GENERAL DISCUSSION AND CONCLUSION
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The physiological role of PRL in male vertebrates remains far from clear, yet this hormone is present in the anterior pituitary gland as well as in the blood of laboratory animals and man (Riddle, 1963; Meites and Nicoll, 1966; Bern and Nicoll, 1968; Frantz et al., 1972; Nicoll and Bern, 1972; Nicoll, 1974). Plasma concentration of PRL increases markedly in male rodents during puberty and decreases after sexual maturation (Negro-Vilar, 1973; Dohler and Wuttke, 1974; Barkley, 1979). Thus the temporal alterations which occur in circulating PRL concentrations suggest that this hormone may influence the development of male reproductive system. The developmental profiles of PRL and testosterone levels in the blood of male rats closely coincide and are marked by a steep increase at the approach of puberty (Marie et al., 1979). An interdependence between androgen and PRL establishes itself at the approach of puberty (Gooren et al., 1980). Localization of specific PRL receptors in the rat testicular interstitial tissue described by Charreau et al. (1977) suggest that the effect of PRL on androgen production is due to direct effect of PRL on Leydig cells. In addition to its effect on testicular _ function PRL has been demonstrated to act synergistically _

In the present study effects of hypo-and hyper-prolactinemia induced by PRL inhibiting/releasing drugs on some of the biochemical markers of testicular function were evaluated in intact prepubertal and adult male rats. Bromocryptine, a well known DA receptor agonist, at a dose of 100 µg significantly decreased the weights of the testes and ventral prostate in both immature and prepubertal rats. Lower doses of 10 and 50 µg however, had no effect. The present results strongly support similar observations reported earlier (Calandra et al., 1982; Sinha and Wandarlaan, 1982; Nag et al., 1983; Stroud et al, 1985). Prolactin or pimozide in both immature and prepubertal rats induced increase in the testes and the ventral prostate weights. The influence of PRL upon prostatic cells is partially attributed to the hormone's stimulatory effect upon testicular androgen production. In addition it is well established that PRL acts directly upon the cells of prostate gland and/or of the seminal vesicles in several species to augment the effects of androgens (Keenan and Thomas, 1975; Nigro-Vilar et al., 1977). Identification of receptors for PRL in male accessory sex organs lends further support to the concept
of the direct role for Prl in the function of these tissues (Barkey et al, 1977; Charreau et al, 1977; Barkey et al, 1979; Keenan et al, 1979).

Glucosamine 6-phosphate synthase activity increases during the development of testes and the high levels of the enzyme are associated with the first wave of proliferation of testicular cells. Administration of Be lowers the (glucosamine 6-phosphate synthase activity in the testes and ventral prostate. A dose of 100 μg/rat was effective in bringing about the reduction in the enzyme activity in both immature rats treated for 15 days and prepubertal rats treated for 5 days. Male accessory reproductive organs are actively involved in the synthesis and secretion of glycoproteins. The presence of high activity of glucosamine 6-phosphate synthase in male accessory reproductive organs and its control by androgens signifies that this enzyme plays a physiologically important role and contributes to the free amino sugars and glycoproteins of semen. It has been observed that the suppression of Prl levels can bring about a decrease in the weight and secretory activity of the ventral prostate (Asano et al, 1971; Bartke, 1974; Hostetter and Piacsek, 1977). Treatment with Prl or Pim on the other hand, increased the enzyme activity of both the testes and ventral prostate supporting the earlier view that Prl is
involved in the sexual maturation and reproductive function of male rats.

Hyaluronidase, a marker enzyme involved in spermatogenesis, was also evaluated in the present study. Bromocryptine treatment caused a decrease in the testicular hyaluronidase enzyme activity. Administration of Pim or Prl, on the other hand, increased the testicular enzyme activity. Both these enzymes, hyaluronidase and glucosamine 6-phosphate synthase are androgen dependent enzymes and the fact that induction of hypo-or hyper-prolactinemia caused a significant decrease or increase, respectively, in these enzyme activities show that Prl may be involved in the androgen biosynthesis thereby influencing testicular and accessory reproductive organ function.

Testosterone has been clearly shown to be reduced to ba-DHT and other 5α-reduced androgens by the male gonads and the sex accessory organs. These transformations occur under the action of at least three physico-chemically well characterised enzymes: 5α-reductase and 3α/p HSD (Monsalve and Blaquier, 1977; Hastings et al, 1980). Few years age Faransworth (1972) postulated that Prl might regulate the activity of the enzymes involved in the conversion of T to 5α-reduced androgens of the prostate
gland. Later a number of reports have shown that Prl is involved in regulating the rate of steroidogenic pathways. Yananaka et al (1975) using prepubertal castrated rats maintained with T observed an increase in prostatic 5α-reductase activity in the Prl treated animals. It has been described that Prl stimulates the activities of 3p and 17p HSD in the testes (Hafiez et al, 1972; Musto et al, 1972). At the time of puberty the levels of androstenedione decreased shifting to the production of T with increased activity of 17p-HSD and a concomitant increase in the Prl synthesis from the pituitary and increased serum Prl levels.

Testicular glucosamine 6-phosphate synthase and hyaluronidase enzyme activities were seen to increase from day 20 to 40 in rats around which time Prl levels were also known to rise (Odell and Swerdloff, 1976; Payne et al, 1977; Piacsek and Goodspeed, 1978; Prasad and Adiga, 1979). Both these enzymes are involved in spermatogenesis and are androgen dependent. In this study the decrease or increase in these two enzymes observed by the induction of hypo-or hyperprolactinemia, respectively, suggest that Prl may be involved in the conversion of either androstenedione to T or T to DHT which is evident by the fact that ba-reductase and 17p-HSD levels increase with the rise in Prl levels and decreases when Prl levels are inhibited.
The alteration observed in the glucosamine 6-phosphate synthase activity in the ventral prostate by hypo- or hyperprolactinemia support the interdependence of androgens and Prl in maintaining the accessory glands of reproduction.

Cholesterol is an obligatory precursor for steroid hormone synthesis and any alteration in the plasma cholesterol is likely to affect the steroid hormone synthesis. Circulating plasma cholesterol levels were also found to alter with changes in Prl secretion brought about by Prl releasing/inhibiting drugs. Plasma cholesterol levels increased in both immature and pre-pubertal rats when treated with Be. This increase was observed in the esterified cholesterol levels, whereas no change was observed in free cholesterol. This may be interpreted as interference of Prl during steroidogenesis leading to the accumulation of the cholesterol in the plasma as the target tissues are unable to utilize the circulating cholesterol for androgen synthesis. On the other hand, both Pim and Prl reduced the plasma cholesterol esters, showing that the rate of steroidogenesis increases with hyperprolactinemia as the cholesterol uptake by the target tissues increases with the increased androgen output. This provides further evidence that Prl may be involved in the maintenance of steroidogenesis in sexually maturing rats.
Baclofen and Muscimol, two known GABA agonists however, did not modify either the weights or the enzyme activity of the testes and the ventral prostate. This may be due to the insensitivity of the GABA receptors to these agonists in young rats. In adult rats there was no change in the testicular or ventral prostate weights or their glucosamine 6-phosphate synthase activity. Testicular hyaluronidase and plasma cholesterol levels also did not show any modification. Similarly treatment of male rats with pituitary grafts failed to elicit gonadal response and the ratio between T and DHT suggesting a normal 5cc-steroid reductase activity in adult male inspite of increased Prl levels (Bartke et al. 1977; Webber et al, 1982; 1983). Although spermatogenesis in immature rats is analogous to spermatogenesis in mature rats with respect to sequence and dynamics (Clermont and Perey, 1957; Hilsher, 1974) the hormonal control of its initiation may show a difference as compared with the established cyclic course in mature rats. Hence the difference in response to Prl levels in immature and prepubertal adult rats.

The present results provide additional support that one of the physiological roles of Prl is concerned with the growth and functional aspects of reproduction and other androgen sensitive organs. These results also
indicate that Prl may facilitate T production by acting at the testicular site and also exert a direct modulatory influence on the androgen action at the level of the target tissue. However, the sensitivity of the various organs to Prl seems to be critically governed by the age and physiological state of the species. In general the androgen dependant tissues of immature and prepubertal rats appear to be more sensitive to alterations in the hormone levels than adult animals. Thus from the present results it appears that one of the functions of Prl in the developing male rodent is concerned with the initiation of puberty.

In another study a similar role for Prl was observed in prepubertal female rats. Bromocryptine treatment in prepubertal female rats reduced the ovarian and uterine weights as well as the uterine glycogen concentration and glucosamine 6-phosphate synthase activity. On the other hand, Pirn a DA receptor antagonist, increased the ovarian and uterine weights as well as the uterine glycogen concentration and glucosamine 6-phosphate synthase activity. Several reports have implicated Prl in the regulation of the timing of puberty (Advis and Ojeda, 1978; Ojeda et al., 1980). These results are in harmony with this view showing a stimulatory role for Prl in the onset of puberty. Blockade of Prl with Be was shown to decrease the ovarian
LH binding sites (Marta et al., 1984) and delayed development of the ovarian follicles (Advis et al., 1980; Shaban and Terranova, 1984). Hyperprolactinemia has been reported to induce precocious puberty in rats (Advis and Ojeda, 1978; Advis et al., 1981). In the present study also injection of Pirn caused onset of precocious puberty in more than 60% of the rats. If vaginal opening is taken as a marker for the onset of puberty, induction of hyperprolactinemia with Pirn advanced the onset, by at least 2 days, in the present studies.

In O VX rats administration of EB was able to restore the uterine weights, glycogen concentration and glucosamine 6-phosphate synthase activity. Pimozide alone was not able to modify any of these parameters. However, Pirn and EB or Be + EB treatment in O VX rats restored the uterine weight, glycogen concentration and glucosamine 6-phosphate synthase activity. Prolactin is shown to participate in the developmental process that leads to puberty by increasing the ovarian steroidogenic response to gonadotrophs. Hyperprolactinemia is shown to advance puberty and shows ovarian follicles in a more advanced phase of development than in the control animals. In hyperprolactinemia more follicles develop to a stage in which the estrogen output is significantly increased (Advis et al., 1984). The changes seen in the
present study induced by hypo-or hyperprolactinemia provides support to the view that Prl influences the estrogen output which is essential for the uterine growth and other estradiol induced parameters.

Finally, the effect of Be on the gestation period, litter size and uterine glucosamine 6-phosphate synthase activity also indicate Prl involvement at some stage. Injection of Be prior to implantation on/or before day b produced inhibition of implantation and subsequent pregnancy. The glucosamine 6-phosphate synthase activity which is known to increase during early pregnancy was decreased with Be treatment on day 5 of pregnancy. This enzyme is predominantly found in the endometrium and the blockade of Prl brought about a decrease in the progesterone synthesis leading to a decrease in the enzyme activity. However, blockade of Prl by Be after implantation on day 9 to 14 or 14 to 20 did not cause any effect on the gestation period or the litter size. Administration of Be on day 7 of pregnancy also did not disturb the pregnancy nor the enzyme levels.

Thus the present study shows that Prl is essential during early pregnancy before implantation as it is necessary for the maintenance of corpus luteum which in turn secretes progesterone for the maintenance of pregnancy.
Blockade of Prl during early pregnancy impairs progesterone secretion thereby causing failure of implantation and subsequent gestation. In the later stages of pregnancy, after the implantation has occurred, the rPL appears which maintains the P secretion from corpus luteum (Yogve and Terkel, 1978; Voogt, 1980). Bromocryptine is a dopamine agonist which blocks the Prl release at the pituitary level and it does not seem to have any effect on the rPL as there was no interference with the pregnancy or the enzyme levels when Be was administered after implantation.