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A Traditional Herbal Medicine, Rikkunshi-To (TJ-43), Prevents Intracellular Signaling Disorders in Gastric Smooth Muscle of Diabetic Rats

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Abstract: Prevention of diabetic gastrointestinal dysfunction is of utmost importance. The present study demonstrated that diacylglycerol kinase (DGK) activity in diabetic gastric smooth muscle in the resting state was approximately 3.5-fold greater than that in controls. However, oral administration of TJ-43 (1% of food intake) or subcutaneous insulin injection (12 units/kg/day) in streptozotocin-induced diabetic rats (DM) for 2 weeks prevented DGK abnormalities based on the control level. Increased DGK activity in the resting state of DM was inhibited significantly by R59022, neomycin or staurosporine; in contrast, these drugs did not affect DGK activity in controls, insulin-treated DM or TJ-43-treated DM. In controls, the endogenous phosphatidic acid (PA) level was inhibited significantly by R59022 or neomycin but not affected by staurosporine. On the other hand, these three drugs significantly inhibited endogenous PA levels in DM, and neomycin significantly inhibited endogenous PA levels in insulin-treated and TJ-43-treated DM. This suggests that TJ-43 could prevent alteration of DGK activity and PA formation without reduction of blood glucose levels. Moreover, these effects were greater than those of insulin treatment. Results suggested that TJ-43 treatment influenced the hyperreactivity of DGK and DAG formation via phospholipase C activity. In conclusion, TJ-43 can be recommended with respect to enhancement of the quality of life in patients displaying diabetic gastrointestinal complications.

Keywords: Diabetes; Herbal Medicine; Diacylglycerol Kinase; Stomach; TJ-43; Liu-Jun-Zi-Tang; Rikkunshi-to; Zingiberis Rhizoma; Glyrrhizae Radix; ZiZyphi Fructus.

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Introduction

A number of studies exist regarding contractile abnormalities in diabetes mellitus (DM) depending on species, regional differences, mode of DM induction, and period following induction of DM. However, abnormal contractile responses are frequently related to altered Ca^{2+} levels, membrane transduction and production and action of intracellular second messengers (Ozturk *et al.*, 1996; Fleischhacker *et al.*, 1999; Sakai and Maruyama, 2000).

The traditional kampo herbal medicine, Liu-Jun-Zi-Tang (Rikkunshi-to, TJ-43), when administered orally for treatment of chronic gastritis, has been identified as an effective drug with respect to dyspeptic symptoms, and is widely used for therapy in patients that suffer from such symptoms (Tatsuta and Iishi, 1993). TJ-43 significantly inhibited gastric mucosal damage caused by absolute ethanol at doses in excess of 500 mg/kg in a dose-dependent manner. This drug displays gastroprotective action in rats, and has been reported to promote gastric adaptive relaxation (Arakawa *et al.*, 1999; Hayakawa *et al.*, 1999). However, the mechanism governing these effects in signal transduction of gastric smooth muscle remains unclear.

Hyperglycemia or elevated glucose levels can increase diacylglycerol (DAG) levels and activate protein kinase C (PKC) activity in various smooth muscles (King *et al.*, 1994; Nobe *et al.*, 1998). Activation of the DAG-PKC cellular signal pathway is probably linked to dysfunction of vascular and gastrointestinal smooth muscle in DM (Nobe *et al.*, 1998; Xia *et al.*, 1994). Subsequent investigations have suggested that the increases in DAG levels are derived from the *de novo* synthesis pathway in response to elevated glucose levels (Shiba *et al.*, 1993). DAG elevation in DM can persist chronically and may be difficult to reverse, lending additional support to the possibility that it may be involved in the development of chronic diabetic complications.

We previously reported that hyperreactive contraction elevated PKC and diacylglycerol kinase (DGK) activities in gastric smooth muscle isolated from streptozotocin-induced diabetic rats in comparison with age-matched control rats (Sakai *et al.*, 1994; Nobe *et al.*, 1998). In order to improve these abnormalities of gastric smooth muscle in insulin-dependent DM, TJ-43 was administered orally or insulin was injected subcutaneously for 2 weeks. Subsequently, the mechanisms of TJ-43 and insulin effects on signal transduction, especially DGK activity, were investigated in gastric smooth muscle of diabetic rats.

Materials and Methods

Materials

Streptozotocin (STZ), carbachol (CCh), atropine sulphate (Atr) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Carrier- and HCl-free [^{32}P]-Pi were acquired from DuPont-New England Nuclear (Boston, MA, USA). DiC8 was obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA). 6-[2-(4-[(4-Fluorophenyl) phenyl-methylene]-1-piperidinyl)ethyl]-7-methyl-5H-thiazolo [3,2-α] pyrimidine-5-one (R59022) was purchased from Janssen Life Science Products (Beerse, Belgium). TLC plates

(silica gel 60 with a concentrating zone) were procured from Merck Inc. (Darmstadt, Germany). TJ-43 (Lot No. 250043010, Liu-Jun-Zi-Tang, Tsumura & Co. Tokyo, Japan) is a spray-dried powder product derived from a hot water extract of a mixture consisting of eight herbs: Zingiberis Rhizoma (0.5 g), Glyrrhizae Radix (1.0 g), ZiZyphi Fructus (2.0 g), Auranti Nobilis Pericarpium (2.0 g), Pineliae Tuber (4.0 g), Hoelen (4.0 g), Attracylodis Lanceae Rhizoma (4.0 g) and Ginseng Radix (4.0 g). This powder was dissolved in distilled water and mixed with powdered food (Oriental Yeast Co., Tokyo, Japan) for feeding. All other chemicals and materials were of reagent grade.

Preparation of Experimental Diabetic Rats

Experimental diabetes was induced in rats by treatment with STZ as previously reported (Sakai *et al*., 1991). STZ (60 mg/Kg body weight) in citrate buffer was injected into the lateral vein of eight-week-old Wistar rats (male, 220–260 g body weight). Age-matched controls were injected with citrate buffer. Rats were decapitated 6–7 weeks after injection. During this period, randomly selected diabetic rats were treated with insulin or TJ-43. In TJ-43-treated STZ rats, TJ-43 was added to serve as 1% of 1-day food intake for 2 weeks. Other selected diabetic rats received insulin subcutaneously daily following confirmation of the development of diabetes 35 days after injection of STZ (insulin-treated diabetics) as described below. On each of the first 4 days, a dosage of 9.6 units/kg/day of insulin was injected; subsequently, treatment dosage was increased to 12 units/kg/day for 10 days; total insulin treatment was conducted for 2 weeks. Blood glucose level was determined on a Tidex glucose analyzer (Bayer-Sankyo, Tokyo, Japan).

Preparation of Smooth Muscle Tissues

Rats were sacrificed in an ether-saturated chamber and smooth muscle tissues were replaced as previously described (Nobe *et al.*, 1998). Following dissection, fat and connective tissues were removed from the specimens. Internal surfaces of gastric smooth muscle tissues (mucosa) were removed. Tissues were then equilibrated in physiological salt solution (PSS) supplemented with 118 mM NaCl, 5.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.4 mM NaH_2PO_4, 21.4 mM $NaHCO_3$ and 11.1 mM glucose aerated with 95% O_2 and 5% CO_2 at 37°C.

Assay of DGK Activity Utilizing DiC8 in Tissue

In order to measure DGK activity, a cell-permeable species of short-chain DAG, diC8, was utilized as an exogenous substrate as previously described (Nobe *et al.*, 1994). Upon penetration of the cell membrane by DiC8, [^{32}P]-dioctanoyl-phosphatidic acid ([^{32}P]-diC8-PA) was formed by DGK. DiC8 did not originally exist in the tissue; therefore, changes in [^{32}P]-diC8-PA accumulation were considered to reflect changes in DGK activity.

Following homogenization of samples, [^{32}P]-diC8-PA and [^{32}P]-PA were extracted with 1 ml of $CHCl_3$ and 1 ml of 0.1 mM HCl solution. Samples were then spotted on TLC plates, which were developed employing an organic phase consisting of ethyl acetate/isooctane/acetic acid/water (7:5:2:1, v/v/v/v). The spots corresponding to [^{32}P]-diC8-PA and [^{32}P] PA, as localized by autoradiography (–20°C, overnight), were scraped and radioactivity was measured by liquid scintillation spectrometry.

Data Analysis

The data were expressed as means ± SEM. Statistical differences were determined by one-way analysis of variance (ANOVA) followed by the *Bonferroni* t-test for multiple comparisons.

Results

Characteristics of Experimental Rats

Basic conditions were verified in rats at 6 to 7 weeks after STZ injection (Table 1). In DM animals, body weight decreased to approximately three-quarters of the controls; additionally, blood glucose, as well as water consumption and urine volume combined increased to levels approximately 3.5-fold and eight-fold greater than those of controls, respectively. These changes in diabetic rats recovered significantly upon treatment with insulin. However, no significant changes were observed with TJ-43 treatment.

Table 1. Characteristics of Experimental Rats

Experimental Group	Animals	Body Weight (g)	Body Glucose (mg/dl)	Water Intake (ml/day)	Urine Volume (ml/day)
Control	16	311.5 ± 9.7	122.1 ± 11.2	24.0 ± 1.7	18.0 ± 2.3
Diabetic	10	218.4 ± 10.2*	431.0 ± 28.1*	192.0 ± 12.5*	142.0 ± 3.5*
Diabetic + Insulin	5	279.8 ± 12.0*,†	158.2 ± 14.6†	74.0 ± 3.5*,†	30.0 ± 4.1*,†
Diabetic + TJ-43	7	220.8 ± 8.5*	551.7 ± 17.4*	174.0 ± 6.8*	128.0 ± 4.4*

Note: Values in control and STZ (diabetic) rats were measured at 6 to 7 weeks following the injection. Insulin and TJ-43 were administered in this period as described in "Materials and Methods."
$^{*}p < 0.05$ versus control rats and $^{\dagger}p < 0.05$ versus diabetic rats.

Changes in DGK Activities Induced by CCh in Each Group

Previously, diC8, a cell-permeable species of short-chain DAG, was utilized as an exogenous substrate for measurement of the level of DGK activity in tissues. Following DiC8 penetration of the cell membrane, [^{32}P]-diC8-PA was formed by DGK in [^{32}P]-Pi-pre-labeled tissue. DiC8 was not originally present in the tissue; consequently, changes in [^{32}P]-diC8-PA accumulation were considered to reflect changes in DGK activity (Nobe *et al.*, 1998). In

order to measure DGK activity via this approach, it was necessary to establish whether changes in [^{32}P]-diC8-PA accumulation reflected DGK activity. Incorporation rates of diC8 as an extracellular substrate increased with pre-incubation in a time-dependent manner in gastric smooth muscle. Submaximal values were detected at 90 minutes. At that time, it was confirmed that 67.0%, 21.2% and 11.8% of diC8 existed in the free form, as diC8-PA and additional phospholipids, respectively, involving phosphatidylinositols and phosphatidylcholine (data not shown).

To examine whether the increase in [^{32}P]-diC8-PA accumulation reflected DGK activation, effects of carbachol (CCh) and atropine were measured in each group as depicted in Fig. 1. In controls, DGK activity was approximately three-fold higher than that of resting levels upon treatment with 10 μM CCh for 5 minutes. The muscarinic receptor antagonist, atropine (10 μM), itself did not affect DGK activity in the resting state. However, 10 μM atropine abolished CCh-induced DGK activity. In DM rats, DGK activity in the resting state was 3.5-fold greater than that in controls. CCh (10 μM) did not affect DGK activity in the presence or absence of 10 μM atropine. In the insulin-treated DM, DGK activity during resting conditions was significantly less than that in DM. For example, 10 μM CCh did not accelerate the activity in the presence or absence of 10 μM atropine. In TJ-43-treated DM, DGK activity in the resting state was approximately identical to levels observed in controls. Moreover, 10 μM CCh accelerated DGK activity nearly two-fold in the absence of 10 μM atropine. This increased activity was abolished by 10 μM atropine.

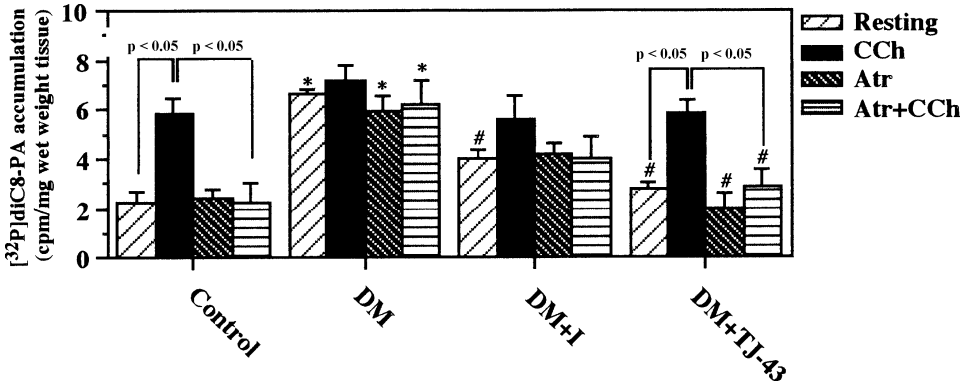

Figure 1. Effects of CCh treatment on DGK activity in diabetic rat gastric smooth muscle. Gastric smooth muscle tissues were isolated from controls and each strain of treated diabetic rat. These tissues were pre-incubated with 2220 GBq/mL [^{32}P]-Pi and 100 μM diC8 at 37°C for 90 minutes. Following washing, tissues were incubated in the presence or absence of 1 μM atropine (Atr) for 5 minutes. Subsequently, 10 μM CCh was added for 5 minutes. [^{32}P]-diC8-PA accumulation was quantified as DGK activity as described in "Materials and Methods." Each value represents the mean ± SEM of at least five independent determinations. $^{*}p < 0.05$ versus control rats and $^{\#}p < 0.05$ versus DM rats.

Changes in DGK Activities Induced by PMA

PKC supposedly participates in both insulin and high glucose concentration-stimulated glucose transport. The possible role of PKC with respect to changes in DGK in DM was investigated by testing the effect of phorbol 12-myristate 13-acetate (PMA) as a specific PKC activator. Therefore, changes in DGK activity were examined under various conditions; changes were compared with resting levels as illustrated in Fig. 2. DGK activity did not change in DM and insulin-treated DM under Ca^{2+}-free conditions (pre-incubation of tissues in Ca^{2+}-free PSS for 5 minutes); furthermore, DGK activity was not altered by 60 mM KCl with or without PMA. DGK activities in all groups were unaffected under Ca^{2+}-free conditions and in the presence of 60 mM KCl. In DM, the resting level of DGK activity was elevated markedly and the value was similar to the maximal response to CCh exhibited by controls. However, in the presence of 60 mM KCl, 1 μM PMA significantly accelerated DGK activity in both the controls and TJ-43-treated DM. In insulin-treated DM, DGK activity was attenuated, but it did not change significantly under the aforementioned conditions. In TJ-43-treated DM, DGK activity closely resembled that observed in controls.

Effects of Various Inhibitors on DGK Activity at the Resting Level

[^{32}P]-PA accumulation was measured simultaneously as an indicator of endogenous PA level. Alteration of DGK activity and endogenous PA level were investigated employing various enzyme inhibitors at resting levels in four groups (Fig. 3). The DGK inhibitor, 5 μM R59022, phospholipase C (PLC) inhibitor, 3 μM neomycin and the PKC inhibitor, 3 μM staurosporine, did not affect DGK activity in controls. However, elevated DGK activity in the resting state

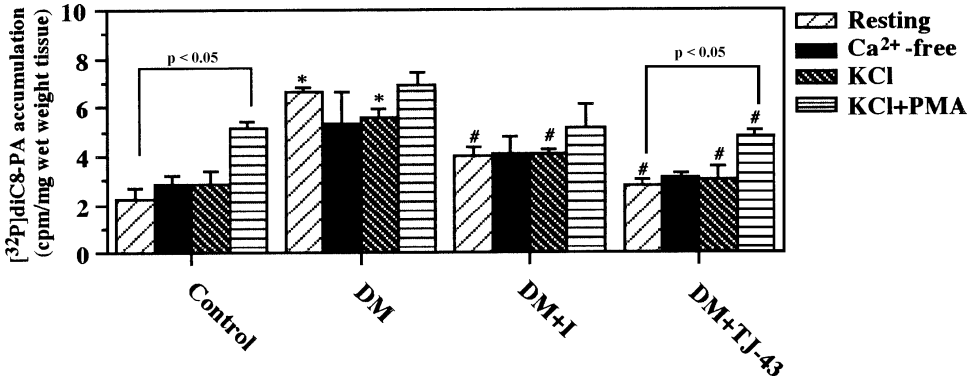

Figure 2. Effects of calcium-free PSS, KCl and/or PMA treatments on DGK activity in diabetic rats. Fresh tissues were isolated from controls and each strain of treated diabetic rat. These tissues were pre-labeled with [^{32}P]-Pi and 100 μM diC8; subsequently, tissues were incubated in the presence or absence of calcium-free PSS (Ca^{2+}-free), 60 mM KCl and/or 1 μM PMA for 5 minutes. [^{32}P]-diC8-PA accumulation was quantified as DGK activity as described in "Materials and Methods." Each value represents the mean ± SEM of at least five independent determinations. $^{*}p < 0.05$ versus control rats and $^{\#}p < 0.05$ versus DM rats.

of DM was abolished by R59022 and staurosporine, whereas partial inhibition was observed with neomycin. In insulin-treated and TJ-43-treated DM, resting levels of DGK activity recovered significantly; however, DGK activity was not influenced by R59022, neomycin or staurosporine. On the other hand, in controls, endogenous PA level displayed significant inhibition in the presence of R59022 or neomycin but not affected by staurosprorine. In DM, endogenous PA levels were inhibited significantly by these drugs. In insulin-treated and TJ-43-treated DM, endogenous PA levels were significantly inhibited by neomycin.

Effects of Various Inhibitors on CCh-induced DGK Activity

DGK activity enhanced by 10 μM CCh was approximately the same level in the four groups (Fig. 4). R59022 (5 μM) abolished this activity in all groups. Neomycin inhibited DGK activity significantly, albeit slightly, in controls, DM and insulin-treated DM. Staurosporine also inhibited CCh-induced DGK activity in all groups. The CCh-induced increases in

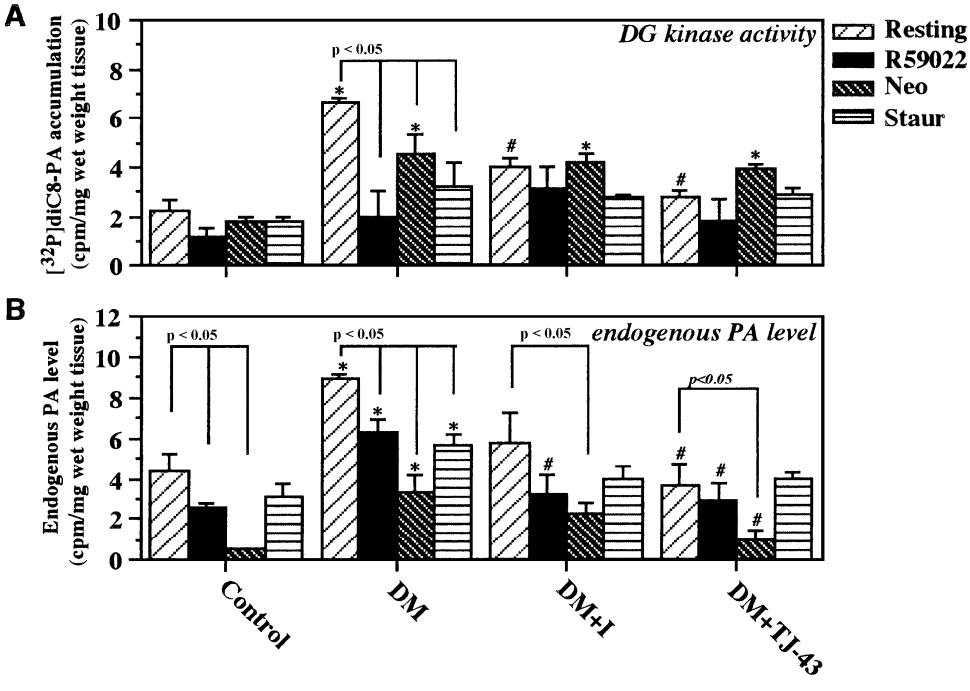

Figure 3. Effects of R59022, neomycin and staurosporine treatments on DGK activity in diabetic rats. Fresh tissues were isolated from controls and each strain of treated diabetic rat. These tissues were pre-labeled with [^{32}P]-Pi and 100 μM diC8; subsequently, tissues were incubated in the presence or absence of 5 μM R59022, 3 μM neomycin (Neo) and 3 μM staurosporine (Staur) for 5 minutes. [^{32}P]-diC8-PA accumulation was quantified as DGK activity; [^{32}P]-PA accumulation was quantified as endogenous PA level as described in "Materials and Methods." Each value represents the mean ± SEM of at least five independent determinations. $^{*}p < 0.05$ versus control rats and $^{\#}p < 0.05$ versus DM rats.

endogenous PA levels in controls and DM were nearly abolished by 5 μM R59022. In controls, endogenous PA levels were significantly inhibited by either R59022 or neomycin but were not affected by staurosporine. In DM, endogenous PA levels were inhibited significantly by these drugs. In insulin-treated and TJ-43-treated DM, endogenous PA levels were significantly attenuated by neomycin.

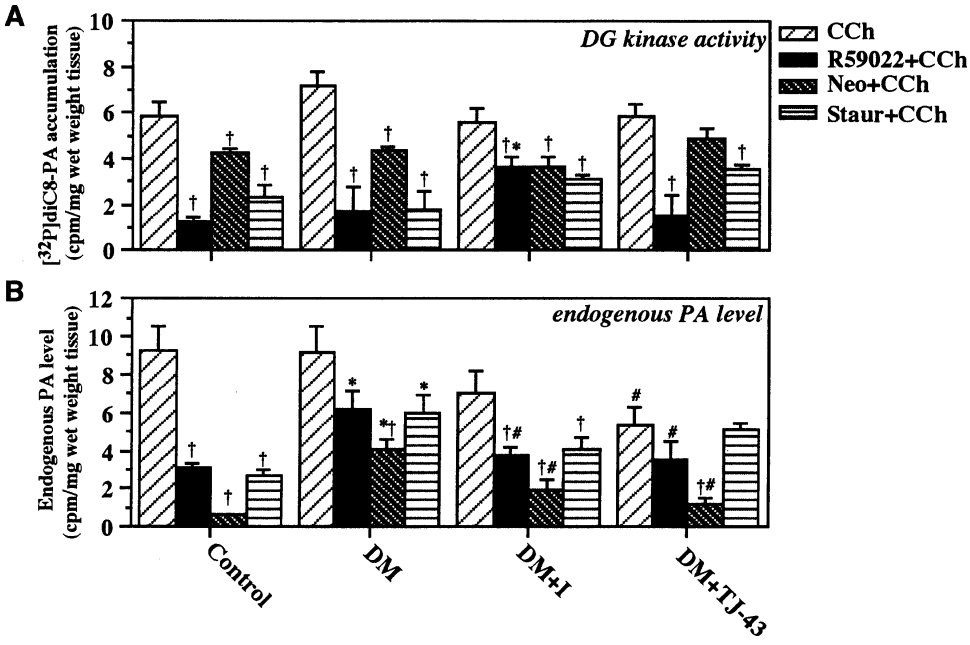

Figure 4. Effects of R59022, neomycin and staurosporine pre-treatments on CCh-induced DGK activation in diabetic rats. Fresh tissues were isolated from controls and each strain of treated diabetic rats. These tissues were pre-labeled with [^{32}P]-Pi and 100 μM diC8; subsequently, tissues were pre-incubated in the presence of 5 μM R59022 (R59022+CCh), 3 μM neomycin (Neo+CCh) and 3 μM staurosporine (Staur+CCh) for 5 minutes. CCh (10 μM) was added for 5 minutes. [^{32}P]-diC8-PA accumulation was quantified as DGK activity; [^{32}P]-PA accumulation was quantified as endogenous PA level as described in "Materials and Methods." Each value represents the mean ± SEM of at least five independent determinations. $^{*}p < 0.05$ versus control rats, $^{\#}p < 0.05$ versus DM rats and $^{\dagger}p < 0.05$ versus single treatment of CCh.

Discussion

Clinical studies have demonstrated that TJ-43 is effective in the symptomatic treatment of dyspepsia, a syndrome that may be related to the disturbance of gastric motility and emptying. The effects of several prokinetic drugs have been evaluated in diabetic patients who present abnormal gastrointestinal motility and dyspeptic symptoms (Tatsuta and Iishi, 1993). The findings of this study may offer a possible mechanism regarding TJ-43 action in abnormal gastric smooth muscle motility in DM.

DGK catalyzes DAG phosphorylation, which is believed to play a major physiological role in the metabolism of the intracellular messenger DAG. A dramatic increase was observed in DGK activity in DM, which leads to changes in DAG levels and smooth muscle dysfunction.

Possible mechanisms governing changes in DGK signaling related to dysfunction of diabetic gastric smooth muscle contractility are displayed in Fig. 5A. TJ-43 prevented this intracellular signaling disorder to a greater extent than did insulin in DM rats. An explanation regarding the mechanisms of TJ-43 action on signal transduction related to DGK activity is presented in Fig. 5B.

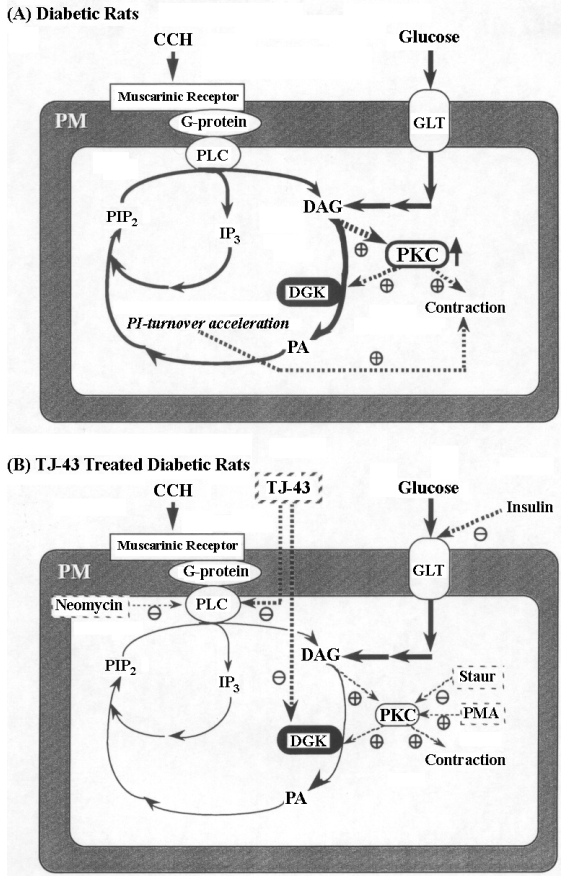

Figure 5. A schematic model depicting DGK activity via PLC pathway in gastric smooth muscle isolated from STZ-induced diabetic rats (A) and TJ-43-treated diabetic rats (B). Plasma membrane (PM) possesses muscarinic receptors linked via G-protein to phospholipase-C (PLC). Glucose is transported by a glucose transporter (GLT). Hyperglycemia led to increased diacylglycerol (DAG) and protein kinase C (PKC) activation in diabetic rats. Diacylglycerol kinase (DGK) converts DAG into phoshatidic acid (PA). Phosphatidylinositol (PI)-turnover is accelerated in diabetic rats. In the diabetic model, solid thick lines denote regions of increased activity. Treatment with TJ-43 in diabetic rats for 2 weeks inhibited acceleration of PI turnover, DGK activity and PKC activity. The solid lines are thinner and arrows are smaller. Neomycin is an inhibitor of PLC, staurosporine (Staur) is a PKC inhibitor, and phorbol 12 myristate 13-acetate (PMA) is a PKC activator.

R59022 and atropine abolished the elevation in DGK activity in the resting state of DM. DGK activity increased significantly upon treatment with 10 μM CCh in controls. Furthermore, CCh-induced DGK activity was abolished by 1 μM atropine or 5 μM R59022 and this change was mediated by receptor stimulation. However, treatment with TJ-43 in DM prevented DGK activity to the same extent as that of controls. These results suggested that enhancement of DGK activity in the resting state of DM was not related to muscarinic receptors. It was reported that no significant difference exists between control and DM rats in total number of [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB) binding sites on gastric smooth muscle cell membranes or in the affinity of [^{3}H]-QNB for the binding sites (Lin *et al*., 2000). Therefore, our findings suggest that DM may induce alterations in signal transduction at downstream receptors in gastric smooth muscle.

A portion of incorporated glucose was converted to DAG in the absence of mediation by the phospholipase C (PLC) pathway under high-glucose conditions, a phenomenon common in DM (Inoguchi *et al*., 2000). Hyperglycemia-induced increases in DAG and PKC activation occur in tissues, i.e. in animal models and in humans with diabetes (Craven *et al*., 1990: Hoffman *et al*., 1991). Smooth muscle cells exposed to high glucose levels *in vitro* accumulate DAG and exhibit higher PKC activity (Xia *et al*., 1994). Insulin inhibited glucose uptake and DAG accumulation, and significantly attenuated DGK activity. Responses to tested agents were similar to those observed in DM (Fig. 1).

Although the mechanism regarding the relation between effects of TJ-43 and cellular DAG level remains unclear, TJ-43 could prevent alterations of DGK activity and PA formation without reduction of blood glucose level (Table 1). Moreover, these effects were greater than those of insulin treatment (Figs. 1 and 2). We concluded that the action of TJ-43 was not related to glucose uptake and DAG accumulation from the pathway of glucose.

Results support the hypothesis that acceleration of the rate of phosphatidylinositol (PI)-turnover, including increased DGK activity and basal activation of PKC in DM. However, the effect of neomycin on diabetic gastric smooth muscle is complex. In DM, DGK activity resembled that of endogenous PA levels and both were inhibited upon treatment with R59022. These findings, which indicate that DGK plays a major role in the removal of endogenous DAG, suggest that DGK activation leads to an increase in the rate of PI turnover. The present data confirmed that PI hydrolysis by PLC is one acceleration route of DAG formation in DM. We have previously demonstrated that smooth muscle dysfunction is related to changes in intracellular signaling systems in diabetic rats (Maruyama *et al*., 1999; Sakai and Maruyama, 2000; Nobe *et al*., 2002b). The relationship between the changes in DGK activity and gastric smooth muscle dysfunction may correspond to PI turnover and PKC activity associated with diabetic complications. Endogenous PA levels in all groups were also inhibited by neomycin, which blocks PI metabolism in the resting state and in the presence of CCh (Figs. 3 and 4). We emphasize that TJ-43 affects DGK activity and PI turnover without inhibition of glucose uptake (Fig. 5B).

Changes in DGK activity and DAG levels modulate PKC-dependent cell cycle events in gastric smooth muscle. Hence, DGK exerts control upon the PKC-activation threshold, which is reflected in PKC-dependent events (Makhlouf and Murthy, 1997; Nobe *et al.*, 1998). The

PKC activator, PMA, affected DGK activity in both controls and TJ-43-treated DM in the presence of KCl. (Fig. 2). Staurosporine inhibited DGK activity in all four groups in the presence of CCh, but it did not affect those in the resting state, with the exception of DM. This activation depends on increases in intracellular Ca^{2+} and PKC activity. PKC activity increased in gastric smooth muscle from DM (Sakai *et al*., 1994). TJ-43 may affect elevated PKC-dependent DGK activity in DM.

It now appears that the increase in DAG selectively activates certain PKC isoforms. For example, Inoguchi *et al*. showed that PKC-β_{II} was predominantly activated in the heart and aorta of diabetic rats, whereas in the retina, both PKC-γ and PKC-β_{II} were activated (2000). We have previously reported the distribution of PKC isoforms in gastric smooth muscle. The PKC-ε isoform increased in cytosol isolated from diabetic rats (Maruyama *et al*., 1999). This phenomenon may be related to the result obtained from that in Ca^{2+}-free PSS (Fig. 2). Increased cellular DAG levels activate PKC; and activated PKC phosphorylates and activates DGK directly. On the basis of this regulation, DGK phosphorylates DAG in a manner which is dependent upon intracellular Ca^{2+}. This mechanism operates as a feedback system with respect to reduction of cellular DAG level and moreover contributes to the acceleration of PI-turnover. Consequently, we hypothesized that DAG level was an important element with respect to alteration of DGK in DM. In actuality, DAG levels increased in the resting state of gastric smooth muscle in DM (Nobe *et al.*, 2002a).

In conclusion, TJ-43 affects the abnormal signal transduction pathway in DM and in particular, it inhibits DGK activity in gastric smooth muscle without altering blood glucose levels in DM. Our observation supports the view that TJ-43 treatment can prevent gastric dysfunction in DM, yet the mechanism is currently unclear. Thus, future studies are warranted. We hypothesize that control of diabetes with TJ-43 treatment improves gastric function through mechanisms that may include inhibition of PI turnover.

Acknowledgments

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