Unit of Renal and Cardiovascular Pathophysiology
Department of Physiology and Pharmacology. University of Salamanca. Spain.

[1] Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL), Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain.
[2] Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain.
[3] Unidad de Fisiopatología Renal y Cardiovascular, Instituto "Reina Sofía" de Investigación Nefrológica, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Salamanca, Spain
[4] Bio-inRen, S.L., Salamanca, Spain.

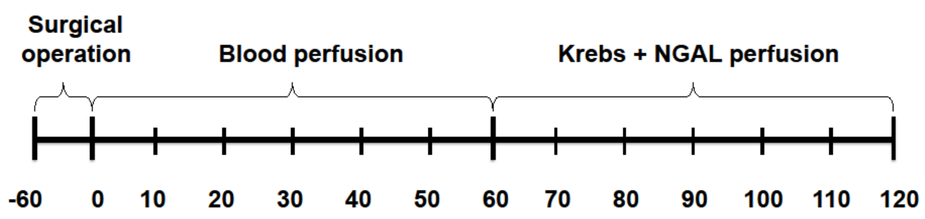
KIDNEYS FROM DIABETIC-HYPERTENSIVE RATS PERFUSED IN SITU WITH NGAL-CONTAINING KREBS SOLUTION EXCRETE MORE NGAL THAN THOSE FROM HYPERTENSIVE RATS

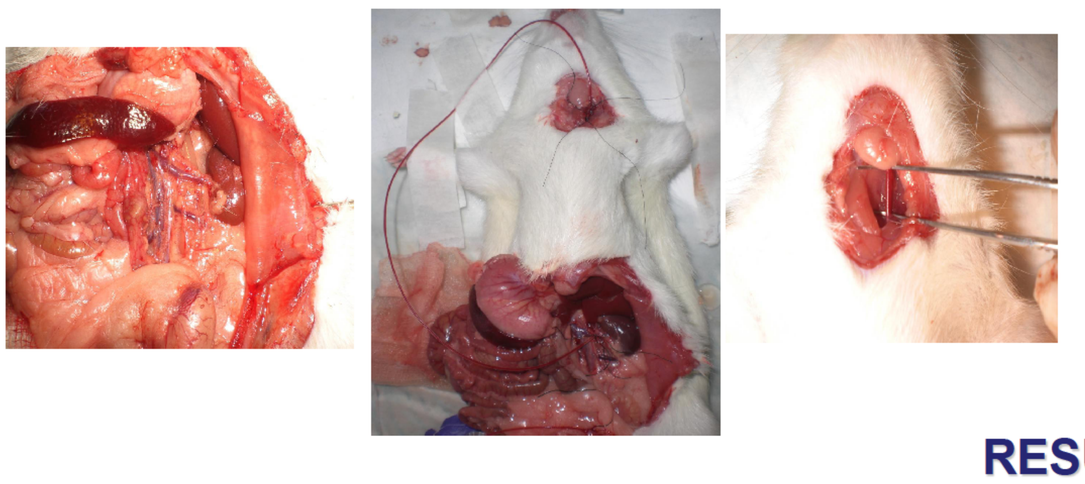
Víctor Blanco-Gozalo, BSc [1,2,4], María Ana M. Blázquez-Medela, PhD[2,3], Omar García-Sánchez, PhD[2,3] , Yaremi Quiros, PhD[2,4], J. Montero, PhD[2], Carlos Martínez-Salgado, PhD[1,2,3], Francisco J. López-Hernández, PhD [1,2,3], **José M. López-Novoa, PhD**[2,3]

BACKGROUND AND AIMS

Hypertension and diabetes are known to eventually damage target organs including the heart, blood vessels and the kidneys. Specifically, hypertension and diabetes lead to a progressive and irreversible loss of the kidneys' function known as chronic kidney disease (CKD). We have previously demonstrated that urinary NGAL (uNGAL) increased as a consequence of the additive action of hypertension and hyperglycemia, but not when only one of these conditions is present. In the present work we aimed at specifically studying the renal handling of NGAL in hypertensive-hyperglycemic and hypertensive rats, through in situ renal perfusion experiments.

EXTRACORPOREAL CIRCUIT

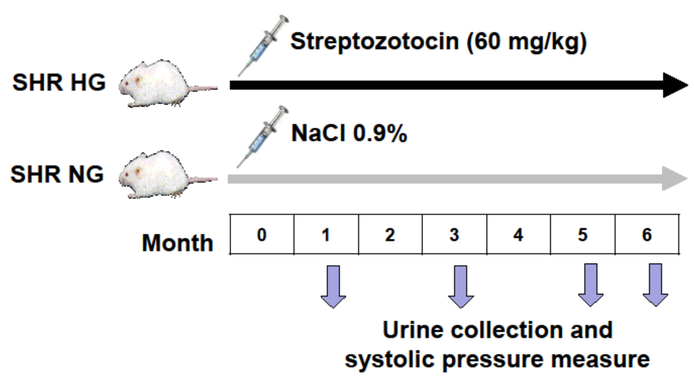




METHODS

Male spontaneously hypertensive rats (SHR) were rendered hyperglycemic (SHR HG) by a single administration of streptozotocin (60 mg/kg), or not (SHR NG) as controls. For glycemic control, diabetic rats were injected daily with the necessary dose of insulin to keep glycemia at about 400 mg/dL. After 6 months, rats were anesthetized and an extracorporeal circuit for kidney perfusion was set up. The renal artery, vein and ureter of the right kidney were ligated. The renal artery of the left kidney and the urinary bladder were canulated. A catheter was placed in the right carotid artery and connected directly to the renal artery. Urine was continuously collected from a catheter placed in the urinary bladder at 10 minute intervals. After 1 hour of renal perfusion with blood from the carotid artery, oxygenated and warm (37 ºC) Krebs-dextran (40 g/L of dextran) in which rat NGAL (42 ng/ml) was added, was perfused through the renal artery at 3 mL/min, and was discarded through the renal vein. NGAL was measured in the different urine fractions.

EXPERIMENTAL PROCEDURE



RESULTS

After one month of coexistence of hypertension and hyperglycemia, urinary NGAL was significantly increased, as compared to control rats, in which urinary NGAL was undetectable. Our previous results showed that when kidneys from hypeglycemic SHR were in situ perfused with Krebs-dextran solution, NGAL disappeared from the urine. In the present work, when exogenous rat NGAL was added to the Krebs solution prefunding the kidney, hypertensive-hyperglycemic rats excreted more NGAL in the urine than hypertensive rats. As a control of the perfusion experiments, NGAL still appeared in the urine in hypertensive-hyperglycemic rats whose kidney was perfused with its own blood. However, urinary NGAL was undetectable in hypertensive rats.

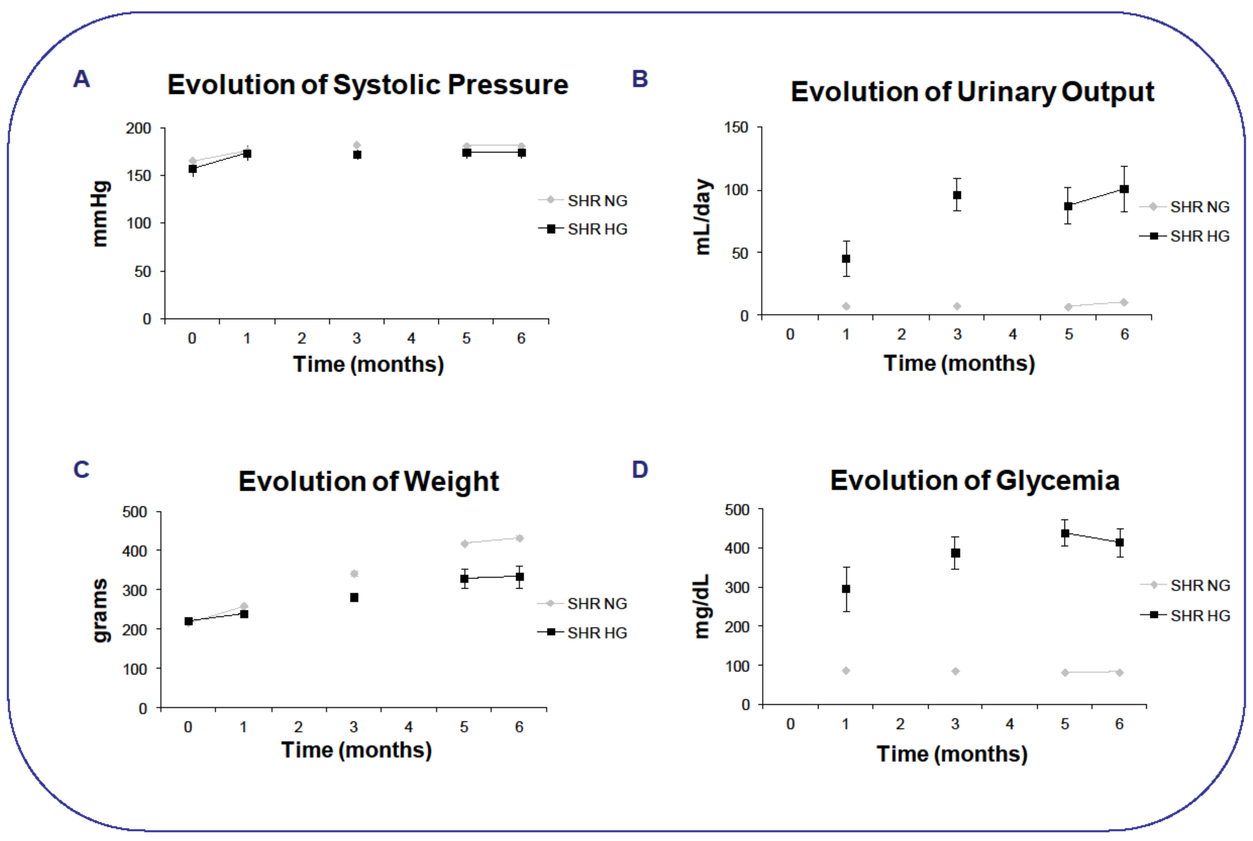


Figure 1. Evolution of systolic blood pressure (a), evolution of urinary output (b), evolution of weight (c) and evolution of glycemia (d) during 6 months of treatment.

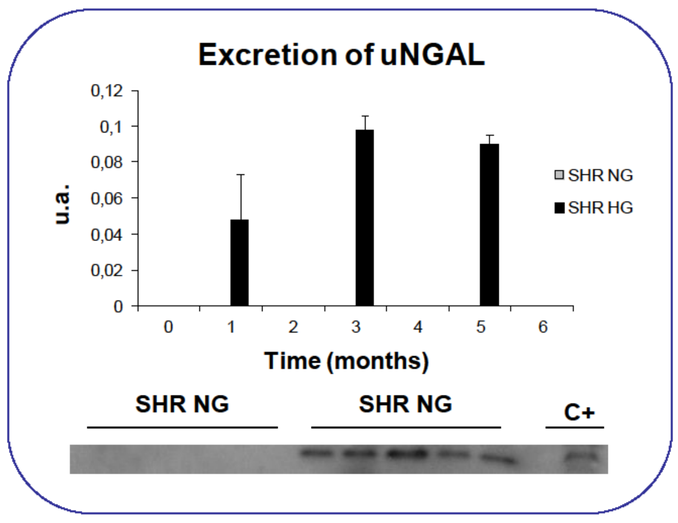


Figure 2. Excretion of urinary NGAL during 6 months of treatment.

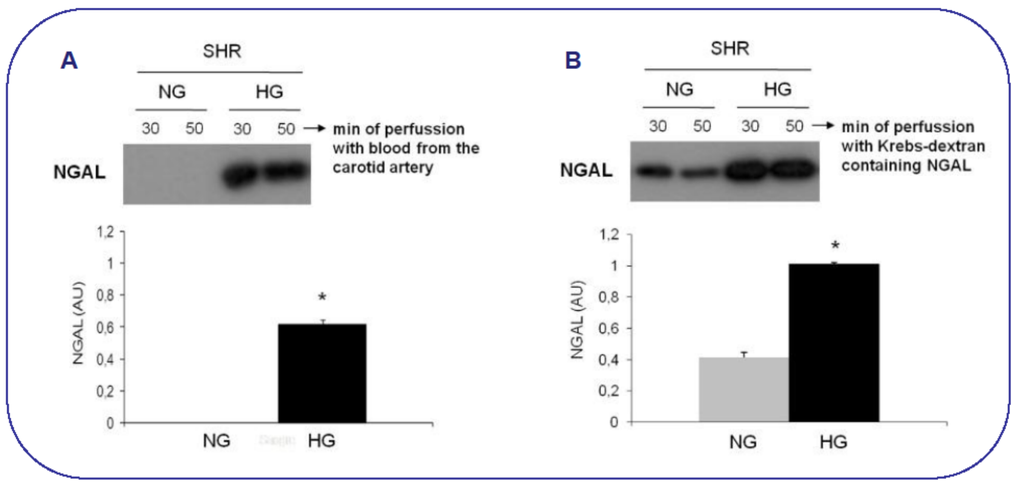


Figure 3. Excretion of urinary NGAL when the kidney is perfused with its own blood (a) or with Krebs-dextran containing NGAL (b) .

CONCLUSIONS

NGAL is a low molecular weight protein freely filtered through the glomerular filtration barrier. Accordingly, our results using a complementary technique reinforce our previous studies, which indicated that the urinary NGAL observed in rats suffering concomitantly from hypertension and hyperglycemia results from its altered tubular handling of this protein, most probably a defect in its tubular re-uptake

DOI: 10.3252/pso.eu.50era.2013