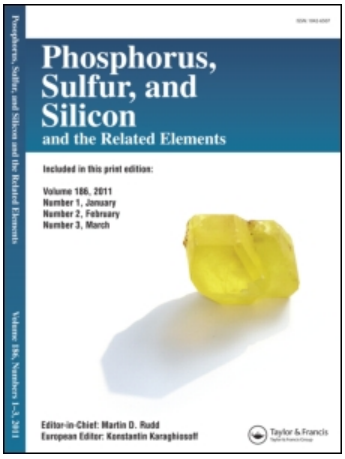

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OVEREXPRESSION OF THE SELENOENZYME PHGPx DEMONSTRATES THE INVOLVEMENT OF HYDROPEROXIDES IN THE IL-1-MEDIATED ACTIVATION OF NFκB.

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An endothelial cell line, ECV 304, overexpressing the seleno- enzyme PHGPx (ECV $^{PHGPx+SelD+}$) was used to investigate the involvement of hydroperoxides in the IL-1-induced activation of NFκB. Even under selenium deficiency ECV $^{PHGPx+SelD+}$ showed a significantly higher PHGPx activity which also contributed to H_2O_2 removal. The enhanced PHGPx activity, i.e. removal of lipophilic hydroperoxides persisted at optimal selenium supply, whereas the H_2O_2-removal capacity was the same as in control cells. These PHGPx overexpressing cells under optimal selenium supply thus provide a model to study the effects of PHGPx selectively. We could show that the IL-1-induced activation of NFκB can be prevented by PHGPx. This demonstrates i. the involvement of hydroperoxides in the IL-1 signaling pathway and ii. a regulatory role of PHGPx therein.

Keywords: IL-1 signaling, hydroperoxides, PHGPx, NFκB

INTRODUCTION

There is growing evidence that reactive oxygen species (ROS), apart from being toxic, play an important role in the signaling pathways of cytokines[1]. Whereas this has been conclusively demonstrated for the activation of nuclear factor κB (NFκB) by tumor necrosis factor-α [2,3] convincing evidence for the involvement of ROS in the IL-1-mediated cellular response is lacking. The production of superoxide

radicals has only been shown in IL-1-treated fibroblasts[4] and mesangium cells[5] but its relevance to signaling has not been studied. Neither has the role of H_2O_2 or lipid hydroperoxides in IL-1 signaling been investigated so far. To explore the role of ROS in IL-1-mediated activation of NFκB we made use of an endothelial cell line stably transfected with the gene for phospholipid hydroperoxide glutathione peroxidase (PHGPx) [6, 7]. Reasonable overexpression was only achieved by co-transfection with the gene for SelD, the selenophosphate synthetase, which provides the selenium donor for the biosynthesis of selenoproteins. The rational for the co-transfection lies in the complex mechanism of eukaryotic selenoprotein biosynthesis reviewed in [8].

All four known selenium-dependent glutathione peroxidases reduce H_2O_2 at the expense of glutathione. In contrast to the cytosolic form (cGPx), PHGPx efficiently reduces also hydroperoxides from complex lipids[9]. By differentially modifying the activities of cGPx and PHGPx it should therefore be possible to see whether a hydroperoxide is involved in a signaling pathway at all and to characterize its nature. By combining overexpression of PHGPx and titrating the activity of all glutathione peroxidases with selenite supplementation, we here show that IL-1-induced NFκB activation is mediated or at least modulated by a hydroperoxide, which is not H_2O_2 but rather a lipid hydroperoxide specifically reduced by PHGPx.

RESULTS AND DISCUSSION

PHGPx activities, measured with the PHGPx-specific substrate phosphatidyl choline hydroperoxide (PCOOH), differed significantly in

control (ECV) and double-transfected cells (ECV$^{PHGPx+SelD+}$) as did total GPx activity, measured with H_2O_2, even when cells were grown in selenium-deficient medium (table 1). This indicates that transfected PHGPx significantly contributes not only to lipid hydroperoxide- but also to H_2O_2-metabolism. In contrast, under optimal selenium supply (50 nM sodium selenite) also endogeneous GPxs are up-regulated (table 1, ECV). Therefore the transfected PHGPx contributes significantly only to the metabolism of lipid hydroperoxides.

TABLE 1 Selenium-dependent GPx activity [nmol ROOH/min x mg protein] in control and double-transfected cells

	ECV		ECV$^{PHGPx+SelD+}$	
Substrate	**H_2O_2**	**PCOOH**	**H_2O_2**	**PCOOH**
- Se	**8.6 ± 2.71**	**1.6 ± 0.93**	**18.1 ± 5.87$^+$**	**6.4 ± 2.16***
+ Se	**120.3 ± 17.53**	**19.8 ± 2.26**	**147.4 ± 22.72**	**28.7 ± 5.56***

For experimental details see [7], + $p < 0.01$, * $p < 0.005$ vs ECV

We thus have created 4 states differing in cGPx and PHGPx activity.

1. In control cells at low selenium, cGPx and PHGPx activity is almost absent. H_2O_2 as well as lipid hydroperoxides can exert putative functions unbalancedly.

2. In control cells at high selenium, both cGPx and PHGPx activity is high. Cells can therefore counteract effects of H_2O_2 as well as of lipid hydroperoxides.

3. In double-transfected cells at low selenium, cGPx activity is almost absent (as in state 1) but due to the enhanced PHGPx activity the effects of H_2O_2 and of lipid hydroperoxides can be inhibited.

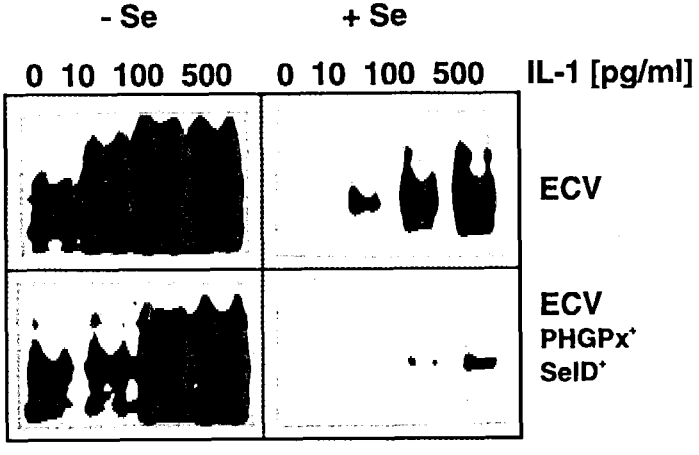
4. In double-transfected cells at high selenium, cGPx activity is enhanced like in state 2. PHGPx activity, however, is again higher

than in state 2. Effects on hydroperoxides can thus be exclusively attributed to PHGPx.

We then tested whether IL-1-induced activation of NFκB was influenced under states 1 - 4.

FIGURE 1 IL-1-induced activation of NFκB. Effect of selenium and overexpression of PHGPx.

Control (ECV) and double transfected (ECV$^{PHGPx+SelD+}$) cells were grown without or with 50 nM sodium selenite supplementation. Cells were stimulated with the indicated concentrations of IL-1 for 30 min, the nuclear proteins extracted and analyzed in an electrophoretic mobility shift assay. For details see[7].



As obvious from fig. 1, the concentration-dependent NFκB activation was inhibited when ECV cells were grown in selenium supplemented medium (state 2). A similar inhibition was observed in unsupplemented ECV$^{PHGPx+SelD+}$ (state 3). The most striking result is the

complete prevention of NFκB activation in selenium supplemented ECV$^{PHGPx+SelD+}$ (state 4). This clearly indicates that PHGPx is able to counteract IL-1 signals probably by a compartmentalized removal of lipophilic hydroperoxides involved in the activation of NFκB.

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