Hydrogen peroxide increases responses to nerve, phenylephrine and potassium stimulation in mouse tail artery by multiple mechanisms

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Reactive oxygen species (ROS) are believed to play an essential role in normal vascular function such as cold-induced reductions in skin blood flow. Excessive ROS production can however induce vascular dysfunction in diseases such as diabetes. Here we have investigated the role of ROS in neural activation of mouse tail artery. The mouse tail artery supplies blood to the skin of the tail and plays a role in thermoregulation similar to that of digital arteries in humans. All animals used in this study were killed by an overdose of isoflurane prior to tissue collection. Proximal segments of the mouse ventral tail artery were mounted in wire myographs and stimulated either by electrical stimulation of the perivascular sympathetic axons with 50 stimuli at 1 Hz or by contractile agents. Hydrogen peroxide (H_2O_2) was used as a source of ROS. H_2O_2 concentration dependently increased the amplitude of nerve-evoked contractions (10 μM, 127 ± 10 %; 30 μM, 176 ± 18 %; 100 μM, 277± 26 %; n = 7). Rho-Kinase has been reported to be involved in ROS-dependent increases in vascular reactivity (Bailey *et al.*, 2004). The Rho-Kinase inhibitor Y-27362 (0.5 μM) decreased nerve-evoked contraction (by 48 ± 3 %; n = 4) but did not change the facilitatory effect of H_2O_2 (H_2O_2, 297 ± 30 %, n = 8; H_2O_2 + Y-27362, 254 ± 39 %; n = 4). H_2O_2 increased the sensitivity of the artery to the α_1-adrenoceptor agonist phenylephrine (n = 4), but did not change the % blockade of nerve-evoked contraction produced by the α_1-adrenoceptor antagonist prazosin (n = 4). These findings suggest that the H_2O_2-induced increased force is due, at least in part, to a marked increase in the α_1-adrenocceptor-mediated component of nerve-evoked contraction and that this change does not involve Rho kinase.

In most experiments, H_2O_2 produced an increase in resting force that was sensitive to α-antagonists, suggesting that H_2O_2 evokes the release of noradrenaline from sympathetic nerves. To further investigate the potential role of nerve-released noradrenaline or other mechanisms in the facilitatory effects of H_2O_2, we investigated K^+-evoked contractions in the absence and in the presence of α-adrenoceptor blockade (0.1 μM prazosin + 0.1 μM idazoxan; n = 10). K^+ concentration dependently increased the force of contractions (20 mM, 0.1 ± 0.0 mN/mm; 25 mM, 1.1 ± 0.3 mN/mm; 30 mM, 3.4 ± 0.2 mN/mm; 50 mM, 4.6 ± 0.1 mN/mm). H_2O_2 increased the amplitude of contractions evoked by 20 - 30 mM K^+ (20 mM, 2.0 ± 0.4 mN/mm; 25 mM, 4.1 ± 0.2 mN/mm; 30 mM, 4.7 ± 0.2 mN/mm) but did not change contractions evoked by 50 mM K^+ (5.0 ± 0.2 mN/mm). α-Adrenoceptor blockade did not significantly change the concentration contraction curves for K^+ in the absence of H_2O_2. In contrast, α-adrenoceptor blockade markedly reduced the facilitatory effect of H_2O_2 on contractions evoked by 20 and 25 mM K^+ (20 mM, 0.5 ± 0.2 mN/mm; 25 mM, 2.7 ± 0.3 mN/mm) but did not significantly change it for contractions evoked by 30 mM K^+ (4.3 ± 0.1 mN/mm). These findings indicate that noradrenaline released from the sympathetic nerve terminals contributes to the facilitatory effect of H_2O_2 on contractions to 20 and 25 mM K^+, whereas at 30 mM K^+ the augmentation of contraction produced by H_2O_2 is primarily mediated by a non-α-adrenoceptor-mediated mechanism. In summary, these data suggest that H_2O_2 increase nerve and K^+-evoked contractions in mouse tail artery by multiple mechanisms. One of these mechanisms appears to involve ROS-induced release of noradrenaline from sympathetic nerves.

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