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Mechanism of Peroxide-Induced Potentiation of Sympathetic Neurotransmission in Bovine Irides: Role of Extracellular Calcium

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Hydrogen peroxide (H_2O_2) and enzymes that regulate its metabolism are present in tissues of the anterior segment of the eye. We have previously shown that *in vitro*, H_2O_2 can enhance sympathetic neurotransmission in irides from several mammalian species. In the present study, we investigated the role of extracellular calcium in H_2O_2-induced potentiation of sympathetic neurotransmission in the bovine isolated iris. Isolated bovine hemiirides were incubated in a bicarbonate-buffered, carbogen-gassed Krebs buffer solution containing [^{3}H]-norepinephrine ([^{3}H]NE) for 60 min. After incubation, tissues were prepared for studies of [^{3}H]NE release using the superfusion method. Release of [^{3}H]NE was elicited by consecutive trains of electrical field stimulation. Removal of calcium from the buffer solution attenuated field-stimulated [^{3}H]NE overflow in isolated, superfused bovine irides without affecting basal tritium efflux. H_2O_2 (1 mM) enhanced evoked [^{3}H]NE release to the same extent in tissues exposed to buffer solutions containing normal calcium (1.3 mM) as in those containing low calcium (0.13 mM) or zero calcium. However, in the presence of zero-calcium buffer solution containing the chelator, EDTA (1 mM), H_2O_2 (1 mM) caused a gradual and sustained increase in basal tritium efflux. In buffer solutions containing high calcium (1.95 mM), the magnitude of H_2O_2-induced increase in field-stimulated [^{3}H]NE release was significantly ($P < 0.05$) attenuated. Although the neuronal calcium channel antagonist ω-conotoxin (20 nM) inhibited [^{3}H]NE by 25%, it had no effect on H_2O_2 (1 mM)-induced potentiation of evoked [^{3}H]NE overflow. We conclude that while trace amounts of extracellular calcium are necessary for H_2O_2-induced enhancement of sympathetic neurotransmission, increasing extracellular (buffer) calcium concentration impaired peroxide-induced enhancement of [^{3}H]NE release. Furthermore, voltage-activated calcium channels may not be directly involved in peroxide-induced alteration of adrenergic neurosecretion in bovine isolated irides.

Keywords: Hydrogen peroxide, extracellular calcium, bovine iris, norepinephrine release, calcium channel antagonists, oxidative stress

INTRODUCTION

It is well established that calcium influx via voltage-gated calcium channels subsequent to depolarization of the sympathetic nerve terminal membrane is the trigger for neurotransmitter

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release by exocytosis (as reviewed by Fried[1]). Although the presence of extracellular calcium is believed to be a prerequisite for neurotransmitter release (reviewed by Augustine *et al.*[2]), there is evidence that neurotransmitter release can also occur in the absence of extracellular calcium.[3,4] Indeed, different pathways have been shown to be involved in the release of neurotransmitters (such as norepinephrine, NE) in calcium-containing versus calcium-free medium.[5]

Removal of calcium from the extracellular medium has been reported to markedly increase lipid peroxidation, mitochondrial and cytoplasmic glutathione depletion, glutathione disulfide formation and efflux of reduced glutathione from rat isolated hepatocytes indicating that extracellular calcium ions may serve a protective role against cellular injury.[6] In the "calcium omission model," Reed *et al.*[7] found that ruthenium red and lanthanum prevented malondialdehyde formation and reduced glutathione and vitamin E loss. Furthermore, omission of calcium caused a profound loss of mitochondrial transmembrane potential that was prevented by ruthenium red, vitamin E and desferroxamine. Reed *et al.*[7] then hypothesized that the absence of extracellular calcium caused mitochondrial calcium cycling which may partially contribute to the observed oxidative stress. Taken together, these studies indicate a role for extracellular calcium in the prevention and/or generation of oxidant-induced injury to cells.

Oxidative stress can also alter the activity of membrane calcium channels. Ascorbate-induced lipid peroxidation has been shown to inhibit binding of calcium channel blocker [^{3}H]PN200-110 to membranes, indicating that lipid peroxidation can lead to modification of voltage-dependent calcium channels.[8] It is, therefore, possible that modification of voltage-dependent calcium channels by oxidative stress could indirectly alter calcium entry into cells. Josephson *et al.*[9] found that nitrendipine protected myocytes against damage to oxidative stress suggesting a role for calcium influx via voltage-sensitive calcium channels in oxidant injury to cells. Thus, by preventing influx of extracellular calcium into cells in response to oxidative stress, calcium channel antagonists may prevent free radical damage to cells. The exact relationship between oxidative stress and changes in extracellular calcium remains to be determined.

In our laboratory, we found that both naturally-occurring and synthetic peroxides enhanced electrically-induced [^{3}H]-norepinephrine release from isolated human, rabbit, rat and bovine irides.[10,11] The mechanisms whereby peroxides induce changes to sympathetic neurotransmission in ocular tissues is, however, unclear. We have evidence that both the cyclic AMP-specific phosphodiesterase inhibitor, RO-201724 and the inhibitor of protein kinase I/II, Rp-cAMPS had no effect on the enhancement of electrically-evoked NE release induced by H_2O_2 suggesting that cyclic AMP is not a mediator of the peroxide response in the bovine iris.[12,13] Since extracellular calcium has been reported to regulate neurotransmitter release from nerves and can also affect the generation free radicals in some tissues, the present study considered the possibility that the potentiation of sympathetic neurotransmission induced by peroxides may be dependent on extracellular calcium homeostasis. The aim of the present study was, therefore, to determine the role of extracellular calcium in hydrogen peroxide-induced potentiation of sympathetic neurotransmission in the bovine isolated iris.

MATERIALS AND METHODS

Studies were performed on bovine eyeballs obtained from local slaughterhouses in Omaha area (Greater Omaha Packing Company and J. F. O'Neill Packing Company). Eyeballs from freshly sacrificed cows were transported to the laboratory in an ice bucket. The anterior segment of each eye was removed and placed immediately in oxygenated Krebs solution, and iris-ciliary bodies were dissected free of the lens,

lens-capsule, sclera and adherent vitreous. Hemiirides (5 mm wide, 25 mm long) were then prepared for *in vitro* superfusion studies.

Studies on [^{3}H]-Norepinephrine Release

Tissues were prepared for analysis of evoked [^{3}H]-norepinephrine [^{3}H]-NE) release as described previously.[11,14] Briefly, isolated irides were mounted between nylon mesh cloth (200 μm pore size) and incubated for 60 min at 37°C in a bicarbonate-buffered, carbogen-gassed Krebs buffer solution containing 2.5 μCi/ml L-[^{3}H]NE (New England Nuclear, Boston, MA; 40.8 Ci/mmol). The Krebs buffer solution had the following composition (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 25; $MgSO_4$, 2.0; and dextrose, 10 (pH 7.4). After incubation, tissues were rinsed (three 5-min washes) in warm Krebs buffer solution to remove excess radioactivity and then transferred to individual, temperature-controlled Plexiglass superfusion chambers. Irides were superfused at 2 ml/min with oxygenated Krebs buffer solution containing the neuronal uptake inhibitor, desipramine (1 μM). Fractions of the superfusate were collected at 4-min intervals and 4-ml aliquots of each fraction were combined with 12 ml of aqueous scintillation cocktail (Ecolume, ICN Radiochemicals, CA) and analyzed for radioactivity by liquid scintillation spectrometry.

After an initial 60 mins of superfusion to establish a stable baseline of spontaneous tritium efflux, release of [^{3}H]NE was elicited by consecutive trains of 300 d.c. pulses (5 Hz, 2 ms pulse duration, 50 V/cm interelectrode distance, 60 s) delivered to the platinum chamber electrodes using a Grass S48 stimulator (Grass Instruments, Quincy, MA). Stimulation-evoked release and overflow of tritium was estimated by subtraction of the extrapolated basal tritium efflux from total tritium released during the 20-min period after the onset of stimulation. Basal (unstimulated) tritium efflux was assumed to decline linearly between pre- and post-stimulation fractions. Chromatographic analysis of the radioactive release products in bovine irides has shown more than 46% of the radioactive material released in response to field stimulation consists of unmetabolized [^{3}H]NE, whereas the spontaneously released radioactivity represents various NE metabolites and oxidation products.[14] Thus, stimulation-evoked tritium efflux was designated as [^{3}H]NE release.

All tissues received two stimulations (S_1 and S_2) 30 min apart. To determine the effect of exogenous agents (e.g. H_2O_2) on [^{3}H]NE release, test compounds were applied 8 min before and during S_2. In some experiments, nitrendipine or ω-conotoxin was applied 20 min before S_1 and was present during both stimulation periods (S_1 and S_2). To determine the role of extracellular calcium on H_2O_2 (0.3–1 mM)-induced potentiation of evoked [^{3}H]NE release, the peroxide was present in the buffer solution containing modified calcium concentrations: high (1.95 mM), low (0.13 mM) or absent (0 mM) and 0 mM plus the chelator, ethylenediaminetetraacetic acid (EDTA, 1 mM) 8 min before and during S_2. Experiments in which tissues were exposed to different calcium concentrations (in the absence of peroxides) at S_2 served as controls for these studies.

Stimulation-evoked release of [^{3}H]NE during S_1 and S_2 was determined graphically, and the ratio of the two peaks (S_2/S_1) was calculated and compared to untreated control preparations. The pattern and total amount of basal and evoked [^{3}H]NE efflux from isolated, superfused tissues can vary remarkably. However, the ratio of evoked [^{3}H]NE overflow between the first (S_1) and second (S_2) stimulation period is near unity for control experiments but varies under test conditions.

Data Analysis

Results are expressed as the absolute S_2/S_1 ratios. Paired iris strips were prepared from each eyeball and four tissues were set up for each series of superfusion experiments with one tissue serving

as a control. Data from different experiments (control and test) were pooled and then subjected to statistical analysis. Except where indicated otherwise, values given are arithmetic means ± S.E.M. Significance of differences between S_2/S_1 values obtained in control and agent-treated preparations were evaluated using analysis of variance (ANOVA) followed by Dunnett's test. Differences with P values < 0.05 were accepted as statistically significant.

Drugs and Chemicals

Hydrogen peroxide, desipramine and, ethylenediaminetetraacetic acid disodium dihydrate salt (EDTA) were purchased from Sigma Chemical Co., St. Louis, MO. L-[^{3}H]Norepinephrine (40–80 Ci/mmol) was purchased from Dupont NEN, Boston, MA. ω-conotoxin GVIA and nitrendipine were purchased from Research Biochemicals International, Natick, MA.

RESULTS

To determine the role of extracellular calcium on peroxide-induced potentiation of adrenergic neurosecretion, we first examined the effect of Krebs buffer solution containing low concentrations of calcium on field-stimulated [^{3}H]NE overflow (Figure. 1, top panels). Figure 1 (panel A) shows the effect of two trains of electrical stimuli (S_1 and S_2) on [^{3}H]NE efflux in buffer containing normal calcium (1.3 mM). In the

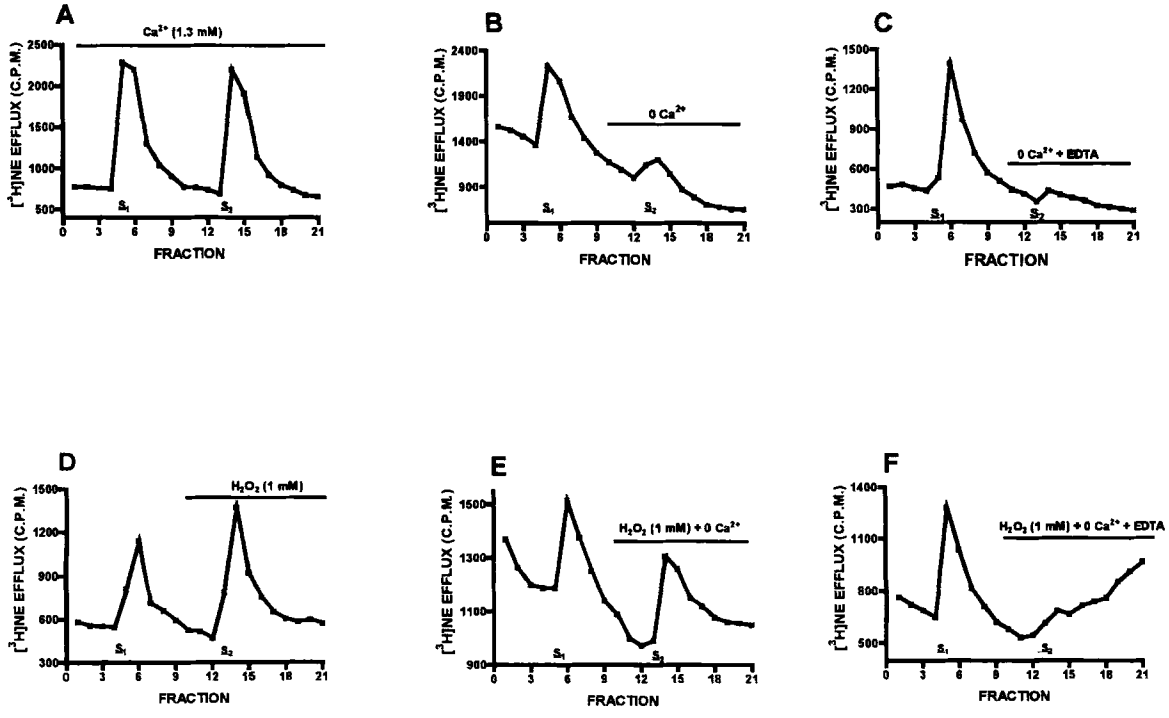

FIGURE 1 Effect of calcium omission from buffer solution on field-stimulated [^{3}H]-norepinephrine ([^{3}H]NE) release from isolated, superfused bovine irides in the absence (top panels) and presence (bottom panels) of H_2O_2 (1 mM). Trains of field stimulation (5 Hz, 2 ms pulse duration, 50 V, 60 sec) were applied at fraction 5 (S_1) and fraction 13 (S_2). Experiments in Panels A and D were performed in the presence of buffer solution containing normal calcium concentration (1.3 mM) at both S_1 and S_2, whereas those in panels B, C, E and F had normal calcium present in the buffer solution at S_1 only. Panel A (normal Ca^{2+}, 1.3 mM); panel B (0-Ca^{2+}); panel C (0-Ca^{2+} plus EDTA, 1 mM); panel D (H_2O_2, 1 mM); panel E (0-Ca^{2+} buffer plus H_2O_2, 1 mM) and panel F (0-Ca^{++} buffer plus EDTA, 1 mM and H_2O_2, 1 mM). Fraction of the superfusate containing [^{3}H]NE were collected at 4-min intervals and analyzed for radioactivity as described under Methods.

absence of calcium in the buffer solution at S_2, there was an attenuation of field-stimulated [^{3}H]NE overflow by 60% (Figure 1, panel B). A reduction of field-stimulated [^{3}H]NE release by 73% was observed with buffer solutions containing no added calcium plus EDTA (1 mM) (Figure 1, panel C).

We next investigated the effects of H_2O_2 on electrically-induced [^{3}H]NE release in the presence of buffer containing normal and low concentrations of calcium (Figure 1, bottom panels). In comparison to controls (Figure 1, panel A), H_2O_2 (1 mM) caused a significant enhancement of evoked [^{3}H]NE overflow in buffer solutions containing normal calcium at S_2 (Figure 1, panel D). When compared to controls with no added calcium (Figure 1, panel B), H_2O_2 (1 mM) increased the overflow of [^{3}H]NE at S_2 (Figure 1, panel E). Interestingly, there was no difference in the magnitude of enhancement of evoked [^{3}H]NE release caused by H_2O_2 in the presence of buffer solution containing normal calcium (1.3 mM) and that produced in the absence of buffer calcium (Table I). When compared to controls with zero-calcium buffer solution containing EDTA (Figure 1, panel C), H_2O_2 (1 mM) caused a gradual and sustained increase in basal tritium efflux of electrically-evoked [^{3}H]NE overflow at S_2 (Figure 1, panel F).

As shown in Figure 2, decreasing external (buffer solution) calcium to 0.13 mM attenuated electrically-evoked [^{3}H]NE overflow by 54% when compared to controls. H_2O_2 (1 mM)

TABLE I Magnitude of hydrogen peroxide-induced enhancement of electrically-evoked [^{3}H]norepinehrine release from the bovine iris in buffer solution without calcium

Experiment	% increase from control	n
H_2O_2 (1 mM)	95.8 ± 17.9*	7
H_2O_2 (1 mM) + 0-Ca^{2+}	74.8 ● 19.7†	6

* Represents an increase in evoked NE release in the presence of buffer solution containing normal calcium (1.3 mM).
† Represents an increase in evoked NE release in the presence of buffer solution without calcium.
n = number of observations.

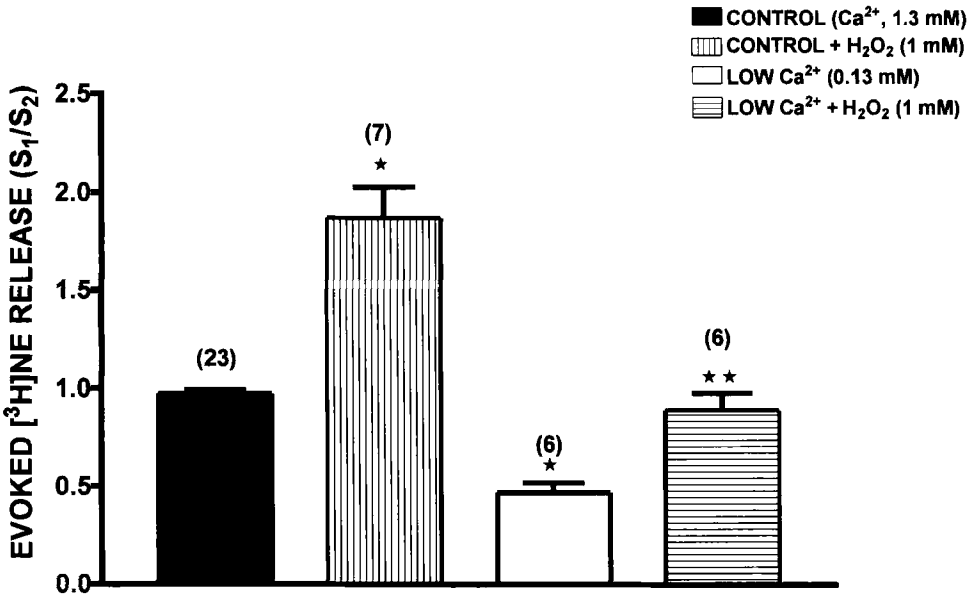

FIGURE 2 Effect of low calcium in the buffer solution on hydrogen peroxide (H_2O_2)-mediated enhancement of evoked [^{3}H]NE release from isolated, superfused bovine irides: control or in the presence of H_2O_2 (1 mM), low calcium buffer solution (LOW Ca^{2+}, 0.13 mM) and LOW Ca^{2+} buffer plus H_2O_2 (1 mM). Vertical bars represent means ± S.E.M., number of observations in brackets. *$P < 0.001$, significantly different from untreated control or **$P < 0.01$, significantly different from LOW Ca^{++} alone.

potentiated field-stimulated [^{3}H]NE release in the presence of low external calcium to the same extent as was observed in the presence of normal calcium (1.3 mM). There was no difference in the magnitude of enhancement of evoked [^{3}H]NE release caused by H_2O_2 in the presence of buffer solution containing normal calcium levels (1.3 mM) and that produced in the presence of low external calcium (0.13 mM) (Table II).

TABLE II Magnitude of hydrogen peroxide-induced enhancement of electrically-evoked [^{3}H]norepinephrine release from the bovine iris in buffer solution containing low concentrations of calcium

Experiment	% increase from control	n
H_2O_2 (1 mM)	95.8 ± 17.9*	7
H_2O_2 (1 mM) + Ca^{2+} (0.13 mM)	99.8 ± 22.9†	6

*Represents an increase in evoked NE release in the presence of buffer solution containing normal calcium (1.3 mM).
†Represents an increase in evoked NE release in the presence of buffer solution containing low calcium (0.13 mM).
n = number of observations.

The effect of H_2O_2 (0.3–1 mM) on evoked [^{3}H]NE overflow was also investigated in the presence of high external (buffer solution) calcium concentration (1.95 mM). Although the presence of high external calcium increased stimulated [^{3}H]NE release by 28%, H_2O_2 (0.3–1 mM) did not cause further enhancements of neurotransmitter release (Figure 3). Under this condition, the magnitude of peroxide-induced increase in field-stimulated [^{3}H]NE release was significantly ($P < 0.05$) attenuated in the presence of H_2O_2 (1 mM) (Table III).

To determine if calcium entry via voltage-sensitive channels are involved in H_2O_2-induced enhancement of stimulated [^{3}H]NE overflow, we examined the effect of the peroxide in the presence of ω-conotoxin (a neuronal calcium channel antagonist) and nitrendipine (a non-neuronal calcium channel blocker). ω-Conotoxin (20 nM) inhibited [^{3}H]NE release by 25% and prevented high external calcium (1.95 mM)-induced increase in neurotransmitter overflow. However, ω-conotoxin had no effect on H_2O_2

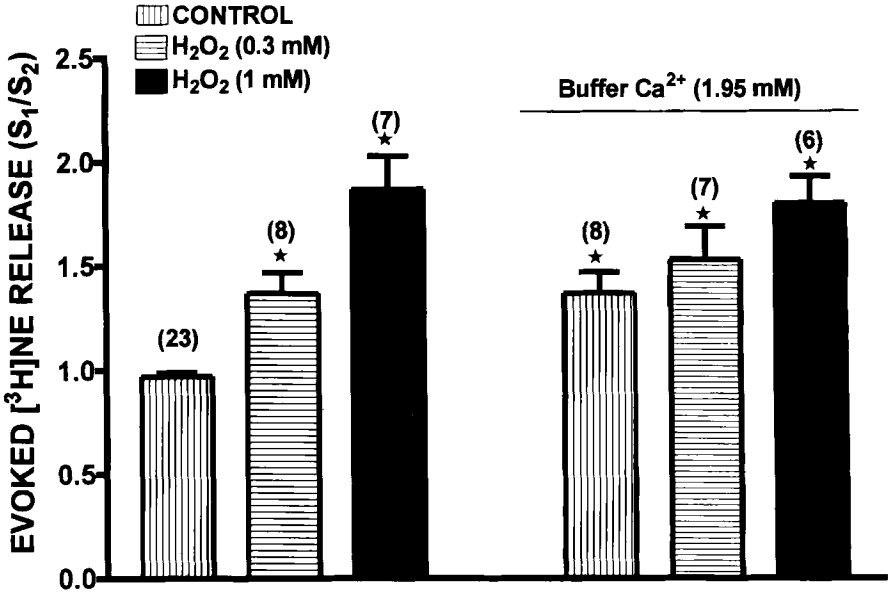

FIGURE 3 Effect of high calcium in the buffer solution on hydrogen peroxide (H_2O_2)-mediated enhancement of evoked [^{3}H]NE release from isolated, superfused bovine irides. Left panel (normal buffer calcium, 1.3 mM) and right panel (high buffer calcium, 1.95 mM): controls or in the presence of H_2O_2 (0.3–1 mM). Vertical bars represent means ± S.E.M., number of observations in brackets. *$P < 0.001$, significantly different from untreated controls.

(1 mM)-induced potentiation of evoked [^{3}H]NE release in bovine isolated irides (Figure 4). Similarly, nitrendipine (1 μM) had no effect on H_2O_2 (1 mM)-induced potentiation of evoked [^{3}H]NE release (Figure 4) nor those induced by high external calcium concentrations.

TABLE III Magnitude of hydrogen peroxide-induced enhancement of electrically-evoked [^{3}H]norepinephrine release from the bovine iris in buffer solution containing high concentration of calcium

Experiment	% increase from control	n
H_2O_2 (0.3 mM)	45.3 ± 10.6*	8
H_2O_2 (1 mM)	95.8 ± 17.9*	7
H_2O_2 (0.3 mM) + Ca^{2+} (1.95 mM)	28.4 ± 13.4†	7
H_2O_2 (1 mM) + Ca^{2+} (1.95 mM)	45.6 ± 11.0†,‡	6

*Represents an increase in evoked NE release in the presence of buffer solution containing normal calcium (1.3 mM).
†Represents an increase in evoked NE release in the presence of buffer solution containing high calcium (1.95 mM).
‡$P < 0.05$, significantly different from H_2O_2 (1 mM) alone.
n = number of observations.

DISCUSSION

Although H_2O_2 is present in tissues of the anterior uvea of the eye and in the aqueous humor at concentrations between 30 and 70 μM, higher concentrations of this oxidant have been reported in the aqueous humor of some patients with cataracts.[15,16] It is possible that the observed increases in H_2O_2 levels in the aqueous humor (as a consequence of oxidative stress) may induce oxidative damage to surrounding tissues of the anterior uvea. Indeed, there is evidence that H_2O_2 is associated with damage due to light and oxygen in the retina.[17] In the eye, both enzymes involved in free radical metabolism and

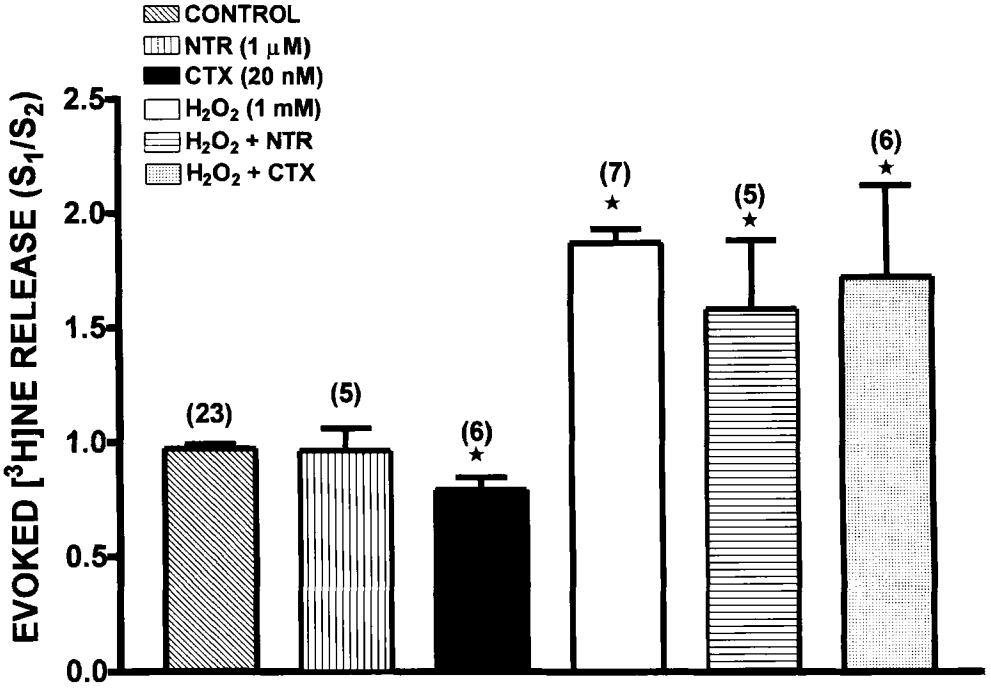

FIGURE 4 Effect of nitrendipine and ω-conotoxin on hydrogen peroxide (H_2O_2)-mediated enhancement of evoked [^{3}H]NE release from isolated, superfused bovine irides: control or in the presence of nitrendipine (NTR 1 μM), ω-conotoxin (CTX, 20 nM), H_2O_2 (1 mM), H_2O_2 plus NTR and H_2O_2 plus CTX. Vertical bars represent means ± S.E.M., number of observations in brackets. *$P < 0.001$, significantly different from untreated control.

antioxidants (such as ascorbate) have been shown to protect ocular tissues against the damaging effect of H_2O_2.[18,19] *In vivo*, injection of H_2O_2 into the anterior chamber of the eye has been reported to cause significant morphological changes in iris and ciliary body,[20,21] and increased lipid peroxidation in iris epithelial cell membranes.[22] Furthermore, intracameral administration of H_2O_2 has been shown to lower intraocular pressure in rabbits.[21] *In vitro*, we have evidence that H_2O_2 can enhance sympathetic neurotransmission in irides from several mammalian species,[11] an effect which is not dependent on the integrity of the cyclic AMP second messenger pathway.[13]

The stated aim of the present study was to determine the role of extracellular calcium in H_2O_2-induced enhancement of sympathetic neurotransmission in mammalian irides. It is well known that the release of NE from synaptic vesicles in sympathetic nerves is dependent on calcium entry into terminals via voltage activated channels of the N-type.[23] Presumably, H_2O_2-induced increases in evoked NE release from the anterior uvea could be dependent on the concentration of calcium in the extracellular compartment in the immediate vicinity of the nerves. Decreasing extracellular calcium concentration from 1.3 mM to 0.13 mM or 0 mM caused a marked reduction in field stimulated-induced overflow of [^{3}H]NE, indicating that neurotransmitter release from sympathetic nerves in the bovine iris is dependent on the concentration of calcium in the extracellular compartment. However, reducing the external (buffer solution) calcium concentration from 1.3 mM to 0.13 mM or 0 mM did not affect the magnitude of potentiation of evoked [^{3}H]NE release caused by H_2O_2 suggesting that the effect caused by the peroxide may be independent of these low levels of extracellular calcium.

We next considered the possibility that even though calcium was absent from the buffer solution, trace amounts of this cation may still be available for sympathetic neurotransmission. Addition of the chelator, EDTA to buffer solutions with no added calcium caused a further attenuation of evoked [^{3}H]NE release when compared with effects observed in zero-calcium buffer solution. In zero-calcium buffer containing EDTA, H_2O_2 caused a gradual and sustained increase in basal tritium efflux after S_2. It is unclear why H_2O_2 caused an increase in baseline efflux of [^{3}H]NE in zero-calcium buffer solution containing EDTA following electrical stimulation at S_2. It may well be that removal of trace amounts of extracellular calcium with EDTA alters intracellular calcium homeostasis to the extent where an attempt to induce exocytotic release of NE in the presence of peroxides leads to a failure of neurotransmission. It is also feasible that when trace amounts of extracellular calcium is diminished by the chelator, the nerve terminals may be rendered more susceptible to oxidative stress. A similar impairment of cumene hydroperoxide-induced increase in intracellular calcium by a zero-calcium buffer solution containing EGTA has been reported in cultured neonatal rat myocytes.[24] Trace amounts of extracellular calcium may, therefore, be required for H_2O_2-induced enhancement of sympathetic neurotransmission in the bovine iris. It is, however, unclear whether the effect observed with peroxides in the presence of EDTA is due to a direct effect of the oxidant on calcium concentration in the extracellular compartment or to an indirect action on the intracellular calcium pool.

Increasing the external (buffer solution) calcium concentration from 1.3 mM to 1.95 mM potentiated field stimulation evoked [^{3}H]NE overflow in the bovine iris. It is pertinent to note that the ability of raised extracellular calcium concentration to enhance evoked NE release mimics the effect of peroxides on sympathetic neurotransmission. We found that both low and medium concentrations of H_2O_2 did not cause further enhancements of induced [^{3}H]NE release in the presence of high external calcium concentration. It is surprising that increasing external calcium concentration did not potentiate H_2O_2 effects on sympathetic neurotransmission (as

would have been expected from a combined effect of both treatments on NE release).

Indeed, the magnitude of H_2O_2-induced potentiation of [^{3}H]NE release was reduced for both concentrations of the oxidant tested in the presence of high external calcium. These results indicate that high levels of external calcium concentration can impair peroxide-induced potentiation of sympathetic neurotransmission. The mechanism which underlies the attenuation of H_2O_2-induced enhancement of stimulated NE release in the presence of high external (buffer solution) calcium is unclear, but it may be due to the protective effect of the high concentration of extracellular calcium against peroxide-induced damage.[6,25]

Apart from the absolute levels of extracellular calcium, influx of calcium via voltage-dependent channels may affect the ability of peroxides to enhance sympathetic neurotransmission in the bovine iris. Indeed, Ebersole and Moloniff[8] showed that ascorbate-induced lipid peroxidation inhibited the binding of a calcium channel antagonist to membranes indicating that oxidative stress could lead to a modification of voltage-dependent calcium channels. In the present study, both ω-conotoxin (a blocker of specific voltage-activated N-type calcium channels) and nitrendipine (a blocker of L-type calcium channels) had no effect on H_2O_2-induced potentiation of evoked [^{3}H]NE overflow. A similar observation has been made by Ueda and Shah in renal tubular epithelial cells where verapamil had no effect on H_2O_2-induced increase in intracellular calcium and failed to protect these cells against H_2O_2-induced cytotoxicity.[26] Thus, the peroxide-induced enhancement of stimulated [^{3}H]NE in the bovine iris does not appear to depend directly on the influx of extracellular calcium via voltage-sensitive calcium channels.

We conclude that while trace amounts of extracellular calcium are necessary for H_2O_2-induced enhancement of sympathetic neurotransmission in bovine isolated irides, increasing extracellular (buffer) calcium concentration impaired peroxide-induced enhancement of [^{3}H]NE release. Furthermore, voltage-activated calcium channels are not directly involved in peroxide-induced alteration of adrenergic neurosecretion in bovine irides. It remains to be determined whether intracellular calcium homeostasis plays any role in peroxide-induced enhancement of NE release from tissues of the anterior uvea of the eye.

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