NO Is Not Exclusively Generated by the Reaction of L-Arginine and NOS and Is Poorly Identified by the Griess Reaction or Clark-Type Electrodes

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Dear Sir,

We read with great interest the report by Dr. Rivas-Cabañero et al. [1] published as a letter to the editor in Nephron last year. The authors found that nitrite and nitrate, stable oxidative products of NO ($\text{NO}_2 + \text{NO}_3 = \text{NO}_x$) actually increase during the administration of the nitric oxide synthase (NOS) inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), in the long-term incubation of isolated glomeruli. In fact, the concentration of NO_x in the incubation medium containing L-NAME is higher than that of controls after 8 h of incubation. The Griess reaction was employed to measure NO_x in the study. The authors speculated that the stimulated NO production is derived from enzymatic synthesis of L-arginine from L-NAME. On the other hand, a recent publication by Dr. Heyman et al. [2] demonstrates, using a Clark-type electrode to measure NO, a paradoxical increase in renal medullary NO production. These authors report that outer medullary NO electrode current did not fall but rose following the addition of L-NAME to renal perfusate despite a rise in blood pressure. They hypothesized that the NO current may reciprocally increase during a reduction in tissue oxygenation, as less NO is being scavenged by oxygen and reactive oxygen species. The possibility of a renal substance that interferes with the electrode current has also been considered.

Recently, we reported that NO_x , detected by the Griess reaction, increases following the reaction of hydrogen peroxide and D-arginine, L-arginine, L-canavanine, or even L-NAME. However, when we used chemiluminescence for the detection of NO, it was detected only from the reactions of hydrogen peroxide and D- or L-arginine. We conclude that there is a pathway for NO synthesis from D- and L-arginine not dependent upon the action of NOS and that the use of NO_x as a marker of NO generation is unreliable because nitrite and/or nitrate rather than NO could be released from L-canavanine and L-NAME through the action of hydrogen peroxide [3]. In addition, in a study monitoring NO generation by Clark-type electrode, we found that the existence of hydrogen peroxide alone

resulted in the detection of an electric current and that the level is higher than that of the current derived from equimolar amounts of NO donor, NOC 5 [4].

Considering our findings [3, 4], it is possible and more reasonable to interpret the findings of Dr. Rivas-Cabañero et al. [1] to indicate that NO_x and not NO is released from the reaction of L-NAME and hydrogen peroxide generated under the peroxidative conditions of long-term incubation of glomeruli. Also, it is possible to interpret the results of Dr. Heyman et al. [2] such that increased NO electrode current is derived from hydrogen peroxide generated under the hypoxic conditions of the renal medulla. It would appear appropriate at this time to re-evaluate the current methods for the detection of NO and to explore alternative mechanisms for NO generation besides NOS pathway.

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