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Increased susceptibility of gastric mucosa to ulcerogenic stimulation in diabetic rats – role of capsaicin-sensitive sensory neurons

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1 We examined the gastric mucosal blood flow (GMBF) and ulcerogenic responses following barrier disruption induced by sodium taurocholate (TC) in diabetic rats and investigated the role of capsaicin-sensitive sensory neurons in these responses.

2 Animals were injected streptozotocin (STZ: 70 mg kg^{-1}, i.p.) and used after 5, 10 and 15 weeks of diabetes with blood glucose levels of >350 mg dl^{-1}. The stomach was mounted on an *ex-vivo* chamber under urethane anaesthesia and exposed to 20 mM TC plus 50 mM HCl for 30 min in the presence of omeprazole. Gastric transmucosal potential difference (PD), GMBF, and luminal acid loss (H^+ back-diffusion) were measured before and after exposure to 20 mM TC, and the mucosa was examined for lesions 90 min after TC treatment.

3 Mucosal application of TC caused PD reduction in all groups; the degree of PD reduction was similar between normal and diabetic rats, although basal PD values were lower in diabetic rats. In normal rats, TC treatment caused luminal acid loss, followed by an increase of GMBF, resulting in minimal damage in the mucosa.

4 The increased GMBF responses associated with H^+ back-diffusion were mitigated in STZ-treated rats, depending on the duration of diabetes, and severe haemorrhagic lesions occurred in the stomach after 10 weeks of diabetes.

5 Intragastric application of capsaicin increased GMBF in normal rats, but such responses were mitigated in STZ diabetic rats. The amount of CGRP released in the isolated stomach in response to capsaicin was significantly lower in diabetic rats when compared to controls.

6 The deleterious influences on GMBF and mucosal ulcerogenic responses in STZ-diabetic rats were partially but significantly antagonized by daily insulin (4 units rat^{-1}) treatment.

7 These results suggest that the gastric mucosa of diabetic rats is more vulnerable to acid injury following barrier disruption, and this change is insulin-sensitive and may be partly accounted for by the impairment of GMBF response associated with acid back-diffusion and mediated by capsaicin-sensitive sensory neurons.

Keywords: streptozotocin; diabetic rat; gastric mucosa; taurocholate mucosal blood flow; lesion; capsaicin

Introduction

Experimental studies show that prolonged diabetic conditions have deleterious influences on various functions in the gastrointestinal tract (O'Reilly & Long, 1987; Takehara *et al*., 1997). In the streptozotocin (STZ)-induced diabetic rat, an accepted model of insulin-dependent diabetes, it has been reported that overnight fasting of diabetic rats resulted in development of gastric lesions by an insulin-sensitive mechanism, suggesting that STZ-diabetes predisposes rats to gastric ulceration (Piyachatrurawat *et al*., 1991; Takeuchi *et al*., 1994a; Goldin *et al*., 1997). However, the mechanism of the increased mucosal susceptibility in diabetic rats has not yet been studied.

It is well known that neuronal dysfunction develops in association with pathophysiological alterations in the diabetic conditions, a change that could also contribute to the aggravated vulnerability of the gastric mucosa. Clinical studies have shown that long-standing insulin-dependent diabetes reduces the acid secretory response to sham-feeding, suggesting vagal neuropathy in these patients (Feldman *et al*., 1979). A decrease in the number of neuropeptide-containing nerve fibres including calcitonin gene-related peptide (CGRP) in the stomach and intestine was reported in diabetic rats (Lincoln *et al*., 1984; Belai & Burnstock, 1987; Belai *et al*., 1991). CGRP is a neurotransmitter of capsaicin-sensitive sensory nerves that are known to mediate gastric hyperaemic response induced by acid back-diffusion after barrier disruption and are important in modulating gastric mucosal integrity (Holzer *et al*., 1991a; Li *et al*., 1991; 1992). Indeed, functional ablation of these neurons worsens the mucosal ulcerogenic response to a variety of noxious agents and impairs the healing of gastric lesions (Holzer *et al*., 1991b; Takeuchi *et al*., 1993; 1994b). Therefore, it is possible that the gastric mucosa of diabetic animals is more vulnerable to acid, because of impairment of capsaicin-sensitive sensory neurons.

In the present study, we investigated using STZ-diabetic rats, gastric mucosal blood flow (GMBF) and ulcerogenic responses induced by acid back-diffusion following barrier disruption caused by sodium taurocholate (TC), especially in relation to capsaicin-sensitive sensory neurons.

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Methods

Male Sprague-Dawley rats (260–280 g: Charles River, Shizuoka, Japan) were used. The animals were fed standard rat chow and tap water *ad libitum*. One week after purchase, they were given streptozotocin (STZ: 70 mg kg^{-1}, i.p.) and fed normally thereafter. Control animals received an equal volume of saline.

General procedures

The experiments were performed in the following five groups of rats; normal rats 8 weeks and 23 weeks old, and STZ-treated rats after 5 weeks, 10 weeks, and 15 weeks of diabetes. Blood was sampled from the tail vein and blood glucose levels (BGL) were determined by Glucostar-Glucostix (Miles-Sankyo Co., Ltd., Tokyo, Japan). The STZ-treated animals with BGL of less than 350 mg dl^{-1} under nonfasting conditions were excluded from the study. Some of the diabetic rats (10 weeks) were injected once daily with zinc insulin with a long duration of action (4 units rat^{-1}, s.c.) for 9 weeks starting 1 week after STZ treatment (Takeuchi *et al*., 1994a; 1997). In these groups of rats, the effects of mucosal application of sodium taurocholate (TC) plus HCl on gastric transmucosal potential difference (PD), gastric mucosal blood flow (GMBF), luminal acid loss (acid back-diffusion), and the gastric mucosa were examined under urethane anaesthesia. In some cases, the effect of mucosal application of capsaicin on GMBF was examined in both normal and STZ-treated rats at various periods of diabetes. In a separate study, the amount of calcitonin gene-related peptide (CGRP) released in response to capsacin stimulation was determined in the isolated stomach of normal and STZ-diabetic rats *in vitro*. In all studies, the animals were deprived of food but allowed free access to tap water for 18 h before the experiment.

Determination of PD, GMBF, and acid back-diffusion

Animals were anaesthetized with urethane (1.25 g kg^{-1}, i.p.), and the trachea was cannulated to ensure a patent airway. Acid secretion was completely inhibited by pretreatment with omeprazole (60 mg kg^{-1}, i.p.). Simultaneous measurement of PD, GMBF, and acid back-diffusion was performed in the chambered stomach as described previously (Takeuchi *et al*., 1993; Miyake *et al*., 1996). Briefly, the abdomen was incised, and the stomach was exposed and mounted on an *ex-vivo* chamber (area exposed, 3.14 cm^2). At the beginning of each experiment, the mucosa was rinsed several times with a solution of 50 mM HCl plus 100 mM NaCl. When the gastric exudate became clear, 2 ml of the acid solution was instilled in the chamber, and 15 min later the gastric contents were recovered from the chamber. This procedure was repeated every 15 min, three times before and six times after exposure of the mucosa to 20 mM TC for 30 min. PD was determined using two agar bridges, one positioned in the chamber and the other in the abdominal cavity. GMBF was measured by a laser Doppler flowmeter (Advance Model ALF 21, Tokyo, Japan), placing the probe gently on the corpus mucosa using a balance, and changes in GMBF were continuously monitored on a two-channel recorder (U-228, Tokai-Irika, Tokyo, Japan) simultaneously with those of PD (Matsumoto *et al*., 1992). On the other hand, luminal acid loss (acid back-diffusion) was determined from the analyses of the collected acid solution. Each sample was analysed for volume and acid concentration, which was determined by automatic titration of an aliquot against 0.1 N NaOH to pH 7.0 (Autoburette, Comtite-7, Hiranuma, Tokyo, Japan). The amount of acid back-diffusion was calculated as the difference between the product of the final volume and concentration and the product of initial volume and concentration. Positive values indicate that the net flux of H^+ was from the mucosa to the lumen, and the results were expressed as μEq (15 min^{-1}).

Ninety minutes after exposure to TC, the mucosa was examined for haemorrhagic lesions under a dissecting microscope with a square grid ($\times 10$). To prevent bias the observer measuring the lesion was unaware of the treatment. Tissue samples were then immersed into 10% formalin for histological observation, processed for routine light microscopy, sectioned at 5 μm, and stained with haematoxylin and eosin.

In separate experiments, we also examined the effect of capsaicin on GMBF responses in normal and STZ-treated animals after various periods of diabetes. For this purpose, the stomach was perfused with saline at a flow rate of 1.0 ml/min. After both PD and GMBF had well stabilized, the perfusion was discontinued, and capsaicin (2 ml of 0.1 mg ml^{-1}) was applied topically to the mucosa for 10 min, and GMBF was measured continuously before, during and after the mucosal exposure to capsaicin.

Determination of CGRP in isolated stomachs

Capsaicin-induced CGRP release from the isolated rat stomach was determined according to the method of Inaba *et al*. (1994). Briefly, under ether anaesthesia, the stomachs of normal (23 week-old) and STZ-diabetic rats (15 weeks) were isolated. After rinsing the stomach with cold saline, both the forestomach and antrum were dissected out, and the corpus mucosa was inverted (mucosal side out). Then, the tissues were incubated in test tubes containing 2 ml of a modified Krebs-Henseleit solution gassed with 95% O_2-5% CO_2 maintained at 37°C. Capsaicin (1×10^{-6} M) dissolved in 10% ethanol (0.1 ml) was added to the incubated tissues. Thirty minutes later, the incubated solution was centrifuged at 3000 r.p.m. for 5 min at 4°C. The CGRP content of the supematant was determined by chemiluminescent enzyme immunoassay.

Preparation of drugs

Drugs used were urethane (Tokyo Kasei, Tokyo, Japan), streptozotocin (Wako, Osaka, Japan), taurocholate Na (Difco Lab., Detroit, Michigan, U.S.A.), capsaicin (Nacalai Tesque, Kyoto, Japan), zinc insulin (Novo, Tokyo, Japan) and omeprazole (Hassel Co., Mondal, Sweden). Omeprazole and capsaicin were suspended in 0.5% carboxymethylcellulose (CMC) (Nacalai, Kyoto, Japan) solution, while other drugs were dissolved in saline. Each agent was prepared immediately before use and administered i.p. or s.c. in a volume of 0.5 ml per 100 g body weight, or applied topically to the chamber in a volume of 2 ml per rat.

Statistics

Data are presented as the means ± s.e. from 5–8 rats per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of $P < 0.05$ were regarded as significant.

Results

Blood glucose concentrations after STZ treatment

Blood glucose levels (BGL) under nonfasting conditions were increased after STZ injection, reached significantly high levels

at 1 week (384.5 ± 7.9 mg dl^{-1}) relative to basal values (138.0 ± 5.2 mg dl^{-1}) and remained significantly elevated for 15 weeks thereafter (Table 1). Normal rats receiving saline showed stable BGL during the test period (127.0–141.5 mg dl^{-1}). Daily injection of insulin (4 units rat^{-1} day^{-1}), starting 1 week after STZ administration, significantly decreased high BGL levels in diabetic rats, although the values still remained significantly high compared to normal rats. Since STZ-treated rats kept consistently high BGL up to 15 weeks, the following studies were carried out using STZ-treated rats after various periods of diabetes, i.e., 5 weeks, 10 weeks, and 15 weeks after the administration of STZ.

Gastric functional responses and mucosal injury induced by mucosal application of taurocholate

PD Response: Under chambered conditions in the presence of omeprazole (inhibition of acid secretion) and exogenous acid (50 mM HCl plus 100 mM NaCl), the stomachs of control rats generated a PD of $-50 \sim 55$ mV (mucosa negative) and maintained relatively constant GMBF ($140 \sim 200$ mV: arbitrary unit) during a 2-h test period (Figure 1). These values were almost the same in control groups of 8 and 23 week old-rats (not shown). Exposure of the normal rat mucosa (23 week-old) to 20 mM TC for 30 min caused a marked reduction of PD from -54.1 ± 2.8 mV to -32.8 ± 1.1 mV, but after the exposure the reduced PD gradually returned to basal values within 1 h, the degree of PD recovery being $76.1 \pm 4.6\%$ at 90 min post-treatment (Figure 2a and b). On the other hand, STZ-diabetic rats exhibited lower PD at basal conditions as compared to normal rats; the values were $-35 \sim -45$ mV and remained in similar ranges after 5, 10 or 15 weeks of diabetes. The mucosal application of TC caused a similar degree of PD reduction in STZ-diabetic rats; ΔPD was 18.5 ± 3.8 mV in 15 week-STZ rats. However, the recovery of PD seen after exposure to TC was significantly delayed in these diabetic rats; the degree of PD recovery at 90 min post-treatment was $44.8 \pm 5.1\%$ in 15 week-STZ rats, which was significantly lower than in control rats injected with saline. Daily injection of insulin for 9 weeks to STZ rats significantly enhanced the basal PD values and the recovery of PD ($65.7 \pm 6.1\%$) after exposure to TC, although it did not affect the maximal reduction of PD in response to TC (ΔPD: 20.3 ± 4.5 mV).

GMBF response: The GMBF was significantly elevated during exposure of the normal rat stomach (23 week-old) to TC, reaching a peak value of $84.0 \pm 7.6\%$ increase, and remained elevated even after removal of TC from the chamber in the presence of 50 mM HCl (Figure 3a). Even at 90 min after treatment, the GMBF showed a significant increase ($30.4 \pm 3.8\%$) as compared to the preexposure values. This hyperaemic response caused by TC was significantly mitigated in STZ-treated rats, and the degree of inhibition was dependent on the duration of diabetes (Figure 3b). In particular, this hyperaemic response of the rat stomach following TC treatment was almost totally attenuated in 15 week diabetic rats, and the values of GMBF during and after exposure to TC were significantly lower than those observed in control rats. When STZ-diabetic animals were treated with insulin to decrease BGL, the impaired GMBF responses to TC plus HCl were totally normalized; the peak increase in GMBF was 87.3 ± 7.3 %, even higher than that observed in saline-injected control animals.

Acid back-diffusion: When the gastric mucosa was exposed to 50 mM HCl in the absence of acid secretion due to omeprazole

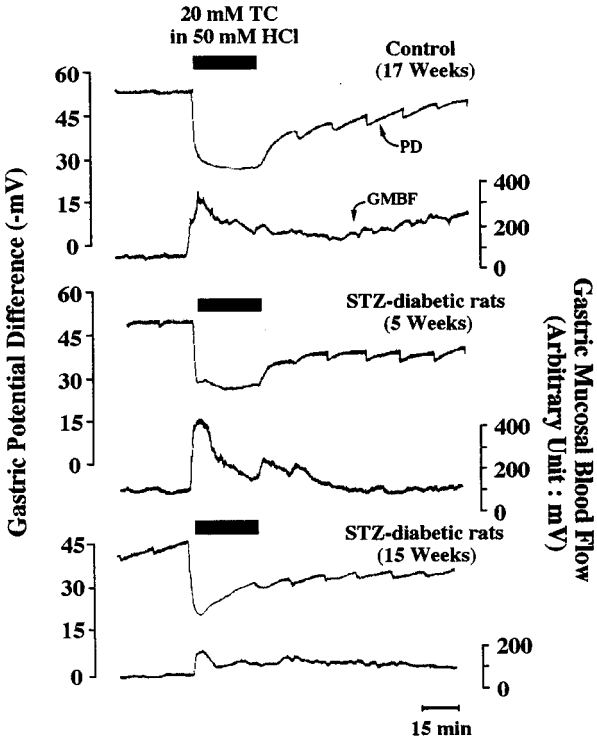

Figure 1 Representative recordings showing changes in transmucosal PD and GMBF after exposure of the stomach to TC plus HCl in a normal rat (18 week-old) and STZ-treated rats at 5 and 15 weeks of diabetes. The stomach was exposed to 20 mM TC plus 50 mM HCl (2 ml) for 30 min, and 50 mM HCl (2 ml) was applied to the stomach every 15 min before and after TC treatment. Note that the gastric hyperaemic response induced by TC was mitigated in the 15 week diabetic rat, despite that gastric PD was reduced similarly as compared to a normal rat.

Table 1 Changes in blood glucose levels in STZ-diabetic rats

Group	*Normal*	*STZ*	*STZ plus insulin*
	Blood glucose levels (mg dl^{-1})		
8 (0) week	139.2 ± 7.4	138.0 ± 5.2	147.3 ± 9.8
9 (1) week	141.5 ± 5.2	384.5 ± 7.9^{a}	382.3 ± 8.6^{a}
13 (5) weeks	136.7 ± 3.7	385.3 ± 7.0^{a}	$204.3 \pm 2.5^{a,b}$
18 (10) weeks	138.8 ± 2.6	392.0 ± 2.9^{a}	$181.5 \pm 12.7^{a,b}$
23 (15) weeks	127.0 ± 3.9	395.2 ± 2.2^{a}	ND

All values are presented as the means $\pm$ s.e. from 5–8 rats per group. Eight week-old rats were injected STZ (70 mg kg^{-1}, i.p.), and the experiments were performed using these rats after various periods of diabetes. Insulin (4 units rat^{-1}, s.c.) was injected once daliy for 9 weeks, starting 1 week after STZ treatment. Statistically significant different at $P < 0.05$; [a]from normal rats; [b]from STZ alone. The number of weeks in parenthesis indicates the diabetic period after STZ injection. ND: not determined.

treatment, a small but significant loss of luminal H^+ was consistently observed in control rats under normal conditions; ΔH^+ was less than 20 μEq (15 min)$^{-1}$ (Figure 4). Following the mucosal application of TC, the loss of H^+ was significantly increased and reached the maximal values immediately after the exposure, followed by a gradual decrease to the preexposure levels 90 min later. The maximal ΔH^+ value was 58.2 ± 3.2 μEq (15 min)$^{-1}$ in control rats (23 week-old). Although we measured luminal H^+ loss before and after exposure to TC in 8 week-old and 23 week-old control rats, no significant difference was observed in the values between these two groups (not shown). The mucosal permeability to H^+ in STZ-treated rats was significantly greater in basal conditions as compared to control rats, and the luminal acid loss [ΔH^+: 30–40 μEq (15 min)$^{-1}$ was 2–2.5 times the control value. The luminal acid loss in STZ-diabetic rats was further increased in response to TC, similar to control rats; the magnitude of H^+ loss observed immediately after TC treatment was 62.5 ± 9.1–68.3 ± 7.2 μEq (15 min)$^{-1}$ in the animals at 5 to 15 weeks of diabetes, which was not significantly different from that observed in control rats. The increased mucosal H^+ permeability responses to TC in diabetic rats were almost at the pre-exposure levels 90 min later, similar to normal rats. Insulin supplement to 10 week-STZ rats did not modify the degree of acid back-diffusion after TC treatment, but significantly reduced the mucosal permeability to H^+ in basal conditions.

Mucosal injury: Mucosal application of TC or acid solution (50 mM HCl) by itself did not induce gross damage in the gastric mucosa (not shown), but these treatments (TC plus 50 mM HCl) applied together produced haemorrhagic damage in the mucosa of the age-matched normal rats (23 week-old), the lesion score being 8.7 ± 2.9 mm^2 (Figure 5). These lesions were significantly worsened in STZ-treated rats, depending on the duration of diabetes: the lesion score was 26.0 ± 5.4 mm^2,

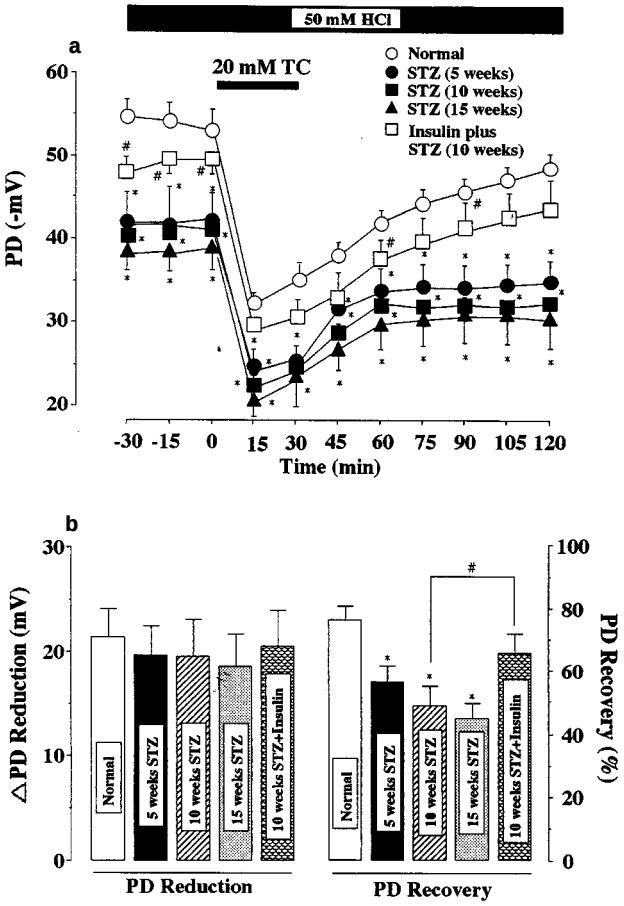

Figure 2 Changes in PD after exposure of the stomach to TC plus HCl in control rats (23 week-old) and STZ-treated rats at 5, 10, and 15 weeks of diabetes. (a) The stomach was exposed to 20 mM TC plus 50 mM HCl (2 ml), and 50 mM HCl (2 ml) was applied to the stomach every 15 min before and after TC treatment. Insulin (4 units rat^{-1}) was injected s.c. once daily for 9 weeks, starting 1 week after STZ treatment. ○: normal rats; ●: STZ rats (5 weeks); ■: STZ rats (10 weeks); ▲: STZ rats (15 weeks); □: insulin plus STZ rats (10 weeks). Data are the means ± s.e. of values determined every 15 min from 5–8 rats. (b) values indicate ΔPD reduction (maximal PD reduction: mV) and PD recovery (% recovery within 90 min after TC treatment) in each group, and are presented as the means ± s.e. from 5–8 rats. Statistically significant difference at $P<0.05$; * from normal rats; # from 10 week STZ rats.

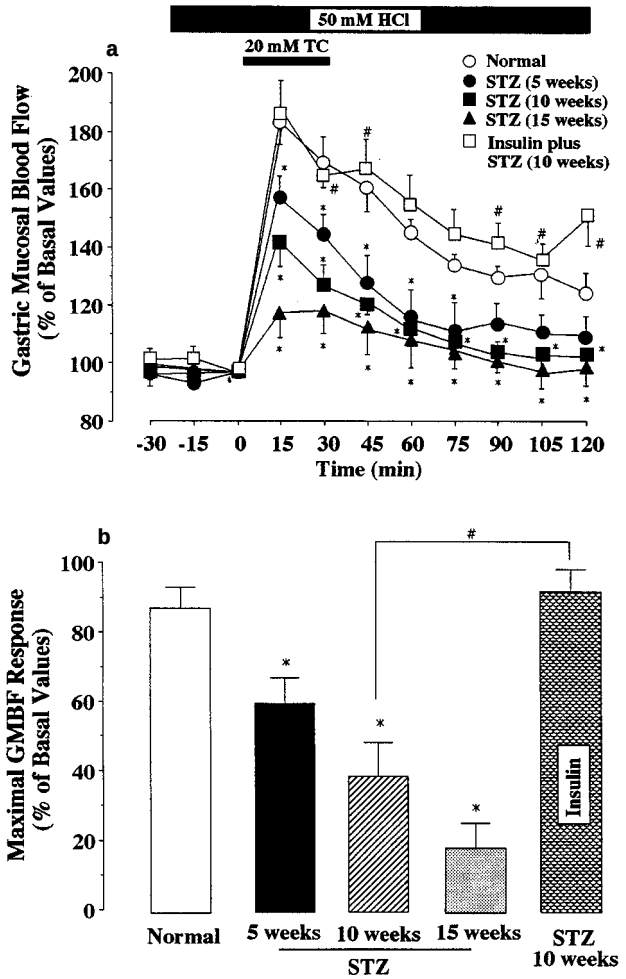

Figure 3 GMBF responses induced by TC plus HCl in control rats (23 week-old) and STZ-treated rats at 5, 10, and 15 weeks of diabetes. (a) The stomach was exposed to 20 mM TC plus 50 mM HCl (2 ml), and 50 mM HCl (2 ml) was applied to the stomach every 15 min before and after TC treatment. Insulin (4 units rat^{-1}) was injected s.c. once daily for 9 weeks, starting 1 week after STZ treatment. ○: normal rats; ●: STZ rats (5 weeks); ■: STZ rats (10 weeks); ▲: STZ rats (15 weeks); □: insulin plus STZ rats (10 weeks). Data are expressed as % increase from basal values observed immediately before TC treatment and represent the means ± s.e. of values determined every 15 min from 5–8 rats. (b) shows the peak responses in GMBF (% increase from basal values) in each group, and values are the means ± s.e. from 5–8 rats. Statistically significant difference at $P<0.05$; * from normal rats; # from 10 week STZ rats.

42.6 ± 5.6 mm^2 and 61.8 ± 4.8 mm^2, respectively, in 5 week, 10 week and 15 week diabetic rats. Histologically, the stomachs in control rats responded to TC by widespread disruption of epithelial cells without deep damage, but in diabetic rats the damage was deeper into the mucosa with severe haemorrhage (not shown). This increased gastric ulcerogenicity in STZ-diabetic rats was prevented by insulin treatment for 9 weeks starting 1 week after STZ injection, and the lesion score was decreased to 23.3 ± 3.0 mm^2, which was significantly lower than that observed in 10 week-STZ rats.

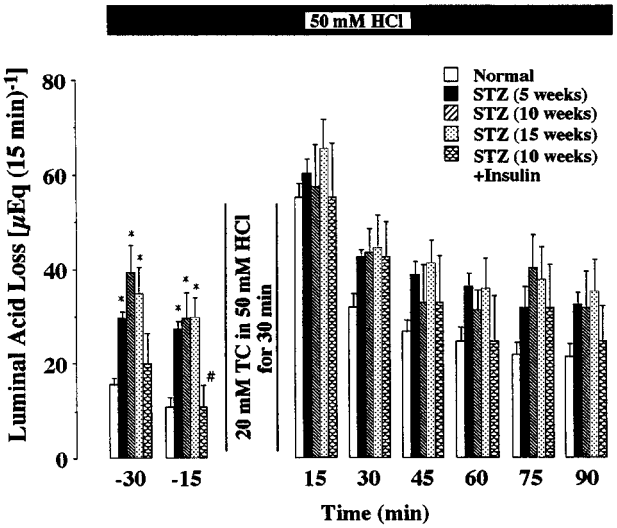

Figure 4 Amount of acid back-diffusion (luminal acid loss) after exposure of the stomach to TC plus HCl in control rats (23 week-old) and STZ-treated rats at 5, 10, and 15 weeks of diabetes. The stomach was exposed to 20 mM TC plus 50 mM HCl (2 ml), and 50 mM HCl (2 ml) was applied to the stomach every 15 min before and after TC treatment. Insulin (4 units rat^{-1}) was injected s.c. once daily for 9 weeks, starting 1 week after STZ treatment. Data are the means $\pm$ s.e. of values determined every 15 min from 5 $\sim$ 8 rats. Statistically significant difference at $P<0.05$; * from normal rats; # from 10 week STZ rats.

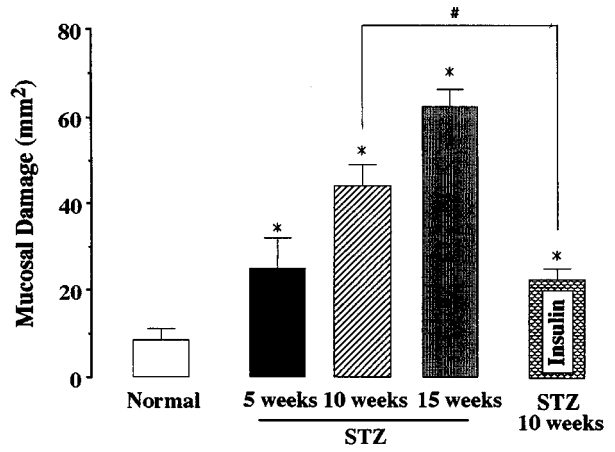

Figure 5 Development of gastric mucosal lesions after exposure of the stomach to TC plus HCl in control rats (23 week-old) and STZ-treated rats at 5, 10, and 15 weeks of diabetes. The stomach was exposed to 20 mM TC plus 50 mM HCl (2 ml), and 50 mM HCl (2 ml) was applied to the stomach every 15 min before and after TC treatment. Insulin (4 units rat^{-1}) was injected s.c. once daily for 9 weeks, starting 1 week after STZ treatment The animals were killed 90 min after TC treatment. Data are the means $\pm$ s.e. from 5–8 rats. Statistically significant difference at $P<0.05$; * from normal rats; # from 10 week STZ rats.

GMBF responses induced by mucosal application of capsaicin

To further investigate the different GMBF responses in STZ-diabetic rats, we compared the effects of intragastric capsaicin on GMBF in control and STZ-treated rats after various periods of diabetes. Mucosal application of capsaicin (0.1 mg ml^{-1}) caused a marked increase of GMBF in control rats (23 week-old), the peak response (% increase) being $98.2 \pm 8.9\%$ (Figure 6). However, the GMBF responses induced by capsaicin were less marked in STZ-treated animals and diminished depending on the duration of diabetes; the peak response (% increase) was $40.3 \pm 10.1\%$ and $30.8 \pm 3.8\%$, respectively, in 10 week and 15 week diabetic rats. This capsaicin treatment did not cause any effect on PD in either group of rats (not shown). The impaired GMBF response to capsaicin in 10 week-STZ rats was partially but significantly reversed by daily injection of insulin; the peak response was $74.6 \pm 6.4\%$, which was significantly higher than that in age matched STZ rats.

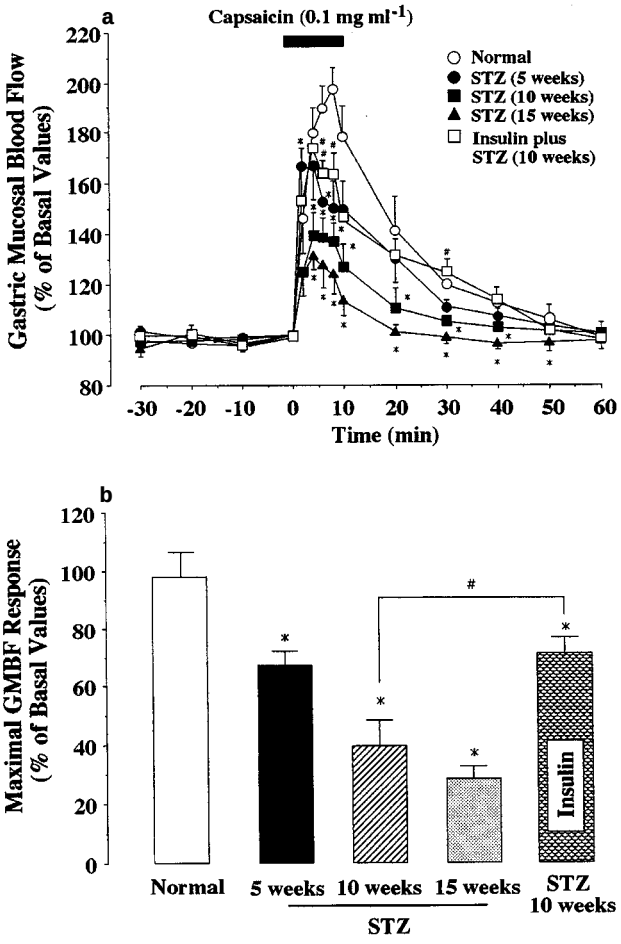

Figure 6 GMBF responses induced by intraluminal capsaicin in control rats (23 week-old) and STZ-treated rats at 5, 10, and 15 weeks of diabetes. (a) Capsaicin (0.1 mg ml^{-1}) was topically applied to the mucosa for 10 min, and the stomach was perfused with saline before and after capsaicin treatment. Insulin (4 units rat^{-1}) was injected s.c. once daily for 9 weeks, starting 1 week after STZ treatment. ○: normal rats; ●: STZ rats (5 weeks); ■: STZ rats (10 weeks); ▲: STZ rats (15 weeks); □: insulin plus STZ rats (10 weeks). Data are expressed as % increase from basal values observed immediately before capsaicin treatment and represent the means $\pm$ s.e. of values determined every 15 min from 5–6 rats. (b) shows the peak responses in GMBF (% increase from basal values) in each group, and values are the means $\pm$ s.e. from 5–6 rats. Statistically significant difference at $P<0.05$; * from normal rats; # from 10 week STZ rats.

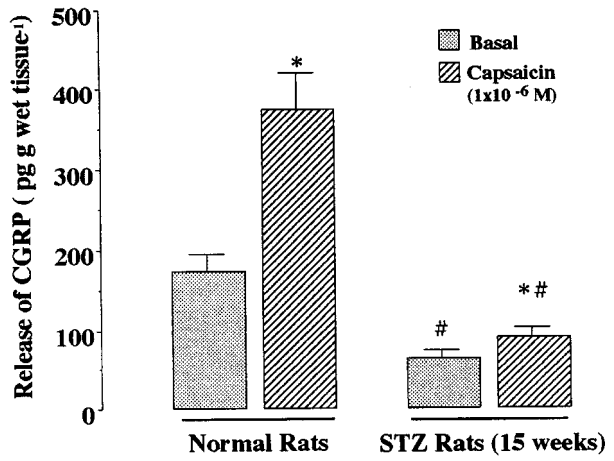

Figure 7 CGRP release in response to capsaicin in isolated stomachs of normal (23 week-old) and diabetic rats (15 weeks). The corpus mucosa was incubated for 30 min in the absence or presence of capsaicin (1×10^{-6} M). The CGRP content of the supernatant was determined by chemiluminescent enzyme immunoassay. Data are presented as the means ± s.e. from 5–6 rats. Statistically significant difference at $P<0.05$; * from basal values; # from normal rats.

CGRP release in response to capsaicin in isolated stomach

The basal release of CGRP for 30 min in the control rat stomach was 172.5 ± 20.9 pg (g wet tissue weight^{-1}). Incubation of these stomachs with capsaicin (1×10^{-6} M) for 30 min caused a significant increase of CGRP release; the value was 379.5 ± 45.7 pg (g wet tissue weight^{-1}), about 220% of basal release that occurred spontaneously (Figure 7). In 15 week diabetic animals, however, the basal release of CGRP from the stomach was significantly lower than in control rats, and capsaicin stimulation also caused a significantly less increase of CGRP release from the stomach, the values being 88.1 ± 12.6 pg (g wet tissue weight^{-1}), which is only 133.7% of basal release [65.9 ± 10.3 pg (g wet tissue weight^{-1})].

Discussion

We found in the present study that STZ-induced diabetes impairs the gastric hyperaemic response induced by acid back-diffusion following barrier disruption and leads to increased mucosal susceptibility to acid injury. This phenomenon is associated with dysfunction of capsaicin-sensitive sensory neurons, because capsaicin, a selective stimulus of sensory neurons, failed to elicit release of CGRP in the stomach and to increase GMBF in STZ-diabetic animals. Insulin treatment corrected the impaired GMBF response to normal levels, suggesting that the GMBF impairment may be insulin-dependent but not due to nonspecific effects of STZ.

STZ is known to possess diabetogenic properties and cause selective destruction of pancreatic β-cells. As expected, all STZ-treated animals developed a persistent hyperglycemia, which was observed one week after STZ injection. In STZ-diabetic rats we found characteristic changes in mucosal H^+ permeability under basal conditions without any treatment; a decrease in basal PD values and an increase in mucosal H^+ permeation, depending on the duration of diabetes, in agreement with the findings by Hung (1994). In this study, the enhanced mucosal H^+ permeation manifested itself as an increase of spontaneous acid back-diffusion. These results account for the lowered PD values and are in agreement with the increased mucosal permeability in STZ-diabetic rats when determined by luminal appearance of Evans blue (Takeuchi *et al*., 1994a). Although the mechanism underlying the increase of mucosal permeability in diabetic rats remains unknown, this may also be a factor contributing to the increased susceptibility of the mucosa to ulcerogenic stimuli.

Exposure of the stomach to TC caused disruption of the gastric mucosal barrier as reflected by PD reduction, followed by a marked loss of luminal acid and an increase of GMBF. The stomachs of diabetic animals similarly responded to TC, resulting in a PD reduction and an occurrence of acid back-diffusion, yet the accompanying hyperaemic response was markedly diminished in these animals. In a previous study, we showed that the gastric hyperaemic response induced by TC plus HCl was almost totally attenuated in sensory deafferented animals by pretreatment with a large dose of capsaicin (Takeuchi *et al*., 1993). Holzer *et al*. (1991a,b) reported that these sensory nerves signal for an increase of GMBF in association with acid back-diffusion. On the basis of these findings, we speculate that the failure of GMBF response in diabetic animals is at least partly due to dysfunction of these sensory neurons. Indeed, intragastric application of capsaicin failed to increase GMBF in STZ-diabetic rats, although this agent caused a marked hyperaemia in the normal rat stomachs. Certainly, the increased GMBF responses caused by intragastric capsaicin are attenuated by sensoy deafferentation following capsaicin pretreatment (Holzer *et al*., 1991a; Matsumoto *et al*., 1992). In addition, because the impaired GMBF response in STZ-diabetic rats was significantly restored by daily injection of insulin, it is likely that such GMBF impairment is insulin-dependent but not due to a nonspecific effect of STZ.

Li *et al*. (1991) reported that CGRP is a neurotransmitter involved in the mechanism of GMBF response induced by intragastric capsaicin. The same authors also showed that gastric hyperaemic response associated with acid back-diffusion is mediated by CGRP (Li *et al*., 1992), supporting the contention that capsaicin-sensitive sensory nerves signal for an increase of GMBF in the face of pending acid injury. A decrease in the number of neuropeptide-containing nerve fibres (CGRP, vasoactive intestinal peptide, acetylcholine, etc) in the stomach and intestine has been reported in STZ-treated rats after 8 weeks of diabetes (Lincoln *et al*., 1984; Belai & Burnstock, 1987; Belai *et al*., 1991). Lincoln *et al*. (1984) showed that the density of CGRP-immunoreactive fibres in the intestine was markedly decreased in diabetic rats and that the CGRP release in response to electrical stimulation was decreased in these animals. We confirmed in the present study that the amount of CGRP released in the isolated stomach in response to capsaicin was significantly lower in STZ-diabetic animals than in normal rats. These data together also support notion that there is a dysfunction of capsaicin-sensitive afferent neurons in the gastric mucosa of diabetic rats. We previously reported that duodenal HCO_3^- secretion in response to 16,16-dimethyl prostaglandin E_2 and vasoactive intestinal peptide as well as mucosal acidification was decreased in STZ-diabetic rats, suggesting a decreased sensitivity of the mucosa to mediators of secretion (Takehara *et al*., 1997). It might be possible that a decreased sensitivity of the vasculature to CGRP is also involved in the mechanism of GMBF impairment in STZ-diabetic rats.

It is generally accepted that GMBF plays a role in maintaining gastric mucosal integrity by supplying oxygen and nutrients and by removing toxic substances, in addition to

buffering hydrogen ions, and contributes to mucosal repair as well as mucosal protection. As expected, the process of PD recovery after TC treatment was significantly delayed in diabetic rats, resulting in a marked aggravation of gastric lesions. We have observed similar results after TC treatment in sensory deafferented rats; chemical ablation of sensory nerves significantly delayed the process of PD recovery and worsened gastric mucosal lesions after exposure of the stomach to TC plus HCl (Takeuchi *et al*., 1993). Holzer *et al*. (1991b) also demonstrated that the attenuation of gastric hyperaemic response by sensory deafferentation potentiated the mucosal lesions induced by ethanol plus HCl. These results may indicate a similarity of deleterious influences on the mucosal integrity between diabetes and sensory deafferentation. Thus, it is likely that the potentiation of TC-induced gastric lesions in STZ-diabetic rats is at least partly accounted for by the impairment of GMBF response mediated by capsaicin-sensitive sensory neurons.

Several studies showed a deficiency in the mechanisms underlying the nitric oxide (NO)-mediated vascular response in diabetic rats (Pieper *et al*., 1992; Diederich *et al*., 1994; Busse & Fleming, 1996). NO is a vasodilator substance that increases GMBF, similar to CGRP, and this substance is important in the modulation of gastric mucosal integrity, through interaction with sensory neurons (Whittle *et al*., 1990). The increase of GMBF caused by intragastric capsaicin is mitigated by N^G-monomethyl L-arginine (Whittle *et al*., 1992), and the gastric cytoprotection induced by capsaicin is also attenuated by the NO synthase inhibitor (Lambrecht *et al*., 1993). Furthermore, gastric hyperaemia caused by acid back-diffusion or CGRP is mediated partly by endogenous NO (Lippe & Holzer, 1992). It is possible that the impaired GMBF response in diabetic rats is due to a decreased release and or production of endogenous NO, in addition to dysfunction of capsaicin-sensitive sensory neurons. Additionally, it has been reported that the vascular contractility and sensitivity to thromboxane and its mimetics were changed in diabetic animals, the phenomenon probably being associated with alteration of its receptor and smooth muscle cell proliferation, and these changes were reversed by insulin therapy (Morinelli *et al*., 1993). Thus, alteration in the vasculature itself should also be considered as the background for the increased mucosal susceptibility in diabetic rats.

Taken together, the present results demonstrated that the gastric mucosal hyperaemic response induced by acid back-diffusion following barrier disruption was mitigated in diabetic animals and that this phenomenon is insulin-sensitive and may be associated with dysfunction of capsaicin-sensitive afferent neurons, leading to an increase of the mucosal susceptibility to acid injury.

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