Modification by Hydroxyl Radicals of Functional Reactivity in Rabbit Lingual Artery

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ABSTRACT To understand the direct involvement of hydroxyl radical ('OH) in the modification of functional reactivity in isolated rabbit lingual artery ring preparations, this study was undertaken to examine the effect of 'OH generated from dihydroxy fumarate (DHF) plus Fe3+-ADP or from H2O2 plus FeSO4. When vasodilators (acetylcholine and nitroglycerin) were given after the 'OH-generating system was removed from the organ chamber, the earlier 'OH exposure produced an attenuation of the ring relaxation induced by acetylcholine but not that by nitroglycerin. Moreover, the earlier 'OH exposure attenuated caffeine-induced contraction and depressed the phasic response, but potently enhanced the tonic response of norepinephrine-induced contraction. Both the enhanced tonic response of KCl-induced contraction produced by earlier 'OH exposure and norepinephrine-induced contraction was inhibited by nisoldipine. These results are consistent with the view that 'OH radicals can potentiate the voltage-dependent influx of Ca. It is also postulated that 'OH may damage sarcoplasmic reticulum (SR) function in the smooth muscle cells, thus reducing Ca release from the SR (this may be reflected by the attenuation of the phasic response), and may selectively attenuate endothelium-dependent relaxation as opposed to endothelium-independent relaxation.

Keywords: Electron spin resonance, Endothelium, Lingual artery, Oxygen free radical, Vascular smooth muscle

Alterations in oxidant-antioxidant balance are a factor in the development of the vascular injury that occurs following exposure to ischemia and then reperfusion (1). Under this condition, it is believed that ischemia causes accumulation of purine catabolites (hypoxanthine) and a protease-dependent conversion of tissue xanthine dehydrogenase to oxygen radical-producing xanthine oxidase. Thus, when reperfusion supplies oxygen, hypoxanthine and xanthine oxidase can generate oxygen free radicals, causing cell damage.

The possibility that xanthine oxidase contributes to vascular damage is supported by a number of observations. First, xanthine oxidase forms superoxide (O2-) and hydrogen peroxide (H2O2), which react with iron (Fe2+) to form hydroxyl radical ('OH) in vitro (1). 'OH is one of the most potent oxidants known and has the capacity to damage key cell structures. Second, xanthine oxidase is strategically concentrated in endothelial cells (2). Third, xanthine oxidase depletion reduces injury in a variety of models of vascular damage (3-5).

In some of the reactive oxygen intermediates (ROI)-induced pathophysiological conditions, the first target of oxygen free radicals is the vascular system (6). O2- is capable of inhibiting the action of endothelium-derived relaxing factor (EDRF), as observed by Griffith et al. (7) who found that EDRF inhibitors such as phenidone, BW755C, dithiothreitol, and hydroquinone inhibit the action of EDRF released from the endothelial cells; the inhibitory action of these compounds is attenuated by concomitant application of superoxide dismutase (SOD). This suggests that EDRF inhibitors can inactivate EDRF via generation of O2-. Furthermore, this postulation was confirmed by demonstrating that another generator of O2-, pyrogallol, inhibits the action of EDRF and that cytochrome c, an O2- scavenger, potentiates the action of EDRF (8). However, the direct effect of 'OH on contractility and reactivity of blood vessels has received less attention. We, therefore, designed the following study to assess the direct effect of 'OH on the contractility of isolated rabbit lingual artery. We used in vitro oxygen free radi-

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cal-generating systems, including H$_2$O$_2$ plus FeSO$_4$ and dihydroxy fumarate, as well as Fe$^{3+}$-ADP (FeCl$_3$ plus ADP) for the generation of 'OH.

**MATERIALS AND METHODS**

**Materials**

The following pharmacological agents were used: dihydroxy fumarate (DHF), ADP, caffeine, indomethacin, superoxide dismutase (SOD), catalase (from bovine liver, 40,000 U/mg protein), acetylicholine hydrochloride (ACh), and norepinephrine hydrochloride (Sigma, St. Louis, MO, USA); nisoldipine (Bayer, Leverkusen, FRG); hydrogen peroxide (H$_2$O$_2$) and dimethyl sulfoxide (DMSO) (Aldrich, Milwaukee, WI, USA); nitroglycerin (Nihon Kayaku, Tokyo); FeCl$_3$, FeSO$_4$ and l-ascorbic acid (Wako Chemicals, Osaka); and 5,5-dimethyl-l-pyrroline-N-oxide (DMPO; Labotec, Tokyo). All these agents except nisoldipine and indomethacin were prepared in distilled water and diluted in Krebs-Ringer solution before being added to the organ chamber. Nisoldipine was dissolved in DMSO. The indomethacin stock solution was prepared by dissolving three parts of indomethacin and one part of sodium bicarbonate in distilled water. The concentrations of agents are expressed as the final concentrations in the organ chamber. The timed sequence of reagent addition is described in the Results.

**Vascular preparation and isometric tension recording**

In accordance with our institutional Animal Care Committee guidelines, lingual arteries were taken from male albino (New Zealand) rabbits (2.0–2.5 kg) after exsanguination under anesthesia with diethyl ether. Fat and other nonvascular tissue were gently removed from the blood vessels, which were cut into rings (1-mm width, 540–610 pm OD and 440–470-pm ID) without disturbing the intimal layer, after immersion in ice-cold modified Krebs-Ringer solution (0.05 mM indomethacin to prevent ROI induced release of vasoactive prostanoids from endothelium, 0.05 mM EDTA and 0.03 MM L-ascorbic acid to stabilize norepinephrine, 118.0 mM NaCl, 4.7 mM KCl, 1.18 mM MgSO$_4$, 2.5 mM CaCl$_2$, 1.18 mM KH$_2$PO$_4$, 25.0 mM NaHCO$_3$ and 5.5 mM glucose, aerated with 95% O$_2$–5% CO$_2$, pH 7.2–7.3).

The rings were suspended in a 5-ml water-jacketed organ chamber (37°C ±0.4°C) with one end tied to a fixed point and the other connected to a force transducer (Nihon Kohden JB-612T, Tokyo); changes in isometric force were recorded with an amplifier (Nihon Kohden AP-601G) attached to a recorder (Nihon Kohden RM-6000). Before the start of the experiment, the rings were stretched to a passive tension of 0.5 g (9). The tissue preparations were contracted with 10$^{-5}$ M norepinephrine, and the functional integrity of the endothelium was checked by the presence of relaxation induced by ACh (10$^{-5}$ M).

**Hydroxyl radical (OH)-generating system**

Autooxidizing DHF was chosen as a chemical source of oxygen radicals at physiological pH. The reaction sequence of the autooxidation of DHF under physiological conditions is

\[
\text{DHF} + O_2 \rightarrow \text{DHF}^* + O_2 \tag{1}
\]

\[
2H^+ + \cdot O_2^- + \text{DHF} \rightarrow \text{DHF}^* + H_2O_2 \tag{2}
\]

\[
\text{DHF}^* + O_2 \rightarrow \text{DHF} + \cdot O_2^- \tag{3}
\]

where DHF$^*$ is a free radical formed by the loss of one electron from DHF and DKS is diketosuccinate.

Once formed, 'O$_2^-$ leads to the generation of other active oxygen species such as 'OH, H$_2$O$_2$, and singlet oxygen ('O$_2$) through the nonenzymatic dismutation reaction sequence:

\[
\text{O}_2^- + \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \tag{4}
\]

\[
H_2O_2 + \cdot O_2^- \rightarrow O_2^- + \cdot OH + OH^- \tag{5}
\]

\[
H_2O_2 + \cdot O_2^- \rightarrow \cdot OH + OH^- + O_2 \tag{6}
\]

Reaction [5] may be catalyzed by Fe$^{3+}$-ADP as

\[
\text{Fe}^{3+} + \cdot O_2^- + \text{ADP} \rightarrow \text{Fe}^{2+} + \text{ADP} \tag{7}
\]

\[
\text{Fe}^{3+} + \text{H}_2\text{O}_2 + \text{ADP} \rightarrow \text{Fe}^{2+} + \text{ADP} + \text{OH}^- + \cdot \text{OH} \tag{8}
\]

O$_2$ uptake is completely inhibited by superoxide dismutase (Eq. [1]) added any time during the reaction (10). For the 'OH-generating system, H$_2$O$_2$ and FeSO$_4$ were also used.

**Electron spin resonance (ESR) analysis**

The production of oxygen free radicals by DHF and Fe$^{3+}$-ADP or by H$_2$O$_2$ and FeSO$_4$ was verified by ESR spectroscopy. ESR detection of spin adducts was performed by a JES-RE2X, X-band spectrometer (Jeol, Tokyo) at the following instrument settings: 334.9 mT field set, 5 mT scan range, 100 kHz modulation frequency, 0.1 mT modulated amplitude, 2 x 100 receiver gain, 0.1 sec time constant, 2 min scanning time, and 8 mW microwave power. DMPO (15 μl of original liquid) was used as the spin trap. The desired reaction mixtures (200 μl) were prepared in glass tubes and transferred to a quartz ESR flat cell (130 μl), which was in turn placed in the cavity of the ESR spectrometer. Sequential ESR scans were then started 45 sec after the addition of DMPO to the reaction mixture at 25°C. To quantitate the DMPO spin adducts detected, the Mn$^{2+}$ standard ESR spectrum (MnO) was obtained. Preliminary experiments demonstrated that antioxidant ascorbic acid at the concentration used in the present study had no effect on ESR signals produced by DHF/Fe$^{3+}$-ADP or H$_2$O$_2$/FeSO$_4$, indicating
that our experimental system is a valid means for assessing the direct effect of \( \cdot \text{OH} \).

**Statistical analyses**

The data are shown as means±S.E. Unless otherwise noted, each experimental group consisted of at least five blood vessels taken from different rabbits. For statistical evaluation, multiple analysis of variance was carried out, and Duncan’s multiple range test was used to determine differences between the means within the population. Differences were considered to be statistically significant when \( P < 0.05 \).

**RESULTS**

**Spin-trapping of hydroxyl radical**

In preliminary experiments, oxygen free radical species generated from DHF/Fe\(^{3+}\)-ADP or \( \text{H}_2\text{O}_2/\text{FeSO}_4 \) under the conditions employed in the free radical exposure studies were verified by using highly sensitive ESR spectroscopy and the spin-trap DMPO in the absence of the tissue preparations. The 1:2:2:1 quartet (the hyperfine splittings were \( A_N = A_H = 1.49 \text{ mT} \)), characteristic of the DMPO-\( \cdot \text{OH} \) spin adduct (11), was observed (Figs. 1A and 2A). Previously, we have shown that the signal produced by DHF/Fe\(^{3+}\)-ADP was replaced by the ethanol-radical adduct, characteristic of ethanol scavenged \( \cdot \text{OH} \) radical, verifying that the DMPO-\( \cdot \text{OH} \) signal was, in fact, spin-trapped \( \cdot \text{OH} \) radical (12).

The data of Figs. 1 and 2 further document the concentration-related effect of DHF (Fig. 1B) and Fenton’s reagent (\( \text{H}_2\text{O}_2 \) and \( \text{FeSO}_4 \); Fig. 2, B and C). The calculated relative signal intensity of the DMPO-\( \cdot \text{OH} \) adduct produced from DHF in the presence of a fixed concentration of Fe\(^{3+}\)-ADP (43 nM FeCl\(_3\) plus 1.56 \( \mu \text{M ADP} \)) increased in a concentration-dependent manner. In the presence of \( 2 \times 10^{-4} \text{ M FeSO}_4 \), \( \text{H}_2\text{O}_2 \) also produced the DMPO-\( \cdot \text{OH} \) signal adduct concentration-dependently (Fig. 2B); \( \text{FeSO}_4 \)-induced DMPO-\( \cdot \text{OH} \) signal production was concentration-dependent, and the maximum relative signal intensity was obtained at \( 2 \times 10^{-4} \text{ M FeSO}_4 \) concentration in the presence of \( 3 \times 10^{-4} \text{ M H}_2\text{O}_2 \) (Fig. 2C). The relative signal intensity of the DMPO-\( \cdot \text{OH} \) adduct immediately reached the maximum value after the addition of \( 2.4 \text{ mM DHF/Fe}^{3+}-\text{ADP} \) or \( 3 \times 10^{-4} \text{ M H}_2\text{O}_2/2 \times 10^{-4} \text{ M FeSO}_4 \) into the bathing media, and then showed a sharp decline during the first 30 min (data not shown); thus the first 30 min of exposure is sufficient to assess the effect of \( \cdot \text{OH} \). Based on this, the exposure conditions to the ring preparations were chosen. We decided to use 30 min of exposure to \( 2.4 \text{ mM DHF/Fe}^{3+}-\text{ADP} \) (43 nM FeCl\(_3\) plus 1.56 \( \mu \text{M ADP} \)) or \( 3 \times 10^{-4} \text{ M H}_2\text{O}_2/2 \times 10^{-4} \text{ M FeSO}_4 \), which was repeated three times in some experiments.

**Effects of DHF/Fe\(^{3+}\)-ADP and \( \text{H}_2\text{O}_2/\text{FeSO}_4 \) reactions on endothelium-dependent and -independent relaxation**

The ring preparations confirmed to have the ability of endothelium-dependent relaxation were exposed to the \( \cdot \text{OH} \)-generating system using DHF/Fe\(^{3+}\)-ADP or \( \text{H}_2\text{O}_2/\text{FeSO}_4 \) for 30 min; the rings were serially washed and reequilibrated for 15 min; and then after contraction with norepinephrine (10\(^{-5}\) M), relaxations were generated using ACh (10\(^{-5}\) to 10\(^{-3}\) M). ACh relaxed the norepinephrine-contracted ring preparations in a concentration-dependent manner. Exposure to DHF/Fe\(^{3+}\)-ADP or \( \text{H}_2\text{O}_2/\text{FeSO}_4 \) attenuated these responses with a rightward shift of the concentration-relaxation curves and a decrease in the maximum relaxation (Fig. 3). Nitroglycerin (10\(^{-6}\) M), an endothelium-independent vasodilator, produced a relaxation in normal as well as DHF/Fe\(^{3+}\)-ADP or \( \text{H}_2\text{O}_2/\text{FeSO}_4 \)-exposed rings (Fig. 4).
Fig. 2. Electron spin resonance (ESR) spectra and signal intensity obtained from Fenton’s reaction. A: ESR spectra of DMPO-OH produced by Fenton’s reagent (0.3 mM H$_2$O$_2$ plus 0.2 mM FeSO$_4$) in the bathing media. Signals appearing at both sides of the ESR spectra correspond to Mn$^{2+}$ (MnO) installed in the ESR cavity as a reference. The ESR spectrum was recorded 45 sec after the addition of DMPO to the reaction mixture. B: Relative intensity of the DMPO-OH signal produced by 3 x 10$^{-7}$ to 3 x 10$^{-3}$ M H$_2$O$_2$ in the presence (●) or absence (□) of 2 x 10$^{-4}$ M FeSO$_4$. The relative signal intensity was calculated as described in Fig. 1B. C: Relative intensity of the DMPO-OH signal produced by 2 x 10$^{-1}$ to 2 x 10$^{-3}$ M FeSO$_4$ in the presence (■) or absence (□) of 3 x 10$^{-4}$ M H$_2$O$_2$. The relative signal intensity was calculated as described in Fig. 1B.

Fig. 3. Effect of prior exposure (30 min) to DHF/Fe$^{3+}$-ADP (A) or H$_2$O$_2$/FeSO$_4$ (B) on the endothelium-dependent relaxation induced by ACh (10$^{-8}$ to 10$^{-5}$ M) in lingual arteries that were precontracted by norepinephrine (NE, 10$^{-5}$ M). When added, the doses of the agents were as follows: 2.4 mM DHF, 43 nM FeCl$_3$, 1.56 nM ADP, 3 x 10$^{-4}$ M H$_2$O$_2$, and 2 x 10$^{-4}$ M FeSO$_4$. Four ring preparations (for time-matched non-control, ○; DHF or H$_2$O$_2$ alone, □; Fe$^{3+}$-ADP or FeSO$_4$ alone, ■; and complete 'OH-generating system, ◆) obtained from the same vessel were studied in parallel, and one concentration-response curve to ACh was made per ring preparation. The rings were exposed to DHF, Fe$^{3+}$-ADP, H$_2$O$_2$, FeSO$_4$ or to the complete 'OH-generating system for 30 min; and then they were serially washed and reequilibrated for 15 min. After contraction with NE, relaxations were generated by ACh. The maximum relaxations induced by 10$^{-5}$ M ACh in the time-matched none-control experiments are taken as 100%, and other data are plotted in relation to it. The points represent the mean, and vertical lines show S.E.M. (n=5); n refers to the number of rabbits from which the lingual artery was taken. *Significantly (P < 0.05) different from the corresponding value for the control.
Fig. 4. Effect of prior exposure to DHF/Fe³⁺-ADP or H₂O₂/FeSO₄ on the relaxation induced by nitroglycerin (10⁻⁶ M) in lingual arteries that were precontracted by norepinephrine (NE, 10⁻⁷ M). When added, the doses of the agents were as follows: 2.4 mM DHF, 43 nM FeCl₃, 1.56 pM ADP, 3 x 10⁻⁴ M H₂O₂, and 2 x 10⁻⁴ M FeSO₄. Two ring preparations (for DHF/Fe³⁺-ADP and H₂O₂/FeSO₄) obtained from the same vessel were studied in parallel, and one 'OH-exposing time-response curve (30 to 90 min) was determined per ring preparation. The ring preparations confirmed to have the ability of endothelium-independent relaxation (induced by 10⁻⁶ M nitroglycerin) were exposed to DHF/Fe³⁺-ADP or H₂O₂/FeSO₄ for 30 min; the rings were serially washed and reequilibrated for 15 min, and then after contraction with NE, relaxations were generated by nitroglycerin. This procedure was repeated two or three times (giving 60 min and 90 min of total exposure to the 'OH radicals, respectively). Data are expressed as a percentage of the precontraction and shown as the mean; vertical lines indicate S.E.M. (n=4); n refers to the number of rabbits from which the lingual artery was taken.

Effects of DHF/Fe³⁺-ADP and H₂O₂/FeSO₄ reactions on the phasic and tonic responses of norepinephrine-induced contraction and on caffeine-induced contraction

Figure 5 shows the effect of prior exposure to DHF/Fe³⁺-ADP or H₂O₂/FeSO₄ on the norepinephrine-induced contraction (10⁻⁵ M). Norepinephrine generated the phasic and tonic responses of the ring preparations (Fig. 5A-a). The prior exposure to DHF/Fe³⁺-ADP (Fig. 5B) or H₂O₂/FeSO₄ (Fig. 5C) significantly inhibited the phasic response but potently enhanced the tonic response of the norepinephrine-induced contraction. At the concentrations tested, DHF, Fe³⁺-ADP, H₂O₂ and FeSO₄ alone had no effect on this system. The observed effect of DHF/Fe³⁺-ADP or H₂O₂/FeSO₄ at 30 min of prior exposure on norepinephrine-induced contraction was inhibited by SOD or catalase, respectively (Fig. 6).

It is postulated that the phasic response of vascular smooth muscle is evoked by the release of Ca from the storage site following activation of the influx of Ca, and that the tonic response is generated by the amount of free Ca, balanced by the Ca influx, release and uptake at the storage site and also the Ca efflux. Furthermore, Haessler et al. (13) suggested that norepinephrine and caffeine may release Ca from the same Ca storage site, the sarcoplasmic reticulum (SR), in rabbit mesenteric artery. To describe further the effect of 'OH on vascular smooth muscle contractility, the caffeine-induced contraction in the ring preparations previously exposed to H₂O₂/FeSO₄ for 30 min was studied. As indicated in Fig. 7, the caffeine-induced contraction was attenuated by H₂O₂/FeSO₄ but not by H₂O₂ alone, suggesting that 'OH can indeed reduce Ca release from the SR.

Effect of nisoldipine on KCl-induced contraction of DHF/Fe³⁺-ADP- and H₂O₂/FeSO₄-exposed ring preparations

If the view that 'OH can enhance the tonic response of norepinephrine-induced contraction via a mechanism by which the voltage-dependent influx of Ca is activated is correct, the KCl-induced contraction should be also enhanced, inasmuch as KCl-induced contraction is known to be generated mainly by activation of the voltage-dependent influx of Ca (14). We tested this hypothesis by application of nisoldipine, a selective voltage-dependent Ca channel blocker (15-17), to our experimental system. As expected, the tonic contraction evoked by 60 mM KCl of the ring preparations was enhanced when rings were exposed to DHF/Fe³⁺-ADP or H₂O₂/FeSO₄ before the addition of KCl (Fig. 8); DHF, Fe³⁺-ADP, H₂O₂ or FeSO₄ alone had no effect on the KCl-induced contraction. The observed effect of the 'OH radical generating system was inhibited by nisoldipine; enhanced tonic contraction was reversed to near normal by 10⁻¹¹ M nisoldipine.

DMSO is known to be an 'OH radical scavenger; however, the amount of DMSO, which is used to dissolve nisoldipine, in the organ chamber had no detectable effect on the DMPO-OH signal produced by the 'OH radical generating system used (data not shown). Therefore, this rules out the possibility that the effect of nisoldipine is elicited via the scavenging effect of the solvent DMSO.

Effect of nisoldipine on norepinephrine-induced contraction of DHF/Fe³⁺-ADP- and H₂O₂/FeSO₄-exposed ring preparations

Finally, to assess the influence of voltage-dependent influx of Ca on the changes in norepinephrine-induced contraction caused by prior exposure to 'OH, additional experiments were carried out in the presence of nisoldipine. Nisoldipine (10⁻¹¹ M) significantly inhibited the enhanced tonic response of norepinephrine-induced contraction produced by DHF/Fe³⁺-ADP- or H₂O₂/FeSO₄-exposure (Fig. 9). Nisoldipine had no effect on any of the changes induced by earlier DHF/Fe³⁺-ADP- or H₂O₂/FeSO₄-exposure in the phasic response of norepinephrine-induced contraction (data not shown), suggesting that the nisoldipine-inhibitable component of Ca influx in smooth muscle cells is sensitive to early 'OH radical exposure.
DISCUSSION

The ESR spectra of DHF/Fe^{3+}-ADP and H_{2}O_{2}/FeSO_{4} reactions revealed a DMPO-OH signal (Figs. 1 and 2); prior exposure to DHF/Fe^{3+}-ADP or H_{2}O_{2}/FeSO_{4} was found to produce an attenuation of the endothelium-dependent relaxation induced by ACh in the ring preparations of lingual artery (Fig. 3), but did not affect the endothelium-independent relaxation caused by nitroglycerin (Fig. 4), in the present study. It appeared from these findings that 'OH can damage endothelium-dependent relaxation. Both EDRF and nitroglycerin are reported to produce vascular relaxation by increasing the production of guanosine 3',5'-cyclic monophosphate (cGMP), which...
inhibits the smooth muscle contractile process (18). Because prior exposure to \(^{1} \text{OH}\) generated from \(\text{DHF/Fe}^{3+}\)-ADP or \(\text{H}_2\text{O}_2/\text{FeSO}_4\) only inhibits the actin of \(\text{ACh}\), and not that of nitroglycerin, its action may be at some site(s) between the endothelial site of interaction and the smooth muscle production of cGMP. The cellular mechanisms by which nitrite and subsequently nitric oxide are produced from nitroglycerin have not been elucidated completely; however, in vitro studies have demonstrated that nitroglycerin is a substrate of the glutathione S-transferases (19, 20). Yeates et al. (21) have reported that inhibition of glutathione S-transferase activity by bromosulfophthalein results in decreased nitroglycerin-induced relaxation of precontracted rabbit thoracic aorta strips; the susceptibility of the processes of cGMP production mediated by nitroglycerin in smooth muscle cells to \(^{1} \text{OH}\) radicals may differ greatly from that mediated by EDRF. Thus it is likely that \(^{1} \text{OH}\) radicals may selectively damage endothelium function.

The mechanisms of the effect of ROI on vascular smooth muscle at the cellular level have not been extensively studied. Recently, the Ca-transport activity (22) and Ca-adenosinetriphosphatase (ATPase) activity (23) of vascular smooth muscle SR have been shown to be inhibited by ROI. Our laboratory has provided evidence that a major target organelle attacked by ROI is the
system that regulates Ca delivery (SR and sarcolemma) to the contractile proteins and not the contractile proteins per se in cardiac muscle (24–27). In the present study, prior exposure of the ring preparations to DHF/Fe^{3+}-ADP or H_2O_2/FeSO_4 depressed the phasic response of the norepinephrine-induced contraction; and in contrast, the tonic response was potentiated (Fig. 5). The observed effect of DHF/Fe^{3+}-ADP or H_2O_2/FeSO_4 was SOD or catalase-inhibitable, respectively (Fig. 6). SOD had a protective effect for two reasons. First, it may have eliminated 'O_2 and prevented the reduction of Fe^{2+} to Fe^{3+}, thereby ruling out the possibility of 'OH formation by Fenton's reaction. Second, it may have inhibited autooxidation of DHF, thereby reducing the formation of free radicals.

Fig. 8. Enhanced tonic response of the KCl-induced contraction produced by early exposure to 'OH radical and effect of nisoldipine. The ring preparations were exposed to DHF/Fe^{3+}-ADP (B) or H_2O_2/FeSO_4 (C) for 30 min; the rings were serially washed and reequilibrated for 15 min. This procedure was repeated three times (to give 90 min of total exposure to 'OH radicals), and then 10 min after the addition of nisoldipine (10^{-12} to 10^{-9} M), contractions were generated by 60 mM KCl. The amplitude of the tonic response of contraction evoked by KCl in each ring preparation before the start of the experiment was taken as 100%. Five ring preparations (for "no nisoldipine" time-matched DHF/Fe^{3+}-ADP or H_2O_2/FeSO_4-exposure study, and experiments with 10^{-12}, 10^{-11}, 10^{-10} and 10^{-9} M of nisoldipine in DHF/Fe^{3+}-ADP or H_2O_2/FeSO_4-exposed ring preparations) obtained from the same vessel were studied in parallel, and one determination, in the presence or absence of nisoldipine, of the response to KCl was performed per ring preparation exposed to the DHF/Fe^{3+} or H_2O_2/FeSO_4 reaction. The amplitude of the tonic response in each ring preparation before the start of the experiment was taken as 100%. Each column represents the mean, and vertical lines show the S.E.M. (n=7); n refers to the number of rabbits from which the lingual artery was taken. *P<0.05, **P<0.01: significantly different from the corresponding value for "no nisoldipine."

Fig. 9. Effect of nisoldipine (10^{-12} M) on enhanced tonic response produced by early exposure to 'OH radical of norepinephrine-induced contraction. The experimental conditions were identical to those described in Fig. 8. except that the contractions were generated by 10^{-5} M norepinephrine. Four ring preparations (for "no nisoldipine" time-matched DHF/Fe^{3+}-ADP and H_2O_2/FeSO_4-exposure studies, and experiments on nisoldipine in DHF/Fe^{3+}-ADP and H_2O_2/FeSO_4-exposed ring preparations) obtained from the same vessel were studied in parallel, and one determination, in the presence or absence of nisoldipine, of the response to norepinephrine was performed per ring preparation exposed to the DHF/Fe^{3+}-ADP or H_2O_2/FeSO_4 reaction. The amplitude of the tonic response in each ring preparation before the start of the experiment was taken as 100%. Each column represents the mean, and vertical lines show S.E.M. (n=6); n refers to the number of rabbits from which the lingual artery was taken. *P<0.01: significantly different from the corresponding value for "no nisoldipine."

The protective effect of catalase is due to H_2O_2 scavenging. We, therefore, postulated that prior exposure to 'OH may damage SR function in the smooth muscle cells, thus reducing Ca release from the SR (this may be reflected by the attenuation of the phasic response), and may produce an increased voltage-dependent influx of Ca which is reflected by the enhanced tonic response of the norepinephrine-induced contraction. This postulation is further inferred by the following significant observations: 1) earlier exposure to 'OH (generated from H_2O_2/FeSO_4 reaction) attenuates the caffeine-induced contraction...
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