The mammary gland of the rat undergoes profound metabolic changes after parturition. The rate of oxygen uptake, the respiratory quotient (1), and the activity of the phosphogluconic acid oxidative pathway increase markedly (2, 3). These alterations disappear within 1 or 2 days after the suckling young are weaned. The hormonal “environment” also changes at these times. After parturition, the source of placental hormones is lost, there is probably a release of prolactin from the pituitary (4), and there is a release of oxytocin in response to suckling (5). The hormonal changes after weaning are not as well documented, but there is certainly less reflex stimulation for the release of oxytocin.

Consideration of the changes in oxytocin release which parallel the changes in mammary metabolism occasioned the investigation reported here, in which an effect of oxytocin, vasopressin, and acetylcholine on glucose oxidation has been demonstrated in rat mammary gland slices.

**EXPERIMENTAL PROCEDURE**

**Methods**

Lactating rats of the Sprague-Dawley strain were separated from their young after 3 to 17 days of suckling. Twelve hours later, the adult females were killed by decapitation, the mammary tissue excised, and slices made with a Stadie-Riggs microtome. Where indicated, a similar procedure was performed on pregnant rats, pregnant or lactating albino rabbits, or lactating mice of the DBA/2 strain.

Slices of the tissue were weighed, incubated in flasks containing center-wells, and capped with rubber “vaccine” stoppers. The incubation medium was Krebs-Ringer-bicarbonate buffer. The gas phase was 95% oxygen-5% carbon dioxide, and the incubation was carried out in a metabolic shaker at 37°C. Each flask contained 0.1 μCi of radioactive glucose, with varying concentrations of carrier glucose. Hormones were added from aqueous stock solutions.

Incubations were terminated by injecting 6 N sulfuric acid to a final concentration of 0.6 N. C14O2 was collected in hyamine base (6) introduced into the center-wells, and radioactivity was determined with a liquid scintillation spectrometer using diphenyloxazole as the phosphor.

Glucose-1-C14 and glucose-6-C14 were obtained from Volk Radio-Chemical Company. Three preparations of oxytocin were studied: the partially purified ovine hormone, Pitocin (Parke, Davis and Company); the purified synthetic oxytocin, Syntocinon (Sandoz Chemical Works, Inc.); and crystalline synthetic oxytocin, the generous gift of Dr. Vincent Du Vigneaud. The three preparations were found to be of equal potency under the conditions of these experiments. Two preparations of synthetic lysine vasopressin were studied, one made by Sandoz Chemical Works, Inc., the other the gift of Dr. Du Vigneaud, and they exerted equal effects in parallel experiments. The insulin preparation used was crystalline bovine zinc insulin supplied by Eli Lilly and Company. Acetylcholine chloride was obtained from Nutritional Biochemicals Corporations.

**RESULTS**

Effect of Individual Hormones on Glucose Oxidation—Oxytocin increased the oxidation of glucose-1-C14 and glucose-6-C14 to C14O2 in mammary gland slices from lactating rats. The maximal effect was obtained at an oxytocin concentration between 10^-5 m and 10^-4 m, the saturating concentration varying from animal to animal. The mean effect on glucose-1-C14, at a hormone concentration of 10^-4 m was 49% (twelve experiments), with a range from 8% to 91%, and the mean effect on glucose-6-C14 was 18% (seven experiments), with a range from 12% to 24%.

Vasopressin and acetylcholine also increased the oxidation of glucose-1-C14 and glucose-6-C14. Vasopressin, at 5 × 10^-8 m, produced a mean stimulation of glucose-1-C14 oxidation of 39% (eight experiments), with a range from 20% to 65%. Acetylcholine, at a concentration of 10^-4 m, exerted a mean effect on glucose-1-C14 of 60% (five experiments), with a range from 26% to 92%, and a mean effect on glucose-6-C14 of 35% (two experiments, 19% and 52%). Insulin, at a concentration of 10^-8 m, elicited a mean stimulation of 75% in glucose-1-C14 oxidation (nine experiments), with a range from 23% to 182%.

A 20% effect on glucose-1-C14 oxidation was detected at an oxytocin concentration as low as 10^-3 m (0.001 μg per ml, or 0.5 milliunits per ml). At that concentration, the presence of 3.0 × 10^-12 moles of hormone resulted in an increase of 3.6 × 10^-4 moles of glucose oxidized to CO2. The lowest insulin or acetylcholine concentration at which a 20% effect was observed was

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1 Obtained through the courtesy of Dr. Rudolph Bircher.
2 Obtained through the courtesy of Dr. W. R. Kirtley.
10⁻⁸ M (equivalent to 0.0018 μg per ml of acetylcholine chloride, and 1.5 milliliters per ml of insulin).

When oxidation of the two labeled substrates was compared in parallel experiments, the absolute increment in glucose-1⁻¹⁴C oxidation caused by oxytocin, vasopressin, or acetylcholine was consistently greater than that of glucose-6⁻¹⁴C, but no reproducible ratio was observed. In several experiments, with oxytocin or vasopressin, the % stimulation of glucose-6⁻¹⁴C oxidation exceeded that of glucose-1⁻¹⁴C oxidation.

Effect of Hormone Combinations on Glucose Oxidation—In order to investigate possible differences in the mechanism of action of the hormones, experiments were performed with them in various combinations. Parallel dose-response curves were determined for the individual hormones to ascertain whether one of the hormones in the combination experiment was present at a saturating concentration. Several typical experiments, with the use of mammary gland tissue from animals in late lactation, are listed in Table I. Oxytocin and insulin had effects on the oxidation of glucose-1⁻¹⁴C which were additive, i.e. the addition of oxytocin to a saturating concentration of insulin caused a further increase in the yield of C⁴Ο₂, and vice versa. Similarly, the effects of vasopressin and acetylcholine were additive to that of insulin. However, neither vasopressin nor acetylcholine increased the stimulatory effect of oxytocin. In some experiments the effect of the combination of hormones exceeded the arithmetic sum of the individual effects, i.e. there was an apparent synergism of insulin with the other hormones.

Glucose Concentration and Hormone Effects—Table II presents the results of experiments in which the effects of oxytocin, vasopressin, and acetylcholine on glucose-1⁻¹⁴C oxidation were determined in media containing various concentrations of glucose. It can be seen that the base-line oxidation was elevated by increasing concentrations of glucose. Furthermore, the % stimulation of oxidation as a result of hormone action was greater at the higher concentrations of glucose.

Oxytocin Effects in Other Species—Oxytocin increased oxidation of glucose-1⁻¹⁴C in mammary slices from pregnant rats and rabbits, and from lactating rabbits and mice. There were no significant differences in the magnitude of the oxytocin effect in these species, or in the slices from rats at various stages of pregnancy and lactation ranging from 2 days before parturition to the 17th day of lactation. Oxytocin exerted no detectable effect on the oxidation of glucose-1⁻¹⁴C to C⁴Ο₂ in slices from rat liver.

**DISCUSSION**

Oxytocin is known as the “milk let-down factor” or “milk ejection factor” because of its stimulatory effect on the contractile cells which surround milk-secreting alveoli. Several workers have also noted increased milk yields and other beneficial effects of injected oxytocin in lactating animals (7). These effects have been attributed to more efficient emptying of milk ducts or release of anterior pituitary hormones by circulating oxytocin, or both. The results reported here suggest that part of the physiological effect of oxytocin on the mammary gland may relate to a direct stimulation of metabolism in secreting cells. It is unlikely that the observed effects on glucose metabolism in mammary slices are the result of stimulation of contractile cells or adipose tissue alone. The contractile cells constitute a small

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**Table I**

Effects of combinations of hormones on glucose-1⁻¹⁴C oxidation in rat mammary gland slices

Approximately 100 mg of tissue were incubated for one hour in 3.0 ml of Krebs-Ringer-bicarbonate buffer in 25-ml flasks, with 10.8 mg of glucose and 0.1 μc of C⁴⁻¹⁴C-labeled glucose (specific activity, 2 to 6 μc per mg). C⁴Ο₂ was collected and radioactivity determined as described under "Methods." Osmotic pressure was measured by water resorption and from lactating rabbits and mice. There were no significant differences in the magnitude of the oxytocin effect in these species, or in the slices from rats at various stages of pregnancy and lactation ranging from 2 days before parturition to the 17th day of lactation. Oxytocin exerted no detectable effect on the oxidation of glucose-1⁻¹⁴C to C⁴Ο₂ in slices from rat liver.

**Table II**

Hormonal stimulation of glucose-1⁻¹⁴C oxidation in rat mammary gland slices and effect of glucose concentration

Procedure as described in legend to Table I, but varying amounts of nonspecific glucose were added to incubation flasks. The specific activity of glucose-1⁻¹⁴C ranged between 4.0 and 7.0 μc per mg.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Glucose concentration</th>
<th>Base-line radioactivity in C⁴Ο₂</th>
<th>Amount of glucose oxidized to C⁴Ο₂</th>
<th>Effect of hormone on C⁴Ο₂ production</th>
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</thead>
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<td>20</td>
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<td>+20</td>
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+ 38 +79
fraction of the total cellular mass of mammary tissue. Adipose tissue is prominent in mammary glands early in lactation, but is almost entirely replaced by secretory tissue in late lactation. Since oxytocin exerted comparable effects on mammary slices at both stages of lactation, it is likely that oxytocin stimulates glucose oxidation by the secretory epithelium, whether a comparable effect is exerted on other cellular elements is not known.

The minimal effective concentration of oxytocin in these experiments, $10^{-9} \text{M}$, is of the same order of magnitude as the estimated concentration of oxytocin in human blood (8), and 20 times higher than the smallest concentration detected by the rat-uterus bioassay (9).

In the experiments reported here, the hormone effects were “catalytic,” i.e., the molar increments in glucose-$1\text{C}^{4}$ and glucose-$6\text{C}^{4}$ oxidation far exceeded the quantities of oxytocin, vasopressin, acetylcholine, or insulin which elicited them.

With the use of a rabbit bioassay, Cross and Van Dyke (10) showed that vasopressin exerted a milk-ejecting activity one-sixth that of oxytocin. The results of our experiments show that vasopressin is also similar to oxytocin in its stimulation of glucose oxidation in mammary slices. Acetylcholine, like oxytocin and vasopressin, stimulates contractile cells in the appropriate target organs. Thus, acetylcholine stimulates uterine contraction in vitro (9), and exerts milk-ejecting activity (11). From the results reported here, it can be concluded that acetylcholine has the further property of stimulating glucose metabolism in rat mammary slices. Acetylcholine has been found to stimulate the metabolism of other secretory tissues in vitro. Eggman and Hokin (12) have shown that it increases the incorporation of $\text{P}^{32}$ into phosphatides in slices from pancreas, salivary glands, pigeon esophageal mucosa, and adenohypophysis. Pastan et al. (13) have observed a stimulation of glucose metabolism by acetylcholine in slices from thyroid, brain, pancreas, and liver.

The present observations on the stimulation by insulin of glucose-$1\text{C}^{4}$ oxidation in mammary slices confirm those of Abraham, Cady, and Chaikoff (14) and McLean (15). The ability of a hormone further to stimulate glucose oxidation in the presence of a saturating concentration of a second hormone is considered to be evidence that the two hormones act by different mechanisms. On this basis the mechanisms of action of oxytocin, vasopressin, and acetylcholine are different from that of insulin in rat mammary slices.

High concentrations of glucose increase the base line rate of oxidation of glucose-$1\text{C}^{4}$. This confirms the results of McLean (15), and is analogous to the findings of Jeanrenaud and Renold (16) who used rat epididymal fat pads. In addition, we have shown that the effects of oxytocin, vasopressin, and acetylcholine are enhanced by high glucose concentrations. In other words, a high concentration of glucose acts in synergism with these hormones to increase glucose oxidation.

It would appear that, with increasing availability of glucose as substrate, the hormones are capable of eliciting greater stimulation of the oxidation of this substrate. Experiments designed to delineate the nature of such hormonal action are in progress.

### Summary

1. Oxytocin, vasopressin, and acetylcholine increase the oxidation of glucose-$1\text{C}^{4}$ and glucose-$6\text{C}^{4}$ in mammary gland slices from lactating rats which have been separated from their young. Insulin has a similar effect on glucose-$1\text{C}^{4}$ metabolism.

2. The effects of oxytocin, vasopressin, and acetylcholine on glucose oxidation are observed in the presence of saturating concentrations of insulin.

3. The effects of oxytocin, vasopressin, and acetylcholine are enhanced by increasing glucose concentration.

### References

Effects of Oxytocin, Vasopressin, and Acetylcholine on Glucose Metabolism in Mammary Tissue \textit{in Vitro}

Theodore L. Goodfriend and Yale J. Topper


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