

ABSTRACT

Properties of the Insulin Receptor in the Lactating Rat Mammary Gland

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This study aimed to determine binding characteristics of insulin receptors of lactating rat mammary gland acini. It was assumed that the use of monocomponent tyrosine A14 iodinated insulin would yield better data than that hitherto available.

Initial studies confirmed existence of specific, high affinity, saturable insulin binding at 4°C (incubated for 18h) but not at 37°C due to considerable degradation of insulin. Dissociation of bound insulin from partially filled receptors (1 - 12%) at 4°C was a slow, approximately first order process ($t_{1/2}$ approx. 2h) and was not significantly accelerated by the presence of unlabelled insulin.

Binding data from lactating mammary gland, analysed by Scatchard and Hill plots yielded unusually complex curves not obtained for rat liver or human erythrocytes. Data from binding curve of the mid-lactating gland was interpreted as a composite of binding by three classes of receptor sites -- high

affinity $K_d = 0.2 \text{ nM}$ and number $(n_h) = 1.5 \times 10^9$ sites/mg protein, intermediate and low affinity sites of total number (n_t) approximately 15×10^{10} sites/mg protein. Intermediate affinity sites contributed significantly to binding in the region $0.1 - 10 \text{ nM}$ free insulin and were detectable following 2 days starvation and prolactin deprivation, at 4 days lactation but not at day 0 and after weaning. n_h was highest (3.3×10^9 sites/mg protein) at day 0 and n_t highest at day 4 (19×10^{10} sites/mg protein). On weaning all parameters declined significantly, n_h to 0.4×10^9 sites/mg protein, n_t to 6×10^{10} sites/mg protein. 48h starvation resulted in an increase of n_h to 3×10^9 sites/mg protein without a change of K_d whereas 48h prolactin deprivation diminished n_h relative to matched controls both at 4 days and mid-lactation.

These effects are broadly consistent with the hypothesis that metabolic adaptation of the mammary gland during lactation is dependent on the occupancy of high affinity insulin receptors/unit tissue (with a possible contribution from receptors of intermediate affinity) involving a subtle interplay of insulin receptor number and affinity and also plasma insulin concentration.

Wheat germ agglutinin (5ug/ml) did not appear

to affect the general shape of the Scatchard curve, however it substantially increased both n_h and n_t (to 5×10^9 and 25×10^{10} sites/mg protein respectively) as well as Kd_h to 0.3nM. 1mM-Vanadate ion had complex effects on insulin binding depending on the nutritional state of the animal:- Acini from fed animals showed a small decline of n_h (to 1×10^9 sites/mg protein) without significant changes of n_t or Kd_h ; whereas acini from starved animals showed an increase of n_h (to 11×10^9 site/mg protein) and Kd_h to 0.8nM. Intermediate affinity sites were not detected in either case. Increased phosphorylation of the β -subunit of the insulin receptor may have contributed to these effects.