PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 171, 285–288 (1982)

Effect of Prolactin on Galactose-Induced Cataractogenesis in the Rat (41512)

O. GONA*,[1] AND S.-C. J. FU†

*Department of Anatomy and †Department of Biochemistry, University of Medicine and Dentistry–New Jersey Medical School, 100 Bergen Street, Newark, New Jersey 07103

Abstract. The hypothesis was tested that the hormone prolactin, a noncataractogenic agent, can influence cataractogenesis in rats fed on a D-galactose-rich diet. This hormone accelerated the cataractogenic process in females but was without significant effect in males. It was also found that the galactose diet itself induced cataract formation more rapidly in females than in males. Thus, prolactin may play a hitherto unsuspected role in acceleration of cataractogenesis, and this role is apparently influenced by the sex of the individual.

Both in humans and in animal models, a wide variety of conditions is known to stimulate the development of cataracts in the mammalian lens. Although the initiating factors may be different, one common feature of many types of cataract is a dramatic change in lens hydration (1). A number of hormones have actions which directly or indirectly affect water balance and, as a consequence, may affect the lenticular opacification triggered by other agents. One such hormone is prolactin, which is secreted by the anterior pituitary gland and which influences water and/or electrolyte balance in a large number of vertebrates (2–4). This hormone has not been implicated in the cataractogenic process. However, we decided to test the hypothesis that prolactin, of itself a noncataractogenic agent, can influence the progression of cataractogenesis in an animal system where lens opacification is induced by a D-galactose-rich diet. The results of our investigations are reported here.

Materials and Methods. The experiment described was performed twice using identical groupings and treatments. In each run, a total of 36 Sprague–Dawley rats (18 males and 18 females), initial body weight 88–104 g, were used. The animals were separated by sex and randomly assigned to one of two groups. They were kept, three per 50 × 50 × 30-cm suspended wire cage, at 22 ± 1° under a 12-hr light/12-hr dark lighting regimen. All animals were fed *ad libitum* on a diet of 30% D-galactose and 70% ground Purina Rodent Laboratory chow without preservative. The diet was freshly prepared once a week by pulverizing the commercial food pellets and mixing it with D-galactose in a blender. The prepared diet was stored at 4°. A powdered food chow was not used in order to avoid the possible effect of preservatives (e.g., the antioxidant 2, (3)-t-butyl-4-hydroxyanisole) in the diet.

Ovine prolactin (NIH-P-S12, relative potency 35 IU/mg) was dissolved in 0.9% normal saline at a concentration of 10 IU/0.1 ml and stored at 4°. Small amounts were prepared each time so that the storage time did not exceed one week. Half of the animals (nine males and nine females) in each run received daily intraperitoneal injections of 10 IU prolactin. The other half received 0.1 ml of the saline vehicle only. The animals were weighed seven times during the experiment: on the first day, on the seventh day, and every 3 days thereafter. Food consumption was determined by weighing the food dishes every 2 or 3 days.

The eyes of all the animals were examined daily under 200 W fluorescent light. The degree of opacity was assessed subjectively and staged in accordance with the

[1] To whom all correspondence should be addressed.

0037-9727/82/110285-04$01.00/0

progression of lens opacity as described by Sippel (5).

The assessment of lens opacity was made by an experienced observer without knowledge of the experimental grouping. Two other observers also assessed the condition of the lens before a rat was returned to its cage. In cases where there was disagreement, the lower numerical stage was always used.

We expanded Sippel's scale as shown in Table I in order to minimize the chances of over- or underestimating the progress of cataractogenesis. These stages of cataractogenesis have been correlated with gradually occurring protein changes (6). For quantitation, assigned opacity scores (Table I) for both eyes of all animals were used in the statistical analyses. The experiment was terminated on the 24th day.

Results. During both runs of the experiment, cataractogenesis progressed most rapidly in prolactin-injected females. Because of the novelty of the findings from the first run of the experiment, we repeated the experiment (randomizing examination of animals and scoring lens opacity more precisely) to substantiate our results. The data from the second experimental run are presented here.

The first distinct changes in lenticular opacity were observed in two prolactin-treated females on the 12th day of treatment. By the 15th day, every animal had developed some nuclear opacity in at least one eye. At the termination of the experiment, cataracts had developed to stage 5 in only three animals, all of which were prolactin-treated females.

After 18 days of treatment, it was obvious, even to the casual observer, that cataract progression was more advanced in one of the groups than in the others (Fig. 1). This group consisted of the prolactin-treated females. Statistical analysis by group comparisons of the assigned scores corroborated the subjective assessment that there were significant differences among the four groups. These differences were apparent as early as Day 15 (analysis of variance F value = 7.28; $P = 0.0007$) and continued until the day the experiment was terminated ($F = 16.05$; $P = 0.0001$). Because the variance indicated that scores were influenced by both prolactin and sex, one-tailed t tests were performed to assess the effect of each of these parameters separately. At every time period from the 15th day onward, scores of the prolactin-treated females were found to be significantly different ($P \leq 0.045$) from the saline-injected control females. Furthermore, significant differences existed between the scores of prolactin-treated males and prolactin-treated females at every time period ($P \leq 0.011$). Only the prolactin-treated female group was consistently different from the other groups. Control male and female scores were not significantly different until Day 23. However, the mean daily scores of control males tended to be lower than those of females throughout the experiment and were significantly lower on Days 23 and 24 ($P \leq 0.03$). Prolactin appears to have little effect on the progress of cataractogenesis in males and had no effect on food consumption and body weight gain (data not presented).

Discussion. The results clearly show that prolactin accelerates cataractogenesis in female rats fed on a 30% galactose diet. Since prolactin has not been implicated

TABLE I. STAGES OF OPACITY: EQUIVALENT CATARACTOUS STAGES AS DESCRIBED BY SIPPEL (5) AND ASSIGNED OPACITY SCORES

Stages of opacity	0	1^-	1	1^+	2^-	2	2^+	3^-	3	3^+	4^-	4	4^+	5^-	5	5^+
Sippel (5) equivalent	Normal	1–A	1–B	1–C		2			3			4			5	
Assigned opacity score	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

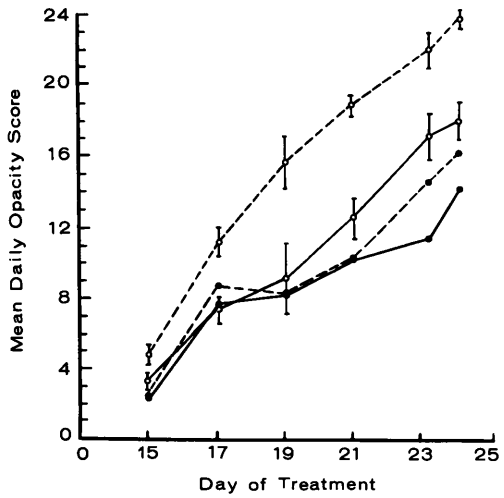

FIG. 1. Progression of lenticular opacification in 30% galactose-fed rats treated with prolactin. The mean daily opacity scores for each group (group N = 9) are shown. Mean daily opacity score represents the mean for two eyes per animal. Differences between prolactin-treated males and females, as well as differences between prolactin-treated and control females, are statistically significant (t test, $P \leq 0.045$) at all time periods. Differences between control males and females are statistically significant (t test, $P \leq 0.03$) on Days 23 and 24. (●– – –●) Prolactin-treated males; (●——●) control males; (○– – –○) prolactin-treated females; (○——○) control females. Vertical bars = standard error of mean.

previously in cataractogenesis, our findings add to the already long list of reported prolactin actions, many of which derive from the influence of prolactin on osmoregulatory physiology (2–4). Although we have not demonstrated the mechanisms by which prolactin accelerates galactose-induced cataractogenesis, we believe that this effect is also mediated by the prolactin influence on ion and water balance.

Prolactin is reported to alter water and ion movement in a number of vertebrates (2–4, 7–10) and to affect sugar and amino acid transport by the rat intestine (11). Additionally, there is evidence that prolactin may affect human glucose tolerance under certain pathologic conditions (12, 13). In galactose-induced cataractogenesis, the osmotic change that results from sugar alcohol accumulation is followed by increased lens permeability to amino acids and cations (1). Thus, although prolactin is not, by itself, a cataractogenic agent, it may accelerate the failure of the cation pump in this animal model by its action on water and cation (or even sugar and amino acid) balance.

The present studies also indicate that female rats are more susceptible to galactose-induced cataracts than are males. This cataractogenic propensity of the female was unexpected and the present experiment does not provide any basis for an explanatory hypothesis. In 1968, however, Lambert (14) reported that progestins and estrogens increase lens permeability to cations *in vitro*. The exaggerated response seen in prolactin-treated females may have been due to stimulation of prolactin secretion by estrogen (15) or it may indicate a synergistic interaction between prolactin and estrogen. Interactive effects between prolactin and other hormones are well known (4). Whatever the mechanism is that accounts for our findings, the present studies suggest that sex and prolactin are potentially important factors in cataractogenesis.

The authors wish to thank Ms. M. Pascavage for typing the manuscript and Dr. W. Rhoten for constructive criticism during preparation of this manuscript. The prolactin was generously supplied by the Endocrinology Study Section of the NIH. Supported by USPHS NIH Grant EY 01156.

1. Kinoshita JH. Mechanisms initiating cataract formation. Invest Ophthalmol 13:713–724, 1974.
2. Nicoll CS. Role of prolactin in water and electrolyte balance in vertebrates. In: Jaffe R, ed. Current Endocrinology. New York, Elsevier/North-Holland, pp127–166, 1981.
3. Bern HA. Prolactin and osmoregulation. Amer Zool 15:937–948, 1975.
4. Ensor DM. Comparative Endocrinology of Prolactin. London, Chapman & Hall, 1978.
5. Sippel TO. Changes in the water, protein, and glutathione contents of the lens in the course of galactose cataract development in rats. Invest Ophthalmol 5:568–575, 1966.
6. Ocken PR, Fu S-CJ, Hart R, White JH, Wagner BJ, Lewis KE. Characterization of lens protein. I. Identification of additional soluble fractions in rat lenses. Exp Eye Res 24:355–367, 1977.
7. Mainoya JR. Effects of bovine growth hormone,

human placental lactogen and ovine prolactin on intestinal fluid and ion transport. Endocrinology **96**:1165–1170, 1975.

8. Mattheij JA, Sterrenberg L, Swarts JH. Current concepts. III. Prolactin and calcium metabolism in the rat. Life Sci **27**:2031–2035, 1980.
9. Bisbee CA. Prolactin effects on ion transport across cultured mouse mammary epithelium. Amer J Physiol **240**:C110–C115, 1981.
10. Pang PKT. Pituitary and prolactin influences on calcium regulation in the mud puppy, *Necturus maculosus*. Gen Comp Endocrinol **44**:524–529, 1981.
11. Mainoya JR. Effect of prolactin on sugar and amino acid transport by the rat jejunum. J Exp Zool **192**:149–154, 1975.
12. McGarry EE, Beck JC. Biological effect of non-primate prolactin and human placental lactogen. In: Wolstenholme GEW, Knight J, eds. Lactogenic Hormones. Edinburgh, Churchill–Livingstone, p36, 1972.
13. Landgraf R, Landgraf-Leurs MM, Weissmann A, Hörl R, von Werder K, Scriba PC. Prolactin: A diabetogenic hormone. Diabetologica **13**:99–104, 1977.
14. Lambert BW. The effects of progestins and estrogens on permeability of the lens. Arch Ophthalmol **80**:230–234, 1968.
15. Stone JP, Holtzman S, Shellabarger CJ. Neoplastic responses and correlated plasma prolactin levels in diethylstilbestrol-treated ACI and Sprague–Dawley rats. Cancer Res **39**:773–778, 1979.

Received June 1, 1982. P.S.E.B.M. 1982, Vol. 171.