Krebs Cycle Kinetics in Rat Hearts by $^{1}$H-($^{13}$C) HMQC-TOCSY

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INTRODUCTION:
Numerous studies have shown that $^{13}$C NMR can be used to estimate Krebs cycle flux ($V_{TCA}$) in brain in vivo and in the isolated perfused heart. A second flux parameter ($V_x$), included in all kinetic models of the Krebs cycle, reflects either transaminase exchange or mitochondria ↔ cytosol transport of metabolites. There is considerable interest in NMR methods because this parameter, $V_x$, is difficult to assess by other techniques. Because of limited sensitivity of direct $^{13}$C NMR, we compared measurement of $V_{TCA}$ and $V_x$ using standard $^{13}$C observe to a recently-reported 2D NMR method, HMQC-TOCSY. The temporal appearance of glutamate $^{13}$C isotopomers was measured in extracts of rat hearts perfused ± aminooxycetate (a nonspecific inhibitor of transaminase enzymes). A kinetic analysis of the two types of isotopomer data revealed no significant differences in calculated metabolic fluxes.

METHODS:
After general anesthesia, rat hearts were rapidly excised and perfused using standard Langendorf methods at a column height of 70 cm H2O. Before adding [2-$^{13}$C]acetate (2 mM), hearts were perfused for 30 min with recirculating KH buffer (control group) or KH buffer containing 0.5 mM AOA. This period ensured equilibration of the inhibitor into all cellular compartments. Perfusion with [2-$^{13}$C]acetate were allowed to continue for 6 different time periods (3, 6, 9, 12, 15, 45 min.) + AOA. Hearts were then freeze-clamped, extracted with 3.6% PCA, neutralized with KOH, freeze-dried, and dissolved in 0.6 ml H2O. The pH was adjusted to 7.2 for $^{13}$C NMR spectra and to 2.75 for HMQC-TOCSY spectra. HPLC and enzymatic assays were used to measure Krebs cycle intermediate pool sizes in extracts. Pool sizes, fractional enrichments, and HMQC-TOCSY and $^{13}$C NMR multiplet data were used in a kinetic analysis of the Krebs cycle.

RESULTS AND DISCUSSION:
HMQC-TOCSY spectra of 6 min. perfused control and AOA inhibited rat hearts are compared in Figure 1.

![HMQC-TOCSY spectra](image)

Fig.1. HMQC-TOCSY spectra of hearts perfused for 6 min either without (left) or with (right) AOA.

2D cross peaks were more intense in spectra of control hearts versus hearts perfused with AOA, reflecting more incorporation of $^{13}$C in control hearts at 6 min. $^{13}$C NMR multiplets, for the same early time points, show that multilabeled glutamate isotopomers (e.g., as given by the ratios C3T/C3D and C4D34/C4S) are more abundant in the hearts perfused with AOA than in control hearts. A lower fractional enrichment and higher relative abundance of multilabeled glutamate isotopomers is consistent with transaminase inhibition by AOA. Kinetic analysis of the HMQC-TOCSY and the $^{13}$C NMR multiplets (C4H4D vs CH3S) (Figure 2) provided the flux values reported in the table.

<table>
<thead>
<tr>
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<th>$V_{TCA}$ [5%-95%]</th>
<th>$V_x$ [5%-95%]</th>
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<tbody>
<tr>
<td>Controla</td>
<td>10.9 [10.0-11.9]</td>
<td>14.0 [11.0-17.9]</td>
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<tr>
<td>AOAa</td>
<td>19.5 [16.7-22.7]</td>
<td>1.5 [1.3-1.7]</td>
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<tr>
<td>AOBb</td>
<td>16.3 [13.8-19.2]</td>
<td>2.0 [1.3-3.0]</td>
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$^{13}$C-NMR multiplets; HMQC-TOCSY multiplets.

REFERENCES: