SULFHYDRYL GROUPS OF THE TISSUES OF THE SUBMANDIBULAR SALIVARY GLANDS AFTER INTERFERENCE WITH THEIR PARA-SYMPATHETIC INNERVATION

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It was shown in an earlier publication from this laboratory, dealing with the biochemistry of secretion, that, following prolonged secretion (2 hours) by the submandibular salivary gland of cats which had been given pilocarpine, the free sulfhydryl group content of the proteins of this gland fell considerably, while that of its non-protein constituents rose [3]. This process coincided in time with fall in mucin content of the tissues, and with increase in them of other proteins, soluble in 0.2% KH_2PO_4, and not precipitated together with mucin [5]; certain changes in posphorus metabolsim were also associated with the process [1]. These effects were not observed when secretion was inhibited by the action of atropine.

In the present paper we examined the effects on tissue metabolsim of abolishing central parasympathetic control of the submandibular gland, by division of the chorda tympani. Changes in the activity of the submandibular glands following this operation, in particular, their enhanced sensitivity to a number of pharmacologically active agents, have been reported by a number of authors [6, 7, 9, 10]. Strömblad [10] found a considerable fall in the activity of certain enzymes, and in the respiratory rate of the gland tissue, following division of the chorda tympani.

METHODS

The chorda tympani of cats was severed at the point where its fibers merged with the lingual nerve to proceed to the submandibular salivary gland. The operation was similar to that performed by Strömblad in his experiments. As a result of the operation central parasympathetic impulses no longer reach the gland, and certain afferent connections are abolished; the gland is no longer capable of reflex secretion. So-called paralytic secretion was not observed. Secretion in response to pilocarpine was not abolished, although its nature was somewhat altered—more protein was secreted, and the amount of saliva fell, as has been shown by G. V. Chernysheva [5]. The operation was performed unilaterally on some of the cats, and bilaterally on others. As controls we took the glands of healthy, intact cats. For unilaterally operated cats, we examined the metabolism both on the operated and on the contralateral side, with intact innervation. The glands were taken for analysis 7-8, 14-15 and 20-21 days after the operation, by which time the secretory process showed the greatest deviation from normal. At all these times, the glands were analyzed both in the resting state, and after pilocarpine stimulation.

The animals were killed by decapitation, and the glands were quickly removed, frozen in carbon dioxide snow, group up to a powder and portions of powder were taken for analysis. The free sulfhydryl group content was

Content of Free Sulfhydryl Groups and of Nitrogenous Constituents in Submandibular Salivary Gland Tissue after Abolishing Parasympathetic Innervation

Secretory conditions	Weight of gland (mg)	Dry weight of tissue (%)	SH-group content (mg per g dry weight)				Nitrogen content (mg per g dry weight)			Protein N / NPN	Total SH-groups / total nitrogen	Protein SH-groups / protein nitrogen	Non-protein SH-groups / non-protein nitrogen
			total SH-groups	protein SH-groups	non-protein SH-groups	protein SH-groups / non-protein SH-groups	total N	protein N	NPN				
Intact glands													
Resting	915	25,0	2,281	2,250	0,031	72,5	97,1	84,4	12,7	6,6	0,0225	0,0267	0,0024
Pilocarpine	887	20,0	1,740	1,678	0,062	27,2	—	—	—	—	—	—	—
Unilateral denervation													
Resting — contralateral	916	22,5	3,272	2,978	0,2942	10,1	84,2	64,2	19,98	3,8	0,0384	0,0464	0,0148
Resting — denervated	666	21,05	2,987	2,784	0,239	11,7	104,8	83,4	21,4	4,4	0,0286	0,0303	0,0111
Pilocarpine — contralateral	1 012	21,7	2,961	2,7073	0,2537	10,7	106,38	94,3	12,08	7,72	0,0279	0,0286	0,0230
Pilocarpine — denervated	913	16,0	2,860	2,675	0,185	14,4	125,9	107,4	18,5	5,83	0,0238	0,0249	0,0103
Bilateral denervation													
Resting	788	23,6	2,622	2,345	0,277	8,5	98,7	80,9	17,8	4,53	0,0266	0,0290	0,0155
Pilocarpine	749	18,7	2,761	2,654	0,108	24,6	123,4	99,3	24,1	3,82	0,0224	0,0267	0,0044

determined according to Tsiperovich and Loseva [4], and the non-protein sulfhydryl content according to Grunert and Phillips [8]; the protein sulfhydryl group content was given as the difference between these two values (the analytical error amounted to about 10%).

Protein and non-protein nitrogen was determined by precipitation of proteins with trichloroacetic acid, followed by Kjeldahl digestion. The dry content of the tissues was also determined. The content of components was calculated per gram of fresh tissues, as well as per gram of dry tissue, in view of fluctuations in the water content of the glands. The values given in the tables represent the means of 10-15, and in some cases of 20 analyses, for each of which two cats were taken.

RESULTS

The weight of the glands fell continuously following division of the chorda tympani, being 27.5-33% less on the 20-21st day after operation than the contralateral gland (see table). The dry content of the glands also fell somewhat after denervation, from 24-25 to 21-22%; the greatest difference was found after two hours of pilocarpine-induced secretion, when it fell from the resting value of 21% to 16-18%, this difference being greater than for intact glands following denervation was reflected by a rise in their water content (on the average, by 12.4%).

The water content of both intact and denervated glands rose after administration of pilocarpine, by 5.94--6.4%, on the average. This effect was not found, however, in the contralateral glands of unilaterally denervated cats; there was scarcely any change in their water content, although their secretory activity was the same as in intact animals.

After unilateral denervation, the protein nitrogen content of resting denervated gland tissue varied within the range 84.4-90.9 mg per g dry weight (see table), while the contralateral gland contained 64.2 mg per g dry tissue. The non-protein nitrogen content rose after denervation from 12.7 to 18.5-21.5 mg per g of dry tissue, both on the denervated and the contralateral side.

The protein nitrogen content of the tissues rose following pilocarpine-induced secretion, very slightly in the glands of intact animals, but markedly more so in operated animals, particularly in the contralateral glands. Non-protein nitrogen fell in all cases following secretion, except for bilaterally operated animals.

Correspondingly, the value of the ration $\frac{\text{protein N}}{\text{NPN}}$ rose steeply after secretory activity of intact glands (from 6.61 to 11.1, i.e., by 75.5%), and even more so in the contralateral glands of operated animals (from 3.8 to 7.72, i.e., by 103%). The value of this ratio rose much less in the denervated gland of a unilaterally operated animal (from 4.4 to 5.83, i.e., by 32.5%), while after bilateral denervation the value even fell. This last finding is in accordance with G. V. Chernysheva's results [5]; she reported that the soluble protein content of these glands fell very steeply following pilocarpine-induced secretion. It would appear that during pilocarpine-stimulated secretion of the glands of intact animals the loss of soluble proteins is compensated to a large extent by their synthesis.

As far back as 1890, I. P. Pavlov drew the following conclusion from the results of his investigations of the nitrogen balance of the submandibular salivary gland: "When the secretory nerve of the gland is stimulated, breakdown and restitution proceed side by side" [2].

This process is adversely affected when the parasympathetic links between the gland and the central nervous system are severed, in particular when the chorda tympani is divided bilaterally.

The content of protein sulfhydryl groups rose somewhat in denervated glands (see table), in particular on the 21st day after the operation (on the average 2.748 mg per g of dry tissue), as compared with 2.250 mg for intact glands. High levels (2.978 mg) were, however, also found in the intact contralateral glands of operated animals. This rise in the protein SH-group level proceeded gradually, beginning with the 14-15th day after operation, and in particularly clearly shown when calculated for the whole gland. The content of non-protein SH-groups rose after denervation, this effect being particularly evident by the 20-21st day. The ratio of protein to non-protein SH groups fell correspondingly after denervation.

As reported by us earlier, the contents of both protein and non-protein sulfhydryl groups changed consider-

ably during pilocarpine-stimulated secretion by intact glands. This suggested that reconstruction of proteins took place in the gland during its secretory activity. We have now examined this process in glands deprived of central parasympathetic innervation. The changes taking place in denervated and contralateral glands following administration of pilocarpine are of particular interest. Following unilateral denervation, the protein SH-group content of the denervated gland scarcely changed at all after secretion, while the content of non-protein SH-groups not only did not rise, as is the case in intact animals, but it even fell appreciably, from 0.239 to 0.185 mg per g of dry tissue. The corresponding changes found after administration of pilocarpine to bilaterally denervated animals are also significant. The content of protein SH-groups rose from 2.345 to 2.654 mg after the action of pilocarpine, while that of non-protein SH-groups fell from 0.277 to 0.108 mg. Thus the ratio of protein to non-protein SH-groups not only did not fall after pilocarpine-stimulated secretion of denervated glands, as is the case in intact glands (-65%), but, on the contrary, it increased, by 59% for unilaterally denervated animals, and by 188% for bilaterally operated ones. The changes in the value of this ratio following secretion by the contralateral glands, with intact innervation, are very distinctive. The content of free sulfhydryl groups, both protein and non-protein, rose in the resting gland, while after pilocarpine-stimulated secretion (according to G. V. Chernysheva [5], this secretion does not differ, qualitatively or quantitatively, from that found in intact cats) a fall was observed in the contents of both protein and non-protein free SH-groups.

As indices of the state of the gland proteins, we also derived the ratios of the amounts of free protein and non-protein SH-groups of gland tissue to its protein and non-protein nitrogen contents (see table). The ratio of protein SH-groups to portein N was 0.0303 for unilaterally denervated glands, and 0.0290 with bilateral denervation; these values fell after pilocarpine-stimulated secretion. The ratio fell more steeply in contralateral glands, actively secreting saliva of normal composition. An appreciable fall in the ratio of non-protein SH-groups to non-protein N was found for secreting denervated glands, especially for bilaterally operated animals, whereas the value of the ratio rose in the contralateral glands of unilaterally operated cats.

The differences in the content of free sulfhydryl groups in the tissues of denervated glands must be related to differences in their functional state following unilateral or bilateral severance of central parasympathetic connections, the effects of which include changes in their metabolism, in particular, their protein metabolism, and in the activity of their functional groups.

Under these conditions, the secretory processes of these glands, their tissue metabolism, and their contents of protein and non-protein free SH-groups, are also differently affected by pilocarpine stimulation.

The changes found in the contents of free protein SH-groups (the ratio $\frac{\text{protein SH}}{\text{protein N}}$) and of non-protein nitrogenous compounds (the ratio $\frac{\text{non-protein SH}}{\text{NPN}}$) of glandular tissue, both following denervation, and after pilocarpine-stimulated secretion, constitute evidence of the existence of qualitative changes in the tissue proteins of the glands in the pathological states under examination.

The differences in the metabolic disturbances found after unilateral and bilateral denervation suggest the presence of mutual regulatory mechanisms acting between these paired organs. The characteristic changes observed in the contralateral glands may be supposed also to be related to such mechanisms.

Our findings point to the importance of the role of central parasympathetic control in maintaining physiological levels of secretion and of metabolism of the submandibular salivary glands.

SUMMARY

The metabolism of the submaxillary salivary gland tissue (particularly the content of protein and retention nitrogen, free protein and nonprotein sulfhydryl groups) is seen to change following the exclusion of the central parasympathetic effect by chorda tympani division. These changes develop gradually and are distinctly seen on the 20th-21st day after the operation. They are similar for unilateral and bilateral denervation, but each type of denervation has its characteristic features. With unilateral denervation the metabolism of the contralateral gland is also altered; however, these changes are not marked and differ qualitatively from those in the denervated glands.

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