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Original Article

Fatty acids carried on albumin modulate proximal tubular cell fibronectin production: a role for protein kinase C

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Abstract

Background. Proteinuric renal disease is associated with accumulation of tubulointerstitial matrix proteins. Human proximal tubular cells (PTCs) produce fibronectin in response to serum proteins but not albumin alone. It has been suggested that renal toxicity of filtered albumin depends on its lipid moiety. We therefore investigated the functional consequences of different fatty acids (FAs) carried on human albumin after exposure to human PTCs in culture.

Methods. Confluent human PTCs were exposed to recombinant human serum albumin (rHSA) or palmitate (P)-, stearate (S)-, oleate (O)-, and linoleate (L)-complexed rHSA. In all experimental conditions, test media contained 1 mg/ml rHSA alone or carrying 100 mmol FAs. Mitogenic response was assessed by [^{3}H]thymidine incorporation. Cell culture supernatants were assayed for fibronectin. Protein kinase C (PKC) activity was assessed in cell lysates.

Results. Apical exposure to rHSA alone or the O–rHSA complex stimulated a significant increase in [^{3}H]thymidine incorporation, whereas the L–rHSA complex was markedly inhibitory to human PTC growth. The L–rHSA complex was associated with severe cytotoxicity as assessed by lactate dehydrogenase release. Among all conditions, O–rHSA was the only test media that significantly increased fibronectin levels over control conditions ($150.1 \pm 10.6\%$ over control, $P < 0.05$, $n = 3$). Pre-treatment of PTCs with PKC inhibitors before O–rHSA exposure resulted in a dose-dependent decrease in fibronectin secretion. O–rHSA activated PKC significantly compared with controls.

Conclusions. We conclude that rHSA has a mitogenic effect on human PTCs, but fibronectin secretion was only induced by O-complexed rHSA and the O–rHSA effect was mediated via PKC activation. Involvement of PKC signal transduction pathway may be a novel therapeutic target for ameliorating proteinuria-induced tubular injury.

Keywords: albumin; fibronectin; lipids; protein kinase C; proteinuria; renal failure

Introduction

The cause of the relentless progression of chronic renal failure remains largely unexplained and is likely to be multifactorial. Clinical and experimental evidence suggest that the rate of progression is closely correlated with the pathological changes seen in the tubulointerstitium of kidney. These pathological changes are manifest as an inflammatory cell infiltrate and abnormal accumulation of extracellular matrix (ECM) proteins eventually resulting in tubulointerstitial fibrosis (TIF) and tubular atrophy. This final common pathway of TIF occurs independently of the initial insult and predicts long-term renal prognosis. The exact mechanisms of this effect are yet to be determined.

The major perturbation in the tubular milieu in many progressive renal diseases is severe and prolonged proteinuria. Moreover, patients with nephrotic range proteinuria are more likely to have chronic interstitial disease and to progress to end stage. Abnormal filtration of proteins through damaged glomeruli may exert toxic effects on proximal tubular cells (PTCs), initiating and perpetuating the inflammatory and scarring response [1,2]. Indeed, experimental data have shown that exposure to serum proteins and proteinuric urine stimulates aberrant expression of chemokines, cytokines, and vasoactive molecules in PTCs [2].

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Fibronectin is an ECM protein that is present in the tubulointerstitium of scarred kidneys. Previous work from our laboratory demonstrated that apical exposure to serum proteins increases fibronectin secretion by human PTC in culture. Fractionation of the serum demonstrated that the stimulus to fibronectin secretion was resident in a fraction of molecular weight (MW) 40–100 kDa. The two major components of this fraction, albumin (MW 67 kDa) and transferrin (MW 76 kDa), however, failed to mimic serum effect when added alone to the culture. This observation has led to the hypothesis that non-protein molecules may be responsible for the nephrotoxicity of proteinuric urine [3].

One current theory suggests that the renal toxicity of filtered albumin depends on its lipid moiety [4]. Indeed, albumin is the major carrier molecule for fatty acids (FAs), binding over 99% of plasma FAs. Interestingly it has been shown that tubular re-absorption of FA-bearing albumin, but not FA-free albumin causes release of a novel lipid with chemotactic activity [5]. Furthermore, exposure of opossum kidney PTCs to different FAs carried on albumin results in major perturbation in the cellular lipid pools [6]. Lipids play a vital role in cell structural, metabolic, and signalling functions, and alterations in prevailing lipid levels are likely to have a profound impact on the cell phenotype and function. In this study, therefore, we investigated the functional consequences of different FAs carried on albumin on exposure to human PTC in culture.

Subjects and methods

Materials

Yeast recombinant human serum albumin (rHSA) was a generous gift of Delta Biotechnology, Notthingham, UK. All other chemicals, unless otherwise stated, were obtained from Sigma Chemical Company, Dorset, UK.

Cell culture

Human PTCs were isolated as reported previously [3], by a modification of the method described by Detrisac *et al.* [7]. This procedure has been approved by the Leicestershire Research Ethics Committee, and all patients gave their informed consent. In brief, the outer cortex was dissected from the normal pole of kidneys removed for the treatment of renal carcinoma. The fibrous capsule of the kidney was removed and tissue from the cortex was cut into small pieces and digested in type II collagenase (1.0 mg/ml) at 37°C for 30 min. After digestion, the cell suspension was passed through a series of sieves (BDH Merck Ltd, UK) of diminishing mesh size and the glomeruli removed on the top of the 90-mm mesh. Tubular fragments passing through the sieves were seeded into 75 cm^2 flasks (Costar, UK), which had been coated with bovine collagen type I and fetal calf serum proteins. The cells were grown in serum-free DMEM:F12 (Life Technologies, UK) with the addition of 25 mM HEPES buffer, insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), triiodo-thyronine (4 pg/ml), hydrocortisone (36 ng/ml), benzyl penicillin (100 IU/ml), and streptomycin (50 μg/ml). The cells reached confluence in 10–14 days and were sub-cultured. Proximal tubular origin of cultured cells were characterized as reported previously [3], by their typical morphology, positive staining for cytokeratin (Dako, UK) but not von Willebrand factor (Dako), and cAMP response to parathyroid hormone.

All experiments were done in passage 2 cells as fibronectin secretion increases in cultured human PTCs with an increase in passage number (unpublished observations). Passage 2 cells were seeded into 12 wells (Costar) coated with bovine collagen type I, with a cell density of 1×10^4 cells/cm^2. Confluent cells were made quiescent by 24-h incubation in serum-free DMEM (Life Technologies) containing benzyl penicillin (100 IU/ml) and streptomycin (50 μg/ml). This quiescence medium contains no FAs and a physiological concentration of glucose (5.5 mM), as supraphysiological concentrations of glucose have been shown to stimulate matrix protein production by tubular cells [8]. This quiescence medium was used as control medium and as a vehicle for the different test conditions.

Cell viability was measured following exposure of cells to each of the test conditions for 48 h using both Trypan blue and lactate dehydrogenase (LDH) release. Trypan blue was applied to confluent cell monolayers and number of dead cells (uptake of blue dye) was calculated as percentage of the total cell number counted with a haemocytometer. LDH release into the apical media (as a percentage of total LDH) was measured using a commercially available (LDH Assay Kit, Sigma, UK) enzyme kinetic method, which measures the rate of oxidation of nicotine adenine dinucleotide by a change in optical density.

Preparation of FA–albumin complexes

FA–albumin preparations were produced by a saponification technique defined by Goldstein *et al.* [9], using palmitate (C 16:0), stearate (C 18:0), oleate (C 18:1) and linoleate (C 18:2) for complexing to rHSA, which is essentially FA-free. These four FAs were chosen, as they constitute approximately 80% of the total FA load on HSA [10]. Briefly, FAs were dissolved in ethanol in a siliconized Erlenmayer flask and saponified with sodium hydroxide. The sodium salt was dried under nitrogen, resuspended in saline, and heated for 5 min at 60°C. While the solution was still warm, 25% (w/v) rHSA was added and the mixture was stirred at room temperature for 4 h to allow the FAs to bind to the albumin. Palmitate and stearate were sonicated at low power (20 W for 30 s, Jencons Sonicator) prior to stirring in order to break up the soap flakes. The rHSA–FA complexes (6.4 mmol/l FA:0.9 mmol/l rHSA, molar ratio 7:1) were then sterile filtered, aliquoted, and frozen for future use. In the majority of experimental settings, test media contained 1 mg/ml rHSA alone or carrying 100 mmol FA. These concentrations were chosen to mimic alterations of FA:albumin ratios observed in nephrotic syndrome, and to allow comparison with the few other published studies examining the effects of FA–albumin complexes on PTC function [6]. For dose response studies the rHSA concentration was kept constant whilst varying the FA concentration.

Measurement of cell proliferation

Cell proliferation was determined by [^{3}H]thymidine incorporation using standard methodology. Briefly, confluent, quiescent human PTCs were exposed to rHSA and rHSA–FA

complexes. After 48 h, the supernatants were removed and replaced with fresh medium containing 2 mCi/ml [^{3}H]thymidine (Amersham, UK). The cells were then incubated at 37°C for 2 h after which the supernatants were discarded and the wells rinsed once with HBSS; 0.1 mM cold thymidine in DMEM was added to each well to remove non-specific binding and incubated for 20 min. The cells were then washed once with ice-cold saline, three times with ice-cold 10% (v/v) trichloroacetic acid (TCA), and once with saline again. The TCA precipitate was scraped from wells by a rubber policeman into 10% (v/v) perchloric acid (PCA) and centrifuged at +4°C. This mixture was heated to 70°C for 20 min to solubilize the DNA. [^{3}H]Thymidine counts per min (c.p.m.) were measured by adding supernatant of cell digest to Ecoscint A scintillation fluid (National Diagnostics, Kimberley Research, UK) and counted by using an LKB 1219 liquid scintillation counter. The pellet was dissolved in 0.5 M sodium hydroxide, and the protein concentration was measured using a Lowry assay.

Measurement of fibronectin secretion

Culture supernatants were assayed for fibronectin after 48 h exposure to rHSA or rHSA–FA complexes using a sandwich ELISA as described previously [3]. In brief, the plates were coated with a rabbit polyclonal anti-human fibronectin antibody (1:1000 in coating buffer). The diluted samples were incubated overnight. A mouse monoclonal anti-human fibronectin (1:500) was added for 1 h and this was followed by horseradish peroxidase (HRP)-conjugated anti-mouse immunoglobulin (1:1000) for 1 h. HRP was detected using *o*-phenylenediamine that produces a yellow colour. The reaction was stopped by addition of 1 M H_2SO_4 and absorbance was read at 492 nm using a Multiscan Plate Reader (Titertek, Labsystems, Finland). The concentration of fibronectin was determined from the standard curve.

Effect of protein kinase C inhibitors on fibronectin secretion

To study the effect of protein kinase C (PKC) activity on fibronectin production in human PTCs, various concentrations two PKC inhibitors (Go 6976 and Ro-31-8220, Calbiochem, UK) were added to cell monolayers 30 min before addition of rHSA or rHSA–FA complexes. The cells were then incubated for 48 h. At the end of the incubation period, media were collected and assayed for fibronectin by ELISA.

PKC assay

To determine PKC enzyme activity in human PTC, confluent cells grown in 6 wells (Costar) were exposed to different experimental conditions for 24 h. The cells were scraped into ice-cold PBS and approximately 1.0×10^7 cells were collected for PKC assay by using a non-radioactive, enzyme-linked immunosorbent assay that utilizes a synthetic PKC pseudo-substrate and a monoclonal antibody that recognizes the phosphorylated form of the peptide exactly according to the manufacturer's instructions (Calbiochem Biosciences, UK).

Protein concentrations in each sample were determined by using Bradford dye-binding assay (Bio-Rad, UK). The PKC activities were expressed in arbitrary units after correcting for the amount of protein in cell lysates.

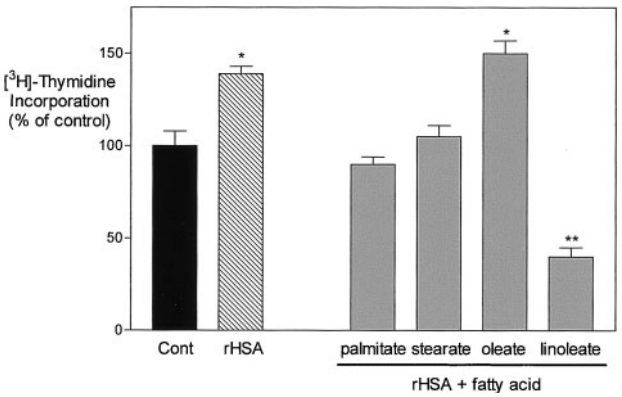

Fig. 1. Proliferation of human PTCs measured as [^{3}H]thymidine incorporation in response to rHSA or palmitate (P)-, stearate (S)-, oleate (O)-, and linoleate (L)-complexed rHSA. Cells were incubated for 48 h following a 24-h quiescence. Each condition was performed in triplicate and results are expressed as percentages ± SEM of control ($n = 6$). *$P < 0.05$ *vs* control (Cont), P + rHSA, S + rHSA, L + rHSA; **$P < 0.05$ *vs* all other conditions (ANOVA with Bonferroni correction for post-tests).

Statistics

The data were analysed using SPSS v. 6.0 for Windows (SPSS Inc.). Results were expressed as mean ± SEM. *n* refers to the number of experiments carried in each case. Each group of experiments was performed in triplicate in at least two kidney preparations. Results were analysed by analysis of variance with Bonferroni correction for post-test multiple comparisons. Differences were considered significant at $P < 0.05$.

Results

Cell characterization

The morphology of the cells was epithelial with dome formation. The cells stained positively for cytokeratin but negatively for von Willebrand factor antigen. Production of cAMP on exposure to parathyroid hormone increased by 10-fold compared with control ($P < 0.001$). These studies were identical to our previous observations [3] and indicate that the cultured cells were predominantly of proximal tubular origin.

Effects of albumin and FAs on the growth of PTCs

The effects of rHSA alone, or rHSA complexed with either palmitate (P), stearate (S), oleate (O), or linoleate (L) on the proliferation of human PTC was assessed by measuring [^{3}H]thymidine incorporation (see the Subjects and Methods). As shown in Figure 1, incubation of human PTCs with rHSA for 48 h resulted in a significant increase in cell proliferation (139 ± 4% over control, $P < 0.05$). Incubation of PTCs with both P–rHSA and S–rHSA did not stimulate any significant change in proliferation compared with the control, these FAs being able to attenuate the positive proliferative effect observed with rHSA alone.

Conversely, when human PTCs were incubated with O–rHSA, the mitogenic effect of rHSA was facilitated ($150 \pm 7\%$ over control, $P < 0.05$). Incubation of human PTCs with L–rHSA, however, inhibited thymidine incorporation to below that observed in controls ($40 \pm 5\%$ compared with control, $P < 0.05$).

Cell viability after 48 h exposure to either rHSA or FA–rHSA was assessed semi-quantitatively by Trypan blue exclusion and quantified functionally by measuring the release of LDH from cells into the culture medium. By Trypan blue exclusion the percentage of dead cells was less than 7% for human PTCs incubated for 48 h with rHSA, P–rHSA, S–rHSA, and O–rHSA. However, when cells were incubated similarly with L–rHSA group cells showed a significant increase in cell death was observed (22%, $P < 0.01$). Measurement of LDH released into the media confirmed that L–rHSA was the only cytotoxic FA–albumin complex (Figure 2).

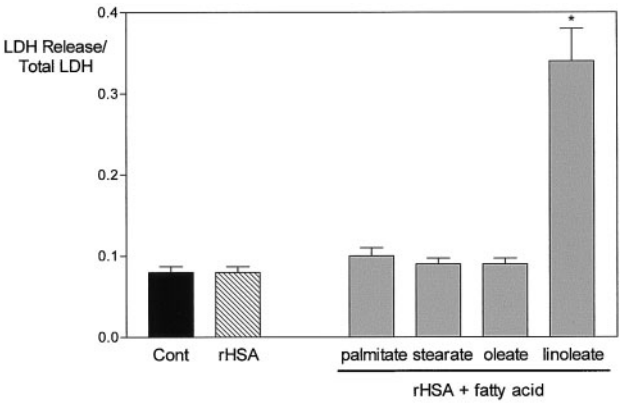

Fig. 2. Effect of rHSA or palmitate (P)-, stearate (S)-, oleate (O)-, and linoleate (L)-complexed rHSA on the release of LDH into the culture medium. Cells were incubated for 48 h following a 24-h quiescence. Each condition was performed in triplicate and results are expressed as ratio ± SEM of LDH released to culture supernatants to the total LDH in cell lysates control ($n = 3$). $^{*}P < 0.01$ *vs* all other conditions (ANOVA with Bonferroni correction for post-tests).

Effects of albumin and FAs on fibronectin secretion by PTCs

Human PTCs in culture produce the ECM protein fibronectin. Under control conditions for 48 h the fibronectin concentration in culture supernatants was 2.31 ± 0.14 ng/mg cell protein (Figure 3). Apical exposure to rHSA, P–rHSA, and S–rHSA did not stimulate any significant alteration in fibronectin secretion compared with control, although rHSA enhanced fibronectin secretion compared with P–rHSA ($P < 0.05$). When PTCs were exposed to O–rHSA complexes, however, fibronectin secretion was significantly enhanced ($150.1 \pm 10.6\%$ compared with controls). O–rHSA was the only condition that enhanced fibronectin secretion compared with the control. Fibronectin secretion was also higher in the O–rHSA condition compared with P–rHSA and S–rHSA. Cells exposed to L–rHSA demonstrated modestly increased fibronectin in culture supernatants compared with P–rHSA and S–rHSA, but these values were not significantly greater than controls. This increase in fibronectin concentrations in L–rHSA condition was attributed to an increased number of dead cells in the media due to cytotoxic effects of this FA–albumin complex.

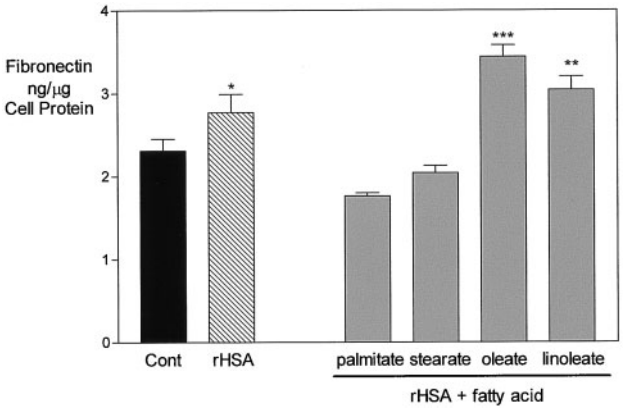

Fig. 3. Effect of rHSA or palmitate (P)-, stearate (S)-, oleate (O)-, and linoleate (L)-complexed rHSA on apical secretion of fibronectin into the culture medium. Cells were incubated for 48 h following a 24-h quiescence. Each condition was performed in triplicate and results are expressed as means ± SEM of control ($n = 4$). $^{*}P < 0.05$ *vs* P + rHSA, $^{**}P < 0.05$ *vs* P + rHSA and S + rHSA, $^{***}P < 0.05$ *vs* control (Cont), P + rHSA and S + rHSA (ANOVA with Bonferroni correction for post-tests).

Effects of PKC inhibitors on O–albumin-stimulated fibronectin secretion by PTCs

As PKC activation is known to enhance fibronectin production in mesangial cells [11], we tested whether pre-treatment with PKC inhibitors was able to abolish the O–rHSA-induced increase in fibronectin production by human PTC. As shown in Figure 4A, addition of increasing doses of a selective PKC inhibitor (Ro-31-8220) together with O–rHSA resulted in a dose-dependent decrease in fibronectin concentrations in culture supernatants after 48 h. Fibronectin concentrations were decreased to 105.0 ± 9.7 and $100.8 \pm 12.5\%$ of the control, respectively, at 10 and 20 nM Ro-31-8220, at which concentrations fibronectin production was no different from controls. Ro-31-8220 had no effect on fibronectin secretion by cells growing under control conditions. Pre-treatment with Go 6976 was also able to attenuate fibronectin secretion stimulated by O–rHSA (Figure 4B). Although these results did not reach statistical significance a clear dose-dependent reduction in fibronectin secretion is apparent.

The addition of PKC inhibitors in these concentrations did not alter cell viability as assessed by Trypan blue exclusion.

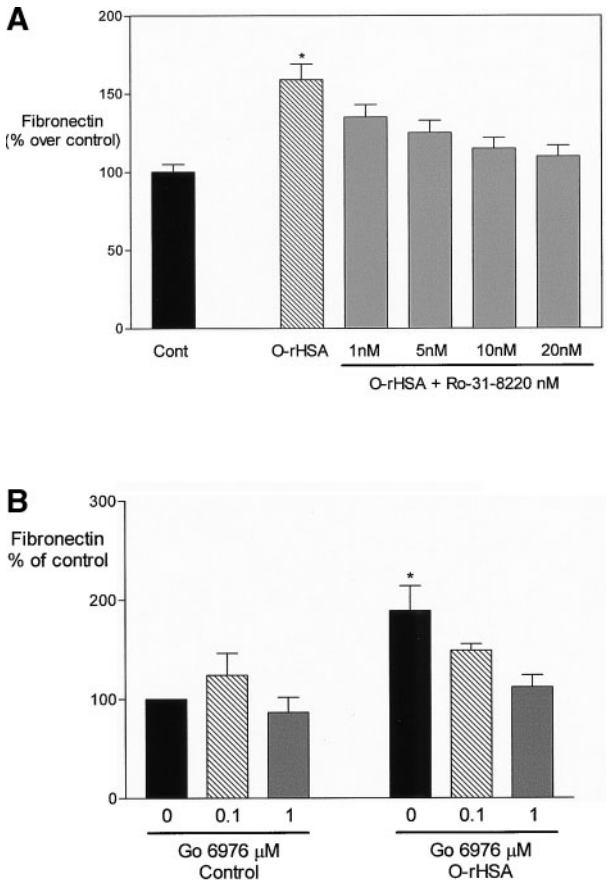

Fig. 4. Effects of PKC inhibitors on fibronectin production by PTCs. (**A**) Effect of pretreatment with increasing doses of a selective PKC inhibitor (Ro-31-8220) on O+rHSA-induced fibronectin secretion by human PTCs. Ro-31-8220 was added 30 min before additon of O+rHSA and then cells were incubated for 48 h. Each condition was performed in triplicate and results are expressed as percentages ± SEM of control ($n=3$). *$P<0.05$ *vs* control (Cont), O+rHSA/10 nM Ro-31-8220 and O+rHSA/20 nM Ro-31-8220 (ANOVA with Bonferroni correction for post-tests). (**B**) Effect of pretreatment with increasing doses of a selective PKC inhibitor Go 6976 on O+rHSA-induced fibronectin secretion by human PTCs. Go 6976 was added 30 min before additon of O+rHSA and then cells were incubated for 48 h. Each condition was performed in triplicate and results are expressed as percentages ± SEM of control ($n=3$). *$P<0.05$ *vs* control 0 μM Go 6976, and $P<0.01$ *vs* control Go 6976 1 μM (ANOVA with Bonferroni correction for post-tests).

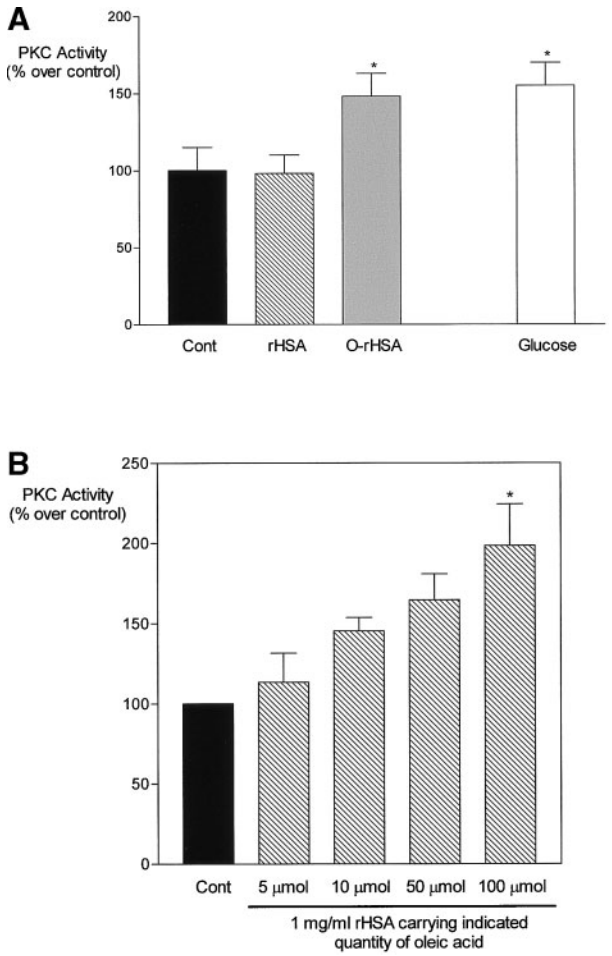

Fig. 5. Stimulation of PKC activity in PTC by O-rHSA. (**A**) Effect of rHSA and oleate-complexed rHSA (O+rHSA) on PKC activity. Cells were incubated with rHSA, O+rHSA and 25 mM glucose (GLU, positive control) for 24 h. Each condition was assessed in triplicate. Results are expressed as percentages ± SEM over control ($n=4$). *$P<0.05$ *vs* control (Cont), and rHSA (ANOVA with Bonferroni correction for post-tests). (**B**) Dose response effect of oleate-complexed rHSA (O+rHSA) on PKC activity. Cells were incubated with rHSA loaded with various concentrations of oleic acid. Each condition was assessed in triplicate. Results are expressed as percentages ± SEM over control ($n=3$). *$P<0.05$ *vs* control, and 5 μmol oleate (ANOVA with Bonferroni correction for post-tests).

Effects of O–albumin and albumin on PKC activity of PTCs

We next determined whether rHSA and O–rHSA complexes were able to activate PKC in human PTCs. As shown in Figure 5A, incubation of PTCs for 24 h with O–rHSA significantly induced PKC activity (147.9 ± 36.8% of control, $P<0.05$) giving values similar to 25 mM glucose (158.2 ± 15.1% of control, $P<0.05$) which has been demonstrated previously to stimulate PKC activity, and was therefore used as positive control [12]. PKC activity in response to rHSA alone was similar to control conditions (99.3 ± 35.1% of control). The stimulation of PKC activity was dependent on the concentration of O–rHSA (Figure 5B).

Discussion

Renal fibrosis is the final common pathway for almost all forms of kidney disease that progress to end-stage renal failure. The strong association between proteinuria, tubulointerstitial scarring and rate of progression in various experimental and clinical studies has led to the hypothesis that either proteinuria *per se* or some other unidentified bioactivity in nephrotic glomerular ultrafiltrate may play an important role in the development of renal fibrosis [1,2,13]. Despite the abundant evidence indicating that abnormal trafficking of proteins may damage the tubulointerstitium, the actual component(s) of the filtered protein carrying

nephrotoxic and fibrogenic potential and the operative process(es) are only beginning to be explored.

Albumin constitutes the majority of filtered protein in nephrotic urine, typically about 80%. Physiologically, albumin carries different hydrophobic substances including lipids, which confers upon albumin the properties of a lipoprotein. In health over 99% of plasma FAs are bound to albumin with a FA–albumin molar ratio of less than 1 [14]. In human proteinuric glomerular disease the FA load per molecule is markedly increased, elevating the FA:albumin molar ratio to approximately 6 [15]. Thus, in nephrosis, albumin presents lipid molecules to PTCs at concentrations far in excess of that determined by their solubility in aqueous solution.

Incubation of PTCs with lipidated albumin has profound effects on cell lipid metabolism [6]. Furthermore, PTCs exposed to lipidated albumin release a lipid macrophage chemotactic factor that has a chromatographic pattern identical to a lipid factor extracted from urine of rats with protein-overload proteinuria [5]. Lipidated albumin also alters cell surface integrin expression in human PTCs, which may modulate the relationship of PTCs with the interstitium in a potentially detrimental manner [16].

Consequently, in this study, we have investigated the potential pathophysiological roles of individual long chain FAs carried by albumin on PTCs. We have used an all-human system working with primary human PTC culture and rHSA. Yeast rHSA is sterile, pyrogen-free, uncontaminated by bioactive blood-derived molecules, and devoid of attached ligands. Our results demonstrate that apical exposure of human PTCs to either rHSA or FA–rHSA complexes elicits disparate mitogenic and fibrogenic responses.

Growth alterations in PTCs may represent a maladaptive response to various injurious stimuli, which result in eventual progressive renal TIF. In this study, rHSA was found to stimulate human PTC proliferation. This finding confirms our previous observations in opossum kidney cells where proliferation was stimulated by both bovine serum albumin [17] and rHSA [18] and extends this finding to human PTCs. More importantly, the current study demonstrates that different FAs carried on rHSA have markedly distinct effects on human PTC growth. Both P–rHSA and S–rHSA complexes prevented rHSA-stimulated PTC proliferation. Conversely O–rHSA facilitated the proliferative effect of albumin in PTCs whereas L–rHSA was frankly toxic. The mechanism of the growth inhibitory and toxic effects of linoleate, stearate, and palmitate are unclear, but intracellular free FAs have been implicated in the pathogenesis of tubular damage in acute renal failure [19].

Matrix protein synthesis and secretion by PTCs has been implicated in the development of renal fibrosis in the presence of proteinuria [3]. By selectively re-lipidating albumin with individual FAs we have shown that the addition of only oleic acid but not other FAs to albumin enhances the fibrogenic properties of PTCs in a manner identical to that produced by serum as shown in our earlier work [3].

Oleate is a potent activator of PKC in some different cell types [20]. Furthermore, activation of PKC in response to high glucose and phorbol myristate acetate mediates up-regulation of fibronectin expression in rodent mesangial cells [11,12]. PKC has been implicated in the progression of renal disease forming a link between hyperglycaemia and diabetic nephropathy [11]. We, therefore, sought to examine whether oleate stimulated PKC activity in PTC was responsible for the observed induction of fibronectin production in PTCs after treatment with O–rHSA. The current data clearly indicate a key role for PKC in regulating PTCs fibronectin secretion in response to O–rHSA. Whilst Ro-31-8220 is a general PKC inhibitor, Go 6976 is more selective for the Ca^{2+}-dependent isozymes PKC_{α} and $PKC_{\beta 1}$. Although the inhibition of fibronectin secretion observed with Go 6976 failed to reach statistical significance the dose-dependent reduction observed suggests that this is a biologically relevant effect. It seems likely, therefore, that the PKC responsible for the mediation of O–rHSA effects is a classical Ca^{2+}-dependent isozyme.

The present data demonstrate for the first time that the PTC biological effects of albumin are significantly modified by bound FAs. In particular, FAs can abolish albumin-induced PTC proliferation, thus potentially disturbing PTC homeostasis and survival [18]. In addition, albumin-bound FAs may evoke a fibrogenic phenotype in PTCs and provide a crucial stimulus to the accumulation of interstitial matrix and the development of tubulointerstitial scarring.

Ghiggeri *et al.* [21] have established that urinary albumin in minimal change disease patients is markedly lower in FA content, particularly linoleic and oleic acids, than urinary albumin collected from patients with other nephrotic conditions. In the current study, linoleic acid was the most tubulo-toxic and oleic acid pro-fibrogenic, and thus the reduction of these FAs in the urinary albumin of minimal change disease may explain the generally non-progressive nature of this condition. Further clinical studies are clearly warranted to measure the FA content of urinary and serum albumin in a large group of patients with different progression characteristics.

In conclusion, the FA content of filtered albumin may condition its potential to induce TIF characteristic of proteinuric states. The involvement of PKC signal transduction pathway in O–rHSA induced Fn production from human PTC may be a novel therapeutic target for ameliorating protein-induced tubular injury.

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References

1. Burton C, Harris KPG. The role of proteinuria in the progression of chronic renal failure. *Am J Kidney Dis* 1996; 27: 765–775
2. Abbate M, Benigni A, Bertani T, Remuzzi G. Nephrotoxicity of increased glomerular protein traffic. *Nephrol Dial Transplant* 1999; 14: 304–312
3. Burton CJ, Combe C, Walls J, Harris KPG. Fibronectin production by human tubular cells: the effect of apical protein. *Kidney Int* 1996; 50: 760–767
4. Thomas ME, Schreiner GF. Contribution of proteinuria to progressive renal injury: consequences of tubular uptake of fatty acid bearing albumin. *Am J Nephrol* 1993; 13: 385–398
5. Kees-Folts D, Sadow JL, Schreiner GF. Tubular catabolism of albumin is associated with the release of an inflammatory lipid. *Kidney Int* 1994; 45: 1697–1709
6. Thomas ME, Morrison AR, Schreiner GF. Metabolic effects of fatty acid-bearing albumin on a proximal tubule cell line. *Am J Physiol* 1995; 268: F1177–F1184
7. Detrisac CJ, Sens MA, Garvin J, Spicer SS, Sens DA. Tissue culture of human kidney epithelial cells of proximal tubular origin. *Kidney Int* 1984; 25: 383–390
8. Ziyadeh FN, Snipes ER, Watanabe M, Alvarez RJ, Goldfarb S, Haverty TP. High glucose induces cell hypertrophy and stimulates collagen gene transcription in proximal tubule. *Am J Physiol* 1990; 259: F704–F714
9. Goldstein JL, Basu SK, Brown MS. Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. *Methods Enzymol* 1983; 98: 241–260
10. Saifer A, Goldman L. The free fatty acids bound to human serum albumin. *J Lipid Res* 1961; 2: 268–270
11. Struder R, Craven P, DeRubertis F. Role for protein kinase C in the mediation of increased fibronectin accumulation by mesangial cells grown in high-glucose medium. *Diabetes* 1993; 42: 118–126
12. Ayo SH, Radnik R, Garoni JA, Troyer DA, Kreisberg JI. High glucose increases diacylglycerol mass and activates protein kinase C in mesangial cell cultures. *Am J Physiol* 1991; 261: F571–F577
13. Wang S-N, Hirschberg R. Tubular epithelial cell activation and interstitial fibrosis. The role of glomerular ultrafiltration of growth factors in the nephrotic syndrome and diabetic nephropathy. *Nephrol Dial Transplant* 1999; 14: 2072–2074
14. Spector AA. Plasma albumin as a lipoprotein. In: Scanu AM, Spector AA, eds. *Biochemistry and Biology of Plasma Lipoproteins. Vol 2: The Biochemistry of Disease*. Marcel Dekker, New York, NY, 1986; 247–279
15. Shafrir E. Partition of unesterified fatty acids in normal and nephrotic syndrome serum and it's effect on serum electrophoretic pattern. *J Clin Invest* 1958; 37: 1775
16. Peruzzi L, Trusolino L, Amore A *et al*. Tubulointerstitial responses in the progression of glomerular diseases: albuminuria modulates a_{vb5} integrin. *Kidney Int* 1996; 50: 1310–1320
17. Burton CJ, Bevington A, Harris KPG, Walls J. Growth of proximal tubular cells in the presence of albumin and proteinuric urine. *Exp Nephrol* 1994; 2: 345–350
18. Dixon R, Brunskill NJ. Activation of mitogenic pathways by albumin in kidney proximal tubular epithelial cells: implications for the pathophysiology of proteinuric states. *J Am Soc Nephrol* 1999; 10: 1487–1497
19. Humes HD, Nguyen VD, Cieslinski DA, Messana JM. The role of free fatty acids in hypoxia induced injury to renal proximal tubular cells. *Am J Physiol* 1989; 256: F688–F696
20. Touny S, Khan W, Hannun Y. Regulation of platelet protein kinase C by oleic acid. Kinetic analysis of allosteric regulation and effects on autophosphorylation, phorbol ester binding, and susceptibility to inhibition. *J Biol Chem* 1990; 265: 16437–16443
21. Ghiggeri GM, Ginevri F, Candiano G *et al*. Characterization of cationic albumin in minimal change nephropathy. *Kidney Int* 1987; 32: 547–553

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