Regulation of Xanthine Oxidase by Nitric Oxide and Peroxynitrite*

(Received for publication, October 1, 1999, and in revised form, November 2, 1999)

Chang-il Lee‡§, Xiaoping Liu‡, and Jay L. Zweier‡¶

From the §Molecular and Cellular Biophysics Laboratories, Department of Medicine, Division of Cardiology and the Electron Paramagnetic Resonance Center, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21224 and the ¶Department of Pharmacology and Electron Spin Resonance Center, Kanagawa Dental College, 82 Inaoka-cho Yokosuka, Kanagawa, Japan 238-0003

Xanthine oxidase (XO) is a central mechanism of oxidative injury as occurs following ischemia. During the early period of reperfusion, both nitric oxide (NO) and superoxide (O$_2^-$) generation are increased leading to the formation of peroxynitrite (ONOO$^-$); however, questions remain regarding the presence and nature of the interactions of NO$^-$ or ONOO$^-$ with XO and the role of this process in regulating oxidant generation. Therefore, we determined the dose-dependent effects of NO$^-$ and ONOO$^-$ on the O$_2^-$ generation and enzyme activity of XO, respectively, by EPR spin trapping of O$_2^-$ using 5-(diethoxyphosphoryl)-5-methyl-1-pyrrrole-3,2-nitroxy and spectrophotometric assay. ONOO$^-$ markedly inhibited both O$_2^-$ generation and XO activity in dose-dependent manner, while NO$^-$ from NO$^-$ gas in concentrations up to 200 μM had no effect. Furthermore, we observed that NO$^-$ donors such as NOR-1 also inhibited O$_2^-$ generation and XO activity; however, these effects were O$_2^-$ dependent and blocked by superoxide dismutase or ONOO$^-$ scavengers. Finally, we found that ONOO$^-$ totally abolished the Mo(V) EPR spectrum. These changes were irreversible, suggesting oxidative disruption of the critical molbydENUM center of the catalytic site. Thus, ONOO$^-$ formed in biological systems can feedback and down-regulate XO activity and O$_2^-$ generation, which in turn may serve to limit further ONOO$^-$ formation.

It has been demonstrated that oxygen free radical generation is a critical mechanism causing injury in postischemic cells and tissues. Xanthine oxidase (XO),$^1$ a metalloflavoprotein, is an important source of oxygen free radicals (1). The enzyme catalyzes the reduction of O$_2^-$, leading to the formation of superoxide (O$_2^-$) and H$_2$O$_2$, and it has been proposed as a central mechanism of oxidative injury (2, 3). Both direct and spin trapping EPR techniques have demonstrated markedly increased oxygen free radical generation in tissues, such as heart, following postischemic reperfusion (4–6). XO has been shown to be the primary source of the oxygen radical generation, with this process largely triggered by increased formation of the XO substrates, xanthine and hypoxanthine, due to ATP degradation during ischemia (2, 7–9). During ischemia and reperfusion, increased nitric oxide (NO$^-$) formation also occurs, and this can interact with XO-derived O$_2^-$, leading to the formation of peroxynitrite (ONOO$^-$) (10). However, questions remain concerning the effects of NO$^-$ and ONOO$^-$ on XO itself.

The free radical, NO$^-$, is generated in biological tissues and is an important regulator of a wide range of biological functions (11). It is of critical importance in modulating vascular tone and was identified as the mediator of endothelium-derived relaxation (12–14). NO$^-$ also inhibits the enzyme activity of a number of enzymes including glutathione peroxidase (15), cytochrome c oxidase (16), and NADPH oxidase (17, 18). Major mechanisms attributed to explain NO$^-$-mediated inhibitory effects involve heme binding or destruction of enzyme Fe-S centers to yield inactive Fe-S-NO derivatives and thiol oxidation (19, 20). Recent reports have shown that O$_2^-$ plays a critical role in NO$^-$-induced toxicity, and previously proposed mechanisms for both O$_2^-$ and NO$^-$-mediated tissue injury now include a role for their combined reaction product, ONOO$^-$ (21). The radical-radical reaction between O$_2^-$ and NO$^-$ is extremely fast and almost diffusionally limited in rate (2 $\times$ 10$^4$ M$^{-1}$ s$^{-1}$) (22).

ONOO$^-$ is a potent oxidant that can attack a wide variety of biological molecules and is produced in diverse inflammatory and pathological processes including postischemic injury (10), septic shock (23), chronic tissue rejection (24), multiple sclerosis (25), amyotrophic lateral sclerosis (26, 27), Alzheimer’s disease (28), cardiomyopathy (29, 30), and atherosclerosis (31, 32). ONOO$^-$ can directly oxidize sulfhydrils (33) and also reacts by either one- or two-electron oxidation reactions with various biological target molecules (34). In acid conditions, the homolysis of peroxynitrous acid (HOONO) (pKa, 6.8) gives reactive intermediates with OH-like properties that can oxidize DNA and proteins (33, 35, 36). ONOO$^-$ directly oxidizes an active site methionine, resulting in inactivation of α1-antiprotease (37).

During the early period of reperfusion, both NO$^-$ and O$_2^-$ generation are increased, leading to formation of ONOO$^-$ (10). Following the postischemic burst of ONOO$^-$ generation, it has been observed that XO activity is decreased in the early period of reperfusion in heart tissue (9). It has been suggested that either NO$^-$ or ONOO$^-$ could feedback and inhibit XO, however, controversy remains regarding the presence and nature of interactions of NO$^-$ or ONOO$^-$ with XO and the role of this process in regulating oxidant generation. NO$^-$ could inhibit and regulate endothelial cell xanthine dehydrogenase/XO activity (38, 39), and a recent paper reported that XO and xanthine dehydrogenase are inactivated by NO$^-$ under anaerobic conditions (40). However, other studies reported that ONOO$^-$ inactivates the enzyme, while NO$^-$ has no effect (41).

To resolve the controversy regarding the effects of NO$^-$ and ONOO$^-$ on XO activity, we have determined the dose-dependent effects of NO$^-$ and ONOO$^-$ on XO activity and O$_2^-$ genera-
tion from purified XO. EPR spin trapping techniques were applied to quantitate $O^2_2$. We demonstrate that ONOO$^{-}$ induces a dose-dependent loss of activity, while its precursor NO$^+$ does not. The mechanism of this inactivation is further shown to be due to disruption of the critical molybdenum center of the enzyme.

**EXPERIMENTAL PROCEDURES**

**Materials**—XO (grade III; from buttermilk, chromatographically purified, in 2.3 m (NH$_4$)$_2$SO$_4$, 10 m sodium phosphate buffer (pH 7.8), containing 1 m EDTA and 1 m sodium salicylate) was obtained from Sigma. The salicylate was removed chromatographically with Sephadex G-25 prior to use. Xanthine, uric acid, and superoxide dismutase were also obtained from Sigma. The ONOO$^{-}$ decomposition catalyst (5,10,15,20-tetraphenylporphyrinato Fe(III)) (FeTPP) was obtained from Monsanto Corp. (St Louis, MO). The NO donor NOR-1 was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA).

ONOO$^{-}$ Preparation—The ONOO$^{-}$ was synthesized from acidified nitrite and hydrogen peroxide according to Beckman et al. (34). Alternatively, similarly prepared ONOO$^{-}$ was obtained from Alexis Corp. (San Diego, CA). The concentration of ONOO$^{-}$ was checked by optical absorbance measurements at 302 nm at the time of synthesis and again just prior to each experiment, and only ONOO$^{-}$ that was $>95\%$ of the original concentration was used. To assure that there was no significant pH change upon the addition of alkaline ONOO$^{-}$ to the reaction mixture, we also monitored the pH and limited the amount of alkaline ONOO$^{-}$ stock used so that the final pH did not significantly change.

NO$^+$ Gas Solution—NO$^+$ was scrubbed of higher nitrogen oxides by passage through a trap with solid NaOH pellets and a second trap with NaOH. 500 ml of phosphate-buffered saline, pH 7.4 (PBS), was deaerated by bubbling with argon for 30 min and then bubbled with argon for 30 min and then bubbled with scrubbed NO$^+$ for 30 min (42, 43). To further verify the precise NO$^+$ concentration from NO$^+$ gas-equilibrated solution, electrochemical measurements of NO$^+$ concentrations were carried out at 25 °C using a CH 832 electrochemical detector with a Faraday cage (CH Instruments, Inc., Cordova, IN) and WPI NO$^+$ electrode.

**Photometric Measurements**—UV-visible absorption spectra of XO and assays of XO activity were performed with a Varian Cary 300 UV-visible spectrophotometer equipped with a temperature-controlled circulator. To remove the salicylate and other low molecular weight compounds, XO (0.1 µM) was passaged through Sephadex G-25 pre-equilibrated with PBS, pH 7.4. XO activity was assayed at 25 °C in PBS after the addition of xanthine (360 µM) by measurement of uric acid production from absorbance change at 295 nm ($ε$ = 37,800 m$^{-1}$ cm$^{-1}$). We determined the effects of ONOO$^{-}$, NO$^+$, and NOR-1 on XO activity as follows. We confirmed that the various concentrations of the alkaline ONOO$^{-}$ stock used were neutralized to pH 7.4 and quantitated the ONOO$^{-}$ concentration spectrophotometrically at 302 nm. As previously reported, ONOO$^{-}$ rapidly decays at pH 7.4 with a half-life of <1 s (34), and after 1 min no detectable ONOO$^{-}$ remains. Therefore, ONOO$^{-}$ (0–200 µM) was added with a gas-tight syringe to the XO reaction mixture in PBS, and after 1 min the reaction mixture was transferred to the spectrophotometer cuvette, and xanthine (380 µM) was added for measurement of enzyme activity. Dissolved NO$^+$ gas solutions were used to determine the effects of NO$^+$ on XO activity. The dissolved NO$^+$ gas (0–200 µM) was preincubated with XO for 10 min in 0.1 m PBS (pH 7.4), after which xanthine was added, and enzyme activity was measured. Electrode measurements confirmed that after a 10-min preincubation, no detectable NO$^+$ remained, ensuring that NO$^+$ would not significantly scavenge $O_2$ generated from the XO-xanthine system. For the NO$^+$ donor NOR-1, the NOR-1 (0–100 µM) and xanthine were added simultaneously to the XO reaction mixture in 0.1 m PBS, and the rate of uric acid production was immediately measured. For the anaerobic experiments, ONOO$^{-}$ (100 µM) and NO$^+$ (100 µM) were added to the XO reaction mixture under argon for 10 min. After continued argon purging to remove any remaining NO$^+$, the enzyme activity was measured with the addition of xanthine (360 µM) and exposure to air. Anoerobic solutions of the reaction buffer with XO, ONOO$^{-}$, and xanthine were prepared by purging with argon prior to use.

**EPR Measurements**—All EPR measurements were performed using a Bruker ER 300 spectrometer operating at X-band with a TM110 cavity. The microwave frequency was measured with an EIP model 575 micro-

![Fig. 1. Effect of ONOO$^{-}$ on the $O_2$ generation and activity of XO. A, EPR spin trapping measurement of $O_2$ generation from XO (0.1 µM) and xanthine (380 µM) in 0.1 m PBS (pH 7.4). a, in the absence of ONOO$^{-}$, a prominent spectrum of DEPMPO–OH with only a small signal of DEPMPO–OOH is seen with relative intensities of 92 and 8%. b, with preexposure of XO to ONOO$^{-}$ (100 µM), the DEPMPO–OOH signal was decreased 5-fold, and the DEPMPO–OH adduct was increased with relative intensities of 44 and 56%. In B, are shown the time course of $O_2$ generation from either control untreated or ONOO$^{-}$ (100 µM)-pretreated XO measured by EPR spin trapping following the addition of xanthine at time 0 (a) and the kinetics of XO activity determined by uric acid production from spectrophotometric assay at 295 nm (b).]

---

**Fig. 1.** Effect of ONOO$^{-}$ on the $O_2$ generation and activity of XO. A, EPR spin trapping measurement of $O_2$ generation from XO (0.1 µM) and xanthine (380 µM) in 0.1 m PBS (pH 7.4). a, in the absence of ONOO$^{-}$, a prominent spectrum of DEPMPO–OH with only a small signal of DEPMPO–OOH is seen with relative intensities of 92 and 8%. b, with preexposure of XO to ONOO$^{-}$ (100 µM), the DEPMPO–OOH signal was decreased 5-fold, and the DEPMPO–OH adduct was increased with relative intensities of 44 and 56%. In B, are shown the time course of $O_2$ generation from either control untreated or ONOO$^{-}$ (100 µM)-pretreated XO measured by EPR spin trapping following the addition of xanthine at time 0 (a) and the kinetics of XO activity determined by uric acid production from spectrophotometric assay at 295 nm (b).
To determine $O_2$ generation, EPR spin trapping studies were performed using the spin trap 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) (44). The instrument settings used in the spin trapping experiments were as follows: modulation amplitude, 0.32 G; time constant, 0.16 s; scan time, 60 s; modulation frequency, 100 kHz; microwave power, 20 milliwatts; microwave frequency, 9.76 GHz. The samples were placed in a quartz EPR flat cell, and spectra were recorded at ambient temperature (25 °C). The component signals observed in these spectra were identified and quantified as reported (46). The double integrals of DEPMPO-OOH experimental spectra were compared with those of a 1 mM TEMPO sample measured under identical settings to estimate the concentration of $O_2$.

Measurements of the Mo(V) EPR signal of XO were performed as follows. The samples of native XO (10 μM), XO plus xanthine, ONOO$^-$-treated XO, ONOO$^-$-treated XO plus xanthine (30 μM), and ONOO$^-$-treated XO plus dithionite (1 mM) were prepared as described (45). The reaction mixture (700 μl) in bicine buffer (pH 8.2) was frozen in a 3-mm quartz EPR tube and measured at 77 K. The instrument settings were as follows: modulation amplitude, 5.0 G; time constant, 0.32 s; scan time, 60 s; modulation frequency, 100 kHz; microwave power, 10 milliwatts; microwave frequency, 9.54 GHz.

Statistical Analysis—All of the experiments were performed in triplicate and repeated a minimum of three times. Results are expressed as means ± S.E. Statistical analysis was performed by Student’s t test or a one-way analysis of variance. Statistical significance was defined at a level of $p < 0.05$.

RESULTS

Effects of ONOO$^-$ and NO$^+$ on $O_2$ Generation and XO Activity—We investigated the effects of ONOO$^-$ and NO$^+$ on XO-mediated $O_2$ generation measured by EPR spin trapping with DEPMPO. XO activity was assayed under these same experimental conditions. As reported previously (44), after the addition of xanthine to XO, we observe primarily a characteristic DEPMPO-OOH adduct spectrum with hyperfine splitting giving rise to 12 resolved peaks (Fig. 1A, a). In addition to the large signal of DEPMPO-OOH, a small signal of DEPMPO–OH was observed. The $O_2^*$-derived DEPMPO-OOH...
adduct comprised 92% of the total intensity, and the DEPMPO-OH adduct comprised 8%. With ONOO⁻ (100 μM) pretreatment of XO and the subsequent addition of xanthine, the DEPMPO–OOH signal was decreased by more than 5-fold, and a larger signal of DEPMPO–OH was seen (Fig. 1A, b). These data indicate that ONOO⁻ markedly inhibits O₂⁻ generation from XO and either forms OH⁻ or hydroxylates DEPMPO in this system. With NO⁻ treatment, however, no alterations in the EPR spectrum were seen with identical DEPMPO–OOH (92%) and DEPMPO–OH (8%) spin adducts as in the absence of NO⁻ (Fig. 2A). Measurements of XO activity confirmed that ONOO⁻ strongly inactivated the enzyme with decreased initial rates of uric acid formation (Fig. 1B, b), while NO⁻ had no effect (Fig. 2B, b). The dose-dependent effects of ONOO⁻ and NO⁻ on O₂⁻ generation and XO activity were measured (Fig. 3). It was clearly observed that ONOO⁻ decreased both O₂⁻ generation and XO activity in a dose-dependent manner with more than 25, 50, and 90% inhibition with ONOO⁻ levels of 10, 30, and 200 μM, respectively (Fig. 3A); however, NO⁻ in concentrations up to 200 μM did not decrease either O₂⁻ generation or XO activity (Fig. 3B).

Effects of NO⁻ Donors on O₂⁻ Generation and XO Activity—While NO⁻ in aerobic solution has a short concentration-dependent half-life of only a few seconds, longer sustained NO⁻ levels can be achieved with NO⁻ donors. It was previously reported that NO⁻ released from NO⁻ donors can inhibit XO function (38, 39); however, it is unknown if this effect is mediated by NO⁻ or ONOO⁻ generated from the reaction of NO⁻ with XO-derived O₂⁻. The time course of NO⁻ release from NOR-1 was measured by the NO⁻ electrode (see “Experimental Procedures”). In contrast to NO⁻ gas, with which a rapid fall in NO⁻ concentration to near zero within 10 min is observed, with NOR-1, NO⁻ concentrations in solution initially increase and then persist for more than 15 min (Fig. 4). When NOR-1 was

**FIG. 4.** Time course of decline in NO⁻ concentration in aqueous solutions after the addition of NO⁻ gas or the NO⁻ donor NOR-1. NO⁻ concentration was measured by polarigraphic electrode. A, at time 0, either 200, 100, 30, 10, or 1 μM NO⁻ (top curve to bottom curve) from NO⁻ gas–equilibrated solutions was added to PBS (pH 7.4) at 25 °C. B, at time 0, either 100, 50, or 10 μM (a, b, and c, respectively) NOR-1 was added to PBS (pH 7.4) at 25 °C. While the NO⁻ concentration from NO⁻ gas falls to near 0 (<25 nM) by 10 min, the NO⁻ donor NOR-1 provides sustained NO⁻ release and measurable solution NO⁻ concentrations for more than 15 min.

**FIG. 5.** Effect of the NO⁻ donor NOR-1 on the O₂⁻ generation and activity of XO. A, EPR spin trapping measurement of O₂⁻ generation from XO (0.1 mM) and xanthine (360 μM) in 0.1 M PBS (pH 7.4). a, in the absence of NOR-1 a prominent spectrum of DEPMPO–OOH with only a small signal of DEPMPO–OH is seen as in Fig. 1; b, with the addition of NOR-1 (100 μM) the DEPMPO–OOH signal was decreased about 3-fold. In B are shown the time course of O₂⁻ generation from XO and xanthine in the presence or absence of NOR-1 (100 μM) (a) and the XO activity assayed from the kinetics of uric acid production monitored at 295 nm (b).
Inhibition of Xanthine Oxidase by Peroxynitrite

In the oxidized state, the molybdenum is present as Mo(VI), which is EPR-silent; however, with reduction to Mo(V), various EPR signals have been reported (45, 46–48). Studies of the Mo(V) EPR signal of XO have provided important information regarding the mechanism of enzyme catalysis and have shown that the oxidative hydroxylation of xanthine to uric acid takes place at the molybdenum center. As previously reported, we observe that native XO exhibits the rapid signal Mo(V) EPR 

Effects of ONOO$^-$ and NO$^+$ on $O_2^*$ Generation and XO Activity under Anaerobic Conditions—Under anaerobic conditions, NO$^+$ is stable and persists for longer periods of time so that anaerobic incubation of XO with a given concentration of NO$^+$ could exert more pronounced effects than those seen with aerobic exposure. Since NO$^+$ from gas-equilibrated solution ($\approx 200 \mu M$) did not significantly decrease XO activity under aerobic conditions, we examined effects of NO$^+$ and ONOO$^-$ on enzyme activity under anaerobic conditions. Similar to the results under aerobic conditions, only ONOO$^-$ significantly decreased XO activity, while there was no significant effect on enzyme activity with NO$^+$ preexposure (Fig. 8).

**Effects of ONOO$^-$ on the Molybdenum Center—**To examine the effects of ONOO$^-$ on the critical molybdenum center of XO, EPR measurements were performed on frozen enzyme at 77 K. In the oxidized state, the molybdenum is present as Mo(VI), which is EPR-silent; however, with reduction to Mo(V), various EPR signals have been reported (45, 46–48). Studies of the Mo(V) EPR signal of XO have provided important information regarding the mechanism of enzyme catalysis and have shown that the oxidative hydroxylation of xanthine to uric acid takes place at the molybdenum center. As previously reported, we observe that native XO exhibits the rapid signal Mo(V) EPR spectrum (Fig. 9B) in Bicine buffer (pH 8.2), and the intensity

Fig. 6. Dose-dependent effects of NOR-1 on $O_2^*$ generation and XO activity (A) and effect of superoxide dismutase (SOD) on NOR-1-mediated inhibition (B). A, NOR-1 (0–100 \mu M) and xanthine (360 \mu M) were added to XO (0.1 \mu M) in 0.1 M PBS (pH 7.4) at 25 °C, and $O_2^*$ generation was then immediately measured by EPR spin trapping with DEPMPO, while in matched experiments enzyme activity was measured from urate product monitored at 295 nm. In B, experiments were performed as in A, but superoxide dismutase (250 units/ml) was added to the XO before the addition of NOR-1 (100 \mu M) and xanthine (360 \mu M). Data are presented as mean ± S.E. of triplicate experiments. *, significance of $p < 0.01$ for the difference from the corresponding control value; †, significance of $p < 0.01$ for the difference from the value with NOR-1.

Fig. 7. Effect of the ONOO$^-$ scavenger urate and ONOO$^-$ decomposition catalyst FeTMPS on NOR-1-mediated inhibition of XO activity. NOR-1 (100 \mu M) and xanthine (360 \mu M) were added simultaneously to the XO (0.1 \mu M) in 0.1 M PBS, and XO activity was immediately measured. ONOO$^-$ (100 \mu M) was preincubated with XO for 1 min, and then xanthine was added. The assays of XO activity were performed by measurement of uric acid production at 295 nm. XO was preincubated with urate (10 \mu M) (A) or with FeTMPS (10 \mu M) (B) for 3 min before the addition of NOR-1 and xanthine or the addition of ONOO$^-$ data. Data are presented as mean ± S.E. of triplicate experiments. *, significance of $p < 0.01$ for the decrease from the corresponding value of control; †, significance of $p < 0.01$ for the increase from the corresponding value of XO treated with NOR-1; ‡, significance of $p < 0.05$ for the increase from the value of XO treated with ONOO$^-$.
of this signal further increases after the addition of xanthine (Fig. 9C) (45). Following the addition of ONOO$_2$, however, the rapid signal of the Mo(V) EPR spectrum is almost totally abolished (Fig. 9D). The loss of this signal could not be reversed by the addition of xanthine (Fig. 9E) or dithionite (Fig. 9F). These observations suggest that ONOO$_2$ irreversibly oxidizes and disrupts the molybdenum center of XO.

**DISCUSSION**

XO is an important source of oxygen free radicals in biological cells and tissues and has a particularly important role in oxygen radical generation and pathogenesis of injury following postischemic reperfusion (2). More recently, we have also demonstrated that there is markedly increased NO$_2$ generation and accumulation in ischemic and postischemic tissues, such as the heart, and this NO$_2$ reacts with O$_2$ generated during the early period of reperfusion, resulting in the formation of ONOO$^-$ (10). However, questions and controversy remain regarding whether NO$_2$ or ONOO$^-$ modulates XO function and XO-mediated free radical generation. Most prior studies assessed the effects of NO$_2$ and ONOO$^-$ on XO activity measured by urate production; however, there was a lack of direct monitoring of the concentrations of NO$_2$ present along with the process of XO-mediated O$_2$ generation. This has led to the present controversy and confusion regarding the role of NO$_2$ and ONOO$^-$ on XO function. Therefore, we have performed studies to assess the effects of NO$_2$ and ONOO$^-$ on XO activity and O$_2$ generation using electrochemical and EPR methods to monitor NO$_2$ concentrations and O$_2$ generation directly. Our studies provide direct evidence that ONOO$^-$ down-regulates O$_2$ generation from XO. ONOO$^-$ was shown to decrease both XO activity and O$_2$ generation in a dose-dependent manner, while NO$_2$ had no significant effect (Fig. 3).

We found that NO$_2$ does not directly inactivate XO but must first react with O$_2$ to form ONOO$^-$ (Fig. 3). Since in the process of assaying XO activity with the addition of xanthine or other substrates the enzyme generates O$_2$, any NO$_2$ present at the time of enzyme activation can be converted to ONOO$^-$.

Although the NO$_2$ donor NOR-1 is reported to provide rapid release of NO$_2$, this release was measured to persist for more than 10 min as determined by either polarigraphic electrode or EPR spin trapping methods. While NO$_2$ itself was ineffective...
In conclusion, we have demonstrated that ONOO\textsuperscript{-} inhibits the O\textsubscript{2}\textsuperscript{=} generation and activity of XO in a dose-dependent manner, while NO\textsuperscript{•} only exerts significant inhibition in the presence of O\textsubscript{2}\textsuperscript{=} conditions in which ONOO\textsuperscript{-} is formed. This ONOO\textsuperscript{-}-mediated XO inhibition could be suppressed by urate. ONOO\textsuperscript{-} inhibited XO function primarily by oxidative disruption of the molybdenum catalytic site. Taken together, ONOO\textsuperscript{-} in biological systems can feedback and down-regulate XO activity that in turn may serve to limit further ONOO\textsuperscript{-} formation and oxidant-derived injury.

Acknowledgments—we thank Dr. P. Kuppusamy, Dr. A. Samouilov, Dr. A. F. Vanin, and Dr. Russ Hille for helpful advice.

REFERENCES

1. Hille, R., and Nishino, T. (1995) FASEB J. 9, 995–1003
2. McCord, J. M. (1985) N. Engl. J. Med. 312, 159–163
3. Zweier, J. L., Kuppusamy, P., and Lutty, G. A. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4046–4050
4. Zweier, J. L., Fhlaiby, J. C., and Weisfled, M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1404–1407
5. Zweier, J. L. (1988) J. Biol. Chem. 263, 1353–1357
6. Zweier, J. L., Kuppusamy, P., Williams, R., Rayburn, B. K., Smith, D., Weisfled, M. L., and Fhlaiby, J. C. (1989) J. Biol. Chem. 264, 18890–18896
7. Abd-Elfattah, A. S., Jessen, M. E., Lekven, J., Doherty, N. E. D., Brunsting, L. A., and Wechsler, A. S. (1988) Circulation 78, 224–235
8. Jennings, R. B., and Steenbergen, C. J. (1985) Annu. Rev. Physiol. 47, 727–749
9. Xia, Y., and Zweier, J. L. (1995) J. Biol. Chem. 270, 18797–18803
10. Wang, P., and Zweier, J. L. (1996) J. Biol. Chem. 271, 29223–29230
11. Moncada, S., Palmer, R. M., and Higgs, E. A. (1993) Pharmacol. Rev. 43, 109–142
12. Furchgott, R. F., and Vanhoutte, P. M. (1989) FASEB J. 3, 2007–2018
13. Ignarro, L. J., Byrns, R. E., Buga, G. M., and Wood, K. S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 866–879
14. Palmer, R. M., Ferrige, A. G., and Moncada, S. (1987) Nature 327, 524–526
15. Asahi, M., Fujii, S., Suzuki, K., Sea, H. G., Kuzuya, T., Mori, H., Tada, M., Fujii, S., and Taniguchi, N. (1995) J. Biol. Chem. 270, 21035–21039
16. Cleeter, M. W., Cooper, J. M., Darley-Usmar, V. M., Moncada, S., and Schapira, A. H. (1994) FEBS Lett. 345, 50–54
17. Clancy, R. M., Lees-Miller, J. J. D., and Abramson, S. R. (1992) J. Clin. Invest. 90, 1116–1121
18. Fujii, H., Ichimori, K., Hoshiaki, K., and Nakazawa, H. (1997) J. Biol. Chem. 272, 32773–32779
19. Jia, L., Bonaventura, C., Bonaventura, J., and Stamler, J. S. (1996) Nature 380, 221–228
20. Tarpey, M. J., Beckman, J. S., Ischiropoulos, H., Gore, J. Z., and Brock, T. A. (1995) FEBS Lett. 364, 314–318
21. Hui, R. E., and Padmaja, S. (1993) Free Radic. Res. Commun. 18, 195–199
22. Szabo, C., Salzman, A. L., and Ischiropoulos, H. (1995) FEBS Lett. 372, 229–232
23. MacMillan-Crow, L. A., Crow, J. P., Kerby, J. D., Beckman, J. S., and Thompson, J. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11853–11858
24. Hooper, D. C., Baggsa, O., Marini, J. C., Zborek, A., Ohiishi, S. T., Kean, R., Champion, J. M., Sarker, A. B., Boberksi, L., Farber, J. L., Akaite, T., Maeda, H., and Koprowski, H. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2525–2533
25. Brujin, L. L., Beal, M. F., Becher, M. W., Schulz, J. B., Wong, P. C., Price, D. L., and Cleveland, D. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7606–7611
26. Abe, K., Pan, L. H., Watanabe, M., Konno, H., Kato, T., and Itoyama, Y. (1997) J. Neurosci. 17, 2535–2547
27. Nutt, B., Clark, S. G., Giulian, A. D., and Fuchs, L. C. (1997) J. Pharmacol. Exp. Ther. 282, 1643–1649
28. Koo, N. W., Lewis, S. J., Royall, J. A., Ye, Y. Z., Kelly, D. R., and Beckman, J. S. (1997) Crit. Care Med. 25, 812–819
29. Beckman, J. S., Ye, Y. Z., Anderson, P. G., Chen, J., Accavitti, M. A., Tarpey, M. M., and White, C. R. (1994) Methods Enzymol. 233, 229–240
30. Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., and Freeman, B. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1620–1624
36. Salgo, M. G., Bermudez, E., Squadrito, G. L., and Pryor, W. A. (1995) *Arch. Biochem. Biophys.* **322**, 500–505
37. Moreno, J. J., and Pryor, W. A. (1992) *Chem. Res. Toxicol.* **5**, 425–431
38. Hassoun, P. M., Yu, F. S., Zulueta, J. J., White, A. C., and Lanzillo, J. J. (1995) *Am. J. Physiol.* **268**, L609–L617
39. Cote, C. G., Yu, F. S., Zulueta, J. J., Vosatka, R. J., and Hassoun, P. M. (1996) *Am. J. Physiol.* **271**, L669–L674
40. Ichimori, K., Fukahori, M., Nakazawa, H., Okamoto, K., and Nishino, T. (1999) *J. Biol. Chem.* **274**, 7763–7768
41. Houston, M., Chumley, P., Rahi, R., Rubbo, H., and Freeman, B. A. (1998) *Arch. Biochem. Biophys.* **355**, 1–8
42. Liu, X., Miller, M. J. S., Joshi, M. S., Thomas, D. D., and Lancaster, J. R., Jr. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 2175–2179
43. Liu, X., Miller, M. J. S., Joshi, M. S., Sadowska-Krowicka, H., Clark, D. A., and Lancaster, J. R., Jr. (1998) *J. Biol. Chem.* **273**, 18709–18713
44. Roubaud, V., Sankarapandi, S., Kuppusamy, P., Tordo, P., and Zweier, J. L. (1997) *Anal. Biochem.* **247**, 404–411
45. Malthouse, J. P., Williams, J. W., and Bray, R. C. (1981) *Biochem. J.* **197**, 421–425
46. Bray, R. C. (1961) *Biochem. J.* **73**, 189–195
47. Bray, R. C., Palmer, G., and Beinert, H. (1964) *J. Biol. Chem.* **239**, 2667–2676
48. Bray, R. C., and Lowe, D. J. (1997) *Biochem. Soc. Trans.* **25**, 762–768
49. Zweier, J. L., Wang, P., and Kuppusamy, P. (1995) *J. Biol. Chem.* **270**, 304–307
50. Zweier, J. L., Wang, P., Samouilov, A., and Kuppusamy, P. (1995) *Nat. Med.* **1**, 804–809

### Inhibition of Xanthine Oxidase by Peroxynitrite

- Liu, X., Miller, M. J. S., Joshi, M. S., Thomas, D. D., and Lancaster, J. R., Jr. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 2175–2179
Regulation of Xanthine Oxidase by Nitric Oxide and Peroxynitrite
Chang-il Lee, Xiaoping Liu and Jay L. Zweier

J. Biol. Chem. 2000, 275:9369-9376.
doi: 10.1074/jbc.275.13.9369

Access the most updated version of this article at http://www.jbc.org/content/275/13/9369

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 50 references, 23 of which can be accessed free at http://www.jbc.org/content/275/13/9369.full.html#ref-list-1