Induction of EGFR Tyrosine Kinase in the Gastric Mucosa of Diabetic Rats (44391)

AHMED J. KHAN,† SUZANNE E. G. FLIGIEL,*,‡ LEI LIU,† RICHARD JASZEWSKI,*,† ARUN CHANDOK,* AND ADHIP P. N. MAJUMDAR*,†,§,¶,[1]

Veterans Affairs Medical Center and Departments of Internal Medicine,† Pathology,‡ Biochemistry and Molecular Biology,§ and Center for Molecular Medicine and Genetics,¶ Wayne State University School of Medicine, Detroit, Michigan 48201*

Abstract. **Diabetes mellitus is associated with spontaneous gastric mucosal injury and enhanced susceptibility of the mucosa to damaging agents. Little information is available about the biochemical changes that occur in the gastric mucosa of diabetes mellitus. Evidence is accumulating that tyrosine kinases, particularly the EGF-receptor (EGFR), are involved in regulating a variety of structural and functional properties of the gastric mucosa. The primary objectives of this investigation were to determine whether diabetes induces morphological changes in the gastric mucosa, and if so, whether these changes are associated with alterations in EGFR tyrosine kinase. Diabetes-induced changes in gastric mucosal morphology were also examined. Diabetes was induced in 3- to 4-month-old male Fischer-344 rats by streptozotocin (STZ; 45 mg/kg; i.v.). Four weeks after induction of diabetes mellitus, the gastric mucosa of overnight-fasted rats was found to be slightly atrophic. A reduction in gastric mucosal thickness with deposition of fibrous tissue above the muscularis layer was observed in the stomach of overnight-fasted diabetic rats. These changes were associated with a marked stimulation in tyrosine kinase activity and protein expression of EGFR. The relative concentrations of several precursor forms of TGF-α in both membrane and cytosolic fractions from the gastric mucosa of overnight-fasted diabetic rats were also found to be significantly above the corresponding controls. This suggests that endogenous TGF-α may play a critical role in regulating mucosal EGFR tyrosine kinase through a juxtacrine/paracrine mechanism.** [P.S.E.B.M. 1999, Vol 221]

Diabetes mellitus affects the structural and functional integrity of many organ systems, including the gastrointestinal tract. Although gastroparesis diabeticorum is the most common complication of diabetes in the digestive tract (1), clinical studies have demonstrated that diabetic ketoacidosis is often associated with abdominal pain, nausea, and vomiting (2–5). This could partly be the result of upper gastrointestinal hemorrhage, the prevalence of which is increased in diabetic ketoacidosis (3, 4). In addition, results from experimental models of diabetes have revealed that diabetes is associated with spontaneous gastric ulceration and enhanced susceptibility of the gastric mucosa to exogenous damaging agents as well as to stress-induced gastric ulcer (6–8). Food deprivation in streptozotocin (STZ)-induced diabetic rats has been shown to produce severe gastric lesions (7, 8). However, the gastric mucosa possesses a remarkable inherent capacity to repair promptly, which could be attributed to mucosal adaptation to injury (9). In view of this, it is reasonable to speculate that the spontaneous gastric mucosal injury resulting from diabetes will also induce the reparative events in the gastric mucosa. The present investigation will test this hypothesis.

Recent results from this and other laboratories suggest a role for tyrosine kinases (Tyr-ks), particularly the enzyme associated with EGF-receptor (EGFR), in gastric mucosal reparative processes after acute and chronic injury (9–13). For example, we have reported a marked rise in tyrosine kinase activity and expression of EGFR in the gastric mucosa during the initial as well as the late reparative period after hypertonic saline–induced mucosal injury in rats (10,

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[1] To whom requests for reprints should be addressed at Research Service-151, VA Medical Center, 4646 John R, Detroit, MI 48201. E-mail: A.Majumdar@wayne.edu

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11). In addition, we have demonstrated that in rats, doses of tyrphostin, which inhibit the tyrosine kinase activity of EGFR, but not $pp60^{c-src}$, markedly attenuate gastric mucosal repair after acute injury (10). A similar phenomenon has also been noted during gastric ulcer healing (13). These and other relevant observations prompted us to suggest that EGFR tyrosine kinase may play a critical role in mucosal repair (9). To determine whether diabetes also affects EGFR tyrosine in the gastric mucosa, we examined tyrosine kinase activity and expression of EGFR in the gastric mucosa of STZ-induced diabetic rats and their euglycemic counterparts. Furthermore, since ligand-induced activation of EGFR is one of the primary causes of induction of its intrinsic tyrosine kinase activity, we also evaluated the role of endogenous TGF-α in regulating EGFR tyrosine kinase activity in the gastric mucosa.

Materials and Methods

Induction of Diabetes Mellitus and Processing of the Gastric Tissue. Diabetes mellitus was induced by injecting streptozotocin [STZ; 45 mg/kg in 0.1 ml of citrate buffer (pH 4.5)] in the tail vein of 3- to 4-month-old male Fischer-344 rats. The control animals were similarly given an equivalent volume of the vehicle. Body weight of the animals was recorded each week. Three weeks after administration of STZ, blood glucose levels were determined by applying a drop of blood to a One-Touch glucometer (Boehringer Mannheim, Indianapolis, IN). Blood was obtained by cutting a small part of the tail. The animals were used 4 weeks after induction of diabetes. Only those animals with blood glucose levels between 350 and 450 mg/dl were used. Mean blood glucose level of STZ-treated rats was 389 ± 65 mg/dl. Mean body weight for the euglycemic controls and STZ-induced diabetic rats at the end of the experimental period was 305 ± 10 g and 289 ± 18 g, respectively. All rats were maintained on *ad libitum* food and water.

The rats were sacrificed (overexposure to CO_2) after an overnight fast. The stomach was removed, opened along the greater curvature, and rinsed thoroughly with cold normal saline; small portions of the oxyntic gland area were fixed in either buffered formalin or Bouin's fixative. Mucosal scrapings were obtained from the rest of the oxyntic gland area and stored at –90°C in small aliquots.

Mucosal Histology. Small sections of formalin-fixed gastric tissues were dehydrated in graded alcohol and embedded in paraffin to yield the full thickness of the mucosa. The paraffin-embedded tissues were serially sectioned at 4-μm intervals and stained with hematoxylin and eosin for histologic evaluation by light microscopy.

Immunofluorescent Staining of EGFR. Bouin's-fixed gastric tissues were embedded in paraffin and sectioned as stated above. Sections were collected on poly-L-lysine-coated slides. After deparaffinization and rehydration, sections were blocked with 10% chick serum, 10% porcine serum, and 0.5% BSA in phosphate-buffered saline (PBS) for 1 hr at room temperature. After washing with PBS twice, the slides were incubated overnight at 4°C with rabbit anti-EGFR antibody (polyclonal antibody, 2 μg/ml, Santa Cruz Biotechnology, Santa Cruz, CA). Rabbit γ-globulin (2 μg/ml; Sigma Chemical Co., St. Louis, MO) in PBS was used as a negative control. After extensive washing in PBS, the slides were incubated with FITC-conjugated-$F(ab')_2$ fragment of antirabbit IgG (Jackson Immuno Research, West Grove, PA) for 1 hr at room temperature (20 μg/mL). After washing in PBS for 3–4 times (5 min each), the slides were mounted with Vectashield mounting medium (Vector Laboratories, Inc., Burlingame, CA) and sealed with nail polish. The slides were examined using a fluorescent microscope (Olympus System, Model BX60, Olympus America Inc., Lake Success, NY).

Tyrosine Kinase Activity. The enzyme activity was determined as previously described (15, 16). Briefly, aliquots of mucosal scrapings were homogenized in lysis buffer [20 m*M* sodium phosphate, pH 7.4, 0.5% Triton X-100, 0.5% Nonidet P-40, 50 m*M* NaCl, 5 m*M* EDTA, 1 m*M* phenylmethylsulfonyl (PMSF), 1 m*M* Na_3VO_4, 10 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 m*M* 1,10-phenanthroline] using a Dounce Homogenizer. The homogenate was stirred in a mechanical rotator for 30 min at 4°C, and subsequently centrifuged at 10,000*g* for 10 min at 4°C. The supernatant, after diluting with an equal volume of homogenizing buffer A (HEPES, pH 7.4, 150 m*M* NaCl, 1 m*M* PMSF, 1 m*M* Na_3VO_4, 10 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 m*M* 1,10-phenanthroline), was used as the source for tyrosine kinase. Protein content was measured using the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA).

For determination of tyrosine kinase activity of EGFR, aliquots of mucosal lysate containing 350 μg protein were diluted with an equal volume of lysis buffer containing 1% Triton X-100, 0.1% SDS, and 0.5% sodium deoxycholate. The samples were incubated overnight at 4°C with 1 μg of polyclonal antibody to EGFR (UBI, Lake Placid, NY). The immune complexes were precipitated with heat-denatured Pansorbin and washed several times with RIPA/homogenizing buffer. The immunoprecipitates were resuspended in 30 μl of buffer A containing 0.1% Triton X-100 and assayed for tyrosine kinase activity by measuring ^{32}P incorporation from [γ-^{32}P]ATP into acid-denatured enolase (16). In all immunoprecipitation studies, protein concentration was standardized among the samples.

Western Blot Analysis of EGFR andTGF-α. EGFR was immunoprecipitated from detergent-solubilized gastric mucosa containing 3 mg protein with EGFR antibodies as stated above. In the case of TGF-α, mucosal homogenate (mucosa homogenized in homogenizing buffer) was centrifuged at 30,000*g* to obtain crude membrane and cytosolic fractions. TGF-α was then immunoprecipitated from the membrane and cytosolic fractions containing 3 mg protein. All immunoprecipitates were subjected to SDS-PAGE (17). Following electrophoresis, proteins were transferred electrophoretically onto nitrocellulose membranes

(Bio-Rad, Richmond, CA) using a transfer cell (International Corp. Mount Prospect, IL). Membranes were incubated for 1 hr at room temperature with blocking buffer [PBS containing 5% nonfat dry milk (Sanalac^{R}), 0.25% BSA, and 0.1% Tween-20] with gentle agitation. After washing the membranes with PBS containing 0.1% Tween-20, they were incubated overnight at 4°C with either EGFR (UBI) or TGF-α (monoclonal antibody from Santa Cruz Biotechnology, Santa Cruz, CA) antibodies at a final concentration of 1:2500 in PBS containing 0.25% BSA. The membranes were washed again, and subsequently incubated with horseradish peroxidase–linked anti-mouse antibody conjugates (ICN). After washing, the protein bands were visualized by enhanced chemiluminescence (ECL) detection system (Amersham) according to the manufacturer's instructions. The membranes containing the electrophoresed proteins were exposed to X-Omat film, and the density of the protein bands was analyzed by a digital image analysis system (Molecular Dynamics Strom 860, Sunnyvale, CA).

Results

Histologic evaluation of the stomach from euglycemic control rats revealed normal gastric pits and glands with abundant mucus-secreting surface epithelial cells and deep gastric pits surrounded by thin, tall villous projections (Fig. 1A,C). Induction of diabetes mellitus by streptozotocin (STZ), on the other hand, resulted in reduction in mucosal thickness with deposition of fibrous tissue above the muscularis layer (Fig. 1B). In diabetic rats, the gastric pits were noted to be shallow, and there was an overall flattening of the mucosal surface accompanied by extensive loss of intracytoplasmic mucus from surface epithelial cells (Fig. 1B). Overall, the gastric mucosa was observed to be mildly atrophic (Fig. 1D), and showed morphologic alterations similar to those found in aged rats (9). These changes were accompanied by a marked rise in tyrosine kinases in the gastric mucosa. However, whereas overall mucosal tyrosine kinase activity in the gastric mucosa of diabetic rats was increased by 34%, [enzyme activity expressed as pmole ^{32}P incorporated/mg protein; control = 964 ± 97; diabetic = 1460 ± 216, $P < 0.02$], the enzyme associated with EGFR revealed 126% higher activity when compared with the respective controls (Fig. 2). Since autophosphorylation of EGFR is one of the prerequisites for activation of the intrinsic tyrosine kinase activity of EGFR (18), we also examined the extent of EGFR autophosphorylation. As expected, the extent of autophosphorylation of EGFR in the gastric mucosa of diabetic rats was found to be about 40% higher than in controls (Fig. 3).

To determine whether the observed induction EGFR tyrosine kinase activity in diabetic gastric mucosa could be due partly to increased levels of the enzyme, a Western-immunoblot was performed to assess the relative concentration of EGFR. Under the present experimental condition, the EGFR band was hardly detectable in the gastric mucosa from euglycemic controls (Fig. 4) In contrast, the EGFR band was very prominent in mucosal samples from STZ-diabetic rats (Fig. 4).

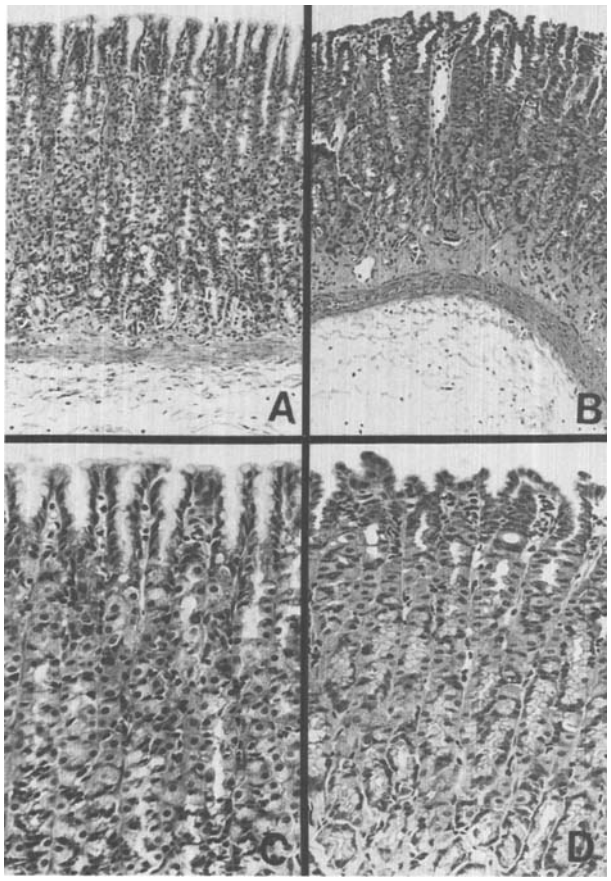

Figure 1. Photomicrographs of the gastric mucosa from (A, C) euglycemic control and (B, D) STZ-induced diabetic rats. Sections of the stomach under low-powered focus (A; magnification 100x) from control rats demonstrate normal gastric pits and gland, whereas under high-powered focus (C; magnification 400x) show abundant mucus-secreting surface epithelial cells and deep gastric pits surrounded by thin, tall villous projections. (B) Low-powered focus of the stomach from diabetic rats demonstrates decreased mucosal thickness with deposition of fibrous tissue above the muscularis mucosae layer. The gastric pits are shallow, and there is an overall flattening of the mucosal surface. (D) High-powered focus of the diabetic stomach shows marked atrophy of gastric pits, mild atrophy of the gastric glands, and an extensive loss of cytoplasmic mucus from the surface epithelial cells. There are numerous dilated and congested superficial mucosal capillary vessels.

Immunocytochemical analysis was then performed to further evaluate cellular distribution and relative abundance of EGFR in the gastric mucosa. Results are shown in Figure 5. In general, the number of EGFR immunoreactive cells in the gastric mucosa of diabetic rats was substantially higher than in their euglycemic controls. Furthermore, there was also a marked difference in cellular distribution of EGFR in the gastric mucosa between diabetic and control rats. In control rats, EGFR immunoreactive cells were primarily located in the surface and neck regions, whereas in diabetic rats they extended from the neck to the bottom of the gastric gland. EGFR staining intensity was also considerably stronger in diabetic rats than in their euglycemic controls.

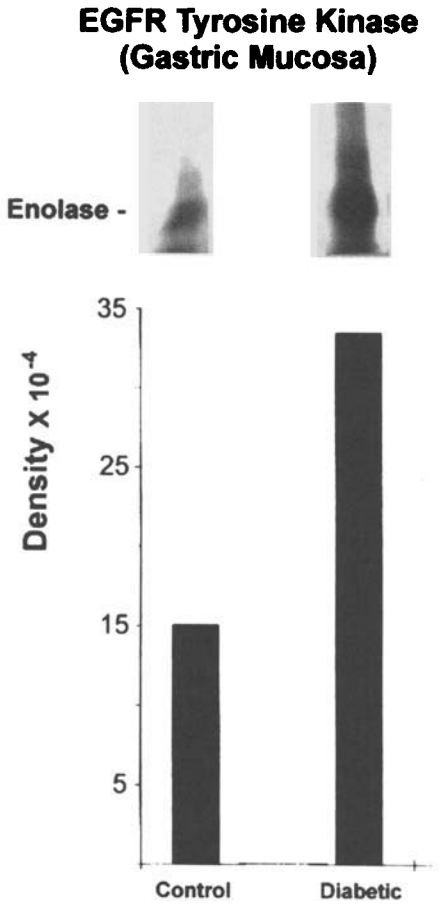

Figure 2. Representative autoradiograph showing changes in EGFR tyrosine kinase activity in the gastric mucosa of euglycemic control and STZ-induced diabetic rats. EGFR tyrosine kinase activity was determined by the extent of enolase phosphorylation. The histogram shows changes in the extent of enolase phosphorylation. The experiment was repeated three times with similar outcomes.

Ligand binding to the extracellular domain of EGFR is one of the primary causes for activation of intrinsic tyrosine kinase activity of the receptor (18). However, TGF-α, one of the primary ligands of EGFR, is a membrane-anchored peptide (19), and in most cases the peptide is present on the cell surface in its precursor form(s), which also activates EGFR (14, 20, 21). Therefore, to determine the involvement of endogenous TGF-α in modulating EGFR tyrosine kinase activity in the gastric mucosa of STZ-induced rats, mucosal membrane and cytosolic fractions from both groups of rats were analyzed for TGF-α levels by Western-immunoblot. Membrane and cytosolic fractions from both groups of rats showed two prominent molecular forms of TGF-α with M_r of 18 and 20 kDa (Fig. 6). The relative concentration of both forms of TGF-α in the membrane and cytosol from diabetic rats was higher, when compared with their corresponding controls (Fig. 6). Phosphorimager analysis of the 18-kDa band in the cytosolic and membrane fractions from diabetic rats revealed 350% and 200% higher levels, respectively, when compared with the corresponding controls (Fig. 6).

Discussion

Evidence has been accumulating that diabetes mellitus, in addition to causing gastroparesis diabeticorum, also induces spontaneous gastric mucosal injury and enhances susceptibility of the mucosa to damaging agents (1, 6–8, 22). Although the underlying biochemical mechanism(s) for the prevalence of spontaneous mucosal injury in diabetic animals remains to be fully elucidated, data from earlier studies suggest that decreased thickness of the surface epithelium could be a contributing factor. Watanabe *et al.* (23) reported a decreased surface epithelial cell layer in the gastric mucosa of diabetic rats. In the current investigation, we have also noted the mucosal thickness to be comparatively lower in overnight-fasted STZ-diabetic rats than in their healthy counterparts. There was also a loss of intracytoplasmic mucus from the surface epithelial cells in the gastric mucosa of diabetic rats. These changes are likely to enhance susceptibility of the mucosa to various endogenous and exogenous

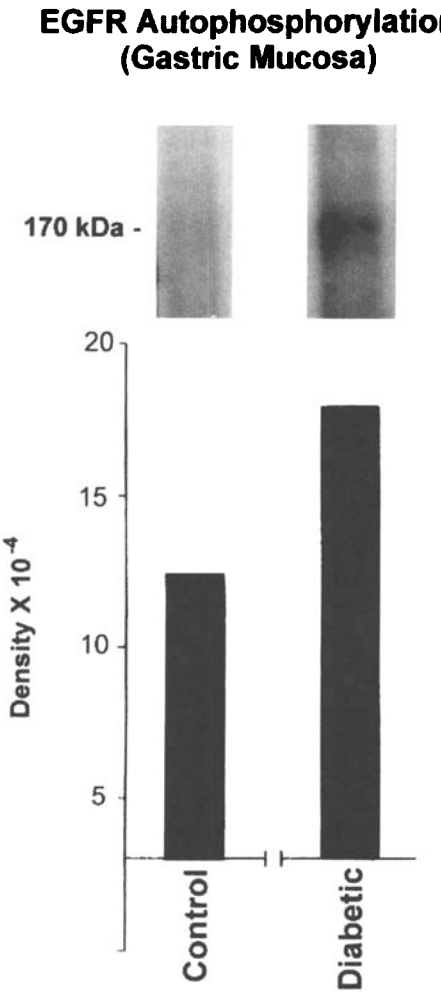

Figure 3. Representative autoradiograph showing changes in autophosphorylation of EGFR in the gastric mucosa from euglycemic control and STZ-induced diabetic rats. The histogram shows the extent of EGFR autophosphorylation as analyzed by a Phosphorimager.

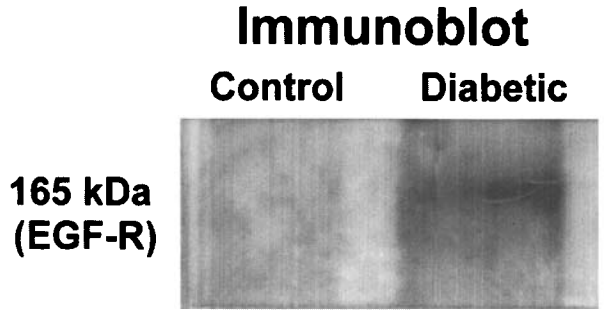

Figure 4. Representative Western-blot showing changes in relative concentration of EGFR in the gastric mucosa of euglycemic control and STZ-induced diabetic rats. The experiment was repeated twice with similar outcomes.

injurious agents. Although in the current investigation we made no effort to evaluate the extent of mucosal injury, food deprivation has been shown to increase the extent of injury in the gastric mucosa of diabetic rats (7). Since all rats were fasted overnight before sacrificing, it is reasonable to speculate that some of the morphological changes, such as the loss of surface cells observed in diabetic rats, could be the result of injury. Although Piyachaturawat *et al.* (6) earlier suggested a damaging effect of STZ on the gastric mucosa, Goldin *et al.* (8) recently demonstrated that the extent of gastric mucosal injury in 24-hr-fasted pancreatectomized diabetic rats is similar to that observed in STZ-induced diabetes. Taken together, the results suggest that diabetes enhances susceptibility of the gastric mucosa to spontaneous injury.

Results from this and other laboratories have demonstrated that in normal adult rats, gastric mucosal injury, whether acute or chronic, results in a marked stimulation in tyrosine kinase activity and expression of EGFR (9–13). This induction of the EGFR signaling pathway is thought to be essential for initiating the subsequent reparative processes, since inhibition of tyrosine kinase activity of EGFR, but not $pp60^{c-src}$ by tyrphostin greatly attenuates mucosal repair in rats (10). In view of these observations, it is tempting to speculate that the marked stimulation of EGFR tyrosine kinase activity in the gastric mucosa of STZ-diabetic rats could be the result of mucosal adaptation to reparative processes. Increased tyrosine kinase activity and tyrosine-specific phosphorylation of a number of proteins have also been observed in jejunal mucosa of STZ-diabetic rats (24). However, as opposed to what has been observed in the gastric mucosa, STZ-induced diabetes is associated with hyperplasia in the small intestine (24, 25). Although the reasons for the morphological differences between the gastric mucosa and small intestine in diabetic animals are unknown, gastric mucosal atrophy noted in diabetic rats could be due partly to the loss of surface mucosal cells resulting from spontaneous mucosal injury.

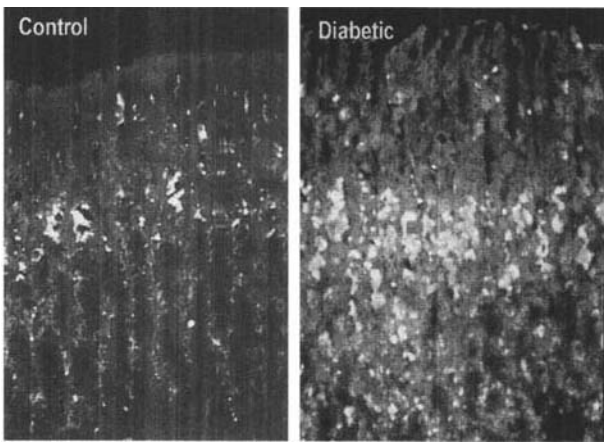

Figure 5. Representative photomicrograph showing changes in EGFR immunoreactivity in the gastric mucosa of euglycemic controls and STZ-induced diabetic rats (magnification 400x).

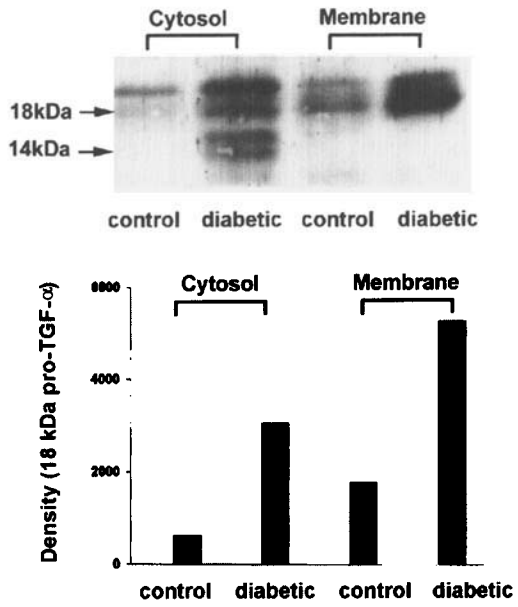

Figure 6. Representative Western-blot showing changes in relative concentration of TGF-α in the membrane and cytosolic fractions from the gastric mucosa of euglycemic controls and STZ-induced diabetic rats. The histogram shows changes in density of protein bands as analyzed by a Phosphorimager.

Among many factors that modulate the intrinsic tyrosine kinase activity of EGFR, binding of the ligand to the extracellular domain of EGFR is considered to be one of the primary causes for activation of the enzyme activity (18). Earlier studies have demonstrated that in rats, the levels of TGF-α, but not EGF, are greatly elevated in the gastric mucosa and gastric juice shortly after acute injury (11, 26). Such an observation suggests that endogenous TGF-α, but not EGF, may play a critical role in regulating the intrinsic tyrosine kinase activity of EGFR in the gastric mucosa after injury through an autocrine/paracrine mechanism. However, TGF-α is a membrane-anchored peptide, and in most cases it is present on the cell surface in its precursor form(s), which can also activate EGFR (14, 20, 21). We have reported that the increased EGFR tyrosine kinase activity in the gastric mucosa, whether the result of ageing or injury, is associated with a concomitant rise in several precursor forms of TGF-α (15) indicating that the membrane-bound

forms of the peptide may partly be responsible for modulating EGFR tyrosine kinase through a juxatcrine/paracrine mechanism. Our current observation that induction of diabetes mellitus by STZ also results in a marked rise in several precursor forms of TGF-α in both cytosolic and membrane fractions suggests that the peptide might partly be responsible for regulating EGFR tyrosine kinase activity in diabetic rats. Whether the increased TGF-α levels in both cytosolic and membrane fractions of the gastric mucosa of diabetic rats are the result of increased synthesis of the peptide remain to be determined.

In summary, our current data demonstrate that STZ-induced diabetes mellitus in rats causes marked morphological changes in the gastric mucosa as reflected by the loss of mucus-containing surface epithelial cells and moderate atrophy of the mucosal layer, which may partly be responsible for reported spontaneous gastric mucosal injury and increased susceptibility of the mucosa to injurious agents. We have also observed that STZ-induced diabetes results in a marked stimulation in EGFR tyrosine kinase activity and protein expression of the receptor. The fact that the levels of TGF-α in both membrane and cytosolic fractions of the gastric mucosa are greatly elevated, compared to the corresponding controls, prompted us to suggest that endogenous TGF-α may play a critical role in regulating mucosal EGFR tyrosine kinase through a juxtacrine/paracrine mechanism.

1. Feldman M, Schiller LR. Disorders of gastrointestinal motility associated with diabetes mellitus. Ann Intern Med **98:**378–384, 1983.
2. Siperstein MD. Diabetic ketoacidosis and hyperosmolar coma. Endocrinol Metab Clin North Am **21:**415–432, 1992.
3. Barrett EJ, Sherwin RS. Gastrointestinal manifestations of diabetic ketoacidosis. Yale J Biol Med **56:**175–178, 1983.
4. Katz LA, Spiro HM. Gastrointestinal manifestation of diabetes. New Engl J Med **275:**1350–1361, 1966.
5. Campbell IW, Duncan LJP, Innes JA, MacCuish AC, Munro JF. Abdominal pain in diabetic metabolic decompensation, clinical significance. JAMA **233:**166–168, 1975.
6. Piyachaturawat P, Poprasit J, Glinsukon T. Gastric mucosal secretion and lesions by different doses of streptozotocin in rats. Toxicol Lett **55:**21–29, 1991.
7. Takeuchi K, Ueshima K, Ohuchi T, Okabe S. Induction of gastric lesions and hypoglycemia responses by food deprivation in streptozotocin-diabetic rats. Dig Dis Sci **39:**626–634, 1994.
8. Goldin E, Ardite E, Elizalde JI, Odriozola A, Panes J, Pique JM, Fernandez-Checa JC. Gastric mucosal damage in experimental diabetes in rats: Role of endogenous glutathione. Gastroenterology **112:**855–863, 1997.
9. Majumdar AP, Fligiel SE, Jaszewski R. Gastric mucosal injury and repair: Effect of aging. Histol Histopathol **12:**491–501, 1997.
10. Majumdar AP, Fligiel SE, Jaszewski R, Tureaud J, Dutta S, Chelluderai B. Inhibition of gastric mucosal regeneration: Evaluation of the role of EGF-receptor tyrosine kinases. J Lab Clin Med **128:**173–180, 1996.
11. Relan NK, Fligiel SEG, Dutta S, Tureaud J, Chauhan DP, Majumdar AP. Induction of EGF-receptor tyrosine kinase during early reparative phase and effects of aging. Lab Invest **73:**717–726, 1995.
12. Liu L, Turner JR, Yu Y, Khan AJ, Jaszewski R, Fligiel SE, Majumdar AP. Differential expression of EGFR during early reparative phase of the gastric mucosa between young and aged rats. Am J Physiol **275:** G943–G950, 1998.
13. Pai R, Ohta M, Itani RM, Sarfeh IJ, Tarnawski AS. Induction of mitogen-activated protein kinase signal transduction pathway during gastric mucosal ulcer healing in rats. Gastroenterology **114:**706–713, 1998.
14. Massague J. Epidermal growth factor-like transforming growth factor. II. Interactions with epidermal growth factor receptors in human placenta membranes and A431 cells. J Biol Chem **258:**13614–13620, 1983.
15. Tureaud J, Sarkar FH, Fligiel SE, Kulkarni S, Jaszewski R, Reddy K, Yu Y, Majumdar AP. Increased expression of EGFR in gastric mucosa of aged rats. Am J Physiol **273:**G389–G398, 1997.
16. Majumdar AP, Goldenring JR. Localization and significance of pp55, a gastric mucosal membrane protein with tyrosine kinase activity. Am J Physiol **274:**G863–G870, 1998.
17. Majumdar AP, Moshier JA, Arlow FL, Luk GD. Biochemical changes in the gastric mucosa after injury in young and aged rats. Biochim Biophys Acta **992:**35–40, 1989.
18. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell **61:**203–212, 1990.
19. Podolsky DK. The epidermal growth factor family. In: Johnson LR, Ed. Physiology of the Gastrointestinal Tract. New York: Raven Press, pp129–167, 1994.
20. Brachmann R, Lindquist PB, Nagashima M, Kohr W, Lipari M, Derynck R. Transmembrane TGF-α precursors activates EGF/TGF-α receptors. Cell **56:**691–700, 1989.
21. Pandiella A, Massague J. Cleavage of membrane precursor for transforming growth factor α is a regulated process. Proc Natl Acad Sci U S A **88:**1726–1730, 1991.
22. Takeuchi K, Takehara K, Tajima K, Kato S, Hirata T. Impaired healing of gastric lesions in streptozotocin-induced diabetic rats: Effect of basic fibroblast. J Pharmacol Exp Ther **281:**200–207, 1997.
23. Watanabe T, Asanuma A, Tanaka M, Akiba T, Koga T. Morphological study on the gastric mucosa in diabetes mellitus rats induced by streptozotocin. Exp Anim **43:**693–696, 1995.
24. Younoszai MK, Parekh VV, Stephen TC, Hoffman JL. Relation of ornithine decarboxylase and tyrosine kinase activity in the jejunal mucosa *in vivo*. Proc Soc Exp Biol Med **211:**339–345, 1996.
25. Parekh VV, Hoffman JL, Younoszai MK. Effects of diabetes and difluoromethylornithine treatment on hyperplasia, activity of MAP-kinase, and activity and association with cyclin B of p34cdc2 kinase in rat jenunal mucosa. J Invest Med **46:**76–81, 1998.
26. Polk WH, Jr., Dempsey PJ, Russell WE, Brown PI, Beauchamp RD, Barnard JA, Coffey RJ. Increased production of transforming growth factor α following acute gastric injury. Gastroenterology **102:**1467–1474, 1992.