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Role of the general base Glu268 in nitroglycerin bioactivation and mechanism-based superoxide formation by aldehyde dehydrogenase-2

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Background

Mitochondrial aldehyde dehydrogenase (ALDH2) plays an essential role in nitroglycerin (GTN) bioactivation, resulting in formation of nitric oxide (NO) or a related activator of soluble guanylate cyclase (sGC) and consequently in cGMP-mediated vasorelaxation [1]. ALDH2 denitrates GTN to 1,2-glyceryl dinitrate (1,2-GDN) and nitrite but also catalyzes reduction of GTN to nitric oxide (NO) [2]. To elucidate the mechanism of ALDH2-catalyzed GTN bioactivation in relation to the established ALDH2 activities (dehydrogenase, esterase), we compared the function of the wildtype (WT) enzyme with a mutant lacking the general base Glu268 (E268Q).

Results

Despite low dehydrogenase and esterase activities (<3% of WT) the E268Q mutant exhibited virtually unaffected rates of GTN denitration (133 ± 11% of WT). The nucleotide cofactor NAD caused a pronounced increase in the rates of 1,2-GDN formation by WT-ALDH2 from 1.21 ± 0.18 to 8.73 ± 0.09 nmol × min^{-1} × mg^{-1}, but inhibited the reaction catalyzed by the E268Q mutant to about 3% of WT. In contrast to WT-ALDH2, the E268Q mutant generated detectable NO measured with a Clark-type electrode even in the absence of superoxide dismutase (SOD). The apparent initial rate was 2.1 ± 0.31 nmol × min^{-1} × mg^{-1} and the peak concentration of NO was 0.17 ± 0.03 μM. Purified sGC was activated by GTN in the presence of increasing amounts of WT-ALDH2, but the effect reached a plateau of about 30% of maximal sGC activity at 50–100 μg of ALDH2 (9.0 ± 0.38 μmol cGMP × min^{-1} × mg^{-1}). Superoxide dismutase markedly potentiated the effect of ALDH2, resulting in maximal sGC activation with 25 μg of protein. With E268Q-ALDH2, maximal sGC activation was observed with 100 μg of protein even in the absence of SOD. In the presence of SOD, the effect of the mutant was virtually identical to that of WT-ALDH2. Formation of superoxide was confirmed by determination of hydroethidine oxidation that was inhibited by SOD and the ALDH2 inhibitor chloral hydrate. E268Q-ALDH2 exhibited about 50% lower rates of superoxide formation than the WT enzyme.

Conclusion

Our results suggest that E268 is involved in the structural organization of the NAD binding pocket but is not required for GTN denitration. Mechanism-based superoxide formation by ALDH2 may essentially account for oxi-

dative stress in GTN-exposed blood vessels contributing to nitrate tolerance.

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