Role of GSH in the modulation of NOS-2 expression in the weaned mammary gland

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Abstract
GSH delivery to the lactating mammary gland is essential for the maintenance of lactation as its decrease leads to apoptosis and involution of the mammary gland. In fact, it has already been demonstrated that some of the changes in gene expression found in the lactating mammary gland after forced weaning are reproduced in rats treated with buthionine sulfoximine to deplete GSH levels. An oligonucleotide microarray experiment would give us a better knowledge of the mRNA expression patterns during lactation and after weaning and the possible functions of GSH in the modulation of these events.

Importance of GSH during lactation
The intertissue flux of GSH is an important mechanism of l-cysteine supply to the lactating mammary gland, which lacks γ-cystathionase activity. The supply of l-cysteine to the mammary tissue is used for the synthesis of protein and other non-protein compounds such as GSH, and it also appears as a free amino acid in milk. The maintenance of the GSH concentration in the gland is essential for milk production [1] and its decrease leads to apoptosis and involution of the mammary tissue [2].

The GSH redox pair is an index of oxidative stress. The GSH/GSSG ratio is at equilibrium with thiols and disulphides in proteins. The GSH depletion in apoptosis is due to two factors: an increased efflux of the reduced form, which is a non-oxidative loss, and an oxidation of GSH to form GSSG [3]. The apoptotic process that takes place after litter removal is characterized by a decrease in GSH and an increase in GSSG in the secretory cells. GSSG levels in mitochondria from the lactating mammary gland are also higher during weaning. In addition, oxidative damage to mtDNA (mitochondrial DNA) is higher in apoptotic cells than in control acini and the peroxide production in mitochondria isolated from weaned mammary glands is 300% higher than that in lactating glands. In fact, there is a direct relationship between mtDNA damage and the GSH/GSSG ratio, which supports the role of mitochondria oxidative stress in the induction of apoptosis during weaning [3].

GSH depletion in the lactating mammary gland mimics the gene expression profile found after 8 h of weaning
The most significant physiological response of the mammary gland to weaning is a decline in the rate of milk secretion. Previous results obtained in our laboratory reproduced this cessation of milking after GSH depletion, which suggests an essential role of this molecule in the maintenance of lactation [1]. A decrease in GSH levels in vivo induces an increase in the expression of p53, p21, p27, c-Jun and pJNK [phosphorylated JNK (c-Jun N-terminal kinase)], triggering apoptotic epithelial cell death. All these changes are also found in the lactating mammary gland when pups are removed, which is responsible for mammary gland involution [2]. To gain a better understanding of the changes in gene expression that regulate involution of the mammary tissue after either weaning or GSH depletion, oligonucleotide microarray experiments have been performed. The expression of approx. 100 genes was affected after 8 h of weaning when compared with control lactating mammary gland. Among those genes, it is interesting to point out that IκB (inhibitory κB) expression is significantly up-regulated [4]. This expression pattern is reproduced when lactating mammary glands are depleted of GSH (results not shown).

Activation of NF-κB (nuclear factor κB) is one of the most rapid transcription factor responses in the involution of the mammary gland [5]. Our results show that, after 8 h of weaning, there is a degradation of the NF-κB major inhibitor, IκBα, followed by an activation of NF-κB, which translocates to the nucleus where it binds to specific target genes. Among them, we found an increase in the expression of IκBα, which could modulate the NF-κB action, and also in inducible NOS-2 (nitric oxide synthase 2) mRNA, which is known to have NF-κB binding sites on its promoter. Our results show that NF-κB is bound to the NOS-2 promoter during weaning, resulting in the actual transcription of the gene as demonstrated by the RNA polymerase II binding to the coding region of the NOS-2 gene.

Nitric oxide production in the lactating mammary tissue
NOS activity has already been reported in the mammary gland of different species by immunohistochemical techniques [6]. During lactation, NO is generated at a low rate

Key words: glutathione, lactation, mammary gland, milatation, nitric oxide (NOS), nitric oxide synthase (NOS).
Abbreviations used: IκBα, inhibitory κB; mtDNA, mitochondrial DNA; NF-κB, nuclear factor κB; NOS, nitric oxide synthase.
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constitutively in the mammary gland, mainly by the endothelial NOS (NOS-3) isoform. NO may have a role in mammary gland differentiation and in regulatory functions, such as the regulation of the blood flow to this tissue; however, the protein levels of NOS-3 in the gland declined after 24 h of weaning, whereas NOS-2 levels increased [4]. A down-regulation of NOS-3 and an increase in NOS-2 expression in the rat mammary gland after treatment with lipopolysaccharide has already been reported [6]. The switch in the regulation of both isoforms is directly related to the involution of the mammary gland. The increased production of NO by NOS-2 might trigger the apoptotic process, either by causing DNA strand breaks and thus inducing p53 activation or by activating the mitochondrial apoptotic pathway [7].

High concentrations of NO produce peroxynitrite, a reactive molecule that can nitrosylate or nitrate proteins, producing a post-translational modification that might alter protein function and signal transduction. Western-blot analysis demonstrated that protein nitration was increased in the mammary gland during weaning, but was limited to a few specific tyrosine-nitrated proteins. A decrease of GSH in the mammary gland at the peak of lactation partially mimics these findings and emphasizes the role of NO as a potential signal that triggers involution [4] (Scheme 1).

**Physiological implications**

Previously, it has been shown that GSH is crucial for the maintenance of lactation [1,2]. It is interesting to point out the fact that NO increases during weaning and after GSH depletion, which suggests that NO plays a potential role in the induction of programmed cell death and gives support to the importance of keeping the GSH levels within a physiological range.

**Scheme 1** The increased production of NO during weaning is reproduced after buthionine sulphoximine (BSO) treatment of rats at the peak of lactation

-INOS, inducible NOS (NOS-2).

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**References**


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