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SECRETION OF KALLIKREIN AND ITS ROLE IN VASODILATATION IN THE SUBMAXILLARY GLAND

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SUMMARY

1. The effects of parasympathetic (chorda) and sympathetic nerve stimulation on the concentration and output of kallikrein secreted in saliva from the cat's submaxillary gland were compared. Sympathetic stimulation always produced a much higher concentration (up to 500 times) and output (up to 390 times) of kallikrein than parasympathetic stimulation. In the dog, in which sympathetic nerve stimulation produces little or no secretion from the submaxillary gland, there was also a marked increase in the secretion of kallikrein when sympathetic was superimposed on parasympathetic secretion. This effect did not occur, however, in the rabbit's submaxillary gland.

2. It was possible to deplete the cat's submaxillary gland of kallikrein, either by ligation of the duct for several days or by duct ligation and sympathetic nerve stimulation, so that it was undetectable either in the gland or in saliva after stimulation of the chorda. Such glands, nevertheless, responded to chorda stimulation with a normal atropine-resistant vasodilatation.

3. There is a close parallelism between the rate of secretion of saliva and vasodilatation over a range of frequencies of chorda stimulation, but the output (and concentration) of kallikrein in saliva is distinctly different for the same frequencies of nerve stimulation.

4. Our results are consistent with the view that vasodilator nerves exist in the parasympathetic nerves to the submaxillary gland. We suggest that they are cholinergic in nature despite the fact that chorda vasodilatation is resistant to atropine. It is further suggested that neither the kallikrein–kinin system nor adrenergic vasodilator nerve fibres play a significant role in chorda vasodilatation.

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INTRODUCTION

Salivary kallikrein was first described by Werle & Roden (1936) and shortly afterwards was shown to exert its hypotensive and other pharmacological effects by releasing an active fragment from a protein substrate in plasma apparently by a proteolytic action (Werle, Götze & Keppler, 1937). Ungar & Parrot (1936) immediately suggested that salivary kallikrein was released during salivary secretion and that it was the mediator of the atropine-resistant vasodilatation produced by parasympathetic (chorda) nerve stimulation in the submaxillary gland. This suggestion extended Barcroft's (1914) view that functional vasodilatation was caused by the secretory metabolic activity of the gland cells, by implicating kallikrein as the hitherto unidentified metabolic mediator of vasodilatation. The view that kallikrein released into the interstitial fluid during secretion was the physiological mediator of functional vasodilatation not only in the salivary glands, but also in sweat, pancreatic and other glands, has since been maintained by some workers (Hilton & Lewis, 1955*a*, *b*; 1956; 1958; Fox & Hilton, 1958; Hilton & Jones, 1963) and opposed by others (Bhoola, Morley & Schachter, 1962; Bhoola, Morley, Schachter & Smaje, 1965; Morley, Schachter & Smaje, 1966; Webster, 1966; Skinner & Webster, 1968; Schachter & Beilenson, 1967).

The present experiments were undertaken to establish new facts which might be relevant to the physiological role of kallikrein in the submaxillary gland. They have provided new information in regard to the following: (*a*) the influence of the sympathetic and parasympathetic nerves in regulating the secretion of kallikrein, (*b*) the vasodilatation in a submaxillary gland which has been depleted of kallikrein, and (*c*) the quantitative relationship between rate of salivary secretion, degree of vasodilatation, and concentration or output of kallikrein in saliva.

METHODS

Cats were anaesthetized with chloralose (80 mg/kg I.V.) for acute experiments. Sodium pentobarbitone (35 mg/kg I.P.) was used as anaesthetic in aseptic operations in which the submaxillary duct was tied. Dogs were anaesthetized with sodium pentobarbitone (30 mg/kg I.V.) and rabbits with urethane (1·5 g/kg I.V.). Arterial blood pressure was recorded with a Grass pen recorder from a femoral artery connected to a Statham transducer. Intravenous injections were made through a cannula in a femoral vein. Heparin (10 mg/kg) was injected intravenously when required.

Collection of saliva. In cats and dogs the submaxillary duct was exposed and cannulated with a fine glass cannula about 5 mm rostral to where the chorda-lingual nerve crossed the duct. In rabbits, dissection of the duct is more difficult and saliva was collected from a fine polyethylene cannula inserted into the papilla of the duct in the mouth.

Nerve stimulation. The cervical sympathetic and chorda-lingual nerves were dissected, cut, and their distal ends mounted on bipolar platinum electrodes. Nerves and electrodes

were immersed in a pool of warm liquid paraffin. The nerves were stimulated supramaximally (7–10 V) with square wave pulses of 0·4 msec duration and at frequencies of 20/sec, unless otherwise stated. Stimulation of the cervical sympathetic nerve was usually intermittent (10 sec stimulation, 20 sec interval) to prevent the progressive reduction in secretion which is probably related to the protracted vasoconstriction that occurs with maintained sympathetic stimulation. Stimulation was automatically interrupted for the interval desired by a Londex clock inserted in the circuit.

Substrate. The potencies of the kallikrein solutions (saliva or extract of the gland) were determined by assaying the amount of kinin which they released from the substrate. A variety of substrates were tested to ensure that kinin release was not confined to a single substrate. Thus, a similar qualitative release of kinin was observed with unheated and heated plasmas of the cat, dog and man, as with 'heated globulin substrate' (see Werle *et al.* 1937; Holdstock, Mathias & Schachter, 1957) but since the most satisfactory and reproducible assays were obtained with 'heated dog globulin' as substrate, the latter was used in all the quantitative work. It was prepared as described for ox globulin by Holdstock *et al.* (1957). This substrate was prepared in bulk, and kept frozen in small vials until required, and then thawed; such preparations kept their activity for months in the frozen state, and one batch did not differ significantly from another. The heated dog globulin substrate, by itself, did not affect the guinea-pig ileum, nor did activity develop on its contact with glass or with dilution.

Kallikrein standard. A standard kallikrein powder was prepared by removing twenty unstimulated submaxillary glands from ten anaesthetized cats. These were frozen immediately in cold acetone-solid CO_2 and freeze-dried. The dried glands were extracted several times with distilled water and centrifuged; the supernatant solution was dialysed overnight at 4° C against large volumes of distilled water. The dialysed extract was then freeze-dried and kept as a standard preparation of kallikrein. One microgram of this preparation arbitrarily contained one kallikrein unit (KU). Under our conditions, 1 KU released the equivalent of 0·01 μg synthetic bradykinin when assayed after 1 min incubation with excess substrate at room temperature. The unknown solutions were assayed against our standard kallikrein preparation.

Conditions of assay. The assay preparation, a loop of isolated guinea-pig's ileum, was suspended in an 8 ml. bath in Mg^{2+}-free Tyrode solution (g/l: NaCl, 8·0; KCl, 0·2; $NaHCO_3$, 1·0; NaH_2PO_4, 0·05; $CaCl_2$, 0·2; glucose, 1·0) at 30–32° C and aerated with 95% O_2–CO_2 mixture. Atropine and mepyramine (2×10^{-8} g/ml.) were present in the Tyrode solution. Contractions of the ileum were recorded on a smoked drum with a frontal writing lever. A 4 min dose cycle was used.

Appropriate volumes of the kallikrein solutions to be assayed were added to 0·4 ml. substrate to make a final volume of 1·0 ml. which was incubated at room temperature for 1 min before assay. When the test solution contained high concentrations of kallikrein (e.g. saliva collected during sympathetic stimulation) it was necessary to dilute it as much as 100-fold before its addition to the substrate in order to ensure an excess of the latter.

For each assay, four doses of the test solution were sandwiched between four doses of the standard kallikrein preparation. The kallikrein concentration of the unknown solution was obtained by plotting the log. dose–response curves for the unknown and standard solutions of kallikrein, and calculating the kallikrein content of the unknown solution in terms of units of the standard. With the concentrations used for assay, there was a consistent linear correlation between the log. of the kallikrein concentration of the incubation mixture and the amount of kinin formed.

When no kinin could be detected in an incubation mixture of substrate and sample of saliva or gland extract, this inactive mixture was further incubated with small amounts of the kallikrein standard. It was then concluded that the smallest amount of kallikrein standard which released detectable amounts of kinin from this mixture was more than that present in the ineffective sample.

Under the conditions of assay, and using dog globulin as substrate, there was no kinin or other activity in the substrate, saliva, kallikrein standard, or gland extracts, appropriately diluted to 1·0 ml. with Tyrode solution, unless *both* substrate and one or other of these were incubated together.

Blood flow. Blood flow through the submaxillary gland of the cat was measured using the open-circuit venous outflow method as described by Bhoola *et al.* (1965), but with minor adaptations required to record with a Grass pen recorder rather than with a smoked drum and kymograph.

Duct ligation. The right submaxillary and sublingual ducts were exposed under aseptic conditions and a double nylon ligature tied around both of them, 5–10 mm rostral to where the chorda-lingual nerve crossed them. Acute experiments were performed 3–5 days after ligation of the ducts.

Drugs. Atropine was used as sulphate and mepyramine as maleate and weights are expressed as these salts.

RESULTS

Kallikrein content of saliva and gland in relation to parasympathetic and sympathetic nerve stimulation

In the cat

Saliva obtained by stimulation of parasympathetic nerve (chorda). In fourteen cats the chorda-lingual nerve was stimulated for 1–5 min to collect the first sample. Successive samples of saliva were then collected. (The first three or four drops of saliva were discarded to eliminate contamination of the chorda sample by secretion intially present in the ducts.) In these cats, the concentration of kallikrein in the first sample of saliva varied widely, from 600 to 33,000 KU/ml. (mean, 8540 KU/ml.). The corresponding values for the production rate of kallikrein ranged from 180 to 19,800 KU/min (mean, 4830 KU/min). In all these experiments, successive samples of saliva had progressively lower concentrations and outputs of kallikrein (Fig. 1), both of which fell by 85–95 % after 2–5 min of stimulation, reaching a 'plateau' after 5–15 min of stimulation. In nine experiments the mean output of kallikrein during the plateau was 56 KU/min. After a resting period of 20–40 min there was some increase, but in only one of six experiments were the initial output and concentration of kallikrein restored.

Saliva obtained by stimulation of sympathetic nerve. The concentration and rate of production of kallikrein in saliva secreted during stimulation of the sympathetic nerve varied widely in different cats. In nine experiments, the kallikrein concentrations in the *first* samples ranged from 13,500 to 475,000 KU/ml. (mean, 149,000 KU/ml.). The corresponding production rate ranged from 4100 to 112,000 KU/min (mean, 54,400 KU/min). The concentration and output of kallikrein in successive samples also fell progressively. The periods of sympathetic nerve stimulation in these experiments ranged from 0·5 to 3·3 min to collect the first sample.

Sympathetic nerve stimulation was always far more effective than parasympathetic in releasing kallikrein (Fig. 2). This was true whether the sympathetic nerve was stimulated before, together with, or after stimulation of the chorda-lingual nerve. Even when the kallikrein content of saliva had fallen markedly after prolonged stimulation of the chorda-lingual nerve, stimulation of the sympathetic nerve could increase the kallikrein concentration as much as 500-fold, and its output as much as 390-fold.

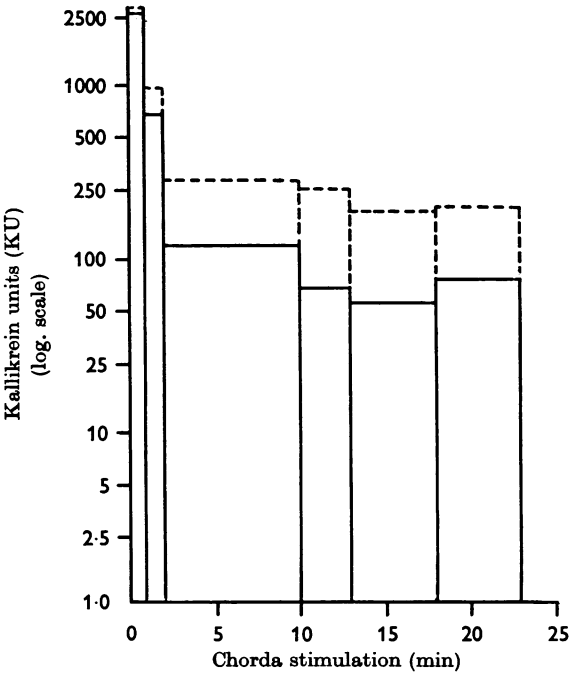

Fig. 1. Progressive diminution in the concentration and output of kallikrein in successive samples of saliva obtained from a cat's submaxillary gland during 23 min of continuous chorda-lingual nerve stimulation. There is a rapid decrease in the first few minutes of stimulation which is followed by a 'plateau'. ⬚ Kallikrein concn. KU/ml.; □ Kallikrein output, KU/min.

In two cats, sympathetic nerve stimulation failed to produce any secretion of fluid. Nevertheless, when sympathetic was superimposed on chorda stimulation in these cats, it caused an increase in both the concentration and output of kallikrein (Fig. 3). Thus, the output of fluid and the secretion of kallikrein appear to be regulated independently of one another.

Kallikrein content of the gland after sympathetic and parasympathetic nerve stimulation. The submaxillary gland could almost be depleted of kallikrein by relatively brief sympathetic nerve stimulation. For example,

if the sympathetic nerve was stimulated for less than 3 min, the kallikrein concentration of the gland fell by 87 %. On the other hand, when the chorda-lingual nerve was stimulated for 25 min there was no significant difference in the kallikrein concentrations of the stimulated and unstimulated glands (Table 1).

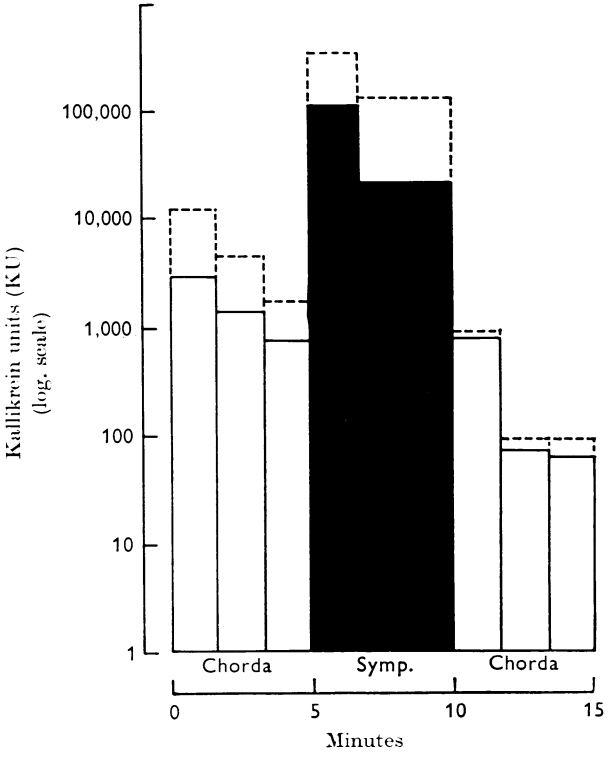

Fig. 2. Comparison of the effects of chorda-lingual and sympathetic nerve stimulation on concentration and output of kallikrein in saliva from a cat's submaxillary gland. (Five drops of saliva were rejected between samples 3 and 4, and between 5 and 6.) Chorda-lingual and sympathetic nerve stimulation were stopped for 20 sec after each 10 sec period of stimulation. Abscissae indicate durations of actual nerve stimulation, excluding intervals of interruption. ⬚ Kallikrein concn. KU/ml.; ▭ or ■ kallikrein output, KU/min.

In the dog

The effect of sympathetic nerve stimulation in the dog differed from that in the cat in that it regularly produced either little or no secretion from the submaxillary gland. Also, the concentration and production rates of kallikrein in the saliva produced by chorda stimulation were lower in the dog than in the cat.

Nevertheless, as in the cat, when sympathetic was superimposed on

chorda stimulation, there was a marked increase in the secretion of kallikrein (Fig. 4).

In five experiments, in which the periods of nerve stimulation ranged from 1 to 5 min, the concentrations of kallikrein in the first chorda sample

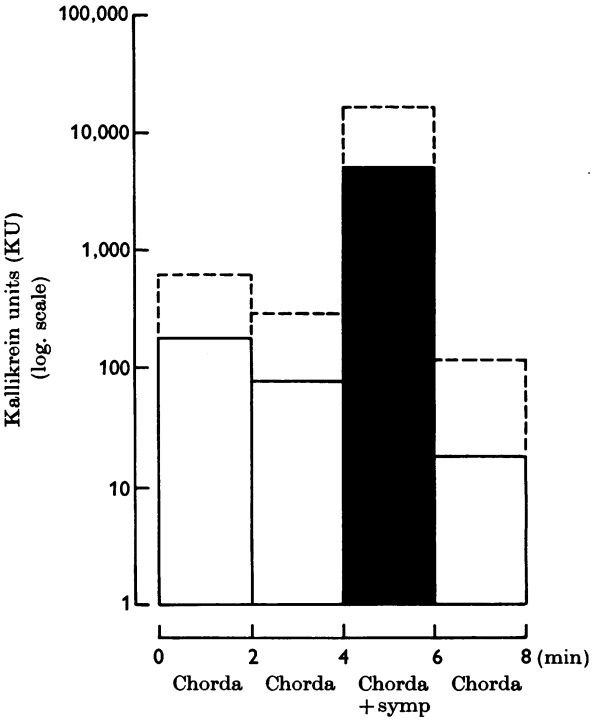

Fig. 3. Effect of superimposing sympathetic on chorda-lingual nerve stimulation. Chorda-lingual stimulation, continuous; sympathetic stimulation, interrupted: 10 sec on, 20 sec off. In this cat, sympathetic nerve stimulation, by itself, failed to cause salivation. Nevertheless, it increased the concentration and output of kallikrein in chorda saliva approximately 100-fold when superimposed on chorda stimulation. ⬚ Kallikrein concn. KU/ml.; □ or ■ kallikrein output, KU/min.

TABLE 1. Effect of nerve stimulation (chorda and sympathetic)* on kallikrein content of the submaxillary gland in four cats

Expt.	Duration of nerve stimulation (min)		Kallikrein content of gland (KU/mg dry gland)		Decrease in kallikrein content (%)
	Chorda	Symp.	Control†	Stimulated	
1	25	0	330	330	0
2	38·5	2·7	670	87	87
3	27	6·8	200	26	87
4	40	47·2	750	4·5	99·4

* Chorda stimulation: Expt. 1, 5 min on, 5 min off; Expts. 2–4, continuous. Sympathetic stimulation: Expts. 2–4, 10 sec on, 20 sec off.

† Control gland is the contralateral unstimulated gland.

of saliva ranged from 53 to 1320 KU/ml. (mean, 715 KU/ml.), and the production rate in the same samples ranged from 80 to 5025 KU/min (mean, 1920 KU/ml.). In contrast, the concentrations in response to sympathetic plus chorda stimulation varied from 2550 to 34,500KU/ml (mean, 12,000 KU/ml.) and the production rates from 580 to 42,600KU/min (mean, 16,000 KU/min). In the latter experiments, the time of combined sympathetic and chorda nerve stimulated ranged from 0·33 to 1·67 min.

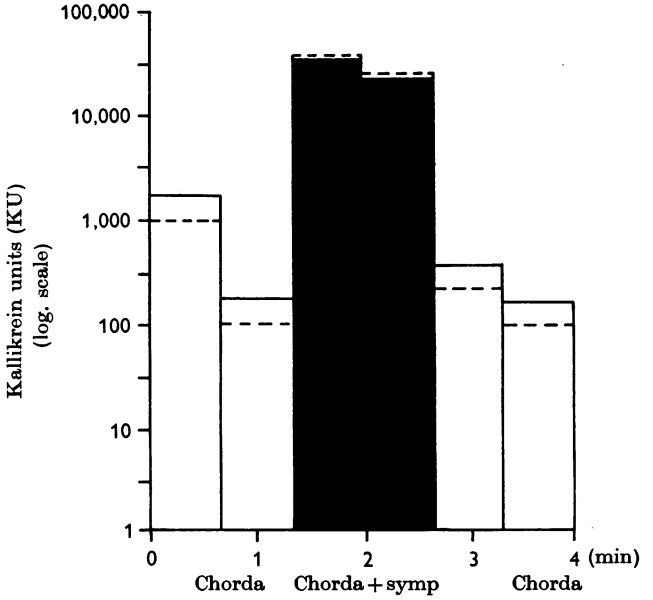

Fig. 4. Comparison of the effects of stimulation of the chorda-tympani nerve and simultaneous stimulation of the chorda-tympani and sympathetic nerves on concentration and output of saliva from a dog's submaxillary gland. Chorda-tympani and sympathetic nerve stimulation, 10 sec on, 20 sec off. ⬚ Kallikrein concn. KU/ml.; □ or ■ kallikrein output, KU/min.

In the rabbit

Sympathetic nerve stimulation failed to cause secretion from the submaxillary gland. In contrast to the dog and cat, in the rabbit there was no increase in secretion of kallikrein when sympathetic was superimposed on parasympathetic nerve stimulation.

Relationship between frequency of chorda stimulation and salivation, vasodilatation, and kallikrein production in the cat's submaxillary gland

Samples of saliva from the submaxillary gland were collected while the chorda-lingual nerve was stimulated at varying frequencies in five cats,

and the kallikrein production rate (KU/min) was determined by assaying the respective samples of saliva. The rate of flow of saliva (ml./min) for the different samples was also calculated. The nerve was stimulated for periods of 1–5 min (sufficiently long to obtain suitable volumes of saliva for kallikrein determinations and flow-rate calculations). The sequence of the different frequencies of stimulation was randomized in each experiment. Similar experiments were performed on four additional cats in which saliva flow-rate alone was measured.

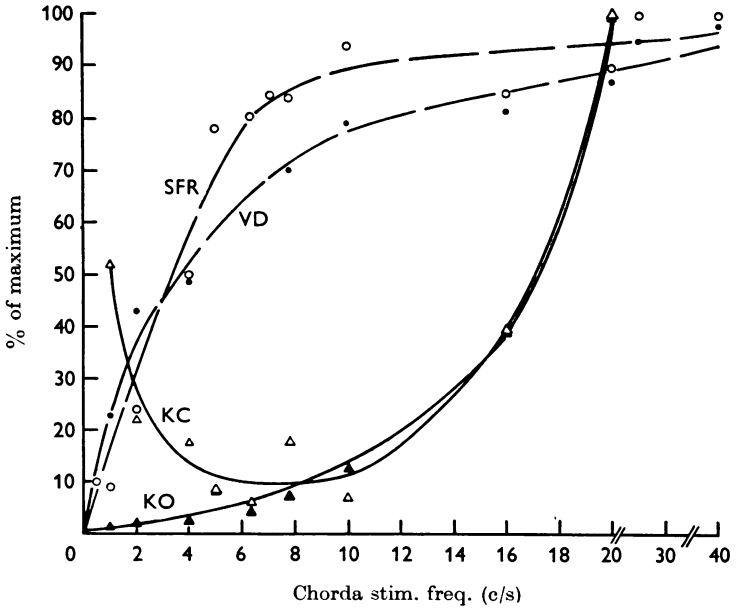

Fig. 5. Cat's submaxillary gland. Relationship between frequency of chorda nerve stimulation and (○) saliva flow rate, SFR, (●) vasodilatation, VD, (▲) kallikrein output in saliva, KO, and (△) kallikrein concentration in saliva, KC. The sequence of the different frequencies of stimulation was randomized in each experiment. SFR (ml./min), each measurement is the mean of nine experiments. VD (ml./min), each measurement is the mean of five experiments. KO and KC (KU/min and KU/ml. respectively), each measurement is the mean of five experiments. See text for details.

Experiments were also carried out on five cats in which the vasodilatation resulting from chorda stimulation for 20–30 sec at different frequencies was measured.

The results of these experiments are illustrated in Fig. 5, which shows that there is a close parallelism between the rate of secretion of saliva and vasodilatation over a range of frequencies of chorda stimulation, but that the output (and concentration) of kallikrein in saliva is distinctly different for the same frequencies of nerve stimulation.

Vasodilatation in the cat's submaxillary gland after depletion of kallikrein

After combination of two procedures, viz. ligation of Wharton's duct for 3–5 days (Mattioli & Mattioli, 1947*a*, *b*; Werle, Vogel & Lentrodt, 1960) and subsequent sympathetic nerve stimulation (approx. 5 min), neither the submaxillary gland nor saliva collected during stimulation of the chorda-lingual nerve contained detectable amounts of kallikrein.

In twelve cats, ligation of Wharton's duct for 3–5 days did not cause a significant change in the dry weight of the gland. The mean dry weight of 'ligated' glands was 252 mg (range 190–332 mg) compared with a mean weight of the contralateral control gland of 262 mg (range 210–336 mg). The kallikrein content of the ligated gland, however, was reduced markedly, and in some instances kallikrein was undetectable; also, there was a marked reduction in the amount of kallikrein in saliva obtained by chorda and sympathetic nerve stimulation as shown in Fig. 6, which compares the secretion of kallikrein in the 'ligated' and contralateral normal gland in the same cat. The rates of flow of saliva in response to chorda stimulation were reduced to 30–90 % (mean 62 %) of the control glands after duct ligations in four cats; in one experiment, however, the flow rate after ligation was 143 % of the control gland.

The effect of chorda and sympathetic nerve stimulation on blood flow was studied in eight experiments after duct ligation. In all these experiments chorda stimulation produced a marked vasodilatation, which was as great as, or possibly even greater than, that seen in normal glands. In two experiments on ligated glands, in which some kallikrein was subsequently still detectable in the saliva on chorda stimulation, stimulation of the sympathetic nerve for 5 min 'depleted' the gland, and kallikrein was undetectable in subsequent saliva collected during chorda-lingual nerve stimulation (< 1 and < 10 KU/ml.). Figure 7 shows the pronounced vasodilatation in the submaxillary gland in response to chorda stimulation in one of the cats. In this experiment the response to nerve stimulation in fact increased when the kallikrein concentration fell from 40 KU/ml. to < 1 KU/ml. after sympathetic nerve stimulation. As in normal glands, the chorda vasodilatation in kallikrein-depleted glands is atropine-resistant (Fig. 7).

The effects of kallikrein depletion in 'ligated' glands on the variable after-dilatation which follows the pronounced sympathetic vasoconstriction (Bhoola *et al.* 1965) was not so clear-cut. In general, the sympathetic vasoconstriction was normal, but the after-dilatation was generally reduced and occasionally absent. In one cat, however, a marked after-dilatation was observed although the kallikrein concentration in the

ligated gland was 0·1 % of that in the contralateral control gland and the relative concentration in the saliva even less.

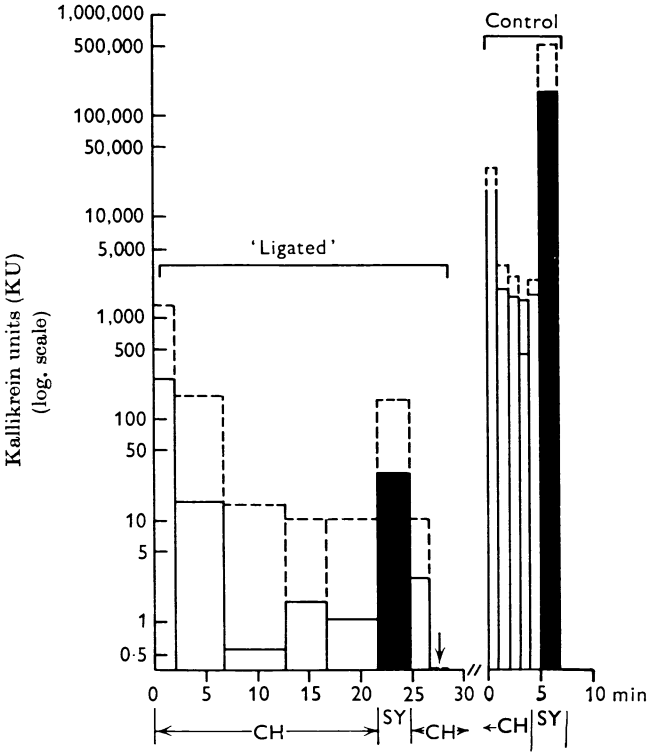

Fig. 6. Cat's submaxillary gland. Comparison of concentrations and outputs of kallikrein in response to chorda and sympathetic stimulation of a gland with Wharton's duct ligated for 3 days and of the contralateral control gland. Chorda stimulation continuous; sympathetic stimulation intermittent, 10 sec on, 20 sec off. Kallikrein content of 'ligated' gland after experiment, < 0·43 KU/mg (undetectable); control gland, 170 KU/mg. ⬚ Kallikrein concn. KU/ml.; □ or ■ kallikrein output, KU/min. ↓ = kallikrein undetectable.

DISCUSSION

The present experiments provide further evidence, consistent with the view of Bhoola *et al.* (1965), that the kallikrein–kinin system plays little or no role in the chorda vasodilatation in the submaxillary gland. They found that stimulation of the chorda-lingual nerve could still produce a maximal vasodilatation even when the gland had been perfused for as long as 3 hr with horse serum, from which cat saliva fails to release kinin. The present experiments further demonstrate that chorda stimulation still produces a maximal vasodilatation in a gland with natural circulation when both the gland and chorda saliva contain no detectable kallikrein. These experiments,

in our view, clearly demonstrate that a maximal chorda vasodilatation can occur in the submaxillary gland under conditions where release of kinin is unlikely.

The finding that there is a distinct dissociation between the rate of flow of saliva and vasodilatation, on the one hand, and kallikrein output (or concentration) in the saliva, on the other, when the chorda-lingual nerve is stimulated at different frequencies, provides new evidence suggesting that

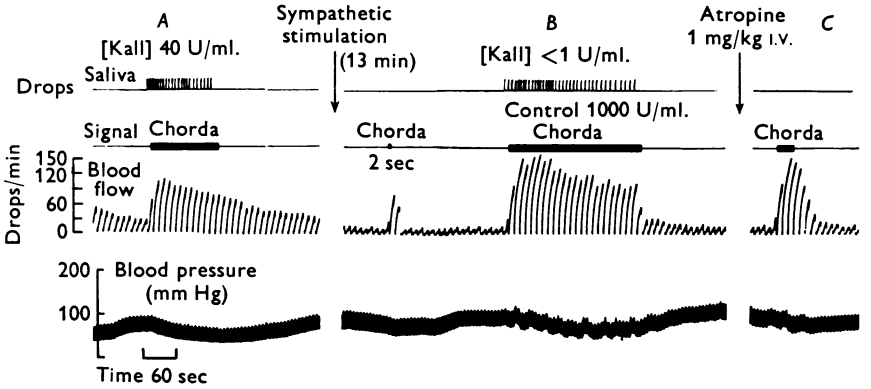

Fig. 7. Blood flow and salivary secretion in a cat's submaxillary gland after depletion of kallikrein.

A. Record of salivary secretion and blood flow 3 days after duct ligation. Chorda saliva contains only 40 KU/ml. but the accompanying vasodilatation is normal.

B. Between *A* and *B* the sympathetic nerve was stimulated for 13 min to further deplete the gland of kallikrein. A marked vasodilator response is obtained in response to only 2 sec of chorda stimulation. A more prolonged chorda stimulation produces a maximal vasodilatation although kallikrein is undetectable (< 1 KU/ml.) in the accompanying secretion. Saliva obtained from the contralateral gland at the same time contains 1000 KU/ml.

C. The vasodilatation in response to chorda stimulation in the gland depleted of kallikrein is atropine-resistant (as in the normal gland) but salivary secretion is blocked.

the secretion of kallikrein is unrelated to the vasodilatation. Figure 5 shows that whereas the secretory flow rate and vasodilatation are reaching their maxima when the nerve is stimulated at a frequency of 8 pulses/sec, the kallikrein output and concentration in the saliva are both near their minimal values at the same frequency. The relatively high *concentration* of kallikrein in saliva secreted at very low frequencies of nerve stimulation suggests that there is a 'basal' secretion of kallikrein.

Our observation that kallikrein is mobilized predominantly by sympathetic nerve stimulation, which causes *vasoconstriction*, lends no support to the view that kallikrein mediates *vasodilatation*. In fact, the discrepancy between nerve stimulation and the appropriate vasoactive compound

secreted in the saliva has been extended recently by Moriwaki, Beilenson & Schachter (1968), who found that chorda, but not sympathetic, stimulation causes the secretion of sialotonin, a potent vasoconstrictor compound. The injection of sialotonin into the submaxillary gland, close-arterially, produces a brief but marked constriction (C. Moriwaki, S. Beilenson & M. Schachter, unpublished).

The recent observations of Skinner & Webster (1968) may now be added to those of Bhoola *et al.* (1965) and the present ones indicating that kallikrein does not play a significant role in chorda vasodilatation. Skinner & Webster (1968) showed that the vasodilator effects of chorda stimulation were unaffected in cats pre-treated with carboxypeptidase B (a potent inactivator of kallidin or bradykinin), whereas the equivalent vasodilator effects of kallidin injected close-arterially were abolished.

Recent histochemical and electronmicroscopic experiments of Garrett (1966) support the view of Schachter and his colleagues that cholinergic vasodilator nerves are present in the chorda tympani nerve despite the fact that the vasodilatation is atropine-resistant. Garrett interprets his anatomical observations after selective, or after combined, sympathetic and parasympathetic nerve section and degeneration, to indicate that the blood vessels of the parotid and submaxillary glands in the cat receive a dual innervation. He states, 'One cannot imply function from the present morphological studies, but it is reasonable to presume that such parasympathetic "fibres" to blood vessels are vasodilatory. Thus we have come the full circle back to Claude Bernard's views.'

A new explanation of the atropine-resistant vasodilator effects of chorda stimulation in the cat's submaxillary gland has recently been offered by Skinner & Webster (1968). These authors suggest that the major part of chorda vasodilatation is due to the presence of adrenergic neurones in the chorda tympani nerve which cause vasodilatation via β-adrenergic receptors which are present in the blood vessels. According to Skinner & Webster the vasodilatation produced by chorda-lingual nerve stimulation is suppressed by propranolol (a β-receptor blocking drug) in parallel with the block by propranolol of the vasodilatation produced by isoprenaline injected into the gland close-arterially. This view is questioned by Schachter & Beilenson (1968), who in part confirm the observations of Skinner & Webster (1968) with propranolol but maintain that, although large doses of propranolol can reduce chorda vasodilatation, they also reduce the action of acetylcholine in parallel. Furthermore, Schachter & Beilenson (1968) have shown that, in a cat pre-treated with reserpine, the secretory and vasomotor actions of sympathetic stimulation on the submaxillary gland are absent, whereas chorda vasodilatation is normal. Similar results were obtained by Bhoola *et al.* (1965) after guanethidine.

The facts that the protein concentration of sympathetic submaxillary saliva is 4–13 times greater that that of chorda saliva and that the proteins in each differ in their solubility in acetone and trichloroacetic acid, have long been known (Langstroth, McRae & Stavraky, 1938; Komarov & Stavraky, 1940). We have now shown that there is a marked difference in the concentration of a *specific* protein, kallikrein, in the respective salivas. Compared with a 4- to 13-fold increase in protein in sympathetic saliva reported by Stavraky and his colleagues, in our experiments the concentration of kallikrein increased up to 500 times and the output up to 390 times. This secretion of a specific protein by selective nerve stimulation is further demonstrated by the contrasting finding that sialotonin, a pressor protein, is secreted in submaxillary saliva with chorda stimulation but not with sympathetic nerve stimulation (Moriwaki *et al.* 1968).

It is generally considered that at least some cells of the submaxillary gland receive a dual innervation (Babkin, 1950; Burgen & Emmelin, 1961). If so, the 'selective' actions of sympathetic and parasympathetic stimulation on secretion of specific proteins as discussed above might have to be explained by actions of the respective transmitters on the extrusion of different intracellular substances or granules. The possibility also exists, however, that different types of cells are innervated by chorda or sympathetic fibres respectively, and that different cells are activated by the respective transmitter substances. Our experiments do not distinguish between these possibilities or, indeed, whether both exist. We have shown, however (S. Beilenson & M. Schachter, unpublished) that the injection of adrenaline (like sympathetic stimulation) releases high concentrations of kallikrein in the saliva but no sialotonin, whereas injection of pilocarpin (like chorda stimulation) produces saliva which contains sialotonin but relatively low concentrations of kallikrein.

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