EFFECT OF AMMONIA ON INTRACELLULAR ENERGETIC STATUS*

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Background and Aim: The cellular energetic status is closely related with oxidation efficiency of TCA cycle intermediate metabolites. Ammonia affects such an oxidation efficiency primarily through cataplerosis of α-ketoglutarate. However, it remains open how ammonia affects concentrations of individual TCA cycle intermediate metabolites, as well as ATP and GTP. Part of intracellular GTP pool is produced by succinyl-CoA synthetase reaction in the TCA cycle.
We studied the effect of ammonia on the concentrations of these metabolites to determine how ammonia affects cellular energetic status.
Materials and Methods: Mouse embryonic fibroblasts were cultured in DMEM containing 0 (control), 2, 4, and 8 mM ammonium chloride for 0 min, 60 min and 120 min, respectively. Metabolite concentrations were quantified by LC-MS/MS. Acetyl-CoA and oxaloacetate were not determined for technical reasons.
Results and Discussion:
In control dishes, which received no ammonia, at 120 min compared to 60 min, citrate+isocitrate (Cit+Isocit) tended to decrease, presumably due to cataplerotic extraction. While α-ketoglutarate (α-KG), fumarate (Fum) and malate (Mal) tended to increase, suggesting that they were supported in part by anaplerosis. The concentrations of cis-aconitate (cis-Aco) and succinate (Suc) showed no substantial differences, and that of succinyl-CoA (Suc-CoA) exhibited greater variability than the difference in mean values. ATP concentration tended to increase, while GTP concentration showed tendency to decline.
While in ammonia-loaded cultures, compared to control which received no ammonia, concentrations of Cit+Isocit, cis-Aco, α-KG, Suc, Fum and Mal tended to decrease at most of the ammonia concentrations loaded at both 60 min and 120 min of culture. Despite decrease in α-KG, the concentration of Suc-CoA appeared to be independent of ammonia load, implying that succinyl-CoA synthetase reaction is supported by anaplerosis. Both ATP and GTP concentrations exhibited no significant change by the ammonia load. L/P ratio tended to decline at 120 min at 4 and 8 mM of ammonia, which could be linked with enhanced reductive amination reaction by glutamate dehydrogenase.
Conclusion: Determinations of TCA cycle intermediate metabolite concentrations revealed that cataplerotic extraction and anaplerotic replenishment could be one of the factors that

affect their concentrations, and addition of ammonia decreased them generally in a dose-dependent manner and rendered the intracellular milieu more oxidized. Suc-CoA appeared to be replenished by anaplerotic supply when α-KG decreased by the addition of ammonia. Ammonia addition had no consistent effect on the concentrations of either ATP or GTP under the conditions employed.

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