CHAPTER IV

POSSIBLE β-ADRENERGIC INFLUENCE ON GLYCOGEN METABOLISM IN SUBMANDIBULAR GLAND OF MALE RATS

Salivary glands are organs in which appreciable translocation of fluid and electrolytes occurs. Stimulation of autonomic nerves supplying the salivary glands is physiologically important in that it regulates secretion of saliva. Schneyer and Hall (1965) have reported that use of adrenergic agents or stimulation of sympathetic fibres to submaxillary or parotid glands of rat causes maximal flow of saliva. However, saliva evoked by action of adrenergic mediation is reported to be generally higher in organic content and certain inorganic salts (Mc Clanahan and Amberson, 1935, Langstroth et al., 1938; Schneyer and Schneyer, 1961; Yoshida et al., 1967). Schneyer (1962) in his experiment on rats employing high doses of isoproterenol to evoke salivary secretion showed that (K^+) of submaxillary saliva reached high levels characteristic for intracellular fluid. B-adrenergic influence on salivary composition could be observed even when the parasympathomimetic agent pilocarpine was administered simultaeneously (Schneyer, 1965; Schneyer and Hall, 1965). Explanation of this is evident in the earlier reports, wherein it was shown that higher doses of pilocarpine not only directly stimulate cholinergic receptors in the gland but also stimulate post-ganglionic adrenergic pathways as a result of an action through the superior cervical ganglion (Dale and Laidlaw, 1912; Trendelenburg, 1954).

Chronic administration of β-adrenergic agonist, isoproterenol, has been known to produce hypertrophic and hyperplastic enlargement of submandibular glands in rat (Schneyer, 1962). Recently, it has been shown by Baum et al. (1984) that epinephrine stimulation led to an increase in the protein synthesis by submandibular gland cells. Further, Wells and Beher (1985) and Yagil et al. (1986), employing a specific β-adrenergic agonist - isoproterenol - observed that this drug induced synthesis specifically of a series of proline-rich proteins in the submandibular gland.

As far as the parotid glands are concerned, Chapola et al. (1985) have shown that stimulation with IPR leads to rise in the amylase activity in mice. As against this it has been reported by Watson et al. (1984) that chronic reserpinization of mice and rabbit led to a significant reduction in parotid amylase release alongwith elevation in the c.AMP and c.GMP levels.

All these reports were based on chronic studies with adrenergic or cholinergic drugs. Hence, the present investigation was taken up to have an insight of the possible influence of acute β-adrenergic stimulation on the metabolism of submandibular glands of male albino rats. Presently, it was decided to confine the work to certain aspects of glycogen metabolism only; in the light of several reports on the influence of IPR on this aspects as described hereunder. It was reported by Yorek et al. (1980) that in rabbit hepatic glycogen breakdown was solely related to β-adrenergic

function. Katz et al. (1987) have shown that hepatic glycogenolysis is mediated via β-adrenergic responsive adenylate cyclase system. According to Thibault et al. (1979) repeated administration of IPR led to a decrease in the skeletal muscle glycogen content of the rats seemingly by enhancing in vivo response of phosphorylase kinase and glycogen phosphorylase activities. A dose-dependent decrease in the glycogen levels of posterio-lateral walls of the left ventricle of dogs was reported by Todd et al. (1979). These diverse studies provide enough justification for the view expressed above in respect of concentrating on aspects of involvement of β-adrenergic receptors in the regulation of glycogen metabolism.

In order to get a generalized idea about the influence of possible involvement of β-adrenergic receptros under IPR stimulation, and especially in the light of various physiological alterations in diverse tissues/organs (loc cit.) reported by several workers, the following parameters of glycogen metabolism were studied:- glycogen content as well as the enzyme activities of glycogen synthetase, glycogen phosphorylase, c.AMP-specific phosphodiesterase, aldolase, total as well as Na^{+}-K^{+}-dependent ATPase and succinate dehydrogenase.

M A T E R I A L A N D M E T H O D S

1. All parameters were assayed in the submandibular salivary glands from 12 normal intact animals.

2. In all 36 rats were injected with 0.5 ml of vehicle (normal saline) and 12 were sacrificed at each of the three intervals of 5, 10 and 60 min.

3. Each of the 108 rats were administered a single 0.5 ml i.p. dose of IPR dissolved in normal saline. All injections were given at 9.00 hrs. These were divided into three equal groups. Group I was administered a dose of 15 mg/Kg b.w., the II - 25 mg/Kg b.w. and the III - 35 mg/Kg b.w. From each of these three groups 12 rats were sacrificed each at 5, 10 and 60 min intervals Tissue glycogen percentage was assayed only at 60 min interval by the anthrone method of Seifter et al. (1950).

4. The following enzyme activities - glycogen synthetase, glycogen phosphorylase, c.AMP specific phosphodiesterase (PDE), total ATPase, Na^+-K^+-dependent ATPase and SDH were assayed as per the methods described in Chapter II

All the experimental animals were healthy adult male rats weighing 120-140 g.

R E S U L T S

1. The results obtained (Table - 1) during the present study revealed reduction of glycogen levels in the submandibular gland 60 min after IPR administration with all the three selected doses.

2. The glycogen synthetase enzyme activity was found to be reduced at 10 min interval with two lower doses whereas the higher dose

Showing influence of isoproterenol administration on glycogen metabolism in submandibular glands

Isoproterenol administration (i.p.)								
15 mg/Kg b.w.			25 mg/Kg b.w.			35 mg/Kg b.w.		
Post injection intervals								
5 min	10 min	60 min	5 min	10 min	60 min	5 min	10 min	60 min
		0.018*** ±0.001			0.017*** ±0.002			0.015*** ±0.008
0.014 ±0.001	0.010§ ±0.001	0.014 ±0.002	0.013 ±0.001	0.008*** ±0.001	0.011 ±0.002	0.013 ±0.001	0.014 ±0.001	0.019* ±0.002
44.21*** ±1.11	31.02§ ±2.20	33.59*** ±0.43	33.33*** ±0.89	55.16*** ±0.61	30.11** ±1.01	49.60*** ±0.90	51.25*** ±1.69	40.50*** ±1.54
2.49 ±0.09	1.88*** ±0.03	1.31*** ±0.09	2.53 ±0.17	1.69*** ±0.12	1.78*** ±0.11	2.47 ±0.15	1.54*** ±0.26	1.58*** ±0.15
17.52 ±0.89	26.40*** ±0.92	29.19*** ±1.09	18.95§ ±1.39	25.81*** ±1.33	26.16*** ±1.49	16.50 ±1.02	25.90*** ±1.15	23.84*** ±0.73
54.02 ±1.52	73.52*** ±1.96	49.80 ±1.42	65.33** ±2.19	71.63*** ±2.03	46.58 ±1.45	56.44 ±1.05	64.70*** ±1.05	36.20*** ±1.31
25.94 ±1.35	26.82 ±1.58	28.56 ±1.37	25.09 ±1.45	25.88 ±1.59	23.47 ±1.14	23.80 ±0.75	28.35 ±1.01	28.58 ±1.08
0.629*** ±0.024	1.153*** ±0.045	1.661*** ±0.148	0.709 ±0.053	0.995 ±0.075	1.955*** ±0.08	0.259** ±0.054	1.565*** ±0.127	1.681*** ±0.046
17.66 ±1.08	19.20 ±0.19	19.64 ±1.28	17.07 ±1.25	21.02 ±1.36	19.56 ±0.88	18.09 ±1.33	19.65 ±0.87	19.87 ±1.03

(35 mg/Kg b.w.) increased it above that of the normal at 60 min interval.

3. In general, phosphorylase and PDE enzyme activities exhibited an inverse relation. Phosphorylase activity levels were maintained above the normal ones with all three doses as well as intervals. On the other hand, PDE did not show any change within 5 min of IPR administration with all three doses. However, it was seen to decrease considerably at the next two post administration intervals.

4. From the data presented in table - 1 it was clear that IPR administration significantly stimulated the Na^{+}-K^{+}ATPase enzyme activity and a concomitant but less marked enhancement to total ATPase was noticeable.

D I S C U S S I O N

As observed during the present study the IPR administration decreases the glycogen levels in the submandibular glands, more through activating the total phosphorylase enzyme system than suppressing the glycogen synthetase system (Table - 1). The total phosphorylase is known to be activated as a result of increased intracellular c.AMP levels (Shapiro and Wertheimier, 1943). The phosphorylase activity was influenced within 5 min of IPR administration, whereas the PDE was affected only after 10 min. This could mean that the phosphorylase activity initially could have been stimulated by the β-adrenoreceptors directly. The reduction

in PDE could have contributed later (10 min) to the increase in c.AMP levels which in its turn could have led to sustained increase in phosphorylase activity level. Such elevation of intracellular c.AMP under the influence of IPR has been reported by Kashiwagi et al., 1983); in cases of human adipocytes, with reference to mouse thymus by Durant (1986); in rat ventral prostate (Carmena et al., 1985); in rat parotid gland (Schneyer et al., 1986), in mice and rabbit parotid gland (Watson et al., 1984). According to John et al. (1986) incubation of rat adipocytes with IPR was reported to lead to a decrease in glycogen synthetase system. Similar depletion in the glycogen synthetase was observed in the submandibular gland 10 min after IPR treatment. However, during the course of present work on submandibular gland it was observed that the effect of acute IPR administration on glycogen synthetase system waned of by 60 min and that a higher dose, on the contrary stimulated the activity even above the normal level.

The aldolase activity revealed an increase 10 min after IPR treatment. The breakdown of the glycogen in the submandibular could lead to release glucose molecules. Glucose molecules thus released would normally be expected to get oxidised via TCA cycle, which does not seem to hold true in present case, as the SDH activity (one of the key enzymes of TCA cycle) was not altered by the experimental treatment employed here. The increase obtained in the aldolase activity could possibly be due to increased glycolysis. However, the pyruvate so formed at the end of glycolytic pathway could not

have entered TCA cycle. This means that TCA cycle was not influenced by IPR administration.

As has been known that Na^{+}-K^{+}-ATPase enzyme facilitates transport of molecules across the cell membrane against concentration gradient (Skou, 1965) any change in the enzyme activity would reflect the flux of various substances, including glucose across the cellular membrane from the submandibular gland. In phase with increase in Na^{+}-K^{+}-ATPase activity; there is also enhancement of total ATPase activity at least upto 10 min, however, the latter was found to exhibit tendency towards recovery. The increase in total ATPase activity could mean an increase in any one of its components - Na^{+}-K^{+}-ATPase, Mg^{++}-ATPase and Ca^{++}-ATPase activity. The Mg^{++}-ATPase component is known to be a mitochondrial enzyme. As has been observed here that another mitochondrial enzyme - SDH has hardly been altered by IPR administration, it can be surmized that IPR is less effective at the mitochondrial level. As is obvious that Na^{+}-K^{+}-ATPase enzyme activity is significantly influenced by IPR administration within 5 min whereas total ATPase activity showed a gradual increase which was highest at 10 min time interval. On the basis of this it could be said that the increase in total ATPase activity could be mainly due to increase in Na^{+}-K^{+}-ATPase component. Taking into account the results obtained here one could think about the following consequences: there is enhanced breakdown of glycogen leading to release of intracellular glucose, increased Na^{+}-K^{+}-ATPase activity could either facilitate efflux of glucose from the gland to the

blood which is physiologically not tenable. Alternatively, it may help in increased uptake of glucose from the blood.

Such situation would lead to intracellular flooding with glucose. It is a matter of conjecture to explain the possible physiological significance of such high level of intracellular glucose. In the light of present observations as well as those on chronic IPR effect it could be suggested that glucose molecules may either be actively incorporated into complex - carbohydrate moieties which are known to be part of salivary solids, or the glucose molecules may be subjected to direct oxidation via HMP-shunt pathway and thereby providing needed metabolic energy and also increasing the feed to the pentose pool, which could be a preparatory acute physiological response to IPR administration that is known (on chronic administration) to lead to cell proliferation needing precursors of nucleic acids. The later activity might have been a consequential attribute of enhanced glycogen degradation induced by IPR administration.

The total protein content was not altered by IPR administration. As it is a known fact that by such early time intervals incresed protein content need not be a manifestation of increased protein synthesis.

Thus, it would not be out of place to mention here that stimulation of β-adrenergic receptors of submandibular gland of rat leads to a significant depletion of glandular glycogen content with fleeting changes in a few important concerned enzymes studied. Whatever be the mechanism, it is reasonable to accept at present that the agonistic action of IPR on β-adrenergic receptors at least initially (5 min) noticeably influences the glycogen metabolism of this gland directly and later through the sustained intracellular level of c.AMP.