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CHANGES IN TISSUE AND SERUM SIALYLTRANSFERASE ACTIVITIES AS RELATED TO PROLIFERATION AND INVOLUTION OF THE RAT MAMMARY GLAND

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Summary

This study, which was performed on rats, demonstrates that the changes in both mammary tissue and serum sialyltransferase activities parallel the growth of the mammary gland, as measured by the DNA content of the tissue. An increase in the specific activity of the enzyme was evident during rapid cellular proliferation, followed by a return to basal values upon cessation of growth and involution of the glands. This pattern occurred under 3 different experimental conditions: (a) pregnancy-lactation-weaning, (b) pseudopregnancy induced by stimulation of cervix, and (c) daily administration of 1 μg estradiol and 3 mg progesterone to promote mammary development equivalent to that of late pregnancy and subsequent decay of mammary gland after withdrawal of hormonal treatment. Although it is difficult to differentiate whether the increase in tissue sialyltransferase is dependent on hormonal stimulation or as a consequence of growth, the elevation in serum sialyltransferase seems to be closely related to the degree of cellular proliferation.

Introduction

Glycosyltransferases are a group of enzymes that catalyze the sequential addition of individual monosaccharides to the appropriate glycoprotein or glycolipid acceptors. These enzymes are present in many mammalian tissues not only as a membrane-bound component associated with both Golgi apparatus [1] and cell surface [2—4], but also in the circulation in a soluble form [5]. The tissue origin and the physiologic significance of these enzymes in the serum still remain unclear. Increased circulating levels of sialyl-, galactosyl-,

and fucosyl-transferases have been reported in patients with liver diseases [6—8] and advanced neoplastic lesions [8—11].

We have reported in a previous publication [12] that in rats with a similar tumor load, the magnitude of increase of serum sialyltransferase and galactosyltransferase was significantly higher in those carrying a fast growing tumor than in those carrying a slow growing tumor. This observation suggests that the level of enzyme activity may be a function of the growth rate of the tumors. The association between rapid cellular proliferation and elevation in serum sialyltransferase activity is further supported by our finding that partial hepatectomy of rats was followed by an immediate rise in serum enzyme level, paralleling the wave of DNA replication in the regenerating liver [13]. The increase in serum sialyltransferase is tentatively attributed to the enhanced synthesis and release of enzyme from the liver as a result of compensatory growth after injury. In the present study, we have investigated the correlation between tissue and serum sialyltransferase activities (CMP-*N*-acetylneuraminate : D-galactosylglycoprotein *N*-acetylneuraminyltransferase, EC 2.4.99.1) and proliferation of the mammary gland in rats during pregnancy-lactation, pseudopregnancy and in vivo hormonal treatment.

Materials and Methods

Treatment of animals

Female Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 220—250 g were used in all experiments. They were housed in a temperature- and light-controlled room. Estrus cycles were determined by the vaginal smear technique. At least 2 normal cycles were followed before mating or initiation of pseudopregnancy.

In the breeding experiment, a male rat was put in a cage with 2—3 females every afternoon. Vaginal smears were taken from each female the next morning. Those which proved to be sperm positive were then segregated and the date of conception recorded. Initiation of pregnancy was further confirmed by the absence of normal cycle during subsequent daily smearing. Animals were killed on day 7, 14 or 20 of gestation. Those that were allowed to carry their pregnancy to term and nurse the pups were killed 2, 7, 14, 20, 25 or 30 days post-partum. Mothers with less than 5 pups in the litter were discarded. Pups were weaned 21 days after delivery.

Pseudopregnancy was induced by stimulation of the cervix with a glass rod on the day of estrus. The duration of pseudopregnancy was first ascertained by daily smears until normal cycles were reestablished. The last day of pseudopregnancy was determined by a positive proestrus smear.

Bilaterally ovariectomized rats were used in the in vivo hormonal treatment study. One week after the operation, the rats were given daily subcutaneous injections of 1 μg estradiol benzoate and 3 mg progesterone in 0.2 ml corn oil for 7, 14 or 20 days in order to promote mammary gland development. They were killed on the day of the last injection. Another group of ovariectomized animals was treated similarly for 20 days, after which the hormonal injection was discontinued and the rats were sacrified either 5 or 10 days later.

DNA determination in mammary tissue

At autopsy, three of the abdominal-inguinal mammary glands on one side were excised and frozen immediately. The tissue was then thawed, minced and defatted in chloroform/methanol (2 : 1, v/v), followed by ether extraction as described by Ferreri and Griffith [14]. The lipid-free tissue was dried, weighed and reduced to powder. The recorded weight was multiplied by 2 so as to obtain the total weight of the 6 abdominal-inguinal glands. DNA was extracted from 30 mg sample of mammary powder with hot trichloroacetic acid and estimated by the diphenylamine method of Burton [15]. The concentration of DNA is expressed as μg per mg of dry fat-free mammary tissue.

Sialyltransferase assay in mammary tissue and serum

The contralateral abdominal-inguinal mammary glands were used for sialyltransferase assay. The frozen tissue was pulverized and homogenized in 0.9% NaCl. The crude homogenate was centrifuged at $1200 \times g$ for 10 min to remove cell debris and nuclei. The supernatant was further centrifuged at $105\,000 \times g$ for 1 h and the particulate fraction was then resuspended in 0.9% NaCl and used as the enzyme source.

Rat blood, collected by vacutainers via heart puncture, was allowed to clot. Red blood cells were removed by centrifugation to obtain serum. The samples were stored at $-70°$C until ready for assay.

The method for the sialyltransferase assay has been described in detail by the author previously [13]. The sugar donor used in the reaction was CMP-[4,5,6,7,8,9-^{14}C]sialic acid (Amersham, spec. act. 4.2 Ci/mol). The exogenous acceptor was desialated fetuin, prepared by mild acid hydrolysis as described by Spiro [16]. Sialyltransferase activities are expressed as nmol of sialic acid transferred per mg of protein per hour. Protein was determined by the method of Lowry et al. [17], with crystallized bovine serum albumin as standard.

Statistics

Results are given as means ± S.E. Statistical comparisons were determined by Student's *t*-test, paired 2-tailed analysis. Differences between means are considered significant when the *P* value is less than 0.05.

Results

As shown in Table I, the weight of the dry, fat-free mammary tissue as well as the DNA concentration increased gradually during pregnancy and lactation. Involution of the mammary glands ensued upon weaning of the pups 21 days post-partum. A slight, although insignificant increase ($0.05 < P < 0.1$) in the specific activity of mammary tissue sialyltransferase was detected on day 7 of pregnancy. The enzyme activity continued to rise and reached a peak (approximately 5-fold increase) just prior to weaning. This was followed by an abrupt decline in tissue enzyme level when the pups were weaned. Determination of serum sialyltransferase activity in these animals showed a parallel pattern, with a maximum increase of 3.5-fold at the end of the lactation period. A return to near normal level in the serum was observed 9 days after weaning (30 days post-partum). Thus the fluctuations in serum sialyltransferase activity followed

TABLE I

EFFECT OF PREGNANCY AND LACTATION ON DNA CONTENT AND SIALYLTRANSFERASE ACTIVITY OF MAMMARY GLAND AND SERUM SIALYLTRANSFERASE ACTIVITY IN RATS

Group	Day	No. of rats	Weight of mammary tissue (mg)	DNA (μg/mg of tissue)	Sialyltransferase activity (nmol/mg protein/h)	
					Mammary tissue	Serum
Virgin control	—	10	357 ± 22	20.1 ± 0.9	4.3 ± 0.3	0.25 ± 0.01
Normal pregnancy	7	8	479 ± 52 *	27.4 ± 2.3 *	5.4 ± 0.5	0.28 ± 0.03
	14	8	528 ± 67 *	32.6 ± 3.1 *	8.7 ± 0.9 *	0.49 ± 0.04 *
	20	7	675 ± 57 *	37.3 ± 2.6 *	10.6 ± 1.2 *	0.58 ± 0.05 *
Post-partum (pups weaned on day 21)	2	7	693 ± 74 *	43.2 ± 3.8 *	10.3 ± 1.4 *	0.61 ± 0.07 *
	7	7	884 ± 92 *	54.2 ± 5.1 *	16.1 ± 1.4 *	0.65 ± 0.07 *
	14	7	962 ± 95 *	62.7 ± 5.5 *	16.5 ± 1.5 *	0.83 ± 0.10 *
	20	6	1054 ± 97 *	75.6 ± 6.4 *	20.6 ± 2.1 *	0.85 ± 0.11 *
	25	7	682 ± 73 *	51.0 ± 6.2 *	14.7 ± 1.9 *	0.50 ± 0.06 *
	30	8	444 ± 50	25.1 ± 2.9	6.6 ± 0.8 *	0.30 ± 0.05
Pseudopregnancy	10—12	14	418 ± 40	25.9 ± 2.5 *	6.0 ± 0.7 *	0.36 ± 0.04 *

* Significantly different ($P < 0.05$) from the corresponding value for the virgin controls.

the growth and involution of the mammary glands.

In order to assess the effect of the fetuses on the changes in serum sialyltransferase activity during normal pregnancy, pseudopregnancy was induced in female rats of the same age and the results were compared with those that had undergone normal pregnancy. Rats that were pseudopregnant for 10—12 days

TABLE II

EFFECT OF ESTRADIOL BENZOATE AND PROGESTERONE ADMINISTRATION ON PROLIFERATION OF MAMMARY GLAND AND SIALYLTRANSFERASE ACTIVITIES OF MAMMARY GLAND AND SERUM IN RATS

Bilaterally ovariectomized rats were used in Groups 1, 2 and 3. EB, estradiol benzoate; Pg, progesterone. One week after the operation, they were given daily subcutaneous injections of EB + Pg for a period indicated below. The time of killing was denoted in the table as the day after the start of injections.

Group	Treatment	Day	No. of rats	Weight of mammary tissue (mg)	DNA (μg/mg of tissue)	Sialyltransferase activity (nmol/mg protein/h)	
						Mammary tissue	Serum
1	Corn oil vehicle alone	20	10	328 ± 27	19.2 ± 1.1	3.9 ± 0.5	0.23 ± 0.02
2	1 μg EB + 3 mg Pg daily	7	8	352 ± 31	19.5 ± 1.2	3.8 ± 0.4	0.25 ± 0.02
		14	8	490 ± 52 *	29.1 ± 3.0 *	7.5 ± 0.8 *	0.39 ± 0.04 *
		20	8	622 ± 75 *	35.3 ± 3.7 *	8.1 ± 0.8 *	0.47 ± 0.05 *
3	1 μg EB + 3 mg Pg daily for 20 days then discontinued until death	25	8	441 ± 49 *	26.4 ± 3.3 *	5.4 ± 0.6	0.38 ± 0.05 *
		30	8	345 ± 38	21.0 ± 2.5	4.1 ± 0.5	0.24 ± 0.02
4	None, 20-day pregnant control	—	6	653 ± 56 *	38.1 ± 3.1 *	9.9 ± 1.1 *	0.55 ± 0.05 *

* Significantly different ($P < 0.05$) from the corresponding value in Group 1 (corn oil injected controls).

showed a significant increase in both mammary tissue and serum sialyltransferase levels, suggesting that the presence of the fetuses is not a prerequisite for the elevation in serum enzyme activity (Table I).

In the second experiment shown in Table II, ovariectomized rats were injected with 1 μg estradiol benzoate and 3 mg progesterone for various lengths of time in order to promote mammary gland development equivalent to that of pregnancy (Group 2). Groups 1 and 4 were the ovariectomized corn oil injected controls and the 20-day pregnant controls, respectively. It can be seen that the mammary gland proliferated gradually during the course of hormal treatment, as indicated by the weight and DNA content of the tissue. After 20 days of estradiol benzoate and progesterone administration, growth of the mammary gland in these rats was comparable to that of rats pregnant 20 days. Elevation of mammary tissue and serum sialyltransferase activities were also apparent in these treated animals, although the magnitude of increase seemed to be smaller when compared to the 20-day pregnant rats. Discontinuation of hormonal treatment led to an immediate involution of the mammary tissue as well as a decline in both tissue and serum sialyltransferase levels in these rats (Group 3). Thus results of this experiment further support the hypothesis that elevation in serum sialyltransferase activity parallel the proliferation of the mammary tissue.

Discussion

We have demonstrated in this study that the changes in both mammary tissue and serum sialyltransferase activities coincided with the development of the mammary tissue. An increase in enzyme level was evident during rapid cellular proliferation, followed by a return to basal values upon cessation of growth and subsequent involution. This pattern occurred under 3 different experimental conditions: pregnancy-lactation-weaning, pseudopregnancy, and hormone-induced growth and subsequent involution after withdrawal of treatment.

This is the first report on the elevation of serum sialyltransferase during pregnancy and lactation in rats. Through personal communication, it was learned that Bernacki has made similar observation in mice. Pseudopregnancy, although resulting in a smaller growth of the mammary tissue as reported by Anderson and Turner [18] and confirmed in this study, nonetheless led to a significant increase in both tissue and serum sialyltransferase levels. The combination dose of estradiol and progesterone used in the second experiment was based on the work of Moon et al. [19] in which they reported that a ratio of 1 : 3000 produced a mammary gland DNA concentration equivalent to that observed in normal late pregnancy after 19 days of treatment. The finding that pseudopregnancy or hormonal administration alone resulted in an enhancement in both tissue and serum sialyltransferase activities suggests that the presence of the fetuses and the lactational process have no permissive effect on the induction of sialyltransferase.

In addition to mammary gland, increases in tissue glycosyltransferases have been reported under conditions of growth and proliferation. These include embryonic development [20], mid or late S phase in the mitotic cycle of syn-

chronized cells [21], invasive tumors [9,11,22] and regenerating liver [13,23, 24]. An increase in various circulating glycosyltransferases has also been documented in hosts (both animal and human) with neoplastic diseases [8—12, 25] and in rats with regenerating liver [13,26].

It is impossible under the present circumstance to determine whether the increase of sialyltransferase in the mammary tissue is dependent on hormonal stimulation or as a consequence of cellular proliferation, since regulation of growth and lactogenesis in situ is closely controlled by pituitary as well as steroid hormones [27]. The rise in serum sialyltransferase presumably is due to release of the enzyme from the developing mammary gland, although the mechanism is far from understood. However, it cannot be ruled out that the increase in tissue sialyltransferase may be a coincidence rather than a determinant factor in the concomitant increase of enzyme in the serum. We have reported earlier that daily injection of hydrocortisone to adenalectomized rats led to a 3-fold elevation in liver sialyltransferase but failed to elicit any change in the corresponding enzyme in the serum [13]. These observations suggest that cellular proliferation may be more important in the regulation of serum sialyltransferase activity.

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