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MEETING ABSTRACT

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Mechanism of sustained vascular smooth muscle relaxation by nitroglycerin

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Background: Biotransformation of nitroglycerin (glyceryltrinitrate, GTN) to nitric oxide (NO) is catalyzed by aldehyde dehydrogenase-2 (ALDH2). Since the active-site cysteine of ALDH2 is oxidized in the course of the reaction, thiols are required to sustain catalysis. While dithiothreitol (DTT) may perform this function *in vitro*, a similarly effective reductant has not been reported in cells. Within the context of ALDH2-catalyzed GTN biotransformation, the development of nitrate tolerance has been tentatively explained by irreversible turnover-dependent ALDH2 inactivation. However, the occurrence of this phenomenon in cells has not been investigated.

Methods: In the present study we expressed a recently developed fluorescent intracellular NO probe in vascular smooth muscle cells (VSMC) that also overexpress a double ALDH2 mutant (C301S/C303S). This mutant exhibits higher NO formation rates and shows a greater tendency towards irreversible inactivation than the wild-type enzyme. In these cells we determined the kinetics of formation and decay of GTN-derived NO and compared the results with GTN-induced relaxation of rat thoracic aortas.

Results: As expected, ALDH2 catalyzed sustained formation of NO in the presence of DTT, but only a short burst of NO, corresponding to a single turnover of ALDH2, in its absence. However, even without DTT the burst phase was followed by low nanomolar NO generation, suggesting slow regeneration of reduced ALDH2 by an endogenous reductant. In addition to the thiol-reversible oxidation of ALDH2, thiol-refractive, turnover-dependent inactivation was observed as well. Nevertheless, organ bath experiments with rat aortas showed that GTN caused longer-lasting relaxation than the NO donor diethylamine NONOate.

Discussion: Our results demonstrate that an endogenous reductant allows sustained generation of GTN-derived NO in the low nanomolar range that is sufficient for vascular relaxation. On a longer time scale, turnover-dependent irreversible inactivation of ALDH2 may render blood vessels tolerant to GTN. These results suggest that there may not be an efficient intracellular reductant of oxidized ALDH2 and that the inefficiency of ALDH2 reactivation may actually allow the enzyme to generate low but sufficient concentrations of NO over longer periods.

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