

C H A P T E R V I I I

EFFECTS OF NEUROENDOCRINE MANIPULATIONS ON
SIALIC ACID CONTENT OF SUBMANDIBULAR
GLAND OF MALE RATS

In animal tissues sialic acid (SA) occurs as constituent of mucoproteins, mucolipids and lipoprotein-carbohydrate complexes. SA occurs as important components of complex carbohydrate moieties with varied significant biological properties. At the functional level SA cannot be regarded as the counterpart in animals of the bacterial muramic acid. As a rule cells of the animal body do not possess rigid cell walls. The significance of sialo-mucoproteins as components of the membrane of certain animal is obscure. Wide distribution of sialo-mucoproteins in animal secretions and excretions would indicate a protective rather than a structural function. Sialo-mucoproteins are regular components of the viscous mucins covering the epithelial surfaces of the respiratory, digestive and urogenital tracts.

Considerable studies have been conducted on the influence of various androgenic agents on SA in various mammalian tissues. Administration of various antifertility agents have been known to reduce the sialic acid content in testis, epididymis, seminal vesicles of rats and gerbil (Dixit, 1977 and Nag *et al.*, 1977) and of hamsters (Bose *et al.*, 1977). Rajalakshmi *et al.* (1970) have shown that administration of a non-steroidal triphenyl derivative of

chlorotrianisene-chlomiphene led to depletion of SA levels of epididymis and Cowper's gland of rats. Androgen deprivation decreased the levels of SA in spermatozoa, vas deferens and epididymis of hamster (Bose and Prasad, 1975). Injection of α -chlorohydrin to dogs reduced SA levels in the testis and epididymis (Dixit, 1976).

Ravetto et al. (1965) reported on the occurrence of sialic acid in the submaxillary glands of rats. Poddar and Jacob (1978) demonstrated histochemically the presence of carboxylated sialomucins in mongoose salivary glands. Various researchers have shown that the submandibular glands of rodents are androgen sensitive. Additionally, it has also been well documented that the submandibular glands of rat are influenced by the β -adrenergic receptors. The saliva evoked by action of adrenergic mediators are generally higher in organic content than saliva evoked by cholinergic stimulation. The higher organic content of sympathetically evoked saliva reflects elevated levels of proteins, which include glycoproteins, digestive enzymes, etc. The levels of ions as well as levels of proteins are highest with sympathetic stimulation that involves an action of β -adrenergic receptors. SA constitute predominant portion of the carboxylated mucosubstances (Smith and Frommer, 1975). Hence, the present study was carried out to have an insight of influence of sex steroids as well as β -adrenergic agonist and antagonist on sialic acid content of submandibular glands of male rats.

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

1. All the experiments were carried out on healthy young adult male albino rats weighing 140 ± 20 g.
2. A total of 168 rats were utilized for investigating the influence of sex steroids on the sialic acid content of the gland. These animals were grouped according to the hormonal manipulation in the manner described under (3) and (4).
3. 36 rats were administered exogenously (i.m.) $100 \mu\text{g}$ testosterone propionate while other 36 received $100 \mu\text{g}$ of 17β -estradiol. At intervals of 1, 2 and 4 hr after both types of hormone treatment a batch of 12 rats at each of intervals were sacrificed.
4. 96 rats were bilaterally castrated under mild anaesthesia. 48 hr after castration 42 rats were administered TP, 42 were administered estradiol and the remaining 12 rats served as 48 hr castrate controls. At a time 12 rats replaced with either of the hormones were sacrificed at 1, 2 and 4 hr intervals after hormone administration.
5. A group of 144 intact rats were utilized to study the effects of administration of adrenergic agents. Half of these rats were injected intraperitoneally with isoproterenol in three different doses (15, 25 and 35 mg/Kg b.w.) to 24 individual/dose levels. Isoproterenol was dissolved in normal saline.
6. The remaining 72 rats were divided into three equal groups which received intraperitoneally propranolol at the following dose levels - 25, 35 and 45 mg/Kg b.w.

7. At a given time 12 animals treated with either of the drugs were sacrificed at the intervals of 1 and 2 hr.
8. The submandibular glands were excised immediately after sacrificing under mild ether anaesthesia, freed of connective tissue, weighed accurately (upto 0.1 mg). The glandular tissue was hydrolysed by weak sulphuric acid. From the hydrolysate the total SA was eluted successively over Dowex-2 and -50 columns and assayed colorimetrically as per the method of Svennerholm (1958). The total SA content was expressed as u moles SA/100g fresh tissue weight. Statistical analysis was carried out employing Student's 't' test.

R E S U L T S

Hormone treatment

1. Exogenous administration of TP was seen to lead to a significant ($P < 0.001$) depletion in the SA content of the gland. 17β -estradiol was noted to enhance in a time dependent manner rise in glandular SA content.
2. The SA content was observed to get reduced almost to 1/3 the normal level due to castration.
3. Replacement with TP showed a tendency towards recovery at 1 and 2 hr intervals but by 4 hr this effect seemingly waned off.
4. Replacement with 17β -estradiol remarkably increased the SA as compared to normal level, the maximal (about 4 fold) increase being at 2 hr interval. However, by 4 hr there was some reduction but still it was twice that of normal.

Table 1

Showing alterations in Sialic Acid content ($\mu\text{g SA}/100 \text{ g}$) of rat submandibular gland under different experimental regimes.

Normal	Normal intact animals injected with 100 $\mu\text{g TP}$ (i.m.)				48 hr castrates injected with 100 $\mu\text{g TP}$ (i.m.)			
	Post injection intervals				Post injection intervals			
	1 hr	2 hr	4 hr		1 hr	2 hr	4 hr	
357.24 ± 11.70	178.32*** ± 5.87	296.11*** ± 6.50	253.62*** ± 3.76	130.74*** ± 7.16	195.02*** ± 3.44	200.17*** ± 8.59	136.20*** ± 7.45	
	Normal intact animals injected with 100 $\mu\text{g Estradiol-17}\beta$ (i.m.)				48 hr castrates injected with 100 $\mu\text{g Estradiol-17}\beta$ (i.m.)			
618.40*** ± 11.10	799.56*** ± 11.48	977.07*** ± 28.36			548.70*** ± 18.99	1310.42*** ± 107.04	660.66*** ± 11.78	
	Isoproterenol administration (i.p.)				Isoproterenol administration (i.p.)			
Control	15 mg/Kg b.w.	25 mg/Kg b.w.	35 mg/Kg b.w.					
	Post administration intervals				Post administration intervals			
364.87 ± 5.17	60 min	120 min	60 min	60 min	120 min	60 min	120 min	
	278.74*** ± 14.38	283.93*** ± 10.48	264.23*** ± 10.67	302.99*** ± 2.45	247.39*** ± 12.36	225.21*** ± 13.83		

5. Administration of isoproterenol (β -agonist) led to a significant ($P < 0.001$) depletion of SA content with all the three doses as well as at the two early intervals.
6. Propranolol, a β -adrenergic antagonist, exhibited effect opposite to that of IPR, the glandular SA content being significantly elevated above that of normal value.

All the observed values in respect to above items are presented in Tables 1 (for items 1 to 4) and 2 (for items 5 & 6).

D I S C U S S I O N

For the sake of convenience the observations reported herein will be discussed in two separate parts and then an attempt will be made to look for any possible relation between the two. As is apparent, the experimental regimes themselves were of separate nature. One was to understand, on a comparative basis the influence of testosterone and estradiol on the SA content of the submaxillary glands of male rats. The other was to know, in a similar manner, the effects of administration of a β -adrenergic agonist and an antagonist on SA levels. Both were tried on a short-term basis.

It is well known that oral glands were primarily of mucous type in the vertebrate series but later in mammals these evolved into complex seromucoid type. SA being an important constituent of mucoid secretions protecting the epithelial surfaces of the body, stands out as an interesting parameter of such secretions. Among the three major paired salivary glands of mammals, the

submaxillary glands contribute substantial percentage of mucoproteins of the saliva. Some of the chief salivary mucoproteins of interest are mucins of general nature, lysozyme and blood group substances contributed by submaxillary glands of white rats.

As is evident from the data obtained here the lack of androgen due to orchidectomy led to substantial reduction in sialic acid level of the glands. Administration of 0.1 mg of TP could facilitate a slight degree recovery and that too, transitorily. On the other hand, administration of similar doses of TP to intact rats, contrary to expectation, also significantly suppressed the SA content within an hour of injection and though, there was some waning of this decremental influence, it was not recovered to normalcy even upto 4 hr. Such a paradoxical situation is rather difficult to explain. Nevertheless, what could be suggested is this that SA content is a parameter highly sensitive to fluctuations in circulating levels of androgens and that neither lower nor higher levels of the male sex hormone are conducive to maintenance of normal SA contents. There may exist a certain critical dynamic balance of male sex hormone underlying the mechanism of SA regulation in submaxillary gland of the male rats, at least at such short intervals. The alterations of SA may reflect more probably those in general mucins, however, changes in lysozyme or blood group substances cannot be ruled out. In the absence of direct assessment of other mucoid substances, no conclusive comments can be offered, however, by way of generalization, it could be suggested that under the experimental conditions employed here, both hypo- or hyper-physiological androgen titres may induce secretion of more of watery saliva.

With respect to the second part of experimental design, involving administration of adrenergic drugs, it could be clearly seen that β -adrenergic receptors do play a role in the regulation of SA content of submandibular glands of male white rats in highly significant manner. That a β -agonist lowered the levels of SA and that an antagonist led to enhancement; is proof enough to support the above contention. It would be logical to think that β -adrenoreceptors are probably normally active only under a certain critical titre of androgenic compounds so as to facilitate elaboration and probably release also, of SA in case of the submandibular glands. As opposed to this either, lower (castration) or higher levels of androgen adversely influence β -adrenoreceptor functions in an entirely different way as yet not reported in literature, so far as the author is aware of.

On the basis of the results obtained it can be seen that the influence of TP administration to the intact or castrates leads to an influence which is parallel to that shown or exhibited after isoproterenol administration. Hence it could be said that testosterone either in hyper- or hypo- levels act in a manner similar to the influence of isoproterenol administration. The situation with 17β -estradiol administered intact as well as castrated male rats was different in that a highly significant rise in SA content could be observed. So, it seemed that the effect of estradiol administration happens to be similar to a remarkable extent to the response shown to propranolol injections. It therefore, appears that estradiol in males may exert a β -blocking influence, whereas

either sub-normal or hyper-normal androgenic titres bring about the enigmatic effect, as noted above, on submandibular SA content by affecting β -adrenergic function. It was shown in earlier chapter (III), that β -adrenergic action leads to reduction of PDE, thereby allowing the hormone induced intracellular c.AMP levels to enhance phosphorylase activity within the short intervals. The increment of c.AMP indirectly be responsible for lowering SA content. As regards the case of estradiol influence, which was observed to be similar to β -blocker type, it may act by facilitating PDE, and thereby exhibiting contrary effect upon glandular SA levels.

By now it is a well proven fact that sexual differences in mice and rats salivary glands are very obvious. There is another fact of life related to these rodents that they have a vivid licking habit, more so with female sex. Higher content of mucin in saliva of female rodents or those males administered female sex hormones may well suit this licking habit. Licking of self body parts and the developing young ones by females providing a potent antimicrobial action through lysozyme (a mucoprotein) may be a significant fact. In this light, the reduction of salivary SA content in males due to the double edged enigmatic influence of less or more androgen then constitutes secretion of a more watery saliva that is poor in mucoproteins which is not suiting to licking habit. This seems to be a transitory physiological disturbance, at least at such short intervals employed here. Hence it may tentatively be suggested that either lower or higher levels of testosterone are less conducive to normal β -adrenergic function in case of submandibular glands

of male rats. If one considers the results obtained with isoproterenol and propranolol administration together with this suggestion, then it cannot be assumed that the androgenic levels under consideration act either as β -blocking or enhancing factors. Decidedly, these exploratory findings warrant a more closer as well as intensive investigation so as to enable us understand the situation in a desirably better light.