THE PROBLEM OF THE EFFECT OF PARASYMPATHETIC DENERVATION ON THE PROTEIN METABOLISM OF THE SUBMAXILLARY SALIVARY GLANDS OF THE CAT

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The problem of the effect of various sections of the nervous system on the secretory function of the salivary glands has been studied in considerable detail. Less studied has been the effect of the nervous system on the metabolic processes which occur in the gland tissue [2, 4, 5, 19, 23-27]. This is especially true of protein metabolism, which plays an important part in the secretory processes of the gland [7, 8, 10, 21]. In addition, the study of nitrogen metabolism of the salivary gland and its regulation by the nervous system is of great value for the understanding of the mechanism of secretion.

The problem of the present work is the investigation of protein metabolism in the submaxillary salivary gland and its comparison with the secretory function of this gland after destruction of its connection with the central nervous system by section of the chorda tympani.

EXPERIMENTAL METHOD

The experiments were carried out on the submaxillary salivary glands of cats. We ran several series of experiments with 20-30 animals in each. Parasympathetic denervation (one or both sides) was carried out in the same way as in a number of other studies by sectioning the chorda tympani where it is given off from the lingual nerve and goes to the submaxillary gland [13, 22, 24, 26], thus breaking basically the parasympathetic and partially the afferent connection with the center. The study was carried out on the tissues of the salivary gland and on the saliva. Nitrogen content of the saliva was studied in cats with a submaxillary gland fistula. Exposure of the duct was carried out by the method of A. M. Ugolev [9]. Tissue proteins were studied in the gland removed rapidly after decantation of the fluid. The gland was weighed, frozen with dry ice (to reduce the action of tissue enzymes) and ground to a fine powder. All further treatment of the tissue was carried out in the cold. A sample of tissue was ground in a mortar, homogenized in a glass homogenizer in a 0.2% solution of KH_2PO_4 with pH 5.33 (2.5 ml of solution per g of wet tissue). Then the homogenate was centrifuged for 10 minutes at 5000 rpm. In the resulting water-salt extract we determined the total nitrogen (by Kjeldahl), mucin nitrogen (by the method of Anrep [10] and Kazakova [6]), and the amount of nitrogen in other proteins, not precipitated with mucin. For precipitation we used 7% CCl_3COOH.

To characterize the protein fractions we determined in them after hydrolysis with 2 N HCl at 100° for 4 hours the content of amino sugars by the method of Elson and Morgan [12] and the pentosuria method [3], and also the amount of reducing substances by the Hagedorn method.

RESULTS

The study of the tissues and saliva of the submaxillary gland after unilateral denervation was carried out on the 8th, 14th, and 21st days and after bilateral denervation, only on the 21st day after sectioning the chorda tympani. We chose the latter period because at this time there occur in the gland the greatest changes in its secretion [2, 11, 18, 20].

Sectioning the chorda tympani caused a short reflex formation of saliva on eating meat. In our experiments, as in those of Emmelin and Muren [13-16] the "paralytic" secretion of saliva was absent.

In the table we give the average data for each series of experiments. As controls for the denervated gland we used the glands of intact cats.

The content of nitrogenous substances was calculated per g of dry tissue and per weight of the whole gland. As the table shows, on the 8th to 9th day after unilateral sectioning of the chorda tympani the denervated gland does not show any marked differences in weight, amount of solid residue, or nitrogen content of its tissues. Later on after the denervation there was progressive fall in weight of the gland. The amount of solid residue decreased on the 14-15th day and remained small on the 21st day after denervation. In the tissues of the denervated gland the content of total nitrogen and mucin nitrogen, calculated per g of dry weight, was higher than in the tissues of the glands from intact cats (50.1 mg of mucin nitrogen on the 14th day after denervation, 45.6 mg on the 21st day, and 38.6 mg in the intact cats). The content of protein which did not precipitate with the mucin decreased on the 14-15th day after denervation. On the 21st day it was within normal limits.

Change in Chemical Composition of Tissues of Submaxillary Glands of Cats under the Influence of Denervation

Experimental conditions			Weight of one gland, mg	Solid residue, %	Total nitrogen, mg		Mucin nitrogen, mg		Protein nitrogen without mucin, mg		Ratio of protein N without mucin to mucin nitrogen, %	Amino sugars		Reducing substances		No. of animals		
					per g dry tissue	in one gland	per g dry tissue	in one gland	per g dry tissue	in one gland		mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	No. of animals
In resting state			980	23.4	52.4	12.25	38.6	7.7	3.6	0.7	10.0	37.2	—	84.7	2.4	0.5	0.02	43
After injection of pilocarpine			887	20.0	51.0	9.1	27.8	5.5	9.8	2.1	26.8	21.5	0.6	78.0	5.4	0.4	0.03	34
After unilateral denervation	on 8–9th day	contralateral gland	991	24.1	61.5	14.6	48.4	11.5	2.9	0.7	6.2	26.0	0.2	—	—	3,4	—	15
		denervated gland	914	23.3	52.0	11.0	39.0	8.2	3.0	0.6	6.8	20.0	1.0	—	—	1.7	0.32	15
	on 14–15th day	contralateral gland	1 017	23.4	59.6	14.2	46.45	11.2	3.7	0.8	11.2	124.4	0	—	—	—	—	11
		denervated gland	711,7	20.7	62.8	9.25	50.15	7.35	2.0	0.3	5.5	87.1	—	—	—	0.6	—	11
	on 21st day	contralateral gland	916	22.5	66.1	13.3	44.9	9.8	4.8	1.3	13.7	—	—	78.7	4.3	—	—	37
		denervated gland	666	21.1	61.5	9.3	45.6	6.6	4.1	0.6	10.5	—	—	90,7	3.9	0.6	0.22	37

Change in Chemical Composition of Tissues of Submaxillary Glands of Cats under the Influence of Denervation (continued)

Experimental conditions			Weight of one gland, mg	Solid residue, %	Total nitrogen, mg		Mucin nitrogen, mg		Protein nitrogen without mucin, mg		Ratio of protein N without mucin to mucin nitrogen, %	Amino sugars		Reducing substances		Pentoses		No. of animals
					per g dry tissue	in one gland	per g dry tissue	in one gland	per g dry tissue	in one gland		mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	
With injection of pilocarpine in unilateral denervation	after 1 hour action	contralateral gland	876	22.5	50.0	9.65	30.8	5.1	17.1	1.9	45.9	15.0	1.6	—	—	0.5	0.36	14
		denervated gland	764	18.35	48.3	6.4	32.4	4.3	10.8	1.1	50.1	8.5	—	—	—	0.6	0.8	14
	after 2 hour action	contralateral gland	1 071	21.7	62.5	13.9	43.35	9.95	8.7	2.1	23.6	16.4	0.2	52.0	6.5	—	—	16
		denervated gland	913	16.0	70.0	10.9	42.2	7.2	13.1	2.0	30.1	35.4	—	61.6	5.6	—	—	16
After bilateral denervation		denervated gland	726	23.6	64.8	10.6	52.5	9.2	2.9	0.4	4.4	12.8	—	100	2.4	—	—	23
With iniection of pilocarpine in bilateral denervation	after 2 hour action	denervated gland	749	18.0	50.5	7.0	29.3	4.2	6.7	1.0	23.4	39.5	17.7	—	—	2.0	11.6	16

We must note that although the contralateral gland differed little in weight and quantity of solid residue from the gland of the intact cats, the content of mucin nitrogen in the extracts of these tissues was considerably higher than in the tissues from the glands of intact cats.

The increased content of nitrogenous substances which we found in tissues of denervated glands on the 21st day after unilateral sectioning of the nerve was still more marked in this period with bilateral denervation. The content of mucin nitrogen in the tissues of these glands rose to 52.5 mg (in unilateral denervation, 45.8 mg; in intact cats, 38.6 mg).

Thus, from all these experiments with unilateral and bilateral sectioning of the chorda tympani we can demonstrate the accumulation of mucin nitrogen in the tissues of the denervated glands. Evidently this is due to the absence of consumption of protein, since after denervation there is neither reflex nor "paralytic" secretion.

In order to follow the manner in which denervation acts on the function of the gland, we studied the secretion of this gland after injection of pilocarpine (0.75 mg per kg weight of animal) before and after section of the chorda tympani.

The study was carried out on 12 cats with fistulas of the submaxillary glands. Saliva was collected with a small funnel attached by Mendeleev cement at the point of emergence of the duct. Every 30 min we observed the amount of saliva and determined its content of total nitrogen and mucin nitrogen. In these experiments the change in secretory activity of the denervated gland was most sharply shown with bilateral section of the chorda tympani.

In the figure we give different typical experiments. The action of pilocarpine on glands with severed chorda tympani decreased the latent period to 3-5 min compared to a latent period of 14-15 min in these glands before denervation. The latent period of the contralateral glands as a result of unilateral denervation did not change and was the same as in glands which retained their innervation (14-15 min).

When pilocarpine acted on the denervated glands it was found, just as in the experiments of Pierce and Gregersen [22], that there was a shift in maximum formation of saliva: before sectioning the secretory nerve, the greatest quantity of saliva in response to a pilocarpine injection occurred in the second half hour of secretion; and, after sectioning, in the first half hour. The total amount of saliva formed after injection of pilocarpine after $1\frac{1}{2}$ hr secretion was the lower, the greater the period of time which passed after denervation (see figure, black columns). This was also observed by Vulpian [28] and by Langley [20]. However, the amount of total nitrogen (see figure) and mucin nitrogen isolated from saliva in the time of secretion after injection of pilocarpine was considerably greater in the entire period of denervation (8th, 14th, 21st day) than before denervation of these glands under the same action of pilocarpine.

The increase in content of mucin nitrogen in unilateral and bilateral denervation probably is connected with an increase in sympathetic influence which, as is known, occurs in parasympathetic denervation of the glands [2, 15, 17].

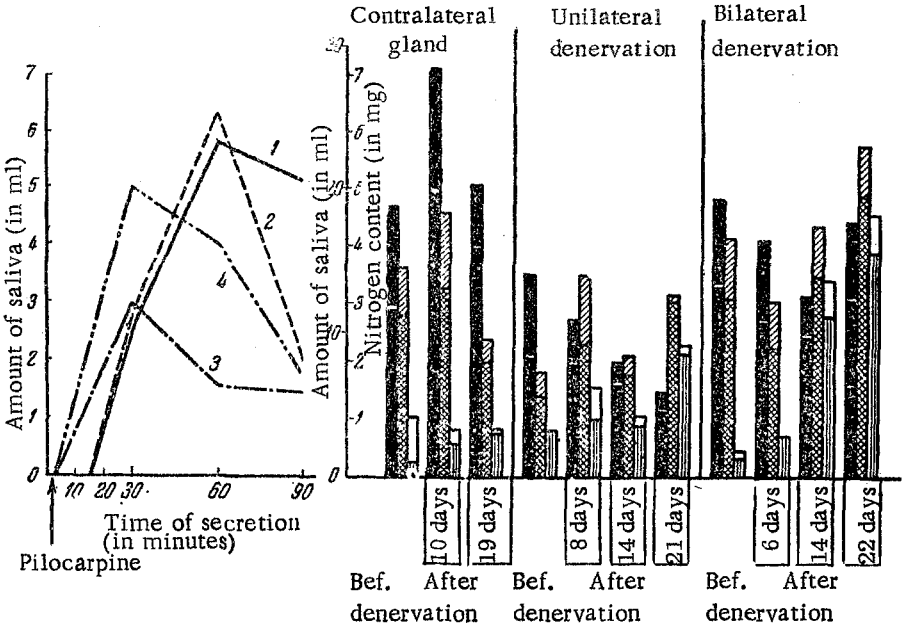
Thus, the study of the secretory function of the denervated glands has shown that the glands after sectioning of the chorda tympani in response to the action of pilocarpine form less saliva, but saliva with a greater content of nitrogenous substances than before the operation.

The high content of protein in the saliva formed after injection of pilocarpine into denervated glands leads to an understanding of the amount in the tissues of these glands. The more mucin that is excreted with the saliva, the less of it remains in the gland tissue. This is particularly clear after bilateral denervation of the gland (the content of mucin nitrogen in the tissues falls from 52.5 mg to 29.3 mg) and after an hour's action of pilocarpine on the denervated gland of an animal with unilateral sectioning of the nerve (from 45.6 to 32.4 mg). After two hours action, the content of mucin nitrogen in the tissues of denervated glands with unilateral denervation decreases only slightly (from 45.6 to 42.2 mg). This is apparently connected with the fact that the excretion of mucin with the saliva occurs especially in the first hour of secretion (see figure).

During the second hour of secretion, with injection of pilocarpine there is formed a liquid low in the nitrogenous substances of saliva (see figure, upper part of the columns). These facts allow us to suggest that the content of protein in the tissues after, two hours' secretion on pilocarpine, strongly reflects the restoration of the mucin lost in the first hour of secretion.

Under the influence of pilocarpine on the denervated glands, in their tissues, just as in the tissues which have retained their innervation, there is a strong increase in the amount of protein nitrogen which does not precipitate with mucin (see table). We showed previously [1] that the appearance of this protein is connected with strengthened secretory activity of the gland. This protein gives reactions characteristic for glucoproteins, to which group submaxillary mucin belongs, but in its ability to be precipitated and in its content of amino sugars, pentoses, and total reducing substances, it differs from mucin (see table). The increased content of this protein can be compared with the data of histological study of denervated glands after the action of pilocarpine [16]. The authors have shown an increase of serous cells and a raised content in them of secretory granules.

Thus, all of the experiments run with the action of pilocarpine on denervated glands show that denervation does not decrease the synthesis of proteins and the excretion of the salivary glands, but, as compared with intact glands, changes the intensity of metabolic processes



Change in secretion and amount of nitrogenous substances of saliva after injection of pilocarpine before and after denervation of the submaxillary gland. 1) Secretion of saliva (in ml) before denervation; 2) secretion of saliva by the contralateral gland; 3) secretion of saliva after unilateral denervation; 4) secretion of saliva after bilateral denervation; Black columns—total amount of saliva formed after two-hour secretion with pilocarpine. Slanted line columns—content of total nitrogen (in mg) in this saliva. White columns—content of mucin nitrogen. Lower part of all columns (lower cross-hatched part)—amount of saliva, total nitrogen, and mucin nitrogen formed in first-hour secretion with pilocarpine.

and their relation to each other in response to the same stimulation.

SUMMARY

The author studied the influence of unilateral and bilateral section of the chorda tympani on the protein metabolism and the secretory function of the submaxillary salivary glands in cats. The reflex and the "paralytic secretion in the denervated glands was absent. The nitrogen content of mucin in the tissues of these glands increased.

Changes in protein metabolism and in the secretory activity of the glands were more marked with bilateral denervation. Denervation did not stop secretion and synthesis of protein. Administration of pilocarpine caused typical changes in the salivary secretion and in the content of nitrogenous substances in the saliva and in the tissue of the salivary glands: 1) the latent period decreased; 2) the maximum quantity of the secreted saliva was changed; 3) after denervation the total amount of saliva secreted in 2 hours decreased (the content of the nitrogenous substances, however, was much higher than before denervation); 4) after pilocarpine the nitrogen content of mucin in the tissues of the denervated glands decreased and the content of protein which was not precipitated with mucin and which is typical of an actively secreting gland increased.

Thus, the parasympathetic denervation alters protein metabolism and the secretory function of the submaxillary salivary glands in cats.

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