CHAPTER VI

EFFECT OF EXOGENOUSLY ADMINISTERED TESTOSTERONE PROPIONATE ON SOME ASPECTS OF METABOLISM OF RAT SUBMANDIBULAR GLAND

Pioneering studies on rodent salivary glands by Lacassagne (1940) described the sexual dimorphism and further that the sex hormones produce morphological changes in the submandibular gland. The submandibular glands of male have been reported to show more concentration of granular tubules than that of females. Reversal of structural changes due to administration of sex hormones of opposite sex in mice and rats have been demonstrated by Chaulin-Servinere (1942); Shafer and Muhler (1953); Bixler et al. (1955); Cassano (1958) and Atkinson et al. (1959). Berkman and Kronman (1970) have shown that administration of testosterone to mice induced enlargement of granular tubules. Sex-dependent variations in biochemical and histochemical distribution in the submandibular glands of various rodent species have already been reported viz. - for alkaline- and acid-phosphatase (Junqueira, 1949; Junqueira et al., 1949), protease (Sreebny et al., 1955), acid mucopolysaccharides (Shackleford and Klapper, 1961), amylase (Raynaud and Rebeyrotte, 1949), arginase (Kochakian et al., 1955) and tryptophan and tyrosine (Junqueira, 1949; Kronman, 1963). Though such studies have been conducted on submandibular glands, the effects of endocrine manipulations on metabolic patterns of the glandular tissue have not been

adequately explained so far. Hence the present investigation was planned to characterize some metabolic changes in the submandibular gland of male rat after exogenous administration of testosterone. In a comparative study on relative potencies of testosterone and its metabolite in restoring some morphological and functional characteristics of murine submandibular gland by Minetti et al. (1985) it was reported that testosterone elicited its effect on submandibular gland, without prior conversion to DHT, and that, it is possibly due to receptor-independent mechanisms whereby α- and β-diols exert trophic or metabolic effects on the gland. Curbello et al. (1987) have shown that administration of testosterone propionate (TP) to castrated and noncastrated male mice hypertrophies the tubular portion of submaxillary gland without affecting the acini.

Various reports are available on the effect of testosterone administration on accessory reproductive organs as well as non-target tissues. Hosoi et al. (1978) showed that liver enzymes of mouse were affected by androgens. Based on their work on rat seminal vesicle and epididymis, Higgins et al. (1981) have reported that androgens may principally control RNA degradation. TP was required to maintain normal levels of total protein, sialic acid and alkaline-phosphatase activity in epididymis of langurs (Gupta and Dixit, 1981). Teruo et al. (1987) observed that replacement with testosterone enanthate in castrated rats did not fully restore the activity of γ-glutamyl transpeptidase in the epididymis. Rate of incorporation of ^{3}H Leucine into total protein and into androgen binding

phosphodiesterase (PDE), aldolase, total ATPase, Na^{+}-K^{+}-ATPase and succinate dehydrogenase (SDH) were assayed.

4. Methods employed for assaying these parameters were same as stated in Chapter I and II
5. Student's 't' test was carried out to know the stastical significance of the data.

R E S U L T S

1. The results obtained (Table - 1) showed a significant increase in the total lipid, cholesterol and AA content of the submandibular gland.
2. The glycogen content was observed to increase significantly ($P < 0.02$) 1 hr after TP administration but by 2 hr it was restored to almost normal level and sustained so upto further time lapse.
3. The glycogen synthetase activity was found to register a significant ($P < 0.001$) increase by 1 hr and was noticed to show further stepwise marked increase at subsequent two intervals while total phosphorylase activity was found to be noticeably suppressed initially but tend towards recovery was apparent.

4. It was noticed that exogenous administration of TP led to varying degrees of suppression of PDE, Na^{+}-K^{+}-ATPase and total ATPase activity levels in that ascending order after 1 hr however, this trend was reversed in a significant manner at the subsequent

Table 1

Showing the influence of TP administration by way of exogenous dose to intact male rats on various biochemical parameters

	Normal	Normal intact animals injected with 100 µg TP (i.m.) Post injection intervals 1 hr	2 hr	4 hr
GLYCOGEN mg/100 mg tissue	0.062 ±0.003	0.091* ±0.009	0.074 ±0.008	0.071 ±0.005
GLYCOGEN SYNTHETASE µ moles UDP formed/mg protein/ 15 min	0.014 ±0.001	0.113*** ±0.010	0.360*** ±0.056	0.428*** ±0.043
PHOSPHORYLASE µg PO4 released/mg protein/ 30 min	25.84 ±0.54	20.72§ ±1.03	21.40 ±1.36	23.67 ±1.27
c.AMP PHOSPHODIESTERASE µg PO4 released/mg protein/ 30 min	2.91 ±0.05	2.05 ±0.29	4.27* ±0.34	3.08 ±0.26
$Na^{+}.K^{+}$-ATPase µg PO4 released/mg protein/ 10 min	13.50 ±1.74	8.44§ ±0.61	21.62** ±1.18	21.15** ±0.94
TOTAL ATPase µg PO4 released/mg protein/ 10 min	52.54 ±2.33	24.75*** ±1.27	46.70 ±2.22	45.69** ±0.88

two intervals. In the latter case the escalation of PDE activity was of the highest order followed by that of Na^{+}-K^{+}-ATPase and total ATPase.

5. Activity levels of SDH and aldolase showed a reduction at all the intervals. In case of aldolase maximum suppresssion occurred at 2 hr while in case of SDH at 4 hr.

D I S C U S S I O N

Looking into the overall results obtained due to exogenous administration of TP; it can be said that an initial increase in the glycogen content was observed within an hour with concomitant increase in glycogen synthetase activity and decrease in phosphorylase activity. But by 2 and 4 hr though the glycogen synthetase activity is enhanced the rate of glycogen synthesis is depleted. A marginal depletion in phosphrylase activity could have led to the observed glycogen decrement. Further, there is decided increase in the synthesis of lipid, cholesterol and AA. Djoseland et al. (1980) have shown that variation in the PDE activity is part of modulation of overall androgenic response. Adrenal steroid therapy has been shown to decrease PDE activity levels in rat fat cells (Vincent and Vaughan, 1972 & 1973, Ross et al., 1977; Elks et al., 1983). Androgens have been shown to elevate c.AMP levels of sex accessory organs (Singhal and Valadares, 1968; Santti and Villee, 1971; Singhal et al., 1971 and Mangan et al., 1973). Contrary to such observations, a marked increase in the PDE activity was obtained

during the present course of study, leading to breakdown of c.AMP to AMP. Such low levels of available c.AMP would lead to depletion in phosphorylase activity and increase in the glycogen synthetase activity which is noticeable by 2 and 4 hr. Breakdown of c.AMP would release more of AMP which in turn is known to activate phosphorylase and phosphofructokinase activity - Activation of phosphorylase yields glucose-1-phosphate which has to enter glycolytic pathway. As observed glycogenesis is not apparent at interval of 2 and 4 hr of TP administration though glycogen synthetase activity is seemingly very high. Whatever quantitiy of glucose is thus released should find its way either through glycolysis or HMP shunt pathway. Glucose has been implicated as a major source for lipid synthesis (Winegard and Renold, 1958). Glucose contributes carbons both to the formation of glycerol-3-phosphate and after glycolysis, to the synthesis of fatty acids. It is clear from the data that glucose is not getting oxidised through TCA as evidenced by suppression of aldolase and SDH activity. In this circumstances it is obvious that glucose is diverted toward HMP shunt which may provide NADH necessary for lipogenesis. Fructose 1,6-diphosphate aldolase has been shown to be involved in lipid synthesizing tissues like mammary gland (Abraham and Chaikott, 1959) adipose tissue (Hollifield and Parson, 1961; Weber et al., 1961) and skin (Takayasu and Adachi, 1970 and Sato et al., 1981). During the present course of study, the aldolase activity was of reduced tune. One would expect this activity to be enhanced as there is increased lipogenesis observed. Such paradoxical result could be explained on following basis:

The enzyme aldolase is known to have 3 isozymes viz. Aldolase A, B and C (Markert, 1974). Primary role of aldolase A has been reported to be preferentially for glycolysis, facilitating breakdown of fructose-1,6-diphosphates to triose phosphates, while aldolase B, which is prominent in liver, has been proved to possess higher affinity for triose phosphate consistent with its role in favouring conversion to hexose diphosphate and thereby aiding the reversal of E-M pathway. Though overall aldolase activity was observed to be reduced, probably the aldolase A involoved in glycolysis was suppressed in preference to B isozyme and the latter was not apparently influenced by exogenous TP. This would lead to accumulation of triose phosphate molecules favouring greater feed for lipid synthesis by providing more of glycerophosphates (Lehninger, 1984).

It has been shown that castration reduces the state of mitochondrial respiration and the mechanism of active transport in various androgen dependent glands (Edelman et al.,1963; Javery et al., 1963; Ahmed and Williams-Ashman, 1969; Doeg et al., 1971 and Brooks, 1978 & 1979). One of the mitochondrial enzymes, SDH, was observed to decrease, which means that the TCA cycle was functioning at a reduced rate. This could have possibly led to rise of citrate concentration in the mitochondria, which must have escaped to the cytosol. Higher cytosolic citrate level is known to facilitate formation of acetyl-CoA which leads to biosynthesis of long chain fatty acids. Consequently, these fatty acids are esterified as more of glycerophosphates are made readily available, as stated earlier. Further, it is known (Harper, 1988) higher intracellular

levels of citrate and acetyl-CoA may also favour cholesterol synthesis.

Testosterone has been documented to exert stimulatory effects on lipogenesis in sebaceous analogs (Huggins et al., 1955; Freinkel, 1963; Archibald and Shuster, 1969; Takayasu and Adachi, 1970; Ebling, 1974; Pochi and Strauss, 1974 and Mesquita and Coimbra, 1981). Thus, increase in cholesterol and lipid contents was obtained due to exogenous TP administration finds support.

It has been observed that Na^{+}-K^{+}-ATPase activity is reduced during first hour of TP administration. This suggests that though the membrane transport phenomenon are not influenced by TP administration within first 60 min, of later intervals of 2 and 4 hr there was a marked rise in the Na^{+}-K^{+}-ATPase activity. This may lead to enhanced uptake of glucose from circulaing blood. However, the rising levels of intracellular glucose apparently did not get reflected in enhanced rate of glycogen. In this circumstance, it could be said that the glucose was increasingly channelized into HMP shunt. On the other hand, total ATPase activity was observed to be depleted at all the time intervals. This could possibly mean that exogenous TP induces lowering of cytosolic ionic flux, which may get reflected in the paradoxical alterations in various enzyme activities (Stubbs and Mc Kerman, 1967). Stubbs et al. (1967) and Khandwekar et al (1973) have shown lower hepatic AA values due to castration and such low levels have been correlated with the deficit of testosterone. An increase in the glandular AA could be due to hyper-

physiological dose of TP. Comparatively high AA content of the gland probably might reflect either enhanced de novo biosynthesis or its utilization at a reduced level.

Kronman and Spinale (1965) have shown that testosterone induced enlargement of granular tubules of submandibular gland of mice. Assumingly, the increase in total lipid and cholesterol obtained during the present course of study could be attributed to such an enlargement of granular tubules, rather than the acinar components in case of rats. From such disturbed metabolic patterns obtained due to TP administration and from the data discussed in Chapter I and II it can be concluded that the normal circulating androgens do have a definite regulatory effect on submandibular gland of rats, and that super normal levels of androgens are responsible for disturbances in the patterns of glandular metabolism.