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Serum Prolactin Levels and Prolactin Binding Activity in Adrenals and Kidneys of Male Rats After Dehydration, Salt Loading, and Unilateral Nephrectomy (38769)

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Prolactin (PRL) has been shown to have a multiplicity of actions in teleost fish, amphibia, reptiles, birds, and mammals, including a role in salt and water regulation (1). PRL was reported to decrease urinary Na excretion in the rat (2). Recent work by Relkin (3) indicated that infusion of hypotonic saline solutions reduced serum PRL levels, whereas hypertonic solutions increased serum PRL while reducing pituitary PRL. In man PRL has been reported to influence renal function by decreasing water and Na excretion (4). Plasma osmolar changes were shown to influence secretion of PRL (5) with serum PRL levels paralleling changes in serum osmolarity. It also has been observed that patients with chronic renal failure had elevated levels of PRL in the serum (6, 7).

Recently, PRL receptor binding was demonstrated in a variety of target organs including the kidneys and adrenals (8, 9). The present study was undertaken to determine the effects of unilateral nephrectomy, dehydration, and salt loading in the rat, all of which alter the osmoregulatory system, on serum PRL levels and PRL binding activity in the kidneys and adrenals.

Materials and Methods. Mature, male Sprague–Dawley rats weighing 225–250 g each were obtained from Spartan Research Animals (Haslett, MI). They were allowed to acclimate to their surroundings for 3 days. The rats were housed in a temperature-controlled (25 ± 1°) and artificially illuminated room (lights on from 5:00 AM until 7:00 PM daily), and received food and water ad lib.

On the morning of the third day after their arrival, treatments were begun as follows: the first group was given 1.5% NaCl ad lib. in place of tap water. No water was given to the second group. The third group was unilaterally nephrectomized under light ether anesthesia and given tap water ad lib. Intact control rats were permitted access to tap water ad lib. All groups had free access to Purina Lab. Chow. At the end of 48 hr all animals were bled via cardiac puncture under light ether anesthesia to obtain samples for determination of serum PRL. The animals were then killed by decapitation and their adrenals and kidneys were removed immediately, placed on dry ice and stored frozen until assayed for PRL binding activity. In the unilateral nephrectomized group, the adrenals and remaining kidney were removed for assay of PRL binding activity.

Both kidneys from each rat were homogenized in 0.3 *M* sucrose and the microsomal membranes were collected according to the methods of Shiu, Kelly, and Friesen (10). In the nephrectomized group, the remaining kidney was homogenized. Each kidney membrane sample used for assay contained 1000 μg of protein as determined by the method of Lowry *et al.* (11). The adrenal glands from two animals were pooled and homogenized in a similar manner, and each membrane sample used in the assay contained 200 μg of protein.

Ovine PRL was iodinated by a lactoperoxidase method using the method of Gelato *et al.* (12). The specific binding of ^{125}I-labeled prolactin to the microsomal membranes was determined by methods previously described by Shiu, Kelly, and Friesen (10), with minor modifications, i.e., the incubation was performed in Dispo Culture Tubes in quadruplicate at 4° for 48 hr, after which time 3 ml of 0.1% Bsa–Tris–Ca^{2+} buffer was added and centrifuged at low speed to yield a discernible pellet. Pellets were then counted in a Nuclear-Chicago 1185 automatic gamma counter. Total binding refers to counts bound to the pellet in the absence of unlabeled hormone, whereas nonspecific binding refers to binding to the pellet

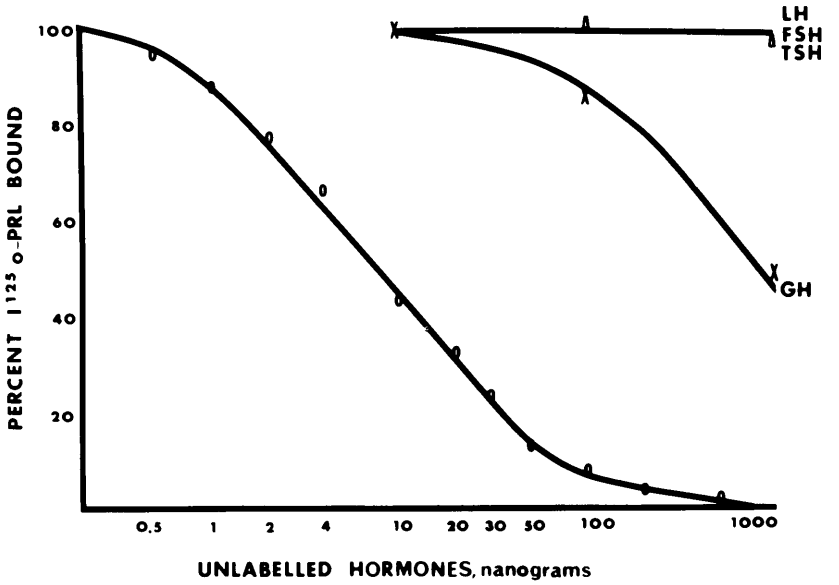

FIG. 1. Binding of ^{125}I-labeled PRL to rat kidney membranes with LH, TSH, FSH, and GH. The abscissa denotes the concentration of unlabeled *o*-PRL and the ordinate represents the amount of radioactivity bound to the membranes expressed as a percentage of the control incubations in which no unlabeled hormone was present.

with an excess of unlabeled hormone. Specific binding is the total binding minus nonspecific binding.

The following hormones were used to test cross reactivity with the labeled PRL: ovine PRL (NIH-S10; 25.6 units/mg); ovine GH (NIH-S11; 0.56 IU/mg); ovine LH (NIH-S5; 0.99 USP units/mg); ovine TSH (NIH-S6; 2.47 USP units/mg); and ovine FSH (NIH-S7; 30.6 IU/mg).

Plasma PRL concentration was determined by the double-antibody radioimmunoassay method described by Niswender *et al.* (13). The standard reference preparation was NIAMD-rat prolactin RP-1. One-way analysis of variance was used for all computations, and the means were compared by using Duncan's Multiple Range Test (14).

Results. Figures 1 and 2 depict the specific binding of ^{125}I-labeled PRL to kidney and adrenal microsomal membrane pellets. LH, TSH, FSH, or GH were not able to displace the labeled PRL from the pellets. Ovine GH showed a slight cross reactivity at the 1-μg level, but this could be accounted for by the stated contamination of the NIH preparation with PRL. The specificity of PRL binding to kidney and adrenal tissues was demonstrated by the ability of nonlabeled PRL to readily displace the labeled PRL.

PRL binding activity in the kidney homogenates (Table I) was significantly reduced ($P < 0.05$) after 2 days of water deprivation, whereas a 1.5% saline solution and unilateral nephrectomy were without effect. Dehydration significantly increased PRL binding to adrenal homogenates ($P < 0.01$) as compared with controls (Table II). Salt-loaded animals also showed a significant rise in PRL binding ($P < 0.05$) as compared with the controls.

Serum PRL levels were significantly elevated after dehydration ($P < 0.01$) and unilateral nephrectomy ($P < 0.05$) (Table III). Salt loading had no significant effect on serum PRL values, although a small increase was seen.

Discussion. These results demonstrate that PRL binding by the kidneys and adrenals is specific and does not cross react with other anterior pituitary hormones. They also show that the sensitivity of the binding sites to displacement by unlabeled PRL is within the range of the endogenous levels of PRL found in the rat. Thus, levels of .5 ng or more of unlabeled oPRL readily displaced ^{125}I ovine PRL (Fig. 1).

The changes found in PRL receptor binding activity in the kidneys as a result of dehydration lends further support to the hy-

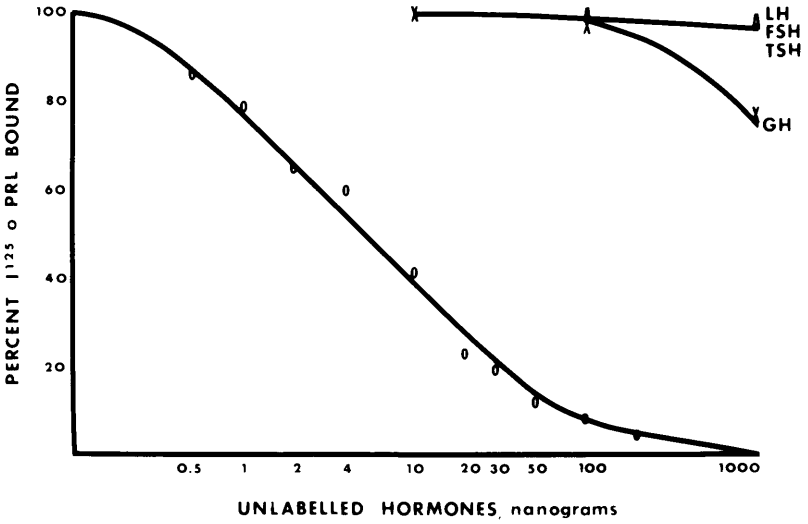

FIG. 2. Binding of ^{125}I-labeled prolactin to rat adrenal membranes with LH, TSH, FSH, and GH. The abscissa denotes the concentration of unlabeled *o*-PRL and the ordinate represents the amount of radioactivity bound to the membranes expressed as a percentage of the control incubations in which no unlabeled hormone was present.

TABLE I. PRL BINDING ACTIVITY IN KIDNEY HOMOGENATES OF DEHYDRATED, SALT-LOADED (1.5% NaCl IN DRINKING WATER), AND UNILATERALLY NEPHRECTOMIZED MALE RATS.

Treatment and no. of rats/group	% Specific binding of ^{125}I-*o*PRL
1 Controls (10)	5.47 ± 0.37[a]
2 Dehydration (8)	4.13 ± 0.38[b]
3 Salt load (9)	6.00 ± 0.45
4 Unilateral nephrectomy (12)	5.12 ± 0.50

[a] Standard error of mean.
[b] $P < 0.05$ as compared to controls.

TABLE II. PRL BINDING ACTIVITY IN ADRENAL HOMOGENATES OF DEHYDRATED, SALT-LOADED (1.5% NaCl IN DRINKING WATER), AND UNILATERALLY NEPHRECTOMIZED MALE RATS.

Treatment and no. of rats/group	No. of pooled samples/ group	% Specific binding of ^{125}I-*o*PRL
1 Controls (10)	5	4.78 ± 0.43[a]
2 Dehydration (10)	5	6.78 ± 0.42[b]
3 Salt load (10)	5	6.09 ± 0.23[c]
4 Unilateral nephrectomy (12)	6	4.99 ± 0.24

[a] Standard error of mean.
[b] $P < 0.05$ as compared to controls.
[c] $P < 0.01$ as compared to controls.

TABLE III. PLASMA PRL LEVELS IN DEHYDRATED, SALT-LOADED (1.5% SALINE AS DRINKING WATER), AND UNILATERALLY NEPHRECTOMIZED MALE RATS.

Treatment and no. of rats/group	Plasma PRL levels (ng/ml)
1 Controls (10)	12.93 ± 1.17[a]
2 Dehydration (10)	31.04 ± 0.43[b]
3 Salt load (10)	15.01 ± 1.91
4 Unilateral nephrectomy (13)	19.58 ± 0.90[c]

[a] Standard error of mean.
[b] $P < 0.05$ as compared to controls.
[c] $P < 0.01$ as compared to controls.

pothesis that of PRL is involved in salt and water regulation by some action on the kidneys (2, 4), although the nature of this action is unknown at present. PRL binding activity in the kidneys previously had been demonstrated by using whole-body autoradiography and microautoradiography of the kidneys, after administering lactoperoxidase ^{125}I oPRL (15). After injection, the strongest labeling was clearly concentrated in the proximal tubular cells.

The changes in PRL receptor activity in the adrenals may reflect alterations in osmoregulation by the aldosterone system. A report by Relkin and Adachi (16) indicates that PRL may enhance aldosterone secretion

in the Na-deprived rat. Work is presently in progress to test this hypothesis.

In human patients with chronic renal failure, elevated levels of plasma PRL were observed (8, 9), which is similar to the rise in serum PRL found after unilateral nephrectomy in our rats. However, salt loading had no significant effect on plasma PRL values, although a small rise was found. It is possible that use of a greater salt load would have resulted in a significant elevation in serum PRL. Dehydration of female rats for 12 hr was found to produce a decrease in pituitary PRL levels (17), which may reflect an increase in release of PRL. It is concluded that PRL has a role in salt and water metabolism in the rat, and that prolactin receptors in the kidneys and adrenals play some part in this regulatory system.

Summary. Serum prolactin (PRL) levels and PRL binding activity in microsomal membranes from kidneys and adrenals were measured in control, water-deprived, unilaterally nephrectomized, and salt-loaded male rats. Unilateral nephrectomy and water deprivation increased serum prolactin levels significanty. Unilateral nephrectomy did not alter PRL binding activity in the kidneys, but significantly increased it in the adrenal glands. Salt loading had no effect on serum prolactin levels or PRL binding in the kidneys, but significantly increased PRL binding in the adrenal glands. Inhibition curves and tests of cross reactivity with LH, FSH, TSH, and GH showed that binding of PRL to its receptors in the kidneys and adrenals was specific. These observations suggest that PRL has a role in salt and water metabolism and that PRL receptors in the kidney and adrenals participate in this regulatory system.

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