Pitfalls in the measurement of tissue DDAH activity: is DDAH sensitive to nitrosative and oxidative stress?

Kidney International (2008) **74,** 969; doi:10.1038/ki.2008.360

To the Editor: Tain and Baylis[1] recently reported on a dimethylarginine dimethylaminohydrolase (DDAH) activity assay for kidney homogenate. Using this assay, which is a modification of the Prescott–Jones colorimetric assay,[2] Tain and Baylis[1] found that nitrite, the nitric oxide (NO) donor diethylamine NONOate and the superoxide forming agent 2,3-dimethoxy-1,4-naphthoquinone inhibited (at 0.1 and 1 mM) cortical DDAH activity *in vitro*.[1] In our opinion, this work does not provide evidence of DDAH sensitivity to NO and oxidative stress because of methodological problems which we would like to discuss here.

The inhibition of DDAH activity by nitrite[1] is very surprising. A possible explanation for the apparent inhibitory effect of nitrite and diethylamine NONOate could be interference by these species and nitrate in the DDAH assay, for example at the derivatization step. Indeed, Prescott and Jones[2] reported that nitrate inhibited color development in their assay. We are unaware of interference studies by the substances tested in the paper by Tain and Baylis.[1] That diethylamine NONOate was less effective than nitrite,[1] argues against a sensitivity of DDAH activity to nitrosative stress. In addition, inhibition of DDAH activity seen by nitrite and the NO donor diethylamine NONOate[1] could have resulted from artifactual *S*-nitrosation of DDAH SH-groups by nitrous acid (pK_a 3.4). Using a fully validated and interference-free gas chromatography–mass spectrometry assay,[3] we found no inhibition of DDAH activity by nitrite or nitrate *in vivo* in humans[3] and *in vitro* (Figure 1).

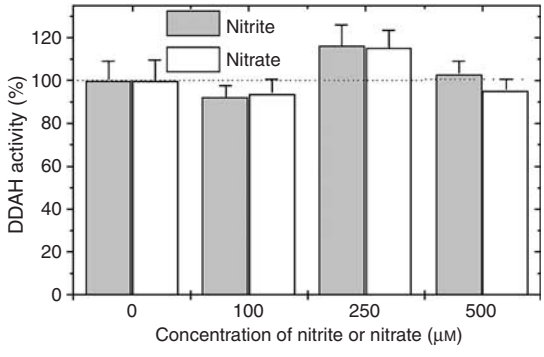

Figure 1 | Effect of sodium nitrite and nitrate on DDAH activity *in vitro* in rat liver homogenate (0.1 M phosphate buffer, pH 7.2). DDAH activity was determined by measuring dimethylamine (DMA) formation from ADMA (100 μM) by GC–MS.[3] Data are presented as mean ± standard deviation from triplicate incubations. DDAH activity of 100% corresponds to 17.8 ± 1.7 pmol DMA per min × mg protein. Nitrite and nitrate (0–1 mM) did not interfere with the GC–MS measurement of DMA in rat liver homogenate in the absence of ADMA (data not shown).

Like *N*-ethylmaleimide,[4] 2,3-dimethoxy-1,4-naphthoquinone is a potent SH-groups alkylating agent. The inhibitory effect of 2,3-dimethoxy-1,4-naphthoquinone on DDAH activity[1] could have resulted from the ability of 2,3-dimethoxy-1,4-naphthoquinone to alkylate rather than to oxidize SH-groups of DDAH.

Albeit generally accepted, there is no solid evidence of the sensitivity of DDAH to nitrosative and oxidative stress *in vitro* and *in vivo*. Investigations addressing this issue should: (1) avoid potential methodological pitfalls; (2) involve use of specific superoxide-producing agents or enzymes; and (3) last but not least important, involve use of drugs at (patho)physiologically or pharmacologically relevant concentrations.

1. Tain YL, Baylis C. Determination of dimethylarginine dimethylaminohydrolase activity in the kidney. *Kidney Int* 2007; **72**: 886–889.
2. Prescott LM, Jones ME. Modified methods for the determination of carbamyl aspartate. *Anal Biochem* 1969; **32**: 408–419.
3. Chobanyan K, Thum T, Suchy MT *et al.* GC–MS assay for hepatic DDAH activity in diabetic and non-diabetic rats by measuring dimethylamine (DMA) formed from asymmetric dimethylarginine (ADMA): evaluation of the importance of *S*-nitrosothiols as inhibitors of DDAH activity *in vitro* and *in vivo* in humans. *J Chromatogr B* 2007; **858**: 32–41.
4. Ogawa T, Kimoto M, Sasaoka K. Purification and properties of a new enzyme, N^G,N^G-dimethylarginine dimethylaminohydrolase, from rat kidney. *J Biol Chem* 1989; **264**: 10205–10209.

Dimitrios Tsikas[1] and Kristine Chobanyan[1]
[1]*Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany*
Correspondence: *Dimitrios Tsikas, Institute of Clinical Pharmacology, Hannover Medical School, Carl-Neuberg-Strasse 1, D-30625 Hannover, Germany. E-mail: tsikas.dimitros@mh-hannover.de*

Response to 'Pitfalls in the measurement of tissue DDAH activity: is DDAH sensitive to nitrosative and oxidative stress?'

Kidney International (2008) **74,** 969–970; doi:10.1038/ki.2008.370

We have the following responses to the comments made by Dr Chobanyan and Dr Tsikas, regarding the quality of our recent publication 'Determination of dimethylarginine dimethylaminohydrolase [DDAH] activity in the kidney'.[1]

Nitrite is a source of nitric oxide synthase-independent nitric oxide production under hypoxic and normoxic conditions[2,3] and that was the reason we used nitrite in these studies. Regarding possible background effects of nitrite (and any other agent that we used), every reagent in our assay was run with a corresponding blank (that is asymmetric dimethylarginine (ADMA) omitted) see supplement, published online.[1] Neither nitrite nor diethylamine NONOate had any effect on the blank value, demonstrating that the decrease of color formation by nitrite is not due to interference. The lack of an effect of nitric oxide on DDAH activity in the study by Tsikas and co-workers[4] may be related to their use of a different tissue