Prolactin-induced Modulation of Tissue Growth Rate Patterns in Albino Rats During Puberal Transition

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The role of prolactin (PRL) in the puberal transition of male albino rats has been investigated. It was found that PRL elevated total organ growth rates, tissue somatic indices, and dry weights of reproductive and nonreproductive tissues. Analysis of the growth rate patterns of these tissues suggests that PRL induces changes that lead to the initiation of function of the testis and accessory reproductive glands in the prepuberal male rat.

Key words: prolactin, growth rate curves, tissue somatic indices, advancement of puberty, accessory male reproductive glands.

Prolactin (PRL) regulates several processes involved in male reproductive functions (Bartke, 1966, 1967; Bartke and Lloyd, 1970; Woods and Simpson, 1971; Hafiez et al, 1972a, 1972b; Bartke, 1974; Johnson, 1974; Walvoord et al, 1976; Negro-Vilar et al, 1977; Bartke et al, 1977). There are conflicting reports on the ability of PRL to induce changes in weights of male reproductive tissues. Administration of PRL was reported to increase seminal vesicle weight without altering testis weight in adult rats (Sheriff and Govindarajulu, 1975), to elevate prostate gland weight in rats and mice (Yamanaka et al, 1975; Thomas and Manadhar, 1975; Walvoord et al, 1976), and to increase ventral prostate weight without inducing a significant change in the weight of the dorsolateral prostate, testis, or seminal vesicle in rats (Dattatreyamurthy et al, 1975). Information pertaining to the impact of PRL on the growth pattern of reproductive tissues during puberal transition is very limited, and there is no information on the effects of PRL on the growth of nonreproductive tissues. Since PRL can increase testicular weight and simultaneously augment fertility (Bartke, 1966, 1967; Bartke and Lloyd, 1970), it is of interest to study the influence of PRL on the growth rate patterns of reproductive and nonreproductive tissues in immature animals. Hence the aim of the present study is to elucidate the effects of PRL on the growth rate patterns of reproductive and nonreproductive tissues in male albino rats, with relation to puberal transition.

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Material and Methods

Immature (21-day-old) male albino Wistar rats were used for the present study. Endogenous PRL levels at this age are very low (Negro-Vilar et al, 1973; Bartke, 1976). The animals were obtained from the Indian Institute of Science, Bangalore, India, and were maintained at 27 ± 2 C in 12 hours/light:12 hours/darkness. A standard rat diet obtained from Hindustan Lever Ltd., Bombay, India, and water were provided *ad libitum*.

Animals in the experimental group received subcutaneous injections of 1.0 μg of ovine PRL/gm body weight (supplied by NIH, Bethesda, Maryland) (Hafiez et al, 1972a, 1972b) in saline, daily, for five days. Control rats received an equivalent volume of physiologic saline alone. The rats were decapitated on posttreatment day one, and testis, epididymis, seminal vesicle, prostate gland, brain, liver, kidney, and gastrocnemius muscle were carefully removed and immediately frozen for the following analyses.

Tissue Somatic Indices (TSI)

Tissues were weighed on a monopan electric balance (Sartorius-Werke, Germany), and TSI were calculated as percent ratios of tissues to body weight.

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TABLE 1. Prolactin-induced Gravimetric Changes in Reproductive and Nonreproductive Tissues of Immature Albino Rats

Tissue	TSI		Dry Weight		Percent Water	
	Control	Experimental	Control	Experimental	Control	Experimental
Testis	0.405 ± 0.034	0.480 ± 0.013	24.66 ± 2.65	26.00 ± 2.28	82.28 ± 6.00	84.02 ± 1.31
	+18.52		+5.43		+2.10	
	$P < 0.001$		NS		NS	
Epididymis	0.137 ± 0.021	0.190 ± 0.025	13.00 ± 2.7115	15.00 ± 2.89	65.09 ± 3.29	78.96 ± 6.51
	+38.68		+15.38		+21.30	
	$P < 0.001$		$P < 0.05$		$P < 0.001$	
Seminal vesicles	0.049 ± 0.007	0.076 ± 0.005	4.00 ± 0.89	5.67 ± 1.211	73.06 ± 3.81	75.49 ± 4.02
	+55.10		+41.75		+2.56	
	$P < 0.001$		$P < 0.05$		NS	
Prostate gland	0.069 ± 0.001	0.086 ± 0.007	6.33 ± 0.51	7.83 ± 0.75	71.60 ± 3.59	76.61 ± 3.64
	+24.64		+23.69		+6.99	
	$P < 0.001$		$P < 0.01$		$P < 0.01$	
Brain	2.627 ± 0.205	3.087 ± 0.215	177.50 ± 8.40	190.16 ± 12.35	79.36 ± 1.06	81.77 ± 1.08
	+17.51		+7.13		+2.52	
	$P < 0.001$		NS		NS	
Liver	3.403 ± 0.314	3.904 ± 0.248	324.50 ± 43.27	369.50 ± 30.70	69.23 ± 0.93	71.07 ± 2.41
	+14.72		+13.86		+2.60	
	$P < 0.001$		$P < 0.05$		NS	
Kidney	1.239 ± 0.116	1.356 ± 0.022	98.83 ± 7.78	109.00 ± 10.48	72.65 ± 3.17	78.66 ± 2.34
	+9.44		+11.30		+8.27	
	$P < 0.01$		$P < 0.05$		$P < 0.001$	
Muscle	0.653 ± 0.058	0.769 ± 0.082	52.16 ± 4.92	56.66 ± 3.50	72.27 ± 2.17	78.45 ± 0.66
	+17.76		+6.73		+8.55	
	$P < 0.05$		$P < 0.05$		$P < 0.001$	

Values are means ± standard deviation of the means of six observations. + and − indicate percent increase and decrease over control; C = control rats treated with saline; E = experimental rats treated with PRL.

Growth Rate Curves

Tissues from untreated male albino rats (hereafter referred to as "normal rats") of different ages, ranging from 25 to 70 days (using six animals for each age group), were removed and weighed. Growth rate curves were plotted, with weight of the tissues against age.

Acetylcholinesterase (AchE)

Tissues from control and treated rats were isolated, and 2% homogenates were prepared in ice-cold 0.25 M sucrose solution. The levels of AchE activity were estimated according to the method of Metcalf (1951).

Results

Tissue somatic indices of all organs were elevated in response to PRL administration (Table 1). Dry tissue weights also increased following treatment with PRL; the maximum difference was seen in the seminal vesicles. Water content was elevated significantly only in the epididymis and prostate among reproductive tissues and the kidney and muscle among nonreproductive tissues. The activity levels of AchE were increased in all tissues from treated animals, although the elevation was more pronounced in reproductive tissues (Table 2).

Figures 1 and 2 represent the growth rate patterns for reproductive and nonreproductive tissues in normal rats. The intercepts of average weights of tissues from treated rats indicate the age at which normal rats would be expected to

TABLE 2. Prolactin-induced Changes in the Levels of Acetylcholinesterase Activity in Reproductive and Nonreproductive Tissues of Immature Albino Rats

Tissues	Control	Experimental	Percent Change
Testis	143.25 ± 1.59	236.36 ± 4.18	+65.00
			$P < 0.001$
Epididymis	72.33 ± 2.57	175.03 ± 2.77	+148.98
			$P < 0.001$
Seminal vesicles	157.20 ± 3.66	174.13 ± 5.10	+10.77
			$P < 0.001$
Prostate gland	114.43 ± 1.25	175.03 ± 3.14	+52.96
			$P < 0.001$
Brain	404.37 ± 2.19	458.16 ± 8.14	+13.35
			$P < 0.001$
Liver	134.66 ± 2.72	142.81 ± 1.49	+6.05
			$P < 0.001$
Kidney	86.34 ± 3.31	141.75 ± 2.64	+64.17
			$P < 0.001$
Muscle	114.17 ± 2.97	167.98 ± 1.77	+47.13
			$P < 0.001$

Values are means ± standard deviation of the means of six observations. + and − indicate % increase and decrease over control; C = control rats treated with saline; E = experimental rats treated with PRL.

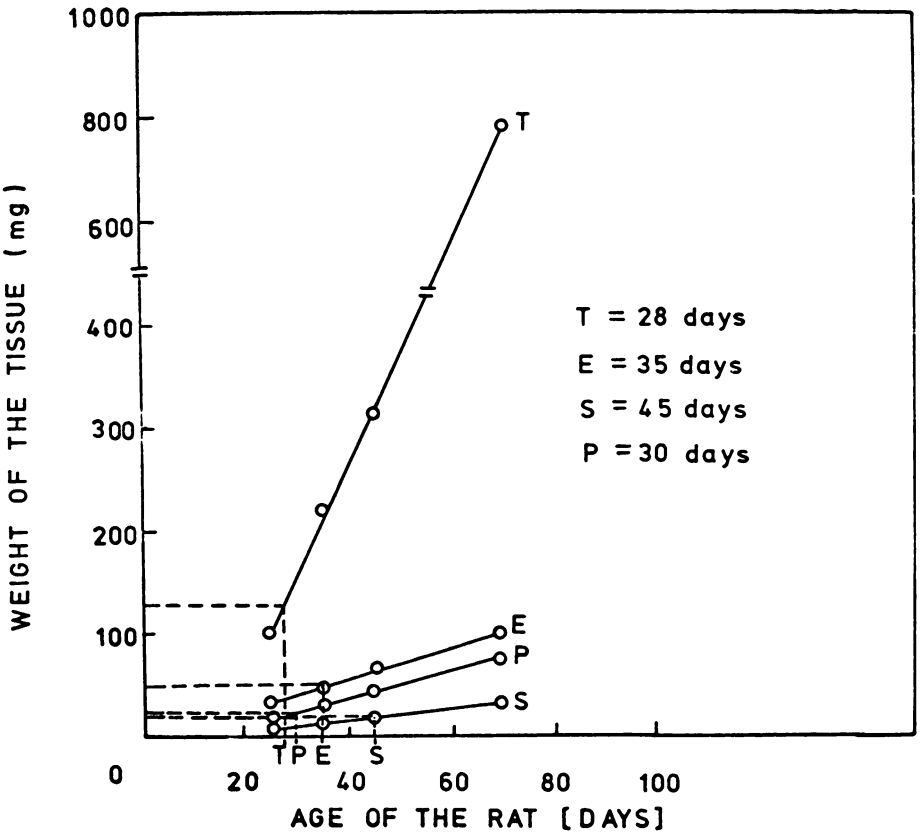

Fig. 1. Growth rate curves of normal rats: pattern of growth of reproductive tissues. The intercepts on the curves are based on the weights of tissues of PRL-treated rats. T = testis; E = epididymis; S = seminal vesicles; P = whole prostate gland.

exhibit the same weights. Thus, the testis, epididymis, seminal vesicle, and prostate gland from 26-day-old, PRL-treated rats exhibited weights which would be found in normal rats at the ages of 28, 35, 45, and 30 days, respectively. The brain, liver, kidney, and muscle showed weights which would be found in 28-, 30-, 28-, and 30-day-old normal rats, respectively.

Discussion

Administration of PRL to immature male rats resulted in an overall elevation in TSI of reproductive and nonreproductive organs, thus exhibiting an anabolic influence. Since PRL enhances androgen production (Hafiez et al, 1972a, 1972b) and androgens exhibit anabolic influences (Keenan and Thomas, 1975; Yamanaka et al, 1975; Walvoord et al, 1976; Thomas et al, 1976), it is likely that the combined effects of the GH-like activity of PRL and its androgen-mediated anabolic action might be responsible for the growth of the tissues in animals treated with PRL.

Reproductive tissues showed higher elevations in TSI than did nonreproductive tissues, suggesting greater responsiveness of reproductive tissues to PRL. A possibility of heightened responsiveness of reproductive tissues to PRL administration is also suggested by the greater increase in AchE activity in these tissues. Higher levels of elevation in TSI of accessory reproductive glands suggest the possibility that initiation of function of these glands might occur prior to the onset of testicular activity. Elevated TSI of the testis in response to PRL suggests the possibility that PRL might normally affect testicular growth before sexual maturation. The present findings are in agreement with those of earlier works using mature rats, wherein PRL-induced elevation of reproductive tissue weights have been reported (Keenan and Thomas, 1975; Yamanaka et al, 1975; Hostetter and Piacsek, 1977).

Elevated TSI in response to PRL might be due to synthetic activities leading to increased dry matter content or due to accumulation of water, since PRL alters osmoregulatory properties of tissues (Holt and Perks, 1975; Debnam and Snart, 1975; Falconer et al, 1977). The accessory reproductive

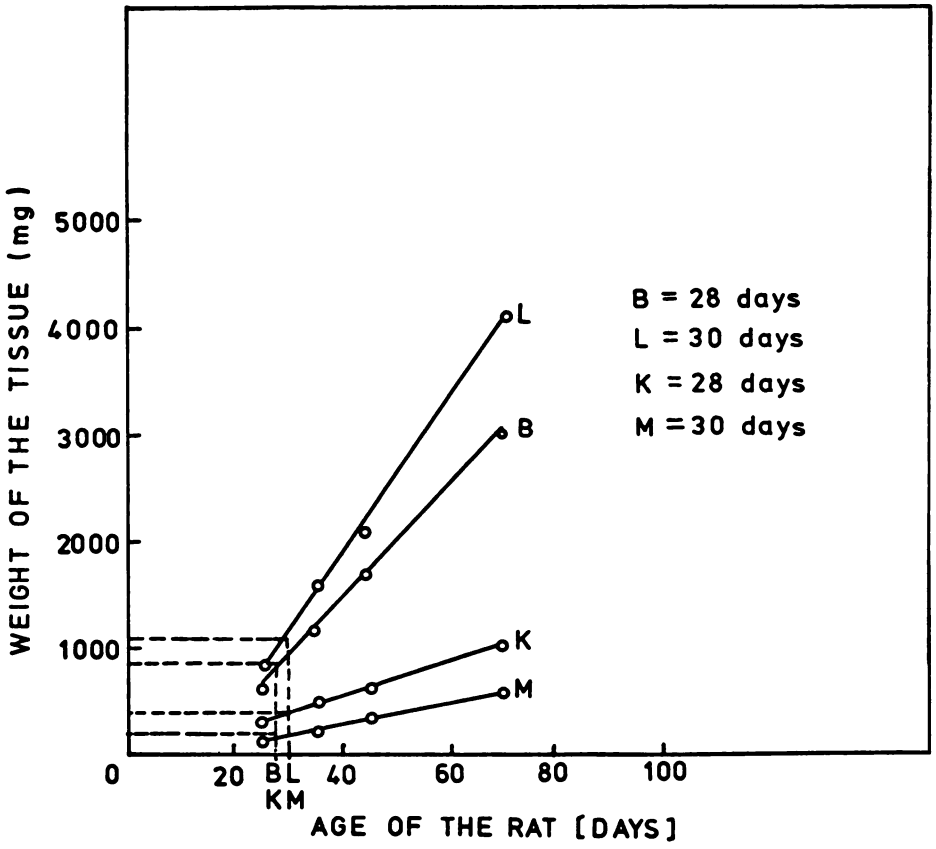

Fig. 2. Growth rate curves of normal rats: pattern of growth of nonreproductive tissues. The intercepts on the curves are based on the weights of tissues of PRL-treated rats. B = whole brain; L = liver; K = kidney; M = muscle.

glands manifested more pronounced elevations in dry matter weight than did the testis, suggesting a greater augmentation in net growth of the accessory glands than in that of the testis.

Since TSI are elevated by PRL administration, PRL-mediated modulations in growth rate patterns of the tissues can be proposed. Hence PRL-induced changes in tissue growth rates are compared with the normal tissue growth rate patterns. Tissue weights in PRL-treated rats were comparable to those in older normal rats. The seminal vesicles of the PRL-treated, 26-day-old rats were equal in weight to those of 45-day-old normal rats. Similarly, weights of epididymis, prostate and testis in treated rats at 26 days of age were equal to the weights of these organs in 35, 30, and 28-day-old normal rats, respectively. These observations suggest that treatment with PRL may induce a higher growth rate in reproductive organs than can be found in normal rats. This could lead to the initiation of structural and functional activation of these tissues before the age when these events take place in the course of normal sexual maturation. Similarly, non-reproductive tissues also indicate the possibility of advanced functioning in response to PRL, since the extent of tissue growth in PRL-treated animals was greater than that found in normal rats.

It can therefore be concluded that PRL is involved in the processes leading to the early development of reproductive functions in the immature male rat.

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References

Bartke A. Influence of prolactin on male fertility of dwarf mice. J Endocrinol 1966; 35:419–420.

Bartke A. Prolactin deficiency in genetically sterile dwarf mice. Mem Soc Endocrinol 1967; 15:193–199.

Bartke A. Effects of inhibitors of pituitary prolactin release on testicular cholesterol stores, seminal vesicles weight, fertility and lactation. Biol Reprod 1974; 11:319–325.

Bartke A. Pituitary testis relationships: role of prolactin in the

regulation of testicular function. Sperm Action Prog Reprod Biol 1976; 1:136–152.

Bartke A, Lloyd CW. Influence of prolactin and pituitary isografts on spermatogenesis in dwarf mice and hypophysectomized rats. J Endocrinol 1970; 46:321–329.

Bartke A, Smith MS, Michael SD, Person FG, Dalterio S. Effects of experimentally induced chronic hyper-prolactinaemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology 1977; 100:1.

Dattatreya MB, Raghavan VP, Purandara TV, Sheth AR, Shanta S, Rao. Synergistic action of prolactin with hCG on rat ventral prostate. J Reprod Fertil 1975; 44:555–558.

Debnam ES, Snart RS. Water transport responses of toad bladder to prolactin. Comp Biochem Physiol [A] 1975; 52:75–76.

Falconer IR, Rowe JM. Effect of prolactin on sodium and potassium concentrations in mammary alveolar tissue. Endocrinology 1977; 101:181–186.

Hafiez AA, Bartke A, Lloyd CW. The role of prolactin in the regulation of testicular function: the synergistic effects of prolactin and luteinizing hormone on the incorporation of c^{14} acetate into testosterone and cholesterol by testis from hypophysectomized rats, *in vitro*. J Endocrinol 1972a; 53:223–230.

Hafiez AA, Lloyd CW, Bartke A. The role of prolactin in the regulation of testis function: the effects of prolactin and luteinizing hormone on the plasma levels of testosterone and androstenedione in hypophysectomized rats. J Endocrinol 1972b; 52:327–332.

Holt WF, Perks AM. The effect of prolactin on water movement through the isolated amniotic membrane of guinea pig. Gen Comp Endocrinol 1975; 26:153–164.

Hostetter MW, Piacsek BE. The effect of prolactin deficiency during sexual maturation in the male rat. Biol Reprod 1977; 17:574–577.

Johnson DC. Temporal augmentation of LH by prolactin in stimulation of androgen production by the testis of hypophysectomized male rats. Proc Soc Exp Biol Med 1974; 145:610–613.

Keenan EJ, Thomas JA. Effects of testosterone and prolactin or growth hormone on the accessory sex organs of castrated mice. J Endocrinol 1975; 64:111–115.

Metcalf RL. Methods in biochemical analysis. Vol. 5. Glick D, ed. New York: Inter Science Pub., 1951.

Negro-Vilar A, Krulich L, McCann SM. Changes in serum prolactin and gonadotropins during sexual development of the male rat. Endocrinology 1973; 93:660–664.

Negro-Vilar A, Saad WA, McCann SM. Evidence for a role of prolactin in prostate and seminal vesicle growth in immature rats. Endocrinology 1977; 100:729–737.

Sheriff D, Sultan, Govindarajulu P. Prolactin and testicular lipids. Horm Res 1975; 7:146–151.

Thomas JA, Manandhar M. Effects of prolactin and/or testosterone on nucleic acid levels in prostate glands of normal and castrated rats. J Endocrinol 1975; 65:149–150.

Thomas JA, Manandhar MS, Keenan EJ, Edwards WD, Klase PA. Effects of prolactin and dihydrotestosterone upon the rat prostate gland. Urol Int 1976; 31:265–271.

Walvoord, Douglas J, Resnick I, Grayhack, JT. Effect of testosterone and dihydrotestosterone, estradiol, and PRL on the weight and citric acid content of lateral lobe of rat prostate. Invert Urol 1976; 14:60–65.

Woods MC, Simpson ME. Pituitary control of hypophysectomized rat. Endocrinology 1971; 69:91–125.

Yamanaka H, Kiradani RY, Saroff J, Murphy GP, Sandberg AA. Effects of testosterone and prolactin on rat prostatic weight, 5α-reductase and arginase. Am J Physiol 1975; 229:1102–1109.