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Prolactin: a Hormonal Regulator of the Neonatal Tissue Water Reservoir

DAVID M. COULTER[33]

Department of Pediatrics, Harvard Medical School, and Department of Pediatrics, Boston University School of Medicine, Boston, Massachusetts, USA

Summary

Groups of newborn rabbits were treated with exogenous prolactin, with fluphenazine (a stimulant of endogenous prolactin secretion) with bromocriptine (a blocker of endogenous prolactin secretion), or with bromocriptine plus exogenous prolactin, and lean body hydration in these animals was compared with that of untreated controls. Animals treated with prolactin or fluphenazine retained more water than did the controls. Bromocriptine-treated animals retained less water than did the controls. Exogenous prolactin abolished the effect of bromocriptine.

Abbreviations

GH, growth hormone
LBW, lean body water
TBW, total body water

The human neonate loses 7–11% of total body water during the first 3 days of life (12, 22, 29). This loss occurs whether or not the infant receives an adequate fluid intake (18). It has been suggested that the water lost represents "a reserve for excretory needs," which might explain the unique ability of newborns to tolerate severe restriction of fluid intake (18). In an earlier study we examined neonatal water loss in the rabbit (7). The animals lost 11.6% of TBW during the first 72 h of life. More detailed analysis of these data revealed an unanticipated relationship between postnatal weight gain at 72 h, an index of fluid and calorie intake, and lean body hydration. Animals, whose intakes were high, retained substantially less water than did those whose intakes were low. There was a highly significant, inverse, linear correlation between fluid intake, as reflected in weight gain, and LBW content. We inferred from these data that mobilization of neonatal tissue water stores is controlled in response to the adequacy of fluid intake. We speculated that this process might be regulated by one or more of the group of hormones whose levels are elevated in the fetus during late gestation and decline during the first days of postnatal life. Included in this group are estrogen, progesterone, and aldosterone (4), renin (15), cortisol (25), and prolactin (1, 14, 32).

We chose first to investigate the effect of prolactin on lean tissue hydration at 72 h of life. Groups of neonatal rabbits were treated with exogenous ovine prolactin, with fluphenazine, a drug which stimulates endogenous prolactin secretion (9), with bromocriptine, a drug which blocks prolactin secretion (13), or with bromocriptine plus exogenous prolactin. Lean tissue water content at 72 h of age in these treated animals was compared with that of untreated or saline-treated controls. Preliminary results of these investigations have been published in abstract form (6).

MATERIALS AND METHODS

Animals. Healthy, timed gestation, New Zealand white rabbit does at 28–29 days gestation were obtained from local breeders. They were housed in standard cages with hay-filled nesting boxes under continuous fluorescent light conditions and were fed Purina Rabbit Chow. They were observed at least twice daily to determine the time of parturition. When pups were discovered in the cage, we weighed them immediately, gave the appropriate treatment, and returned them to the does for care.

Fluid intake was estimated from postnatal weight gain. The alternative approach was to administer fixed volumes of artificial formula to each pup via tube feedings. This alternative was rejected because of the probability that excessive handling and the trauma of tube feedings would increase serum prolactin levels (2, 11). The treatment for a given litter was determined randomly. Based on our previous experience, we planned to compare treatment and control groups of 20–30 surviving pups from 3–5 different litters. Any animals which appeared dehydrated or sick at 72 h of age were eliminated from the study.

Controls. Twenty-two animals from six litters were sacrificed at birth for baseline water measurements. Twenty animals from five litters were returned to the does and were not handled again until sacrifice at 72 h of age. Seventeen animals from three litters were treated with buffered saline, 0.05 ml subcutaneously on the ventrolateral thoracic wall every 12 h. This was the same solution which we used to dissolve the prolactin.

Prolactin treatment. Thirty-five animals from five litters were treated with exogenous prolactin. Each pup was injected subcutaneously on the ventrolateral thoracic wall with 1 international unit (IU) ovine prolactin (0.05 ml solution) every 12 h until sacrifice at 72 h of age. The ovine prolactin, 32 IU/mg (Sigma Corporation) was stored in a freezer until needed. The manufacturer states that this preparation is free of oxytocin, antidiuretic hormone, and all anterior pituitary hormones except GH. GH is present in a quantity less than 0.03 units per mg or 1 unit per 1000 units prolactin. On the morning of parturition it was dissolved in sterile buffered saline (0.85% NaCl, 0.1 M $NaHCO_3$; pH 8.8–9.0) (17) to a final concentration of 20 IU/ml. The solution was stored in individual plastic syringes until needed and was never refrozen.

Phenothiazine treatment. Fourty-seven pups from eight litters were treated with fluphenazine. The preparation used was fluphenazine enanthate in sesame oil, 25 mg/ml (Prolixin, E.R. Squibb and Sons). This is a "depot" formulation, which is administered to human patients every 2 wk. This type of preparation was chosen to minimize handling of the pups. The dose employed (0.03 ml = 0.75 mg) is the equivalent per kg to the dose for human adults. It was administered subcutaneously on the ventrolateral thoracic wall when the pups were weighed at birth.

Bromocriptine treatment. Twenty-seven pups from four litters were treated with bromocriptine (2Br-α-ergocriptine mesylate; CB-154, Sandoz Pharmaceuticals). The dose used was 0.067 mg/day, approximately 1.25 mg/kg/day. The drug and an equal weight of tartaric acid were dissolved in absolute ethanol and then mixed with 0.85% saline to the desired concentration. Solutions were prepared on the morning of parturition and stored refrigerated in plastic syringes until used. Each pup received 0.05 ml daily, subcutaneously on the ventrolateral thoracic wall.

Bromocriptine plus prolactin. Fifteen pups from two litters received 0.67 mg/day bromocriptine plus 1 IU prolactin twice daily. Because we were only interested in low weight gain animals, both litters were separated from the does at 36 h of age to restrict their fluid intake.

Tissue water determinations. The pups were reweighed at 72 h of age, decapitated, and exsanguinated. A portion of dorsal skin was immediately removed, weighed, and minced with scissors. Then, in rapid sequence, both quadriceps femoris muscles and the brain were removed, weighed, and minced. The remainder of the carcass was homogenized in a blender. The carcass and organ specimens were then dried in a 93C, forced air oven to constant weight. The carcass specimen was pulverized in a mortar and pestle and an aliquot taken for fat extraction. This aliquot and the organ specimens were then repeatedly extracted with petroleum ether and air dried again to constant weight in the oven. Fat and water content were calculated by the method of differences.

Data analysis. Grouped data were compared using the *t* statistic for 2 means program for the Hewlett-Packard 67 calculator. Linear regressions were calculated by the method of least squares using the HP-67 Stat Pac program. Regression lines were compared with an analysis of covariance (30).

RESULTS

Survival and postnatal growth. Mortality did not differ significantly between treated groups and untreated controls. As in our previous report, weight gain over the first 72 h of life varied markedly, both within and among litters (13% loss to 75% gain). These values and the main weight gain of 30% of birthweight in control animals are similar to reported growth standards for rabbits (8, 16). At sacrifice the animals which gained little weight were uniformly deficient in interscapular, subcutaneous, and hepatic fat. There was little or no milk present in the gastrointestinal tract. In contrast, the animals who gained large amounts of weight had partially digested milk in the stomach and intestines and had copious amount of body fat. Body fat content was linearly related to weight gain ($r = 0.872$, $n = 28$, $P < 10^{-9}$). It is apparent that weight gain at 72 h is directly related to milk intake and, therefore, to fluid and calorie intake.

Saline-treated controls. These animals were virtually identical in postnatal weight gain to the untreated controls (Fig. 1). The two groups have been combined for comparisons with treated groups.

Exogenous prolactin treatment. The hydration of prolactin-treated animals was significantly greater than that of untreated controls ($P < 0.00001$). The effect of treatment was negligible at low weight gains and increased as weight gain increased (Fig. 2).

Fluphenazine treatment. Treated animals were active, vigorous, and showed no signs of sedation. The pattern of response was similar to that of exogenous prolactin. Tissue water was greater than in control animals, particularly at higher weight gains (Fig. 3). The effect at high weight gain was less than that of prolactin (t- 5.287, df-37, $P < 0.00001$).

Bromocriptine. The mean weight gain in this group was only 18.7 g. There was usually significant erythema and occasionally skin necrosis at the injection site. These animals were compared only with controls who gained <30% of birthweight (Fig. 4). Lean tissue hydration of these treated animals was significantly lower than that of untreated controls (t-3.12, df-37, $P < 0.005$). This difference was abolished in the animals treated with both bromocriptine and exogenous prolactin.

DISCUSSION

Exogenous prolactin or fluphenazine, a drug which stimulates endogenous prolactin secretion, increased tissue hydration at 72 h of age. The effect was most apparent in animals with relatively large weight gains and, by inference, relatively large fluid intakes. These animals normally mobilize their neonatal water stores rapidly (7). Conversely, treatment with bromocriptine, a blocker of endogenous prolactin secretion, reduced lean tissue water content in animals of low weight gain/fluid intake, animals who normally retain larger amounts of fluid (7). Exogenous prolactin abolished this effect. These observations suggest that prolactin may be a regulator of the neonatal tissue water reservoir.

In our initial report we identified a significant, inverse, linear relationship between postnatal weight gain and tissue water content at 72 h of age, and from this observation inferred a regulatory process for postnatal water loss (7). We postulated that if there were hormonal regulators of this process, they might be found in the group of hormones whose levels are elevated in the fetus

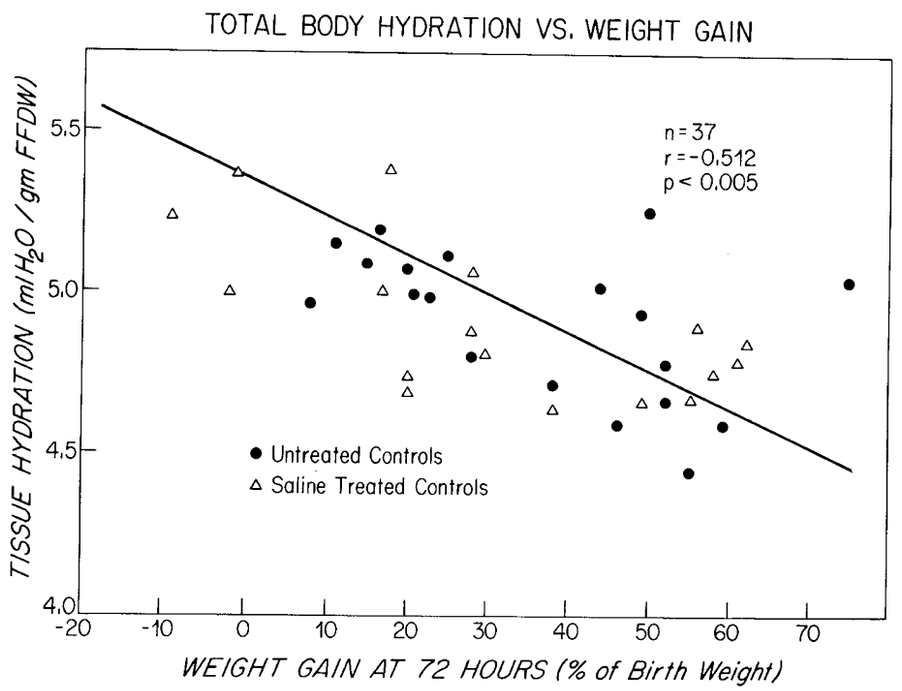

Fig. 1. The relationship between total body hydration at 72 h and weight gain from birth. Weight gain is expressed as % of birth weight. Untreated controls are compared to saline-treated controls. The regression line is calculated for the combined groups.

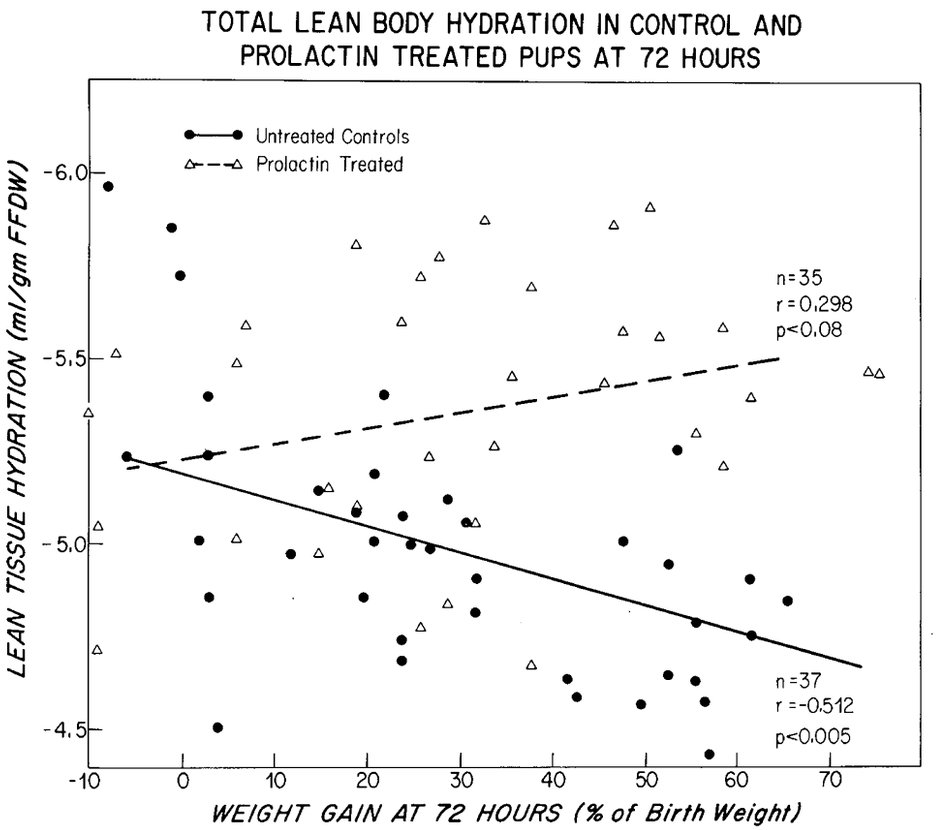

Fig. 2. The regression of total body hydration at 72 h against weight gain in control animals (——) and those treated with prolactin (----). Test of common line f = 11.677, d.f. = 68, $P = 0.001$; test of common slope f = 42.381, d.f. = 69, $P < 0.001$.

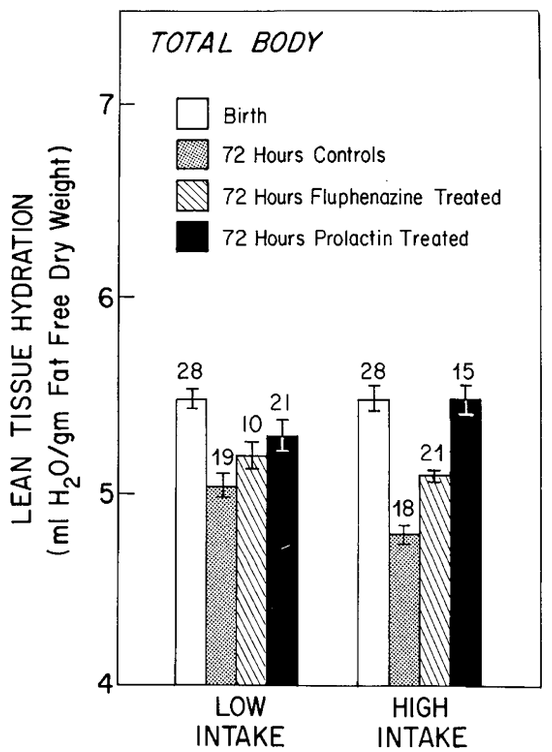

Fig. 3. Tissue hydration at 72 h in prolactin- and fluphenazine-treated animals, separated into groups gaining more than (*high intake*) and less than (*low intake*) 30% of the birth weight (the mean weight gain of control animals) and compared to control groups of similar weight gain and to levels in animals sacrificed at birth. Bars represent standard error of the mean. Numbers in parentheses are number of animals in each group.

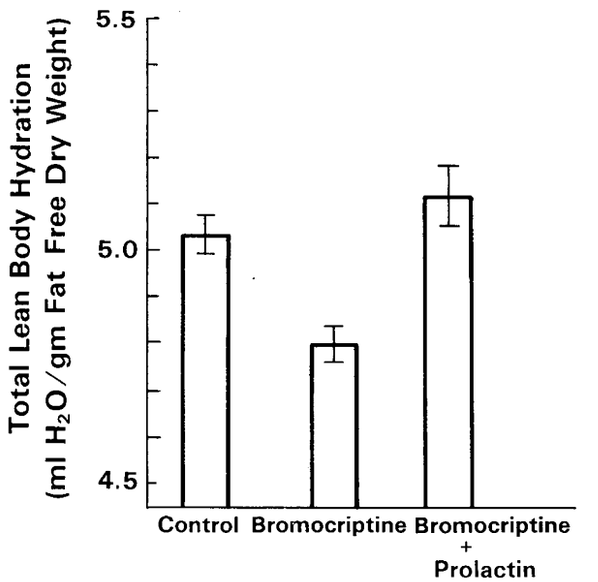

Fig. 4. Blockade of endogenous prolactin secretion with bromocriptine reduced total lean body hydration below control values ($P < 0.004$). Concomitant treatment with exogenous prolactin restored body water to control levels.

during late gestation and fall in the first days of life. As noted above, this group includes renin, aldosterone, progesterone, estrogen, cortisone, and prolactin. All of these hormones could affect body water economy. The renin-angiotension-aldosterone system regulates sodium balance and extracellular fluid composition. Progesterone counters the renal effects of aldosterone and promotes sodium excretion (5). Cortisol has mineralocorticoid activity

and, in addition, acts directly on the kidney to increase glomerular filtration and to promote free water excretion at the tubular level (31). Estrogens have been implicated in the genesis of pregnancy edema (4).

Prolactin plays an important role in fluid and electrolyte homeostasis throughout vertebrate phylogeny (10, 26). Treatment of adult rabbits with prolactin causes retention of water and salt (3). It would also appear to be important during ontogeny. It is a regulator of fluid transport across the amniotic membrane (21, 23), and has been shown to regulate fluid movements between the monkey fetus and the surrounding amniotic fluid (19). Fetal serum prolactin levels follow similar patterns of change in different mammalian species (1, 14, 24, 28). They are low during the first two-thirds of gestation. At the beginning of the third trimester they rise abruptly. A more gradual rate of increase then persists until parturition. Human fetal serum prolactin levels are unrelated to maternal or amniotic fluid levels (27). They rise rapidly early in the third trimester, peak at term, and fall rapidly during the first week of life (1, 32). Prolactin receptors have been identified in most fetal primate tissues (20). A number of factors including, the early third trimester rise in fetal serum prolactin levels, their rapid fall during the neonatal period, the ubiquity of prolactin receptors in fetal tissues, the short serum half-life of the hormone, and its possible role in fetal osmoregulation, led us to test the hypothesis that prolactin is a regulator of neonatal tissue water stores. The data presented herein support that hypothesis. They do not, however, exclude a role for other hormones.

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33. Requests for reprints should be addressed to the author, Neonatal ICU, Primary Children's Medical Center, Salt Lake City, Utah 84103
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