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


To the Editor: Tain and Baylis1 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 Baylis1 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.3 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 nitrite1 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 Jones2 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 NONOate1 could have resulted from the ability of 2,3-dimethoxy-1,4-naphthoquinone to alkylate rather than to oxidize SH-groups of DDAH.

Like N-ethylmaleimide,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 activity1 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.


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

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 conditions2,3 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-workers4 may be related to their use of a different tissue.
(liver) and/or different nitric oxide donors/doses, and/or may reflect inherent problems with the assumption that the gas chromatography–mass spectrometry assay of dimethylamine (DMA) formation gives a direct measure of ADMA breakdown (see below). We did find that nitrate reduced color formation in our DDAH assay (unpublished) as also reported by Prescott and Jones; however, we did not add nitrate to the assay in our study.

We agree that 2,3 dimethoxy-1,4-naphthoquinone may have other actions in addition to generation of superoxide. It would be interesting to see a comparison between different oxidizing agents, however, Tsikas and co-workers do not assess the impact of 2,3 dimethoxy-1,4-naphthoquinone (or other superoxide generators) using their gas chromatography–mass spectrometry assay.

Dr Tsikas and Dr Chobanyan suggest that their DMA production assay provides a definitive DDAH activity assay, but lacking is any direct evidence that 1 U of DMA produced = 1 U of ADMA consumed under any experimental conditions. The wide range in reported urinary DMA:ADMA ratios could reflect alterations in ADMA synthesis, and/or DMA production through other pathways, and/or different handling of DMA and ADMA in specific nephron segments as well as differences in the proportion of urinary ADMA excretion vs DDAH metabolism of ADMA. In fact, although a promising tool for study of ADMA metabolism, DMA production rate, like citrulline production rate, provides only a surrogate measure of ADMA breakdown and requires validation against the ‘gold standard’ of ADMA degradation in every in vivo and in vitro experimental condition.

Although we disagree with the criticisms of Dr Chobanyan and Dr Tsikas regarding the quality of our study, we are in agreement that the question of impact of oxidative stress on DDAH activity remains to be resolved. Indeed, we recently reported that whereas vitamin E supplementation was successful in reducing renal cortical oxidative stress (as measured by reduced nicotinamide adenine dinucleotide phosphate oxidase-dependent superoxide production), renal DDAH activity and plasma ADMA level was not altered in a rat model of kidney disease. As Dr Vaziri points out, oxidative stress is extremely heterogenous, may originate from many locations and in response to many different stimuli. It is possible, therefore, that not all ‘oxidative stress’ is the same in terms of impact on ADMA levels.


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Does pioglitazone provide a better renoprotective effect than insulin in diabetic patients?


To the Editor: In a recent issue of Kidney International, Ohtomo et al.1 showed that pioglitazone (PGZ), one of these currently used thiazolidinediones, could provide better renoprotection than insulin in an obese, hypertensive, type 2 diabetes mellitus rat model. However, a few questions should be considered for this animal study. First, many diabetic patients have insulin as their main treatment for glycemic control, as the author proved PGZ has better renoprotection, would combination treatment with insulin and PGZ be a better regimen for diabetic patients? If the answer is yes, why didn’t the author add another group treated by both insulin and PGZ in their study design. Second, the glycemic control was not better in rats treated with PGZ than those with insulin. Is it possible that the dose of PGZ was too low to achieve a better glycemic status? Third, how could we explain the finding that insulin had a renoprotective effect in the first 8 weeks but it increased urine protein later gradually? According to Schena and Gesualdo, insulin can reduce oxidative stress with prevention of accumulation of toxic advanced glycation end products, which may provide renoprotection in the first 8 weeks. However, in the later period, insulin may induce production of matrix proliferation and results in glomerular sclerosis, thus renal function may decline with increased urine protein excretion.

Finally, the authors only provided a comparison of clearance of creatinine in each group at 26 weeks. I would like to have a look at the change of clearance of creatinine in each group in a time-course manner. There might be an initial increase of clearance of creatinine in the first 8 weeks but with a subsequent decrease of it in insulin treated diabetic rats. This might provide some evidence for combination treatment with both insulin and PGZ because...