Regulation of Transglutaminase Activity by Polyamines in the Gastrointestinal Mucosa of Rats (43672)

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Abstract. **Transglutaminases catalyze the covalent cross-linking of protein and are involved in the mechanism of polyamine-dependent mucosal healing. The current study examined the effect of polyamines on transglutaminase activity in gastrointestinal mucosa. Rats were fasted 22 hr before experiments and enzyme activity was measured as the Ca^{++}-dependent covalent incorporation of [^{3}H]-putrescine into acid-precipitable protein. In some of the experiments, mucosal ornithine decarboxylase (ODC) activity and polyamine levels were also examined. Transglutaminase activity in both gastric and duodenal mucosa increased significantly after polyamine administration. Treatment with α-difluoromethylornithine (DFMO) decreased both basal ODC activity and putrescine levels in the duodenal mucosa. DFMO also significantly decreased mucosal transglutaminase activity. In stress or hypertonic NaCl-induced gastric mucosal injury models, increased polyamine biosynthesis was associated with increased transglutaminase activity, which was completely prevented by DFMO. Exogenous polyamines returned transglutaminase activity toward control levels in the presence of DFMO. In conclusion, these results indicate that: (i) luminal polyamines increase transglutaminase activity in gastric and duodenal mucosa; (ii) polyamine depletion caused by the inhibition of ODC is accompanied by a significant decrease in transglutaminase activity; and (iii) exogenous polyamines significantly reverse the decrease in transglutaminase activity caused by polyamine depletion.**

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Transglutaminases catalyze the covalent cross-linking of proteins and are involved in the post-translational modification of proteins by establishing ε-(γ-glutaminyl)-lysyl isopeptide bonds between protein-bound glutamine and lysine residues (1, 2). The enzyme activity occurring in both intracellular and extracellular compartments is the result of the activity of biochemically and immunochemically different forms of transglutaminases encoded by distinct genes (3–5). Tissue transglutaminase is a cytosolic enzyme related to the interactions between cells and the extracellular matrix (5, 6). Recent studies (5, 7, 8) have shown that transglutaminase cross-linking matrix proteins are implicated in cellular adhesion, proliferation, and differentiation.

Polyamines, spermidine and spermine, and their precursor, putrescine, are organic cations found in all eukaryotic cells and may serve as natural substrates for transglutaminases (9, 10). Since the Km for the incorporation of polyamines into protein in the transglutaminase reaction is in the range of concentrations that are found *in vivo*, it is likely that this reaction is involved in the biological action of polyamines (11). Transglutaminases catalyze the covalent conjugation of polyamines to proteins by facilitation of γ-glutamyl-putrescine, -spermidine or -spermine (10), and N,N-bis-γ-glutamyl-spermine bridges (12). The condensation of a polyamine with a specific glutamine residue could modify the structure and activity of proteins. On the other hand, because the conjugated polyamine contains a terminal amino group, it may be a substrate for a second conjugation reaction (13).

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In the gastrointestinal mucosa, two different processes increase intracellular polyamines. First, ornithine decarboxylase (ODC) catalyzes the decarboxylation of the amino acid ornithine to form putrescine (14). The first rate-limiting step in polyamine synthesis is induced by a number of hormones and growth factors, and also specifically inhibited by α-difluoromethylornithine (DFMO) (9, 14). Second, intestinal epithelial cells accumulate polyamines by absorption from the lumen (15). A series of studies involving the administration of luminal polyamines has demonstrated that these compounds play central roles in mucosal healing following damage and in the regulation of normal mucosal growth (16, 17). We recently have shown that polyamines accelerate repair of the damaged mucosa, at least partly, by influencing transglutaminase activity and protein cross-linking (18, 19). To our knowledge, however, no studies concerning the role of polyamines in the regulation of transglutaminase activity have been documented.

The purpose of the current study was to determine whether polyamine levels regulated transglutaminase activity. We examined this, first, by determining whether luminal polyamines altered enzyme activity; second, by using DFMO to deplete mucosal polyamines and observing the effect of depletion on transglutaminase activity; by examining the effect of increased mucosal polyamine synthesis on transglutaminase activity; and finally, by elucidating whether exogenous polyamines could substitute for the newly synthesized compounds in regulating enzyme activity. Some of these data have been published in abstract form (20).

Material and Methods

Materials and Experimental Design. L-[1-^{14}C] ornithine (sp act 51.6 mCi/mmol) and [2,3-^{3}HCN] putrescine dihydrochloride (sp act 40.3 Ci/mmol) were obtained from New England Nuclear (Boston, MA). DFMO was a kind gift of the Merrell Dow Research Institute (Cincinnati, OH). Putrescine, spermidine, and spermine as hydrochloride salts were purchased from Sigma Chemical Co. (St. Louis, MO) and suspended in normal saline, 0.9% NaCl immediately before use.

Male Sprague-Dawley rats weighing between 125–150 g were housed in wire-bottomed raised cages and given water and standard laboratory rat food *ad libitum*. All animals were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The animals' quarters were maintained at a temperature of 22 ± 1°C with a 12-hr light-dark cycle. Animals were deprived of food but allowed free access to tap water for 22 hr before the experiments. Each study was carried out using six to eight rats per group. These studies were conducted under protocol number 549 approved by the University of Tennessee Health Science Center Animal Care and Use Committee on August 12, 1992.

In the first study, the effects of treatment with a single dose of putrescine, spermidine or spermine, on transglutaminase activity in gastric oxyntic gland and duodenal mucosa were examined. Polyamines were given intragastrically at a dose of 100 mg/kg, and at different intervals after treatment animals were killed by anesthesia with excess methoxyflurane. The stomach and duodenum were removed and opened, and the oxyntic gland and duodenal mucosa were scraped from the underlying smooth muscle with a glass slide. The mucosal scrapings were used for the measurement of enzyme activity.

In the second study, the influence of inhibition of polyamine biosynthesis by α-difluoromethylornithine (DFMO) on transglutaminase activity of gastric and duodenal mucosa was determined. DFMO was given intraperitoneally at the dose of 500 mg/kg, and mucosal polyamine levels, ODC and transglutaminase activities were measured 1, 2, 3, 4, and 6 hr after administration of DFMO. In some of the experiments, we also examined the effects of different doses of putrescine on transglutaminase activity in DFMO-treated rats. Putrescine was given intragastrically at doses of 50 and 100 mg/kg 30 min following DFMO, and the enzyme activity was assayed 3 hr after treatment with putrescine.

In the third study, we investigated the changes in transglutaminase activity in stress or hypertonic NaCl-induced gastric injury and examined whether luminal polyamines could substitute for these endogenously produced by ODC, preventing the inhibition of transglutaminase activity in rats treated with DFMO. The method for the production of gastric stress erosions was basically that described originally by Takagi and Okabe (21). In brief, rats were placed in restraint cages and immersed vertically to the level of the xiphoid process in a water bath (23°C) for 6 hr. DFMO was administered intraperitoneally 30 min before stress and repeated 1 hr after 6 hr stress. Spermidine was given intragastrically immediately after the period of stress. Animals were killed 0 and 4 hr following stress, and gastric and duodenal mucosa were treated as described above. When injury was induced by hypertonic NaCl, DFMO was injected intraperitoneally 10 min before orally giving 1 ml of 3.4 *M* NaCl (19). Spermidine was given intragastrically at a dose of 100 mg/kg 2 hr after treatment with 3.4 *M* NaCl, and the vehicle alone was given to the control animals. The animals were killed 3 hr after administration of spermidine.

Enzyme Assays. Transglutaminase activity was assayed as the Ca^{++}-dependent covalent incorporation of [^{3}H]-putrescine into acid-precipitable protein by the modified method of Lorand *et al.* (22). Tissue

samples were collected as above and placed in 1.0 ml of 20 m*M* tris (hydroxymethyl) aminomethane (Tris) buffer (pH 7.5) containing 1 m*M* EDTA, 0.1% mercaptoethanol. The mucosa was homogenized for 20 sec, sonicated for 15 sec, and then centrifuged at 30,000-g at 4°C for 30 min. The reaction mixture consisted of 0.1 m*M* putrescine, containing 3 μCi ^{3}H-putrescine, 5 mg/ml casein, and 12.5 m*M* $CaCl_2$ in 50 m*M* Tris-HCl buffer (pH 9.0) with 0.25% mercaptoethanol. After 15 min of incubation at 37°C in a shaking water bath, 50 μl were spotted onto 3MM Whatman paper filters and immediately plunged into 10% ice-cold trichloroacetic acid for 15 min. Two consecutive 5-min washings were performed in 5% ice-cold trichloroacetic acid followed by a brief washing in 95% ethanol. The radioactivity present in the dried paper filters was measured by liquid scintillation spectroscopy (LS 5000 TA, Beckman, Fullerton, CA). Aliquots of the 30,000-g supernatant were assayed for total protein, using the method described by Bradford (23). Enzymatic activity was expressed as picomoles of putrescine per milligram of protein per hour.

The ODC activity was measured by a radiometric technique in which the amount of $^{14}CO_2$ liberated from L-[1-^{14}C] ornithine was determined (24). Tissues were collected as above and placed in 1.0 ml (pH 7.4) of 67 m*M* sodium-potassium phosphate buffer containing 0.02% lauryl ether, 0.5 m*M* NAF, 0.1 m*M* pyridoxal phosphate, 10 μ*M* EDTA, and 2 m*M* dithiothreitol (DTT). ODC activity is dependent on pyridoxal phosphate, and DTT stabilizes the enzymatic activity (25). The mucosa was homogenized, and centrifuged at 30,000-g at 4°C for 30 min. A 200 μl aliquot of the supernatant was incubated in a stoppered test tube in the presence of 3.8 nmol L-[^{14}C]-ornithine for 15 min at 37°C. The liberated $^{14}CO_2$ from the decarboxylation of ornithine was trapped on a piece of filter paper impregnated with 20 μl of 2.0 *N* NaOH, which was suspended in a center well above the reaction mixture. The reaction was stopped by the addition of trichloroacetic acid to a final concentration of 10%. The $^{14}CO_2$ trapped in the filter paper was measured, and enzymatic activity was expressed as picomoles $^{14}CO_2$ per hour per milligram of protein.

High-Performance Liquid Chromatographic (HPLC) Analysis of Mucosal Polyamines. The mucosal polyamine content was determined as we have previously described (16). Mucosal scrapings were collected in glass tubes and frozen at −85°C until analysis. The samples were placed in 0.4 *N* perchloric acid, homogenized for 20 sec, ultrasonicated for 15 sec, and then centrifuged at 1,600-g for 10 min. This supernatant was collected and neutralized to pH 7.0 with 3 *M* KOH, and centrifuged to remove the precipitate. A 0.5 ml aliquot of the solution was delivered to clean Sp glass tubes with screw caps. After addition of 0.25 ml saturated Na_2CO_3 and 0.5 ml dansyl chloride solution (10 mg/ml acetone), the reaction was allowed to proceed while heating at 70°C for 30 min, and the mixture was then added to 1.5 ml toluene. After mixing and centrifugation, the organic portion was collected and dried by vacuum centrifugation. To the residue, 300 μl methanol was added and filtered, and an aliquot of 200 μl was used for HPLC analysis. A Waters (Milford, MA) HPLC system was used, which included a 710 Wisp automatic sample injector, two 6000 A solvent delivery units, a 680 solvent programmer, and a Novapack C_{18} column in a radial compression module. Solvent A and B were composed of acetonitrile, water, glacial acetic acid, and triethylamine in the proportions of 80:20:0.02:0.001 and 95:5:0.02:0.005, respectively. The mobile phases used in this separation consisted of a linear gradient starting at 68% solvent A and 32% solvent B, and increasing in solvent B linearly to 100%. Each sample was run for 23 min and the equilibration delay between injections was 2 min. Sufficient mobile phases (A and B) were prepared fresh before starting the automatic injector. Measurements of the polyamines putrescine, spermidine, and spermine were made by comparing ratios of polyamines to 1,10-diaminodecane peak areas with a standard curve. Samples for a calibration curve were obtained by adding known amounts of standards to 1 ml of glass distilled water followed by extraction and dansylation as described above. Protein was dissolved in 1 *M* NaOH and measured by Bradford (23) method. The quantities of polyamines are expressed as nanomoles per milligram protein.

Statistics. Data are presented as mean ± SEM of six to eight rats per group. Statistical analysis was performed using a two-tailed Dunnett's multiple comparison test (26), and values of $P < 0.05$ were regarded as significant.

RESULTS

Effects of Polyamines on Transglutaminase Activity. Basal transglutaminase activity in both gastric oxyntic gland and duodenal mucosa was significantly increased by oral administration of polyamines (Figs. 1 and 2). Maximum increases in the enzyme activity of gastric mucosa were observed 3 hr after a single treatment with spermidine and represented 1.6 times control values. By 6 hr, mucosal transglutaminase activity had returned to normal levels. In the same animals, transglutaminase activity of the duodenal mucosa increased significantly 2 hr after exposure to spermidine and remained significantly elevated 4 hr after treatment. Enzyme activity peaked between 2 to 3 hr after spermidine at a level ~1.9 times control. Increases in transglutaminase activity were linear with doses of spermidine ranging from 50 to 125 mg/kg, and the threshold dose of spermidine for stimulating gas-

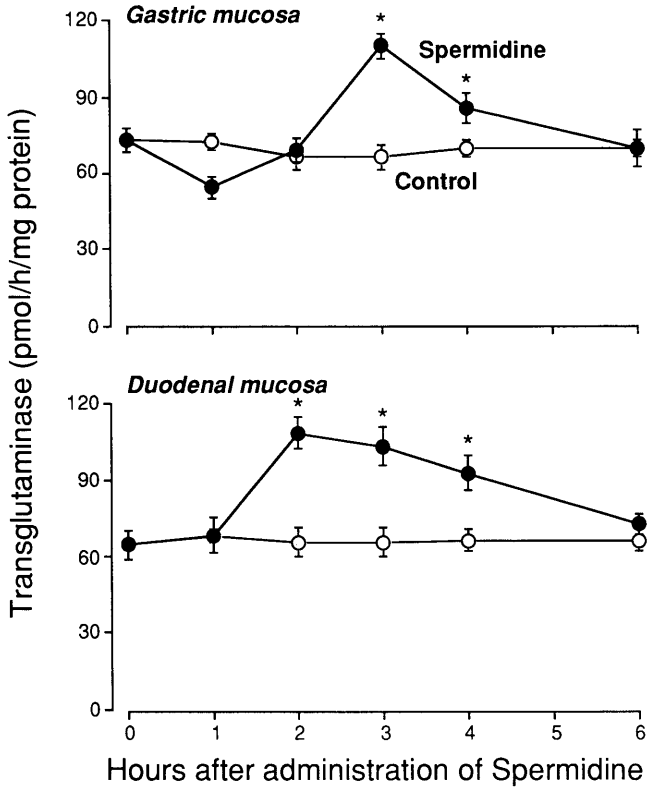

Figure 1. Time-course changes of transglutaminase activity in oxyntic gland and duodenal mucosa after administration of spermidine. Spermidine was given intragastrically at dose of 100 mg/kg. Animals were killed at indicated time points, and mucosa was collected for the enzyme determination. Values represent means ± SEM for six rats per group. *$P < 0.05$ compared with control group.

tric and duodenal transglutaminase was 75 mg/kg (data not shown). Administration of putrescine and spermine, like spermidine, also significantly stimulated mucosal transglutaminase activity in the stomach and duodenum (Fig. 2).

Effects of DFMO on Mucosal Transglutaminase, ODC Activity, and Polyamine Levels. Administration of DFMO at a dose of 500 mg/kg significantly decreased transglutaminase activity in the duodenal mucosa, although it had no effect on the identical enzyme in the gastric oxyntic gland mucosa (Fig. 3). The decrease in transglutaminase activity of duodenal mucosa was significant 1 hr and maximal 2 and 4 hr after treatment with DFMO. In the gastric mucosa, transglutaminase activity in DFMO-treated rats was indistinguishable from that of controls (without DFMO).

A single treatment with DFMO was also without effect on basal ODC activity and polyamine levels in the gastric mucosa (Fig. 4 and Table I). There were no significant differences in gastric mucosal ODC activity and putrescine, spermidine, and spermine levels between controls and rats treated with DFMO. On the other hand, duodenal mucosal basal ODC activity was almost completely inhibited by DFMO. The inhibition of enzyme activity began 1 hr and lasted 6 hr after the

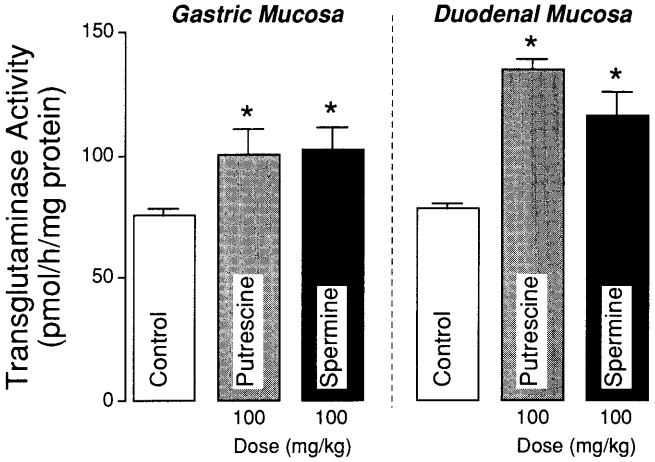

Figure 2. Transglutaminase activity in oxyntic gland and duodenal mucosa after administration of putrescine and spermine. Putrescine or spermine was given intragastrically at a dose of 100 mg/kg, and animals were killed 3 hr after treatments. Values represent means ± SEM for six rats per group. *$P < 0.05$ compared with control group.

intraperitoneal administration of DFMO. As can be seen in Table I, decreased duodenal ODC activity was paralleled by decreased in mucosal putrescine levels. Maximal decreases in putrescine levels in the duodenal mucosa occurred at 2 and 4 hr after treatment with DFMO and were 14%–19% of control levels. Neither

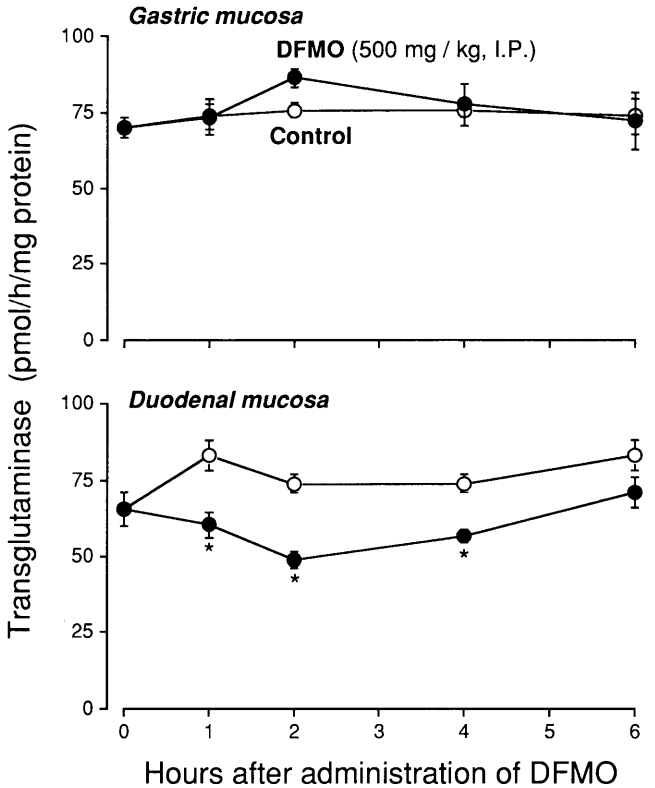

Figure 3. Transglutaminase activity in oxyntic gland and duodenal mucosa after treatment with α-difluoromethylornithine (DFMO). DFMO was given intraperitoneally at the dose of 500 mg/kg, and ODC activity was measured at indicated time points. Values represent means ± SEM for six rats per group. *$P < 0.05$ compared with control groups.

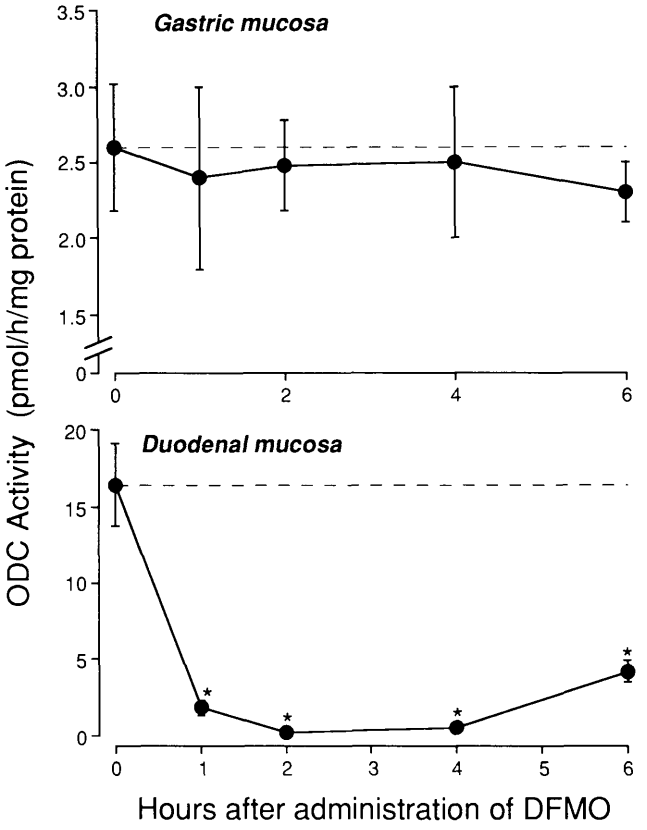

Figure 4. Ornithine decarboxylase (ODC) activity of oxyntic gland and duodenal mucosa from rats described in Figure 3. Values represent means ± SEM for six rats per group. *$p < 0.05$ compared with time 0.

spermidine nor spermine in the duodenal mucosa was significantly decreased in DFMO-treated rats.

Effects of Putrescine on Transglutaminase Activity in DFMO-Treated Rats. Oral administration of putrescine completely prevented the decreases in transglutaminase activity in the presence of DFMO. Figure 5 shows that DFMO at a dose of 500 mg/kg significantly decreased basal transglutaminase activity in the duodenal mucosa but had no effect on enzyme activity in the gastric mucosa. The combined administration of putrescine and DFMO reversed the inhibitory effects of DFMO on the activity of duodenal mucosal transglutaminase. In the case of the duodenum, the decreased enzyme activity in DFMO-treated rats returned toward control levels when putrescine was given at the dose of 50 mg/kg. When 100 mg/kg of putrescine were administered together with DFMO, however, transglutaminase activity was increased 21% in the gastric mucosa, 23% in the duodenal mucosa, respectively, compared with the controls. Neither of these doses of putrescine had an effect on ODC activity in the absence of DFMO (data not shown).

Effects of DFMO and Spermidine on Transglutaminase Activity in Stress or Hypertonic NaCl-Induced Mucosal Injury. We used stress and hypertonic NaCl-induced damage to examine whether transglutaminase activity in gastric oxyntic gland and duodenal mucosa could be influenced by increased endogenous polyamine synthesis. Our previous studies have demonstrated that stress or hypertonic NaCl-induced mucosal lesions are accompanied by significant increases in ODC activity and tissue polyamine levels (18, 27). As shown in Figure 6, transglutaminase activity in the gastric and duodenal mucosa increased significantly after 6-hr stress and remained elevated over that of the corresponding control for 4 hr into recovery. The increase in enzyme activity was totally prevented by administration of DFMO at a dose of 500 mg/kg. During recovery, treatment with spermidine at doses of 50 or 100 mg/kg significantly decreased the inhibition of transglutaminase activity in both tissues of rats treated with DFMO.

Gastric mucosal ODC and transglutaminase activities increased significantly after exposure to 3.4 *M* NaCl for 5 hr (Fig. 7). These increases in ODC and transglutaminase activities were completely prevented when DFMO at a dose of 500 mg/kg was administered intraperitoneally. The combined administration of spermidine at a dose of 100 mg/kg with DFMO had no influence on the decreased ODC activity. In the same animals, however, the transglutaminase activity in the gastric mucosa was significantly increased by the dose of spermidine.

Discussion

Transglutaminases are a group of enzymes that have been isolated, cloned, and sequenced (28, 29), but little is known about the regulation of their activity *in vivo*. We are particularly interested in the effect of polyamines on transglutaminase activity in the gastrointestinal mucosa, since these compounds serve as natural substrates for the enzymes and also play a physiological role in the regulation of mucosal growth (30). The current experiments clearly demonstrate that polyamines, either supplied luminally or synthesized endogenously, modulate transglutaminase activity. Oral administration of polyamines, putrescine, spermidine, and spermine, significantly increased transglutaminase activity in both gastric oxyntic gland and duodenal mucosa (Figs. 1 and 2). Inhibition of polyamine biosynthesis by DFMO was associated with the reduction of transglutaminase activity in normal rats and those stressed or treated with hypertonic NaCl solution (Figs. 3, 6, and 7). Furthermore, exogenous polyamines effectively substituted for tissue polyamines and reversed the inhibitory effect on the activity of transglutaminase in DFMO-treated rats (Figs. 5–7).

Transglutaminases have been found in a wide variety of tissues, cells and body fluids (2, 31, 44). The enzyme activity was first demonstrated in the liver and in some other tissues of the guinea pig (2). There are

Table I. Polyamine Levels of Oxyntic Gland and Duodenal Mucosa After a Single Treatment with α-Difluoromethylornithine (DFMO)[a]

Hours after DFMO	Putrescine		Spermidine		Spermine	
	(nmol/mg, protein)	(% Change)	(nmol/mg, protein)	(% Change)	(nmol/mg, protein)	(% Change)
Gastric mucosa						
0	0.13 ± 0.001		7.19 ± 0.21		6.55 ± 0.45	
1	0.10 ± 0.05	−23.1	7.07 ± 0.24	11.7	6.42 ± 0.30	−2.0
2	0.11 ± 0.01	−15.4	8.57 ± 0.24	19.2	7.35 ± 0.46	12.2
4	0.15 ± 0.01	15.4	8.95 ± 0.79	24.5	8.65 ± 0.84	32.1
6	0.13 ± 0.01	0	9.34 ± 0.23	29.9	8.48 ± 0.29	29.4
Duodenal mucosa						
0	2.15 ± 0.16		7.34 ± 0.21		3.51 ± 0.18	
1	0.63 ± 0.04*	−70.7	8.31 ± 0.46	13.2	3.61 ± 0.29	2.8
2	0.40 ± 0.02*	−81.4	7.83 ± 0.38	6.6	3.65 ± 0.38	4.0
4	0.31 ± 0.02*	−85.6	7.38 ± 0.36	0.5	3.59 ± 0.48	2.2
6	0.51 ± 0.01*	−76.3	7.47 ± 0.32	1.8	3.54 ± 0.38	1.0

[a] Values represent means ± SEM for 6 rats per group. DFMO was given intraperitoneally at the dose of 500 mg/kg, and polyamine levels were measured at indicated time points.
* $P < 0.05$ compared with time 0.

three different forms of transglutaminase, plasma factor XIIIa, the membrane-bound epidermal enzyme, and the tissue forms (2–4). The extracellular transglutaminases, Factor XIII and the enzyme of seminal plasma, catalyze the polymerization of gamma and alpha chains of fibrin during the clotting reactions of plasma proteins (31). The membrane-bound epidermal transglutaminase, restricted to skin and squamous epithelium, may be related to terminal differentiation of keratinocytes (32). Tissue transglutaminase is involved in the interaction between cells and extracellular matrix (33). The induction of tissue transglutaminase activity and protein cross-linking are involved in cellular adhesion, endocytosis, neoplasia, and cell proliferation (5, 8).

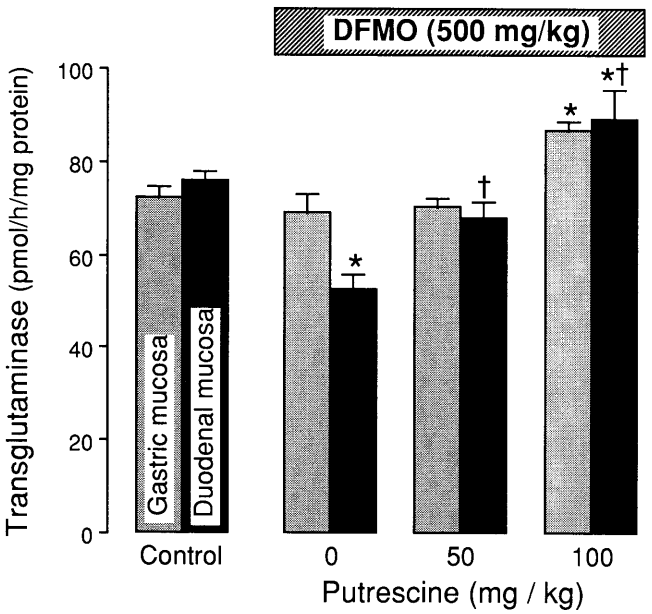

Figure 5. Effect of α-difluoromethylornithine (DFMO) and different doses of putrescine together with DFMO on transglutaminase activity in both oxyntic gland and duodenal mucosa. DFMO was given intraperitoneally at the dose of 500 mg/kg 30 min before oral administration of putrescine. Animals were killed 3 hr after treatment with putrescine. Values represent means ± SEM for six rats per group. *$P < 0.05$ compared with control. †$P < 0.05$ compared with DFMO-treated group.

A striking characteristic of transglutaminases is that polyamines can serve as natural substrates for the enzyme (10). These reactions occur both in extracellular fluid, as in the formation of the cervical plug in rat seminal plasma (31), and in cells, as in the case of hepatocyte nuclear protein (34). At physiological pH, putrescine, spermidine, and spermine possess two, three, and four positive amine groups, respectively, and are readily conjugated to proteins by transglutaminase *in vitro* as well as *in vivo* (7, 34). The products of transglutaminase-catalyzed reactions are γ-glutamyl-putrescine, -spermidine, or -spermine. Several studies (2, 6, 7) have shown that polyamine conjugation not only modifies specific glutamine residues, but that the free amino group of the polyamine can then form a peptide bond with a glutamyl residue on a second protein, cross-linking the two proteins.

Polyamines are small aliphatic nitrogenous bases that function as organic cations in eukaryotic cells (9, 14). These substances have been shown to be involved in many cellular processes by virtue of their effects on enzyme activities, nucleic acid synthesis and stabilization, protein synthesis, and membrane structure, although the mechanisms at the molecular level have not been elucidated (9, 35). Polyamines are intimately involved in, and required for, cell growth and differentiation (35, 36). We recently have shown that polyamine synthesis plays an important role in normal re-

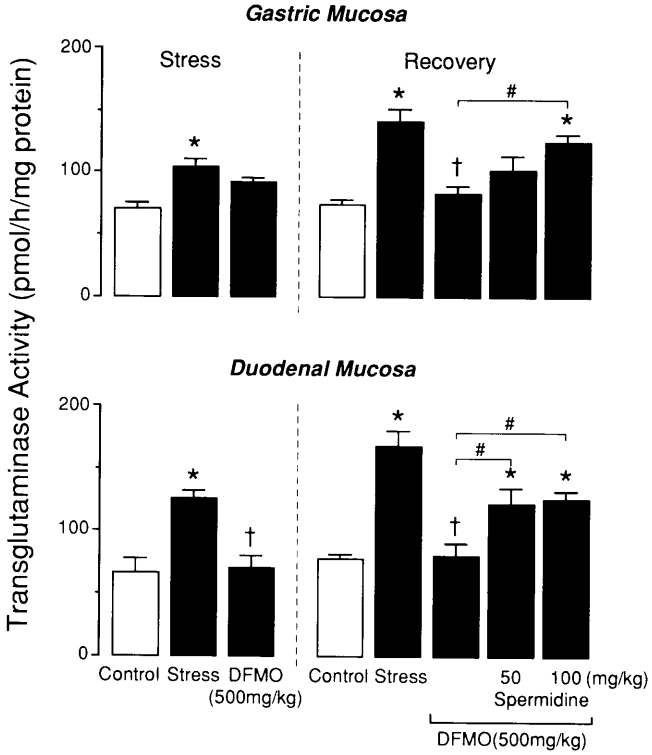

Figure 6. Transglutaminase activity in oxyntic gland and duodenal mucosa from control, stressed, stressed plus α-difluoromethylornithine (DFMO), and stressed treated with DFMO and spermidine groups. Rats were placed in restraint cages and immersed to the level of the xiphoid process in a water bath (23°C) for 6 hr. DFMO was administered intraperitoneally at the dose of 500 mg/kg 30 min before stress and repeated 1 hr after stress. Spermidine was given intragastrically after the period of stress. Animals were killed 0 and 4 hr after 6-hr stress. Values represent means ± SEM for six or eight rats per group. $^{*}P < 0.05$ compared with control; $\dagger P < 0.05$ compared with stress group; $^{\#}P < 0.05$ compared with rats stressed and treated with DFMO.

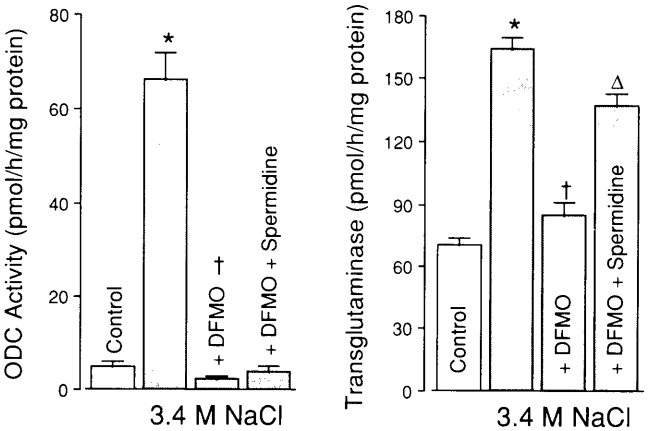

Figure 7. Ornithine decarboxylase (ODC) and transglutaminase activities in oxyntic gland mucosa after exposure to 3.4 *M* NaCl with and without treatment with α-difluoromethylornithine (DFMO) or with DFMO plus spermidine. DFMO was administered intraperitoneally 10 min before orally giving 1 ml of 3.4 *M* NaCl. Spermidine was given intragastrically at a dose of 100 mg/kg 2 hr after treatment with 3.4 *M* NaCl, and animals were killed 3 hr after administration of spermidine. Values represent means ± SEM for eight rats per group. $^{*}P < 0.05$ compared with control; $\dagger P < 0.05$ compared with rats treated with 3.4 *M* NaCl; $^{\Delta}P < 0.05$ compared with rats treated with 3.4 *M* NaCl and DFMO.

pair of gastric and duodenal mucosal stress erosions, and that transglutaminase and protein cross-linking may be part of the mechanism requiring polyamines for healing of the damaged mucosa (16, 18).

To our knowledge, the current study is the first report on the effects of luminal polyamines and endogenously synthesized polyamines on transglutaminase activity in the gastrointestinal mucosa. The properties and the activity of gastric and duodenal mucosal transglutaminase reported in this paper are similar to those described in other tissues (8, 37). Our previous study (19) has shown that the activity of the enzyme in the gastrointestinal mucosa absolutely requires Ca^{++} and that maximal activity of transglutaminase is observed at pH 9.0. In the preliminary experiments for the current study, we demonstrated that enzyme activity was linear with respect to time and homogenate protein concentrations. The Km and Vmax for the enzyme in the gastric and duodenal mucosa are consistent with those described for other tissues (37, 38).

As shown in Figures 1 and 2, the activity of transglutaminase in the gastric and duodenal mucosa was markedly increased after oral administration of polyamines. There are no significant differences in effect on the enzyme activity between spermidine and putrescine or spermine. Maximal increases in transglutaminase activity occurred 3 hr after a single treatment with a polyamine. Furthermore, decreasing tissue polyamine biosynthesis by treating rats with DFMO to decrease ODC, significantly decreased transglutaminase activity in the duodenal mucosa but had no effect on the enzyme activity in the stomach (Fig. 3). Administration of DFMO also failed to inhibit basal ODC activity and polyamine levels in the gastric mucosa, although it almost completely inhibited ODC and significantly decreased putrescine levels in the duodenum (Fig. 4 and Table I).

It is difficult to evaluate the changes in gastric mucosal polyamine biosynthesis because of the presence of a nonspecific decarboxylase and the apparent restriction of specific ODC to the mucous neck cells, which account for only a small fraction of the total mucosal cell population (39). For example, the increased ODC in the oxyntic gland mucosa after feeding or gastrin goes undetected by conventional biochemical assay involving the release of $^{14}CO_2$ from carboxyl-labeled ornithine. This induction, however, is apparent by using the techniques of immunocytochemistry and antibody dilution (39). Treatment with DFMO failed to decrease basal ODC and polyamine levels in the gastric mucosa, but completely prevented increases in the gastric enzyme activity and polyamines stimulated by stress or corticosterone (40, 41). The lack of effect of DFMO on gastric mucosal transglutaminase probably results from the fact that the DFMO does not deplete basal putrescine (Table I).

Figures 6 and 7 show that increasing polyamine biosynthesis in the gastrointestinal mucosa by stress or by exposure to hypertonic NaCl solution significantly increases transglutaminase activity. These results confirmed our previous findings (18, 19) that stress and hypertonic NaCl-induced mucosal lesions are associated with an induction of transglutaminase activity. Since pretreatment with DFMO completely prevented increases in both ODC and transglutaminase activity, the current data are the first indicating that increased transglutaminase activity in the damaged mucosa follows an increase in mucosal polyamines. Furthermore, combined administration of spermidine together with DFMO prevented most of the reduction of transglutaminase activity.

The involvement of polyamines in the regulation of transglutaminase activity of gastrointestinal mucosa may be biologically significant due to the role of the enzyme in protein cross-linking reactions and cellular adhesiveness (5, 7). Early mucosal restitution proceeds primarily by the migration of cells from undamaged areas to cover and seal areas of lamina propria exposed by the loss of cells during damage (42). Polyamines are required for this early phase of repair *in vivo* (16, 42) and are also required for the migration of IEC-6 cells (a small intestinal crypt cell line) *in vitro* (43), suggesting that the cell migration that takes place to repair mucosal damage also requires polyamines. Tissue polyamines are rapidly increased during the repair process by either synthesis or uptake from the luminal side (16, 40). The current findings indicate that as natural substrates of transglutaminase, polyamines significantly stimulate the enzyme activity for protein cross-linking reactions in the damaged mucosa. Increased polyamines during the mucosal repair process might enhance mucosal restitution by both stimulating transglutaminase activity and supplying additional substrate to the serosal surface to participate in cross-linking reactions. This process could stabilize interactions between cells and with the extracellular matrix after cell migration into damaged areas.

In summary, these results show that luminal polyamines significantly increase transglutaminase activity. Treatment with DFMO has no effect on basal transglutaminase in the gastric mucosa, but totally prevents the induction of transglutaminase in the stomach and duodenum. Luminal polyamines can reverse the inhibitory effect of DFMO on transglutaminase activity. These results strongly suggest that polyamines regulate transglutaminase activity in gastrointestinal mucosa.

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