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Cellular Membrane Phospholipids Act as a Depository for Quaternary Amine containing Drugs thus competing with the Acetylcholine / Nicotinic Receptor

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Abstract

We previously demonstrated that ammonium- or guanidinium- phosphate interactions are key to forming non-covalent complexes (NCXs) through salt bridge formation with G-protein coupled receptors (GPCR), which are immersed in the cell membrane's lipids. The present work highlights MALDI ion mobility coupled to orthogonal time-of-flight mass spectrometry (MALDI IM oTOF MS) as a method to determine qualitative and relative quantitative affinity of drugs to form NCXs with targeted GPCRs' epitopes in a model system using, bis-quaternary amine based drugs, α - and β - subunit epitopes of the nicotinic acetylcholine receptor' (nAChR) and phospholipids. Bis-quaternary amines proved to have a strong affinity for all nAChR epitopes and negatively charged phospholipids, even in the presence of the physiological neurotransmitter acetylcholine. Ion mobility baseline separated isobaric phosphatidyl ethanolamine and a matrix cluster, providing an accurate estimate for phospholipid counts. Overall this technique is a powerful method for screening drugs' interactions with targeted lipids and protein respectively containing quaternary amines and guanidinium moieties.

Keywords

Drug-protein interactions; Drug-lipid interactions; MALDI-IM-TOF MS; Noncovalent complexes Phospholipids; Quaternary amines; guanidinium

Introduction

It is a well known fact that many drugs are stored in adipose tissue, thus the importance of understanding the intricacies of lipid-drug interactions is critical for drug development¹. Lipids can affect drug binding properties, as seen in the case of chlorisondamine and the nicotinic acetylcholine receptor, and other target proteins². Determination of protein-protein, protein-drug³⁻⁵ or lipid-drug⁶⁻¹¹ interactions are important for ADMET (absorption, distribution, metabolism, elimination and toxicology) in pre-clinical studies. Lipid-drug

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molecular interactions have been widely studied by fluorescence³, NMR¹²⁻¹³, and surface plasmon resonance¹⁴⁻¹⁵.

The neuronal nicotinic acetylcholine receptor (nAChR) consists of five subunits (e.g., α , β , γ , and δ) and has been well characterized¹⁶⁻¹⁸. The nAChR is allosteric and contains multiple agonist-binding sites (e.g., between an α and γ subunits or an α and δ)¹⁸. Two acetylcholine molecules, each containing a quaternary amine, combine with nAChR to initiate excitatory post-synaptic potential in nerves or end plate potential in muscles. The acetylcholine – receptor interaction lasts about one millisecond¹⁹ and is followed by acetylcholinesterase rapidly hydrolysing acetylcholine to acetate and choline, to return the nAChR to its resting potential or to re-polarize. For this study non-phosphorylated and phosphorylated versions of the nAChR $\alpha 2$ isoform epitope (residues 388-403, GEREETEEEEEEEDEN) and the $\beta 2$ isoform (residues 439-450, SEDDDQSVSED) were used to test bis-quaternary amine compounds' interactions with these nAChR epitopes²⁰.

Most nicotinic cholinergic receptor blockers such as chlorisondamine or modulators, contain quaternary or bis-quaternary amines²¹. The drugs decamethonium, hexamethonium, and succinylcholine are bis-quaternary amines²². Decamethonium (DCM) is a partial agonist to nAChR. Unlike the neurotransmitter acetylcholine, DCM continues to strongly bind to nAChR after depolarizing the motor end plate. Hexamethonium (HXM) is a nAChR antagonist. Succinylcholine (SCH) is a depolarizing skeletal muscle relaxant, which depolarizes the cholinergic receptors of the motor end plate and is the shortest lived depolarizing agent as pseudocholinesterases breaks it down²². Decamethonium and hexamethonium are not metabolized, but are slowly excreted from the body.

Phospholipids are one of the major lipid groups in cellular membranes and represent the majority of brain lipid content²³. A phospholipid contains two acyl groups at *sn1*, *sn2* position and one phosphate group at position *sn3*. The different phospholipid classes are shown in Scheme 1. PC contains a fixed positive charge via the quaternary amine, whereas all of the other phospholipids preferentially ionize in negative ion mode. PG and PI tend to be the most acidic as reflected in mass spectrometric studies²⁴⁻²⁵. The negatively charged phosphate group in a phospholipid can form a strong and stable non-covalent complex (NCX) with the positively charged quaternary amine group²⁶⁻²⁷. This same ionic bond stability manifests itself in the electrostatic interaction between the amino acid arginine guanidinium group and the phosphate of a phosphorylated residue^{26, 28-29}.

In this present study we used a matrix assisted laser desorption ionization – ion mobility – mass spectrometer (MALDI IM MS)³⁰ to determine qualitative and relative quantitative affinity of phospholipid-bis-quaternary amine drug and nAChR epitope-bis-quaternary amine drug interactions. Ion mobility mass spectrometry quickly separates and detects biomolecules originating from complex samples and has had a wide array of applications including metabolomics, proteomics, and beyond³¹⁻³⁴. Isobaric analytes from specific biological families fall along distinct, familial “trend lines”, facilitating the identification of species³⁰. This analytical technique was previously used to study biomolecular interactions³⁵⁻³⁶. Woods et al. have previously shown the tetrachlorobenzene portion of the drug chlorisondamine, which is an nAChR antagonist, forms a stable NCX with the lipid

sphingomyelin^{2,38}. The bis-quaternary amine portion of chlorisondamine forms stable NCXs with acidic phospholipids³⁷ and with the nAChR epitopes³⁸. The formation of NCXs indicates the affinity of bis-quaternary amine based drugs for non-phosphorylated and phosphorylated target epitopes of the nAChR in the presence of phospholipids.

Experimental

Samples

Phospholipids—Six C36:2 (C18:1 / C18:1) phospholipids, (phosphatidic) acid (PA), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG), phosphatidyl inositol (PI), and phosphatidyl serine (PS) were purchased from Avanti Polar Lipids (Alabaster, AL). The prepared phospholipid stock solutions were 10 or 1 nmol / μ L in 2:1 chloroform methanol, and then diluted to the appropriate concentration just before deposition.

Receptor Peptides—The α 2 subunit nicotinic / acetylcholine receptor epitope consisted of GEREETEEEEEEEDEN (MW=1981.7) and GEREEpTEEEEEEEEDEN (p=phosphorylated residue, MW=2061.7). The β 2 subunit nicotinic / acetylcholine receptor epitope is EDDDQSVSED (ppt3, MW=1138.4) and diposphorylated serine is pSEDDDDQpSVSED (MW=1385.4). All peptides were synthesized at the Johns Hopkins School of Medicine Peptide Synthesis Core Facility. The peptide epitope stock solutions were prepared between 1 and 4 nmols per μ L in doubly distilled, 18 M Ω water and then diluted to the appropriate concentration just before deposition.

Quaternary Amines—Decamethonium bromide (DCM, MW = 258.48), hexamethonium chloride (HXM, MW=202.24), and succinylcholine chloride (SCH, MW=290.98) were the bis-quaternary amines and acetylcholine hydrogen chloride (ACH, MW=146.23), a neurotransmitter containing one quaternary amine, were all purchased from Sigma (St. Louis, MO). Stock solutions were dissolved in 20 % water / 80 % ethanol to 35 nmols per μ L. The final quaternary amine concentration was half of the total concentration of phospholipids and peptides.

Sample Preparation

All analytes were mixed with the supernatant of a saturated 2,4,6 – trihydroxy acetophenone (THA, 28 mg per mL saturated solution) in 50 % water : 50 % ethanol.

Six phospholipids and one bis-quaternary amine were mixed in a 1:1:1:1:1:1:3 ratio. The non-phosphorylated and phosphorylated acidic peptides and bisquaternary amine were mixed as a 1:1:1 ratio. The six phospholipids, non-phosphorylated and phosphorylated acidic peptides, and bis-quaternary amine were mixed as a 1:1:1:1:1:1:5:5:8 ratio. For acetylcholine experiments, it was added at the same concentrations as the bis-quaternary amines.

Mass Spectrometer

Data were acquired with a periodic focusing MALDI IM TOF MS instrument using positive ion mode (Ionwerks Inc., Houston, TX). Samples were accessed via an X-Y sample stage (National Aperture Inc., folded micro-stage, model MM-3M-F-2) that provided 1 μm accuracy in beam positioning and sample scanning. Ions were generated by a Nd:YLF UV laser (Spectra Physics, Irvine, CA; $\lambda = 349 \text{ nm}$ at 100 Hz) and extracted into an 18cm, stacked ring mobility cell operated at 1700 V across it and filled with $\sim 1.2 \text{ Torr } 99.999\%+$ helium gas. Calibration standards routinely achieved mobility resolution of 25 (FWHM of drift time) and a mass resolution of 2800 for m/z 1060, were more than adequate for the purpose of distinguishing the complexes we were looking for.

Mobility separation of the individual analytes and non-covalent complexes took hundreds of microseconds, whereas the flight times within the mass spectrometer took tens of microseconds. Therefore, multiple (10-30) mass spectra could be obtained for every laser pulse to capture the mobility profile. An interleave³⁷ equal to 25 provided ample points across the mobility peaks.

Data are presented as 2D contour plots, which are a function of drift time (y-axis), m/z (x-axis), and ion intensity that is designated using a color scheme where green is the lowest relative intensity signal, red is intermediate and white is the most intense signal. In addition, summed 1D ion mobility spectrum and 1D mass spectrum are shown. In 2D contour plots of drift time versus m/z , compounds having the same molecular weight but different structures are observed to have different slopes or trend lines. The reported m/z and drift time values are based upon a centroid calculation of the (boxcar-averaged) smoothed 2D signal, giving the center-of-mass for a particular 2D ion intensity distribution. All contour plots were produced using IDL software (Research Systems, Boulder, CO).

Results

A. Non-covalent interactions between phospholipids and bis-quaternary amines

PA, PC, PE, PG, PS, and PI were mixed with bis-quaternary amines DCM, HXM, and SCH (Figures 1a, 1b, and 1c, respectively) to assess their tendency to form NCXs. PA, PE, PG, PS, and PI added one bis-quaternary amine to form singly, positively charged NCXs (Table 1). Intact PC did not form an NCX with any of the bis-quaternary amines. A minor NCX formed between each bis-quaternary amine and the PC fragment resulting from loss of its quaternary amine.

Phospholipid and bis-quaternary amine NCX percentages in Table 2 were calculated by dividing NCX counts over total protonated [m/z 786], sodiated [m/z 808], potassiated [m/z 824], and sodiated minus the tertiary amine [m/z 749] PC counts. Generally, PI and PG were the most likely to form an NCX with a bis-quaternary amine followed by PE, PA, and PS. HXM formed the most abundant NCXs, followed by DCM and SCH.

B. Non-covalent interactions between α or β subunit peptides and decamethonium

Equimolar amounts of DCM, GEREETEEEEEEEDEN, and GEREEpTEEEEEEEEDEN were mixed (Figure 2a). DCM formed an NCX with the non-phosphorylated epitope (m/z 2+ = 1119, 1+ = 2238) and with the phosphorylated epitope (m/z 2+ = 1159, 1+ = 2318). Using protonated plus sodiated GEREETEEEEEEEDEN counts as the base, the ratio of counts of DCM-phosphorylated peptide NCX was more abundant at 29.3% versus 18.8% for the non-phosphorylated NCX. Protonated and sodiated GEREEpTEEEEEEEEDEN were 74 % of the base GEREETEEEEEEEDEN peaks.

Equimolar amounts of DCM, EDDDDQSVSED, and pSEDDDDQpSVSED were mixed (Figure 2b). Both EDDDDQSVSED (m/z 2+ = 698, 1+ = 1396) and diphosphorylated pSEDDDDQpSVSED (m/z 2+ = 821 1+ = 1641, 1898) epitopes formed NCX with DCM. Only pSEDDDDQpSVSED formed an NCX with two DCM. Using protonated and sodiated EDDDDQSVSED counts as the base, the ratio of counts of DCM plus phosphorylated peptide NCX was more abundant at 85.6% versus 59.3% for non-phosphorylated peptide NCX. Though these ions are not isobaric, the mobility dimension provided ample separation for EDDDDQSVSED + DCM (m/z 1396, slower mobility) and the faster acidic, protonated pSEDDDDQpSVSED (m/z 1385) peaks.

C. Competition for decamethonium between phospholipids and α or β subunit epitopes

DCM was mixed with PA, PC, PE, PG, PI, PS, GEREETEEEEEEEDEN and GEREEpTEEEEEEEEDEN (Figure 3a). PA, PE, PG, PI, and PS each added one bis-quaternary amine to form singly, positively charged NCXs. As expected, PC did not form an NCX due to repulsion of like quaternary amine groups. PI and PG DCM NCXs were the most abundant followed by PE, PS, and PA (Table 3, Figure 3a). One DCM added in equal abundance to non-phosphorylated and phosphorylated α -subunits, but GEREEpTEEEEEEEEDEN was more likely than GEREETEEEEEEEDEN to add two DCM (Table 3, Figure 3a).

DCM was mixed with PA, PC, PE, PG, PI, PS, EDDDDQSVSED and pSEDDDDQpSVSED (Figure 3b). Again, each PA, PE, PG, PI, and PS added one bis-quaternary amine to form singly, positively charged NCXs. PI and PG DCM NCXs were the most abundant followed by PA, PE, and PS. pSEDDDDQpSVSED formed more abundant NCXs with one and two DCM than the non-phosphorylated EDDDDQSVSED peptide.

D. Competition for decamethonium and acetylcholine between phospholipids and α and β subunit epitopes

Acetylcholine (ACh), DCM, PA, PC, PE, PG, PI, PS, GEREETEEEEEEEDEN, and GEREEpTEEEEEEEEDEN were mixed (Figure 4a). Acetylcholine did not form a complex with α -subunit peptides. Acetylcholine only formed a complex with PC (in blue, Figure 4a). PA, PE, PG, PI, and PS each added one bis-quaternary amine to form singly, positively charged NCXs; the relative abundance order was PI, PG, PE, PS, and PA. The abundance of GEREEpTEEEEEEEEDEN DCM NCXs was greater than non-phosphorylated GEREETEEEEEEEDEN. Overall peptide signal was suppressed with respect to data collected without ACh, suggesting an effect of acetylcholine on ionization.

ACh, DCM, PA, PC, PE, PG, PI, PS, EDDDQSVSED, and **pSEDDDDQpSVSED** were mixed (Figure 4b). PA, PE, PG, PI, and PS each added one bis-quaternary amine to form a singly, positively charged NCXs; the relative abundance order was PI, PG, PA, PE, and PS (Table 3, Figure 4b). PC formed an NCX with acetylcholine (in blue, Figure 4b). The relative abundance of **pSEDDDDQpSVSED** – DCM NCXs was greater than EDDDQSVSED – DCM NCXs. Peptides EDDDQSVSED and **pSEDDDDQpSVSED** each formed NCXs with one DCM and one ACh. Overall peptide signal was suppressed with respect to data collected without ACh, suggesting an effect of acetylcholine on ionization.

Discussion

Phospholipids are readily measured using MALDI-TOF MS in positive and negative ion modes. As demonstrated by our data, PC was easily detected in positive ion mode consistent with the results of Jackson et al.⁴⁰, due to its fixed quaternary amine positive charge. However it did not form an NCX with DCM, HXM, or SCH, as NCX formation depends on one of the interacting domain to be negative. The intramolecular interaction of the positively charged choline with the negatively charged phosphate would make PC less acidic and be competitive with interacting non-covalently with DCM, HXM, or SCH. The absence of an NCX between PC and these quaternary amines in our results is consistent with Coulombs' law, as the large positive charge imparted by the quaternary amines in both molecules causes them to repulse each other. PA, PG, PI and PS which are negatively charged phospholipids and are typically detected using negative ion mode, are far more amenable to forming NCX with quaternary amines, thus making these phospholipids detection possible in positive ion mode. PI and PG formed the most abundant NCXs with DCM. As a species becomes more acidic, it is more likely to form a strong electrostatic phospholipid – quaternary amine NCX amidst other competing species. Our observation that PI and PG are the most acidic phospholipids is consistent with negative mode mass spectrometric measurements made by Koivusalo et al.²⁴ and Thomas et al.²⁵. The stability of such interactions was demonstrated by the work of Laskin et al. and Woods et al. When normalizing data, it is preferred to use a phospholipid-NCX to total phospholipid ratio but because PI, PG, PA, PE, and PS do not form protonated ions, the signal of PC was used as the normalization agent. Using PC, we were able to compare across experiments as well to understand relative NCX abundances.

The acidic nAChR subunit epitopes studied all formed NCXs with DCM. The pI of GEREETEEEEEDEN and EDDDQSVSED are 3.2 and 2.6, respectively⁴¹. EDDDQSVSED and **pSEDDDDQpSVSED** have a greater negative charge at the experimental pH, thus it naturally follows that more acidic EDDDQSVSED and **pSEDDDDQpSVSED** form complexes with DCM to a greater extent than GEREETEEEEEDEN and GEREETEEEEEDEN peptides. Nonetheless, the single negative charge on phospholipids competes quite well with these four acidic epitopes for quaternary amines, as evident by the data presented in this paper.

Another observation that the bis-quaternary amine HXM forms more abundant phospholipid – HXM NCXs than either DCM or SCH is in line with a study by Ascher et al.⁴². They noted that the “concentrations [of DCM] needed for a given degree of [nAChR] block were *higher* than those needed for curare and HXM”³⁸ to accomplish the same blockage.

(emphasis added). In this study it follows that strong HXM – nAChR peptide complexes results in strong HXM – phospholipid NCXs. The weaker effects of DCM are consistent with HXM and SCH in that they can all form electrostatic interactions with and can block nAChR via their two quaternary amines. Thus DCM is an appropriate bis-quaternary amine representative for subsequent phospholipid and receptor epitope competition studies presented in this paper. Laskin et al.⁶ studied the binding energies of non-covalent complexes between quaternary amines and phosphate, and demonstrated the stability of such interactions.

The ion mobility method provided separation for classes of compounds, with each class falling along distinct trend lines³⁰. With respect to classes of compounds analyzed in this paper, matrix clusters, phospholipids, phospholipids – bis-quaternary amine NCXs, acidic peptides, acidic peptides –bis-quaternary amine NCXs, and multiple charge state ions each have their own trend line. PE and PC fall along the faster mobility (421 and 439 us, respectively) lipid trendline whereas PA, PE, PG, PS, and PI bis-quaternary amine NCXs have slightly slower mobility (495, 510, 516, 513, and 528 us, respectively). Doubly charged peptide-bis-quaternary NCXs have the fastest mobility of all compounds measured in this experimental set, falling along their own doubly charged state trend line (316 and 325 us, respectively). Acidic peptides have a faster mobility in positive ion mode than acidic peptide-DCM NCXs as seen in Figure 2, presumably because negatively charged portions of the peptide adopt a folded conformation around positive charges. The bis-quaternary amines are similar to phospholipids in that both contain long $-(CH_2)-$ chains linking the amines; both have slow mobilities relative to matrix cluster trendline. Generally the bis-quaternary amine mobility slows down the acidic peptide mobility, as evident in the near baseline separation of protonated pSEDDDDQpSVSED (m/z 1385.4, 471.7 us) and EDDDDQSVSED plus DCM (m/z 1394.7, 507.3 us).

Acetylcholine (ACh), a neurotransmitter for the nAChR, is an agonist for α -subunit epitopes, GEREETEEEEEDEN and GEREETEEEEEDEN. ACh is also an agonist for β -subunit epitopes, EDDDDQSVSED and pSEDDDDQpSVSED. We did not observe any signal corresponding to α -subunit-ACh NCX, however, β -subunit-DCM-ACh NCX was observed. The affinity of β -subunit epitope for ACh is therefore considered stronger than the α -subunit. The increased acidity of the β -subunit with respect to the α -subunit provides the coulombic attraction necessary to add the additional positive charge of ACh. ACh has sufficient interaction with the phosphate of PC to disrupt the intra-molecular interaction between the choline headgroup and phosphate to be observed at 21% and 16% (see Table 3) of the total PC signal suggesting that ACh more readily interacts with the lipid than the epitope peptides present. The data also tells us that peptide epitopes/bis-quaternary amine NCXs are preferred to peptide epitope/ACh NCXs.

A very important aspect of the ion-mobility technology is that the mobility cell provided baseline separation of phospholipid and epitope peptide versus matrix clusters. As expanded upon in Figure 5, protonated PE and a $(THA)_3 + DCM - H_{20} - H$ cluster. Protonated PE has a centroided mobility time of 416.2 us, whereas the cluster appears earlier at 369.2 us. The unique separation capability of ion mobility enabled us to focus only on PE and peptide counts. Without mobility, the matrix cluster signal could not be decoupled from the PE and

epitope peptide signals. This MALDI ion mobility mass spectrometry experimental approach offers a quick, selective method to assess drug binding affinity to target peptides in the midst of cellular membrane phospholipids.

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References

1. Waring MJ. Lipophilicity in Drug Discovery. *Expert Opin Drug Discov.* 2010; 5(3):235–248. [PubMed: 22823020]
2. Woods AS, Moyer SC, Wang HYJ, Wise RA. Interaction of Chlorisondamine with the Neuronal Nicotinic Acetylcholine Receptor. *J Proteome Res.* 2003; 2(2):207–212. [PubMed: 12716135]
3. Romsicki Y, Sharom FJ. The Membrane Lipid Environment Modulates Drug Interactions with the P-Glycoprotein Multidrug Transporter. *Biochem.* 1999; 38(21):6887–6896. [PubMed: 10346910]
4. Trainor GL. The importance of plasma protein binding in drug discovery. *Expert Opin Drug Discov.* 2007; 2(1):51–64. [PubMed: 23496037]
5. Vuignier K, Schappler J, Veuthey JL, Carrupt PA, Martel S. Drug-protein binding: a critical review of analytical tools. *Anal Bioanal Chem.* 2010; 398(1):53–66. [PubMed: 20454782]
6. Laskin J, Yang Z, Woods AS. Competition between covalent and noncovalent bond cleavages in dissociation of phosphopeptide-amine complexes. *Phys Chem Chem Phys.* 2011; 13(15):6936–6946. [PubMed: 21387029]
7. Woods AS, Moyer SC, Jackson SN. Amazing Stability of Phosphate-Quaternary Amine Interactions. *J Proteome Res.* 2008; 7(8):3423–3427. [PubMed: 18578519]
8. Maheswari KU. Lipid bilayer-methotrexate interactions: a basis for methotrexate neurotoxicity. *Current Sci.* 2001; 81(5):571–574.
9. Barratt G, Saint-Pierre-Chazalet M, Luiseau PM. Cellular Transport and Lipid Interactions of Miltefosine. *Current Drug Metabolism.* 2009; 10(3):247–255. [PubMed: 19442087]
10. Seddon AM, Casey D, Law RV, Gee A, Templer RH, Ces O. Drug Interactions with lipid membranes. *Chem Soc Rev.* 2009; 38(9):2509–2519. [PubMed: 19690732]
11. Lucio M, Lima JLFC, Reis S. Drug-Membrane Interactions: Significance for Medicinal Chemistry. *Curr Med Chem.* 2010; 17(17):1795–1809. [PubMed: 20345343]
12. Sujak A. Interactions Between Canthaxanthin and Lipid Membranes-Possible Mechanisms of Canthaxanthin Toxicity. *Cell Mol Biol Lett.* 2009; 14(3):395–410. [PubMed: 19214394]
13. Batchelor R, Windle CJ, Buchouz S, Lorch M. Cholesterol and Lipid Phases Influence the Interactions between Serotonin Receptor Agonists and Lipid Bilayers. *J Biol Chem.* 2010; 285(53):41402–41411. [PubMed: 20961857]
14. Cimitan S, Lindgren MT, Bertucci C, Danielson UH. Early Absorption and Distribution Analysis of Antitumor and Anti-Aids Drugs: Lipid Membrane and Plasma protein Interactions. *J Med Chem.* 2005; 48(10):3536–3546. [PubMed: 15887962]
15. Huber W, Mueller F. Biomolecular Interaction Analysis in Drug Discovery Using Surface Plasmon Resonance Technology. *Curr Pharm Design.* 2006; 12(31):3999–4021.
16. Karlin A. Emerging Structure of the Nicotinic Acetylcholine Receptors. *Nature Rev Neurosci.* 2002; 3(2):102–114. [PubMed: 11836518]
17. Kalamida D, Poulas K, Avramopoulou V, Fostieri E, Lagoumintzis G, Lazaridis K, Sideri A, Zouridakis M, Tzartos S. Muscle and neuronal nicotinic acetylcholine receptors. Structure, function and pathogenicity. *Febs J.* 2007; 274(15):3799–3845. [PubMed: 17651090]

18. Miyazawa A, Fujiyoshi Y, Stowell M, Unwin N. Nicotinic Acetylcholine Receptor at 4.6 Å Resolution: Transverse Tunnels in the Channel Wall. *J Mol Biol.* 1999; 288(4):765–786. [PubMed: 10329178]
19. Taylor, P. Agents Acting at the Neuromuscular Junction and Autonomic Ganglia. In: Hardman, JG.; Limbird, LE., editors. *Goodman and Gilman The Pharmacological Basis of Therapeutics*. Tenth. McGraw-Hill; New York: 2000. p. 193
20. Woods AS, Moyer SC, Wang HY, Wise RA. Interaction of chlorisondamine with the neuronal nicotinic acetylcholine receptor. *J Proteome Res.* 2003; 2:207–212. [PubMed: 12716135]
21. Holladay MW, Dart MJ, Lynch JK. Neuronal Nicotinic Acetylcholine Receptors as Targets for Drug Discovery. *J Med Chem.* 1997; 40(26):4169–4194. [PubMed: 9435889]
22. Koelle, GB. *Goodman and Gilman The Pharmacological Basis of Therapeutics*. Fourth. McGraw-Hill; New York: 1970. Neuromuscular Blocking Agents; p. 601-619.
23. Agranoff, BW.; Benjamins, JA.; Hajra, AK. *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects*. Sixth. Lippincott-Raven; Philadelphia: 1999. Lipids; p. 47-67.
24. Koivusalo M, Haimi P, Heikinheimo L, Kostianinen R, Somerharju P. Quantitative determination of phospholipid compositions by ESI-MS: effects of acyl chain length, unsaturation, and lipid concentration on instrument response. *J Lipid Res.* 2001; 42(4):663–672. [PubMed: 11290839]
25. Thomas MC, Mitchell TW, Blanksby SJ. A Comparison of the Gas Phase Acidities of Phospholipid Headgroups: Experimental and Computational Studies. *J Am Soc Mass Spectrom.* 2005; 16(6):926–939. [PubMed: 15907707]
26. Woods AS. The Mighty Arginine, the Stable Quaternary Amines, the Powerful Aromatics, and the Aggressive Phosphate: Their Role in the Noncovalent Minuet. *J Proteome Res.* 2004; 3(3):478–484. [PubMed: 15253429]
27. Woods AS, Ferre S. Amazing Stability of the Arginine-Phosphate Electrostatic Interaction. *J Proteome Res.* 2005; 4(4):1397–1402. [PubMed: 16083292]
28. Jackson SN, Wang HYJ, Woods AS. Study of the Fragmentation Patterns of the Phosphate-Arginine Noncovalent Bond. *J Proteome Res.* 2005; 4(6):2360–2363. [PubMed: 16335986]
29. Jackson SN, Wang HYJ, Yergey A, Woods AS. Phosphate Stabilization of Intermolecular Interactions. *J Proteome Res.* 2006; 5(1):122–126. [PubMed: 16396502]
30. Woods AS, Ugarov M, Egan T, Koomen J, Gillig KJ, Fuhrer K, Gonin M, Schultz JA. Lipid/Peptide/Nucleotide Separation with MALDI-Ion Mobility-TOF MS. *Anal Chem.* 2004; 76(8):2187–2195. [PubMed: 15080727]
31. Dwivedi P, Schultz AJ, Hill HH. Metabolic Profiling of Human Blood by High-Resolution Ion Mobility Mass Spectrometry (IM-MS). *Int J Mass Spectrom.* 2010; 298:78–90. [PubMed: 21113320]
32. Baker ES, Livesay EA, Orton DJ, Moore RJ, Danielson WF 3rd, Prior DC, Ibrahim YM, LaMarche BL, Mayampurath AM, Schepmoes AA, Hopkins DF, Tang K, Smith RD, Belov ME. An LC-IMS-MS Platform Providing Increased Dynamic Range for High-Throughput Proteomic Studies. *J Proteome Res.* 2010; 9(2):997–1006. [PubMed: 20000344]
33. Bleiholder C, Dupuis NF, Wytenbach T, Bowers MT. Ion mobility-mass spectrometry reveals a conformational conversion from random assembly to β -sheet in amyloid fibril formation. *Nat Chem.* 2011; 3(2):172–177. [PubMed: 21258392]
34. Plasencia MD, Isailovic D, Merenbloom SI, Mechref Y, Novotny MV, Clemmer DE. Resolving and Assigning N-linked Glycan Structural Isomers from Ovalbumin by IMS-MS. *J Am Soc Mass Spectrom.* 2008; 19(11):1706–1715. [PubMed: 18760624]
35. Woods AS, Koomen J, Ruotolo B, Gillig KJ, Russell DH, Fuhrer K, Gonin M, Egan T, Schultz JA. A Study of Peptide-Peptide Interactions Using MALDI Ion Mobility o-TOF and ESI Mass Spectrometry. *J Am Soc Mass Spectrom.* 2002; 13(2):166–169. [PubMed: 11838019]
36. Woods AS, Fuhrer K, Gonin M, Egan T, Ugarov M, Gillig KJ, Schultz JA. Angiotensin II-Acetylcholine Noncovalent Complexes Analyzed with MALDI Ion Mobility-TOF MS. *J Biomol Tech.* 2003; 14(1):1–8. [PubMed: 12901606]
37. Jackson SN, Wang HYJ, Woods AS, Ugarov M, Egan T, Schultz JA. Direct Tissue Analysis of Phospholipids in Rat Brain Using MALDI-TOFMS and MALDI-Ion Mobility-TOFMS. *J Am Soc Mass Spectrom.* 2005; 16(2):133–138. [PubMed: 15694763]

38. Wang HYJ, Taggi AE, Meinwald J, Wise RA, Woods AS. Study of the Interaction of Chlorisondamine and Chlorisondamine Analogues with an Epitope of the α -2 Neuronal Acetylcholine Nicotinic Receptor Subunit. *J Proteome Res.* 2005; 4(2):532–539. [PubMed: 15822931]
39. Ruotolo BT, McLean JA, Gillig KJ, Russell DH. The Influence and Utility of Varying Field Strength for the Separation of Tryptic Peptides by Ion Mobility-Mass Spectrometry. *J Am Soc Mass Spectrom.* 2005; 16(2):158–165. [PubMed: 15694766]
40. Jackson SN, Woods AS. Direct Profiling of Tissue Lipids by MALDI-TOFMS. *J Chrom B.* 2009; 877(26):2822–2829.
41. As calculated by GPMW, version 6.11, 2003.
42. Ascher P, Marty A, Neild TO. The mode of action of antagonists of the excitatory response to acetylcholine in Aplysia neurons. *J Physiol.* 1978; 278(1):207–235. [PubMed: 671288]

Figure 1.

Contour plot of drift time versus m/z of non-covalent complexes of PA, PC, PE, PG, PI, PS with (a) DCM, (b) HXM, and (c) SCH.

Figure 2.

Contour plot of drift time versus m/z of noncovalent complexes (NCX) of DCM with (a) GEREETEEEEEEEDEN and GEREEpTEEEEEEEEDEN and (b) EDDDQSVSED and pSEDDDDQpSVSED.

Figure 3. Contour plot of drift time versus m/z of PA, PC, PE, PG, PI, PS, and DCM with (a) GEREETEEEEEEEDEN and GEREETEEEEEEEDEN and (b) EDDQSVSED and pSEDDQpSVSED.

Figure 4.

(a) Contour plot of drift time versus m/z of PA, PC, PE, PG, PI, PS, DCM and Ach with (a) GEREETEEEEEEEDEN and GEREETEEEEEEEDEN and (b) EDDQSVSED and pSEDDQpSVSED.

Figure 5.
Baseline mobility separation of THA/DCM clusters and protonated PE.

HG = phospholipid head group

Scheme 1.

Table 1
Phospholipid and quaternary amine masses

Phospholipid	MH+/MNa+	M+DCM	M+HXM	M+SCH
PA C36:2	- /722.5	957.9	901.8	989.8
PC C36:2	786.6/808.6	-	-	-
PC C36:2 – N(CH ₃) ₃	749.5	-	-	-
PE C36:2	744.5/766.5	1000.9	944.8	1032.8
PG C36:2	- /797.5	1031.9	975.8	1063.9
PI C36:2	- / -	1120.0	1063.9	1151.9
PS C36:2	- / -	1044.9	988.8	1076.8

Table 2
Percent NCX versus total PC C36:2 signal

Species	DCM	HXM	SCH
PA	18.4	26.3	10.5
PE	27.2	41.1	15.5
PG	45.5	60.0	25.7
PS	13.0	16.7	7.3
PI	50.2	59.6	29.7

Extent to form NCXs is PI>PG>PE>PA>PS

Table 3

Percent NCX versus total PC C36:2 signal

Species	Figure 3a	Figure 3b	Figure 4a	Figure 4b	m/z
PC+H/Na	100	100	100	100	786.6/808.6
PC+ACh	-	-	21.0	15.9	931.7
PE+H/Na	11.2	11.6	7.7	9.4	744.7/766.7
PG+Na	4.6	4.6	2.7	3.8	797.7
PA+DCM	12.7	15.2	7.4	12.7	957.9
PE+DCM	25.2	8.6	19.5	7.9	1000.9
PG+DCM	38.6	20.5	24.3	18.6	1031.9
PS+DCM	16.2	9.1	10.1	6.8	1044.9
PI+DCM	39.3	24.8	30.7	21.9	1120.0
EDDDQSVSED+H/Na	-	77.8	-	25.7	1138.4/1160.4
pSEDDDDQpSVSED+H/Na	-	32.0	-	15.3	1385.4/1407.4
EDDDQSVSED+DCM	-	15.5	-	6.0	698.3
EDDDQSVSED+DCM	-	37.9	-	15.1	1394.7
EDDDQSVSED+DCM+ACh	-	-	-	2.3	1539.8
EDDDQSVSED+2DCM	-	3.7	-	-	1652.0
pSEDDDDQpSVSED+DCM	-	15.1	-	8.0	821.9
pSEDDDDQpSVSED+DCM	-	48.1	-	26.5	1641.7
pSEDDDDQpSVSED+DCM+ACh	-	-	-	1.7	1786.8
pSEDDDDQpSVSED+2DCM	-	10.7	-	4.6	1898.0
GEREETE7DEN+H/Na	31.2	-	6.8	-	1981.7/2003.7
GEREETE7DEN+H/Na	15.5	-	5.2	-	2061.7/2083.7
GEREETE7DEN+DCM	16.1	-	2.8	-	2238.0
GEREETE7DEN+2DCM	2.4	-	0.7	-	2494.3
GEREETE7DEN+DCM	15.9	-	4.8	-	2318.0
GEREETE7DEN+2DCM	6.8	-	2.0	-	2574.3

All relative abundances are calculated against total MH⁺, MNa⁺, MK⁺, and [PC-N(CH₃)₃] PC counts.