# Tissue Kallikrein and Tonin Levels in Submandibular Glands of STZ-Induced Diabetic Rats and the Effects of Insulin

KA-MING CHAN, JULIE CHAO, GORDON B. PROCTOR, JOHN R. GARRETT, DEEPAK K. SHORI, AND LEIGH C. ANDERSON

**The levels of tissue kallikrein, tonin, and other kallikrein-like proteinases were determined in extracts of rat submandibular glands 3 mo after the induction of diabetes with STZ (65 mg/kg i.v.). Total kallikrein-like proteinase activity was assayed catalytically with the fluorogenic substrate DVLR-AFC. Tissue kallikrein was assayed by using the same substrate in the presence of SBTI. Activity of other kallikrein-like proteinases was defined as the difference between the total kallikrein-like activity and that of tissue kallikrein. Tonin was assayed by using the substrate ZVKKR-AFC in the presence of aprotinin. Results were compared with age-matched controls and with diabetic rats that had received daily insulin injections for the last week of the test period. The results showed that all activities were significantly reduced in diabetic glands compared with controls. Insulin treatment restored concentrations of tissue kallikrein activity, whereas the activities of tonin and other kallikrein-like proteinases were unchanged. RIA supported these findings. The results indicate that in rat submandibular glands, insulin affects the synthesis of kallikrein-like proteinases in different ways and, allowing for the slowness of the processes involved, insulin may exert a direct influence on the regulation of tissue kallikrein synthesis but only have indirect effects on the synthesis of tonin and the closely related kallikrein-like proteinases. *Diabetes* 42:113–17, 1993**

From the Department of Oral Pathology, King's College School of Medicine and Dentistry, London, United Kingdom; the Department of Biochemistry, Medical University of South Carolina, Charleston; and the Department of Oral Biology, School of Dentistry, University of Washington, Seattle.

Address correspondence and reprint requests to Prof. J.R. Garrett, Department of Oral Pathology, King's College School of Medicine and Dentistry, The Rayne Institute, 123 Coldharbour Lane, London, UK SE5 9NU.

Received for publication 16 March 1992 and accepted in revised form 27 August 1992.

STZ, streptozocin; DVLR-AFC, D-Val-Leu-Arg-7-amino-4-trifluoromethylcoumarin; SBTI, soybean trypsin inhibitor; ZVKKR-AFC, Z-val-lys-lys-arg-7-amino-4-trifluoromethylcoumarin; RIA, radioimmunoassay; ANG II, angiotensin II; ALX, alloxan; PB, phosphate buffer; BSA, bovine serum albumin; ANOVA, analysis of variance; $T_4$, thyroxine; SDS, sodium dodecyl sulfate; NS, no significance.

Tissue kallikrein (EC 3.4.21.35) belongs to a group of closely related serine proteinases. It occurs in many mammalian species (1) and is found in different tissues and fluids such as the pancreas and pancreatic juice, salivary glands and saliva, and the kidney and urine (1,2). Its best known biochemical activity is the selective cleavage of the plasma protein kininogen to release vasodilator kinins (3). Some researchers have postulated that this property of tissue kallikrein plays a role in the regulation of blood pressure and local blood flow (4), but this is open to debate. Several different kallikrein-like enzymes in rat submandibular glands were first identified and then semipurified by Hopsu-Havu et al. (5,6) in 1967. Subsequently, tissue kallikrein-like activity has been localized enzyme-histochemically and immunohistochemically in the granules of the granular tubules and the periluminal zone of the striated ductal cells (7–9). The physiological functions of tissue kallikrein in submandibular glands and saliva are still unclear, although its release into saliva may influence membrane transport of ions (10). A number of other related serine proteinases also have been localized in the granular tubules of the rat (11–14); among these enzymes, tonin, which can liberate ANG II from angiotensinogen, occured in relatively high concentrations (15).

Immunoreactive concentrations, biological activities, and mRNA levels of different kallikrein-like enzymes in rat submandibular glands are influenced by different circulating hormones, including testosterone, thyroxine, and growth hormone (16–19). Although morphological (20,21) and biochemical (22,23) studies have demonstrated that structural and enzymic changes occur in STZ and ALX diabetic submandibular glands, few studies have demonstrated the effects of diabetes on their content or composition of secretory proteinases. Immunoreactive kallikrein concentrations in submandibular glands were reduced in short-term (2–4 wk) STZ- and ALX-

induced diabetic rats, but on subsequent administration of insulin for 3 days, no recovery in the concentration of kallikrein was detected (24,25).

No documented studies have shown the effects of diabetes and insulin on rat submandibular proteinases in terms of their enzyme catalytic activities or of possible differences between the individual constituent enzymes. This study assessed the effects of experimental diabetes for 3 mo, with or without insulin for the last week, on both of these aspects.

## RESEARCH DESIGN AND METHODS

**Treatment of animals and submandibular glands.** Male Wistar rats (King's strain) originally weighing 170–250 g were fasted overnight. A single injection of STZ (65mg/kg i.v., Sigma, Poole, England), dissolved in ice-cold sterile saline, induced diabetes. Animals demonstrating nonfasting serum glucose levels >15 mM were considered diabetic. In addition to the diabetic animals, a group of age-matched rats from the same batch of animals were kept as controls. All animals were allowed free access to food (hard chow) and water and were kept under the same conditions for 3 mo. One group of diabetic animals received an intermediate-acting insulin (6 U/day im, Human Insulatard, Novo Nordisk A/S, Denmark) for the last week of this period.

Left submandibular glands from all animals were removed under terminal anesthesia (pentobaritone 35 mg/kg i.p., followed by α-chloralose at 75 mg/kg i.v.) and weighed. The tissues were homogenized over 10 min in 0.1 M PB (pH 6.0) containing 2 mM EDTA and 0.02% Triton X100 using an IKA Ultra-Turrax disperser (Janke and Kankel, Staufen, Germany) at maximum speed (30-s burst every 2 min). An aliquot was taken out for DNA assay and the rest centrifuged at 15,000 *g* for 10 min at 4°C. The supernatant was collected and stored at −20°C until analysis.

**Protein and DNA assay.** DNA concentration of homogenates was assayed using Hoechst 33258 according to Cesarone et al. (26). Calf thymus DNA was used as standard. Total protein concentration of the glandular extracts was determined using folin-phenol reagent as described by Petersen (27). A mixture of human serum albumin and gamma globulin was used as standard (Sigma).

**Assay of the activities of tissue kallikrein, other kallikrein-like proteinases, and tonin.** Total kallikrein-like activity in the glandular extracts was assayed using DVLR-AFC (Enzyme System Products, Livermore, California) as substrate. Tissue kallikrein activity was determined by means of the same substrate in the presence of 50 μM SBTI (Sigma) (28). The contribution of the other kallikrein-like enzyme activity (i.e., not including tissue kallikrein) was determined by the difference between these two results. Glandular extracts suitably diluted in 20 mM Tris buffer, pH 8.0, were preincubated with or without inhibitors for 5 min at room temperature. The assay was initiated by the addition of the substrate to a final concentration of 10 μM; fluorescence was measured at room temperature after 5 min using an Ames fluorostat (Miles, Slough, England) fitted with a 405-nm excitation filter and 500-nm emission filter and calibrated with free AFC (0.1–5.0 μM).

Tonin activity was determined using ZVKKR-AFC as substrate in the presence of 1 μM aprotinin (Sigma) as described by Shori et al. (28). Tonin activity of glandular extracts against 10 μM substrate were measured in 50 mM Tris buffer, pH 8.0, containing 100 μg/ml BSA. Extracts with or without inhibitors were preincubated for 5 min at 30°C before the addition of substrate. The fluorescence was read on an Ames fluorostat, following a 10-min incubation at 30°C of the enzyme with the substrate.

**RIA of tissue kallikrein and tonin.** RIA of tissue kallikrein was performed as described by Shimamoto et al. (29), using a polyclonal antibody against rat urinary kallikrein. Tonin immunoreactivity was determined as described by Shih et al. (17).

**Statistical analysis.** Data were expressed as means ± SE. Differences between means were tested for statistical significance by the Student's *t* test and by one-way ANOVA followed by Fisher PLSD. Differences were considered significant at a level of $P < 0.05$. Unless otherwise stated, *P* values referred to Student's *t* test.

## RESULTS

Polyuria, polydipsia, hyperphagia, high serum glucose level (19.7 ± 0.38 mM, $n = 23$), reduced body and submandibular gland weight in STZ-injected rats all reflected that the animals were severely diabetic (Table 1). After insulin treatment for 1 wk, both body weight and submandibular gland weight were significantly higher than those of untreated diabetic rats, although still much lower than those of normal control animals. Mean serum glucose level in insulin-treated diabetic rats (5.98 ± 1.04 mM, $n = 10$) was not significantly different from that of controls (5.77 ± 0.36 mM, $n = 9$).

Total protein concentration was reduced in the diabetic glands, as compared with age-matched controls ($P < 0.001$). The diabetic and insulin-treated diabetic groups showed no differences. The amount of DNA per g wet weight of gland tissue was not different in normal control, diabetic, and insulin-treated diabetic rats (Table 1). We, therefore, can reasonably assume that proteinase activities expressed per gram gland wet weight were equivalent to activities expressed on a per unit DNA (per cell) basis.

Tissue kallikrein, other kallikrein-like proteinases, and tonin activities each were significantly reduced in diabetic glands. Subsequent insulin treatment restored the tissue kallikrein activity per unit mass of tissue to control levels, but the activities of other kallikrein-like proteinases and of tonin remained unchanged after 1 wk of insulin treatment (Table 2).

Immunoreactive tissue kallikrein concentration was significantly reduced in diabetic glands and showed a significant increase after insulin treatment, but still was statistically different from control levels. Tonin immunoreactivity also showed a significant reduction in diabetic

TABLE 1
Body weight, submandibular gland weight, submandibular gland DNA concentration and total protein concentration of control, diabetic, and insulin-treated diabetic rats

| Animal group | *n* | Body weight (g) | Gland weight (g) | DNA concentration (mg/g) | Protein concentration (mg/g) |
|---|---|---|---|---|---|
| Control | 9 | 525.1 ± 11.9 | 0.37 ± 0.02 | 11.14 ± 0.59 | 83.92 ± 1.57 |
| Diabetic | 12 | 228.1 ± 7.5 | 0.19 ± 0.01 | 11.86 ± 0.85 | 67.46 ± 2.99 |
| | | $P < 0.001$* | $P < 0.001$* | NS* | $P < 0.001$* |
| Insulin-treated diabetic | 11 | 279.0 ± 10.5 | 0.22 ± 0.01 | 11.11 ± 0.68 | 69.36 ± 2.48 |
| | | $P < 0.001$† | $P < 0.02$† | NS† | NS† |
| | | $P < 0.001$‡ | $P < 0.001$‡ | NS‡ | $P < 0.001$‡ |

Values are mean ± SE.
*Diabetic versus control.
†Insulin-treated diabetic versus diabetic.
‡Insulin-treated diabetic versus control.

glands, but no recovery was observed after insulin treatment (Table 3).

## DISCUSSION

This study shows that the catalytic activities of tissue kallikrein, tonin, and other kallikrein-like proteinases are all reduced in diabetic submandibular glands (Table 2). However, the extent of these reductions varied (tissue kallikrein 25%, tonin 56%), suggesting that tissue kallikrein formation is not as severely affected as tonin by the metabolic disturbances associated with diabetes. Upon insulin treatment for 1 wk, only the tissue kallikrein concentration recovered to control levels, whereas tonin and the other kallikrein-like proteinases remained at the same level as in the untreated diabetic glands. The relationship between insulin and tissue kallikrein synthesis, therefore, appears to be different from that for other kallikrein-like proteinases or tonin.

Levels of immunoreactive tissue kallikrein were also reduced in diabetes (Table 3), in agreement with previously reported reductions of tissue kallikrein immunoreactivity in the gland after a shorter period of diabetes (24,25). After 1 wk of insulin treatment, the concentration of immunoreactive tissue kallikrein increased, but still was statistically different from controls. A previous study found no recovery of immunoreactive tissue kallikrein after 3 days of insulin treatment (24). Thus, a longer period of treatment may be needed for recovery of tissue kallikrein immunoreactivity to become evident.

In this study, the increase of immunoreactive tissue kallikrein levels resulting from insulin treatment appeared less complete than that from assessments of its catalytic activity. A possible explanation for the observed difference is that cross reaction with other closely related kallikrein-like proteinases was skewing the levels of tissue kallikrein measured by means of a polyclonal antibody. This also could explain why the levels of immunoreactive tissue kallikrein in diabetic glands showed a greater reduction than when assayed catalytically (49 vs. 25%). Our results give no indication of the exact times, after the onset of insulin treatment, when the levels of tissue kallikrein actually recover—only that it had occurred within 7 days. The synthesis of secretory material in granular tubule cells is slow in comparison with that of the secretory proteins of acinar cells (30,31), and the secretory proteinases take 7–10 days to recover to normal levels after massive degranulation in normal animals (32). Therefore, if insulin is capable of influencing the synthesis of tissue kallikrein directly in granular tubule cells, any changes in levels of stored enzyme are unlikely to become detectable for some days. This is supported by the converse finding that STZ-induced diabetic rats took 10 days before a decrease in immunoreactive tissue kallikrein was detected in submandibular glands (24).

TABLE 2
Proteinase activities in submandibular glands of control, diabetic, and insulin-treated diabetic rats

| Animal group | *n* | Tissue kallikrein | Other kallikrein-like proteinases | Tonin |
|---|---|---|---|---|
| Control | 9 | 59.27 ± 2.63* | 91.10 ± 5.57 | 3.49 ± 0.18 |
| Diabetic | 12 | 44.91 ± 2.27 | 35.75 ± 5.20 | 1.53 ± 0.15 |
| | | $P < 0.001$† | $P < 0.001$† | $P < 0.001$† |
| Insulin-treated diabetic | 11 | 61.17 ± 2.33 | 40.73 ± 4.31 | 1.81 ± 0.10 |
| | | $P < 0.001$‡ | NS‡ | NS‡ |
| | | NS§ | $P < 0.001$§ | $P < 0.001$§ |

Submandibular proteinases were assayed according to Shori et al. (1991). Values are mean ± SE. Tissue kallikrein activity equals the SBTI-resistant DVLR-AFC lytic activity. Other kallikrein-like proteinases equal the SBTI-sensitive proteinase activities with DVLR-AFC. Tonin activity is the aprotinin-resistant ZVKKR-AFC lytic activity.
*Activities are expressed as μmol AFC per min per gram gland tissue.
†Diabetic versus control.
‡Insulin-treated diabetic versus diabetic.
§Insulin-treated diabetic versus control.

TABLE 3
Immunoreactive tissue kallikrein and tonin concentrations in control, diabetic, and insulin-treated diabetic rat submandibular glands

| Animal group | *n* | Tissue kallikrein | Tonin |
|---|---|---|---|
| Control | 8 | 12.72 ± 1.10* | 5.84 ± 0.28 |
| Diabetic | 10 | 6.52 ± 0.33<br>*P* < 0.001† | 2.69 ± 0.31<br>*P* < 0.001† |
| Insulin-treated diabetic | 10 | 10.25 ± 0.75<br>*P* < 0.001‡<br>*P* < 0.05§‖ | 3.22 ± 0.40<br>NS‡<br>*P* < 0.001§ |

RIA was performed on rat submandibular gland extracts as described in METHODS. Values are mean ± SE. Tissue kallikrein content as determined by immunoprecipitation with a polyclonal antibody to rat urinary kallikrein. Tonin immunoreactivity as determined by using a monoclonal antibody to rat submandibular tonin.
*Immunoreactivity was expressed as mg/g wet gland weight.
†Diabetic versus control.
‡Insulin-treated diabetic versus diabetic.
§Insulin-treated diabetic versus control.
‖*P* value refers to one-way ANOVA followed by Fisher PLSD. No significant differences were found using Student's *t* test.

Studies with other tissues already suggest that insulin may affect synthesis at the mRNA levels, such as that for amylase in rat parotid glands (33) and tissue kallikrein in the kidney (34). The possibility that insulin may act directly at the mRNA level for tissue kallikrein in rat submandibular glands still needs to be explored.

Immunoassessment of tonin concentrations using a monoclonal antibody showed the same pattern as for its catalytic activity: a large reduction in diabetes and no significant recovery after 1 wk of insulin treatment (Table 3). This reduction in tonin reflects a reduced synthesis of this enzyme by submandibular glands in diabetes. The lack of recovery after 1 wk of insulin treatment suggests that the synthesis of tonin is influenced indirectly by insulin. Tonin in normal rat submandibular glands has been found to be responsive to various circulating hormones, including testosterone, thyroid hormones, and growth hormone (17). Experimental diabetes causes reductions in the plasma concentrations of these hormones and pituitary luteinizing hormone (35–38), with recovery only after extended insulin treatment. For full recovery, growth hormone required 7 days of insulin treatment, $T_4$ took 16 days to recover (39), and pituitary luteinizing hormone and testosterone were restored by 28 days. Because the restoration of these hormones takes time, 7 days of insulin treatment therefore may be insufficient to overcome the observed reduction in tonin activity caused by diabetes.

Total protein concentration in submandibular glandular extracts was significantly reduced in the diabetic animals, and insulin treatment for 7 days failed to bring about a significant recovery (Table 1). In the extracts prepared by our methods, the greatest contribution of protein comes from the granule contents of the granular tubules, as indicated by SDS gel electrophoresis. Our results therefore reflect the state of the granular tubules in these glands. Morphometric findings are in close agreement with our observations; they show a reduction in the diameter and number of granular tubules and in the volume density of their secretory granules in a similar series of diabetic submandibular glands (21).

Previous studies have shown that glandular protein concentration of rat parotid glands was reduced in ALX-induced diabetes, but returned to control levels within 7 days of insulin treatment (40). The turnover of proteins in granular tubule cells of normal rat submandibular glands, however, is much slower than of that in acinar cells (30–32), so granular tubules begin with a low rate of synthesis. Because the reduced protein content in diabetic glands appears to reflect granular tubule numbers and size as well as their granule content, it follows that the effects of insulin on total protein concentration, as measured in this study, may take a longer time to become evident.

With respect to other tissues in STZ-induced diabetic rats, previous studies have found that the concentrations of tissue kallikrein in the pancreas had not decreased at times when tissue kallikrein in the submandibular glands from the same animals was reduced (41). Subsequently, Jaffa et al. (42) have shown immunochemically that, in STZ-induced diabetic rats, reduced levels of active kallikrein were present in the kidney and in urinary excretion, whereas prokallikrein levels were the same, but its synthesis (studied by radioactive methionine incorporation) was reduced. Insulin reversed these changes, and the study concluded that "insulin moderates renal kallikrein production, activation and secretion." Recently, Jaffa et al. (34) have shown that the reduction in renal kallikrein in experimental diabetes is reflected by a similar reduction of kallikrein mRNA, and both effects were prevented by insulin administration. Thus, insulin appears to have different effects on the synthesis of the same enzyme—tissue kallikrein—in the different organs that produce it.

This study indicates that insulin also affects synthesis of individual, closely related, kallikrein-like enzymes in different ways in the same cells of submandibular glands: Insulin is likely to have direct effects on the synthesis of tissue kallikrein but to have only indirect effects on the synthesis of other kallikrein-related enzymes in the same glands.

## ACKNOWLEDGMENTS

This study was supported by the King's Medical Research Trust and National Institutes of Health Grant HL-29397.

## REFERENCES

1. Webster ME: Kallikrein in glandular tissues. *Handb Exp Pharmacol* 25:131–55, 1970
2. Bhoola K, Lemon M, Matthews R: Kallikrein in exocrine glands. *Handb Exp Pharmacol*, (Suppl.) 25:489–523, 1979
3. Fiedler F: Enzymology of glandular kallikrein. *Handb Exp Pharmacol*, (Suppl.) 25:103–61, 1979
4. Orstavik TB: The kallikrein-kinin system in exocrine glands. *J Histochem Cytochem* 28:881–89, 1980
5. Hopsu-Havu VK, Riekkinen PJ, Ekfors TO: Studies on the alkaline trypsin-like enzymes in rat submandibular gland and saliva. *Acta Odont Scand* 25:657–67, 1967
6. Ekfors TO, Riekkinen PJ, Malmihaju T, Hopsu-Havu VK: Four isozymic forms of a peptidase resembling kallikrein purified from the rat submandibular gland. *Hoppe-Seyler's Z Physiol Chem* 348:S111–18, 1967
7. Brandtzaeg P, Gautvik KM, Nustad K, Pierce JV: Rat submandibular

kallikreins: purification and cellular localization. *Br J Pharmacol* 56:155–67, 1976
8. Garrett JR, Smith RE, Kidd A, Kyriacou K, Grabske RJ: Kallikrein-like activity in salivary glands using a new tripeptide substrate, including preliminary secretory studies and observations on mast cells. *Histochem J* 14:967–79, 1982
9. Arnold WH, Orstavik TB, Holck M: A 4-methoxy-2-naphthylamide substrate for the histochemical localization of esteroproteases in the submandibular gland of the rat. *Histochem J* 15:139–46, 1983
10. Nustad K, Orstavik TB, Gautvik KM, Pierce JV: Glandular kallikreins. *Gen Pharmacol* 9:1–9, 1978
11. Orstavik TB, Carretero OA, Hayashi H, Scicli GA, Johansen L: Immunohistochemical localization of tonin and its relation to kallikrein in rat salivary glands. *J Histochem Cytochem* 30:1123–29, 1982
12. Berg T, Holck M, Johansen L: Isolation, characterization and localization of antigen psi, a serine proteinase of the "kallikrein family" in the rat submandibular gland. *Hoppe-Seyler's Biol Chem* 368:1455–67, 1987
13. Simson JAV, Condon J, Fenters R, Chao L, Chao J: Immunocytochemical localization of a kallikrein-like serine protease esterase A. in rat salivary glands. *Anat Rec* 221:475–81, 1988
14. Xiong W, Chen LM, Chao J: Purification and characterization of a kallikrein-like T-kininogenase. *J Biol Chem* 265:2822–27, 1990
15. Boucher R, Asselin J, Genest J: A new enzyme leading to the direct formation of angiotensin II. *Cir Res* (Suppl.) 34–35:203–209, 1974
16. Chao J, Margolius HS: Differential effects of testosterone, thyroxine and cortisol on rat submandibular gland versus renal kallikrein. *Endocrinology* 113:2221–25, 1983
17. Shih HC, Chao L, Chao J: Age and hormonal dependence of tonin levels in rat submandibular gland as determined by a new direct radioimmunoassay. *Biochem J* 238:145–49, 1986
18. Clements JA, Matheson BA, Funder JE: Tissue-specific regulation of the expression of rat kallikrein gene family members by thyroid hormones. *Biochem J* 267:745–50, 1990
19. Clements JA, Matheson BA, Wines DR, Brady JM, MacDonald RJ, Funder JW: Androgen dependence of specific kallikrein gene family members expressed in rat prostate. *J Biol Chem* 263:16132–37, 1988
20. Reuterving CO, Hagg E, Henriksson R, Holm J: Salivary glands in long-term alloxan diabetic rats. A qualitative light and electron-microscopic study. *Acta Path Microbiol Immunol Scand* A95:131–36, 1987
21. Anderson LC, Suleiman AH, Garrett JR: Morphological effects of diabetes on the granular ducts and acini of the rat submandibular gland. *J Em Technique*. In press
22. Murakami H: Changes in activities of several enzymes for carbohydrate metabolism in rat submaxillary gland in response to experimental diabetes and insulin treatment. *J Nihon Univ Sch Dent* 16:91–94, 1974
23. Kamada A, Kawamura M, Funato N, Nakagawa M, Nagasawa S, Sakaki T: Changes in rat submandibular gland N-acetyl-Beta-glucosaminidase activity in streptozotocin-induced diabetes. *J Osaka Dent Univ* 23:15–27, 1989
24. Jaffa AA, Pratt J, Ashford A, Bailey GS: A time course study of submandibular kallikrein, blood glucose and insulin of alloxan-diabetic and streptozotocin-diabetic rats. *Agents and Actions* 15: 478–81, 1984
25. Sakamoto W, Yoshikawa K, Nishikaze O: Glandular kallikrein content in tissues of diabetic and hypertensive rats measured by enzyme immunoassay. *J Clin Chem Clin Biochem* 23:521–23, 1985
26. Cesarone CF, Bolognesi C, Santi LP: Improved microfluorometric DNA determination in biological material using 33258 Hoechst. *Anal Biochem* 100:188–97, 1979
27. Petersen GL: A simplification of the protein assay method of Lowry et al., which is more generally applicable. *Anal Biochem* 83:346–56, 1977
28. Shori DK, Proctor GB, Chao J, Chan KM, Garrett JR: New specific assays for tonin and tissue kallikrein activities in rat submandibular glands; assays reveal differences in the effects of sympathetic and parasympathetic stimulation on proteinases in saliva. *Biochem Pharmacol* 43:1209–17, 1992
29. Shimamoto K, Margolius HS, Chao J, Crosswell AR: A direct radioimmunoassay of rat urinary kallikrein and comparison with other measures of urinary kallikrein activity. *J Lab Clin Med* 94:172–79, 1979
30. Matthews RW: Measurement of protein synthesis in the rat submandibular gland using tritiated tryptophane. *Arch Oral Biol* 19:985–88, 1974
31. Kuijper-Lenstra AH, Kramer MF: Rate of protein synthesis in rat salivary gland cells after pilocarpine or feeding. I. Rate of (Glyco) protein secretion from cells of mixed salivary glands. *Cell Tiss Res* 164:435–46, 1975
32. Shori DK, Proctor GB, Garrett JR, Chan KM: Secretion of multiple forms of tissue kallikrein in rat submandibular gland is influenced by the animal's sex and type of autonomic nerve impulse. *Biochem Soc Trans* 20:98S, 1992
33. Kim SK, Cuzzort LM, McKean RK, Allen ED: Effects of diabetes and insulin on alpha-amylase messenger RNA levels in rat parotid glands. *J Dent Res* 69:1500–504, 1990
34. Jaffa AA, Chai KX, Chao J, Chao L, Mayfield RK: Effects of diabetes and insulin on expression of kallikrein and renin genes in the kidney. *Kidney Int* 41:789–95, 1992
35. Murray FT, Orth J, Gunsalus G, Weisz J, Li JB, Jefferson LS, Musto NA, Bardin CW: The pituitary-testicular axis in the streptozotocin-diabetic male rat: evidence for gonadotroph, sertoli cell and Leydig cell dysfunction. *Int J Andrology* 4:265–80, 1981
36. Benitez A, Diaz JP: Effects of streptozotocin-diabetes and insulin treatment on regulation of Leydig cell function in the rat. *Horm Metab Res* 17:5–7, 1985
37. Carlsson IMS, Clark RG, Skottner A, Robinson ICAF: Growth hormone and growth in diabetic rats: effects of insulin and insulin-like growth factor-I infusions. *J Endocrinol* 122:661–70, 1989
38. Zaninovich AA, Brown TJ, Boado R: Thyroxine metabolism in diabetic rats. *Acta Endocrinol* 86:336–43, 1977
39. Jolin T, Ortiz-Caro J: Secretion and metabolic clearance rates of thyroxine and triiodothyroxine in streptozotocin-diabetic rats. *Acta Endocrinol* 110:395–400, 1985
40. Anderson LC: Effects of alloxan diabetes and insulin in vivo on rat parotid gland. *Am J Physiol* 245:G431–37, 1983
41. Jaffa AA, Rashid Z, Pratt J, Ashford A, Bailey GS: A quantitative study of the levels of glandular kallikrein in normal and diabetic rats. *Biochem Med* 31:42–46, 1984
42. Jaffa AA, Miller DH, Bailey GS, Chao J, Margolius HS, Mayfield RK: Abnormal regulation of renal kallikrein in experimental diabetes. Effects of insulin on prokallikrein synthesis and activation. *J Clin Invest* 80:1651–59, 1987