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Regulation of the Secretory Process of Granular Components from the Convoluted Tubular Cells of the Mouse Submandibular Gland

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Autonomic regulation of the secretion of granule components in the convoluted tubular cells of male mouse submandibular glands was investigated with the use of an agar gel diffusion test using an antisera for male specific components. Whereas the injection of neither a parasympathomimetic agent (pilocarpine) nor a β -adrenergic agent (isoproterenol) decreased the amount of the components in the glands, the injection of α -adrenergic agents (norepinephrine or phenylephrine) significantly decreased the amount of male specific components. Phenoxybenzamine, an α -blocker, completely inhibited these actions of norepinephrine and phenylephrine. These facts suggest that the α -adrenergic receptor participates in the secretion of male specific components present in the granules in the convoluted tubular cells of mouse submandibular glands.

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The marked morphological sexual difference in mouse submandibular glands^{6,11,13} involves the convoluted tubular cells of the glands, which in the male are occupied by a number of serous granules.²¹ The male glands contain other biologically active substances, nerve growth factor¹ and epidermal growth factor.¹⁸ Some enzyme activities, such as proteases⁵ and glucose-6-phosphate dehydrogenase,¹⁷ are also higher in males than in females. These sexual differences suggest that mouse submandibular glands are affected by androgen.^{10,16}

Although none of the components in the granules in convoluted tubular cells of mouse submandibular glands have been purified, there is some indirect evidence suggesting that kallikrein is included in the granules in the striated duct cells of cat glands^{2,9} and rat

glands.^{3,7} Our previous study with immunoelectrophoresis revealed that at least 4 components were present in the granules in the convoluted tubular cells of male mouse submandibular glands.¹⁰

An electron microscope study indicated that the components present in granules in the convoluted tubular cells of mouse submandibular glands are synthesized within the glands.¹² In addition, kinetic analysis of the decrease and increase of granule amounts by castration and testosterone injection indicated that testosterone stimulates the synthesis of granules, but it has little influence on the secretion of granular content.¹⁰ However, the amount of granules in striated duct cells of cat submandibular glands is known to be diminished by electrical stimulation of the autonomic nerve.^{2,9} In rats, the participation of α -adrenergic receptor in the secretory process of granule contents from striated duct cells of the gland was suggested.¹⁵ However, the extent of autonomic regulation of the secretory process of the granular content in the mouse submandibular gland is still unknown. Therefore, in the present study, the autonomic regulation of the secretory process of the granular components in the submandibular glands of mice was investigated with the use of various autonomic agents.

Materials and Methods

Adult male mice (ICR strain) weighing about 35 gm were given an ordinary laboratory diet and water ad libitum. All the drugs used in the present study were dissolved in physiological saline. Parasympathomimetic and sympathomimetic agents (pilocarpine, isoproterenol, norepinephrine, and phenylephrine) were injected into the mice intraperitoneally, and an α -blocker (phenoxybenzamine) was given intravenously. The mice were killed 60 minutes after the injections of autonomic agents, and the submandibular glands were immediately

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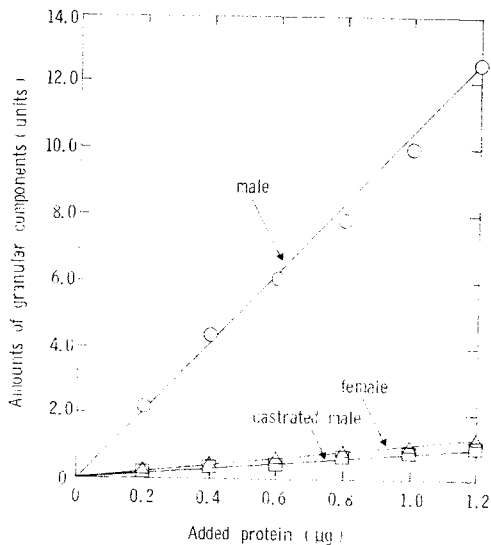


FIG 1.—Standard curves for assay of the components in granules in mouse submandibular glands by agar gel diffusion test. Pooled extracts from the submandibular glands of 5 mice were diluted and 5 μ l of each sample solution was injected into the well of an agar gel plate containing 1% of the antisera. The gel plates were then diffused for 48 hours at room temperature on a horizontal plane. After washing of gel plates with 0.9% NaCl containing 0.02% merthiolate, the precipitated proteins on the gel plates were stained with 0.1% Coomassie brilliant blue in 12.5% trichloroacetic acid for 20 minutes followed by washing with 7% acetic acid.

The radii of rings thus stained were measured. The values were normalized by the area of the well made in the agar plate and areas of precipitate were calculated. \circ , male; \triangle , female; \square , castrated male.

excised. In some cases, the mice were pretreated with phenoxybenzamine 60 minutes before the injection of various autonomic agents.

The excised submandibular glands from each mouse were homogenized with 9 volumes of 20 mM sodium phosphate buffer (pH 7.0) and the resulting homogenate was centrifuged at $23,000 \times g$ for 30 minutes, after which the supernatant was collected. The supernatant thus obtained was used for the measurement of the content of granular components and for protein estimation. Antisera against the extracts of male mouse submandibular glands was prepared in rabbits.

For assaying the amount of granular components in the glands, a somewhat specific anti-

sera to these components was prepared by absorbing the original antisera with extracts of young (21- to 22-day-old) female glands. The antisera thus treated was directed at several male specific components seen previously¹⁰ as well as granular components of the convoluted tubular cells of the glands, when male glands were observed with an immunofluorescent technique.¹⁰ For purposes of this report, material reacting with the antisera will be considered granular components.

The amounts of the granular components in extract from each mouse were measured by an agar gel diffusion test⁸ using the antisera for the granular components described above. The agar gel plate prepared for the experiment contained 1% of this antisera. The radius of the precipitin ring formed in this gel plate was measured and the area of the ring was calculated. One unit was defined as the quantity of the granular components which gives 10 mm² of the precipitin ring in this gel plate (arbitrary unit). Protein content in the extract was measured according to the method of Lowry et al.¹⁴

Results

The standard curves for the assay of granular components are shown in Figure 1. Linear relations were obtained between the added proteins and the areas of precipitin rings in the agar gel plates containing the antisera. It is clear from Figure 1 that quantitative estimation of granular components was available from 0 to 12 units in this agar gel plate. The amount of granular components in the glands of adult male mice was found to be 10 times higher than those in adult females or castrated males. These values are reasonable when compared with the morphological observations of other workers.^{11,17,21}

Adult male mice with many granules in the glands were treated with various autonomic agents, and the amount of granular components in the glands was estimated by the method described above (Table).

Pilocarpine is known as a parasympathomimetic agent. It generally has strong muscarinic action and little nicotinic action. However, it was reported that pilocarpine at the dose of 10 mg/kg significantly stimulates the superior cervical ganglion²⁰ and releases amylase from the parotid glands through the β -adrenergic receptors.^{19,20} Therefore, in the present experiment, large and small doses (3 to 10 mg/kg and 3 to

30 $\mu\text{g}/\text{kg}$) of pilocarpine were administered to mice. However, at all the doses used in the present study, pilocarpine had no effect on the amount of granular components in the submandibular glands of mice. This fact suggests that the secretion of granular components in convoluted tubular cells of mouse submandibular glands is not affected by cholinergic agents, and that, unlike the parotid glands of the rat, pilocarpine at high doses does not stimulate the secretion of the granular components from the mouse submandibular gland by way of the ganglia.

Accordingly, the effect of α - and β -adrenergic agents on the amount of granular components in the glands was estimated (Table). Isoproterenol, which strongly stimulates β -adrenergic receptors, had no effect on the amount of granular components in the glands at doses of 3 to 10 mg/kg. Conversely, both of the α -adrenergic agents tested in the present study (norepinephrine, phenylephrine) decreased the amount at doses of 3 to 10 mg/kg. In addition, the effects of norepinephrine and phenylephrine were completely inhibited by the pretreatment of mice with the α -blocker, phenoxybenzamine (10 mg/kg). In the mice administered phenoxybenzamine without α -adrenergic agents, the amounts of granular components in the glands were almost the same as those in control mice. These facts suggest that the decreases in the amount of granular components in the glands of mice administered α -adrenergic agents were the result of the action of these drugs on the α -adrenergic receptors in the submandibular gland.

Discussion

In the present study, the granular components of the convoluted tubular cells of the mouse submandibular gland appear to be secreted by the stimulation of the α -adrenergic receptors in the gland. This result is in agreement with the previous findings of Matthews,¹⁵ who demonstrated that the convoluted tubules in the submandibular glands of rats responded to α -sympathetic stimulation. Similarly, Byyny et al⁴ have demonstrated that the amount of epidermal growth factor in the glands and in the serum are decreased and increased respectively following the injection of phenylephrine into mice. This fact suggests that epidermal factor, which is known to be present in the convoluted tubular cells of the gland,²² is secreted

TABLE

EFFECTS OF AUTONOMIC AGENTS AND α -BLOCKER ON THE AMOUNT OF GRANULAR COMPONENTS IN MOUSE SUBMANDIBULAR GLANDS IN VIVO

Drug Treatments	Doses (mg/kg Body)	Granular Components (%)
None	100 \pm 12.0
Pilocarpine	0.003	102 \pm 7.1
	0.01	97.7 \pm 9.0
	0.03	99.2 \pm 9.8
	3	103 \pm 11.2
Isoproterenol	10	104 \pm 8.5
	3	97.7 \pm 15.2
Norepinephrine	10	99.6 \pm 6.0
	3	59.6 \pm 13.9
Phenylephrine	10	40.3 \pm 19.4
	3	58.4 \pm 13.2
Phenoxybenzamine	10	38.3 \pm 7.8
	10	96.3 \pm 7.2
Phenoxybenzamine + norepinephrine	10	99.2 \pm 5.4
	10	97.2 \pm 17.4
Phenoxybenzamine + phenylephrine	10	
	10	

The amounts of granular components are expressed as units/ μg protein and represented as percentages of the control values. All results are the mean \pm S.D. of 4 mice.

by stimulation of α -adrenergic receptors in the gland and transferred to the serum.

In the normal state, some granular components are detectable in the saliva of male mice by an immunoelectrophoretic technique (Hosoi, unpublished data). Therefore, the granular components released by the stimulation of the α -adrenergic receptor in the gland seem to appear in the saliva. It is not yet clear how these components affect the oral environment such as the maintenance of a normal status of gingival tissue, oral mucosa, or teeth. Thus, the intracellular localization of kallikrein and epidermal growth factor, and biochemical analysis and characterization of the granular components would seem to be important for the comprehension of the physiological significance of the granules. Accordingly, we are now attempting to isolate the granular components using the antisera. The precise manner of the discharge of these granular components is also now under investigation by electron microscopy.

Conclusion

The amount of granular components in

the convoluted tubular cells of male mouse submandibular glands was decreased, when α -adrenergic receptor site in the gland were stimulated by drugs. This fact suggests that the α -adrenergic receptor participates in the secretion of these components from mouse submandibular glands.

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