Effect of autonomic nerve stimulation on bleb formation in striated duct cells of the rat submandibular gland

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Several previous investigations have shown that blebs form on the apical surface of the striated duct cells of the rat submandibular gland on feeding after starvation. In the present report the influence of autonomic nerve stimulation on bleb formation was studied by electron microscopy. Both parasympathetic and sympathetic stimulation were performed, using electric nerve stimulation. In addition, sympathetic nerve stimulation in combination with alpha- or beta-adrenergic blockers was used. Massive bleb formation took place in response to sympathetic nerve stimulation. This response was almost completely abolished by the administration of alpha- but not by beta-adrenergic blocker. Bleb formation was not seen after parasympathetic nerve stimulation. ☐ *Nerve stimulation; salivary glands; ultrastructure*

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Apical, plasmalemmal modifications of striated duct cells resulting in cellular protrusions of material devoid of cytoplasmic organelles and inclusions, have been reported several times. Such protrusions are commonly known as blebs and are separated from the rest of the cytoplasm by a separating zone consisting of bundles of fine filaments. Blebbing has been described on striated duct cells in the parotid gland of the rat (1), bovine (2), goat (3), cattle (4, 3), baboon (5), sheep (3, 6), and humans (7, 8) and in the submandibular gland of the squirrel monkey (9), nine-banded armadillo (10), rat (11–14), male mouse (15), calf (4), and humans (16).

The origin of blebbing has been discussed by several investigators. Some indicate that they are fixation artifacts (17, 18), whereas others believe that they are indicative of apocrine secretion (11, 12) and not produced by the fixation procedure (19). Furthermore, studies showing bleb formation when rats were starved and then fed (11, 12, 20) indicate that bleb formation is induced as a response to a physiologic stimulus.

The present study was undertaken to investigate further the nature of blebbing by studying the effect of autonomic nerve stimulation on striated duct cells of the rat submandibular gland. The effect of cholinergic and of adrenergic stimulation alone or in the presence of alpha- or beta-adrenergic blockers was studied.

Materials and methods

Female Wistar rats (330–400 g body weight) with food and water ad lib were anesthetized with pentobarbital sodium (Nembutal®, 70 mg/kg body weight) by an intraperitoneal injection and tracheotomized to facilitate breathing. The main excretory duct of the submandibular gland was exposed by removing the digastric and mylohyoid muscles, and the duct was cannulated with a polyethylene cannula. The effect of nerve stimulation was checked by recording the presence of salivary flow and measurements of salivary kallikrein concentration (21). Kallikrein was measured by an immunoradiometric assay for glandular kallikrein (22).

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Autonomic stimulation of the gland was performed in accordance with previous studies (21, 23, 24). Parasympathetic stimulation of the submandibular gland was done on three rats by electric stimulation of the ductal nerve plexus close to the gland hilus, using a bipolar silver electrode connected to a Grass SD9 stimulator (7.5 V, 10 Hz, 2 msec duration). Sympathetic stimulation (four rats) was done similarly by electric stimulation of the cervical sympathetic chain (CN), which was dissected free from the vagal nerve. Sympathetic nerve stimulation was also performed after administration of a beta-adrenergic blocker (propranolol-chloride, 2 mg/kg body weight, followed by a second injection of 2.5 mg/kg body weight, allowing an additional 20 min for the drug to be effective) (four rats), or an alpha-adrenergic blocker (phentolamine, 1.6 mg/kg body weight, again waiting 20 min for the drug to be fully effective) (four rats). Twenty minutes after stimulation the glands were perfused with 5% glucose, followed by 3.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), entering through the carotid artery. After 15 min of perfusion the submandibular glands were extirpated and cut into thin slices under the dissecting microscope. The tissues were fixed for an additional period of 24 h by immersion in the perfusion fixative. Four rats not given any stimulation were perfused and fixed as above and were used as controls. Before being embedded in Vestopal W (25), the tissue slices were rinsed for 10 min in 0.15 M phosphate buffer (pH 7.3), postfixed in 1% osmium tetroxide (4°C, 2 h) (26), and then rapidly dehydrated in a graded series of acetone.

For light microscopic viewing and orientation, 1-µm-thick sections were cut and stained with the Ponceau de Xylidine/Giemsa method (27). Ultrathin sections were cut on an LKB Ultrotome Nova. The sections were collected on copper grids and stained with uranyl acetate for 30 min, followed by lead citrate for 5 min (28). The grids were examined in a Philips 400 electron microscope. Tissue sections from stimulated glands were compared with sections from unstimulated control glands. The extent of bleb formation was determined as the percentage blebbing cells out of the total number of cells observed in one duct. Three to 6 ducts were randomly selected from each rat, and a total of 12–25 different ducts were studied in each group, giving a total number of at least 250 cells. Statistical significance of differences between the various groups was determined by Wilcoxon's two-sample test (29).

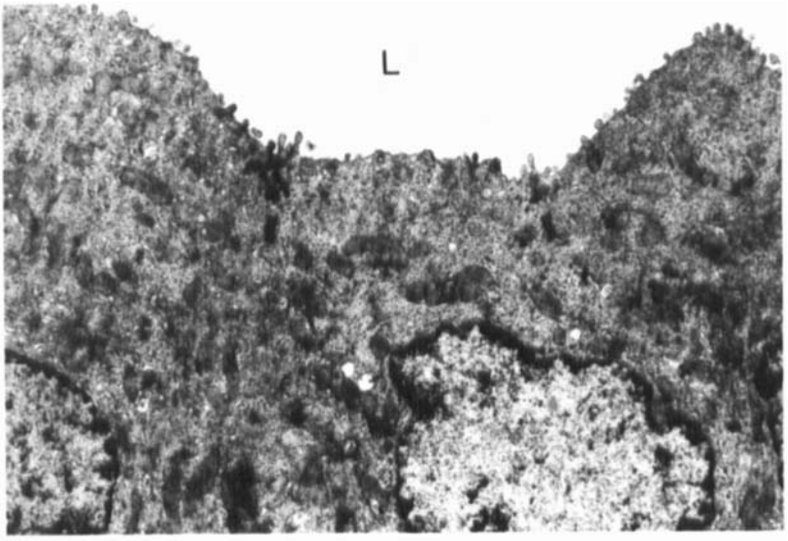

Fig. 1. Luminal part of striated duct cells of submandibular gland subjected to parasympathetic nerve stimulation. The luminal membrane is microvillous with no sign of bleb formation. (Magnification, ×20,000.)

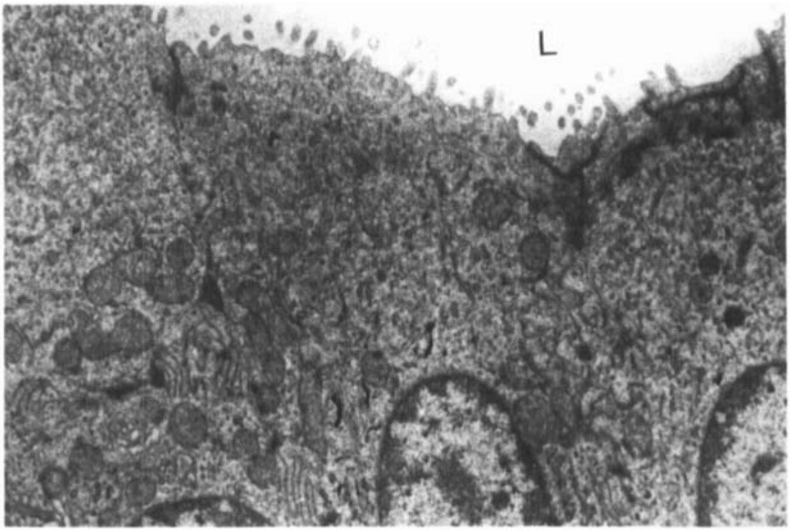

Fig. 2. Striated duct cells of unstimulated rat submandibular glands. Microvilli are observed on the luminal membrane but no blebs could be detected. (Magnification, ×25,000.)

Results

Striated ducts in unstimulated control glands and glands subjected to parasympathetic stimulation did not differ in morphology (Figs. 1 and 2). In the supranuclear region of the cells a limited number of mitochondria was observed, whereas the apical cytoplasm was devoid of electron-dense granules. The luminal membrane was microvillous, and no sign of apical swelling or bleb formation was observed (Table 1). In the submandibular glands subjected to sympathetic nerve stimulation, blebs of different sizes were present on the apical extremity of striated duct cells (Fig. 3, Table 1). The blebs were electron-lucent and had a homogeneous appearance with no cytoplasmic organelles. The blebs were divided from the rest of the cytoplasm by a separating zone or fibrillar material. On the basal side of the separating zone small electron-dense granules appeared in various numbers. The granules were found within a

Table 1. Extent of bleb formation in submandibular glands from control rats, after parasympathetic stimulation and sympathetic stimulation alone or after administration of alpha-adrenergic blocker (phentolamine) or beta-adrenergic blocker (propranolol)

	Ductal cells showing blebbing, %	Salivary secretory rate, μl/min	Salivary conc. of glandular kallikrein, μg/ml
Control	0 ± 0	0	—
Parasympathetic stim.	0 ± 0 (NS)	19 ± 3*	184 ± 58
	*		*
Sympathetic stim.	98 ± 5*	12 ± 1*	11,700 ± 2800
	(NS)		(NS)
Sympathetic stim. + beta-adrenergic blocker	96 ± 5*	10 ± 3*	4320 ± 930
	*		*
Sympathetic stim. + alpha-adrenergic blocker	3 ± 3 (NS)	7 ± 1*	280 ± 90

NS = not significant ($p > 0.05$); *$p < 0.005$, given after the value when compared with control glands and on the line between the two groups compared for other intragroup comparisons. The results are given as the mean value ± standard deviation.

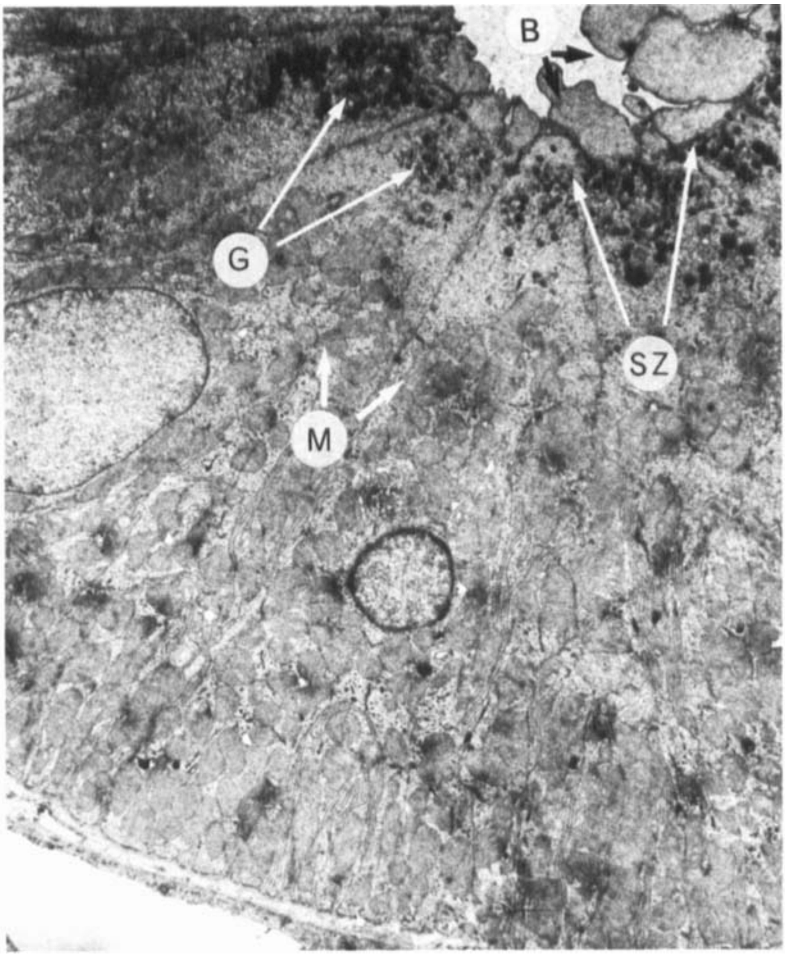

Fig. 3. Striated duct cells of submandibular gland subjected to sympathetic nerve stimulation. Blebs of different sizes present on the apical surface of the cells. A separating zone divides the bleb from the rest of the cytoplasm. Small granules are present on the basal side of the separating zone. B = bleb; SZ = separating zone; G = granules; M = mitochondria. (Magnification, ×8000.)

zone of electron-lucent cytoplasm. The rest of the supranuclear region consisted predominantly of densely packed mitochondria.

In the apical cytoplasm of striated duct cells of glands stimulated sympathetically after administration of alpha-adrenergic blocker, a large number of electron-dense granules were observed (Fig. 4). Bleb formation was significantly reduced (Table 1), and blebs were only occasionally present (Fig. 5). In striated duct cells of submandibular glands stimulated sympathetically in combination with administration of beta-adrenergic blocker, apical blebs developed in most cells (Fig. 6, Table 1). The number of blebbing cells was not significantly different from that seen after sympathetic nerve stimulation alone (Table 1).

The blebs induced after sympathetic stimulation were electron-lucent and of various sizes. They were separated from the rest of the cytoplasm by a separating zone. The cytoplasm adjoining the separating zone was normally devoid of electron-dense granules and appeared electron-lucent with only a few organelles.

The presence of salivary secretion confirmed submandibular gland activation by nerve stimulation (Table 1), and the salivary kallikrein concentration (Table 1) supported the concept that the autonomic nerve stimulation was performed in a pattern similar to that obtained in previous studies (21, 23, 24).

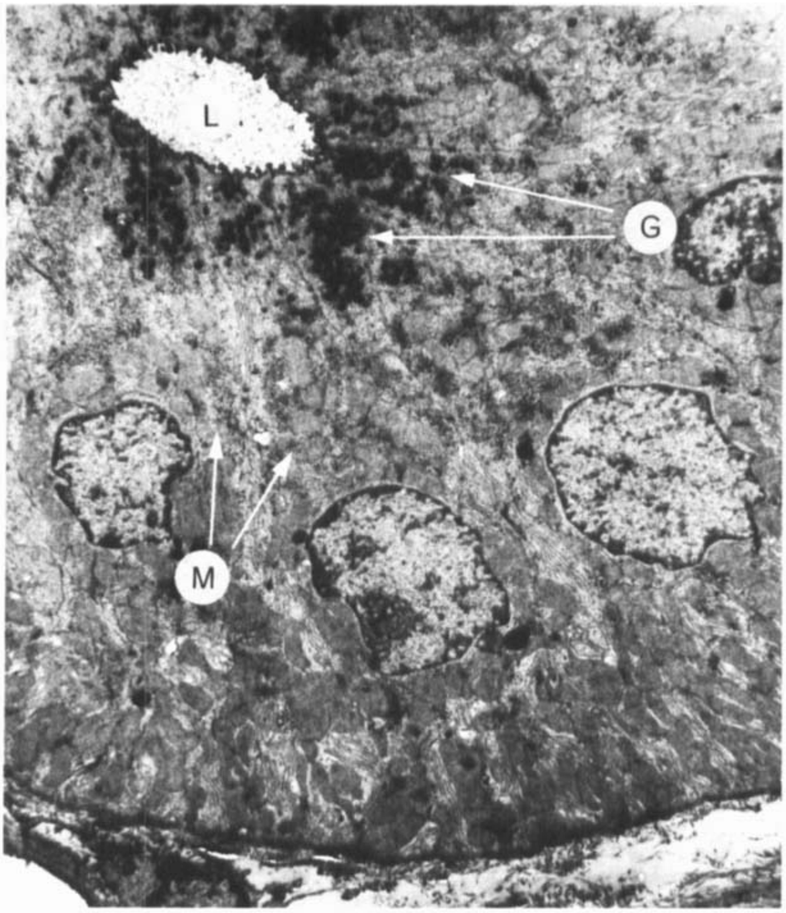

Fig. 4. A striated duct after sympathetic nerve stimulation during alpha-adrenergic blockade. The apical cytoplasm is filled with electron-dense granules. L = lumen; G = granules. (Magnification, ×8000.)

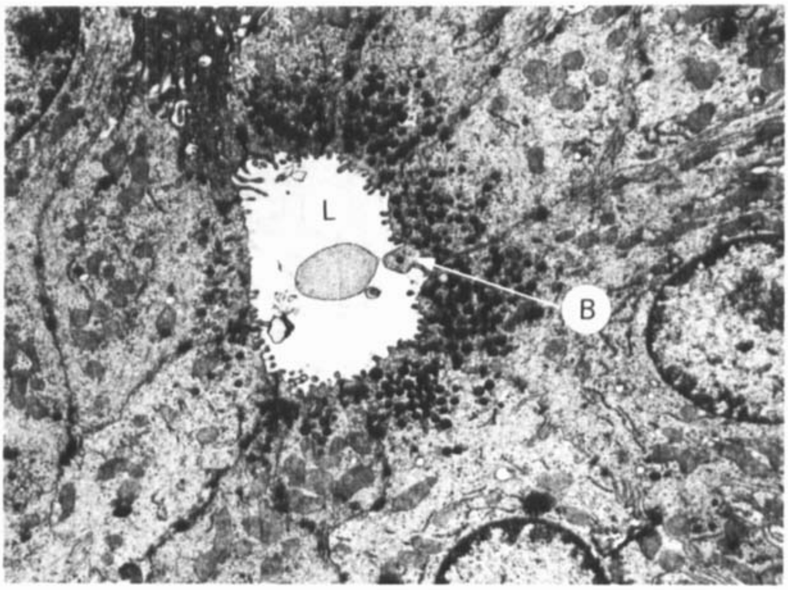

Fig. 5. Striated duct cell after sympathetic nerve stimulation during alpha-adrenergic blockade, showing one single bleb. L = lumen; B = bleb. (Magnification, ×8000.)

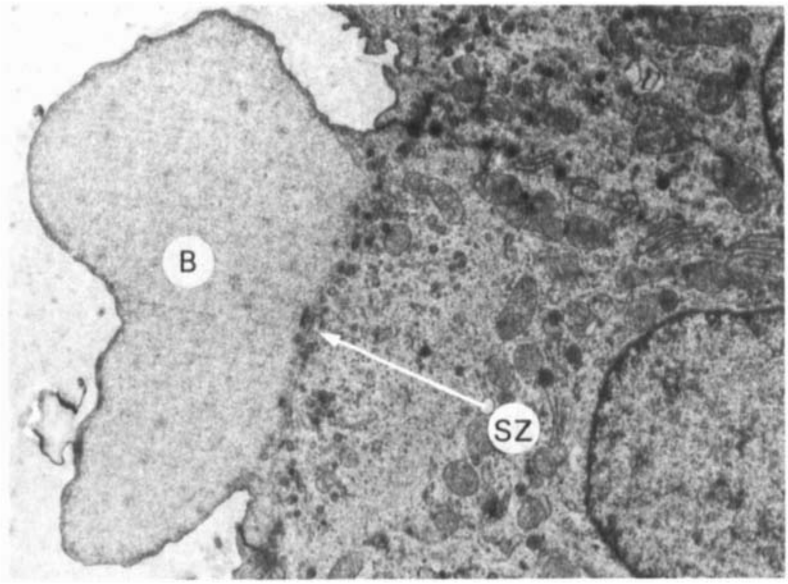

Fig. 6. A typical striated duct cell after sympathetic nerve stimulation during beta-adrenergic blockade. The bleb is separated from the cytoplasm by a separating zone. B = bleb; SZ = separating zone. (Magnification, ×20,000.)

Discussion

Eating and chewing normally involve reflex-induced activation of salivary gland secretion. The autonomic nerve activation involved in this process is not fully known. Bleb formation of striated duct cells of the submandibular gland has previously been shown to take place when rats are fed after an overnight period of starvation and water deprivation (12). The present study shows similar blebbing as a response to nerve stimulation mediated through alpha-adrenergic receptors. Parasympathetic and beta-adrenergic receptor activation did not induce bleb formation. These results may indicate that the reflex bleb formation produced by starvation and feeding may be mediated through adrenergic efferent nerves and alpha-adrenergic receptors. Since starvation alone did not induce blebbing, it seems that it was the process of drinking that activated bleb formation.

The controls included in the present study—that is, measurements of saliva and salivary kallikrein secretion—were in agreement with previous studies (21, 23, 24) and confirmed that the stimulation was carried out as intended. On the other hand, the present study is in contradiction to observations made by Parks (20), who found that in the rat parotid gland, pilocarpine, but not adrenalin, induced bleb formation. It is, however, not known whether the different bleb stimulation patterns were influenced by the change in organ.

The physiologic role of bleb formation is not fully understood. A role as a mechanism for apocrine secretion or an increased epithelial surface for electrolyte transport may be suggested. Neither would be contradictory to the alpha-adrenergic dependence of bleb formation, as observed in the present study. Further investigations are required to elucidate the importance of this phenomenon in salivary gland function.

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