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Activity Patterns of Glucose-ATP Phosphotransferases in Livers and Mammary Glands of Virgin, Pregnant, Lactating and Postlactating Mice.* (30809)

L. KOPELOVICH,† S. ABRAHAM, J. C. BARTLEY‡ AND I. L. CHAIKOFF§
Department of Physiology, University of California, Berkeley

Recent studies have shown that rat(1-7) and guinea pig livers(8) and rat epididymal fat pads(9) contain 2 molecularly distinct glucose-ATP phosphotransferases. These two enzymes have been separated from rat liver by ammonium sulfate fractionation(1,5), and from epididymal fat pads by starch-gel electrophoresis(9). One of them, glucokinase, has a low affinity for glucose, with a K_m of 10 mM; the other, hexokinase, has a high affinity for glucose, with a K_m of 0.01 mM (1). The introduction by Viñuela *et al*(1) of a simple method for assay of these 2 enzymes has facilitated study of their activities in crude homogenate fractions.

The activity of the 2 glucose-ATP phosphotransferases in the particle-free supernatant fraction (100,000 $\times$ *g* for 60 minutes) of rat liver homogenates accounts for practically all of the glucose phosphorylating activity of the whole liver homogenate(5,10). In other tissues, such as brain(5,11), small intestine(5), heart(5), thyroid(12) and lactating rat mammary gland(5,13), glucose phosphorylation has been observed in both the particulate and the particle-free supernatant fraction; but in both cellular locations in these tissues, hexokinase appeared to be the only enzyme concerned with this phosphorylation(5,12,13).

It is well established that the activity of glucokinase in liver, but not that of hepatic hexokinase, is altered by dietary changes, diabetes and insulin administration(1-8). Since other hepatic enzymes that adapt to changes in diet also change with lactation (14,15), we became interested in studying the patterns of activity of glucose-ATP phosphotransferases in the livers and the mammary glands of virgin, pregnant, lactating and postlactating mice.

Experimental. Female mice of the C_3H strain that had been raised and maintained on a nutritionally adequate diet (Purina Lab Chow) were used. Livers and mammary glands were excised from mice in the virgin state; in the prepartum state (17th and 18th day of gestation); in the postpartum state while each mouse was suckling 6 pups (17th and 18th day of lactation); and in the postlactating state (2 days after the pups had been weaned) during which the glands were undergoing regression. The mice were killed by cervical fracture, and their mammary glands and livers were rapidly excised and

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† Present address: Graduate Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

‡ Special Research Fellow of Nat. Inst. of Arthritis and Metab. Dis. (5 F3 AM-11,212-02).

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placed in an ice-cold 0.25 M sucrose solution. All preparative procedures were carried out at 2-4°.

The tissues were sliced to a thickness of 0.4 mm with the McIlwain-Buddle tissue chopper(16). To remove milk from the mammary slices prepared from lactating and regressing glands, the slices were washed 5 times with the sucrose solution. The tissue slices were minced and homogenized, and the homogenates were fractionated by centrifugation as described previously(5). The supernatant layer that formed after the homogenate was spun for 10 minutes at 350 × *g* is designated Fraction I; that obtained by spinning the homogenate for 10 minutes at 10,000 × *g* is Fraction II; and that resulting from centrifugation at 100,000 × *g* for 60 minutes is Fraction III (particle-free supernatant fraction). Mitochondria were isolated as described(12).

Glucokinase and hexokinase activities were measured spectrophotometrically by a modification(5) of the method described by Viñuela *et al*(1). Protein was estimated by the biuret method of Gornall *et al*(17). Enzyme activities are expressed either as mμmoles of TPNH formed per milligram protein per minute (specific activity), or as mμmoles of TPNH formed per gram of tissue per minute (total activity).

Results. Glucose-ATP phosphotransferases in livers of virgin, pregnant, lactating and postlactating mice. Since practically all of the hexokinase and glucokinase activity was detected in the particle-free supernatant fractions, measurements of the activities of these two enzymes were confined to Fraction III. The level of hepatic hexokinase activity was about the same in all 4 groups of mice (Fig. 1). Glucokinase activity in the liver, however, varied with the physiological state of the mouse; in the virgin mouse it was about the same as that in the pregnant mouse, a finding which confirms the observations of Walker and Rao(2) in rats, but during lactation it rose about 3-fold. Two days after weaning of the pups, hepatic glucokinase activity fell to about the levels observed in the livers of the virgin and pregnant mice (Fig. 1). The extent of increase in mouse liver

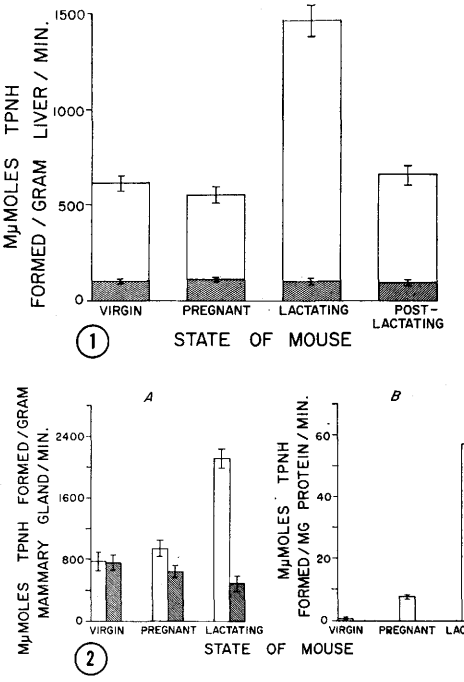

FIG. 1. *Hexokinase and glucokinase activities in liver of virgin, pregnant, lactating and postlactating C₃H mice.* Enzyme assays were performed on the 100,000 x *g* supernatant fractions (Fraction III) of liver homogenates as described in text. Crosshatched columns represent values for hexokinase activity and open columns values for glucokinase activity. Each value is the average, and its standard error (vertical line) of separate determinations with 4 mice in each state.

FIG. 2. *Hexokinase activities in mammary gland of virgin, pregnant and lactating C₃H mice.* Hexokinase activities were determined on (A) 350 x *g* supernatant fractions (Fraction I, open columns), 100,000 x *g* supernatant fractions (Fraction III, crosshatched columns) and (B) mitochondrial fractions of mammary gland homogenates, as described in text. Each value is the average and its standard error (vertical line) of separate determinations with 4 mice in each state.

glucokinase during lactation is similar to that observed in the liver of a fasted rat that had been refed glucose(4,7,18,19), and in the liver of a diabetic rat after insulin treatment(4, 18).

Regardless of the state of the mouse, hepatic glucokinase activity was 5-14 times higher than that of hepatic hexokinase (Fig. 1). A similar relation between the activities of the 2 enzymes has been observed in the livers of rats(1-5,7) and guinea pigs(8). On the other hand, in epididymal fat pads of rats

the activity of glucokinase is only about one-half that of hexokinase(9).

Glucose-ATP phosphotransferases in mammary glands of virgin, pregnant and lactating mice. Our studies indicate that, except for the gland excised from virgin mice, a considerable part of glucose phosphorylating activity in Fraction I could not be accounted for in Fraction III (Fig. 2, A). Since total activities in Fractions II and III were about the same, we inferred that mitochondria, but not microsomes, have glucose phosphorylating activity. Indeed, activity was detected in the mitochondrial fraction isolated from mammary glands of pregnant and lactating mice (Fig. 2, B). Apparently, part of the glucose phosphorylating system in mammary gland of both pregnant and lactating mice is associated with the mitochondrial fraction, the rest being in the soluble fraction of the cell.

About 10% of the glucose phosphorylated in mammary glands of virgin and pregnant mice was apparently due to the activity of glucokinase. The finding of Shatton *et al*(13) that lactating rat mammary glands are devoid of glucokinase activity is confirmed here in the lactating mouse. Thus the pronounced increase in the glucose phosphorylating capacity by lactating mammary tissue can be attributed solely to hexokinase activity.

Glucose phosphorylating activity in Fraction I was much higher in lactating than in nonlactating glands (Fig. 2, A). This activity was lower in Fraction III of mammary glands of lactating mice than in those of either virgin or pregnant mice (Fig. 2, A). The specific activity of the mitochondrial-bound enzyme in lactating mammary tissue was much higher than that in virgin or pregnant mammary gland (Fig. 2, B), a finding that may account for the high activity in Fraction I of glands excised from lactating mice.

Discussion. It has been repeatedly demonstrated that, of the 2 hepatic enzymes concerned with glucose phosphorylation, glucokinase rather than hexokinase responds to various conditions(1-8). It is also well known that some liver enzymes (for example, glucose-6-phosphate dehydrogenase) that adapt to dietary changes also exhibit higher activities with onset of lactation(14,15). It is therefore not surprising that the activity of the glucose phosphorylating system in liver is also increased during lactation and that this increase results solely from increased glucokinase activity.

The significance of the observed increase in glucose phosphorylating capacity of mammary gland during lactation is more difficult to explain than is that of liver, because of the morphological changes which occur in the transition of mammary gland from virgin to pregnant to lactating states. In contrast to liver, hexokinase is responsible for the increase in glucose phosphorylating activity of mammary gland during lactation. This increase in hexokinase activity per gram of mammary gland need not be due to the synthesis of more enzyme per cell, but reflects the higher proportion of glandular tissue in the lactating than in the nonlactating state. If hexokinase of the secretory tissue has a high specific activity and is associated predominantly with the mitochondria, an increase in proportion of secretory elements during lactation concomitant with a decrease in content of adipose tissue, in which both glucose-ATP phosphotransferases occur in the cell sap(9), could readily explain the pattern of enzyme activity observed in the present study. The increase in number of mitochondria per cell in the secretory tissue of mammary gland during the transition from pregnancy to lactation(20) would also contribute to this increased activity of hexokinase per gram mammary gland. Our findings that almost all of the glucose phosporylating activity is in the particle-free supernatant fraction of the virgin gland (largely adipose tissue), whereas most of that of the lactating gland is present in the mitochondria, are in line with the view that new glandular elements are responsible for the hexokinase activity associated with the mitochondria. In addition, the observations that glucokinase as well as hexokinase is present in the mammary glands of virgin and pregnant animals but absent in lactating glands are in agreement with the report that adipose tissue contains both of these enzymes(9).

The possibility cannot be excluded that the synthesis of more hexokinase per cell (enzyme induction) or activation of enzyme present due to the influence of the hormonal milieu during lactation accounts for the increase in hexokinase activity per gram mammary gland.

The total activity (expressed as μmoles per gram tissue per minute) of the glucose phosphorylating systems in the livers of lactating mice was of the same order as that of mammary glands in lactating mice—about 1.6 for liver and 2.1 for mammary gland. Although the rate of glucose phosphorylation by these two tissues is similar, the apparent utilization of glucose by mammary gland slices exceeds that by liver slices. Such findings are undoubtedly related to the fact that, due to the activity of glucose-6-phosphatase, liver produces glucose, whereas mammary gland does not.

Summary. 1. Hexokinase and glucokinase activities were assayed in ultracentrifugally prepared fractions of livers and mammary glands of virgin, pregnant, lactating and 2-days-postlactating mice. 2. Liver contained both enzymes and both were located in the particle-free supernatant fraction. The levels of hepatic glucokinase activities observed in virgin, pregnant and postlactating mice were similar (about 600 mμmoles of TPNH formed per gram tissue), and about one-third of those observed in the lactating mice. 3. In the mammary glands of virgin mice glucose-ATP phosphotransferase activity was present mainly in the particle-free supernatant fraction. But in the glands of pregnant and lactating mice, glucose phosphorylation (solely by hexokinase) was also detected in the mitochondrial fraction. 4. The glands of virgin and pregnant mice contained both enzymes and, in contrast to liver, hexokinase activity exceeded that of glucokinase activity. Glucokinase was not detected in glands of lactating mice. Glucokinase activity in the virgin and pregnant glands probably reflects the presence of large amounts of adipose tissue in these glands. 5. The more than 2-fold increase in glucose phosphorylating activity observed in the mammary glands of lactating mice, as compared to that in the nonlactating mice, is attributed to the increase in activity of hexokinase associated with the mitochondrial fraction. This increase in hexokinase activity is probably related to the increased proportion of secretory elements of the lactating gland.

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