The localization of kallikrein in the dog and guinea-pig submandibular glands[1]

M. Uddin[2] and D. W. Tyler

Department of Physiology, College of Medicine, Department of Oral Biology, College of Dentistry, University of Saskatchewan, Saskatoon (Saskatchewan, Canada S7N OWO), 31 March 1980

Summary. In the dog and guinea-pig submandibular glands kallikrein seems to be present in the striated duct cells. Following sympathetic nerve and in vivo isoproterenol stimulation of the dog and guinea-pig submandibular gland respectively, there is a reduction of kallikrein concentration. Ultrastructurally this reduction corresponds to the decrease of straited duct secretory granules in both species. Parasympathetic stimulation also causes some release of kallikrein from both species.

Kallikreins (kininogenases) are a group of endogenous enzymes present in the salivary glands of many mammals. Although kallikreins were first described in salivary glands some 40 years ago[3], their physiological role in these and other organs remains uncertain. Hilton and Lewis[4-6] suggested that glandular kallikreins are involved in the functional vasodilation of the submandibular gland and the pancreas. However their view was highly contested. In recent years evidence in the kidney has shed some light on the involvement of glandular kallikrein in water and electrolyte balance[7-13]. The presence of detectable amounts of kallikrein in the kidney and the submandibular glands and the morphological similarity between the kidney tubules and the striated duct cells makes it tempting to speculate that in the submandibular gland, kallikrein may also play some role in the regulation of water and electrolyte balance.

Studies[14-20] have demonstrated that kallikrein is present in secretory granules of many mammalian salivary glands. However, the cellular location of kallikrein has only recently been clarified. Earlier studies[21] correlated the light microscope appearance of parasympathetically denervated cat submandibular glands with the kallikrein activity of saliva and concluded that the demilune cells were the source of kallikrein. On the other hand, Bhoola and his co-workers[22] suggested that kallikrein is located in the acinar cell secretory granules of the guinea-pig submandibular gland. More recently, however, the studies[23-31] have indicated that kallikrein is located in the striated duct cells and not in the acinar or demilune cells.

In order to facilitate the localization of kallikrein in the dog submandibular gland, in the present investigation, ultrastructural changes following autonomic nerve stimulation are correlated with the changes in the kallikrein concentration. In addition, in vivo effects of pharmacological agents mimicking autonomic nerve transmitters are studied on the kallikrein content of the guinea-pig submandibular gland. Enzyme decrease is then correlated with the change in the acinar and ductal cell secretory granule population.

Materials and methods. Dogs: Male dogs (11-22 kg) were starved overnight. Anaesthesia was induced with sodium pentobarbital (30-35 mg · kg^{-1}) given i.v.

The autonomic nerve supplying one of the submandibular glands was isolated and stimulated as described by Beilenson et al.[32], the contralateral unstimulated gland was used as a control. The parasympathetic nerve supply to the submandibular gland runs in chorda tympani, a branch of the chorda lingual nerve. The chorda lingual nerve was exposed in the region where it crosses the submandibular and sublingual ducts, and was cut as near as possible to its

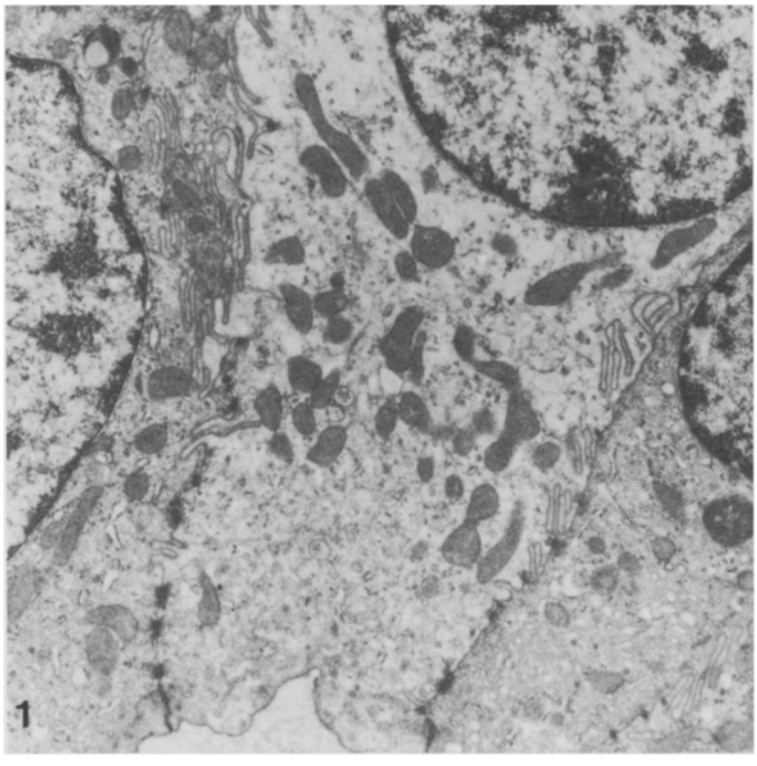

Figure 1 shows the apical region of a striated duct cell following 75 min of sympathetic nerve stimulation. Note the reduction to secretory granule from these cells. × 9600.

point of exit from the skull. The distal end of the nerve was cleared of connective tissue and mounted on a bipolar platinum electrode. After immersion in warm liquid paraffin, the nerve was stimulated supramaximally at 9 V with square wave pulses of 0.4 msec duration and a frequency of 20 Hz. The cervical sympathetic nerve was exposed in the neck region, cut, mounted and stimulated by the same procedure.

Guinea-pigs: Male guinea-pigs ranging from 400 to 800 g were starved overnight. Anaesthesia was induced by sodium pentobarbital (35 mg · kg^{-1} i.p.). Before administration of the pharmacological agents, an incision was made on one side in the neck region and one gland was removed; this gland served as a control. Control glands and glands subjected to pharmacological stimulation were divided for electromicroscopy and for the esterase and protein determinations.

Pharmacological agents: Pilocarpine (20–40 mg · kg^{-1}) was

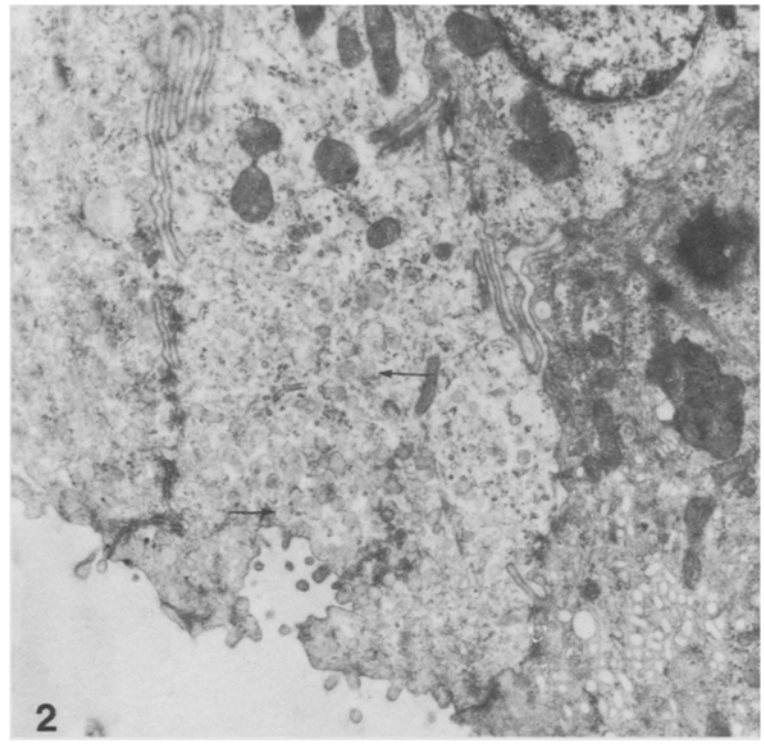

Figure 2 shows an apical region of normal striated duct cells. Note the small size and the number of the secretory granules (arrows). × 9600.

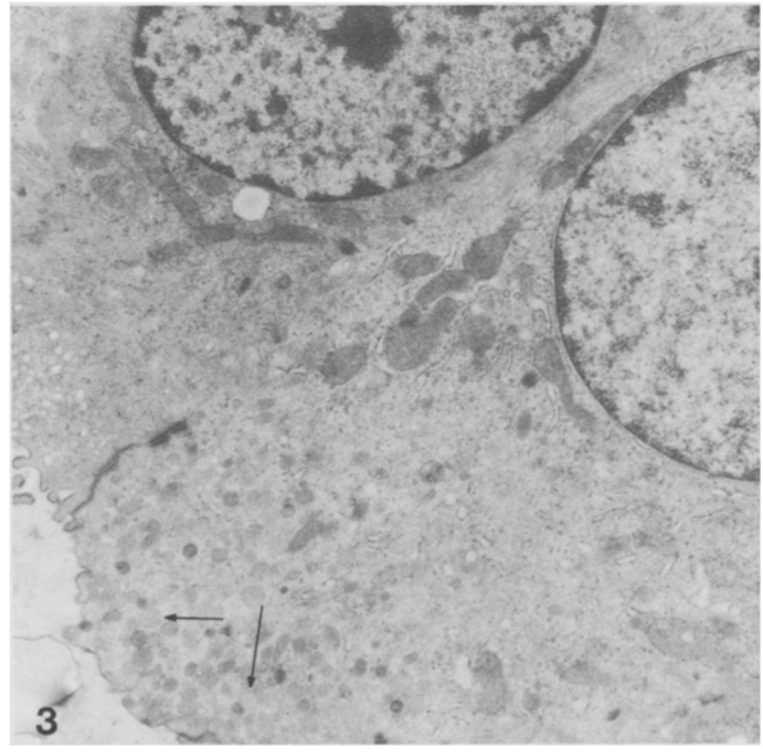

Figure 3. Apical region of a cell following parasympathetic stimulation. Note that the cell does not show depletion of its secretory granules (arrows). Adjacent to a secretory granule containing cell is a vesiculated dark cell. × 9600.

administered in conjunction with the ganglionic blocking agent hexamethonium. Hexamethonium (2–4 $mg \cdot kg^{-1}$) was given 5 min prior to pilocarpine injection. Isoproterenol dose varied between 100 and 300 $mg \cdot kg^{-1}$. All drugs were dissolved in isotonic saline and were administered i.p. A group of 5 guinea-pigs were given hexamethonium alone (4 $mg \cdot kg^{-1}$). Ultrastructural changes and kallikrein activity of these glands were compared with that of normal glands.

Electron microscopy: Small pieces of tissue were removed from different areas of the gland after gentle separation of the lobes. All pieces of the experimental and the control glands were removed while the blood flow was still intact. After removal, these pieces were fixed immediately in a mixture consisting of 4% paraformaldehyde, 2.5% glutaraldehyde and 1% acrolein in 0.1 M cacodylate buffer at pH 7.3. The mixture was kept at 4 °C. After 2 h of fixation and 1 h of postfixation in cold 2% osmium tetroxide in cacodylate buffer at pH 7.3, the tissue was processed as described by Dorey et al.[18]. Thin sections were cut, stained with the uranyl acetate and lead citrate method[33] and examined with a Phillips electron microscope.

Esterase activity: Glands were chopped and lyophilized immediately. An aqueous extract was prepared from the

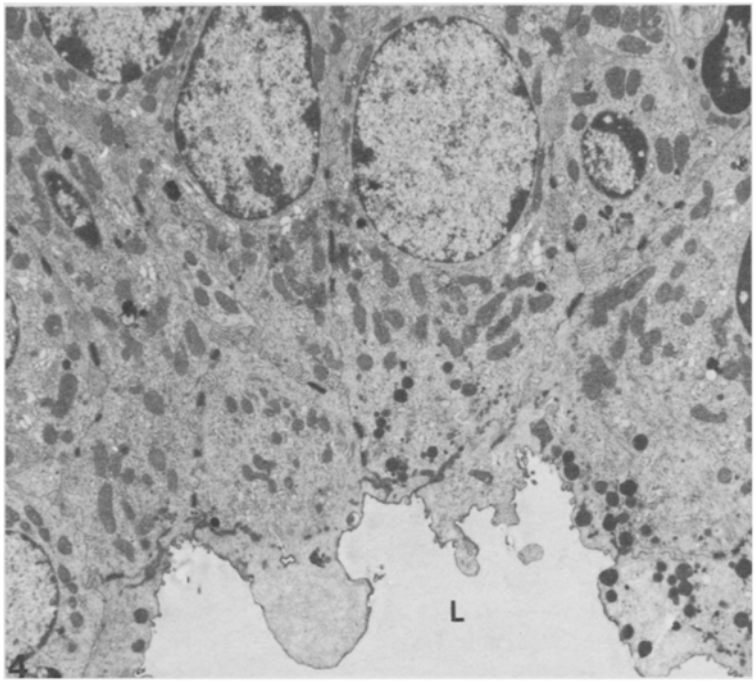

Figure 4. Apical region of striated duct cells following in vivo isoproterenol administration. Majority of secretory granules have disappeared. Lumen. × 3700.

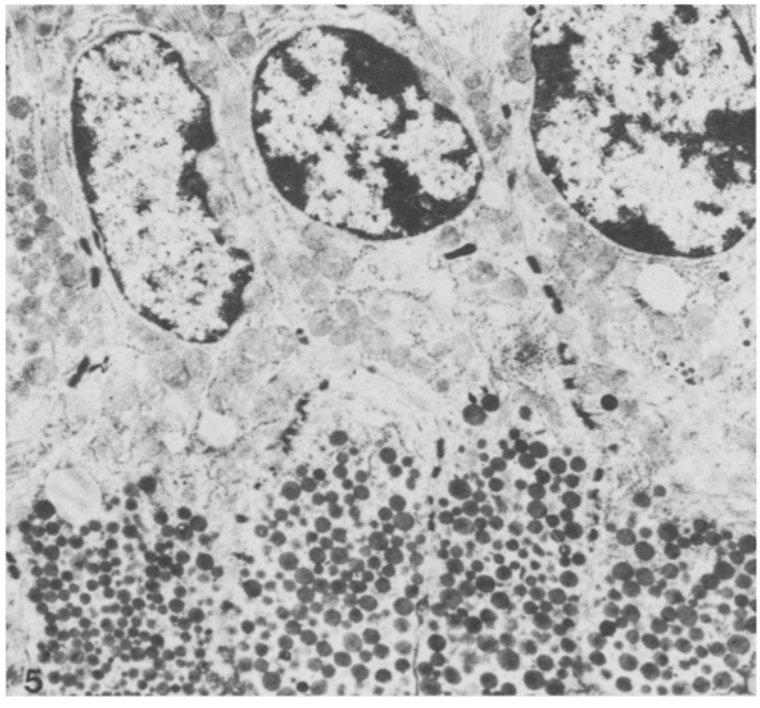

Figure 5. Apical region of normal striated duct cells. Note abundance of secretory granules. × 3700.

lyophilized glands and after centrifugation, the supernatant solution was again lyophilized. The esterase activity was determined from this powder by using the spectrophotometric method of Trautschold[34] utilizing benzoyl-L-arginine ethylester (BAEe) as a substrate.

Proteins: Protein concentrations were determined from the extract used for exterase by the spectrophotometric method of Lowry et al.[35].

Results: Effects of prolonged sympathetic nerve stimulation on the dog submandibular gland. Kallikrein content: Table 1 shows the effect of prolonged sympathetic nerve stimulation on the kallikrein content of the gland. There is a substantial reduction in the relative specific activity, and total kallikrein of stimulated glands when compared to contralateral control glands. No correlation has been observed between the duration of stimulation and the kallikrein decrease from the glands.

Demilune cells: There is an appreciable reduction in the number of secretory granules; cell size is reduced considerably and the nucleus of the cell which usually occupies a position near the base of the cell is displaced more towards the center. In some stimulated glands, these cells show wide variations in their granular content and there seems to be no apparent correlation between the degree of degranulation and kallikrein decrease. Glands which show near complete degranulation of their demilune cells are found to contain substantial amounts of kallikrein.

Acinar cells: The acinar cells show no change in their granular content when compared with their contralateral control acinar cells. In all stimulated glands examined, there is no reduction of acinar cell granules.

Striated duct cells: The granules of striated duct cells are roughly ¼ the size of those of acinar and demilune cells. Depletion of granules is readily detected, but could easily be overlooked when compared to the gross cellular changes observed following degranulation of acinar and demilune cells during various types of stimulation.

Figure 1 is an electron micrograph of striated duct cells following prolonged sympathetic nerve stimulation. The secretory granules of the striated duct cells are depleted as compared to figure 2 which shows striated duct cells from control glands.

Effects of prolonged parasympathetic nerve stimulation on the dog submandibular gland. Kallikrein content: Table 1 also shows the effect of parasympathetic nerve stimulation on the kallikrein content. Shorter stimulation times (55 and 65 min) had little effect, however, longer periods (75 and 85 min) produced substantial reduction.

Demilune cells: The ultrastructural changes include depletion of secretory granules, increased interdigitation of the foot processes of the outer plasma membranes between cells, prominence of nuclei, hypertrophy of the Golgi apparatus and a distinct appearance of the rough endoplasmic reticulum. Variations of these changes were observed in all stimulated glands and no significant difference was observed between different periods of stimulation.

Acinar cells: The most significant feature of the stimulated acinar cells is a dramatic reduction of secretory granules. Other prominent changes include enlargement of the lumen, conspicuous nuclei, mitochondria, rough endoplasmic reticulum and enlarged Golgi apparatus.

Striated duct cells: Straited duct cells showed variable changes in their secretory granule content. The glands which showed the greatest kallikrein decrease also showed the greatest reduction in secretory granules. Figure 3 shows a cell stimulated for 65 min.

Effects of isoproterenol on the kallikrein content of the guinea-pig gland. Table 2 shows that in vivo stimulation by isoproterenol results in a substantial reduction in kallikrein content of the gland as compared to the contralateral

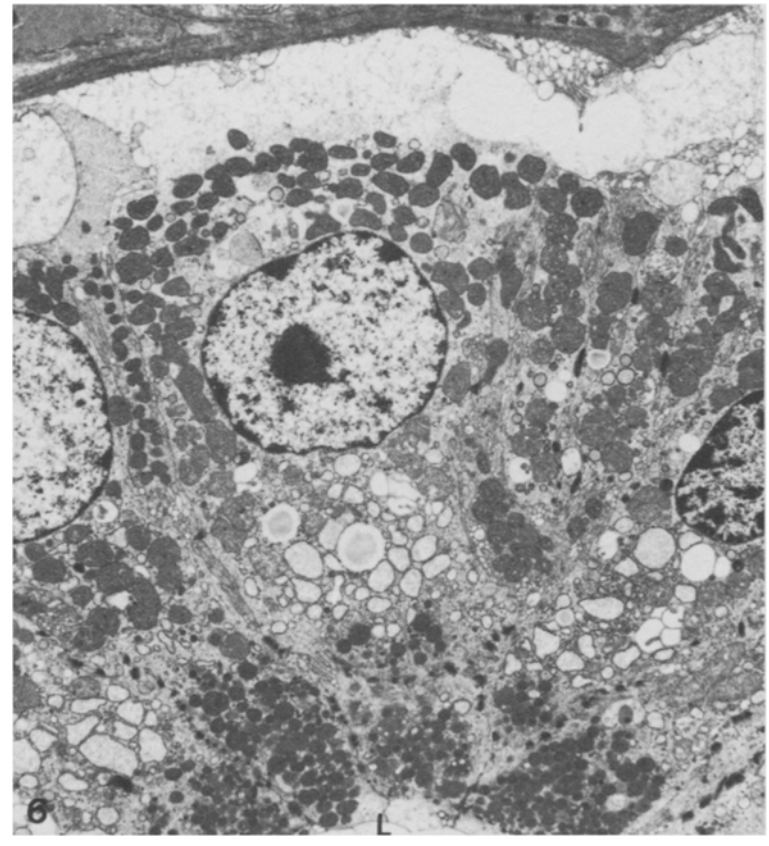

Figure 6. Striated duct cells following in vivo pilocarpine administration. Majority of secretory granules can be seen bordering lumen (L.). There is a general disruption of other subcellular organelles. ×3700.

control gland. Although a considerable degree of variation between glands is observed in the total and the relative specific activity of this enzyme, the overall decrease is 57% and 50% of the control value. In some cases, the concentration was reduced to 90% of the normal value. The degree of reduction of kallikrein content did not seem to be related to the dose of drug administered.

Effects of isoproterenol on cells containing secretory granules. Acinar cells: Isoproterenol caused depletion of acinar cell secretory granules. The decrease, however, was not uniform in all glands. Few glands showed only partial reduction of their acinar cell secretory granules. This reduction of acinar cell secretory granules population, did not correlate with the decrease of kallikrein. Glands which showed complete depletion of their acinar granular content, were found to contain higher amounts of kallikrein. On the other hand, glands with partial reduction of their acinar secretory granules showed lower amounts of kallikrein.

Striated duct: Figure 4 shows part of striated duct cells from the stimulated gland whose acinar cells are also degranulated. It is seen that almost all secretory granules have disappeared. Few granules which are visible, seem to be mobilized and are bordering the lumen. Occasionnally, a few secretory granules can be seen in the lumen, often the apical portion of the cell is protruded into the lumen and few granules are visible in these blebs. For comparative purposes, the striated duct cells of the control gland is shown in figure 5.

Glands which showed lesser degree of striated duct cell degranulation also found to contain higher amounts of kallikrein.

In all stimulated glands, isoproterenol mediated degranulation of acinar cells was always morepronounced than that of the striated duct cell of the same gland.

Effects of pilocarpine on the kallikrein content of the guinea-pig gland. Table 3 shows that the administration of pilocarpine in conjunction with hexamethonium, causes a slight decrease in the kallikrein content of the stimulated glands. Pilocarpine, like isoproterenol, produced variable changes in kallikrein content of the glands. However, the overall reduction of the kallikrein is less than that produced by the isoproterenol.

To exclude the possibility that hexamethonium may be involved in the kallikrein depletion from the gland, a group of 5 guinea-pigs are injected with 4 $mg \cdot kg^{-1}$ hexamethonium alone and the effects of this ganglionic blocking agent on the kallikrein concentration is compared with untreated contralateral control glands. From table 3, it is clear that hexamethonium did not cause any appreciable reduction of the kallikrein content of the gland.

Effects of pilocarpine on the secretory granules containing cells. Acinar: The degranulation of these cells is strikingly similar to that seen following in vivo isoproterenol stimulation. However, the effect of pilocarpine on other subcellular structures seems to be slightly different. Mitochondria are drawn into long rods and appear slender and there seems to be abundant endoplasmic reticulum studded with polyribonucleoprotein particles.

Striated duct: Figure 6 is an electron micrograph of a few striated duct cells from the gland No. 8, table 3. In this gland the degranulation of cells is only slight. Cells show disrupted subcellular structures. The basal plasma membrane infoldings which are arranged in parallel rows per-

Table 1. Effects of sympathetic and parasympathetic nerve stimulation on the kallikrein content of the dog submandibular gland

	Duration of stimulation (min)	Kallikrein concentration $\left(\frac{\text{experimental}}{\text{control}} \times 100\right)$	Relative specific activity
Sympathetic stimulation	60	28.7	28.0
	65	29.7	36.9
	75	31.2	15.8
	75	37.4	58.8
	80	38.3	28.5
	85	45.5	57.7
Parasympathetic stimulation	55	101.8	82.5
	65	90.4	71.3
	75	61.7	45.7
	85	45.7	38.3

Table 2. The effect of isoproterenol on the kallikrein content in the guinea-pig submandibular gland in vivo

Gland No.	Isoproterenol		Kallikrain decrease $\left(1-\frac{\text{experimental}}{\text{control}} \times 100\right)$	
	Dose ($mg \cdot kg^{-1}$)	Duration (min)	Decrease per dry gland weight	Decrease per mg protein
1	300	180	46.9	48.0
2	195	180	53.0	47.5
3	125	135	50.0	49.5
4	225	180	30.9	30.5
5	300	180	81.1	75.0
6	100	180	42.9	35.0
7	300	180	37.5	11.7
8	300	180	96.0	92.4
9	300	180	75.0	64.0
		Mean ± SE	57.0 ± 7.3	50.4 ± 8.1

Table 3. The effects of pilocarpine and hexamethonium on the kallikrein content of the guinea-pig submandibular gland in vivo

Gland No.	Dose ($mg \cdot kg^{-1}$)	Duration (min)	Kallikrein decrease $\left(1-\frac{\text{experimental}}{\text{control}} \times 100\right)$	
			Decrease per dry gland weight	Decrease per mg protein
Pilocarpine* plus hexamethonium**				
1	*30+2**	180	43.6	(6.7)
2	30+2	150	27.3	0.0
3	30+2	170	58.4	53.3
4	30+2	170	54.3	28.8
5	30+2	170	76.8	55.0
6	39+3	135	(8.0)	22.3
7	20+2	110	16.4	23.7
8	40+5	180	35.6	13.7
9	30+2	180	42.5	58.9
		Mean ± SE	38.5 ± 8.3	27.6 ± 7.5
Hexamethonium alone				
	4	180	8.0	19.5
	4	180	0.0	0.0
	4	180	8.2	25.8
	4	180	12.3	13.1
	4	135	9.8	10.8
		Mean ± SE	7.7 ± 2.1	13.8 ± 4.3

( ) indicates increase.

pendicular to the basal plasma membrane infolding in normal cells of the gland are scattered and appear swollen. Stimulated cells also show a dilated endoplasmic reticulum and a few hydrophilic vacuoles.

This particular gland is stimulated with 40 $mg \cdot kg^{-11}$ pilocarpine for 180 min. The kallikrein content of the gland was reduced by 36% and 14% in terms of its total and relative specific activity respectively. In other glands in which there is greater kallikrein decrease, the degranulation and the disruption of other subcellular structures is more pronounced.

Discussion. Following electrical stimulation of the sympathetic nerve in the dog and B-adrenergic stimulation by isoproterenol in the guinea-pig submandibular glands, the kallikrein content is substantially reduced; an observation consistent with the findings of others[27,36]. Barton et al.[27] also showed that brief periods of sympathetic stimulation (10 min) resulted in massive decrease of kallikrein from the cat submandibular gland. In the present study on the dog submandibular gland, however, the decrease of kallikrein is not as dramatic. 60 min of stimulation results in a decrease of approximately $\frac{1}{3}$ of the normal value. Longer stimulation, in general, does not cause further decrease. The difference between the 2 species probably reflects differences in the innervation pattern[37].

Ultrastructurally demilune cells of the dog, acinar cells of the guinea-pig and striated duct cells of both species show reduction in their granular population following sympathetic nerve stimulation and B-adrenergic stimulation respectively. Acinar cell secretory granule population of the dog submandibular gland, on the other hand, show no change following prolonged sympathetic nerve stimulation. Last findings in the dog gland is in confirmation with the results in the cat[27].

Prolonged parasympathetic stimulation of the dog submandibular gland, unlike the cat[27], causes a reduction in glandular kallikrein. However, this reduction required a more prolonged stimulation and is of a lesser magnitude than that seen following sympathetic nerve stimulation.

Ultrastructurally, there is marked degranulation of both demilune and acinar cells. Degranulation of striated duct cells is more difficult to assess due to small size and sparse population of secretory granules, although qualitatively some reduction in these granules is observed in those glands which showed substantial decrease in kallikrein.

The discrepancy between 2 species with respect to changes in kallikrein content and straited duct cell granules mobilization is not surprising because there are differences in the innervation pattern to salivary glands within the same species[37] and even within the same gland[39]. It is quite possible that differences in the innervation between the dog and cat submandibular gland would account for these results.

In vivo pilocarpine stimulation in the guinea-pig submandibular gland also causes release of the kallikrein. Although in some cases the kallikrein decrease is marked, overall decrease is much smaller in comparison to the decrease following in vivo isoproterenol stimulation. In vitro incubation of gland slices in the presence of pilocarpine or isoproterenol indicated a similar pattern of kallikrein decrease[12].

Ultrastructurally, this parasympathomimetic agent, like isoproterenol, results in the depletion of acinar cell secretory granules; an observation similar to that of Bhoola et al.[38]. However, the effects of pilocarpine on the ultrastructure of striated duct cells is quite different to that seen following isoproterenol. The cells show disrupted subcellular organelles and only a small reduction of their secretory granule population. Damaged subcellular structures reflect deleterious effects of pilocarpine on straited duct cells and reduction in their secretory granule population, without excluding other possibilities, would suggest leakage of kallikrein from the gland.

Degranulation of striated duct cells and corresponding decrease in the kallikrein content following sympathetic nerve stimulation and B-adrenergic stimulation by isoproterenol in the dog and guinea-pig submandibular gland, suggest that striated duct cells contain kallikrein-rich secretory granules. This suggestion has been supported by the studies utilizing immunofluorescent techniques for the localization of kallikrein in the cat, rat and the guinea-pig submandibular gland[40] and separation of ductal and acinar fraction in the guinea-pig submandibular gland[29].

Ductal location of kallikrein in the submandibular glands of the dog and guinea-pig, as suggested by this and other studies may have some physiological significance. In the kidney, a correlation has been shown between the excretion of urinary kallikrein which is located in the juxtaglomerular apparatus and ductal cells distal to this point and sodium excretion[7-13]. Also, morphologically, duct cells of the kidney resemble the straited duct cells of the submandibular gland and it has been shown that, like kidney, the straited ducts are also involved in the process of absorption[41]. The morphological resemblance, the demonstration of the process of absorption in the kidney and the submandibular gland, and the existence of the correlation between urinary kallikrein and sodium excretion in the kidney strongly points towards the existence of a similar mechanism in the submandibular gland.

1 Supported by Dean's M.R.C. grant to the College of Dentistry.
2 Present address: Department of Anatomy, McGill University, 3640 University Street, Montreal (Quebec, Canada H3A 2B2).
3 E. Werle and P. von Roden, Biochem. J. *286*, 213 (1936).
4 S.M. Hilton and G.P. Lewis, J. Physiol., Lond. *128*, 235 (1955).
5 S.M. Hilton and G.P. Lewis, J. Physiol., Lond. *129*, 253 (1955).
6 S.M. Hilton and G.P. Lewis, J. Physiol., Lond. *134*, 471 (1956).
7 M.E. Webster and J.P. Gilmore, Am. J. Physiol. *206*, 714 (1964).
8 M.A. Barraclough and H.I. Mills, Clin. Sci. *28*, 69 (1965).
9 J.R. Gill, K.L. Melman, L. Gillespie, Jr, and F.C. Bartler, Am. J. Physiol. *209*, 844 (1965).
10 C.R. Wills, J.H. Ludens, J.B. Hook and E.H. Williamson, Am. J. Physiol. *217*, 1 (1969).
11 M. Marin-Grez, P. Connone and O.A. Carretero, Am. J. Physiol. *223*, 794 (1972).
12 A. Nesjletti, J. Colina-Chourio and J.C. McGiff, Circulation Res. *37*, 59 (1975).
13 A. Adetyibi and I.H. Mills, Lancet *2*, 203 (1972).
14 J. Albano, K.D. Bhoola, P.F. Heap and M.J.C. Lemon, J. Physiol., Lond. *258*, 631 (1976).
15 K.D. Bhoola and C.W. Ogle, J. Physiol., Lond. *184*, 663 (1966).
16 K.D. Bhoola and G. Dorey, J. Physiol., Lond. *203*, 59p (1969).
17 K.D. Bhoola and P.F. Heap, J. Physiol., Lond. *210*, 421 (1970).
18 G. Dorey and K.D. Bhoola, Z. mikrosk.-anat. Forsch. *126*, 320 (1972).
19 E. G. Erdös, L.L. Tague and I. Miwa, Biochem. Pharmac. *17*, 667 (1968).
20 K.M. Gautvik and M. Kriz, Acta physiol. scand. *92*, 95 (1974).
21 N.J. Emmelin and K.G. Henriksson, Acta physiol. scand. *30*, 75 (1953).
22 K.D. Bhoola, G. Dorey and C.W. Jones, J. Physiol., Lond. *235*, 503 (1973).
23 M. Uddin and D.W. Tyler, Life Sci. *22*, 1603 (1978).
24 M. Schachter, B. Maranda and C. Moriwaki, Histochem. J. *26*, 318 (1978).

25 T.B. Orstavik, P. Brandtzaeg, K. Nustad and K.M. Halvorsen, Acta histochem. *54*, 183 (1975).
26 P. Brandtzaeg, K.M. Gautvik, K. Nustad and J.V. Pierce, Br. J. Pharmac. *56*, 155 (1976).
27 S. Barton, E.J. Sanders, M. Schachter and M. Uddin, J. Physiol., Lond. *251*, 363 (1975).
28 M. Schachter, S. Barton, M. Uddin, E. Karpinski and E.J. Sanders, Experientia *33*, 746 (1977).
29 Y. Hojima, B. Maranda, C. Moriwaki and M. Schachter, J. Physiol., Lond. *268*, 793 (1977).
30 B. Maranda, J.A.A. Rodrigues, M. Schachter, T.K. Shinitka and J. Weinberg, J. Physiol., Lond. *276*, 321 (1978).
31 M. Uddin and D.W. Tyler, Experientia *34*, 609 (1978).
32 S. Beilenson, M. Schachter and L.H. Smaje, J. Physiol., Lond. *199*, 303 (1968).
33 E.S. Reynolds, J. Cell Biol. *17*, 208 (1963).
34 I. Trautschold, in: Handbook of Experimental Pharmacology, vol.25, p.71. Ed. E.G. Erdos. Springer, Berlin, Heidelberg, New York 1970.
35 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
36 J.R. Garrett and A. Kidd, Cell Tiss. Res. *161*, 71 (1975).
37 J.R. Garrett, in: Oral Physiology, vol.20. Ed. N. Emmelin and Y. Zotherman. Pergaman, New York 1971.
38 K.D. Bhoola, P.F. Heap and M.J.C. Lemon, in: Experimental Biology and Medicine, vol.28, p.59. Ed. F. Sicuteri, N. Back and G.L. Haberland. Plenum, New York and London 1976.
39 W.T. Yahro, Z. Zellforsch. *110*, 173 (1971).
40 K.D. Bhoola, M.J.C. Lemon and R.W. Matthews, J. Physiol., Lond. *272*, 28p (1977).
41 G. Kaladelfos and J.A. Young, Aust. J. exp. Biol. med. Sci. *52*, 67 (1974).

Circadian and seasonal rhythm in stimulation-produced analgesia

W.R. Buckett[1]

Centre de Recherche Merrell International, 16, rue d'Ankara, F-67084 Strasbourg Cedex (France), 27 March 1980

Summary. The antinociceptive effect induced in mice by peripheral electrical stimulation has been shown to exhibit both circadian (24 h) and seasonal (1 year) rhythms. These findings contribute to an explanation of the variability reported for stimulation-produced analgesia.

In both animals and man it is known that many physiological and pharmacological effects due to endogenous substances and exogenous compounds are under circadian control[2], as well as in many instances being subject to rhythmic influences of much longer duration[3]. It has recently been demonstrated that electrical stimulation-induced analgesia of short duration (ESPA)[4] in mice may represent an endogenous pain control system[5] which is called into play in the face of noxious stimuli, thus permitting the taking of aversive action under antinociceptive cover. This paper provides evidence to show that ESPA in mice is a phenomenon which displays both circadian and seasonal rhythms.

Methods. CD1 female albino mice (20–25 g b.wt) from Charles River, France were housed 20 per cage at 22 °C with a 12-h light cycle commencing at 06.00 h and were allowed rodent diet and water ad libitum. Not less than 1 week after arrival animals were randomly allocated to groups of ten and placed in new cages 4 h before testing. ESPA was induced by caudal stimulation of lightly restrained animals with a bipolar surface electrode (3 mm diameter, P-P-E, Hugo Sachs KG) using a Grass S-48 stimulator and SIU-5 isolation unit. Rectangular wave pulses of 15 msec pulse width were passed at 20 Hz for 30 sec at the threshold voltage for vocalization. ESPA was then measured by immediate transfer within 5 sec of the animals to a hot-plate (22.5 × 11.0 cm surface at 52 °C, with 10 cm high enclosure) and recording the escape latency in seconds. Each mouse was used only once.

Results. Analysis of the results obtained over a 24-h period

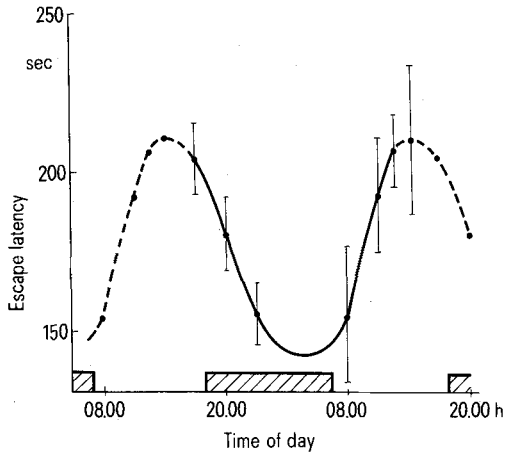

Fig. 1. Circadian rhythm in escape latencies of mice on a 52 °C hot-plate after induction of electrical stimulation-produced analgesia (ESPA). The latencies (mean ± SE, N = 20) in sec are shown against time of day in h. The graph represents mean data from 2 experiments carried out during February and several points are repeated byond 24 h (broken line) so as to emphasize the rhythm. The shaded areas on the abscissa represent the dark phase of the cycle.

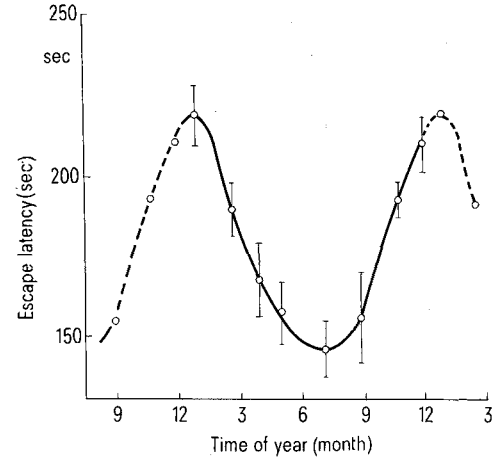

Fig. 2. Seasonal rhythm in escape latencies of mice on a 52 °C hot-plate after induction of electrical stimulation-produced analgesia (ESPA). The latencies (mean ± SE, N = 20–60) in sec are shown against the time of year in months. The graph respresents mean monthly data from experiments carried out from 13 to 17 h during 1 year and several points are repeated (broken line) in order to clearly illustrate the rhythm.