INTRODUCTION

Hydrogen peroxide is produced in many different human cell types, including fibroblast, vascular endothelial, smooth muscle, and inflammatory cells (1). It is known to act as a cellular signaling molecule within blood vessels, and it plays key roles in regulating vascular smooth muscle cell (VSMC) growth, differentiation, migration, and vascular inflammation (1-5). Studies have demonstrated the importance of \( \text{H}_2\text{O}_2 \) in regulating vascular tone, but its role is not well understood since it can modify vascular tone in complex ways. Thus, studies have demonstrated both a contractile and relaxant response to \( \text{H}_2\text{O}_2 \) depending on the species, vascular bed, and contractile state (2, 4, 6, 7). However, the effects of \( \text{H}_2\text{O}_2 \) on the complete renal vasculature are not known. \( \text{H}_2\text{O}_2 \) has been shown to cause constriction in a variety of vascular beds under quiescent conditions, and it can induce vasoconstriction in a number of arteries \textit{in vitro}, including rat aorta (8, 9), vena cava (7, 10) and pulmonary artery (11), canine basilar artery (12), and human placental arteries (13). Several mechanisms contribute to \( \text{H}_2\text{O}_2 \)-induced vasoconstriction in these vessels, including: increase in \( \text{Ca}^{2+} \) influx or \( \text{Ca}^{2+} \) release from intracellular stores in smooth muscle cells (14, 15); formation of cyclooxygenase-derived prostanoids (8, 16); activation of enzymes such as phospholipase A\(_2\) (17), phospholipase C (18), tyrosine kinases (10) and protein kinase C (19); activation of potassium (\( \text{K}^+ \)) channels (7); and generation of hydroxyl radicals (9).

Increased production of free radicals is closely associated with an enhanced arteriolar tone (20, 21), which is in turn associated with an elevation in blood pressure. Thus, Swei et al. (20) reported a correlation between arteriolar tone and plasma hydrogen peroxide levels in hypertensive rats. In mice, vascular overexpression of catalase reduced the pressor response to vasoconstrictor agents (22) and decreased systolic blood pressure and vascular constriction \textit{per se}, indicating the importance of endogenous \( \text{H}_2\text{O}_2 \) as a vasoconstrictor and regulator of blood pressure (23).

The role of hydrogen peroxide in regulating vascular tone would be especially relevant in the kidney, since this organ receives 20% of cardiac output, and renal hemodynamics play an essential role in the control of renal sodium excretion and blood pressure (24). Thus, Chen et al. (25) demonstrated that the short-term administration of \( \text{H}_2\text{O}_2 \) into the renal medulla significantly reduces renal medullary blood flow and sodium excretion, and Makino et al. (26) found that increased renal medullary \( \text{H}_2\text{O}_2 \) leads to hypertension.

Taking account of the above findings on the importance of the tone of the renal vascular bed in the regulation of total...
peripheral resistance, renal sodium excretion, and blood pressure, the present experiments were designed to test the hypothesis that \( \text{H}_2\text{O}_2 \) may play a significant role in the control of renal vascular tone. To this end, the ability of \( \text{H}_2\text{O}_2 \) to contract the complete renal vasculature in the isolated perfused rat kidney was evaluated, studying the main mechanisms responsible for this effect. Gender-associated differences in the renal responsiveness to \( \text{H}_2\text{O}_2 \) were also investigated.

MATERIALS AND METHODS

Animals and isolated perfused kidney preparation

This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No 85-23, revised 1996). Ninety-six male (300±10 g) and six female Wistar age matched rats (250±6 g) were maintained on standard chow and tap water ad libitum. The isolated perfused kidney preparation was performed following a previously reported procedure (27, 28). The animals were anesthetized with pentobarbital sodium (40 mg/kg, i.p.), the abdomen was opened through a midline incision, and the renal arteries were exposed and cannulated with a beveled 18-gauge needle and secured with ligatures. The kidneys were perfused at a constant flow rate (5 ml/g of kidney weight per min) by means of a roller pump (IPS-4, Ismatec S.A., Zurich) with Tyrode solution (37°C) of the following composition (mM): NaCl, 137; KCl, 2.7; CaCl\text{\textsubscript{2}}, 1.8; MgCl\text{\textsubscript{2}}, 1.1; NaHCO\text{\textsubscript{3}}, 12.0; NaH\text{\textsubscript{2}}PO\text{\textsubscript{4}}, 0.42; and D(+) glucose, 5.6, all aerated with 5% \( \text{CO}_2 \) in \( \text{O}_2 \). The kidney was then dissected clear from its surrounding tissues and placed in a chamber containing the Tyrode solution at 37°C. Renal vascular responses were recorded (TRA-021 transducer connected to a two-channel Letigraph 2000 recorder, Letica S.A., Barcelona) as changes in the renal perfusion pressure downstream from the pump.

Experimental protocol

The experiments evaluated the renal response to \( \text{H}_2\text{O}_2 \) (2.2 to 22 \( \times 10^{-5} \) mol/l) under the following conditions (n=6, each group): 1) no treatment; 2) after administration of catalase (1200 U/ml) to evaluate the specificity of the vasoconstrictor effect; 3) after administration of DMSO/mannitol (7/3 10\textsuperscript{-3} mol/l) as hydroxyl radical scavenger; 4) after endothelium removal; 5) after L-NAME (10\textsuperscript{-4} mol/l) pretreatment to inhibit NO synthesis; 6) after L-NAME (10\textsuperscript{-3} mol/l) pretreatment with tetraethylammonium (3\texttimes10\textsuperscript{-3} mol/l) as non-specific K\textsuperscript{+} channel inhibitor; 7) without calcium plus EGT\textsubscript{A} to evaluate the role of intracellular calcium; 8) after verapamil (10\textsuperscript{-5} mol/l) to evaluate the role of extracellular calcium; 9) without calcium plus four successive bolus doses (10\textsuperscript{-5} g/g kidney) of \( \text{H}_2\text{O}_2 \) to determine \( \text{H}_2\text{O}_2 \) needed to induce near-maximal constriction; 10) after administration of nitroprusside (10\textsuperscript{-4} mol/l), papaverine (10\textsuperscript{-4} mol/l), and diazoxide (10\textsuperscript{-4} mol/l), using these three drugs to decrease the sensitivity to calcium and reduce the contractility; and 11) after this last treatment (condition 16) plus chelerythrine.

The endothelium was removed by passing air through the isolated kidney for 4.5 min. Endothelium removal was assessed by measuring the vasodilator response to a bolus dose of 10\textsuperscript{-6} g/g kidney acetylcholine (ACH) in the vascular bed after its constriction with phenylephrine (10\textsuperscript{-4} mol/l). Preparations with a vasodilator response to ACh of >10% were rejected. The above inhibitors were added to the perfusate after the stabilization period, followed by a 30-min interval before dose-response curves were recorded. The inhibitors were present throughout the experiment. Dose-response curves were constructed by injecting boluses of 50 \( \mu \)l/g kidney of the agonist. Each injection produced a small and transient increase in renal perfusion pressure (RPP) that preceded the agonist-induced response. The minimum time interval between successive doses of an agonist was 10 min, which could be extended when necessary until the previous response had disappeared. Dose-response curves in the presence of the inhibitors were compared with the respective dose-response curves under normal conditions. The peak response was used to construct these curves. Dose-response curves were performed in untreated kidneys throughout the experimental period. From these curves, we randomly took n=6 for statistical purposes as control values. The responsiveness to \( \text{H}_2\text{O}_2 \) was also determined in isolated kidneys from female rats in order to explore any gender-related differences in the vasoconstriction induced by this agent.

Drugs

The following drugs were used: l-phenylephrine hydrochloride, acetylcarnitine, catalase, dimethyl sulfoxide (DMSO), \( \text{H}_2\text{O}_2 \), indomethacin, mannnitol, tetraethylammonium chloride (TEA), diazoxide, L-phenyllephrine hydrochloride, N\textsuperscript{-}nitro-L-arginine methyl ester (L-NAME), ethyleneglycol-bis (\( \beta \)-aminoethyl ether)N\textsuperscript{1},N\textsuperscript{3}-tetraacetic acid (EGTA), acetyl methyl ester of bis (\( \alpha \)-aminophenoxy)ethane-N\textsuperscript{1},N\textsuperscript{3},N\textsuperscript{5},N\textsuperscript{7}-tetraacetic acid (BAPTA-AM), genistein, chelerythrine, sodium nitroprusside, papaverine hydrochloride, and verapamil hydrochloride, purchased from Sigma-Aldrich Quimica S.A. (Alcobendas, Madrid, Spain); pentobarbital sodium, purchased from Serva (Heidelberg, Germany); and ODYA, from Cayman Chemical (Ann Arbor, MI, USA). The drugs were prepared daily in deionized water from stock solutions kept at 20°C. Stock solutions were prepared in water, with the exception of indomethacin, which was prepared in absolute ethanol and BAPTA-AM in NaHCO\text{\textsubscript{3}} (0.3N). \( \text{H}_2\text{O}_2 \) was purchased as 30% w/w concentrated solution and subsequently diluted in distilled water.

Statistical analysis

Dose-response curves in the presence or absence of the inhibitors were compared using a three-factor random block design (rat, dose, and treatment). When the results of the analysis of variance were significant, the Tukey’s t and Neumann-Keuls tests were applied (29).

RESULTS

The baseline RPP value was 42±2 mmHg (n=96). Baseline RPP values were not significantly modified by any treatment with the exception of endothelium removal, which was followed by a transient elevation of vascular tone that returned to baseline values within 15 min. The control dose-response curve to \( \text{H}_2\text{O}_2 \) is the same in the different Figures.

\( \text{H}_2\text{O}_2 \) induction of vasocontriction in the isolated perfused kidney and influence of sex

Fig. 1A shows that \( \text{H}_2\text{O}_2 \) elicited a concentration-dependent vasoconstriction of isolated rat kidney. The concentration of \( \text{H}_2\text{O}_2 \) needed to induce near-maximal constriction was 22\texttimes10\textsuperscript{-5} mol/l.
The maximal peak response to H2O2 was 74±5% of the peak response to phenylephrine.

A sexual dimorphic pattern of response to H2O2 was observed. Renal vasculature from female rats showed markedly reduced responsiveness to H2O2 (Fig. 1B). The concentration-response curve was characterized by a shift towards the right, with decreased responses to medium concentrations and a similar maximal response.

Effect of catalase and of hydroxyl radical scavengers on the renal response to H2O2

The treatment of isolated kidneys with 1200 u/ml catalase, which decomposes H2O2, produced an almost complete disappearance of the pressor response to H2O2 (Fig. 2A). Experiments were carried out in the presence of DMSO plus mannitol (hydroxyl radical scavengers) to examine the possible role of hydroxyl radical formation in the vasoconstriction effects of H2O2. These effects on the renal vascular bed were not affected by administration of DMSO plus mannitol (Fig. 2A), suggesting that the contractile response to H2O2 is not associated with the production of hydroxyl radicals.

Effects of endothelium removal, nitric oxide blockade, and K⁺-channel blockade on the renal response to H2O2

Analysis of the dose-response curves showed a significantly greater vasoconstriction at medium doses in kidneys with endothelium removed than in those with intact endothelium. Administration of the NO inhibitor L-NAME did not significantly modify the dose-response curve to H2O2, whereas administration of the non-specific K⁺-channel inhibitor tetraethylammonium produced a significant shift to the left in the dose-response curve to H2O2 (Fig. 2B).

Effects of indomethacin and CYP-450 inhibition on H2O2-induced vasoconstriction in the isolated kidney

Administration of the prostaglandin inhibitor indomethacin did not modify the dose-response curves to H2O2 in the isolated kidneys (Fig. 2C). The effects of the P450 cytochrome inhibitor ODYA were tested to investigate the possible involvement of the metabolites of arachidonic acid generated by cytochrome P450-dependent enzymes. As shown by Fig. 5, the presence of ODYA had no effect on the vasoconstrictor response to any of the tested concentrations of H2O2 (Fig. 2C).

Involvement of Ca⁺⁺ in H2O2-induced vasoconstriction of rat kidney

Results obtained demonstrated that extracellular Ca⁺⁺ is required for the vasoconstriction produced by increasing concentrations of H2O2. The response of isolated kidney to H2O2 was assessed after equilibrating the renal vasculature in Ca²⁺-free Tyrode solution containing 2.10⁻⁴ mol/l EGTA for at least 30 min before the experiments (Fig. 3A) and after calcium channel blockade with verapamil (Fig. 3B). Removal of Ca²⁺ from the extracellular medium and verapamil administration both had an appreciable inhibitory effect on the vasoconstriction caused by all tested concentrations of H2O₂.

For the intracellular Ca²⁺-buffered experiment, the membrane-permeable Ca²⁺ chelator BAPTA was added to the medium in the presence of verapamil. The role of intracellular Ca²⁺ stores was also analyzed by administering four doses of phenylephrine (10⁻⁵ g/g kidney), which led to the disappearance of the response to this agonist. The pressor response to H2O₂ in the isolated kidney was not significantly modified by intracellular chelation or by store depletion (Fig. 3A and B), and intracellular chelation with BAPTA did not significantly modify the inhibitory effect produced by verapamil, suggesting that intracellular Ca²⁺ does not play a role in the H2O₂-induced vasoconstriction observed.

Role of PKC and protein tyrosine phosphorylation in H2O₂-induced vasoconstriction in the isolated kidney

The protein tyrosine kinase inhibitor genistein were tested to investigate the involvement of PKC and/or protein tyrosine phosphorylation in the H2O₂-induced vasoconstriction observed. Contractile responses of the arteries to H2O₂ were suppressed by
Fig. 2. (A) Effects of the administration of catalase (1200 U/ml) and hydroxyl radical scavengers DMSO/mannitol (7/3 10⁻³ mol/l), (B) Effects of endothelium removal, L-NAME (10⁻⁴ mol/l), or TEA (3·10⁻³ mol/l) and (C) Effects of indomethacin (10⁻⁵ mol/l) and ODYA (2·10⁻⁶ mmol/l) on the renal response to increasing bolus doses of H₂O₂. Data are expressed as means±SEM (n=6 in each group). Doses of H₂O₂ are expressed per gram of kidney. * P<0.05; ** P<0.001 vs. normal conditions. Data are expressed as means±SEM (n=6 in each group).

Fig. 3. (A). Effects of calcium-free Thyrode plus EGTA (5·10⁻⁴ mmol/l), calcium-free Thyrode plus BAPTA (10⁻⁵ mol/l), and calcium-free Thyrode plus 4 successive bolus doses (10⁻³ g/g kidney) of phenylephrine. (B) Effects of verapamil (10⁻⁵ mol/l) and of verapamil plus BAPTA (10⁻⁵ mol/l) and (C) Effects of chelerythrine (10⁻⁵ mol/l), genistein (10⁻⁵ mmol/l), calcium-free Thyrode plus calcium desensitizing agents (nitroprusside, 10⁻⁴ mol/l; papaverine, 10⁻⁴ mol/l; and diazoxide, 10⁻⁴ mol/l) and calcium-free Thyrode plus chelerythrine plus calcium-desensitizing agents on the renal response to increasing bolus doses of H₂O₂. * P<0.01; ** P<0.001 vs. normal conditions. Data are expressed as means±SEM (n=6 in each group). Doses of H₂O₂ are expressed per gram of kidney.
treatment of the renal vascular bed with chelerythrine but were not modified by genistein treatment (Fig. 3C). These data strongly suggest that PKC activation but not protein tyrosine phosphorylation is an important step in H2O2-induced vasoconstriction in the isolated kidney.

Influence of calcium-desensitizing agents on H2O2-induced vasoconstriction of isolated rat kidney.

To analyze the influence of H2O2 on calcium sensitivity, the response to this agent was studied in the presence of a cocktail of calcium-desensitizing agents composed of papaverine (PKA activator), nitroprusside (PKG activator) and diazoxide (K+ channel activator) in the absence of extracellular calcium. The mixture of desensitizing agents produced an important attenuation in the dose response curve to H2O2 (Fig. 3C). Moreover, the addition of chelerythrine to these agents in the calcium-free solution virtually abolished the pressor response to H2O2 in the isolated kidney (Fig. 3C).

Table 1 summarizes the differences in renal perfusion pressure attained by the highest dose of H2O2 under normal conditions and after the administration of the different inhibitors.

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Maximal response in RPP (mmHg)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>174.2±8.2</td>
</tr>
<tr>
<td>Cabalase (1200 U/ml)</td>
<td>9.0±3.7**</td>
</tr>
<tr>
<td>DMSO/Mannitol (7/3·10−3 mol/l)</td>
<td>146.9±16.4</td>
</tr>
<tr>
<td>-END</td>
<td>184.0±2.1</td>
</tr>
<tr>
<td>L- NAME (10−4 mol/l)</td>
<td>155.7±9.7</td>
</tr>
<tr>
<td>TEA (3·10−3 mol/l)</td>
<td>180.0±5.1</td>
</tr>
<tr>
<td>Indomethacin (10−5 mol/l)</td>
<td>170.6±9.2</td>
</tr>
<tr>
<td>ODYA (2·10−6 mol/l)</td>
<td>171.5±14.6</td>
</tr>
<tr>
<td>-Ca2+EGTA (5·10−4 mol/l)</td>
<td>139.6±5.5*</td>
</tr>
<tr>
<td>-Ca2+BAPTA (10−5 mol/l)</td>
<td>140.0±6.0*</td>
</tr>
<tr>
<td>-Ca2+Bolus Phe (10−5 g/g kidney)</td>
<td>125.4±10.2*</td>
</tr>
<tr>
<td>Verapamil (10−5 mol/l)</td>
<td>121.0±3.7*</td>
</tr>
<tr>
<td>Verapamil (10−5 mol/l)+ BAPTA (10−5 mol/l)</td>
<td>112.7±7.1*</td>
</tr>
<tr>
<td>-Ca2+NPS (10−4 mol/l)+PV (10−3 mol/l)+Diazoxide (10−4 mol/l)</td>
<td>105.3±15.0*</td>
</tr>
<tr>
<td>-Ca2+NPS (10−4 mol/l)+PV (10−3 mol/l)+Diazoxide (10−4 mol/l)+Chelerythrine (10−5 mol/l)</td>
<td>25.5±8.0**</td>
</tr>
<tr>
<td>Chelerythrine (10−5 mol/l)</td>
<td>133.6±10.4*</td>
</tr>
<tr>
<td>Genistein (10−5 mol/l)</td>
<td>169.8±8.7</td>
</tr>
</tbody>
</table>

Data are mean±SEM; * P<0.05; ** P<0.01 vs. controls.

DISCUSSION

H2O2 is known to modify the vascular tone of various preparations, but no data have been published on the effect of H2O2 on the complete renal vascular bed. This study aimed to define the effects of H2O2 as a regulator of vascular tone in the renal vasculature and to gain insight into the mechanisms of H2O2-induced vessel contractility. The isolated perfused rat kidney was selected for the experiments because it comprises the complete renal vasculature including small resistance arteries and arterioles, which are physiologically more relevant to the control of vascular resistance. Results obtained demonstrate that H2O2 produces a concentration-dependent vasoconstriction in the isolated rat kidney. The maximal vasoconstrictor response was around 74% of that obtained with phenylephrine but lasted longer (180% increase in duration). Thakali et al. (30) also observed that the aortic and venous contraction produced by H2O2 was lower than the maximal contraction induced by an adrenergic agonist.

The concentration of hydrogen peroxide used in this and other studies (7-9), which resulted in contraction, could conceivably occur in vivo, especially under certain pathophysiological conditions. H2O2 levels can reach 0.8 mmol/l in rat venular endothelium during neutrophil activation (31), and vascular H2O2 levels in the µmolar range can be found in the plasma of hypertensive patients (32, 33). Antihypertensive therapy is accompanied not only by a decrease in blood pressure but also by a fall in plasma H2O2 levels (33). Moreover, an increased vasoconstrictor response to H2O2 has been reported in genetic (8) and secondary (7) forms of hypertension. Considered alongside the above findings, the present observation that H2O2 is a potent vasoconstrictor in the renal vasculature suggests that H2O2 generated in the vascular wall can modulate renal vascular tone and contribute to the pathogenesis of various vascular diseases. This may be of special importance in hypertension, due to the key role of renal hemodynamics in this disease (24).

Experimental evidence suggests that oxidative stress is greater in the male sex (34). The increased plasma H2O2 levels in hypertensive patients are lower in women than in men (33), and female SHR showed reduced urinary excretion of H2O2 in comparison to male SHR (35). Female sex is also associated with decreased renal vascular reactivity to vasoconstrictors (27). We found a lower pressor responsiveness to H2O2 in the kidneys from female rats than in those from male rats. This reduced...
responsiveness to H2O2 and its lower production might contribute to the reduced blood pressure of female animals. With respect to the mechanism by which H2O2-induced renal vasoconstriction, research into the role of Ca2+ has yielded controversial findings in different preparations. Thus, H2O2-induced contraction was independent of extracellular Ca2+ influx in pulmonary arteries (11, 36) but dependent on extracellular Ca2+ influx and release of intracellular Ca2+ in canine basilar arteries and rat thoracic aorta (15). Thakali et al. (7) observed that aortic H2O2-induced contraction requires extracellular Ca2+ influx but venous H2O2-induced contraction does not. These studies indicate possible species-specific, experimental condition-specific, and vessel-specific differences in the role of extracellular influx of Ca2+ and release of calcium from intracellular stores in H2O2-induced contraction. The present study demonstrated that removal of extracellular Ca2+ significantly depressed the constrictor effects of H2O2 on renal vessels. These contractile responses to H2O2 were not significantly modified when the intracellular Ca2+ in smooth muscle cells was buffered by 10^-4 mol/l BAPTA, a membrane-permeable Ca2+ chelator, or when the internal stores of calcium were depleted by the administration of successive boluses of phloretin. Therefore, our data indicate that extracellular Ca2+ but not Ca2+ release from intracellular stores is required for H2O2-induced vessel constrictions in the isolated kidney.

Oxidants such as H2O2 are known to participate in PKC mobilization in vascular smooth muscle cell membranes (37). PKC is involved in the signaling pathways by which vasoconstrictor stimuli induce activation of vascular smooth muscle. PKC may increase the myofilament force sensitivity to (Ca2+) and myosin light-chain kinase phosphorylation, thereby maintaining vascular contraction (38). In the present study, the PKC inhibitor chelerythrine significantly attenuated the H2O2-induced vasoconstriction of rat renal vasculature, indicating the involvement of PKC in this effect of H2O2. These data are consistent with reports that PKC activation plays an essential role in the development of contractile responses in aortic rings (9) and endothelial cells (39).

Treatment of vascular smooth muscle cells with oxidants, including H2O2, has been reported to enhance protein tyrosine phosphorylation (37). The present results demonstrate that the protein tyrosine kinase genistein did not significantly modify the H2O2-produced constriction of rat renal vasculature, suggesting that protein tyrosine phosphorylation is unlikely to be involved in this vasoconstriction. These findings contrast with a report by Yang et al. (9) that genistein significantly suppressed the H2O2-induced contraction of rat aortic rings. Intrarenal microcirculation is under hormonal, paracrine and neural control, and renal vascular tone results of the interaction of vasoconstrictors and vasodilators (40, 41). In this balance is specially important the contribution of three major members of the vasodilator family: nitric oxide, prostaglandins (40) and epoxides (41), CYP-450 dependent arachidonic acid (AA) metabolites, identified with the endothelium-derived hyperpolarizing factor (EDHF), all released from endothelial cells, to the buffering of the intrarenal vasoconstrictor influences (28). We found a shift to the left of the dose-response curve to H2O2 in endothelium-denuded kidneys with respect to intact kidneys, indicating that the H2O2-elicted vasoconstriction is negatively modulated by endothelium, as previously reported in rat aorta (8, 9). However, the dose-response curve to H2O2 in the renal vasculature was not significantly affected by nitric oxide (NO) blockade. Hence, the negative modulatory role of NO found in the responsiveness to other vasoconstrictors in the isolated kidney (27, 28) is not observed in the vasoconstrictive response to H2O2 possibly because H2O2 can impair NO-mediated signaling in blood vessels (42), stimulate NAD(P)H oxidase in vascular cells (43), reduce levels of tetrahydrobipterin (44), and increase expression of arginase I (45). Moreover, H2O2 decreases NO production by inactivating eNOS cofactors without affecting eNOS activity (46). L-NAME may be ineffective due to these potential anti-NO effects of H2O2, therefore the increased responsiveness to H2O2 produced by endothelium removal would be due to blockade of the endothelium-derived hyperpolarizing factor. In fact, H2O2-induced renal vasoconstriction was significantly enhanced when K+ channels were inactivated with tetraethylammonium in the present study, in agreement with reports that K+ channel activity plays an important role in the vasoconstrictor response to H2O2. Thus, Thakali et al. (7) observed that aorta under quiescent conditions contracted minimally to exogenous H2O2 and that aorta depolarization, which induces K+ channel blockade, potentiated aortic contraction to H2O2; Ardanaz et al. (2) obtained similar results in the abdominal aorta and superior mesenteric artery. K+ channel blockade did not enhance renal H2O2 vasoconstriction to the same degree as reported in aortic tissue (7). These inter-tissue differences in H2O2-responsiveness have been attributed to differences in K+ channel expression (7). Thus, rat aorta expressed low levels of KATP channel mRNA, whereas the vena cava, which is almost unresponsive to K+ channel blockade, had no detectable KATP channel mRNA (47). H2O2 has been shown to induce indomethacin-sensitive contractions in pial arterioles from newborn pigs (48), in strips of guinea-pig trachea (49), and in rat aortic tissue (8, 9). In our experiment, H2O2-induced vasoconstriction of the renal vessels was not significantly affected in the presence of indomethacin, indicating that the contractile effects of H2O2 in the renal vascular bed are not mediated or modulated by an increased metabolism of arachidonic acid via the cyclooxygenase pathway. Previous results in rat aorta suggested a role for the release of cytochrome P450-dependent metabolites in response to H2O2 (9), and the administration of probenecid (an inhibitor of cytochrome P450 monoxygenase inhibitor) markedly potentiated H2O2-induced aortic contractions in rat. However, the administration of ODYA (cytochrome P450 monoxygenase inhibitor) in the present study did not significantly modify the renal response to H2O2, suggesting that CYP-450 derivatives do not play a role.

Several authors have suggested that the generation of hydroxyl radicals contributes to the vascular smooth muscle contraction induced by H2O2 (50), and the hydroxyl radical scavengers deferoxamine and DMSO significantly attenuated the contractile response to H2O2 in aortic rings (9). However, the vasoconstrictor effect of H2O2 in the renal vascular bed was not modified by the association of the radical scavengers DMSO and mannitol, suggesting that the formation of hydroxyl radicals plays no part in this effect. Similar results have been reported in pial arterioles of newborn pigs using deferoxamine (48), and in the aortic tissue using DMSO (40) or DMSO plus mannitol (6).

In summary, this study demonstrates that hydrogen peroxide can induce vasoconstriction in the vasculature of isolated perfused rat kidney. Our results suggest that the vasoconstrictor response to H2O2 in the rat renal vasculature comprises the following components: 1) extracellular calcium influx, 2) PKC activation, and 3) stimulation of pathways leading to the sensitization of contractile elements to calcium. Our data also indicate that no role is played by calcium release from intracellular stores, prostaglandins, CYP-450 products, or tyrosine phosphorylated products in the vasoconstrictor response to H2O2 in the rat renal vascular bed.

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