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Hydrogen peroxide increases nerve-evoked contractions in mouse tail artery by an endothelium-dependent mechanism

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ABSTRACT

Reactive oxygen species contribute to regulating the excitability of vascular smooth muscle. This study investigated the actions of the relatively stable reactive oxygen species, H_2O_2, on nerve-evoked contractions of mouse distal tail artery. H_2O_2 (10–100 μM) increased nerve-evoked contractions of isometrically mounted segments of tail artery. Endothelium denudation increased nerve-evoked contractions and abolished the facilitatory effect of H_2O_2. Inhibition of nitric oxide synthase with L-nitroarginine methyl ester (0.1 mM) also increased nerve-evoked contractions and reduced the late phase of H_2O_2-induced facilitation. H_2O_2-induced facilitation of nerve-evoked contractions depended, in part, on synthesis of prostanoids and was reduced by the cyclooxygenase inhibitor indomethacin (1 μM) and the thromboxane A_2 receptor antagonist SQ 29548 (1 μM). H_2O_2 increased sensitivity of nerve-evoked contractions to the α_2-adrenoceptor antagonist idazoxan (0.1 μM) but not to the α_1-adrenoceptor antagonist prazosin (10 nM). Idazoxan and the α_{2C}-adrenoceptor antagonist JP 1302 (0.5–1 μM) reduced H_2O_2-induced facilitation. H_2O_2 induced facilitation of nerve-evoked contractions was abolished by the non-selective cation channel blocker SKF-96365 (10 μM), suggesting it depends on Ca^{2+} influx. In conclusion, H_2O_2-induced increases in nerve-evoked contractions depended on an intact endothelium and were mediated by activating thromboxane A_2 receptors and by increasing the contribution of α_2-adrenoceptors to these responses.

1. Introduction

Reactive oxygen species are produced in both vascular smooth muscle and endothelial cells, and are generated by NADPH oxidases, xanthine oxidases, mitochondrial respiration and uncoupled nitric oxide synthases (Ardanaz and Pagano, 2006). The reactive oxygen species produced include unstable free radicals such as superoxide ($O_2^{\bullet -}$), and longer-lived non-free radical oxidants, such as hydrogen peroxide (H_2O_2). Because it is relatively stable, H_2O_2 has been considered to function as both an intracellular second messenger and a paracrine factor that modifies vascular contractions (Ardanaz and Pagano, 2006).

This study investigated the actions of H_2O_2 on nerve-evoked constrictions of the mouse tail artery. No studies have reported effects of H_2O_2 on neurovascular transmission, but H_2O_2 elicits vasoconstriction that depends on activity of cyclooxygenases and the generation of constrictor prostanoids that activate vascular muscle via thromboxane A_2/prostaglandin H_2 receptors (Tang and Vanhoutte, 2009). This action of H_2O_2 has been reported to be mediated either via a direct action on vascular muscle (Gao and Lee, 2005) or by triggering release of constrictor prostanoids from the endothelium (Katusic et al., 1993). Importantly, low concentrations of the thromboxane A_2/prostaglandin H_2 receptor agonist U-46619 that do not produce a contraction, increase neurovascular transmission (Vila et al., 2001). It therefore seems possible that H_2O_2 will increase nerve-evoked contractions of mouse tail artery. In addition to prostanoid-mediated contraction, H_2O_2 can contract vascular muscle by releasing Ca^{2+} from intracellular stores (Pourmahram et al., 2008) and by increasing extracellular Ca^{2+} influx (Lin et al., 2007; Shen et al., 2000).

The mouse tail artery is a thermoregulatory vessel and cooling increases its sensitivity to α_2-adrenoceptor agonists (Chotani et al., 2000). This effect of cooling on reactivity to α_2-adrencoeptor agonists depends on reactive oxygen species signalling (Bailey et al., 2005). This conclusion is based on observations that cooling stimulates mitochondrial production of reactive oxygen species and that the cold-induced increase in reactivity to the α_2-adrenoceptor agonist UK 14304 was prevented by inhibiting mitochondrial generation of reactive oxygen species (Bailey et al., 2005). This increased sensitivity to α_2-adrenoceptor agonists is suggested to contribute to cold-induced vasoconstriction by amplifying nerve-evoked contractions (Chotani et al., 2000).

The cold-induced increase in sensitivity to α_2-adrenoceptor agonists in mouse tail artery depends on activity of Rho kinase

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and recruitment of previously "silent" α_{2C}-adrenoceptors (Bailey et al., 2004; Jeyaraj et al., 2012). Rho kinase inhibition reduces H_2O_2-induced contractions of tracheal smooth muscle (Kojima et al., 2007) and pulmonary arteries (Pourmahram et al., 2008). Therefore activation of Rho kinase by H_2O_2 may contribute to modifying nerve-evoked contractions. In pulmonary arteries, H_2O_2-induced contractions were also reduced by inhibition of protein kinase C (Pourmahram et al., 2008).

The present study tested the hypothesis that H_2O_2 increases nerve-evoked contractions of mouse tail artery and demonstrated that this was the case. Further studies investigated involvement of the endothelium in this facilitatory effect of H_2O_2 and whether it involved the production of prostanoids. In addition, we investigated whether H_2O_2 increased the contribution of α_2-adrenoceptors to neural activation of vascular muscle and whether the augmentation of nerve-evoked contractions depends on Ca^{2+} influx and on the activity of Rho kinase or protein kinase C.

2. Materials and methods

2.1. Animals and tissue preparation

All procedures conformed to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the animal ethics committee at the University of Melbourne. Male C57Bl/6 mice aged 9–13 weeks were obtained from the Animal Resource Centre (Perth, Australia). Mice were deeply anaesthetised with isoflurane and then killed by cervical dislocation. Segments of ventral tail artery ~1.75 mm in length were dissected from 60 to 80 mm along the tail. Vessels were maintained in physiological saline solution containing (mM); NaCl, 133; KCl, 4.7; $CaCl_2$, 2.0; $MgCl_2$, 1.2; $NaH_2PO_4H_2O$, 1.3; $NaHCO_3$, 16.3; glucose, 7.8; ethylenediamine tetraacetic acid, 0.02. This solution was bubbled with 95% O_2/5% CO_2 and heated to ~36.5 °C.

2.2. Drugs

Phenylephrine HCl, carbachol (carbamoylcholine chloride), prazosin HCl, nifedipine, L-NAME (L-nitroarginine methyl ester), indomethacin, sodium nitroprusside and polyethylene glycol-catalase were obtained from Sigma-Aldrich Chemical Company (Castle Hill, NSW), UK 14304 (5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline), Y-27632 (*trans*-4-[(1*R*)-1-aminoethyl]-*N*-4-pyridinylcyclohexanecarboxamide dihydrochloride), JP 1302 (*N*-[4-(4-methyl-1-piperazinyl) phenyl]-9-acridinamine dihydrochloride) and NF449 (4,4′,4″,4‴-[carbonylbis(imino-5,1,3-benzenetriyl-*bis*(carbonylimino))]*tetrakis*-1,3-benzenedisulfonic acid, octasodium salt) were obtained from Tocris Bioscience (Bristol, UK), SQ 29548 ([1*S*-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) and U-46619 (9.11-dideoxy-9α,11α-methanoepoxy-prosta-5Z,13E-dien-1-oic acid) were obtained from Cayman Chemical Company (Ann Arbor, USA), H_2O_2 was obtained from Merck Pty Ltd (Kilsyth, Vic, Australia), and tetrodotoxin was obtained from Alamone (Jerusalem, Israel). Prazosin was prepared as a 1 mM stock in 10% (v/v) dimethylsulphoxide (Chem-Supply Pty Ltd, Port Adelaide, SA, Australia) in water. Nifedipine, indomethacin and UK 14304 were prepared as 10 mM stock solutions in ethanol (final working concentration of ethanol $\leq$0.1% (v/v)). SQ 29548 was prepared as a 10 mM stock in dimethylsulphoxide (final working concentration of dimethylsulphoxide was 0.01% (v/v)). U-46619 was purchased as a 28.5 mM stock solution in methyl acetate (final working concentration of methyl acetate was 0.003% (v/v)). All other drugs were made up as $\geq$1 mM stock solutions in water.

2.3. Mechanical responses

Artery segments were mounted isometrically between two stainless-steel wires (40 μm diameter) in a four-chamber myograph (Multi Myograph Model 610M, Danish Myo Technology, Aarhus, Denmark). To normalize the basal conditions, Laplace's equation was used to convert the measured force to the effective transmural pressure exerted on the vessel wall (Mulvany and Halpern, 1977). Initially the arteries were stretched in small steps until the calculated transmural pressure was 13.3 kN/m^2 (100 mmHg), and then lumen circumference measured at this pressure was reduced by 10% (Mulvany and Halpern, 1977). Under these conditions the lumen circumference was 0.74 ± 0.01 mm. The arteries were allowed to equilibrate for 40 min at which time the effective transmural pressure was ~4.6 kN/m^2 (~35 mmHg). All vessels were then stimulated with 2 applications of phenylephrine (2 μM) and when the second contraction had plateaued, carbachol was applied (1 μM) to determine if the endothelium was intact (defined as relaxation to carbachol $>$70%). In some experiments, the endothelium was removed by rubbing the lumen surface of the vessels with a human hair and the success of this procedure was defined as relaxation to carbachol $<$10%. In all experiments, test and control assessments were made in parallel using tissues obtained from the same animal.

2.4. Electrical stimulation

Electrical stimuli were applied through platinum plate electrodes mounted on either side of the artery along its length. The stimulus pulse width was 0.2 ms and the voltage was set at 120% of the minimum voltage required for a maximal contraction to 50 pulses at 3 Hz (typically 12 V). At the end of all nerve stimulation experiments it was confirmed that α-adrenoceptor blockade (with 0.01μM prazosin + 0.1 μM idazoxan) or tetrodotoxin (0.5 μM) completely abolished electrically evoked contractions (establishing that the electrical stimuli did not directly activate the muscle). In rats, postganglionic sympathetic neurons supplying the tail artery typically discharge action potentials at $<$1 Hz, with the level of activity increasing maximally up to about 2 Hz when the body core temperature is lowered (Ootsuka et al., 2004). For this reason we chose to study contractions evoked by trains of stimuli at 1 Hz.

In the experiments investigating the effects of H_2O_2 and other drugs on nerve-evoked contractions, the arteries were stimulated with trains of 50 stimuli at 1 Hz delivered at 8 min intervals. The first series of experiments assessed the concentration dependence of the H_2O_2-induced increase in nerve-evoked contractions. After 2 control responses, H_2O_2 was added cumulatively at increasing concentrations (10, 30 and 100 μM), with each concentration present for 4 contractions. Because the rates of membrane permeation are comparable to the rates at which H_2O_2 is degraded by peroxidases and catalases, the intracellular concentration would be expected to be maximally about 10% of that applied (Antunes and Cadenas, 2000). In all subsequent experiments, 100 μM H_2O_2 was used because this concentration robustly increased nerve-evoked contractions. Unless otherwise stated, the experiments investigating the effects of various drugs on the H_2O_2-induced increase in nerve-evoked contractions consisted of a series of 8 contractions; with 2 under control conditions followed by 2 in the absence (control) or in the presence of a drug and then 4 in the presence of H_2O_2. The effect of the drug on nerve-evoked contraction was determined at the second response in its presence (i.e., after ~16 min application) and is expressed as a percentage of the contraction immediately prior to the application of the drug. Comparisons were made with measures