phosphoethanolamine head group resulting in a lipophilic substitution that folds back into the hydrophobic interior of membranes, thereby stabilizing membranes [1]. In this regard, brain NAPE levels are augmented in models of neuronal injury [2–4].

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Conflict of interests

The author declares no competing financial interests. The funding source also had no role in these studies.

In contrast, NAE which are cleaved from NAPE by phospholipase D (PLD) serve complex signal transduction functions [4, 5]. NAE have been investigated in schizophrenia and been shown to be elevated in CSF [6] but decreased in postmortem prefrontal cortex, hippocampus, and cerebellum [7].

However, in addition to NAE there are a number of N-acyl amino acids generated in the brain and other tissues [8, 9]. An example is N-acylserines (NAS) which are the metabolic products of N-acylphosphatidylserines (NAPS) via PLD [9]. NAPS were first demonstrated in sheep red blood cells [10] and subsequently in pig brain (0.1% of total lipids) and mouse RAW264.7 macrophage cells [11]. The NAS, N-oleoyl serine and N-arachidonyl serine have also been isolated from mouse bone [12] and bovine brain [13], respectively. N-Arachidonyl serine also has been shown to be a potent modulator of calcium-activated potassium channels [14] and of N-type calcium channels [15].

Based on potential roles of NAPS in membrane function and NAS in modulation of synaptic function, we investigated these complex lipids in the *postmortem* frontal cortex of control and schizophrenia subjects.

Materials and Methods

Patient Brain Samples

Brain samples were provided by the UCLA brain bank. Schizophrenia patients were diagnosed based on the Structural Interview for Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV). The demographics of the donors are presented in Table 1.

High Resolution Mass Spectrometry

We undertook a high-resolution mass spectrometric shotgun analysis of N-acylphosphatidylserines (NAPS) and N-acylserines (NAS) in *postmortem* gray matter of the frontal cortex from patients suffering from schizophrenia. Tissue samples were processed utilizing tert-butylmethylether and methanol for extraction of lipids [16, 17]. The extraction solution contained [$^{2}H_{8}$] arachidonic acid, [$^{13}C_{16}$] palmitic acid, [$^{2}H_{31}$] PtdEtn 34:1, [$^{2}H_{54}$] PtdEtn 28:0, NAPS 36:2-N-19:0, and bromocriptine as internal standards. Extracts were dried by centrifugal vacuum evaporation prior to dissolution in isopropanol: methanol: chloroform (4:2:1) containing 7.5 mM ammonium acetate. Shotgun lipidomics were performed utilizing high-resolution (140,000 at 200 amu) data acquisition from 260 to 1450 amu, with sub-millimass accuracy on an orbitrap mass spectrometer (Thermo Q Exactive, Thermo Scientific) in negative ion mode [16,17]. Samples were infused for 1 min at 5 μL/min. followed by successive 500 μL washes of the infusion line with methanol and hexane/ethyl acetate (3:2) to minimize ghost effects. In negative ion ESI (3.2 kV, capillary temp. of 320°C, sheath gas of 10), the anions of NAPS, NAS, and internal standards were monitored.

Lipid identities were validated by MS^2. In negative ion mode utilizing a collision energy of 10, the phosphatidic acid resulting from the loss of NAS was monitored [11]. With a collision energy of 25 the fatty acids of the phosphatidic acid were monitored [11]. This allowed for full elucidation/verification of the structure of each NAPS. Similarly, in negative ion mode

with a collision energy of 25, the structure of NAS could be verified [8]. This included the loss of [-CH_2O], [-CH_2O-H_2O], [-H_2O-CO_2], and [-CH_3O-COOH].

Data Analyses

Data are presented as R values (ratio of the endogenous lipid to the peak area of an appropriate internal standard), corrected for tissue wet weight, in bar graphs ± SEM [17]. Data were analyzed by the Student's t-test.

Results

NAPS

The major NAPS detected in human frontal cortex were NAPS 52:1, NAPS 52:2, and NAPS 54:2. Minor species included NAPS 54:1 and NAPS 54:4. MS/MS experiments verified that NAPS 52:1 was PS 18:0/18:1-N-16:0, NAPS 52:2 was PS 18:1/18:0-N-16:1, and NAPS 54:2 was PS 18:0/18:1-N-18:1 (Fig. 1). In the frontal cortex from schizophrenia subjects all NAPS were significantly elevated (Fig. 2).

NAS

The major NAS detected in human frontal cortex were NAS 16:0, NAS 16:1, and NAS 18:1. These N-acylserines were validated by MS/MS experiments (Fig. 3). In the frontal cortex from schizophrenia subjects the NAS also were significantly elevated (Fig. 2).

Discussion

Despite intensive research efforts to identify biomarkers for early detection of schizophrenia, our knowledge gap in schizophrenia remains large. It is imperative to increase our understanding of the neurodevelopmental factors that contribute to abnormal oligodendrocyte and synaptic function in the limbic cortices of schizophrenia patients [18–23]. Current concepts involve hyper- and hypo-function in both mesolimbic dopaminergic and cortical glutamatergic pathways [18–23]. In this regard, excessive glutamatergic neurotransmission has been demonstrated to increase levels of NAPE and NAE after ischemia in the rat brain [24, 25], after ischemia in the mouse brain [26], with glutamate toxicity in cultured mouse neocortical cells [27], and in the rat hippocampus with *in vivo* kainate-induced neuronal injury [2]. Increased levels of NAPE are hypothesized to represent a neuroprotective mechanism activated by neuronal injury [4]. Therefore, our observations of elevated NAPS and NAS in the frontal cortex of schizophrenia subjects may also be a biomarker of an endogenous neuroprotective mechanism since N-arachidonyl serine is already known to be neuroprotective [9]. Augmentation of NAPS and NAS may represent biochemical responses to excessive glutamatergic neurotransmission [21] and/or altered neuronal development in the cortex [28]. Alterations in PLD which metabolizes NAPS [4, 11] and fatty acid amide hydrolases (FAAH1, FAAH2) which metabolize NAS [29] also need to be investigated for their potential roles in altered NAS levels (Fig. 4). In this regard FAAH has been demonstrated to be a postsynaptic enzyme in the rat hippocampus, cerebellum, and amygdala [30].

In summary, our targeted lipidomics analyses have revealed altered N-acyl amino acid metabolism in the frontal cortex in schizophrenia. However, the effect of sustained NAPS and NAS on neurotransmission remains to be examined since N-acyl amino acids are potent modulators of ion channel function [3, 14, 15]. It also will be important to determine if this may represent a new therapeutic target for

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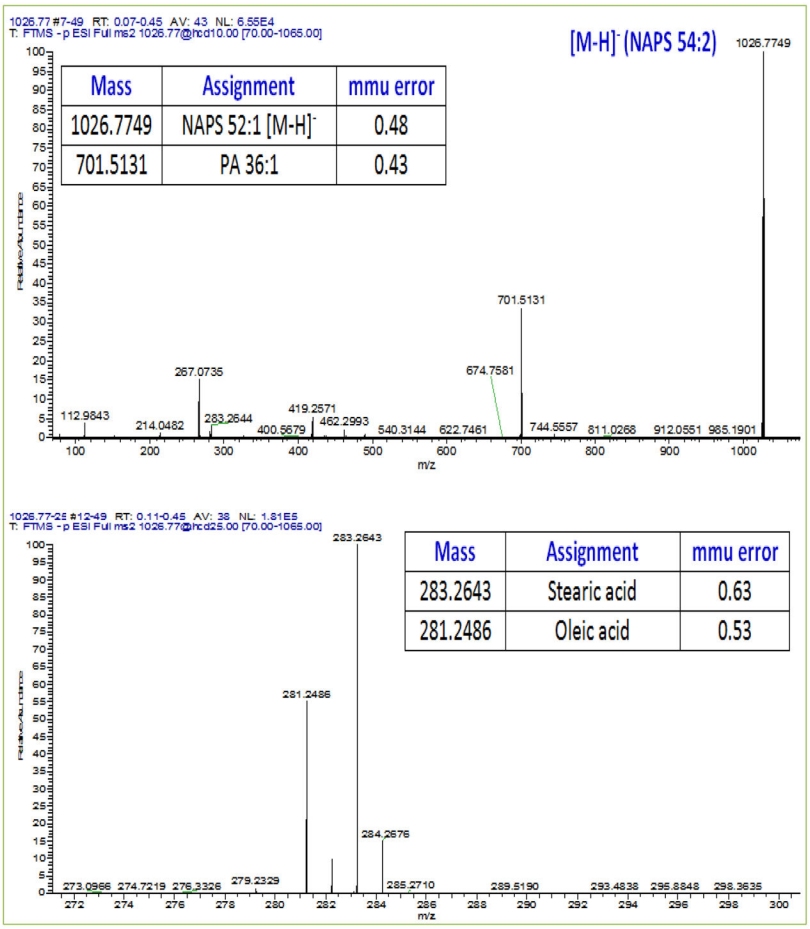


Fig 1. Negative ESI MS^2 spectra for NAPS 52:1 at a collision energy of 10 (upper plot) and at 25 (lower plot) in human frontal cortex lipid extracts

Low collision energies produced phosphatidic acid (PA 36:1) as a result of the loss of N-acyl serine (palmitoylserine). Higher collision energies generated the 2 fatty acid substituents of the PA, namely stearic acid (18:0) and oleic acid (18:1).

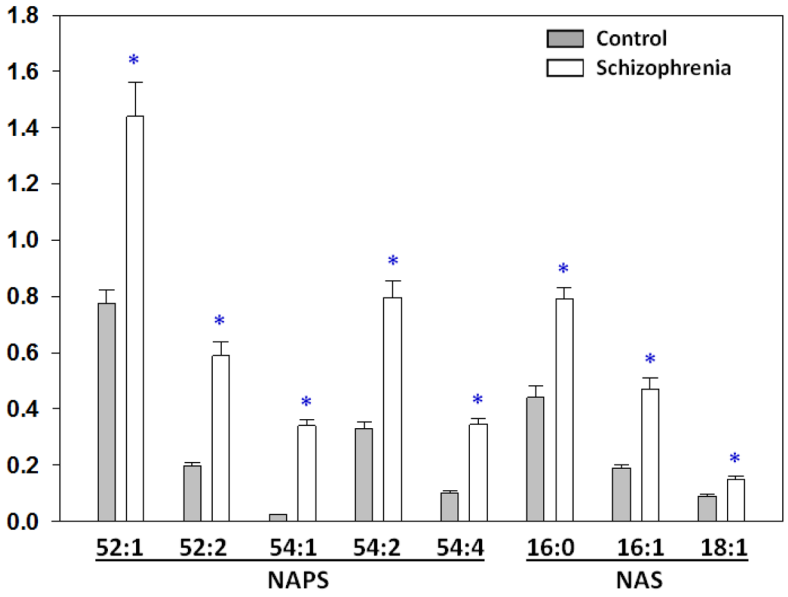
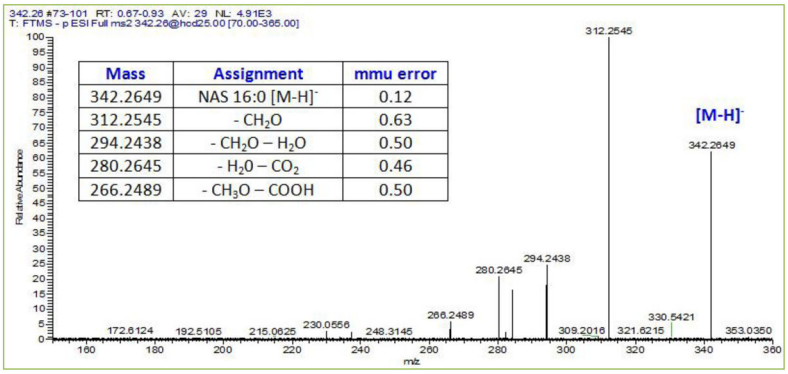


Fig 2. *Postmortem* levels of NAPS and NAS in the frontal cortex of control and schizophrenia subjects

Levels are expressed as the ratio to an appropriate internal standard. Mean ± SEM (N= 9–10). *p< 0.05 vs. control.



Mass	Assignment	mmu error
342.2649	NAS 16:0 [M-H]$^-$	0.12
312.2545	- CH_2O	0.63
294.2438	- CH_2O – H_2O	0.50
280.2645	- H_2O – CO_2	0.46
266.2489	- CH_3O – COOH	0.50

Fig 3. Negative ESI MS^2 spectrum for the NAS, palmitoylserine at a collision energy of 25 in human frontal cortex lipid extracts

The positive ESI MS^2 spectrum was dominated by the $[M+H]^+$ ion for serine 106.0501 (0.22 mmu error; data not shown) further confirming the identity of palmitoylserine.

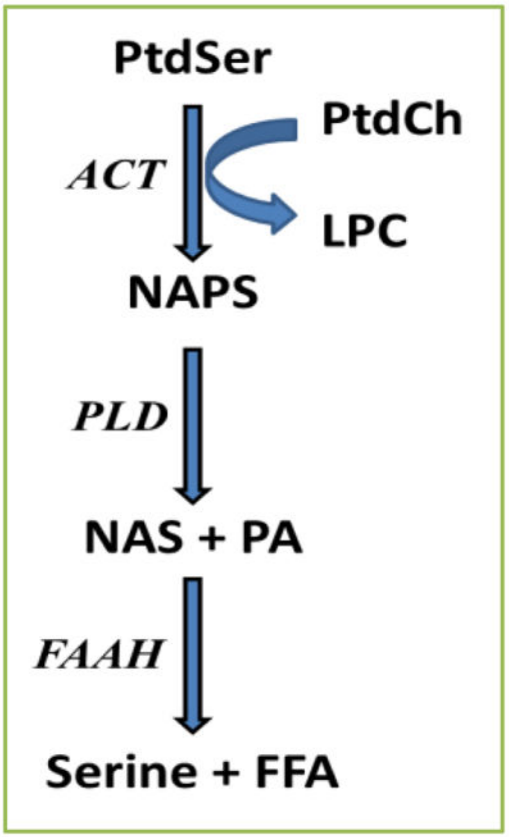


Fig 4. Tentative biosynthetic and degradative pathways for NAPS and NAS

ACT, acyltransferase (fatty acid is from sn-1 of phosphatidylcholine); FAAH, fatty aid amide hydrolase; FFA, free fatty acid; LPC, lysophosphatidylcholine; NAPS, N-acylphosphatidylserine; NAS, N-acylserine; PA, phosphatidic acid; PLD, phospholipase D; PtdCh, phosphatidylcholines; PtdSer, phosphatidylserine. schizophrenia or a biomarker for the early detection of the disease process.

Table 1

Demographics of the donors for the frontal cortex samples.

Diagnosis	Age	Gender	PMI (Hr.)	Neuropathology
Schizophrenia Bipolar	33	M	195	Incidental choroid plexus papilloma
Schizophrenia Bipolar	46	M	21.7	Mildly shrunken CA1 cells
Schizophrenia Bipolar Pancreatic cancer	52	F	15.6	None
Schizophrenia Bipolar Lung cancer	53	M	20.5	None
Schizophrenia Anxiety Depression	55	M	10.7	None
Schizophrenia Stroke	62	F	12.2	Cerebral infarction
Schizophrenia	72	F	13	Old perivascular hemorrhages
Schizophrenia Bipolar CHF	77	F	14.7	None
Schizophrenia Alzheimers	83	F	17	Plaques & tangles
Schizophrenia Depression	85	F	11.5	None
Seizure disorder COPD	35	F	11.8	None
CHF	59	M	20.2	None
Colon cancer	63	M	12	None
Myocardial infarction	68	F	23.7	None
CAD Leukemia	70	M	11.8	None
Pancreatic cancer Diabetes	73	F	18.5	None
Stomach cancer COPD Renal failure	84	M	11.8	None
CHF	87	M	9.3	None
Pancreatic cancer	90	M	19.2	None
Uterine cancer	92	F	23.3	None

CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease