Early Effects of Prolactin on Lactating Rabbit Mammary Gland

Ultrastructural Changes and Stimulation of Casein Secretion

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Summary. Effects of prolactin on the secretion of milk proteins have been investigated by incubating mammary tissue fragments from lactating rabbits. Within 15 min of adding the hormone to the incubation medium, cell morphology is modified: the relative volume occupied by the Golgi region is greatly increased. When prolactin is added immediately after a pulse labelling of proteins (3 min with $^3$H-L-leucine), the amount of labelled caseins secreted during one hour is significantly increased. This increase proceeds neither from an acceleration of intracellular transit of caseins (as shown by electron microscopic autoradiography) nor by an enhancement of amino acid uptake (as measured by incorporation of non-metabolizable amino acids) nor by an increase of overall protein synthesis, during the first hour.

The action of prolactin on the morphology of such subcellular organelles as the Golgi apparatus and its influence on casein secretion are discussed.

Key words: Lactation - Prolactin - Secretion - Caseins - Ultrastructure.

Résumé. Les effets de la prolactine, sur la sécrétion des protéines du lait, ont été étudiés grâce à des incubations de tranches de glandes mammaires de lapines en lactation.

Dès 15 min après l'addition de l'hormone au milieu d'incubation, la morphologie de la cellule est modifiée: le volume relatif des vésicules et des saccules Golgiens est fortement augmenté. Lorsque la prolactine est rajoutée, immédiatement après un marquage en 'pulse' des protéines (pendant 3 min par la L-leucine $^3$H), elle provoque, en une heure, une augmentation de la sécrétion des caséines radioactives dans le milieu d'incubation. Cette augmentation de la

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quantité de caséines sécrétées ne provient ni d’une augmentation du transit intracellulaire des protéines néosynthétisées (étudié par autoradiographie en Microscopie Electronique), ni d’une augmentation d’incorporation des acides aminés (mesurée par l’incorporation d’acides aminés non métabolisables), ni d’une augmentation de la synthèse protéique totale, dans le délai d’une heure. Les relations possibles entre l’action de la prolactine sur l’aspect morphologique d’un organite cellulaire tel que l’appareil de Golgi et sur la sécrétion des caséines sont discutées.

Introduction

Prolactin is the main constituent of the hormonal complex initiating mammary growth and lactation (Folley, 1952). In the rabbit the role of this hormone seems almost exclusive during both these periods (for mammogenesis see Denamur, 1970, 1971; for the lactational stage Cowie, 1969; Cowie et al., 1969; Linzell et al., 1975). Recent experiments with this species (Peaker and Taylor, 1975) indicate that the drug bromoergocryptine (CB 154) which inhibits prolactin release, greatly impairs milk secretion, which may subsequently be reestablished by prolactin injections. Most of these actions imply that prolactin is present for a rather extended period (a few days) and must be mainly attributed to the well studied effects of this hormone on protein and nucleic acid biosynthesis, including mRNA’s for secreted proteins (Gaye and Houdebine, 1975; Houdebine and Gaye, 1975; Shuster et al., 1976). Recent data (Falconer and Rowe, 1975), however report extemporaneous actions of prolactin on mammary tissue in vitro (Increased Na⁺ transport through the Na⁺K⁺ sensitive ATPase), which suggests that, like many other endocrine regulations, the effects initiated by this hormone may be of the “pleiotypic” kind (Hershko et al., 1971). Immediate regulatory steps of this kind, concerning intracellular transport of secreted proteins have also been demonstrated in this laboratory with dBcAMP (Ollivier-Bousquet and Denamur, 1975) and oxytocin (Ollivier-Bousquet, 1976). The present work aims to investigate whether prolactin acts on this parameter of milk secretion by an extemporaneous mechanism demonstrable in vitro; the results indicate a twofold effect of the hormone inducing both a morphological change of the Golgi region and an increase of casein exocytosis.

Materials and Methods

Animals

New Zealand rabbits have been used on the 15th day of lactation; their litters were standardized to 8 animals and the last suckling allowed to take place 3 h prior to the experiment. Bromocryptin (CB 154, Sandoz), when used, was injected subcutaneously (2 x 2 mg a day) in 70% ethanol on days 13 and 14.

Incubation Procedure

Mammary tissue fragments (weighing about 0.1–0.2 mg, total weight per assay 1.5–2.0 mg) were incubated in Krebs-Ringer bicarbonate buffer (pH 7.6, 37°C, atmosphere 95% O₂+5% CO₂)