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Effect of cyclooxygenase inhibitors and nitric oxide on intrarenal haemodynamics in ischaemia–reperfusion injury in the anaesthetised rat

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This study evaluated the usefulness of a non-selective and selective blockade of cyclooxygenases (COX) 1 and 2 with aspirin and Celecoxib, respectively, in moderating the haemodynamic responses over the first 2 h of reperfusion after an ischaemic challenge to the kidney. The role of nitric oxide (NO) in this setting was investigated using NCX4016 (Keeble *et al.* 2001), a combined non-selective COX inhibitor and NO donor.

All procedures were performed under UK Home Office licences. Groups of male Wistar rats (n = 5–7, 250–350g) received vehicle, aspirin (100 mg kg^{-1} day^{-1}), NCX4016 (100 mg kg^{-1} day^{-1}) or Celecoxib (10 mg kg^{-1} day^{-1}), orally for 14 days. On day 14 the rats were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, I.P., 3 mg I.V. when necessary). Cannulae were placed in the femoral artery to measure blood pressure and femoral vein for the infusion of normal saline at 3 ml h^{-1}. The left kidney was exposed, the renal capsule removed and laser-doppler flowmeter microprobes were inserted to a depth of 1.5 mm (cortex) and 5 mm (medulla) with 100 perfusion units (PU) equivalent to 1 V. After 1 h, 2 min baseline readings were taken. The renal artery was clamped for 30 min. Upon clamp removal, 2 min readings were taken every 10 min for 90 min and the animal was killed with an anaesthetic overdose. Means ± S.E.M. were compared using ANOVA and significance taken at $P < 0.05$.

The cortical perfusion responded similarly to a period of ischaemia in the aspirin, Celecoxib and vehicle treated groups. PU decreased from basal levels of 136 ± 20 PU by at least 35 % 10 min after the ischaemic challenge and remained depressed. The NCX4016 treated group however, initially decreased from 160 ± 22 PU but recovered, finishing at 110 % of baseline, which was different from the response in the vehicle group ($P < 0.05$). Medullary responses of the Celecoxib and vehicle groups to the period of ischaemia showed a similar pattern, that is basal levels were 57 ± 5 and 90 ± 12 PU and fell 50 % after 10 min and increased to 60 % of basal after 90 min. In the Aspirin treated group, after an initial decrease to 75 % of basal levels (84 ± 10 PU), it increased to around 85 %. The NCX4016 treated group also displayed a significantly different ($P < 0.05$) response to the vehicle in that it fell to 70 % of 84 ± 15 PU and returned to baseline value at 90 min.

These results indicate that in both the cortex and medulla a combination of COX inhibition and NO release by NCX4016 had a beneficial effect on renal vasculature in comparison to selective COX2 inhibition.

Keeble J *et al.* (2001). *Br J Pharmacol* **133**, 1023–1028.

All procedures accord with current UK legislation.

P92

Role of renal parathyroid hormone-related protein in diabetic nephropathy: evidence from studies in cell culture, in mice and in humans

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Parathyroid hormone-related protein (PTHrP) is present in the kidney, where it modulates glomerular function, and also has a mitogenic effect. Both parathyroid hormone (PTH) and PTHrP bind to a common PTH/PTHrP receptor (PTH1R). In a previous study (Garrido *et al.* 2001) we have found renal PTHrP up-regulation in STZ-induced diabetic nephropathy (DN).

In the present study we analysed the expression of the PTH1R in STZ-induced DN in CD mice as well as the expression of PTHrP and PTH1R in human mesangial cells (HMC) and mice tubular cells (MTC) in the presence and in the absence of high glucose (450 mg dl^{-1}) and/or insulin (10 mg ml^{-1}). Moreover we performed immunohistochemistry for PTHrP and PTH1R in the renal biopsies of six patients with DN, including two patients with type 1 and four patient with type II diabetes mellitus (aged 43 ± 3 years, with 10 ± 3 years of diabetes mellitus). Statistical analysis was performed by Kruskall-Wallis or Mann-Whitney test as appropriate. $P < 0.05$ was considered significant. All the experimetal were previously approved by the local commitee for human and animal reseach and are in agreement with the Declaration of Helsinki. Animals were killed under ether anaesthesia.

In diabetic mice, we observed an increase (of 2.5-fold over the control, $P < 0.05$) PTH1R from the second to the seventh week after the diabetes induction (Western blot and immuno-histochemistry). Both HMC and MCT cells cultured in high glucose showed a PTHrP up-regulation (4- and 3.5-fold over the control, respectively, $P < 0.05$). In addition, in the latter cells we also observed an up-regulation of PTH1R (2.5-fold over the control, $P < 0.05$). In MCT, but not in HMC, insulin treatment was able to prevent the up-regulation of PTHrP induced by high glucose (Western blot and RPA).

In normal human kidney, PTHrP and PTH1R staining was only observed at the tubular level. In contrast, a dramatic PTHrP staining was observed at the glomerular level in all patients studied. Glomerular PTH1R staining was observed in three of the six patients studied.

Our study demonstrates the renal up-regulation of PTHrP and PTH1R in both mice and human with DN. Moreover, *in vitro* findings indicate that PTHrP expression can be modulated by high glucose and/or insulin in these renal cells. Taken together these findings strongly suggest a role of PTHrP in the pathophysiology of diabetic nephropathy.

Garrido P *et al.* (2001). *J Am Soc Nephrol* **12**, 835A.

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All procedures accord with current local guidelines.

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Physiological alterations in rat kidneys in intoxication with lead

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The kidney is more severely affected by lead toxicity than are other tissues because it is the fundamental route for elimination, and thus the main organ providing protection against lead poisoning (Landrigan *et al.* 1984; Nolan & Shaikh, 1992). The process of glomerular filtration followed by reabsorption from the tubules results in the accumulation of large quantities of lead in the tubular epithelium. Direct absorption of lead from the blood (Vostal & Séller, 1968; Victery *et al.* 1979; Ladrón de Guevara & Moya, 1995) causes as much damage to the glomeruli as to the proximal tubules, hence chronic exposure to lead affects glomerular filtration and clearance as well as tubular reabsorption.

To study this effect some physiological parameters were evaluated in two groups of 10 young male rats. One group was treated with lead acetate (300 p.p.m.) in the drinking water during a period of 30 days; the other group received distilled water during the same time and served as controls. After the treatment, rats were humanely killed and the kidneys removed and separated into medulla and cortex. The organosomatic index (kidney wt/body wt ratio), the total quantity of proteins (Bradford, 1976), the concentration of malondialdehyde (Uchiyama y Mihara, 1978), total lipids (Folch, 1957), and the alkaline phosphatase (ALP; Kuftiniec y Miller, 1972) and catalase enzymes were measured.

Statistical analysis showed that in lead-treated rats the weight of the kidney, and its organosomatic index, increased by 21% compared with the control group. Since the weight of the animals did not differ significantly between the groups, this increase could be due to a lead-induced retention of liquid in the kidney. The lead caused a decrease in the values for lipid peroxidation by as much as 51 % and 27 % in the medulla and cortex, respectively. The susceptibility of lead-treated kidneys to lipid peroxidation *in vitro* was 30 % lower than in controls. This suggested the activation of some type of antioxidant defence mechanism in the treated animals and a decrease of 30% in the susceptibility to lipid peroxidation in the medulla. Lead also caused a decrease of 12% in the alkaline phosphatase activity in the medulla.

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All procedures accord with current National and local guidelines.

P94

Distribution, expression and subcellular localization of the serum- and glucocorticoid-induced kinase in the rat kidney

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The serum- and glucocorticoid-induced kinase (SGK) is a serine- and threonine-kinase thought to be of importance for sodium reabsorption in the kidney. The epithelial sodium channel (ENaC), the Na^+–K^+–Cl^- cotransporter (NKCC), and the Na^+,K^+-ATPase increase their activity when co-expressed with SGK in epithelial cells or in *Xenopus* oocytes (Fillon *et al.* 2001). SGK is regulated at two different levels: transcription and activation of the protein by phosphorylation. A variety of stimuli have been shown to enhance transcription of the SGK gene, including serum, gluco- and mineralocorticoids, hypo- and hyperosmolarity and various growth factors. Activation of SGK depends on phosphorylation by 3-phosphoinositide-dependent kinases, PDK1 and PDK2 (Kobayashi & Cohen, 1999).

We have studied the expression and regulation of SGK in the mammalian kidney. Experiments were performed with a newly raised anti-SGK antibody on kidneys from rats with different levels of glucocorticoids and/or mineralocorticoids. Adrenalectomy and dexamethasone replacement were performed as previously described (Stanton *et al.* 1985). Rats were anaesthetized by ether inhalation for surgical preparations. After each treatment rats were anaesthetized by pentobarbital (100 mg per 100 g body weight) and the kidneys were processed for Western blotting or fixed and processed for immunohistochemistry.

The results indicate that SGK expression is restricted to the thick ascending limb (TAL), the distal convoluted (DCT) and cortical collecting tubules (CCT). The distribution of SGK does not correspond to the classical aldosterone-responsive tubule segments. Within cells, SGK localizes to the basolateral plasma membrane in close proximity with the Na^+,K^+-ATPase. Differential centrifugation and Western blotting further confirmed the association of SGK with membranes. Kidneys from control animals express high levels of SGK protein, which are not significantly affected by physiological increases in aldosterone. Adrenalectomy reduces SGK protein expression by 40 % ($\pm$ 0.07, $P < 0.001$, Student's paired t test) and dexamethasone replacement restores SGK levels back to normal.

The constitutive expression of SGK in the TAL, DCT and CCT suggests a permissive effect of the kinase on the activity of ion channels and transporters expressed in these segments of the tubule. The Na^+,K^+-ATPase is the only target that could directly interact with SGK. However, a unifying model for SGK action would require intermediaries to transmit the effects of SGK to proteins located in both the apical and basolateral membranes.

Kobayashi T & Cohen P (1999). *Biochem J* **339**, 319–328.

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Expression and subcellular localization of the serum- and glucocorticoid-induced kinase in the mammalian kidney: a comparative study

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Serum- and glucocorticoid-induced kinase (SGK) is a serine and threonine kinase important for renal function as it is thought to increase the expression and activity of ion channels and transporters involved in sodium reabsorption. Epithelial sodium channels (ENaC) and the Na^+,K^+-ATPase are both known to be activated by co-expression with SGK in cultured cells or in *Xenopus laevis* oocytes (Alvarez de la Rosa *et al.* 1999; Setiawan *et al.* 2002). Adrenal mineralocorticoids and glucocorticoids regulate the expression of SGK in the kidney. Almost nothing is known about the expression of SGK isoforms in the kidney. This study describes the differential distribution of SGK in the kidneys of mice, rats, sheep, humans and marmots and in the adrenal glands of mice, rats and marmots. Immunohistochemistry was performed with a novel antibody directed to the carboxy terminus of SGK that recognizes the SGK-1 isoform.

Rodents were killed by cervical dislocation and the kidneys (with the adrenal glands still attached) were immediately removed and fixed in neutral buffered formalin for 24 h at room temperature. Sheep were humanely killed by injection with 0.75 ml (kg body weight)$^{-1}$ sodium pentobarbitone (Euthatal, Rhône Mérieux, 200 mg ml^{-1}). Archival sections of tumour nephrectomized human kidneys were obtained from St Thomas' Hospital, London with local ethics committee approval. All procedures were carried out strictly in accordance with current legislation.

Tissue sections (8 μm thickness) were subjected to antigen retrieval 0.5% SDS and immunohistochemistry. Expression of SGK varied considerably across the species. In the mouse and rat it was restricted to the thick ascending limb of Henle, the distal convoluted and cortical collecting tubules. In the marmot, SGK expression was significantly lower in the cortex but highly expressed in the medulla. In the adrenal gland SGK was abundantly expressed in the cortex and the highest level of expression was in the zona glomerulosa, the site normally associated with the synthesis of aldosterone. SGK immunostaining was also observed in the zona fasciculata but in lower quantities.

The immunohistochemical data indicate that the restricted expression of SGK in the kidney is not an exclusive feature of rodents, but of mammals with varied diets and different basal kidney function. In most cases the sites of SGK localization do not overlap with the classical aldosterone-sensitive targets. The abundance of SGK in the adrenal cortex suggests that it may play a physiological role in the biosynthesis and secretion of glucocorticoids and mineralocorticoids.

Alvarez de la Rosa *et al.* (1999). *J Biol Chem* **274**, 37834–37839.

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P96

Reptilian respiratory physiology and human pathology

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Respiration in human beings is dependent on multiple factors, mainly adjusted to changing oxygen demands. Other sources of variation, such as the emotional state can also interfere with the control of the blood gas composition. In addition, pathological variations also exist. Between them, the sleep respiratory disturbances constitute an important concern in sleep medicine, particularly the adult's sleep apnoeas as well as those shown by premature infants. A common link between adult and infant apnoeas could exist. In infants, apnoeas seem to be due to immaturity of respiratory control while in adults a neurological problem, for instance brain stroke, could impair the respiratory control in a way similar to that of the immature newborn. Instances of such regressions are not uncommon after degenerative processes. This report deals with the analysis of the respiratory responses of lizards (*Gallotia galloti*) in response to various states and with comparing these responses to those of the immature newborn and those shown in adult central apnoeas.

Twenty animals were implanted with electrodes for recording EEG, ECG and breathing under I.P. barbiturate anaesthesia. After surgery recovery, continuous recordings were performed for at least 48 h. The results were stored in a computer. Linear (amplitude, coherence and FFT) analysis techniques have been used to recognize interacctions between ECG and respiration. In addition, behavioral observation and EEG analysis has been used to determine behavioral states. The analysis has been performed at a body temperature of 20, 25, 30 and 35 °C. The experiments were performed under approval of the local ethics committee for animal experimentation.

Normal lizards showed an important degree of variability depending on (1) emotional state, (2) body temperature and (3) sleep–waking state. Excited animals showed important respiratory changes, in respiratory frequency (four times higher), in pattern (polyphasic movements in front of monophasic ones), in amplitude (about 250% higher) but also showed dramatic apnoeic pauses of up to 200 s. With respect to temperature, the respiratory frecuency was approximately doubled from 20 to 35 °C, irrespective of the behavioural state. Finally, the breathing was regular during waking but the sleep reduced both its amplitude and frequency to 50% of the waking value. In addition, important apnoeic pauses were recorded, lasting for up to 250 s. As an example, at the preferred temperature (30°C) sleeping animals made 14 ± 5 breaths every 40 s, waking animals 20 ± 4 and excited animals 78 ± 22 (means ± S.E.M.).

Interpreting these results using Haeckel's biogenetic law (ontogeny repeats phylogeny), it can be concluded that the central sleep apnoeas observed both in the immature and in the adult human beings can be considered as phylogenetic regressions.

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All procedures accord with current local guidelines.

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Renal angiotensinase activities in rat feed with different dietary fats

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Multiple nutritional factors can be related to blood pressure (BP) control. However, their regulatory mechanisms are not yet understood. Fatty acids (FA), mainly essential fatty acids (EFA), and their derivatives such as eicosanoids, can influence BP and be involved in cardiovascular diseases. Low fat diet, with reduced saturated FA, decreases BP substantially (Das, 2001). In contrast, a positive relationship between BP and high intake of saturated FA has been found (Pérez-Jiménez *et al.* 2002). However, the qualitative effect of different types of fat on BP control is scarcely known.

The renin–angiotensin system (RAS) is an important regulator of BP and water homeostasis. Polyunsatureted FA inhibit ACE and enhance the synthesis of NO (Kumar & Das 1997). This suggests a close interaction between FA and RAS.

There is evidence that local kidney angiotensins (Ang) serve as important paracrine regulators of renal function (Harris & Cheng, 1996). Aminopeptidases (AP) play a major role in the metabolism of RAS (Barret *et al.* 1998).

The aim of this study was to analyse the effect of several fats (with different degrees of saturation and EFA composition) used in the diet on renal angiotensinase activities (AlaAP, ArgAP, GluAP and AspAP). The AP activities were assayed in both soluble (sol) and membrane-bound (MB) fractions obtained from the renal cortex and medulla of adult male rats.

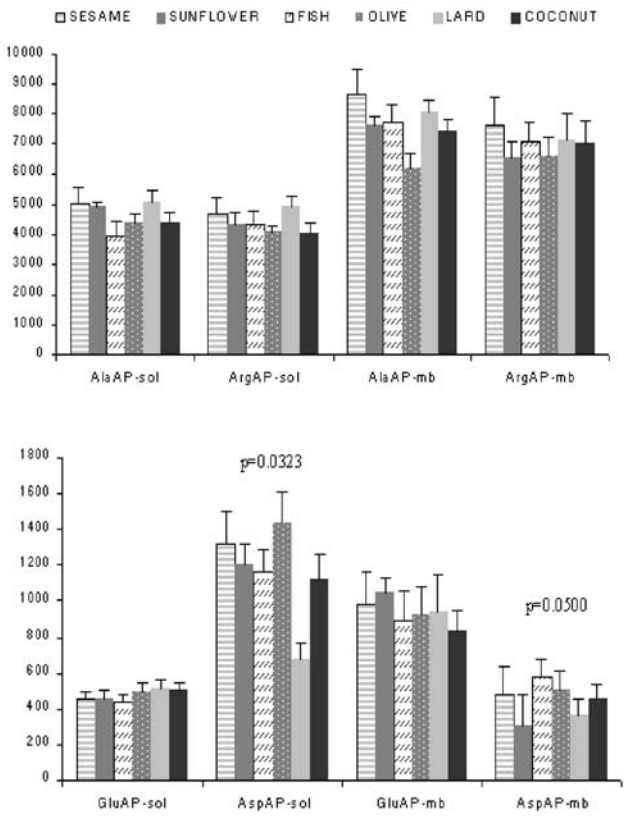

Figure 1.

Male Wistar rats were divided into six groups ($n = 8$) and received for 16 weeks a synthetic diet, adequate with respect to all essential nutrients, and with different sources of fat (10%): sesame (S), sunflower (SF), fish (F), olive (O), coconut (C) oil or lard (L). BP was determined every 2 weeks. After the feeding period the kidneys were removed and the medulla and cortex dissected. Sol and MB fractions were obtained from these samples (Prieto *et al.* 2002). The experimental procedures for animal use and care were in accordance with European Communities Council Directive 86/609/EEC. Data were analysed by one-way analysis of variance. P values below 0.05 were considered significant.

F and L demonstrated significantly lower BP values ($P = 0.0019$) than the rest of groups (Fig. 1). Differences between groups were observed mainly in the renal medulla ($P < 0.001$): The lowest levels of activity were observed with L for sol GluAP, with SF and L for sol AspAP and with SF, O and L for MB AspAP. The present results suggest that dietary fat influences local renal AP activities including angiotensinases.

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P98b

Role of cyclo-oxygenase-2 metabolites in regulating renal function when nitric oxide synthesis is reduced

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Many previous studies have demonstrated that cyclooxygenase-2 (COX-2) is constitutively expressed in the kidney (macula densa, thick ascending limb of Henle, medullary interstitial cells, ...) and that COX-2-derived metabolites are involved in the acute and long-term regulation of renal function (Harris & Breyer, 2001; Roig *et al.* 2002). The objective of this study was to evaluate whether COX-2 is involved in producing the prostaglandins that protect the renal vasculature from the prolonged haemodynamic effects secondary to a reduction in nitric oxide (NO) synthesis.

The study was performed in conscious dogs instrumented as previously described (Gonzalez *et al.* 1998) and with normal sodium intake (70 mEq day^{-1}). Surgery was performed under anaesthesia induced with pentobarbital (30 mg kg^{-1}) and maintained with a 1.5–2 % halothane–O_2 mixture. Experiments were designed according to the national guidelines and the Guiding Principles of the American Physiological Society. We examined the renal haemodynamic and excretory response to the oral administration of a selective COX-2 inhibitor (nimesulide, 5 mg kg^{-1} day^{-1}) during eight consecutive days, with and without the simultaneous intravenous infusion of a NO synthesis inhibitor (L-NAME, 5 μg kg^{-1} min^{-1}). Mean arterial pressure (MAP) and renal blood flow (RBF) were continuously measured with Transonic equipment. Data are given as means ± S.E.M.

The nimesulide administration ($n = 7$) did not modify MAP and glomerular filtration rate (GFR) but induced a significant and continuous reduction in RBF (17 ± 3 %, $P < 0.05$) and a

transitory decrease in urinary sodium excretion ($U_{Na}V$). The prolonged NO synthesis inhibition in the second group of dogs ($n = 6$) elicited a $25 \pm 4\%$ ($P < 0.05$) elevation in MAP, a transitory decrease in RBF ($15 \pm 2\%$, $P < 0.05$) and no changes in GFR and $U_{Na}V$. In the third experimental group ($n = 7$), the continuous NO synthesis inhibition elicited similar changes to those described for group 2. The prolonged administration of the COX-2 inhibitor to these dogs with a reduced NO production elicited a continuous decrease in GFR ($41 \pm 4\%$, $P < 0.05$) and RBF ($37 \pm 4\%$, $P < 0.05$) and a transitory sodium retention that was similar to that found in the dogs only treated with the COX-2 inhibitor.

The results of this study suggest that COX-2-derived metabolites protect the renal vasculature from the prolonged vasoconstriction secondary to a reduction in NO, and that COX-2 is only acutely involved in regulating the renal excretory function when NO synthesis is reduced.

Harris RC & Breyer MD (2001). *Am J Physiol Renal Physiol* **281**, F1–11.

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