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, 250–350 g) 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 selective COX2 inhibition had a beneficial effect on renal vasculature in comparison to combination of COX inhibition and NO release by NCX4016. These results indicate that in both the cortex and medulla a baseline value at 90 min.

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 experimental were previously approved by the local committee for human and animal research 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 immunohistochemistry). 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.
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 & Seller, 1968; Victeryin the tubular epithelium. Direct absorption of lead from the blood (Vostal & Seller, 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 & Mihara, 1978), total lipids (Folch, 1957), and the alkaline phosphatase (ALP; Kufiniec 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.


All procedures accord with current National and local guidelines.
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.


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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 interactions 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 frequency 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.
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. Polyunsaturated FA inhibit ACE and 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 \(\mu\)g 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 significant and continuous reduction in MAP (11 ± 2 mmHg, \(P = 0.0019\)). The MAP reduction was persistent and parallel to the decrease in GFR. The MAP reduction was not affected by the simultaneous intravenous infusion of L-NAME.

Figure 1.

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 \(\mu\)g 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.


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