The effect of bromocriptine and anti-growth hormone serum on the cholesterol economy of the lactating rat mammary gland

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Metabolic activity in the mammary gland is maintained during lactation at a level sufficient to support the increased demands of milk production by the galactopoietic hormones prolactin (PRL) and growth hormone (GH) [1]. Although PRL, rather than GH, appears to be the more important in this respect in rodents, some reports have proposed a role for the latter in lactation in the rat and mouse [2, 3]. Thus it has been estimated that, in the absence of prolactin, only 10–15% of milk production in the rat is dependent on GH [3]. When administered to ruminants, GH has been shown to increase milk yield and milk fat content [4]. The increase in milk fat was not, however, equal across the fat globule membrane [5]. The availability of an antiserum specific for rat GH (anti-rGH) [2], together with the use of the anti-PRL agent bromocriptine, has enabled the effects of GH and PRL on the activities of the three main cholesterol-metabolizing enzymes of the rat mammary gland to be investigated.

Female Wistar rats kept on a constant light cycle (lights on 08.00–20.00 h) were used at 10.30 on days 12–14 of lactation. They were injected with bromocriptine and/or anti-rGH serum with and without PRL and GH at 09.00 and 17.00 h for two successive days. Control animals were given injection carrier alone. On the morning after the last injection, the rats were anaesthetized and the abdominal mammary glands exposed and freeze-clamped. Microsomes were prepared from the glands and assayed for 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase; EC 2.3.1.26) [7] and cholesterol ester hydrolase (CEH; EC 3.1.1.13) [8] and free and esterified cholesterol [9].

HMG-CoA reductase was measured in microsomes prepared both in the presence and absence of phosphatase inhibitors ('expressed' and 'total' activities, respectively). After treatment with anti-rGH, 'total' HMG-CoA reductase activity was reduced relative to the control value (189.9 ± 24.0 versus 105.6 ± 22.1 pmol of mevalonic acid formed/min per mg of protein), while at the same time the remaining enzyme became fully active ('expressed'/'total' ratio = 100%). 'Total' HMG-CoA reductase was reduced to 26% of the control by bromocriptine (49.2 ± 11.6 pmol of mevalonic acid formed/min per mg of protein) and the 'expressed'/'total' ratio decreased to 75%. The combined anti-hormone agents caused a further slight reduction in 'total' HMG-CoA reductase without affecting the 'expressed'/'total' ratio. Administration of prolactin and growth hormone concurrently with the combined anti-hormone agents was only able to produce a small reversal of their effects so that the 'total' HMG-CoA reductase activity was still less than one-third of the original controls (60.3 ± 17.4 pmol/min per mg of protein) although the ratio of 'expressed' to 'total' remained at the control value of 88%.

ACAT activity was not altered by anti-rGH whether assayed with the cholesterol endogenous to the microsomes (control 5.88 ± 0.23 versus 5.82 ± 0.49 pmol of cholesteryl oleate formed/min per mg of protein) or in the presence of an optimal concentration of exogenous cholesterol, added as a suspension with Triton WR1339 (30.44 ± 2.83 versus 28.42 ± 2.81). Bromocriptine caused a > 2-fold increase in ACAT assayed with endogenous cholesterol, but not when the cholesterol concentration was optimized (12.44 ± 1.4 and 33.06 ± 2.39, respectively). This increase correlated with enlargement of the cholesterol and cholesterol ester pool in the microsomes, indicating that it was due to new enzymes molecules, but resulted from the availability of more substrate. Anti-rGH and bromocriptine acted synergistically to give a further increase in ACAT which was doubled in the presence of exogenous cholesterol. Although higher free and esterified cholesterol levels were again observed, this result indicates that new or more active enzyme molecules, as well as increased substrate, were responsible. PRL and GH given concurrently only partially overcame the effects of the anti-hormone agents.

CEH was not affected by anti-rGH, but bromocriptine caused a 22% increase in the enzyme activity (control 315.2 ± 14.4, bromocriptine 384 ± 21.6 pmol of cholesteryl oleate hydrolysed/min per mg of protein). Concurrent administration of the anti-hormone agents showed no appreciable effect on CEH of exogenous cholesterol, added as a suspension with Triton WR1339. This increase was doubled in the presence of exogenous cholesterol. Although higher free and esterified cholesterol levels were again observed, this result indicates that new or more active enzyme molecules, as well as increased substrate, were responsible. PRL and GH given concurrently only partially overcame the effects of the anti-hormone agents.

Abbreviations used: PRL, prolactin; GH, growth hormone; anti-rGH, antiserum to rat GH; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl CoA reductase; ACAT, acyl-CoA:cholesterol acyltransferase; CEH, cholesterol ester hydrolase.

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