

# Neuronal nitric oxide synthase generates superoxide from the oxygenase domain

**Hiroaki Kosaka<sup>1</sup>, Hirohito Yoneyama<sup>1</sup> and Akira Yamamoto<sup>1</sup>**

<sup>1</sup>Second Department of Physiology, Kagawa Medical University

When L-arginine is depleted, neuronal nitric oxide synthase (nNOS) has been reported to generate superoxide. Flavoprotein module construct of nNOS has been demonstrated to be sufficient for superoxide production. In contrast, nNOS was reported not to be involved in superoxide formation, because it occurred in the mixture of the boiled enzyme and redox-active cofactors. We aimed to resolve these controversial issues by examining superoxide generation without addition of redox-active cofactors from recombinant wild-type and C415A-nNOS, which has a mutation in the heme proximal site. In superoxide-sensitive adrenochrome assay, the initial lag period by C415A-nNOS was elongated 2-fold of that by native nNOS. With ESR using the spin trap 5,5-dimethyl-1-pyrroline-N-oxide, prominent signals of superoxide adduct were obtained from wild-type nNOS, whereas the enzyme preparation boiled for 5 min did not produce superoxide. Higher amounts of NaCN, 10mM, decreased superoxide formation by 63%. Though the activity of the reductase domain was intact, superoxide generation from C415A-nNOS markedly decreased to only 50% of that from wild enzyme. These results demonstrate that nNOS truly catalyzes superoxide formation from the oxygenase domain and that the full-length construct of nNOS hinders the reductase domain from producing superoxide..