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REVIEW —*Current Perspective*—

Nitric Oxide and Depolarization Induce Hydroxyl Radical Generation

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ABSTRACT—Nitric oxide (NO) contributes to the extracellular potassium-ion concentration ($[K^+]_o$)-induced hydroxyl radical (˙OH) generation. Cytotoxic free radicals such as peroxinitrite ($ONOO^-$) and ˙OH may also be implicated in NO-mediated cell injury. NO is synthesized from L-arginine by NO synthase (NOS). NOS activation was induced by K^+ depolarization. Oxidative modification of low-density lipoprotein (LDL) is thought to contribute to the production of oxygen derived-free radicals. However, LDL oxidation may be related to noradrenaline-induced ˙OH generation, but LDL oxidation may be unrelated to ˙OH generation via NOS activation. Abnormal levels of extracellular free dopamine (DA) and/or intraneuronal Ca^{2+} triggered by 1-methyl-4-phenylpyridinium ion (MPP^+) may be detrimental to the functioning of dopaminergic nerve terminals in the striatum. Although $[K^+]_o$-induced depolarization enhances the formation of ˙OH product due to MPP^+, the ˙OH generation via NOS activation may be unrelated to the DA-induced ˙OH generation. Depolarization enhances the formation of ˙OH products via NOS activation.

Keywords: Nitric oxide (NO), NO synthase, Depolarization, 1-Methyl-4-phenylpyridinium ion (MPP^+), Free radical

1. Introduction

Endothelium, macrophages and brain synaptosome preparations have been shown to produce nitric oxide (NO) by oxidizing arginine by a calcium-activated NADPH-dependent enzyme (1, 2). NO is a free radical that regulates a variety of biological functions and also has a role of pathogenesis of cellular injury (3 – 5). NO is synthesized from L-arginine by NO synthase (NOS) (6). Highly reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydroxyl radical (˙OH) cause excessive Na^+ entry through the fast Na^+ channel, leading to intracellular Ca^{2+} overload through the Na^+-Ca^{2+} exchange system (7). Intracellular Ca^{2+} overload is then considered to cause cell death under physiological conditions such as ischemia/reperfusion injury (8, 9). The enzyme xanthine oxidase (XO) resulting from xanthine dehydrogenase during ischemia (10) is thought to be a potential source of O_2^-. Although, O_2^- and NO are known to form the stable peroxinitrite ($ONOO^-$) and its decomposition generates ˙OH, these ideas are still being discussed (11). Cytotoxic free radicals such as $ONOO^-$ and ˙OH may also be implicated in NO-mediated cell injury (12). ROS damages biological membranes and cellular components, including DNA, resulting in cell death (13). This review will focus on the mechanism by which the increase in the extracellular potassium-ion concentration, $[K^+]_o$, via NOS activation affects the ˙OH generation.

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2. Detection of hydroxyl radical

Owing to the ultrashort half-life of oxygen free radicals, demonstration of the generation of highly reactive oxidants was previously limited to in vitro studies. Free radicals from in vitro generation of ROS can be trapped and displayed unequivocally by electron paramagnetic resonance (EPR) spin trapping procedures. However, a practical use of EPR spectroscopy for in vivo detection of ROS in biological systems is quite difficult and remains to be improved. Attack of ˙OH radicals, generated by a Fenton system, on salicylate produces 2,3- and 2,5-dihydroxybenzoic acids (DHBA) as major products and catechol as a minor product (14, 15) (Fig. 1). It has been shown that ˙OH free radicals react with salicylate and generate 2,3- and 2,5-DHBA, which can be measured electrochemically in picomole quantity by high performance liquid chromatography with an electrical (HPLC-EC) procedure (16). The ˙OH adducts of salicylate, in particular, 2,5-DHBA, following administration of salicylate have been used as an index of ˙OH generation in heart and brain tissues during

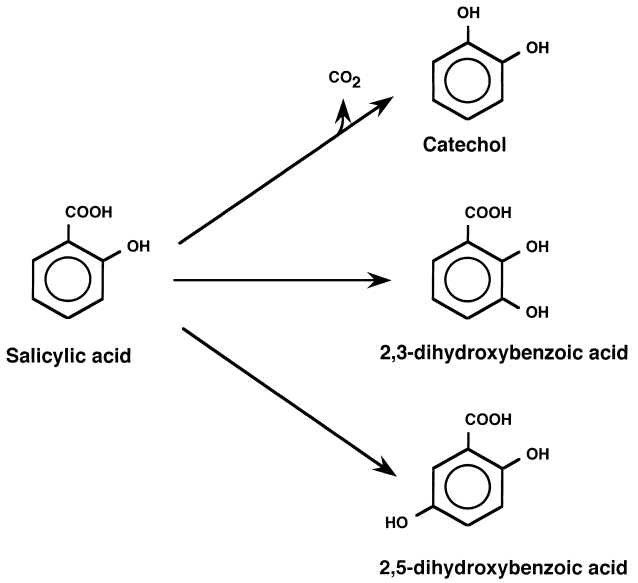

Fig. 1. Products of the attack by ˙OH radicals on the salicylate molecule.

ischemia and reperfusion (17). A cautionary note was raised by Halliwell et al. (14) since 2,5-DHBA can be formed not only by ˙OH adduct but also by hydroxylation catalyzed by liver cytochrome P-450 and microsomal enzymes. Moreover, 2,3-DHBA is non-enzymatically formed by ˙OH adduct and provides an assay for ˙OH formation in vivo. Additionally, it could be used to answer some of the fundamental questions concerning the chemical implications of ROS (i.e., NO and ˙OH) in heart and brain disorders.

In the heart experiment, we designed a system for holding the microdialysis probe which includes loose fixation of the tube and synchronization of the probe with that of the heart. Details of the technique necessary for manipulation of the flexibly mounted microdialysis probe in in vivo rat hearts were described previously (18).

3. Potassium depolarization induces ˙OH generation

It is well known that in the case of acute myocardial infarction or ischemia, there is a marked increase in $[K^+]_o$ and the resulting membrane potential of the ventricular muscle in the infarcted area is remarkably depolarized (19). In heart, the release of norepinephrine was induced by nerve depolarization (20). Catecholamine release contributes to the formation of cytotoxic free radicals. K^+ depolarization enhances Ca^{2+} overload by $[K^+]_o$-induced depolarization and may generate ˙OH radicals in the myocardium. In brain, intracerebral administration of 1-methyl-4-phenylpyridinium ion (MPP^+) elicited an accumulation of Ca^{2+} (21) and sustained increase in striatal dopamine (DA) efflux (22, 23) and produced brain lesions. Abnormal levels of extracellular free DA and/or intraneuronal Ca^{2+} triggered by MPP^+ may be detrimental to the functioning of dopaminergic nerve terminals in the striatum. Release of catecholamines is introduced by depolarization (19). This Ca^{2+}-mediated DA release elicited by MPP^+ was modified by pretreating with $[K^+]_o$-induced depolarization (24). Although the interaction between depolarization and NO remained obscure, NOS activation was induced by $[K^+]_o$-induced depolarization (25, 26). $[K^+]_o$-induced depolarization augmented MPP^+ induced ˙OH formation by NOS activation (24).

4. LDL oxidation and ˙OH generation

Several experimental studies have shown that oxygen radical contributes to myocardial damage induced by ischemia/reperfusion (18, 27). It is well known that ischemia induces depolarization (28, 29). NO may mediate ischemia/reperfusion-induced ˙OH generation via depolarization in ventricular muscle. NO is responsible for tissue damage during ischemia. L-NAME (N^G-nitro-L-arginine methyl ester, a NOS inhibitor) attenuated ˙OH generation by ischemia/reperfusion of rat heart (30). It is known that L-NAME inhibits depolarization-induced NOS activation by Ca^{2+} influx through blockade of the Na^+-Ca^{2+} channel (26). Oxidative modification of low-density lipoprotein (LDL) is thought to contribute to the production of oxygen-derived free radicals (31). Oxidative LDL (Ox-LDL) may be important in neurotoxicity in the brain (32). It is well known that a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor reduces the oxidizability of LDL (33). The inhibitory effect on the susceptibility of LDL oxidation can reduce ˙OH formation. The blockage of LDL oxidation by fluvastatin (an inhibitor of LDL oxidation) can reduce ˙OH generation. However, L-NAME did not affect noradrenaline-induced ˙OH formation. Fluvastatin is associated with a cardioprotective effect due to the suppression of noradrenaline induced ˙OH formation by inhibiting LDL oxidation (Fig. 2) (34). LDL oxidation may be related to noradrenaline-induced ˙OH generation, but LDL oxidation may be unrelated to ˙OH generation via NOS activation.

5. NOS activation and MPP^+-induced ˙OH generation in the striatum

Intracranial administration of MPP^+ elicited an accumulation of Ca^{2+} (21). K^+ depolarization enhances the formation of ˙OH product due to MPP^+ via NOS activation. If indeed the effect of KCl on ˙OH formation is due to NO via $ONOO^-$, $[K^+]_o$-induced depolarization may increase ˙OH formation. NOS inhibition is associated with a protective effect due to suppression of K^+ depolarization-induced ˙OH generation. The ˙OH was generated by the presence of NOS and O_2. Depolarization-induced DA release is well

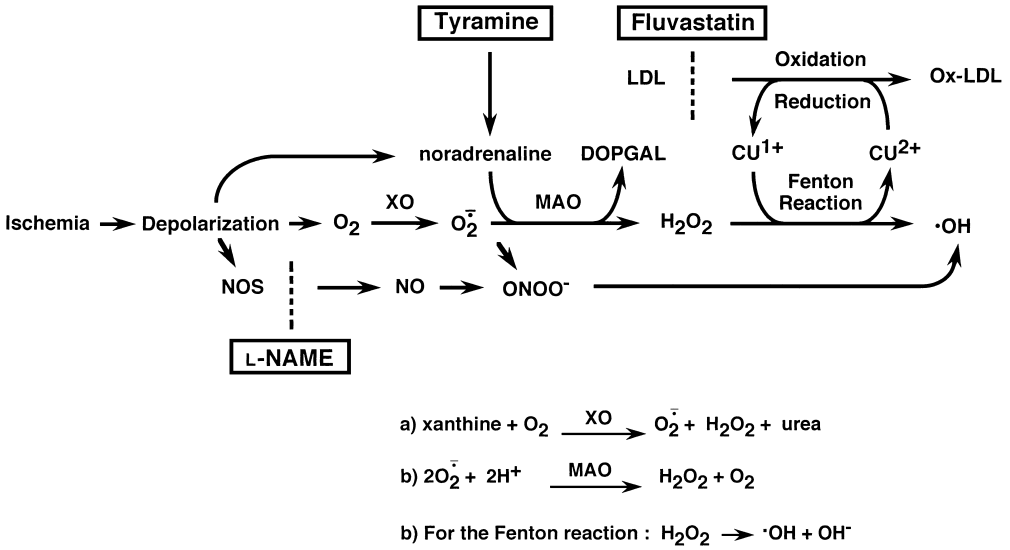

Fig. 2. The reaction pathway in rat heart illustrates the formation of hydroxyl radical by depolarization-induced NO. Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; L-NAME, N^G-nitro-L-arginine methyl ester; XO, xanthine oxidase; O_2^-, superoxide anion; ·OH, hydroxyl radical; MAO, monoamine oxidase; DOPGAL, 3,4-dihydroxyphenylglycolaldehyde; LDL, low-density lipoprotein. (Modified from ref. 34)

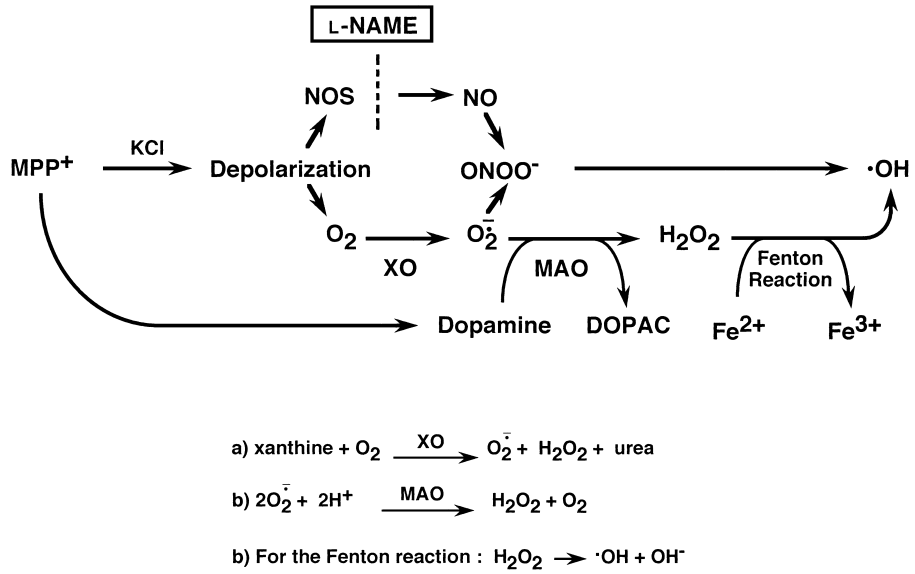

Fig. 3. The reaction pathway in rat brain illustrates the formation of hydroxyl radical by depolarization-induced NO. Abbreviations: NO, nitric oxide; XO, xanthine oxidase; O_2^-, superoxide anion; ·OH, hydroxyl radical; MAO, monoamine oxidase; DOPAC, 3,4-dihydroxyphenylacetic acid; NOS, nitric oxide synthase L-NAME, N^G-nitro-L-arginine methylester; MPP^+, 1-methyl-4-phenylpyridinium ion. (Modified from ref. 24)

known. Therefore, it is possible that endogenous release of DA after KCl stimulation in part contributes to the ·OH formation. Induction of high $[K^+]_o$ or DA significantly increased the MPP^+-induced ·OH formation (24). However, the application of L-NAME abolished the $[K^+]_o$ depolarization-induced ·OH formation with MPP^+, but L-NAME did not change the effect of DA. $[K^+]_o$ induced depolarization enhances the formation of ·OH products due to MPP^+ via NOS activation (24). In accord with the reaction pathway in Fig. 3, ·OH was generated by the presence of NOS and O_2. Based on these studies, the ·OH generation via NOS activation may be unrelated to the DA-induced ·OH generation.

The toxic effects of MPTP are proposed to be mediated via an excessive production of NO (35). Inhibitors of neuronal NOS such as 7 nitioindazole (7-NI) were found to prevent MPTP-induced striata DA depletion and nigral

cell death (36, 37). In addition, 7-NI may react with O_2^- to generate $ONOO^-$ (38) and $^{\bullet}OH$ radicals (12). Di Monte et al. (39) strongly claimed that reduction of MPTP conversion into MPP^+ by inhibition of the extraneuronal B-form of the enzyme monoamine oxidase is a more important factor for the protection of 7-NI than the inhibition of neuronal NOS.

The controversy concerning the possible neurotoxic (40) and/or neuroprotective role of NO in cell cultures has been discussed (41). Chronic or high-dose administration of D-amphetamine elicits NO formation in the striatum of rats and striatal dopaminergic terminal damage ensues (42). Neuronal NOS inhibitors may be useful in the treatment of neurologic diseases in which excitotoxic mechanisms play a role (43). A synthetic nonsteroidal antiestrogen inhibits NOS, leading to interference with consecutive NOS-dependent formation of NO and/or O_2^- in various tissues (44). Rats that lack inducible NOS are resistant to the MPTP-induced decrease in tyrosine hydroxylase-positive neurons, but show no change in DA-depletion. In contrast, glutathione peroxide-homozygote deficient mice and vesicular monoamine transporter 2-heterozygotes showed enhanced MPTP neurotoxicity (45, 46).

6. Conclusion

NO is a free radical that regulates a variety of biological functions and the pathogenesis of cellular injury. NO mediates ischemia/reperfusion-induced $^{\bullet}OH$ generation via depolarization in ventricular muscle. The $^{\bullet}OH$ was generated by the presence of NOS and O_2^-. NOS inhibition is associated with a protective effect due to suppression of $[K^+]_o$ depolarization-induced $^{\bullet}OH$ generation. The $^{\bullet}OH$ generation via NOS activation may be unrelated to the $^{\bullet}OH$ generation by catecholamine.

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