Induction of a variant acetyl-CoA carboxylase mRNA in ovine mammary gland during lactation

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Acetyl-CoA carboxylase, the major flux determining enzyme for lipogenesis is controlled both acutely through reversible phosphorylation [1] and chronically through changes in gene transcription [2]. Regulation of the acetyl-CoA carboxylase gene is complex due to the use of a dual promoter system and alternative exon splicing resulting in transcripts with the same open reading frame but with heterogeneity in the 5' untranslated region [2]. However, transcripts containing a 24 nucleotide deletion potentially giving rise to a protein lacking 8 amino acids upstream of the Serine-1200 phosphorylation site have been characterised in lactating rat mammary gland, in vitro the absence of the 8 amino acids has been implicated in increasing the extent of phosphorylation at this site by cAMP-dependent protein kinase and thus decreasing enzyme activity [3].

Previously we had cloned acetyl-CoA carboxylase from ovine adipose tissue and this cDNA contained the homologous 24 nucleotide within the sequence [4]. Using the polymerase chain reaction with primers flanking this sequence we obtained two amplified products from lactating ovine mammary gland cDNA, one containing the same sequence found in adipose tissue (long form) and the other lacking 24 nucleotides (short form), as in the rat (results not shown). The presence of these transcripts in ovine mammary gland during pregnancy and lactation was investigated further using an RNase protection assay. A 439 nucleotide EcoRI-BamHI fragment (SKBE) overlapping this region was subcloned into pGEM7zf+ from the ovine acetyl-CoA carboxylase cDNA [4]. Antisense transcripts were synthesised from the linearised plasmid and used to hybridise with 20µg samples of total RNA [4], hybridisation to the long form results in a protected fragment of 439 nucleotides and to the short form of fragments of 382 and 33 nucleotides (which is not resolved in this system).

Figure 1 shows that the predominant species in lactating ovine mammary gland corresponds to the short form whereas in adipose tissue RNA the long form predominates. Analysis of RNA from non-pregnant, pregnant and lactating ovine mammary gland demonstrated that total acetyl-CoA carboxylase mRNA increased from 25.2 ± 9.6 (SEM) units per g tissue in non-pregnant animals to 109.8 ± 18.0 units per g tissue (P<0.01) and 154.5 ± 19.9 units per g tissue (P<0.001) in pregnant and lactating animals respectively. This rise in the level of acetyl-CoA carboxylase mRNA was due principally to elevated expression of the short form, increasing from 9.6 ± 4.0 units per g tissue in the mammary gland of non-pregnant animals to 131.7 ± 17.4 units per g tissue in lactation (P<0.001) whilst the long form remained relatively unchanged per mg DNA. Conversely lactation had little effect on the ratio of these variants in adipose tissue and liver with the long form predominating in both cases (results not shown).

Whilst the physiological relevance of an increased acetyl-CoA carboxylase expression during lactation is clear for its role in milk fat synthesis, the explanation for the short form is uncertain given that in other tissues the long form predominates. Nevertheless, it is evident that the expression of the short form is an inducible event in the development of the mammary gland during pregnancy and lactation.

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