Hormonal Actions on Mammary Metabolism

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INTRODUCTION

A number of hormones act and interact in a complex fashion to regulate mammary secretory cell formation, development, and function. Several aspects of these hormone actions have been studied intensively and reviewed in recent years (12, 17, 25, 26, 44). Actions of hormones to regulate metabolism of fully developed mammary secretory cells during lactation, however, have not been studied extensively and have not been specifically reviewed recently. For these reasons, emphasis in this speculative review is upon recent studies which elucidate specific regulatory roles of several lactogenic hormones during lactation. In separate sections, roles of insulin and glucocorticoids in regulation of mammary energy metabolism and lipogenesis; regulation of lactose, protein, and nucleic acid synthesis postpartum; and the possible role of cAMP in the regulation of mammary metabolism will be discussed.

REGULATION OF MAMMARY ENERGY METABOLISM AND LIPGENESIS

Insulin. During lactation insulin apparently acts in two ways. First, it appears that insulin is essential for the maintenance or survival of (nonruminant) secretory cells since several workers (25, 36, 49) have observed that if insulin insufficiency in vivo is prolonged (36 to 48 h), secretory cell losses occur, and administration of insulin thereafter cannot restore normal milk yields. These observations have not been extended even though they imply that one or more functions essential to cell survival are uniquely dependent upon insulin in mammary secretory cells as compared to other cell types. The second action of insulin upon secretory cells is short-term, acute actions affecting glucose metabolism and milk biosynthesis.

These actions have been studied with alloxan diabetic animals, animals injected with anti-insulin serum and tissue slices, and isolated secretory cell techniques.

Several workers have shown that insulin and/or acute insulin insufficiency produce changes in glucose oxidation and milk synthesis in mammary secretory cells (1, 8, 20, 30, 34, 49, 51). The general effects of short-term insulin insufficiency upon mammary metabolism appear to be (1, 8, 20, 30, 34, 49, 51):

1) Decreased glucose-1-14C, -2-14C and -U-14C conversions to 14CO2;
2) No effect upon or a slight increase in glucose-6-14C conversion to 14CO2;
3) Decreased synthesis of fatty acids and/or glyceride glycerol from glucose-1-14C, -2-14C, -6-14C, and -U-14C and acetate-14C;
4) No or only small effects upon pyruvate-1-14C and -2-14C oxidations to 14CO2; and,
5) Decreased lactose, fat, and casein synthesis.

Additions of insulin in vitro to mammary slices or isolated cells and injection of insulin in vivo (limited data) reverse these effects. The general pattern of insulin effects summarized above implies that insulin is essential for optimal rates of milk synthesis, that glucose oxidation is reduced in the absence of insulin, that a relatively greater decrease occurs in glucose oxidation via the pentose phosphate pathway as compared to oxidation via the Embden-Meyerhof and tricarboxylic acid pathways, and that the effects of insulin upon pentose phosphate pathway flux and fatty acid synthesis may be related. In this latter regard, the results of Walters and McLean (50) from the artificial electron acceptor phenazine methosulfate (PMS) imply that a large proportion of the reduction in glucose-1-14C oxidation via the pentose-P pathway is due to a reduction in NADPH2 oxidation attributable to reduced lipogenesis. In evaluating the pattern of responses to insulin insufficiency presented above, it is important to note that magnitudes of effects of insulin insufficiency in vivo and in vitro vary significantly dependent upon the function(s) studied and the experimental system used.

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For example, pup growth, a measure of milk production, decreases to zero within 12 to 24 h after cessation of insulin administration to alloxan diabetic rats (30, 34) while increases in glucose metabolism due to insulin addition to tissue slices in vitro are usually in the 1.2 to 2-fold range. In vitro responses to insulin in isolated secretory cells in glucose metabolism are greater than in tissue slices but still only two to three-fold. Responses by isolated cells to insulin in fatty acid synthesis from pyruvate are three to five-fold. Despite the fact that normal mammary secretory cell function is heavily dependent upon insulin and comparable in magnitude with the dependence of adipose tissue lipogenesis on insulin (40), in vitro responses of mammary tissue to insulin additions are small as compared to responses in adipose tissue in vitro (1, 34, 49, 51). This coupled with the observation above that mammary cells are dependent upon insulin for survival as well as function in vivo while most other mammalian cell types survive well in the absence of insulin, cause concern and reservation as to the adequacy of current concepts regarding mechanisms of insulin action upon mammary tissue.

There are several possible mechanisms of insulin action in regulation of mammary metabolism including: alterations in membrane transport of sugars and amino acids; alterations in hexokinase isozyme distribution; regulation of metabolic pathways directly or indirectly (e.g., cAMP etc.); regulation of tissue enzyme or protein synthesis; and/or control of redox state or energy charge within the cell. Walters and McLean (49) observed reductions in the activities of several important mammary enzymes after induction of diabetes by subcutaneous injection of alloxan on the 4th day of lactation implying that insulin might act, in part, by regulation of mammary enzymes. However, these workers (49) observed similar, though less dramatic, metabolic effects in anti-insulin induced diabetes in the absence of enzyme changes. Martin and Baldwin (34) found in lactating rats made alloxan diabetic prior to pregnancy and lactation with insulin insufficiency induced by cessation of insulin therapy, that lactation was markedly reduced and metabolic changes characteristic of insulin insufficiency were produced in the absence of changes in enzyme amounts. Prolonged insulin deprivation resulted in gland deterioration and reduced enzyme activities (34).

These observations suggest that the primary cause of metabolic changes in insulin insufficiency is not changes in mammary enzymes and, further, that the reductions in mammary enzyme activities after prolonged insulin deprivation might reflect general deterioration of the gland rather than a direct and specific action of insulin in the regulation of enzyme synthesis.

Walters and McLean (50) reported that although total mammary hexokinase was not altered in the diabetic state, the hexokinase isozyme pattern was changed. They suggested with reservation that insulin might act in the regulation of glucose uptake and phosphorylation by mammary secretory cells. Raskin et al. (39) did not observe changes in mammary hexokinase isozyme patterns in rats injected daily with insulin to increase lactational performance. Martin and Baldwin (35) examined the possibility that a primary insulin action was upon nutrient transport by measuring mammary glucose, glucose-6-P, and glutamate in normal, insulin treated, and anti-insulin treated rats (Table 1). Since mammary glucose, glucose-6-P, and glutamate (but not aspartate) were reduced by insulin and increased by anti-insulin injection, they suggested that the most limiting site of insulin action in mammary tissue is not metabolite transport. These workers (35) investigated the effects of insulin and anti-insulin serum injection upon a number of additional mammary intermediate metabolites (Table 1). They found that insulin slightly reduced and anti-insulin increased fructose-1, 6-diphosphate implying that insulin is not a positive effector of the phosphofructokinase reaction although such action might have been masked by the reduction in ATP and increase in AMP and ADP after anti-insulin injection. Triose-P were not affected by treatments while anti-insulin decreased α-glycerol-P. This observation coupled with changes in pyruvate/lactate and acetoacetate/β-hydroxybutyrate ratios was interpreted as reflecting a direct or indirect effect of insulin upon the redox state of the cell toward a more reduced condition. Since the cell energy (TP) charge was reduced, an insulin involvement with electron transport was suggested consistent with remarks by Walters and McLean (49). Subsequent studies (51) have revealed that effects of insulin upon lipogenesis are highly dependent upon cellular redox state since lipogenesis from lactate is reduced by insulin while lipogenesis from pyruvate is stimulated markedly by insulin. Artificial electron acceptors alleviated the depres-
Table 1. Effects of insulin and anti-insulin treatments on metabolites in serum and mammary tissue (35).

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Insulin treated</th>
<th>Anti-insulin treated</th>
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<tbody>
<tr>
<td>Serum glucose</td>
<td>120a</td>
<td>50</td>
<td>280c</td>
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<tr>
<td>Tissue glucose</td>
<td>601b, c</td>
<td>190b</td>
<td>2480c</td>
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<tr>
<td>Glucose-6-phosphate</td>
<td>52b</td>
<td>23b</td>
<td>105c</td>
</tr>
<tr>
<td>UDP glucose</td>
<td>198b</td>
<td>230b</td>
<td>320b</td>
</tr>
<tr>
<td>Glutamate</td>
<td>1165c</td>
<td>593c</td>
<td>1546c</td>
</tr>
<tr>
<td>Fructose-1, 6-di-P</td>
<td>4.1c</td>
<td>3.5c</td>
<td>7.2c</td>
</tr>
<tr>
<td>Fructose-P4</td>
<td>6.8c</td>
<td>6.5c</td>
<td>7.3c</td>
</tr>
<tr>
<td>α-Glycerol-P4</td>
<td>224c</td>
<td>266c</td>
<td>104c</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>58c</td>
<td>56c</td>
<td>38c</td>
</tr>
<tr>
<td>Lactate</td>
<td>1840c</td>
<td>1760c</td>
<td>2640c</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>15.5c</td>
<td>6.3b</td>
<td>16.0c</td>
</tr>
<tr>
<td>β-OH Butyrate</td>
<td>168c</td>
<td>86c</td>
<td>348c</td>
</tr>
<tr>
<td>Lactate</td>
<td>42c</td>
<td>18c</td>
<td>60c</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>4.7c</td>
<td>4.9c</td>
<td>11.7c</td>
</tr>
<tr>
<td>Malate</td>
<td>352c</td>
<td>237c</td>
<td>376c</td>
</tr>
<tr>
<td>2 (ATP) + ADP</td>
<td>.73</td>
<td>.72</td>
<td>.58</td>
</tr>
<tr>
<td>2 (ATP + ADP + AMP)</td>
<td></td>
<td></td>
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1 Expressed as mg/100 ml serum.
2 Expressed as n mol/g tissue.
3 Mean of eight observations. Horizontal values with different superscripts are different (P < .05).

...ision by insulin of lipogenesis from lactate (51).

Insulin acts upon important metabolic functions in the rat mammary secretary cells in addition to possible effects upon membrane transport and, in particular, acts upon fatty acid synthesis and acts upon or interacts with cellular electron transport processes or energy metabolism.

Only limited data are available on possible insulin actions in the regulation of ruminant mammary metabolism. Baldwin et al. (6) reported that chronic insulin administration significantly increased glucose oxidation and conversion to glyceride glycerol in lactating cow mammary tissue in vitro and also increased acetate conversion to fatty acids. This is in contrast with a rat study in which chronic insulin administration did not enhance mammary lipogenesis specifically (18). Bauman et al. (9) reported that insulin had no effect on lipogenesis from acetate at optimal or suboptimal glucose concentrations in cow mammary slices. This contrasts with ruminant adipose tissue where insulin added in vitro in the presence of glucose enhanced lipogenesis from acetate (51). Baldwin et al. (unpublished) confirmed the study of Bauman et al. (9) that insulin added in vitro to cow mammary tissue does not enhance lipogenesis from acetate and found further that insulin added in vitro did not alter patterns of glucose-1-14C, -2-14C, -6-14C or -U-14C metabolism. Thus, it appears that the acute insulin effects upon glucose metabolism and lipogenesis in the rat may not be manifest in the ruminant while chronic insulin administration increases lipogenesis and glucose metabolism in the ruminant and has lesser effects in the rat or nonruminant.

Glucocorticoids. A number of studies recently reviewed (25), have indicated that glucocorticoids are not acute or short-term effectors of mammary metabolism but do act over a longer term such that glucocorticoid insufficiency induced by adrenalectomy results in depressed lactational performance, specific changes in patterns of mammary nucleic acid synthesis and rates of synthesis of several key mammary biosynthetic enzymes, and changes in mammary metabolism. These effects are reversed by glucocorticoid administration.

Greenbaum and Darby (18), Korsrud and Baldwin (22), and Yang and Baldwin (53) have attempted to characterize and identify causes of mammary metabolic changes which develop after adrenalectomy of lactating rats by differing approaches. A key objective of these studies was to determine whether metabolic changes after adrenalectomy are due to reduced enzyme activities or produced by an alternate mechanism(s). The results of Greenbaum and Darby (18) and Yang and
Baldwin (53), largely agree that after adenalec-
tomy glucose catabolism via both the Emb-
den-Meyerhof and pentose phosphate path-
ways is reduced; the decrease in pentose cycle
flux is relatively greater than the decrease in
Embden-Meyerhof flux; fatty acid synthesis
from acetate is not impaired; and cellular ca-
pacity to produce NADPH required for fatty
acid synthesis, although reduced, is not the
factor limiting fatty acid synthesis. Greenbaum
and Darby (18) observed also that pyruvate-
1,14C oxidation and lipogenesis from pyruvate-
2,14C were reduced after adenalec-
tomy implying decreased pyruvate dehydrogenase
function. The reduction in lipogenesis from
pyruvate-2,14C was small. In other studies
(22, 53), only a slight depression of pyruvate-
1,14C oxidation, no decrease in lipogenesis
from pyruvate-2,14C, and no elevation of in-
tracellular pyruvate and lactate concentrations
were observed after adenalec-
tomy. From all the
data, it appears possible that adenalec-
tomy may reduce pyruvate dehydrogenase ca-
pacity or function somewhat but that this is
not sufficient in magnitude to explain the total
decreases in glucose oxidation and conversion
to fatty acids after adenalec-
tomy. A decrease in mammary tissue triose-P after adenalec-
tomy (22) and indirect reasoning based upon
interactions between glucose and pyruvate met-
tabolism in adenalec-tomized as compared to
normal rats imply that a possible limiting site
in glucose catabolism after adenalec-
tomy is phosphofructokinase, an enzyme which does
not decrease after adenalec-
tomy. Further
study is required for clarification of the ef-
et(s) of adenalec-
tomy on glucose metabol-
ism.

The noteworthy result of studies of the
effect of adenalec-
tomy upon mammary metab-
olism is that although the exact metabolic site
of action is not rigorously established, it has
been established clearly that several of the
metabolic changes are not attributable to re-
ductions in enzymes associated with the ef-
ected metabolic functions. The enzyme
decreases which occur after adenalec-
tomy in rats reflect an important physiological
action of the glucocorticoids, but apparently these are
not the cause of decreased glucose utilization
as an energy source and precursor of milk fat.
This observation implies that most enzymes
whose activities are regulated, in part, by
adrenocorticoids are in excess in normal ani-
imals and do not become limiting when re-
duced by adenalec-
tomy. The apparent conclu-
sion is that glucocorticoids are important in
regulation of mammary metabolism and also
are involved in regulation of enzyme synthesis.
Although it was established long ago that
hydrocortisone is essential to maximal lacta-
tional performance in ruminants (12, 17, 25,
26, 44), only limited data are available regard-
ning possible glucocorticoid actions upon mam-
mary function. Ely (16) investigated the ef-
ects of adenalec-
tomy and glucocorticoid
replacement therapy upon enzyme activities
and the metabolism of tissue slices from lactat-
ing sheep. In contrast with the rat, gluco-
corticoid insufficiency did not result in de-
creased enzyme activities in sheep mammary
tissue. In addition, mammary metabolic
changes after adenalec-
tomy in sheep, if present,
were much less dramatic than in rats.
Thus, it appears that ruminant mammary func-
tion is less dependent than rat mammary func-
tion upon glucocorticoids.

HORMONAL REGULATION OF LACTOSE
AND CASEIN SYNTHESIS AND NUCLEIC ACID
METABOLISM

Lactose. As noted above, lactose synthesis
during lactation is depressed by insulin insuf-
fiency and can be restored to normal by in-
sulin both in vivo and in vitro (34, 49).
Similarly, lactose synthesis and lactose syn-
thetase activity are reduced after adenalec-
tomy in the rat and hypophysectomy in the
rabbit and are restored partially by gluco-
corticoid or prolactin therapy, respectively
(14, 19, 22). The mechanism(s) of action of
these hormones in the regulation of lactose
synthesis during lactation have not been
studied extensively. This possibly is due in part
to the key enzyme in the pathway (lactose
synthetase) which is a complex enzyme system
requiring interaction of UDP galactosyltrans-
ferase and α-lactalbumin. This interaction has
not been fully characterized in terms of factors
other than amounts of UDP galactosyltrans-
ferase and α-lactalbumin which might affect
in vivo functions (19, 25, 26, 38). Hence, it
is not known whether the effects of cortisol
and prolactin upon amounts of the two lactose
synthetase proteins or other actions of these
hormones are primary in the regulation of lac-
tose synthesis (19).

Detailed studies (4, 38, 41) of changes as-
associated with lactose synthesis at lactogenesis
indicate that mammary UDP galactosyltrans-
ferase and particularly α-lactalbumin might be
depressed pre-partum by progesterone, thus
preventing synthesis of lactose. The sharp drop
in progesterone at lactogenesis induced by ovariectomy or normal parturition results in increased lactose synthetase proteins and onset of milk synthesis (4, 38, 13). Studies of mammary metabolites indicate quite clearly that the UDPG pyrophosphorylase, P-glucomutase, and UDP galactose-4-epimerase enzymes of mammary tissue catalyze reactions which are close to equilibrium in vivo, thus, cannot be regulatory in lactose synthesis (4, 13). These observations imply that lactose synthetase activity in vivo as influenced by amounts of its component proteins or other factors determines rates of lactose synthesis.

The observations that progesterone represses lactose synthetase proteins (13, 25); that progesterone withdrawal results in increased proteins (24, 25); and that cow mammary lactose precursors such as UDP glucose in vivo are elevated pre-partum (indicating that activity of the terminal enzyme of the pathway is not sufficient or is not functional) suggest that the roles of glucocorticoids and prolactin in regulation of lactose synthesis might be regulation of lactose synthetase. However, other mechanisms of action upon lactose synthetase cannot be ruled out. The possibility that rates of lactose synthesis are regulated by mechanisms not involving synthesis of the lactose synthetase proteins must be considered seriously in the case of insulin since its action is very fast (within an hour) and might be faster than the turnover rates (determinant of time required to produce significant changes in enzyme) would allow (23). Explanation of the action of insulin upon lactose synthesis on the basis of membrane transport or hexokinase function is not tenable in view of elevated tissue glucose, glucose-6-P, and UDP glucose in vivo in anti-insulin induced diabetes (Table 1) (35). On the other hand, the apparent turnover rate of UDP galactosyltransferase may be higher than for most mammary enzymes (23) and the turnover of alpha-lactalbumin may be quite fast due to secretion in milk. Thus, even insulin action via effects on the synthesis of the lactose synthetase proteins cannot be excluded.

Jones and Cowie (19) observed a four-fold decline in lactose synthetase activity in mammary homogenates from rabbits after hypophysectomy. Lactose synthetase was restored to normal by prolactin but not growth hormone therapy. In evaluating these observations, Jones and Cowie (19) reasoned that since the changes in lactose production observed after hypophysectomy and prolactin therapy were much greater than the changes in lactose synthetase activity, the amount of lactose synthetase may not be the main factor controlling lactose output under their conditions.

Nucleic acid and protein synthesis. Insulin, glucocorticoids, and prolactin must be present for maximal rates of nucleic acid and protein synthesis in the mammary glands of lactating animals (2, 25, 26, 37). In recent years extensive literature has developed regarding the actions of insulin, prolactin, and glucocorticoids in vitro in regulating development of secretory activity in mammary explants from, largely, mid-pregnant and pseudo-pregnant rodents. In general, these studies have indicated that prolactin, although not mitogenic in vitro, is required in vivo to "sensitize" secretory cells to insulin so cell proliferation can occur in vitro in the presence of insulin; and that secretory cell proliferation in explants cultured in vitro must occur before the synthesis of casein can be induced by additions of prolactin and a glucocorticoid. In vitro systems, complementary actions of insulin, glucocorticoids, and prolactin involving RNA synthesis and the formation of a functional rough endoplasmic reticulum (RER) are essential to the onset of casein synthesis (1). Studies of the actions of insulin, prolactin, and glucocorticoids in lactating secretory cells have not been extensive, but appear consistent with observations made in vitro systems (2, 25, 37).

Hypophysectomy of lactating ewes and rats results in reductions in rates of RNA, DNA, and protein synthesis; in mammary RNA and DNA; and, in milk synthesis (2, 15). Similar reductions in mammary RNA (RNA/DNA) and in milk production resulted from administration of prolactin anti-sera (46). The decrease in RNA after hypophysectomy appears to be due to a decrease in rates of RNA synthesis and increased RNA catabolism, and precedes the decrease in DNA (15). After hypophysectomy, DNA synthesis is depressed (2, 15) but probably not enough to explain the large decrease in DNA which occurs. Thus, it seems reasonable to speculate that, as is the case with RNA, DNA catabolism or cell death is increased. This possibility emphasizes a point which must be recognized in interpretations of data on effects of hormone insufficiencies upon such as RNA, DNA, and protein synthesis, polyribosome patterns, and enzymes, either in vivo or in vitro. If a particular hormone or group of hormones are essential to (secretory) cell maintenance and cell death.

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occurs in their absence, changes in biochemical parameters might well reflect the time sequence of degenerative events which occurs during cell death and not specific changes reflecting a lack of a hormone acting in a specific fashion upon that characteristic.

In cases where this type of danger in interpretation exists, it must be demonstrated through the use of hormone replacement therapy and under conditions where cell losses are minimal that a hormone has a specific action before the concept that a hormone acts in a specific as compared to general fashion can be accepted. Unfortunately, only limited data are available on effects of hormone replacement therapies upon RNA and DNA metabolism and protein synthesis in mammary secretory cells. These data have been reviewed previously (25) and, hence, need not be discussed in detail here. In general, reasonable mammary RNA and DNA and ratio of synthesis of all types of RNA and of DNA and protein were maintained by prolactin and glucocorticoid therapy after hypophysectomy (2, 3). Prolactin alone maintained DNA synthesis and amounts and rates of synthesis and amounts of nonribosomal RNA, ribosomal RNA, and nuclear RNA. Whether these reflected specific actions of prolactin or a general effect upon cellular integrity was not clear (2, 3).

Glucocorticoids administered alone or with prolactin stimulated RNA synthesis also but may have acted specifically to regulate syntheses of specific classes of nonribosomal and/or nuclear RNA. Both hormones were required for maintenance of normal rates of casein synthesis (2, 3). A role of prolactin in the maintenance of DNA synthesis in mammary cells is consistent with the above mentioned in vitro studies (25) and with studies of lactogenesis (11, 45). The role of prolactin as a general or, largely, nonspecific effector of mammary RNA synthesis is consistent with data with developing explants (25) and, in part, with data with postpartum tissue pieces incubated in vitro (43). The suggestion that glucocorticoids may induce synthesis of specific RNA’s agrees with data in developing explants (25); with observations of effects of adrenalectomy and replacement therapy upon patterns of RNA synthesis (3); and with specific changes in the relative activities of several nuclear RNA polymerases induced by adrenalectomy and replacement therapy (36); but not with data obtained with post-partum mammary tissue pieces incubated in vitro with various combinations of hormones (37).

POSSIBLE ROLE OF cAMP IN REGULATION OF MAMMARY GLAND METABOLISM

Despite cAMP’s importance as an effector of metabolism and an intermediate messenger in the implementation of hormonal signals within most tissues (42), the role that cAMP might fulfill in regulation of mammary metabolism and effects of hormones upon mammary cAMP have been studied only recently and not in appropriate detail. The earliest investigation of the possible involvement of cAMP in mammary development and function was conducted by Majumder and Turkington (31, 32, 33) using developing explants of mid-pregnant mouse mammary. These workers found that insulin and prolactin induced synthesis of two nonparticulate [i.e., not the casein phosphorylating protein kinase of the golgi (10)] protein kinases and of a nuclear cAMP binding protein.

The roles of the two nonparticulate protein kinases isolated from rat and cow mammary glands are not fully understood (10, 31, 32). One of these is specifically activated by cAMP and it has been suggested that by activating this protein kinase, cAMP might induce modifications of nuclear proteins which lead to the changes in patterns of RNA synthesis observed in developing mammary tissue (47). The formation of these protein kinases precedes prolactin induced phosphorylation of specific nuclear proteins (32) and occurs slightly before the increase in RNA which accompanies mammary development (28, 32). Total cytosolic protein kinase activity was not affected by adrenalectomy of lactating rats (29).

Changes during lactation due to hormones in activities of adenyl cyclase and cAMP-phosphodiesterase have been the subject of limited study (27, 28, 43). Adenyl cyclase activity assayed under conditions of maximum activation with NaF in vitro did not change significantly during late pregnancy and lactation. The activity of this important regulatory enzyme was increased after adrenalectomy of lactating rats implying that adrenal corticoids decrease mammary amounts of this enzyme (27, 28, 43). Assayed in vitro, adenyl cyclase activity was increased 1.6, 1.8, 2.4, 3.0, 3.7, and six to seven-fold, respectively, by epinephrine, prolactin, growth hormone, thyroxin, prostaglandin F2alpha, and NaF. Glucagon, insulin, and several additional hormones did not effect adenyl cyclase activity significantly as added to enzyme assay mixtures in vitro (27, 28). Sapaz-Hagar and Greenbaum (43) observed
an increase in the high \( k_m \) phosphodiesterase during lactation and no change in the low \( k_m \) cAMP phosphodiesterase while Louis and Baldwin (28) observed a sharp increase in total cAMP phosphodiesterase activity at lactogenesis, and a slow decline thereafter. Total cAMP phosphodiesterase activity increased after adrenalectomy (29).

Mammary cAMP decreased at lactogenesis leading to the suggestion that cAMP might be an inhibitor of mammary biosynthetic activity (27, 43). Preliminary studies indicated that addition of cAMP to mammary tissue slices is not a valid method for evaluation of effects of cAMP upon metabolism since the effects of cAMP are mimicked by addition of AMP to incubation media (27). Addition of the cAMP phosphodiesterase inhibitor aminophylline caused increases in cAMP in lactating mammary tissue slices and resulted in depressions in glucose oxidation and lipogenesis, also suggestive that cAMP inhibits mammary biosynthetic activity (27). Acute insulin insufficiency in vivo resulted in an increase in cAMP, and adrenalectomy resulted in a decrease in tissue cAMP (27).

A great deal of additional work is required to characterize properly effects of lactogenic hormones upon mammary cAMP and metabolism in mammary tissue and the role of cAMP in regulation of mammary metabolism. Studies of this type should receive a high priority in further investigations of hormone actions in the regulation of mammary metabolism.

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