Functional Innervation of Rabbit Salivary Gland[1] (35664)

MICHAEL E. FRITZ
(Introduced by L. H. Schneyer)
School of Dentistry, Emory University, Atlanta, Georgia 30322

The existence of discrete secretomotor fibers from the autonomic nervous system to the salivary glands was first postulated by Ludwig (1) over 100 years ago, and the innervation of salivary glands has been the subject of extensive research since. Using flow of saliva alone as criterion, electrical stimulation of the parasympathetic nerve always produced significant amounts of saliva in major salivary glands of dog (2), cat (3, 4), rabbit (5, 6), rat (7), sheep (8), and man (9). Stimulation of the sympathetic secretomotor nerves or utilization of sympathomimetic drugs has not produced similar results. For example sympathetic stimulation of parotid gland of cat produced little or no flow of saliva (10, 11), while sympathetic stimulation of the submaxillary gland of cat produced salivary flow (3, 12); on the other hand, stimulation of the sympathetic trunk produced flow of saliva in the parotid gland, but not in the submaxillary gland of the rabbit (5, 6).

Data obtained in the study of membrane potentials of salivary acinar cells have thus far agreed with the data obtained from study of salivary flow characteristics. Thus, secretory cells of cat submaxillary glands respond to both parasympathetic and sympathetic stimulation by displaying a hyperpolarization (3), while secretory cells of cat parotid gland respond to parasympathetic stimulation by hyperpolarizing, but do not respond to sympathetic stimulation (11).

The present study was undertaken to determine the sympathetic secretomotor innervation of acinar cells in parotid and submaxillary glands of rabbit. Simultaneous recording of salivary flow and cell membrane potentials during stimulation of the sympathetic nerve was performed. The hypothesis to be examined was that the rabbit parotid gland, which displays flow of saliva in response to sympathetic stimulation, would display changes in membrane potentials of the acinar cells, while acinar cells in the rabbit submaxillary gland would show no change in membrane potentials in response to sympathetic stimulation.

Methods. The experiments were conducted on 10 albino male rabbits (2.7 to 3.4 kg). The animals were anesthetized with sodium pentobarbital (total dose 30 to 50 mg/kg ip). Tracheostomies were performed and the animals were artificially ventilated at 30 cycles/min with a respiratory pump (Model 944, Harvard Instrument Company, Cambridge, Mass.).

A. Recording of potentials and secretory flow in response to stimulation of autonomic nerves. In 6 rabbits, the parotid and submaxillary glands with their respective excretory ducts were exposed, and in each gland an area of approximately 3 × 3 mm was decapsulated and covered with a layer of 1 mm of a 1% agar in saline solution in preparation for electrophysiological recording. The excretory ducts were each cannulated with a 27-gauge needle adapted to PE 20 polyethylene tubing (Clay Adams, N.Y.), the outflow of which was directed to a drop counter (E-M Instruments) which was in turn coupled to an amplified pen recorder (E-M Instruments).

The preganglionic sympathetic nerves were next exposed. Stimulation of the sympathetic nerves was accomplished by the use of a square wave stimulator (Grass Model S4) connected to a stimulus isolation unit (Grass Model 4678), the output of which was fed to the stimulating electrodes, which were two

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0.015-in. chlorided silver wires embedded in an acrylic shell. The preganglionic trunk of the superior cervical ganglion was stimulated with repetitive stimuli (pulse duration, 1 sec; pulse frequency, 20–40 cycles/sec; train duration, 30 sec). In these experiments the pulse strength was 10 times greater than that required to produce maximal pupillary dilation to ensure that all the postganglionic fibers were excited (13).

The submaxillary and parotid salivary glands were penetrated with 3 *M* KCl-filled microelectrodes (14) which were advanced through the gland with the aid of a hydraulic microdrive (David Kopf Instruments). Resting membrane potentials and potentials in response to supramaximal sympathetic stimulation were recorded between the intracellular electrode and an extracellular reference electrode (chlorided silver wire) by the use of a negative-capacity electrometer (Argonaut Associates), the output of which was led to an amplified pen recorder (E-M Instruments) through a dc preamplifier (E-M Instruments). The tip diameter of all microelectrodes was less than 1 μ, the resistance measured between 5–20 megohms, and the tip potential was less than 5 mV before compensation. Cells of low potential (—12 to —31 mV) on the surface of gland (0–194 μ) were utilized for data analysis. A cell was considered to be penetrated where there was a sudden change in potential which was sustained for at least 30 sec. The data in the present study were gathered only from those situations in which microelectrode resistance was the same on removal from the gland as it was before insertion into the gland.

Following the recording of intracellular potentials in response to supramaxial sympathetic stimulation, an attempt was made to record intracellular potentials in response to parasympathomimetic stimulation. Transmembrane potentials were recorded before stimulation and then methacholine chloride (1 μg/kg) was delivered intra-arterially after isolating and ligating all vessels of the external carotid artery except those supplying the submaxillary and parotid glands. Three recordings of potentials in response to administration of methacholine chloride were obtained on one submaxillary gland and one parotid gland of each rabbit. At the termination of the electrophysiological recording, an aqueous solution of fast green was perfused to ascertain that the drug reached the gland.

B. Staining of cells through the microelectrode. In 4 rabbits, an attempt was made to identify the type of unit from which recording was done. Low potential units (—16 to —28 mV) were recorded, and attempts were made to iontophoretically stain these units by technique of Thomas and Wilson (15). Four attempts were made on one parotid and one submaxillary gland of each animal.

Results. A. Transmembrane potential and flow measurements in response to sympathetic and parasympathetic stimulation. Supramaximal stimulation of the sympathetic nerve never produced flow of saliva from the excretory duct of the submaxillary glands or change in membrane potentials of 66 low potential units (—12 to —31 mV) (Table I). The parotid gland, on the other hand, always exhibited flow of saliva in response to sympathetic stimulation. In addition, 53 of 58 recorded low potential units (—15 to —29 mV) displayed hyperpolarization in response to supramaximal stimulation of the sympathetic nerve (Fig. 1).

Twenty-four low potential (—16 to —28 mV) units and their response to intra-arterial injection of methacholine chloride were re-

TABLE I. Membrane Potentials and Salivary Flow Measurements in Response to Supramaximal Sympathetic Stimulation.

No. of animals	Gland	No. of recorded resting units	Amount of hyperpolarization with sympathetic stimulation (mV; mean ± SE)	Flow of saliva
6	Submaxillary	66	0	Never observed
6	Parotid	53	4.2 ± 0.5	Always observed

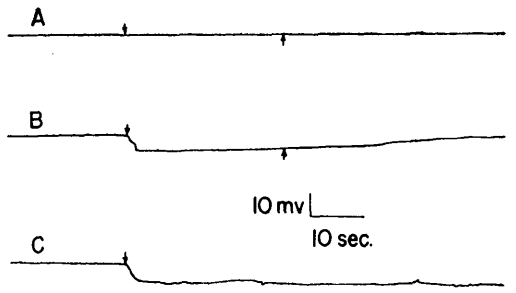

FIG. 1. Typical cellular responses obtained with various modes of stimulation: (A) intracellular recording in submaxillary gland in response to repetitive supramaximal (for pupillary dilation) sympathetic stimulation. (B) intracellular recording in parotid gland in response to repetitive supramaximal sympathetic stimulation. (C) intracellular recording in submaxillary gland in response to methacholine chloride. (A and B, arrows) the start and finish of electrical stimulation of the sympathetic nerves; (C, arrow) the administration of the drug.

corded in the salivary glands of 4 rabbits. All of these units displayed hyperpolarization, and each injection of the drug was accompanied by salivation. The mean hyperpolarization (± SE) recorded in 12 units from the submaxillary glands was 7.7 ± 1.0 mV, while the mean hyperpolarization (± SE) recorded in 12 units from parotid glands was 8.0 ± 0.7 mV. The response to administration of this drug was long, usually lasting 2–4 min following its administration (Fig. 1). The data obtained in 2 rabbits following the administration of methacholine chloride were not utilized for analysis, because leaks in the perfusion system were discovered.

B. Iontophoretic staining of units through the microelectrode. In 4 rabbits positive identification of 21 low potential units (—18 to —30 mV) was made. In the parotid glands, 11 units were recovered and all of these were acinar cells (Fig. 2). In the submaxillary gland, 10 units were recovered, and the dye was found in six cells which would be classified as mucous cells and four cells which would be classified as serous cells.

Discussion. The results obtained in the present study indicate that impulses originating in, or passing through, the superior cer-

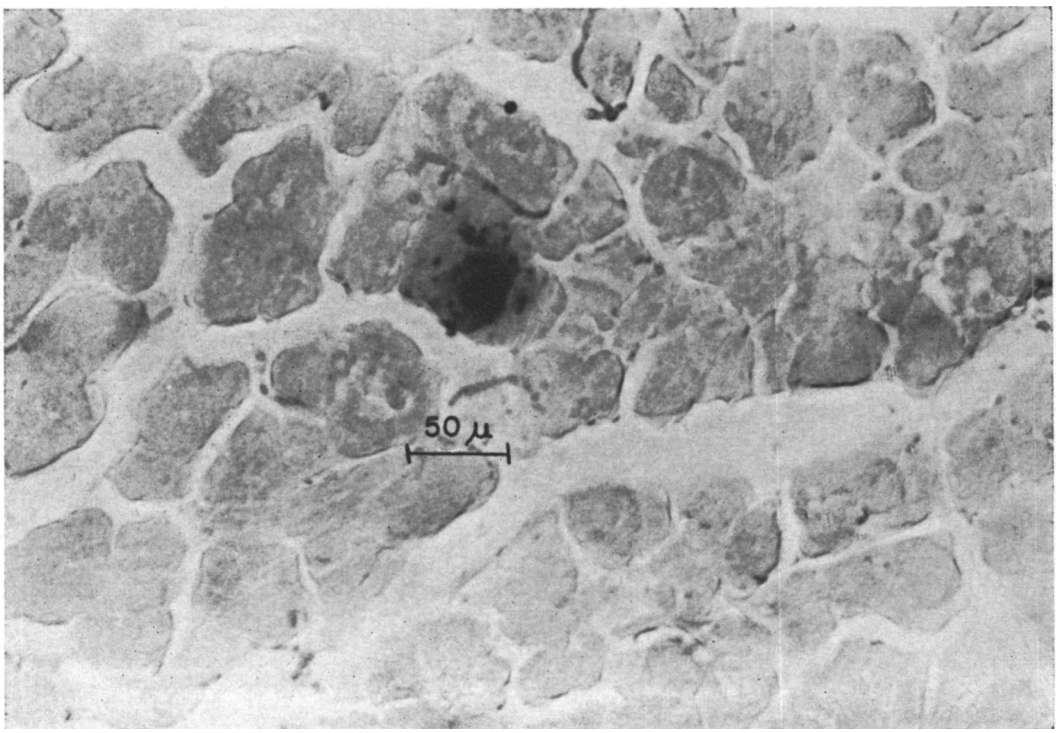

FIG. 2. Parotid gland of rabbit: the cell in the center of the field has been marked by iontophoretic injection of a dye, some of which has leaked out of the cell boundaries.

vical ganglion do not activate acinar cells of the submaxillary gland of rabbit, nor do they cause salivary flow from the rabbit submaxillary gland. On the other hand, sympathetic impulses apparently do activate acinar cells in the parotid gland of rabbit, as evidenced by the recording of sympathetically induced hyperpolarizing potentials from acinar cells and the recording of flow from the salivary ducts. The present findings are compatible with those of other investigators who have demonstrated little or no salivary flow from rabbit submaxillary glands in response to sympathetic stimulation, but increased amounts of saliva in rabbit parotid glands in response to sympathetic stimulation (5, 6).

One can only speculate about the disparity that occurs in sympathetically induced secretion and acinar cell innervation in submaxillary and parotid glands of rabbit in view of recent histochemical findings demonstrating that the distribution of adrenergic nerves is similar in both glands, although there is a slightly more dense sympathetic network in the parotid (16, 17). It would appear that either: (a) the sympathetic innervation of the submaxillary gland arises from another source than the superior cervical ganglion; or (b) adrenergic nerves associated with acinar cells in the submaxillary gland of rabbit may have functions other than the induction of secretion. The former explanation has seemingly been ruled out by the experiment of Ehinger *et al.* (16) demonstrating that unilateral removal of the cervical sympathetic chain in rabbit produced both submaxillary and parotid glands unilaterally devoid of adrenergic nerves. There is no evidence in rabbit to associate sympathetic innervation with functions other than secretion, although sympathetic innervation of myo-epithelial cells has been demonstrated in cat (18).

Submaxillary gland of rabbit is therefore similar to lacrimal (19) and parotid (11) glands of cat, in that there is apparently no sympathetically induced transport of ions and water.

Summary. Acinar cells of submaxillary gland of rabbit are not innervated by impulses passing through the superior cervical ganglion, as evidence by the lack of sympathetically induced secretory potentials and salivary flow. On the other hand, sympathetic impulses produced both salivation and secretory potentials in parotid gland of rabbit.

1. Ludwig, C., Z. Rat. Med. N. F. **1**, 255 (1851).
2. Gregersen, M. E., and Ingalls, E. N., Amer. J. Physiol. **98**, 441 (1931).
3. Lundberg, A., Acta Physiol. Scand. **35**, 1 (1955).
4. Petersen, O. H., and Poulsen, J. H., Acta Physiol. Scand. **70**, 158 (1967).
5. Morley, J., Schachter, M., and Smaje, L. H., J. Physiol. (London) **187**, 595 (1966).
6. Nordenfelt, I., and Ohlin, O., Acta Physiol. Scand. **41**, 12 (1957).
7. Schneyer, C. A., and Hall, H. D., Amer. J. Physiol. **209**, 484 (1965).
8. Coats, D. A., Denton, D. A., Goding, J. R., and Wright, R. D., J. Physiol. (London) **131**, 13 (1956).
9. Diamant, H., Enfors, B., and Holmstedt, B., Acta Physiol. Scand. **45**, 293 (1959).
10. Richins, C. A., and Kuntz, A., Amer. J. Physiol. **173**, 471 (1953).
11. Fritz, M. E., and Botelho, S. Y., Amer. J. Physiol. **216**, 1392 (1969).
12. Emmelin, N., Acta Physiol. Scand. **34**, 11 (1955).
13. Eccles, J. C., J. Physiol. (London) **85**, 179 (1935).
14. Ling, G., and Gerard, R. W., J. Cell. Comp. Physiol. **34**, 383 (1949).
15. Thomas, R. C., and Wilson, V. J., Science **151**, 1538 (1966).
16. Ehinger, B., Garrett, J. R., and Ohlin, P., Experientia **23**, 924 (1967).
17. Freitag, P., and Engel, M., Anat. Rec. **167**, 87 (1970).
18. Emmelin, N., Garrett, J. R., and Ohlin, P., J. Physiol. (London) **200**, 539 (1969).
19. Botelho, S. Y., Hisada, M., and Fuenmayor, N., Arch. Ophthalmol. **76**, 581 (1966).

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