Virchows Arch. A Path. Anat. and Histol. 370, 13—19 (1976)

The Role of Prolactin in the Development of Chronic Progressive Nephropathy in the Rat

B. Richardson and H. Luginbühl

Biological and Medical Research Division, Sandoz Limited, Basle, and Institute of Animal Pathology, University of Bern

Received July 7, 1975

Summary. Prolactin as a factor in the etiology of chronic progressive nephropathy in rats has been studied by exogenous administration and by endogenous inhibition with bromocriptin. Exogenous prolactin caused a significant increase in frequency and severity of the lesions, with accompanying sodium retention. Conversely endogenous inhibition significantly decreased the frequency and severity of the lesions and increased urine volume, pH and sodium excretion. Thus prolactin is important in the etiology of this renal disease in rats.

Key words: Prolactin — Bromocriptin — Nephropathy.

Chronic progressive nephropathy of aging rats is a well known disease, its frequency and severity varying with strain and level of dietary protein (Saxton and Kimball, 1941; Andrew and Pruett, 1957; Snell, 1967). Thus it has been suggested that this condition is caused primarily by the obstruction of collecting ducts with casts formed from precipitated protein (Saxton and Kimball, 1941). However this does not explain the greatly increased frequency and severity of such lesions in rats bearing adrenal or prolactin-secreting pituitary tumors (Gillman et al., 1953; Snell and Stewart, 1959; Furth et al., 1956). As prolactin has direct renal actions in rats (Lockett and Nail, 1965) its probable relation to this nephropathy has been investigated.

Materials and Methods

Both the effects of administering exogenous prolactin and those of inhibiting endogenous prolactin upon the frequency and severity of chronic progressive nephropathy have been studied.

Male albino rats (OFA Sandoz SPF strain) aged 8 weeks, weights 220–300 g were caged separately in a controlled environment with water and a commercial food (NAFAG® 194, Nafag Ltd., Gossau, Switzerland) ad libitum.

Once weekly for 10 weeks 20 rats received 40 i.u./kg ovine prolactin (Ferring Ltd., Düsseldorf, W. Germany) given as a subcutaneous depot in 1 ml aluminium hydroxide adjuvant (Herbert 1970). Twenty controls received adjuvant only. Twenty-four h urine samples were collected from all rats immediately after injections one, six and ten.

In the second study endogenous prolactin was inhibited in 10 rats by mixing the prolactin inhibitor bromocriptin® (Sandoz Ltd., Basle, Switzerland) in the food at a concentration which provided a mean daily intake of 5 mg/kg. At this dose level bromocriptin is known to act as a potent and specific prolactin inhibitor (Billeter and Fluckiger 1971; Fluckiger 1972; Besser et al., 1973). Ten control rats received unmedicated food. This study lasted 52 weeks and 24-h urine samples were collected from 5 treated and 5 control rats after 6, 13, 26 and 52 weeks.

Specific gravity of urine samples was determined by refractometer (American Optical Corporation, Buffalo, N.Y. USA), volume calculated from weight and specific gravity, and pH measured with indicator paper (Merck Ltd., Darmstadt, W. Germany). Sodium and potassium were measured by flame photometer (Ingold Ltd., Zürich, Switzerland) and mean values for treated and control rats compared by Students' t-test. In the case of the bromocriptin study, the mean of the 6- and 13-week values and that of the 26- and 52-week values were taken before statistically analyzing the data.

Following exposure to CO_2 animals were exsanguinated by renal phlebotomy, kidneys removed, freed of extraneous tissue, weighed and surface changes noted. Blocks of right kidneys were fixed in Bouin's fixative and those of left kidneys in 4% formol, embedded in paraffin, cut at 5 μ and stained by hematoxylin and eosin, Van Gieson elastica and PAS.

Sections were examined without knowledge of origin; first to survey for lesions and second to estimate and record degrees of change. Changes were scored on the following scale 0 = none, 1 = mild, 2 = moderate, 3 = severe. Tubular changes were considered primary, and glomerular, interstitial and plevic changes secondary. Therefore values for tubular lesions were doubled in calculating scores. Mean scores for each rat were calculated for nephron, interstitial and pelvic changes by the formula:

$$\text{Mean score} = \frac{\text{Sum of scores for each change}}{\text{number of changes.}}$$

Mean scores for control and treated animals were compared statistically.

Results

Urine

Chronic administration of prolactin induced no significant change in specific gravity, pH, volume or potassium content, although the sodium concentration was significantly decreased in the 10-weeks sample (control 29 ± 4 mEq/l, prolactin 20 ± 2 mEq/l, $P < 0.05$).

The prolactin inhibitor bromocriptin caused significant increases in urine volume, pH and 24-h sodium excretion, with a similar trend in 24-h potassium excretion (Table 1). The effect on sodium and potassium excretion was not merely a reflection of increased urine volume as the concentrations of these ions in the urine was also slightly, but not significantly, increased.

Kidney Weights

Chronic administration of prolactin did not significantly change either the absolute or relative kidney weight. On the other hand, rats with their endogenous prolactin chronically inhibited by bromocriptin showed a significant decrease in both absolute and relative kidney weight (Absolute: Controls 3.93 ± 0.86 treated 3.33 ± 0.47, $P < 0.05$: Relative controls $0.87\% \pm 0.02$; treated $0.65\% \pm 0.01$ $P < 0.001$). The relative kidney weights of rats with inhibited endogenous prolactin were similar to those of 6-month old control rats of this strain ($0.68\% \pm 0.02$).

Macroscopic Lesions

No macroscopic change was seen in kidneys of either control or treated rats from the study where exogenous prolactin was given. In the bromocriptin study four control rats had enlarged kidneys with uneven cortical surfaces and on the cut surface many small cysts as well as pale bands running through the cortices were seen. Such lesions are typical of those commonly encountered in untreated

Table 1. Effects of inhibiting endogenous prolactin with bromocriptin on composition of rat urine

	Mean of weeks 6 and 13 ± s.e.			Mean of weeks 26 and 52 ± s.e.		
	Controls	Bromocryptine	*P*	Control	Bromocryptine	*P*
24-hour vol (ml)	11.4 ± 1.1	17.1 ± 2.2	*<0.05*	9.5 ± 1.1	11.7 ± 1.6	n.s.
pH	5.9 ± 0.1	5.9 ± 0.1	n.s.	6.0 ± 0.1	6.3 ± 0.0	*<0.02*
24-hour Na^+*	87.0 ± 4.7	128.2 ± 11.0	*<0.01*	44.8 ± 7.5	49.0 ± 9.5	n.s.
24-hour K^+*	254.0 ± 15.2	275.5 ± 21.6	n.s.	340.5 ± 53.4	333.1 ± 53.0	n.s.

s.e. = Standard error; n.s. = not significant
* Expressed as μ Eq/100 g body weight/24 h

rats of this age (Saxton and Kimball, 1941; Andrew and Pruett, 1957; Snell, 1967). Only one rat treated with bromocriptin had similar changes.

Microscopic Lesions

Changes in the nephrons of rats treated with exogenous prolactin included thickening of the parietal layer of Bowman's capsule, the presence of protein casts in dilated tubular lumina as well as desquamation and regeneration of tubular epithelium with hyperplasia, hypertrophy and disorganization. Most kidneys had some degree of focal or diffuse chronic inflammatory cell infiltration, with fibrosis of the interstitium (Fig. 1). Occasionally some proliferation of the pelvis epithelium was also observed.

In the bromocriptin study the described changes were more advanced. Proteinaceous material was present in glomerular spaces, there was shrinkage of glomerular tufts, increased dilatation of tubuli, many protein casts, lipofuscin in epithelial and mesenchymal cells and focal tubular degeneration with replacement fibrosis (Fig. 2). Such lesions were more pronounced in control rats, the administration of bromocriptin having clearly inhibited the development of nephron changes in particular. Less protein casts were present and there were fewer inflammatory and sclerotic changes than in kidneys from controls.

Scoring the lesions showed that prolactin administration had produced a significantly increased severity of lesions involving the nephrons ($P < 0.02$ versus controls). Interstitial and pelvic changes showed similar trends. Conversely endogenous prolactin inhibition by bromocriptin resulted in a statistically significant decrease in the severity of nephron lesions ($P < 0.02$ versus controls), with a similar but non-significant effect on interstitial changes. The development of pelvic changes was unaffected by medication (Fig. 3).

Discussion

Weekly subcutaneous depot injections of 40 i.u./kg ovine prolactin to male rats for 10 weeks resulted in a significantly increased severity of progressive nephropathy compared with controls. Prolactin administration especially accelerated the development of nephron lesions, whereby protein casts in the lumen

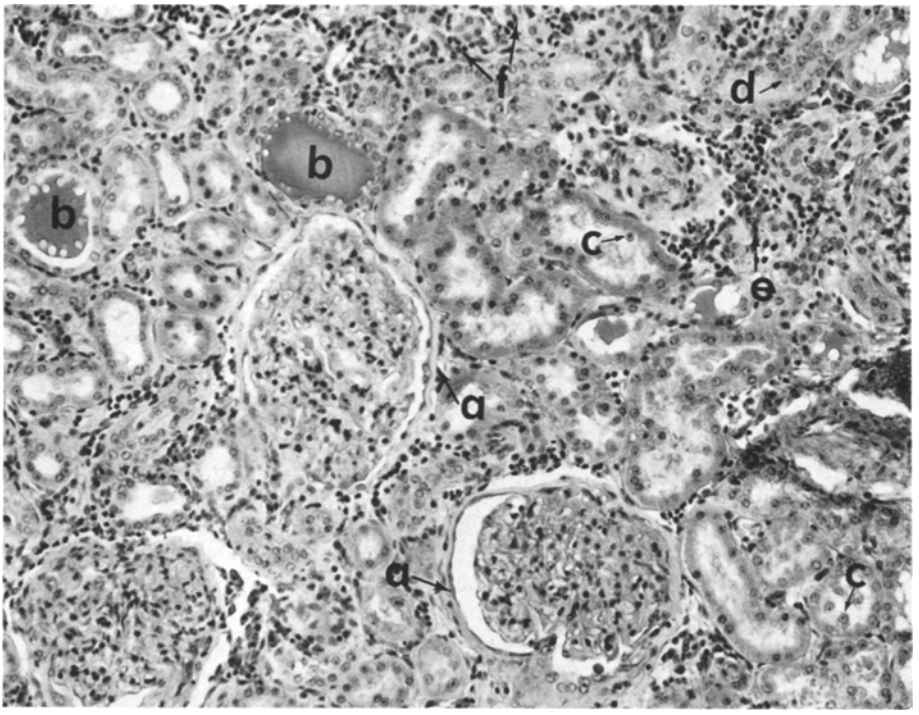


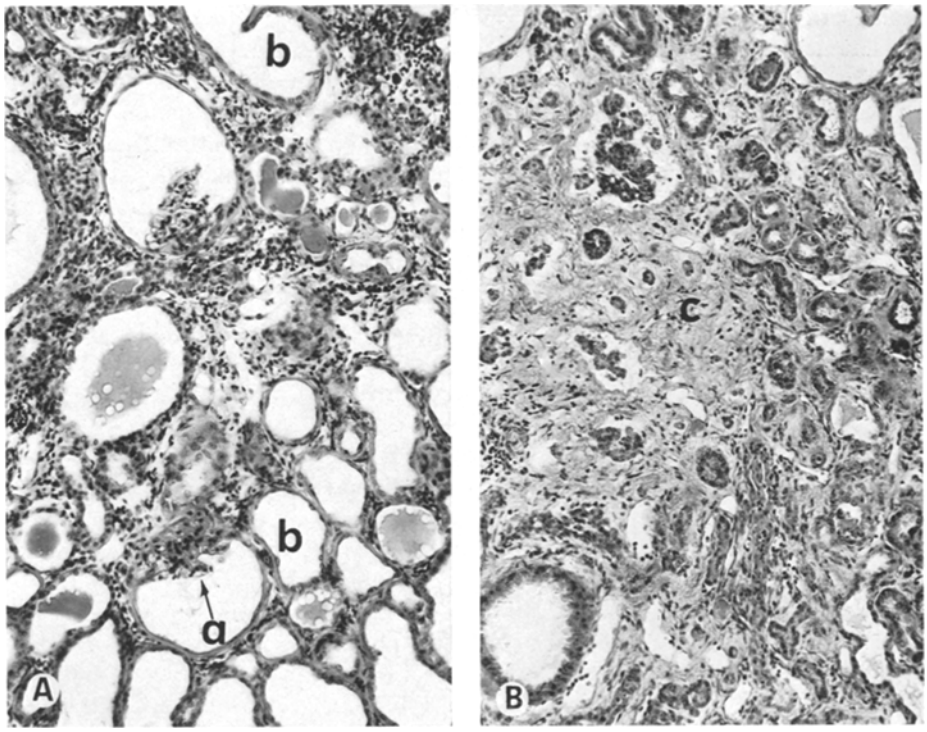
Fig. 1. Mild changes if chronic progressive nephropathy in a control 18-week-old rat. *a* thickening of the parietal layer of Bowman's capsule; *b* protein casts in the tubular lumina; *c* desquamation of tubular epithelium; *d* hyperplasia and disorganization of regenerating tubular epithelium; *e* interstitial chronic inflammatory cell infiltration; *f* interstitial fibrosis. (Hematoxylin and eosin ×300)

and desquamation as well as regeneration of tubular epithelium appeared more commonly than in controls. Thickening of the parietal layer of Bowman's capsule also occurred more commonly, but is considered secondary in nature as it was only seen accompanying tubular lesions, whilst the converse was not the case.

Furth et al. (1956) have observed greatly enlarged kidneys with nephropathy in both rats and mice bearing prolactin-secreting pituitary adenomas. The mechanisms of development of the nephropathy was not clear at that time, but our results strongly suggest that they were attributable to increased prolactin levels.

In contrast to the foregoing, chronic oral administration of the specific prolactin inhibitor bromocriptin significantly reduced the incidence and severity of renal lesions as compared with the control group. The reduction in lesions involving the nephrons was particularly striking. These findings were accompanied by an inhibition of the usual age-related increase in relative kidney weights and there was a prolactin inhibition-related increase in urine volume, pH and sodium excretion opposite to the effects reported after exogenous prolactin administration to rats (Lockett and Nail, 1965).

As bromocriptin is a specific and potent prolactin inhibitor possessing little other pharmacological activity, the beneficial effects of medication on the devel-



Figs. 2A and B. Moderately advanced chronic progressive nephropathy in a control 60-week-old rat. Similar changes are present to those in Fig. 1. In addition: *a* shrinkage of glomerular tuft; *b* increased dilatation of tubuli; *c* replacement fibrosis. (2A Hematoxylin and eosin ×110. 2B Van Gieson elastica ×110)

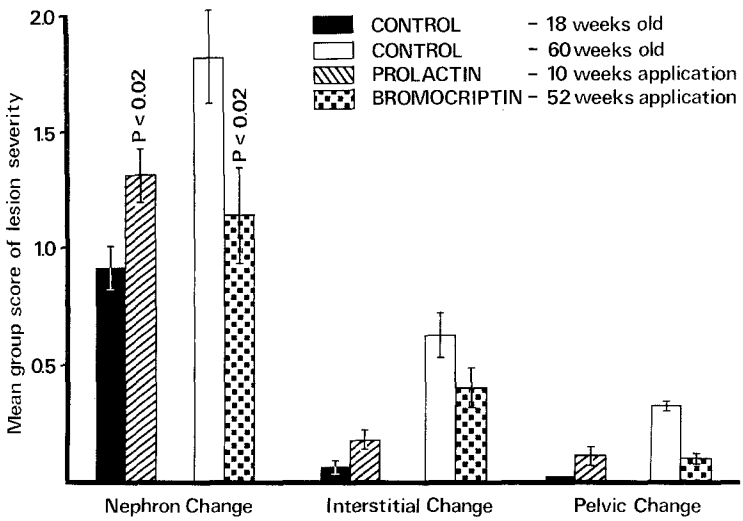


Fig. 3. Comparison between severity of renal lesions occurring in control rats and that present in rats chronically treated with either ovine prolactin or bromocriptin. Vertical bars indicate the standard error of the mean

opment of chronic progressive nephropathy were probably attributable to chronic reduction in serum prolactin levels. Changes in urinary ion content and volume clearly indicated that bromocriptin was having such an effect. It is also possible to speculate as to how chronic prolactin inhibition resulted in such a relatively low incidence of renal lesions. In treated rats there was significantly increased urine volume and pH. As protein is more soluble at high alkalinity, the initial blockage of the collecting ducts with such casts might be at least partially inhibited. The additional flushing action of increased urine volume would act synergistically. Saxton and Kimball (1941) have suggested that the early obstruction of collecting ducts with protein casts is important, if not essential, for the development of the typical lesions of progressive nephropathy in rats. The findings from the present study support this, as a reduction in the number of protein casts in rats with inhibited prolactin was reflected by a similar reduction in secondary inflammatory and sclerotic changes.

Our results thus suggest that prolactin is a very important etiological component in the development of chronic progressive nephropathy in rats. The hormone seems to facilitate the formation of protein casts in the collecting ducts by lowering urinary pH and decreasing urine volume, thereby making the high amounts of protein usually present in rats' urine insoluble.

The relevance of these results to the etiology of renal disease in man is not clear. However, in man as in rats, prolactin produces renal retention of water, sodium and potassium (Horrobin et al., 1971). In addition, serum prolactin levels are greatly elevated in some patients with renal failure (Turkington 1972; Nagel et al., 1973) and albuminuria associated with degenerative glomerular lesions are common complications encountered in the third timester of pregnancy (British Medical Journal, 1970) at a time when serum prolactin levels are elevated (Nokin et al., 1972). Thus in man prolactin may also have significant renal action.

References

Andrew, W., Pruett, D.: Senile changes in the kidneys of Wistar Institute rats. Amer. J. Anat. **100**, 51–80 (1957)

Besser, G. M., Parke, L., Edwards, C. R. W., Forsyth, I. A., McKelly, A. S.: Galactorrhea: Successful treatment with reduction of plasma prolactin levels by bromergocryptine. Brit. med. J. **3**, 669–672 (1972)

Billeter, E., Fluckinger, E.: Evidence for a luteolytic function of prolactin in the intact cyclical rat using 2-bromo-α-ergocryptine. Experientia (Basel) **27**, 464–465 (1971)

British Medical Journal editorial "Treatment of bacteriuria in pregnancy". Brit. med. J. **4**, 631–632 (1970)

Del Pozo, E., Friesen, H., Burmeister, P.: Endocrine profile of a specific prolactin inhibitor: Br-Ergocryptine. Schweiz. med. Wschr. **103**, 847–848 (1973)

Fluckiger, E.: Drugs and the control of prolactin secretion. In: Prolactin and carcinogenesis, 4th Tenovis workshop, p. 162. Cardiff: Alpha Omega 1972

Furth, J., Clifton, K. H., Gadsden, E. L., Buffet, R. F.: Dependent and autonomous mammotropic pituitary tumors in rats: Their somatotropic features. Cancer Res. **16**, 608–616 (1956)

Furth, J., Gadsden, E. L., Clifton, K. H., Anderson, E.: Autonomous mammotropic pituitary tumors in mice: Their somatotropic features and responsiveness to estrogens. Cancer Res. **16**, 600–607 (1956)

Gillman, J., Gilbert, C., Spence, I.: Phaeochromocytoma in the rat: Pathogenesis and collateral relation to comparable tumors in man. Cancer (Philad.) **6**, 494–511 (1953)

Herbert, W. J.: Veterinary immunology. I, p. 196. Oxford: Blackwell Scientific Publications 1970

Horrobin, D. F., Burstyn, P. G., Lloyd, I. J., Lipton, A., Durkin, N., Muiruri, K. L.: Actions of prolactin on human renal function. Lancet **1971 II**, 352–354

Lockett, M. F., Nail, B.: A comparative study of the renal actions of growth and lactogenic hormones in rats. J. Physiol. (Lond.) **180**, 147–156 (1965)

Nagel, T. C., Freinkel, N., Bell, R. H., Friesen, H., Wilber, J. F., Metzger, B. E.: Gynecomastia, prolactin and other petide hormones in patients undergoing chronic haemodialysis. J. clin. Endocr. **36**, 428–432 (1973)

Nokin, J., Vekemans, M., L'Hermite, M., Robyn, C.: Circadian periodicity of serum prolactin concentrations in man. Brit. med. J. **3**, 561–562 (1972)

Saxton, J. A., Kimball, G. C.: Relation of nephrosis and other disease of albino rats to age and modifications of diet. Arch. Path. **32**, 951–965 (1941)

Snell, K. C.: Renal disease of the rat. In: Pathology of laboratory rats and mice. I, p. 105. Oxford and Edinburgh: Blackwell Scientific Publications 1967

Snell, K. C., Stewart, H. L.: Variations in the histologic patterns and functional effects of transplantable adrenal cortical carcinoma in intact, hypophysectomized and new born rats. J. nat. Cancer Inst. **22**, 1119 (1959)

Turkington, R. W.: Human Prolactin: An ancient molecule provides new insights for clinical medicine. Amer. J. Med. **53**, 389–397 (1972)

Dr. B. Richardson
Biological and Medical
Research Division
Sandoz Ltd.
CH-4002 Basle
Switzerland