Indirect Generation of 13C-Hyperpolarized Choline and Lecithin using Parahydrogen

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Introduction

Neurotransmitter disorders are due to several errors of metabolism that affect the production of neurotransmitters. Neurotransmitters have vast CNS effects, controlling aspects of memory and cognition, temperature regulation, pain control, motor function, etc. One of the first neurotransmitters to be discovered was acetylcholine (ACh). ACh acts as a chemical transmitter in both the peripheral nervous system (PNS) and central nervous system (CNS) in many organisms including humans. It is one of many neurotransmitters in the autonomic nervous system (ANS) and the only one used in the somatic nervous system (SNS). ACh is produced by the synthetic enzyme choline acetyltransferase, which uses acetyl coenzyme A and choline as substrates for the formation of ACh. Dietary choline and phosphatidylcholine serve as the sources of free choline for ACh synthesis. Upon release, ACh is metabolized into choline and acetate by acetylcholinesterase, and other non-specific esterases. Release of ACh can be excitatory or inhibitory depending on the type of tissue and the nature of the receptor it interacts with.

Methods and Results

^{13}C-MRI or -MRS are important diagnostic methods to investigate the role of biologically active compounds, but their intrinsically low sensitivity requires some form of signal enhancement such as hyperpolarization derived from Parahydrogen Induced Polarization (PHIP). *In situ* parahydrogenated products of unsaturated precursors namely exhibit up to 10^5-fold ^{13}C-signal enhancement. Certain target compounds, however, such as choline are difficult or even impossible to hyperpolarize directly. Instead, they can be generated via an auxiliary molecule, from which the desired molecule is split off, either chemically or metabolically via an enzyme. An example is succinylcholine, (*'sux'* or *Anectine*), formally the dimer of acetylcholine (ACh). Typically, it is used in emergency medicine and anesthesia to induce muscle relaxation. Here it plays an auxiliary role to provide ^{13}C-hyperpolarized ACh etc., as outline below in Fig.1:

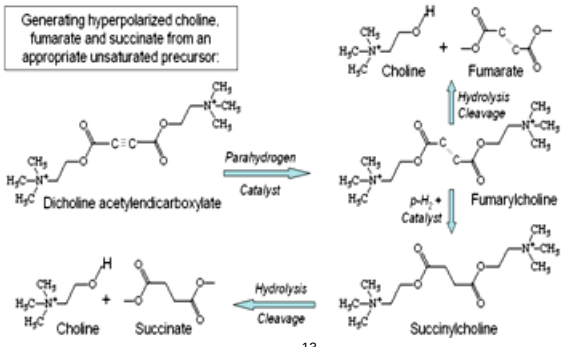

Fig. 1: Reaction scheme leading to ^{13}C-hyperpolarized choline

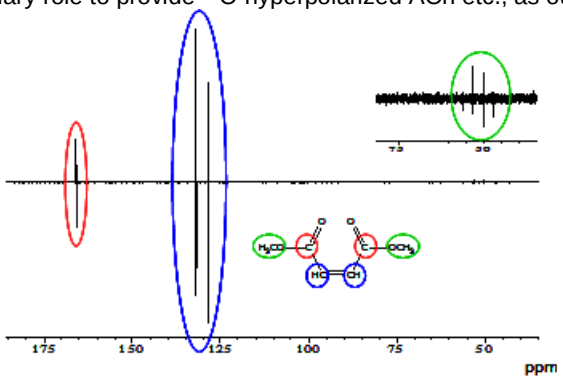

Fig. 2: ^{13}C-hyperpolarization obtained in closely related system

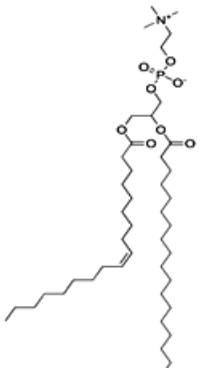

Fig. 3: A lecithin

The resulting succinylcholine imitates the action of ACh at the neuromuscular junction and acts as a neuromuscular blocker. It is not degraded by acetylcholinesterase but by pseudocholinesterase. This hydrolysis is slower than that of ACh. – Fig. 2 depicts the ^{13}C-hyperpolarization obtained in a model compound and closely related system, namely during the parahydrogenation of acetylene dicarboxylate: The ^{13}C-hyperpolarization is spread over all carbon atoms of the molecule and extends even beyond the carboxyl groups.

Furthermore, since the choline moiety is an intrinsic part of phosphatidylcholines, otherwise also known as lecithines, these substrates may become completely ^{13}C-hyperpolarized directly upon parahydrogenating appropriate precursors containing unsaturated functions in their alkyl chains. In this fashion both saturated and unsaturated side groups can be obtained, the latter even with the appropriate stereochemistry upon using the corresponding catalyst, which either yields the cis- or the trans-isomer[3] of the alkyl side chain. The corresponding example outlined in Fig. 3 is a phosphatidylcholine containing an oleyl and a stearoyl side chain. Whereas the oleyl part requires a precursor with a triple bond at the site of the subsequent double bond with cis-geometry, the stearoyl chain can be obtained from an oleyl containing precursor or related one with the double bond in any other part of the alkyl chain. – Choline itself can also be ^{13}C-hyperpolarized directly using a DNP approach. Whereas the DNP approach is a batch process, the PHIP alternative is continuous.

Discussion and Conclusions

We have previously ^{13}C-hyperpolarized dimethyl maleinate[2] starting from acetylendicarboxylate and parahydrogenating this unsaturated precursor at both high and low magnetic fields. The resulting signal enhancement for ^{13}C was > 2580, thereby exceeding the theoretical limit achievable via Dynamic Nuclear Polarization using microwave pumping in the presence of suitable stable free radicals. Similarly, dicholine acetylenedicarboxylate may be parahydrogenated to either fumarylcholine or on to succinylcholine using parahydrogen and appropriate catalysts. The hyperpolarized succinylcholine becomes converted into choline and succinate, whereupon the first one gets acetylated to ACh and the latter is further metabolized in the citric acid cycle. - Fumarylcholine may also be used as a starting point.

References

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