J. Dairy Sci. 99:1–16
http://dx.doi.org/10.3168/jds.2016-11174
© American Dairy Science Association®, 2016.

Sterol regulatory element-binding proteins are regulators of the sodium/iodide symporter in mammary epithelial cells

G. Wen, L. I. Pachner, D. K. Gessner, K. Eder, and R. Ringseis[1]
Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-Universität Gießen, Heinrich-Buff-Ring 26-32, 35392 Gießen, Germany

ABSTRACT

The sodium/iodide symporter (NIS), which is essential for iodide concentration in the thyroid, was reported to be transcriptionally regulated by sterol regulatory element-binding proteins (SREBP) in rat FRTL-5 thyrocytes. The SREBP are strongly activated after parturition and throughout lactation in the mammary gland of cattle and are important for mammary epithelial cell synthesis of milk lipids. In this study, we tested the hypothesis that the *NIS* gene is regulated also by SREBP in mammary epithelial cells, in which NIS is functionally expressed during lactation. Regulation of NIS expression and iodide uptake was investigated by means of inhibition, silencing, and overexpression of SREBP and by reporter gene and DNA-binding assays. As a mammary epithelial cell model, the human MCF-7 cell line, a breast adenocarcinoma cell line, which shows inducible expression of NIS by all-*trans* retinoic acid (ATRA), and unlike bovine mammary epithelial cells, is widely used to investigate the regulation of mammary gland NIS and NIS-specific iodide uptake, was used. Inhibition of SREBP maturation by treatment with 25-hydroxycholesterol (5 μ*M*) for 48 h reduced ATRA (1 μ*M*)-induced mRNA concentration of *NIS* and iodide uptake in MCF-7 cells by approximately 20%. Knockdown of SREBP-1c and SREBP-2 by RNA interference decreased the mRNA and protein concentration of NIS by 30 to 50% 48 h after initiating knockdown, whereas overexpression of nuclear SREBP (nSREBP)-1c and nSREBP-2 increased the expression of *NIS* in MCF-7 cells by 45 to 60%, respectively, 48 h after initiating overexpression. Reporter gene experiments with varying length of *NIS* promoter reporter constructs revealed that the *NIS* 5′-flanking region is activated by nSREBP-1c and nSREBP-2 approximately 1.5- and 4.5-fold, respectively, and activation involves a SREBP-binding motif (SRE) at −38 relative to the transcription start site of the *NIS* gene. Gel shift assays using oligonucleotides spanning either the wild-type or the mutated SRE at −38 of the *NIS* 5′-flanking region showed that in vitro-translated nSREBP-1c and nSREBP-2 bind only the wild-type but not the mutated SRE at −38 of *NIS*. Collectively, the present results from cell culture experiments with human mammary epithelial MCF-7 cells and from genetic studies show for the first time that the *NIS* gene and iodide uptake are regulated by SREBP in cultured human mammary epithelial cells. Future studies are necessary to clarify if the regulation of NIS expression and iodide uptake by SREBP also applies to the lactating bovine mammary epithelium.

Key words: sodium/iodide symporter, sterol regulatory element-binding proteins, mammary gland, iodide transport

INTRODUCTION

The sodium/iodide symporter [Na^+/I^- symporter (**NIS**)] is an integral glycoprotein in the basolateral membrane mediating the efficient uptake of iodide from the bloodstream into cells. The NIS is best known for its essential role in the concentration of iodide in the thyroid, an important prerequisite for the synthesis of iodine-containing thyroid hormones in the thyroid (Carrasco, 1993). The *NIS* gene expression in the thyroid is principally regulated by thyroid-stimulating hormone (**TSH**), the main hormonal regulator of the thyroid (Vassart and Dumont, 1992), via the TSH receptor/cAMP pathway (Laglia et al., 1996; Endo et al., 1997).

Recent evidence indicates that the thyroid *NIS* gene is also subject to regulation by non-TSH signaling pathways (Nicola et al., 2010; Ringseis et al., 2013). In this regard, it has been recently reported that the *NIS* gene is regulated by 2 transcription factors, sterol regulatory element-binding protein (**SREBP**)-1c and SREBP-2 (Hua et al., 1993; Yokoyama et al., 1993), in rat thyrocytes (Ringseis et al., 2013). The SREBP are highly conserved through evolution from fungi to mammals (Osborne and Espenshade, 2009) and play a key role in the regulation of lipid homeostasis. In all mammals, the SREBP-1c isoform preferentially activates genes involved in fatty acid and triacylglycerol synthe-

Received March 16, 2016.
Accepted July 17, 2016.
[1]Corresponding author: robert.ringseis@ernaehrung.uni-giessen.de

ARTICLE IN PRESS

sis, whereas SREBP-2 mainly activates genes involved in cholesterol metabolism (Osborne and Espenshade, 2009). Both, SREBP-1c and SREBP-2 are synthesized as inactive precursors (**pSREBP**) with a molecular weight of about 125 kDa and are located as complexes with other proteins in the endoplasmic reticulum membrane. Upon a decrease of cellular cholesterol content, pSREBP are escorted to the Golgi apparatus, where they are proteolytically processed leading to the release of their active N-terminal domain, called nuclear SREBP (**nSREBP**), with a molecular weight of about 70 kDa (Espenshade et al., 2002; Yang et al., 2002). The nSREBP bind to sterol regulatory elements (**SRE**) in the regulatory region of target genes and, thereby, initiate gene transcription (Horton et al., 2002).

Using molecular biological techniques a functional SRE could be identified in the *NIS* 5′-flanking region indicating that the rat *NIS* gene is a SREBP target gene (Ringseis et al., 2013). Interestingly, transcriptional activation of the *NIS* 5′-flanking region by nSREBP occurred independent of the presence of nucleotide sequences essential for thyroid-specific *NIS* gene regulation like the NIS upstream enhancer region and the proximal promoter (Ringseis et al., 2013), indicating that SREBP-dependent regulation of the *NIS* gene may be of greater importance in extra-thyroidal tissues, in which NIS is also functional but TSH is not the primary regulator. One of these tissues is the mammary gland.

In contrast to the constitutive expression of NIS in the thyroid, investigations in rats and mice showed that mammary gland NIS is functionally expressed in the mammary epithelium from the end of pregnancy and throughout lactation until weaning (Tazebay et al., 2000). In the lactating mammary gland, NIS is responsible for the secretion of iodide into the milk and thus essential for providing iodide for thyroid hormone biosynthesis in the neonate in general and in the newborn calf during the colostral phase in specific. According to studies in laboratory rodents, the inducible expression of NIS in mammary epithelial cells during lactation involves the cooperative action of the lactogenic hormones oxytocin, prolactin, and estrogens (Tazebay et al., 2000), yet the mechanism of *NIS* gene regulation by these hormones is not completely clear. Studies about *NIS* gene regulation and iodide uptake in bovine mammary epithelial cells or bovine mammary gland are completely lacking. However, it has long been known that feedstuff containing glucosinolates, like coproducts from oil production (rapeseed meal, rapeseed press cake) and feed from other cruciferous plants (kale), lowers iodine excretion via the milk of cows (Piironen and Virtanen, 1963). Earlier studies with dairy cows showed that feeding a ration with rapeseed products from old conventional rapeseed varieties with glucosinolate concentrations of 50 to 100 mmol/kg reduced iodine content of cow milk by 50 to 75% (Iwarsson, 1973; Papas et al., 1979) compared with a ration containing no rapeseed but having the same iodine concentration. This effect is mediated by the degradation products of glucosinolates in the animal's body like thiocyanates and isothiocyanates, which are known to competitively inhibit iodide uptake by NIS in the thyroid and the mammary gland (Yoshida et al., 1998; Rillema et al., 2000). Thus, these findings indicate that mammary gland NIS of cattle plays also a role for the provision of iodide to the calf, in modern dairy production at least during the colostral phase. Due to the unique function of mammary gland NIS for iodide transfer into the milk, it is very likely that NIS in the mammary gland of cattle is regulated at least similarly as in other mammals.

Noteworthy, SREBP are strongly induced, activated, or both after parturition and throughout lactation in the mammary gland as shown in cattle (Bionaz and Loor, 2008), sheep (Barber et al., 2003), and mice (Rudolph et al., 2010). The physiological significance of SREBP activation in the lactating mammary gland is to stimulate the synthesis of fatty acids, triacylglycerols, phospholipids, and cholesterol (Rudolph et al., 2007; Rudolph et al., 2010; Mani et al., 2010) and thus to maintain lipid content in the milk and to provide lipophilic nutrients to the suckling neonate. In line with the key function of SREBP-1c for fatty acid and triacylglycerol synthesis, CLA isomers, which cause a reduction of milk fat content in dairy cows (Loor and Herbein, 1998; Perfield et al., 2007) and many other species, such as rats (Ringseis et al., 2004), sheep (Lock et al., 2006), and goats (Lock et al., 2008), were found to inhibit SREBP-1c maturation (Peterson et al., 2004) and decrease expression of SREBP-1c (Harvatine and Bauman, 2006) in mammary epithelial cells. Whether SREBP may also play a role in the secretion of nonlipid nutrients like iodide through regulation of mammary gland NIS and thus are generally important for maintaining the nutrient content of the milk is currently unknown. In view of recent evidence that the *NIS* gene is transcriptionally regulated by SREBP in the thyroid, the present study aimed to test the hypothesis that SREBP are regulators of the *NIS* gene and iodide uptake in mammary epithelial cells. As a mammary epithelial cell model the human breast adenocarcinoma cell line MCF-7 was used because it is a well-established mammary epithelial cell model to investigate the regulation of mammary gland NIS and NIS-specific iodide uptake (Kogai et al., 2000). In contrast, bovine mammary epithelial cell lines, such as BMEG + HM (Schmid et al., 1983), HH2a (Huynh and Pollak, 1995), ET-C (Zavizion et al., 1996), and Mac-T (Huynh et al., 1991), have not been characterized yet for iodide uptake

Download English Version:

https://daneshyari.com/en/article/5542693

Download Persian Version:

https://daneshyari.com/article/5542693

Daneshyari.com