CHAPTER III

OBSERVATIONS ON INFLUENCE OF β-ADRENERGIC AGONIST - ISOPROTERENOL (IPR) - ON LIPID, CHOLESTEROL AND ASCORBIC ACID OF SUBMANDIBULAR GLANDS.

The innervation of the salivary glands of rats comprises of sympathetic adrenergic fibres as well as parasympathetic fibres. Calcium concentration of the submandibular glands of rat was shown to increase 2-3 times, 24 hr after administration of reserpine wherein the increase in the calcium concentration was correlated to a depletion of non-epinephrine (Schneyer et al., 1983). Acute isoproterenol treatment to rats was found not to unalter the calcium concentration of reserpinized submandibular glands but the same was found to decrease in untreated rats (Jirakulsomchok et al., 1984; Schneyer and Jia-Huey, 1984). In rats and mice, enlargement of submandibular salivary gland could be produced experimentally by administering analogs of epinephrine (Selye et al., 1961). This enlarging effect was found to be proportional to the dose administered (Schneyer, 1962; Pohto and Paasonen, 1964). Jia-Huey et al. (1983) showed that substance P, stimulates salivary secretion and that the secretion of fluid, ions and amylase is effected via cholinergic receptors, a subpopulation of substance P-specific receptors.

Putney (1983) has reported that in vitro preparation of rat parotid gland respond to cholinergic and ∝-adrenergic agonists. Further, Putney also reported that the changes in the pohospholipid composition may directly mediate the agonist induced alterations in cellular calcium concentration. Baum et al.(1984) showed that stimulation of ∝-adrenergic nerve of rat parotid gland led to secretion of elevated levels of saliva containing low concentration of proteins. Parasympathetic denervation of the gland decreased the acetylcholine levels, ten days after the surgery (Marvin et al., 1983). Experiments on rats by Mazariegos and Hand (1984) showed that stimulation of parotid glands by IPR caused a transient increase in tight junctional permeability of the gland.

Most of the reports cited above clearly indicate that the functioning of the salivary glands of rat is constantly regulated by sympathetic as well as parasympathetic nerve fibres. The sympathetic acts on the salivary glands via the mediation of catecholamine receptors viz. - ∝ - and β-type. It has been shown that administration of a β-adrenergic agonist (IPR) produces hypertrophy and hyperplasia of the submandibular gland (Schneyer, 1962; Pohto and Paasonen, 1964; Barka, 1966; Wells and Michael, 1985; Yagil and Barka, 1986). β-adrnergic agonist increases sodium absorption, but decreases potassium secretion without changing net water movement (Schneyer and Thavornthon, 1973). Administration of IPR was reported to exhibit a sex difference in DNA synthesis and adenylate cyclase enzyme in rat submandibular gland (Catanzaro et al., 1974).

Many of these studies on adrenergic agents dealt with chronic administration. In light of the above information, it was thought desirable to look into the acute effect of a β-adrenergic agonist (IPR) on submandibular gland of male albino rats. With this view the influence on the metabolic profile of submandibular gland were studied in respect of a few metabolites viz. - total lipids, total cholesterol and total ascorbic acid content. It was shown by Abe et al. (1980) that parotid gland of rat responded to epinephrine in a dose dependent fashion. Schneyer (1962) and Pohto and Paasonen (1964) reported a dose dependent response in case of IPR and aludrine administration on submandibular glands of rat. During the course of present work too, response to three different doses in each case were studied with respect to the metabolites under investigation

MATERIAL AND METHODS

Adult male albino rats weighing 120-140 g were employed for the present experiments. The rats were maintained in the laboratory with food and water ad libitum. Sixty rats were grouped into three:

1. A group of twelve rats served as normal rats.
2. Another group of twelve rats received individually a single dose of 0.5 ml of 0.9% normal saline and these served as control animals.
3. Remaining 36 rats were sub divided into three batches of twelve each, which were administered isoproterenol

(a β-adrenergic agonist) (a) 15 (b) 25 and (c) 35 mg/Kg b.w. for possible dose-dependent response.

Isoproterenol was dissolved in normal saline. The assays in the normal, control and experimental animals were carried out at arbitrarily chosen short intervals of one and two hours post-administration.

At the selected intervals the animals were decapitated under mild anaesthesia. The submandibular glands excised and freed of connective tissue. The glands were immediately weighed and one of the glands was utilized for estimating the total ascorbic acid (AA) content while the total lipid and total cholesterol contents were assayed from the other gland. The total lipids, cholesterol and AA contents in the submandibular gland were determined and expressed as per the methods described in the Chapter I.

R E S U L T S

The present investigation was an attempt to know the involvement of β-adrenergic agonist in influencing the metabolism of submandibular gland with respect to total lipids, cholesterol and AA contents. The results are depicted in Table - 1.

The total lipid content of submandibular gland was found to be significantly ($P < 0.001$) reduced with all the three doses of IPR by 1 hr of its administration. After 2 hr of all the three

doses of IPR administration there was no alteration in the lipid content as compared to 1 hr. A marginal reduction was noticeable at 1 and 2 hr of interval in total cholesterol content with the lower dose of 15 mg/Kg b.w., however, it was statistically non-significant. 25 mg/Kg b.w. dose of IPR significantly ($P < 0.01$) reduced the cholesterol content at the end of 1 hr but by 2 hr interval a highly significant ($P < 0.001$) increase was observed. The higher dose of 35 mg/Kg b.w. of IPR administration reduced significantly ($P < 0.02$) the cholesterol content of the gland within an hour, nevertheless it was restored to the normal levels by 2 hr interval. Thus, the higher dose of IPR seems to reduce the cholesterol content of the submandibular gland by an hour but the effect seems to wane by 2 hr, whereas the lower dose levels apparently do not seem to influence the cholesterol content of the submandibular gland to any noteworthy alteration at both intervals. From table - 1 it is obvious that though the lowest dose of IPR did enhance the AA content of the submandibular gland with increasing doses a suppressive effect was apparent within first 60 min, however, during next 60 min tendency towards recovery was noticeable. In general, it could be said that IPR led to a reduction in the lipid content and increase in cholesterol content. In respect of AA content it could be said that only the lower dose led to an initial increase, but with the higher doses first depletion and then recovery was noted.

Table 1

Showing the influence of IPR on various metabolites of submandibular glands of male rats.

	Normal	Control injected with saline	Isoproterenol administration (i.p.) Post injection interval : 60 min		
			15 mg/Kg b.w.	25 mg/Kg b.w.	35 mg/Kg b.w.
ASCORBIC ACID mg/100 mg tissue	0.022 ±0.001	0.023 ±0.001	0.030** ±0.002	0.040*** ±0.002	0.015*** ±0.001
TOTAL LIPIDS g/100 g tissue	4.491 ±0.211	4.639 ±0.137	1.749*** ±0.130	2.116*** ±0.177	1.262*** ±0.120
CHOLESTEROL g/100 g tissue	0.295 ±0.03	0.290 ±0.001	0.245 ±0.012	0.195** ±0.01	0.197* ±0.018

Values are mean ± SE (not less than 8 animals)
*** - $P<0.001$; ** - $P<0.01$; * - $P<0.02$;

Table 1 (Contd.)

Showing the influence of IPR on various metabolites of submandibular glands of male rats, 120 min after administration

	Normal	Control	Isoproterenol administration (i.p.) 15 mg/Kg b.w.	25 mg/Kg b.w.	35 mg/Kg b.w.
ASCORBIC ACID mg/100 mg tissue	0.022 ±0.001	0.023 ±0.0006	0.034** ±0.003	0.026 ±0.002	0.018* ±0.001
TOTAL LIPIDS g/100 g tissue	4.49 ±0.21	4.63 ±0.13	1.73*** ±0.12	2.01*** ±0.11	1.36*** ±0.07
CHOLESTEROL g/100 g tissue	0.295 ±0.03	0.299 ±0.005	0.233 ±0.018	0.452*** ±0.008	0.228 ±0.028

Values are mean ±SE (not less than 8 animals)
*** - $P<0.001$; ** - $P<0.01$; * - $P<0.02$.

D I S C U S S I O N

Results obtained here indicate that administration if IPR a β-adrenergic agonist led to decisive reduction in the total lipid content irrespective of dosage as well as time lapse. However, the alterations induced due to 25 mg/Kg dose were comparatively erratic. It has been reported that IPR inhibits lipogenesis in the chicken hepatocytes and adipose tissue explants in vitro (Campbell and Scanes, 1985). IPR has been observed to inhibit (^{32}P) Pi incorporation into phosphatidyl inositol in white fat cells of rat (Garcia-Sainz and Fain, 1980) and rat heart (Kiss and Farkas, 1975).

On the other hand, α-adrenergic agonists have been reported to enhance the labelling of phosphatidyl inositol whereas IPR, a β-adrenergic agonist did not elicit any such response in rat parotid slices (Michell and Jones, 1974), rat pineal cultures (Eichberg et al., 1973) and in mouse thyroid gland (Hiroya et al., 1982). Similarly, cholinergic stimulation in guinea pigs was found to lead to enhanced incorporation of ^{32}P into phospholipids in seminal vesicles whereas adrenergic stimulation was ineffective (Lockwood and William Ashman, 1971). Schramm (1967) had shown that IPR administration is ineffective as far as phospholipid synthesis is concerned.

Besides, it was found during the present course of study that administration of IPR led to a significant reduction in the c.AMP-specific phosphodiesterase activity.

This would logically lead to an increase in the cellular c.AMP content. Based on their in vitro and in vivo studies several workers have attributed an antilipogenic role to c.AMP, especially in liver and adipose tissue (Bricker and Levey, 1972; Lakshmanan et al., 1972; Beg et al., 1973; Lee et al., 1973; Capuzzi et al., 1974; Palech et al., 1981.

Synthesis of cholesterol and its esters are known to be reduced by IPR treatment of cultured human fibroblasts (Maziere et al., 1983). From this discussion it is reasonable to suggest that, either phospholipids or cholesterol and its esters being components of total lipid concentration, the reduction in the latter is amply justified as a clear indication of influence of IPR administration on the total lipid profile of the submandibular gland of male white rats.

Earlier works reported from this laboratory on the effect of chronic administration on preputial glands of male rats indicated that exposure to IPR led to metabolic alterations similar to those obtainable after androgen deprivaion. As has been mentioned in Chapter I of the current work it has been shown by several workers that there is close correlation between circulating androgen levels

and tissue AA levels. The observed increase in AA content of submandibular gland under the influence of acute IPR administration could also be due to less availability of androgens to the sensitive tissues. Observed enhancement of AA level in the submandibular gland probably indicates a disturbance with metabolic role, normally regulated by proper levels of androgens. Submandibular gland AA is probably involved in its oxidative metabolism. Such an acute effect observed for an ititial hour could possibly mean that IPR was able to exert its effect at least within an hour of administration, though not over longer period. And this is in consonance with the well known pharmacological fact, that drugs like IPR have very short life under in vivo condition. The limited dose-dependent study conducted here goes to point out that such changes are probably highly sensitive as far as doses are concerned and that any future studies on administration of drugs like IPR should carefully select the dose(s) to get to satisfactorily comparable results in respect of any metabolic fluctuations.