CONCANAVALIN A INDUCED ALTERATIONS IN ^{125}I-LABELED PROLACTIN BINDING

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SUMMARY

^{125}I-labeled ovine prolactin binding to receptors in the liver of female rats is markedly inhibited by Concanavalin A (Con A). *Lens culinaris* agglutinin and succinyl-Con A inhibit binding to a much lesser extent and wheat germ agglutinin and phytohemagglutinin-P are without affect. Con A inhibition is dose related and saturable; however, 30% of prolactin receptors are unaffected by Con A. α-Methylmannoside or α-methylglucoside, both potent inhibitors of Con A binding, reverse the inhibition. Con A also inhibits prolactin binding in other normal target tissues and in neoplastic rat mammary tissue. In contrast, ^{125}I-labeled Con A binding to rat liver is unaltered by prolactin. These results suggest that Con A binding sites may not be identical to the prolactin receptor and that prolactin receptors may exist in two domains on the cell surface - one of which is in close proximity to Con A binding sites and another which is more distant.

The plant lectin Concanavalin A (Con A) binds to specific carbohydrate determinants on the cell surface of a variety of mammalian cells. This interaction can cause a number of diverse biological effects including cell agglutination (1), mitotic induction (2, 3), restoration of contact inhibition of growth (4), and mimicking the effect of polypeptide hormones (5, 6). The mechanism by which Con A causes alterations in cell function and behavior is not well understood; however, it is believed that some of its effects may be mediated through modifications of cell surface topography (7, 8).

Recently, Con A has been shown to bind specifically to rat liver plasma membranes (9, 10), modify the activity of liver membrane 5'-nucleotidase (10) and Na^+-K^+ ATPase (11) and to inhibit insulin binding to liver (5).

To gain insight into the mechanism of prolactin binding to its receptor, we have investigated the effect of Con A on prolactin receptors in the rat liver, a known prolactin target tissue (12). In this report, we show that Con A specifically alters prolactin binding in the rat liver and that the inhibitory

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effects of Con A occur in all normal prolactin target tissues as well as neoplastic rat mammary tissue.

METHODS

Ovine Prolactin Iodination: The iodination reaction was carried out at 22°C in a polystyrene tube (12 x 20 mm) in 50 mM sodium phosphate buffer, pH 7.5. To 70 µl of buffer, 5 µl (5 µg) ovine prolactin and 10 µl lactoperoxidase, (4×10^{-6} M) were added. The reaction was started by the addition of 10 µl ^{125}I (1.0 mCi in 120 µl) followed immediately by 5 µl H_2O_2 (22 µM). Addition of ^{125}I and H_2O_2 was repeated 11 times at 1 min intervals. A final aliquot of H_2O_2 was added and 2 minutes later the reaction was diluted with 10 mM sodium phosphate pH 7.0. Free and protein-bound ^{125}I was separated on a 1 x 16 cm Sephadex G-25 column prepared as previously described (13). The pooled G-25 void volume was applied to a 1 x 6 cm column of bovine serum albumin-saturated DEAE-cellulose (13). After washing the DEAE column with 40 ml of 10 mM sodium phosphate, pH 7.0, the ^{125}I-labeled prolactin was eluted with 40 ml of 30 mM sodium phosphate, pH 7.0. The peak, which contained approximately 40% of the applied radioactivity, was pooled and stored (no more than 2 weeks) at -70°C in small aliquots until use. Specific activity averaged 67 Ci/gm.

Prolactin Receptor Assay: Binding of ^{125}I-labeled prolactin was determined in a 20,000 x g particle fraction of livers from 200 gm virgin female Sprague-Dawley rats with contains 60-80% of the total prolactin binding sites present in liver homogenates (14). Assays were run in 12 x 75 ml polyethylene or polypropylene tubes in 0.5 ml incubation buffer (25 mM sodium phosphate, 10 mM $MgCl_2$, 0.1% bovine serum albumin, pH 7.0) containing 200,000 cpm ^{125}I-labeled prolactin, 50-250 µg liver particles $\pm$ 1 µg unlabeled prolactin. After incubation at 22°C with shaking, 2.5 ml ice-cold incubation buffer was added and the particles sedimented at 20,000 x g for 7 min. The pellets were washed with 2.5 ml ice-cold 10 mM sodium phosphate and then dissolved in 0.5 ml 0.1 N NaOH. Bound radioactivity and protein (15) were determined in the dissolved pellets. Specific prolactin binding to receptors was defined as the difference in bound radioactivity between particles incubated with ^{125}I-labeled prolactin alone and ^{125}I-labeled prolactin plus excess unlabeled prolactin (non-specific binding). Non-specific binding was 5-20% of total binding. Assays for prolactin binding to other target tissues and 7,12-dimethylbenz(a)-anthracene (DMBA)-induced mammary tumors were performed as described above except that microsomal particles were used (16). Mammary tumors were induced in 50-55 day old virgin female Sprague-Dawley rats with 20 mg DMBA dissolved in sesame oil and administered by gavage (17). After tumors appeared, growth was measured twice weekly with calipers and only growing tumors were used.

Con A Binding: Con A was iodinated with Chloramine T as described by Cuatrecasas (18). Iodinated lectin was separated from ^{125}I and purified on Sephadex G-100. Bound ^{125}I-labeled Con A was eluted with 0.2 M acetic acid (18). The purified ^{125}I-labeled Con A solution was neutralized by addition of dry Tris base, diluted with incubation buffer and then added to liver particles. After incubation at 22 C for 3 hours, the particles were sedimented, washed and bound ^{125}I-labeled Con A and protein determined.

Reagents: Lactoperoxidase was kindly supplied by Dr. M. Morrison and was prepared according to Morrison and Hultquist (19). Ovine prolactin (NIH-P-S12) was obtained from NIAMD. Concanavalin A was from Sigma; wheat germ agglutinin, Calbiochem; phytohemagglutinin P, Difco; carrier-free ^{125}I, Schwarz-Mann. Succinyl-Con A was a gift of Dr. Kenneth Noonan and was prepared according to Gunther, *et al.*, (20); and *Lens culinaris* agglutinin, prepared by the method of Howard, *et al.*, (21) was a gift of Dr. David Phillips.

TABLE 1

Effect of Various Lectins on ^{125}I-Labeled Prolactin Binding to Rat Liver

Lectin	Competitor	Percent Inhibition	N
Con A (200 µg/ml)	--	72 ± 3	11
Con A (200 µg/ml)	α-methylmannoside (50 mM)	15 ± 2	9
Con A (200 µg/ml)	α-methylglucoside (50 mM)	26 ± 3	3
Succinyl-Con A (400 µg/ml)	--	32 ± 3	4
Succinyl-Con A (400 µg/ml)	α-methylmannoside (50 mM)	16 ± 3	4
Lens culinaris agglutinin (400 µg/ml)	--	20 ± 3	2
Lens culinaris agglutinin (400 µg/ml)	α-methylmannoside (50 mM)	2 ± 3	2
Wheat germ agglutinin (400 µg/ml)	--	31 ± 6	5
Wheat germ agglutinin (400 µg/ml)	N-acetylglucosamine (200 mM)	24 ± 4	2
Phytohemagglutinin-P (400 µg/ml)	--	20 ± 2	5
Phytohemagglutinin-P (400 µg/ml)	N-acetylgalactosamine (200 mM)	27 ± 6	2

Lectins at the indicated concentrations ± monosaccharide competitors were incubated for 3 hours with liver particles and ^{125}I-labeled prolactin ± 1 µg unlabeled prolactin. Bound radioactivity was determined as described in Methods. Each value is the mean percent inhibition ± S.E. of specific prolactin binding in the absence of lectin for N number of experiments.

RESULTS AND DISCUSSION

Con A markedly inhibited ^{125}I-labeled prolactin binding to rat liver while *Lens culinaris* agglutinin, Succinyl-Con A, wheat germ agglutinin, and phytohemagglutinin-P inhibited binding to a much lesser extent (Table 1). When monosaccharide competitors for each lectin were included in the reaction,

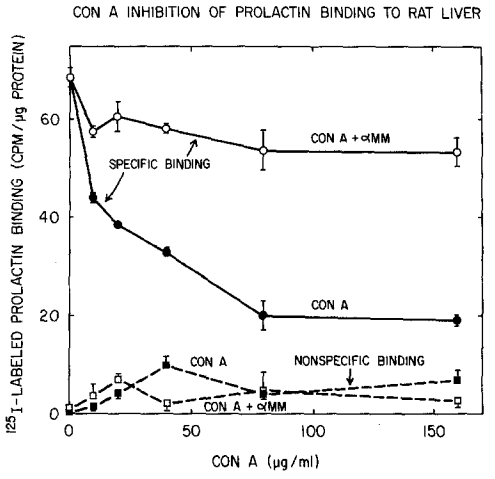

FIGURE 1 - Con A inhibition of ^{125}I-labeled prolactin binding to rat liver receptors. Liver particles were incubated for 3 hrs. with the indicated concentrations of Con A ± 50 mM α-methylmannoside (αMM) and ^{125}I-labeled prolactin ± 1 µg unlabeled prolactin. Bound radioactivity was determined as described in Methods. Each point is the mean ± S.E. for triplicate samples. Protein averaged 230 µg per assay.

only the effect of those lectins which bound to available α-methylmannosyl or α-methylglucosyl residues were reversed suggesting that the effects of the other lectins were non-specific. Maximal inhibition of specific prolactin binding by Con A was observed at 80 µg/ml (Figure 1). Approximately 30% of the specific binding, however, was unaffected by Con A, even at concentrations up to 400 µg/ml. An identical concentration dependent inhibition of prolactin binding by Con A was also obtained with plasma membranes prepared by the method of Lesko, *et al.*, (22) (not shown). Non-specific prolactin binding was usually increased modestly by Con A; however, the increase was not reversed by α-methylmannoside and could not account for the effect on specific prolactin binding. Inhibition of prolactin binding was not a result of ^{125}I-labeled prolactin binding to Con A itself, making it unavailable to interact with receptors, since ^{125}I-labeled prolactin was not retained on Con A-Sepharose 4B columns (not shown).

The Con A effect was not limited to liver prolactin receptors as shown

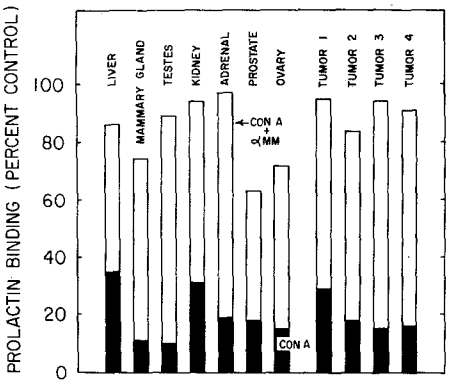

FIGURE 2 - Con A effect on ^{125}I-labeled prolactin binding to target tissues. Microsomal particles prepared from the indicated tissues were incubated in triplicate with 200 μg/ml Con A ± 50 mM α-methylmannoside (αMM) and ^{125}I-labeled prolactin ± unlabeled prolactin. Following incubation for 3 hrs., particles were pelleted, washed and bound radioactivity and protein determined as described in Methods. The height of each bar is the percent of specific prolactin binding in the absence of Con A or αMM.

in Figure 2. Prolactin binding to receptors from all target tissues tested including neoplastic rat mammary tissue was markedly reduced, indicating that the effect was a constant feature of prolactin receptors regardless of the target tissue. Differences were apparent in some tissues in the degree of Con A inhibition and reversal by α-methylmannoside. Whether this is a quality of the particular target tissue or a result of less than optimal assay conditions is not presently known.

To determine if prolactin and Con A were competing for the same binding site, the effect of prolactin on ^{125}I-labeled Con A binding to rat liver was examined (Figure 3). Binding of ^{125}I-labeled Con A was progressively reduced by unlabeled Con A reaching saturation at approximately 100 μg. Prolactin, however, at concentrations ranging from physiological (10 ng) to supraphysiological (1,000 ng) failed to alter Con A binding regardless of the degree of occupancy of the Con A binding sites. Since prolactin was unable to alter ^{125}I-labeled Con A binding, it is possible that the lectin and hormone did not interact at the same binding site in contrast to its interaction with the insulin receptor (5). The existence of a small class of unique Con A binding

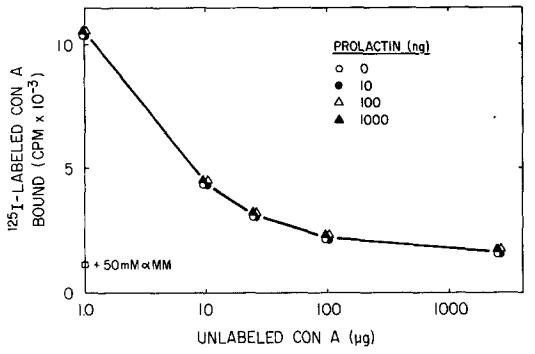

FIGURE 3 - Effect of prolactin on ^{125}I-labeled Con A binding to rat liver. Rat liver particles (56 μg) were incubated with 20,000 cpm ^{125}I-labeled Con A and the indicated amounts of unlabeled Con A and ovine prolactin for 3 hrs. in 0.5 ml incubation buffer. ^{125}I-labeled Con A binding was determined as described in Methods. Each point is the mean of triplicate samples.

sites on the prolactin receptor, however, cannot be totally eliminated by these experiments, since we have found that there are approximately 2,500 more Con A binding sites than prolactin receptors in our liver preparations (unpublished observations). Current experiments to determine if solubilized prolactin receptor binds to Con A affinity columns may clarify this point.

Nonetheless, these findings indicate that occupation of available α-methyl glucosyl or α-methylmannosyl residues on the cell surface specifically inhibits prolactin binding to its receptor. Because a significant proportion of prolactin receptors are resistant to Con A, it is possible that prolactin receptors may reside in two distinct domains on the cell surface - one rich in Con A binding sites and one in which Con A binding sites are sparse. Whether Con A can alter prolactin responsiveness or if these two types of prolactin receptors are responsible for distinct prolactin effects is currently under investigation. The observation that Con A is a much more potent inhibitor than Succinyl-Con A or *Lens culinaris* agglutinin suggests that crosslinking of lectin binding sites by the native tetravalent Con A molecule may be required for maximal inhibition. An analogous mechanism may also account for Con A inhibition of liver plasma membrane 5'-nucleotidase (23).

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