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ENDOGENOUS PEROXYNITRITE PROMOTES ACTIVATION OF MITOCHONDRIAL PHOSPHOLIPASE A_2 AND THIS RESPONSE IS CAUSALLY-LINKED TO PC12 CELL DEATH INDUCED BY *tert*-BUTYLHYDROPEROXIDE

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Peroxynitrite is a strong reactive nitrogen species, produced *in vivo* by the diffusion-limited reaction of superoxide with nitric oxide, that causes an array of deleterious effects including lipid peroxidation, DNA damage, alteration of calcium ion homeostasis, inhibition of enzymes of the mitochondrial respiratory chain and nitration of protein and non-protein sulfhydryl residues.
We recently reported that exposure of PC12 cells to *tert*-butylhydroperoxide (tB-OOH) leads to peroxynitrite-dependent DNA single strand breakage [1], activation of phospholipase A_2 [2] and toxicity [3]. Exogenous, or endogenous (e.g. using tB-OOH), peroxynitrite does not directly oxidize dihydrorhodamine 123. This response is in fact mediated by peroxynitrite-dependent activation of PLA_2 [4]. The mechanism whereby this enzyme activity is increased is also indirect and involves the action of superoxides generated at the level of complex III of the mitochondrial respiratory chain [5]. Finally, the specific PLA_2 isoform activated by superoxides, while calcium-dependent, is different from the cytosolic PLA_2 ($cPLA_2$) and is most likely represented by a low molecular weight PLA_2 localized within the mitochondria ($sPLA_2$) [5].
The results presented in this study are consistent with the notion that activation of $sPLA_2$ plays a pivotal role in the mechanism whereby endogenous peroxynitrite causes toxicity in PC12 cells exposed to tB-OOH. The following lines of evidence support this inference: a) the peroxynitrite-dependent lethal response was blunted by low concentrations of two general PLA_2 inhibitors and by the selective $sPLA_2$ inhibitor indoxam; these effects were downstream to NO and/or peroxynitrite formation since the inhibitors failed to prevent formation of NO and nitration of tyrosine; b) PC12 cells transfected with $sPLA_2$ antisense oligonucleotides, unlike cells transfected with $cPLA_2$ antisense oligonucleotides, were resistant to toxicity mediated by endogenous peroxynitrite; c) nanomolar levels of arachidonic acid (AA) restored the lethal response in nitric oxide synthase- or PLA_2-inhibited cells as well as in cells transfected with $sPLA_2$ antisense oligonucleotides; c) the decline in cellular ATP mediated by endogenous peroxynitrite was prevented by PLA_2 inhibitors and the concomitant addition of exogenous AA reversed this effect.
We conclude that endogenous peroxynitrite stimulates the activity of mitochondrial PLA_2 and that the ensuing AA release mediates toxicity in PC12 cells exposed to tB-OOH.

References

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