Letter to the Editor

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NG-Nitro-L-Arginine Methyl Ester Causes Glomerular Nitrite Production in Long-Term Incubations

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Dear Sir,

NO is synthesized from L-arginine [1] in a reaction catalyzed by nitric oxide synthase (NOS) and is oxidized to nitrite and nitrate in a constant ratio, at a constant pH [2]. Nitrite production has been used as an index of glomerular NO production [3].

Arginine analogs, such as NG-mono-methyl-L-arginine (L-NMMA), NG, NG-di-methyl-L-arginine (ADMA) and Nº-nitro-L-arginine methyl ester (L-NAME), are commonly used as ‘specific’ inhibitors of NO synthesis; however, their effects on NO levels are rarely measured. Some investigators have reported that incubation of arterial rings with L-NMMA causes increases in NO levels [4]. L-NAME has been widely used to investigate the effect of NO synthesis inhibition on renal function. There is no information about the effect of L-NAME on glomerular NO synthesis in long-term incubations. Thus, the aim of this study was to investigate the effect of L-NAME on glomerular NO synthesis, measured by nitrite production.

Female Wistar rats were anesthetized with ether and after washing the kidneys with ice-cold saline through the abdominal aorta, glomeruli were isolated by mechanical sieving [5] in sterile conditions, and plated out in 4 × 6 well plates at a concentration of 7,000 glomeruli per well. Glomeruli were incubated at 37 °C in endotoxin-free RPMI 1640 culture medium (Gibco) supplemented with 10% fetal calf serum. Some wells were added L-NAME 10-4M (Sigma) and some others were used as controls. Additionally, some empty wells were added RPMI medium alone and some others L-NAME and RPMI medium, to assess the effect of RPMI on L-NAME molecule.

1,500

L-NAME E iü Control

0 |

L-NAME E 10-4M

1,000
Glomerular nitrite production after different times of incubation with and without L-NAME. Statistical significance: * p < 0.05 and ** p < 0.01 vs. control wells at the same time.

Discussion
The observed effect of L-NAME on nitrite production seems not to be due to something present in the culture medium. Glomeruli have a great number of endothelial cells, and recent works have shown the capacity of the endothelial cells to metabolize the methylarginines to L-citrulline, and subsequently to L-arginine [6, 7], which can act as a substrate for NO synthesis. Some authors have reported that L-NMMA is a partial agonist for NO synthesis [4]. Moreover, a new enzyme, NG, NG-dimethylarginine dimethylaminohydrolase has been purified to homogeneity from rat kidney [8]. This enzyme catalyzes the hydrolytic liberation of the dimethylamin moiety of dimethyl arginine compounds and forms L-citrulline and dimethylamine. Although this enzyme is rather specific for dimethyl arginine compounds and L-NMMA, it could be possible that it acts in the same way for L-NAME, thus causing an NO release from glomerular cells. In conclusion, after long-term incubations, L-NAME seems to stimulate glomerular NO synthesis. Thus, results obtained after long term treatment with L-NAME should be analyzed with caution.

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