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Biochemical dynamics of the Siberian wood frog in hypoxia and reoxygenation

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Motivation and Aim: The Siberian wood frog *Rana amurensis* Boulenger, 1886 is a unique amphibian capable of surviving several months in water with very low oxygen content (up to 0.2 mg/L) [1]. Our aim was to study metabolomic changes of this species at the onset of hypoxia (1 day), as well as in 1 hour of reoxygenation following long-term hypoxia exposure using ^{1}H nuclear magnetic resonance (NMR).

Methods and Algorithms: Adult individuals of *R. amurensis* were collected in the field. One sample was exposed to 1 day of hypoxia in water (n = 6). Another six individuals were kept for 10 days under hypoxia and then returned to normoxic water for 1 h. Organs (liver, heart, and brain) were extracted as quickly as possible, and quantitative metabolomic analysis was performed using ^{1}H NMR according to a procedure described previously [2]. The results were compared to the samples kept under normal oxygen content, as well as those exposed to 30 days of hypoxia [2, 3].

Results: While succinate in most studied vertebrates accumulates under hypoxia and is believed to undergo rapid conversion upon oxygen restoration, our study revealed a decrease in succinate in the brain in reoxygenation, while it remained unchanged in the liver. This observation suggests the existence of a mechanism that inhibits succinate conversion. Furthermore, we observed intriguing disparities concerning two substances with unclear functions: glycerol and 2,3-butanediol. Glycerol exhibited rapid accumulation during hypoxia and equally swift processing during reoxygenation. In contrast, 2,3-butanediol required an extended period to accumulate, yet persisted after reoxygenation.

Conclusion: Of the identified metabolites pool, only a few molecules exhibited continuous dynamics with rapid accumulation or decrease in response to hypoxia and subsequent reversal upon reoxygenation. Certain substances grouped together into unit with similar patterns. To gain a comprehensive understanding of specific substances, additional time points are necessary.

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