CHAPTER V

EFFECTS OF PROPRANOLOL ADMINISTRATION ON CERTAIN METABOLIC PATTERNS OF SUBMANDIBULAR GLAND OF MALE RAT

It is known that salivary glands of mammals are influenced by adrenergic as well as noradrenergic transmitters (Strombald and Nickerson, 1961; Assorson and Emmelin, 1964; Nordenfelt, 1964; Mangos et al., 1975a & b; Garrett, 1965 & 1966; Schneyer and Hall, 1965; Case et al., 1980; Ekstrom, 1980). It was reported by other workers (Schneyer, 1962; Pohto and Paasonen, 1964) that administration of β-adrenergic agonist leads to hypertrophy of glandular mass. In rats and mice enlargement of salivary glands, comparable to human sialadenosis, can be produced experimentally by administering analogs of epinephrine viz. - isoproterenol and aludrine (Seifert, 1966), however, these effects were noted to be dose-dependent.

According to Norberg and Hamberger (1965) adrenergic nerve fibres have been histologically observed to be in close contact with the acinar cells in the parotid and submandibular glands but the sublingual glands lack in sympathetic secretory innervation. It was suggested that sympathetic dilatation, which follows the marked vasoconstriction in the submandibular gland of the cat is adrenergic in origin (Schachter, et al., 1963, 1965 & 1967). On the basis of their work on cats administered with a β-adrenergic blocking drugs - propranolol and dichlorosioprenaline, Webster

et al. (1967) concluded that atropine-resistant vasodilatation produced by chordalingual stimulation is also adrenergic and the stimulation effect is restricted to parotid and submandibular glands, whereas sublingual glands are not affected. The sympathetic secretory nerve fibres of the salivary glands in rat, except the sublingual, act via the mediation of catecholamine receptors viz. - α- and β-type (Emmelin et al., 1965).

All the above cited observations were mostly made after chronic administration of drugs. On this background, it was thought desirable to look into the acute effects of an adrenergic drug viz. propranolol - on the pattern of glycogen metabolism on salivary glands of rats. Moreno et al. (1984), in their study on rabbit, showed that β-receptors play an important role in the salivary secretory response and the α-adrenoreceptors are involved with the motor one. On the basis of the work done by Schneyer et al. (1986) on rat salivary gland it was shown that acute sympathetic stimulation leads to a change in number of active receptor densities as well as levels of c.AMP and c.GMP. Bloom et al. (1981) have shown that long term super stimulation of rodent salivary glands with β-adrenoreceptor agonist - isoprenaline - induces tremendous enlargement of glands and that this effect was blocked by a β-adrenoreceptor antagonist such as propranolol, suggesting a direct action of drug via the β-adrenoreceptor. The present study was carried out as an attempt to have a better understanding of the acute effect of β-adrenergic blocker - propranolol - on the involvement of β-adre-

nergic receptor functions concerning certain metabolic patterns of submandibular gland.

MATERIAL AND METHODS

1. The submandibular glands were freed of connective tissue and following parameters were assayed - tissue glycogen, total lipids, total cholesterol, total ascorbic acid and enzyme activity levels of total phosphorylase, glycogen synthetase, c.AMP specific phosphodiesterase, aldolase, total ATPase, Na^{+}-K^{+}-ATPase and succinate dehydrogenase.

2. 24 normal intact rats were subjected to the assays for basic reference values of the parameters under investigation.
   In all 72 other normal rats were treated with 0.5 ml of saline for control values.

3. A total of 216 rats were administered a single i.p. dose of propranolol dissolved in normal saline. These animals were divided into three equal groups. Each of the group was administered with propranolol as following doses:- 25 mg/Kg b.w., 35 mg/Kg b.w. and 45 mg/Kg b.w. respectively. 24 animals were sacrificed at intervals of 5, 10 and 60 min. All injections were given at 9.00 hr.
   Student's 't' test was employed to determine the statistical significance of the data.

R E S U L T S

1. The results obtained during the course of present investigation (Table - 1) showed a significant increase in the glycogen, total lipids and total cholesterol content of the submandibular gland by 60 min after the drug administration with all the three selected doses except the highest dose (45 mg/Kg b.w.) revealed no alteration in the total lipid content.
2. The glycogen synthetase enzyme activity was found to be significantly stimulated with all the three doses and selected time intervals, however, only the lowest dose 25 mg/Kg b.w.) did not exhibit any change by 5 min time interval.
3. The phosphorylase activity revealed a dose-dependent suppression at 5 and 10 min time intervals, the maximum being with the lowest dose (25 mg/Kg b.w.). Apparently, the recovery was faster with lower dose than the other two at 60 min time period.
4. On the other hand, the effect of this drug, irrespective of dose level and time interval was seen to lead to sustained elevation of the c.AMP specific PDE activity.
5. The SDH activity did not show any noticeable alteration with propranolol administration of any time interval and also dose level.
6. In the case of aldolase activity it was seen that all the three doses led to suppression by about 10 min, nevertheless signs of recovery were apparent by 60 min.
7. The total ATPase activity registered a significant increase

	Control injected with saline (i.p.)			
		Post injection intervals		
	Normal	5 min	10 min	60 min
GLYCOGEN mg/100 mg tissue	0.062 ±0.002			0.061 ±0.003
GLYCOGEN SYNTHETASE µ moles UDP formed/ mg protein/15 min	0.014 ±0.001	0.013 ±0.001	0.014 ±0.001	0.013 ±0.001
PHOSPHORYLASE µ moles PO4 released/ mg protein/30 min	25.84 ±0.54	25.67 ±0.36	26.18 ±0.33	26.11 ±0.33
c.AMP PHOSPHODIESTERASE µ moles PO4 released/ mg protein/30 min	2.91 ±0.05	2.90 ±0.11	2.81 ±0.25	2.81 ±0.34
$Na^{+}.K^{+}$-ATPase µ moles PO4 released/ mg protein/10 min	13.50 ±1.74	13.09 ±0.99	13.08 ±0.53	13.95 ±0.66
TOTAL ATPase µ moles PO4 released/ mg protein/10 min	52.54 ±2.33	53.71 ±1.99	57.48 ±1.40	55.17 ±1.52
SDH µg formozan formed/ mg protein/30 min	25.99 ±1.29	25.79 ±1.15	25.14 ±1.43	25.17 ±1.09
ALDOLASE µ moles FDP cleaved/ mg protein/60 min	0.871 ±0.042	0.895 ±0.021	0.850 ±0.045	0.905 ±0.070
PROTEIN mg/100 mg tissue	18.84 ±0.69			19.39 ±0.47
ASCORBIC ACID mg/100 mg tissue	0.022 ±0.001			0.023 ±0.0006
TOTAL LIPIDS g/100 g tissue	4.49 ±0.21			4.63 ±0.13
CHOLESTEROL g/100 g tissue	0.295 ±0.030			0.299 ±0.0005

within first 5 min of drug administration, however, at later intervals in case of all doses suppression below normal level of enzyme activity was apparent; the maximum being with 35 mg dose at 60 min (50%).

D I S C U S S I O N

It is obvious from the results obtained here that administration of propranolol leads to an increase in the glandular glycogen content of the rat by 60 min. The glycogen synthetase activity is enhanced significantly within 5 min of drug administration and sustained so at later intervals, too. The total phosphorylase activity is observed to decrease within 5 min of drug administration and aggravating with further lapse of time upto 10 min, however, by 60 min a tendency towards recovery is observable. In phase with this, is the sustained increase of c.AMP-PDE activity with all the doses, in all probability leading to a depletion of intracellular c.AMP level.

Various drugs are known to play an important role in regulation of overall carbohydrate metabolism (Northrup and Parks, 1964; Northrup, 1968; Hornbrook, 1970; Froberg et al., 1975). Hayes et al. (1984) have shown that isoproterenol - a β-adrenergic agonist - increases the activation of c.AMP-dependent proteinkinases and glycogen phosphorylase in cultured cardiac cells. Further it has been corroborated by Newton and Hornbrook (1972) on enhancing

effect of administration of β-adrenergic agonist leading to elevation of phosphorylase activity in rat liver. The adenylate cyclase system was observed to be stimulated by isoproterenol in stria vascularis of the mouse (Schacht, 1985). Administration of β-adrenergic agonist is found to increase cellular c.AMP content in cat thyroid tissue (Joseph and Mills, 1980); in rat myometrium (Do Khac et al., 1986); in mouse thymus (Durant, 1986) and in cardiac myocytes (Shepherd et al., 1986). It is known that increased cellular c.AMP levels lead to enhancement of glycogen phosphorylase activity in various tissues leading to glycogen breakdown (Krebs et al., 1966; Drummond et al., 1969; Rindi, 1971). Propranolol (a β-antagonist) administration was observed to lead to a depletion in branchial c.AMP level in sea water mullet (Djabali and Pic, 1982). Decrease in intracellular cyclic nucelotide is known to suppress glycogenolysis (Moran, 1967; Nickerson and Collier, 1970). On the basis of the work on rabbit hepatocytes Yorek et al. (1980) have reported that epinephrine induced glycogenolysis was inhibited by propranolol and that glycogen breakdown is solely related to β-adrenergic functions.

In the light of the literature cited above and on the basis of results obtained presently it is logical to expect that propranolol would naturally lead to incorporation of glucose into glycogen plausibly by suppression of β-adrenergic receptor induced rise in c.AMP level and hence subsequently the glycogenolysis (Mayer and Moran, 1960; Mayer, 1970; Epstein et al., 1971; Thibault et al

1979 and Katz et al., 1987). Correspondingly, the membrane bound Na^+-K^+-ATPase activity was also enhanced significantly at initial time interval with all doses. Such an increase in activity is indicative of increased uptake of glucose and its incorporation into glycogen which is evident 60 min after administration. It should be noted here that the effect of propranolol on Na^+-K^+-ATPase is transient and later the same gets decreased by 60 min interval, however, stimulation of glycogen synthetase activity is apparently sustained one despite reduction in Na^+-K^+-ATPase activity level, at least upto 60 min though further uptake of glucose is not apparent. The glucose thus taken up in comparatively short spurt is probably sufficient enough to cater to the observed sustained synthesis of glycogen. This contention that the glucose taken up is involved in the synthesis of glycogen content rather than being oxidized finds support in the observed depletion of aldolase activity and negligible alteration in SDH activity. Thus under presently employed regime it can be concluded that acute effect of propranolol administration leads to glycogen synthesis in the submandibular gland of rat more through enhancement of synthetase system rather than suppression of phosphorolytic breakdown.

Simultaneously it was also observed that total lipids and cholesterol content of gland increased due to propranolol administration. Isoproterenol administration has been shown to suppress the activity of lipogenic enzymes, viz. - ATP-citrate-lyase and acetyl

CoA-carboxylase, the enzymes involved in channelizing acetyl CoA towards fatty acid synthesis (Correze et al., 1982) and fatty acid synthetase (Weiss et al., 1980; Gaben et al.,1984). Stimulation of adrenergic β-receptor has been shown to lead to mobilization of cardiac lipids; primarily triglycerides. Obviously it could be expected that administration of propranolol, an antagonist of β-adrenergic receptors, would lead to results contrary to those obtainable with administration of an agonist such as isoproterenol. The elevation in lipid and cholesterol content of the gland observed during the course of present work is thus self-explanatory.

Such overall enhanced rates of synthesis of glycogen and lipids would naturally need greater energy supply which is probably reflected in lowering the AA levels. In this case by way of latter's involvement in energy transfer reaction (Chinoy, 1969, 1970, 1971 & 1973; Chinoy et al., 1974).

It could be surmized from these observations that the initial spurt in the activity of Na^{+}-K^{+}ATPase, is possibly sufficient to provide quick and enough uptake of glucose from the blood to support the observed increase in biosynthesis of lipids and glycogen for at least upto 60 min under the experimental conditions employed here.