Demilune cells of the cat submandibular gland; an unlikely source of kallikrein[1]

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Summary. The functional significance of kallikrein in the salivary gland remains unclear partially because of uncertainty over its precise cellular localization. Kallikrein was thought to originate in acinar cells, until recent evidence from cat and rat localized it primarily to the ducts. The possibility that salivary kallikrein may also be located in demilune cells was investigated in this study. - The total kallikrein content of cat submandibular glands was found to be substantially reduced by sampathetic nerve stimulation; whereas parasympathetic stimulation had no significant effect. These biochemical findings did not correlate with morphological studies that revealed almost complete depletion of the demilune cells secretory granules after stimulation of either division of the autonomic nerve supply. This lack of correlation makes it unlikely that kallikrein is present in the demilune cell secretory granules.

It has been established that kallikrein (kininogenase) is present in the submandibular glands of many mammals[3-9]. The cellular localization remains controversial. Kallikrein was originally thought to be associated with the zymogen granules of the acinar cells[10], however, recent evidence obtained from the cat and the rat indicates a ductal localization[11-14]. The possibility that kallikrein is also present in demilune cells was suggested by Emmelin and Henriksson[15], however, their observation has not been further investigated.

The objective of this study was to determine the association between kallikrein and demilune cells in the cat. Experiments were undertaken to stimulate the sympathetic and parasympathetic nerves supplying the submandibular gland and to correlate ultrastructural changes produced in the demilune cells with kallikrein activity in the gland.

Materials and methods. Cats (2.5-3.7 kg) of either sex were starved overnight. They were anaesthetized with chlorolase (60 mg kg^{-1}, i.v.) after induction with chloroform. The cervical sympathetic and chorda lingual nerves were unilaterally isolated and cut, their distal ends mounted on bipolar platinum electrodes and immersed in a pool of warm liquid paraffin. The nerves were then stimulated supramaximally (7 V) with square wave pulses of 0.4 msec duration at a frequency of 10 Hz.

Maintained sympathetic nerve stimulation leads to vasoconstriction and a progressive reduction in secretion. To avoid this complication the cervical sympathetic nerve was stimulated intermittently. In the 3 experiments, the total stimulation times were 50, 70 and 90 min for the sympathetic nerve and 45, 65 and 90 min, respectively for the parasympathetic nerve.

Small pieces (1-2 mm thickness) of the stimulated submandibular and the contralateral control glands were removed and processed for electron microscopy using the procedure described by Dorey and Bhoola[16]. Thin sections were cut, stained with uranyl acetate and lead citrate and examined with a Philips 200 electron microscope.

The rest of the gland was then extracted, minced and lyophilized. The esterase activity of the lyophilized tissue was measured by the method of Trautschold[17], using benzoyl-L-arginine ethylester (BAEe) as substrate. Protein concentrations were determined by the spectrophotometric method of Lowry, Roseburgh, Farr and Randall[18].

Results and discussion. The table shows the kallikrein activity in unstimulated control glands compared with glands subjected to sympathetic and parasympathetic nerve stimulation.

After 45, 65 and 90 min of parasympathetic stimulation the enzyme activity in the glands are 100.5, 176.0 and 84.1% of that in the control glands, respectively. Thus prolonged chorda nerve stimulation does not alter the kallikrein activity to any significant extent.

Following sympathetic nerve stimulation for 50, 70 and 90 min, enzyme activity is reduced to 24.8, 14.8 and 12.3% of that in contralateral control glands, respectively. Thus it seems clear that sympathetic nerve stimulation casues considerable mobilization of kallikrein.

Figure 1 is an electronmicrograph of a control gland showing demilune cells (DC) packed with secretory granules (SG). In the cat submandibular glands these cells are easily distinguished from acinar cells on the basis of their semilunar arrangement and the presence of an electron dense substructure in their granules. Because of tight packaging of secretory granules the Golgi apparatus is not clearly visible.

Figure 2 shows a demilune cell subjected to parasympathetic stimulation for 65 min. Although kallikrein activity is not reduced (in fact increased) compared to the control gland, the demilune cell is almost completely devoid of its granule content.

Figure 3 is an electromicrograph of a sympathetically stimulated gland showing 2 demilune cells (DC) with their outer membranes interdigitating with each other (→). The lumen (L) is filled with secretory products which are similar in appearance to that seen in secretory granules. A secreto-

Effect of autonomic nerve stimulation on the kallikrein activity in the cat submandibular gland

Cat	Nerve stimulated	Duration of nerve stimulation 7 V, 0..4 msec duration at 10 Hz min	Relative specific activity esterase units mg^{-1} protein* Control gland	Experimental gland	Experimental/ control (× 100)
1	Sympathetic	50	10.02	2.49	24.8
2	Sympathetic	70	5.60	0.83	14.8
3	Sympathetic	90	4.58	0.56	12.3
4	Parasympathetic	45	173.0	174.0	100.5
5	Parasympathetic	65	10.00	17.60	176.0
6	Parasympathetic	90	11.43	9.62	84.1

*Relative specific activity of kallikrein is expressed as the esterase activity using benzoyl-L-arginine ethylester (BAEe) as substrate.

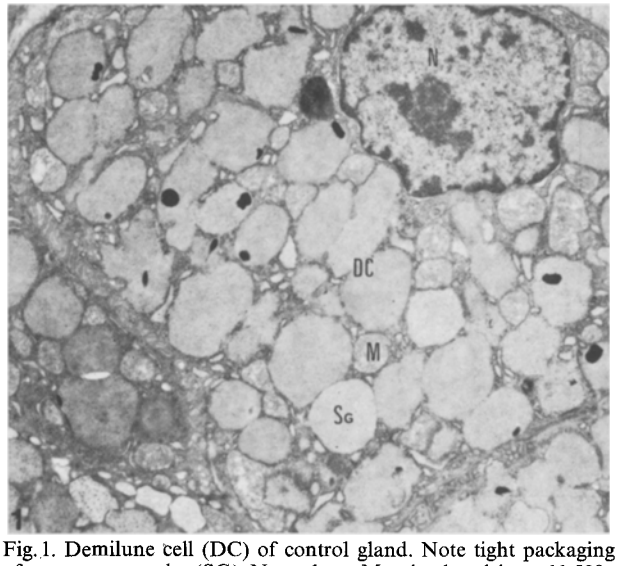

Fig. 1. Demilune cell (DC) of control gland. Note tight packaging of secretory granules (SG). N, nucleus; M, mitochondria. × 11,500.

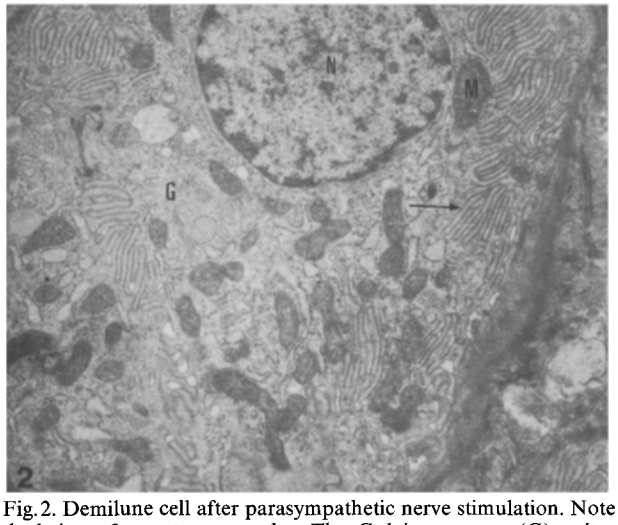

Fig. 2. Demilune cell after parasympathetic nerve stimulation. Note depletion of secretory granules. The Golgi apparatus (G), mitochondria (M) and interdigitation (arrow) are quite prominent. N, nucleus. (Cat No. 5. × 11,500.

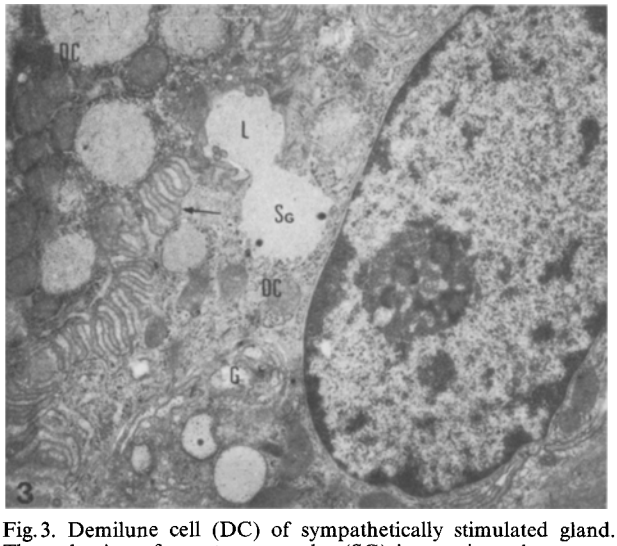

Fig. 3. Demilune cell (DC) of sympathetically stimulated gland. The reduction of secretory granules (SG) is prominent but not as dramatic as seen in figure 2. (Cat No. 1) × 14,000.

ry granule (SG) is in direct contact with the lumen (L) and a few of these granules can still be seen in the cell. Although great numbers of these cells show depletion of their granules, a few cells still contain moderate amounts of secretory granules. The Golgi apparatus (G) is enlarged and clearly visible and its cisternae contains secretory product similar to that in the secretory granules.

These results differ from Barton et al.[11] in that consistent degranulation of demilune cells occurred following prolonged stimulation of the sympathetic and parasympathetic nerves (maximum 90 min). In the previous study, shorter periods of stimulation were utilized (maximum duration 20 min sympathetic; 46 min parasympathetic) which produced unpredictable results. The results of this study indicated that mobilization of demilune cell secretory granules is influenced by both parasympathetic and sympathetic nerves; whereas, mobilization of kallikrein as determined by biochemical measurement of gland depletion is influenced mainly by the sympathetic component. Since parasympathetic nerve stimulation causes almost complete mobilization of demilune cell secretory granules with no obvious reduction in kallikrein activity of the gland, it is highly unlikely that kallikrein is present in demilune cell secretory granules.

Our electronmicroscopic observations are in disagreement with the light microscope observations of Rawlinson[19] and the electron microscope observations of Garrett[20]. They observed that parasympathetic nerve stimulation of the cat submandibular gland generally had a minimal effect on demilune cells. In our investigation, chorda nerve stimulation results in complete depletion of demilune secretory granules.

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3 K.D. Bhoola and C.W. Ogle, J. Physiol. *184*, 663 (1966).
4 K.D. Bhoola and G. Dorey, J. Physiol. *199*, 303 (1969).
5 K.D. Bhoola and P.F. Heap, J. Physiol. *219*, 421 (1970).
6 R.S. Chiang, E.G. Erdös, I. Miwa, L.L. Tague and J.J. Coalson, Circulation Res. *23*, 507 (1968).
7 E.G. Erdös, L.L. Tague and I. Miwa, Biochem. Pharmac. *17*, 667 (1968).
8 F. Geipert and E.G. Erdös, Experientia *27*, 912 (1971).
9 K.M. Gautvik and M. Kriz, Acta physiol. scand. *92*, 95 (1974).
10 J. Albano, K.D. Bhoola, P.F. Heap and M.J.C. Lemon, J. Physiol. *258*, 631 (1976).
11 S. Barton, E.J. Sanders, M. Schachter and M. Uddin, J. Physiol. *251*, 363 (1975).
12 T.B. Ørstavik, P. Brandtzaeg and K. Nustad, J. Dent. Res. *54*, L51 (1975).
13 P. Brandtzaeg, K.M. Gautvik, K. Nustad and J.V. Pierce, Br. J. Pharmac. *56*, 155 (1976).
14 M. Schachter, S. Barton, M. Uddin, E. Karpinski and E.J. Sanders, Experientia *33*, 746 (1977).
15 N. Emmelin and K.G. Henriksson, Acta physiol. scand. *30*, 75 (1953).
16 G. Dorey and K.D. Bhoola, Z. Zellforsch. Mikrosk. Anat. *126*, 320 (1972A).
17 I. Trautschold, in: Handbook of Experimental Pharmacology, vol. 25, p. 71. Ed. E.G. Erdös. Springer Verlag, Berlin, Heidelberg, New York 1970.
18 O.H. Lowry, N.J. Roseburgh, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
19 H.E. Rawlinson, Anat. Rec. *57*, 289 (1933).
20 J.R. Garrett and A. Kidd, J. Physiol. *263*, 198 (1976).