Nitric oxide (NO) formation in cultured rat hepatocytes during simulated ischemia

Jae-Sung Kim[1], Henry D. Connor[1], Ronald P. Mason[2] and John J. Lemasters[1]

[1]Department of Cell & Developmental Biology, University of North Carolina, Chapel Hill, NC 27599; [2]Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Science, Research Triangle Park, NC 27709

Nitric oxide (NO) is an important signaling molecule involved in a variety of biological functions and linked to pathophysiology. NO can be generated during ischemia, but mechanisms of NO formation in ischemic hepatocytes are not known. The AIM of this study was to investigate the effect of ischemia on the generation of NO in hepatocytes. METHODS: Rat hepatocytes were cultured overnight and loaded with 10 mM DAF-FM diacetate, NO-sensitive fluorescent probe, for 30 minutes at 37°C in Krebs-Ringer-HEPES (KRH) buffer at pH 7.4. After washing cells three times with cold KRH, hepatocytes were further incubated for 15 minutes to complete deesterification. The cells in warm KRH were then incubated in air (normoxic) or argon (ischemic) at either pH 6.2 or at pH 7.4 for up to 4 h. Changes in DAF-FM signal were monitored in a fluorescence plate reader. In other experiments, 5 mM arginine, 1 mM N-nitro-arginine methyl ester hydrochloride (L-NAME), an NO synthase inhibitor, or 100 µM S! -nitroso-N-acetylpenicillamine (SNAP), a slow NO donor, was added to the incubation medium. Confocal images of DAF-FM were collected with a Zeiss LSM microscope. For electron paramagnetic resonance (EPR) experiments, freshly isolated hepatocytes were suspended in normoxic or ischemic KRH at a concentration of 106 cells /mL and incubated for 4 h. EPR spectrum was recorded in liquid nitrogen with a Bruker EPR spectrometer. RESULTS: DAF-FM fluorescence increased by 10-fold during ischemia at pH 6.2, but not at pH 7.4. EPR revealed a triplet signal over the magnetic field range from 3300 to 3400 G that was indistinguishable from the spectrum of heme-NO adducts. . NO levels after 4 h anoxia at pH 6.2 were about 10 fold increased, compared to that after 4 h normoxia. Incubation of hepatocytes with arginine or L-NAME did not increase or decrease NO formation during ischemia, although SNAP rapidly enhanced NO levels both in normoxic and ischemic cells at either pH measured by either! DAF-FM or EPR. Confocal imaging of DAF-FM fluorescence also revealed a dramatic increase in intracellular DAF-FM fluorescence during ischemia, not during normoxia. CONCLUSION: Anoxia to hepatocytes under acidotic conditions induces NO formation. The mechanism by which NO is formed during ischemia appears to be independent of nitric oxide synthase. The importance of NO generation to ischemic hepatocellular injury remains to be elucidated.