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OXIDANT STRESS AND GLOMERULAR PROSTANOID PRODUCTION: INFLUENCE OF ANGIOTENSIN CONVERTING ENZYME INHIBITION

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1. The effect of H_2O_2 ($4.7 \times 10^{-9} - 4.7 \times 10^{-3}$ M) on prostanoid production by isolated glomeruli from normotensive (WKY) and, spontaneously hypertensive rats (SHR) has been studied.
2. Oxidant stress significantly increased synthesis of prostaglandin E_2 (PGE_2), I_2 (PGI_2) and thromboxane A_2 (TxA_2) by glomeruli from both strains whereas the ratio ($PGE_2 + PGI_2$)/TxA_2 increased in only SHR.
3. Pre-incubation of glomeruli with the angiotensin converting enzyme inhibitors captopril or lisinopril, had virtually no effect on H_2O_2-induced synthesis of individual prostanoids nor on the ratio ($PGE_2 + PGI_2$)/TxA_2 by glomeruli from either WKY or SHR.
4. The findings suggest that H_2O_2-induced changes in glomerular function may be mediated, in part, by PGs but fail to support the suggestion that the ability of ACEI to protect glomeruli from H_2O_2-induced damage is determined by PGs.

KEY WORDS: Reactive oxygen species, glomerulus, prostaglandins, angiotensin converting enzyme inhibitors.

INTRODUCTION

Angiotensin converting enzyme inhibitors (ACEI) have been shown to limit the severity of glomerular injury associated with certain forms of experimentally-induced glomerular disorders in which reactive oxygen species (ROS) are thought to play a significant pathogenetic role.[1-6] Recently the ACEI captopril and lisinopril have also been shown to attenuate the degree of structural damage which occurs when isolated glomeruli from normotensive Wistar rats (WKY) are incubated with hydrogen peroxide (H_2O_2).[7] Although the mechanisms involved in mediating the protective effect of ACEI are still not clearly understood, previous data suggests that captopril can scavenge ROS via its sulphydryl group.[8] This claim has not however, been supported by recent *in vitro* studies.[9]

Since both ACEI[10,11] and ROS[12,13] influence the synthesis of prostanoids (PG) by whole glomeruli and mesangial cells in culture it would seem possible, that the protective influence of ACEI against H_2O_2-induced glomerular injury may relate to

subtle changes in the relative levels of different tissue PGs. Thus the effects of thromboxane A_2(TxA_2), a potentially cytotoxic compound[14] with vasoconstrictor properties, are offset by changes in PGE_2 and/or PGI_2, both vasodilators which, in addition, have the capacity to limit the cytotoxicity of TxA_2.[14]

The present study was designed to test the hypothesis that the protective effect of ACEI on oxidant-induced glomerular damage results from the effect of these compounds on glomerular PG production and that this might be influenced by either a sulphydryl or non-sulphydryl containing ACEI. Glomeruli from both WKY and SHR were studied since the latter are more vulnerable to both immunologically and non-immunologically determined injury. In addition, the production of prostanoids by glomeruli from the two strains in response to ACEI differs[11] and the protective influence of captopril and lisinopril on H_2O_2-induced structural damage is strain dependent.[7] H_2O_2 was selected as the oxidant since it can modulate prostanoid synthesis by both isolated glomeruli and cultured mesangial cells,[12,13] the concentration can be accurately controlled and the effects of superoxide radical appear to result from its disproportionation into H_2O_2.

MATERIALS AND METHODS

Preparation of Isolated Glomeruli

Preparations of glomeruli were obtained from 16–18 week old WKY and SHR using a modification of the sieving technique described by Fong and Drummond.[10,11,15] Excised kidneys were chilled in ice-cold Krebs–Henseleit buffer (pH 7.4), which was used throughout as a washing buffer. After removing the capsules, the kidneys were bisected longitudinally and the outer cortex was chopped into 1 mm cubes. Cortical tissue was battered, washed with buffer through graded nylon sieves (220 μm, 100 μm) and the filtrate centrifuged for 2 min at 120 g. After discarding the supernatant, the pellet was suspended and washed from a 75 μm sieve. Glomeruli were recovered from the top of the last sieve. The procedure was monitored by examining samples under a light microscope (Leitz $\times$ 125), enabling when necessary a stage of the procedure to be repeated in order to maximise glomerular purity. To ensure the removal of Bowman's capsule, harvested glomeruli were passed under pressure through a 23-gauge needle and the suspension was centrifuged for 4 min at 120 g. Glomeruli were then re-suspended in buffer. All preparative steps were carried out at 4°C. Glomerular suspensions were greater than 85% pure.

Incubation of Isolated Glomeruli with H_2O_2

Suspensions (0.9 ml) of glomeruli (approx. protein 0.25 mg) in Krebs–Henseleit buffer were pipetted into pre-warmed (37°C) polypropylene tubes and after 20 min incubation, H_2O_2 was added to give final concentrations of 0 and 4.7×10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M. After further incubation (20 min 37°C), with frequent gentle agitation, the suspension was centrifuged (2200 g, 4°C, 2 min), the supernatant removed and stored at -20°C until determination of PG concentrations. PG accumulation in the medium was studied after 20 min incubation with H_2O_2 because pilot studies in our laboratory confirmed data previously published by Baud *et al.*[12] showing the PGE_2 production achieved maximum values 10–30 min after

initiation of the oxidant stress. The percentage increases in PGE_2 production by H_2O_2-stressed glomeruli in our studies and, superoxide radical-stressed glomeruli[12] were similar (approximately 60–100%) at 10, 20 and 30 min post stress. The time-courses of accumulation of PGI_2 and TXA_2 were similar to that of PGE_2.

In experiments to study the effects of ACEI, captopril and lisinopril (25 μM) were added to glomeruli 20 min before H_2O_2 as studies from our laboratory have shown the maximum effect of ACEI on PGE_2, PGI_2 and TXA_2 production also occurred after 10–20 min.[10] The dose of ACEI was selected on the basis of previous studies.[7,8,10,11]

Glomerular Prostanoid Production

Glomerular prostanoid production was determined after incubation with H_2O_2 alone or H_2O_2 and ACEI by modifications of previously described methods.[10,11] Results were compared with PG production from glomeruli which had not been exposed to either H_2O_2 or ACEI. PGE_2, PGI_2 (assayed as the stable metabolite 6-keto $PGFI_a$) and TxA_2 (assayed as the stable metabolite TxB_2) were determined in duplicate using radioimmunoassay kits (New England Nuclear Boston, MA, USA) and the results expressed as ng of prostanoid produced/mg glomerular protein.

Statistical Analysis

Data are reported as mean+SEM. The significance of differences between the experimental groups was tested using a one-way analysis of variance for normally distributed data.

RESULTS

Effects of H_2O_2 on Glomerular Prostanoid Production

The effects of different concentrations of H_2O_2 on glomerular PGE_2, PGI_2 and TxA_2 synthesis were compared with basal prostanoid synthesis by unstressed glomeruli from WKY and SHR. Results from unstressed glomeruli are presented as means$\pm$SEM in Table 1. Values of the ratio $(PGE_2+PGI_2)/TxA_2$ were also determined since it was considered that this may be a better indicator of the potential

Table 1 Synthesis of PGE_2, PGI_2 and TxA_2 by isolated WKY and SHR glomeruli

	WKY	SHR
PGE_2	1.53 ± 0.24	4.05 ± 0.84
PGI_2	1.20 ± 0.22	3.20 ± 0.54
TxA_2	1.23 ± 0.28	4.10 ± 1.05

Control isolated glomerular preparations (n = 11) were incubated in Krebs Henseleit buffer without H_2O_2 and the concentrations of PGE_2, PGI_2 and TxA_2 in the medium determined as described in the Materials and Methods. Data shown are nmol prostanoid/mg glomerular protein (mean $\pm$ S.E.M.)

effect of altered prostanoid synthesis on glomerular function and the metabolic and biological activity of glomerular cells than changes in the concentration of indvidual prostanoids.

WKY Glomeruli

Significant increases in the production of PGE_2 of 49.3%, 58.9% and 28.4% were found after incubation of glomeruli with H_2O_2 at concentrations, 4.7×10^{-6} M, 10^{-5} M, and 10^{-4} M respectively (Figure 1); the increase at 4.7×10^{-5} M H_2O_2 being significantly greater ($p < 0.005$) than that at 4.7×10^{-4} M H_2O_2.

A significant increase in PGI_2 production (46.4%) was found with an H_2O_2 concentration of 4.7×10^{-6} M and, in TxA_2 production (31.9% and 51.6%) at 4.7×10^{-8} M and 10^{-6} M respectively. The latter values were not significantly different. Synthesis of the three prostanoids fell after incubation with higher concentrations of H_2O_2 to values similar to those of unstressed glomeruli.

There was no significant difference between the ratio, $(PGE_2 + PGI_2)/TxA_2$ by unstressed WKY glomeruli (mean value 3.2 ± 0.66) and glomeruli exposed to H_2O_2 at any of the concentrations studied.

SHR Glomeruli

Prostanoid production by unstressed and stressed glomeruli from SHR is shown in Figure 1 and Table 1.

Synthesis of PGE_2 was significaantly increased (83.1%, 108.9% and 62.7%) above that of unstressed glomeruli after incubation with 4.7×10^{-6} M, 10^{-5} M and 10^{-4} M

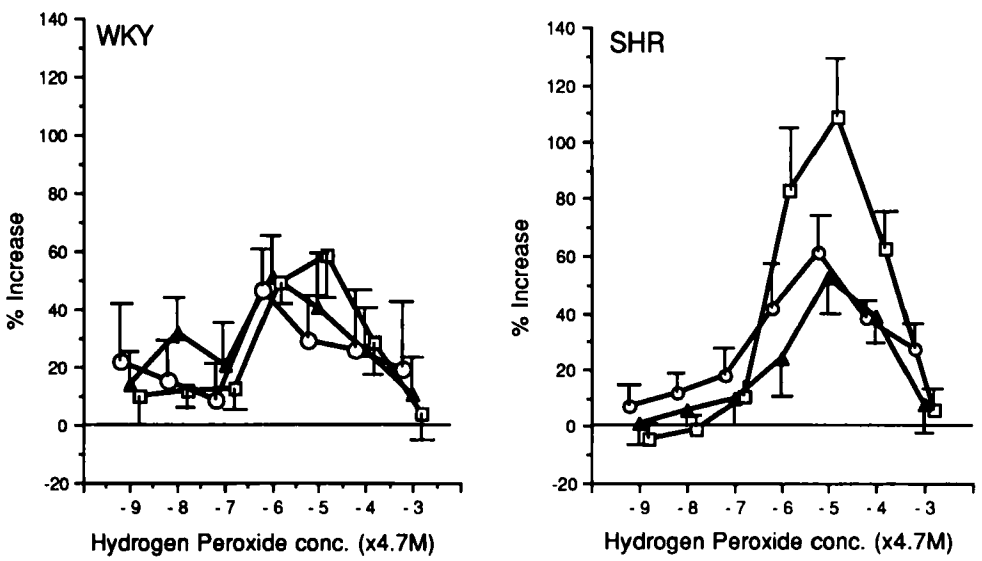

Figure 1 Shows the effect of H_2O_2 on the production of PGE_2 (□), PGI_2 (○) and TxA_2 (▲) by isolated glomeruli from WKY and SHR. Results (mean ± S.E.M.) represent the percentage change in prostanoid synthesis from that in unstressed glomeruli.

H_2O_2 respectively. The proportional increase in PGE_2 following incubation with 4.7×10^{-5} M H_2O_2 was sigificantly greater ($p < 0.005$) than that with 4.7×10^{-4} H_2O_2. Synthesis of PGI_2 was increased by H_2O_2 concentrations of 4.7×10^{-6} M, 10^{-5} M, 10^{-4} M and 10^{-3} M; the proportional increase at 4.7×10^{-5} M H_2O_2 (61.6%) being significantly greater than that at 4.7×10^{-4} M (38.4%) and 10^{-3} M H_2O_2 (27.6%; $p < 0.05$). TxA_2 synthesis was significantly increased after incubation with 4.7×10^{-5} M (52.6%) and 10^{-4} M H_2O_2 (39.0%).

After incubation with 4.7×10^{-6} and 10^{-5} M H_2O_2 the ratio, $(PGE_2 + PGI_2)/TxA_2$, was significantly increased ($p < 0.05$) above that found in unstressed SHR glomeruli (2.2 ± 0.24) with increases of $40.2 \pm 14.4\%$ and $26{,}7 \pm 10{,}2\%$ respectively. There was no significant difference between these values.

Comparison of the Effects of H_2O_2 on Prostanoid Production by WKY and SHR Glomeruli

Production of PGE_2, PGI_2, and TxA_2 by unstressed SHR glomeruli was significantly greater ($p < 0.005$) than that by corresponding WKY preparations (Table 1). The percentage increase in the production of the three prostanoids following incubation with H_2O_2 was similar in the two strains except at a concentration of 4.7×10^{-5} M when the proportional increase in the synthesis of PGE_2 demonstrated by SHR glomeruli was significantly greater ($p < 0.05$) than that of WKY.

The Effects of ACE Inhibitors on Prostanoid Formation by Unstressed and Stressed Glomeruli

Pre-incubation with captopril resulted in a small reduction ($p < 0.05$) in PGE_2 production by glomeruli from WKY at concentration of H_2O_2 of 4.7×10^{-6} M. Otherwise neither ACEI had any significant effect on the synthesis of the three prostanoids, or the ratio of $(PGE_2 + PGI_2)/TxA_2$ by unstressed or stressed glomeruli from either WKY or SHR.

DISCUSSION

This study describes significant H_2O_2-induced increases in the synthesis of PGE_2, PGI_2 and TxA_2 by isolated glomeruli from WKY and SHR. The magnitude of change in individual prostanoid production was such that it resulted in a significant increase in the ratio $(PGE_2 + PGI_2)/TxA_2$ production by glomeruli from SHR but not by those from WKY. Captopril and lisinopril failed to produce any significant changes in either oxidant-induced prostanoid synthesis or the ratio $(PGE_2 + PGI_2)/TxA_2$.

The production of PGE_2, PGI_2 and TxA_2 by glomeruli from unstressed SHR was significantly greater than by preparations of glomeruli from WKY; a finding similar to that previously reported by Harding *et al.*[11] This is the first study demonstrating a difference in PG production by glomeruli from WKY and SHR in response to oxidant stress. It complements, however, a previous study carried out in normotensive rats in which Adler *et al.*[13] describe a biphasic response in PGE_2 synthesis by mesangial cells exposed to H_2O_2 with maximal stimulation occurring at concentrations of 5×10^{-7} M and 5×10^{-5} M H_2O_2; a finding similar to that of the present study in which peak PGE_2 responses to H_2O_2 occurred at concentrations of 4.7×10^{-6} M and 4.7×10^{-5} M.

The mechanism by which H_2O_2 induces increases in prostanoid synthesis is unclear. Although some studies on preparations of isolated glomeruli suggest H_2O_2 increases phospholipase A_2 activity,[12] Adler *et al.*[13] concluded from studies on calcium-ionophore-stimulated mesangial cells that H_2O_2 in concentrations in excess of 5×10^{-4} M may also inactivate cyclooxygenase, the common enzyme in prostanoid synthesis. In the present study the synthesis of all three prostanoids by glomeruli from WKY and SHR exposed to H_2O_2 concentrations greater than 4.7×10^{-5} M was lower than that found with 4.7×10^{-6} M and 4.7×10^{-8} M H_2O_2. Whilst this finding may result from either inhibition of phospholipase A_2, cyclooxygenase or the more specific synthetase enzymes, it could reflect a reduction in the population of viable glomerular cells due to the cytotoxicity of even relatively low concentrations of H_2O_2 (4.7×10^{-6} M). This view is supported by previous studies reported from this laboratory which were designed to examine the influence of different concentrations of H_2O_2 ($4.7 \times 10^{-9} - 10^{-3}$ M) on the viability and structural integrity of isolated glomeruli.[7]

The effects of ACEI on H_2O_2-induced changes in glomerular PG production were modest. Apart from a small captopril-induced reduction in PGE_2 production by glomeruli from WKY at an H_2O_2 concentration of 4.7×10^{-6} M, neither captopril nor lisinopril had any significant influence on prostanoid synthesis or the ratio $(PGE_2 + PGI_2)/TxA_2$ by either unstressed or stressed glomeruli from WKY or SHR. The result of this *in vitro* study contrasts markedly, however with the findings of studies in which the synthesis of PG by glomeruli isolated from WKY and SHR was shown to be significantly influenced by ten days oral administration of captopril.[10,11] The difference between the *in-vivo* and the *in-vitro* effects of ACEI on glomerular PG synthesis in unclear. One could, however, speculate that their *in-vivo* effect might be mediated through non-glomerular renal pathways or through their influence on extrarenal factors such as arginine vasopressin,[16] which is known to modulate glomerular producton of prostanoids.

The functional/biological significance of changes in individual glomerular prostanoid synthesis induced by either H_2O_2 or ACEI during the present studies is difficult to extrapolate to the *in vivo* state. The range of concentrations of H_2O_2 to which glomeruli were exposed, however, reflects both the relatively low levels which result from aerobic metabolism of cells in normal glomeruli, as well as the very much higher concentrations produced by neutrophils and/or macrophages which infiltrate the glomerulus in immunologically or non-immunologically determined glomerular disease. One might speculate therefore that any increase in PGE_2 or PGI_2 induced by ROS or ACEI would have a beneficial effect on the injured glomerulus by maintaining glomerular haemodynamics, reducing basement membrane permeability and/or decreasing cell proliferation whereas, a disproportionate increase in TxA_2, might not only have the opposite effect on glomerular haemodynamics and permeability but may also be either mitogenic[16,17] or cytotoxic.[14] The latter effect of TxA_2 in the pathogenesis of glomerular injury should not be underestimated as significant increases in urinary excretion of TxA_2 have been reported in association with many forms of experimentally-induced glomerulonephritis.[18,19]

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