Correction of abnormal small intestinal cytosolic protein kinase C activity in streptozotocin-induced diabetes by insulin therapy

Ramesh K. WALI, Pradeep K. DUDEJA, Merry J. G. BOLT, Michael D. SITRIN and Thomas A. BRASITUS*

Department of Medicine, The Pritzker School of Medicine of the University of Chicago, Chicago, IL 60637, U.S.A.

Diabetes was induced in rats by administration of a single intraperitoneal injection of streptozotocin (50 mg/kg body wt). After 7 days, one group of diabetic animals was treated with insulin for an additional 5 days. Control, diabetic and diabetic + insulin rats were then killed, their distal small intestines were removed and the epithelial cells were examined and compared with respect to polyphosphoinositide turnover, total protein kinase C activity and cellular distribution, and 1,2-diacylglycerol mass and production. The results of these experiments demonstrated that, compared with their control counterparts, the intestines from diabetic rats had a decreased turnover of polyphosphoinositides, but an increase in 1,2-diacylglycerol mass which was a result, at least in part, of an increase in the synthesis of this lipid *de novo*. Total protein kinase C activity was decreased in the diabetic rats due to a decrease in cytosolic activity, with no significant change in particulate activity. Moreover, insulin administration for 5 days to diabetic animals did not affect their lowered intestinal polyphosphoinositide turnover, but did further accentuate their increased 1,2-diacylglycerol mass and synthesis *de novo*; this treatment also corrected total protein kinase C activity by increasing the cytosolic activity of this enzyme. These results indicate that signalling mechanisms involving polyphosphoinositides, 1,2-diacylglycerol and protein kinase C are abnormal in the intestines of diabetic rats and that some of these biochemical parameters can be modulated by insulin administration *in vivo*.

INTRODUCTION

Considerable evidence exists that a number of structural and functional alterations are present in the small intestines of animals with drug-induced diabetes mellitus (Olsen & Rosenberg, 1970; Leese & Mansford, 1971; Hopfer, 1975; Feingold *et al.*, 1982; Thompson, 1983; Fedorak *et al.*, 1984; Brasitus & Dudeja, 1985). Experimental diabetes has been associated with enhanced intestinal absorption of several nutrients, including glucose and amino acids (Olsen & Rosenberg, 1970; Leese & Mansford, 1971; Hopfer, 1975; Thompson, 1983; Fedorak *et al.*, 1984), as well as increased mucosal mass (Feingold *et al.*, 1982; Brasitus & Dudeja, 1985). Many (Olsen & Rosenberg, 1970; Feingold *et al.*, 1982), but not all (Leese & Mansford, 1971; Brasitus & Dudeja, 1985) of these alterations can be restored to control levels by administration of insulin.

In this regard, increasing attention has been focused on the possible role of protein kinase C (PKC) in mediating certain of the post-receptor actions of insulin in various cell types (reviewed in Klip & Douen, 1989). PKC is a serine/threonine-specific protein kinase which is dependent on phospholipid and Ca^{2+} (Takai *et al.*, 1979). This enzyme is activated by 1,2-diacylglycerol, which is formed in response to extracellular signals by turnover of phosphoinositides (Takai *et al.*, 1979) and other membrane phospholipids (Huang, 1989) as well as by synthesis *de novo* from the glycolytic intermediate dihydroxyacetone phosphate and from glycerol 3-phosphate after stepwise acylation to lysophosphatidic acid and phosphatidic acid (Chiarugi *et al.*, 1989). Activation of PKC involves translocation of the enzyme to the plasma membrane, which, in turn, leads to phosphorylation of target proteins, thereby influencing important cellular processes such as proliferation (Huang, 1989).

It has been proposed that impaired diacylglycerol production

secondary to reduced inositol content could lead to impaired activation of PKC in various tissues in diabetic animals and man, thereby contributing to the known complications of this disease (Greene *et al.*, 1987; Greene & Lattimer, 1987). Although previous studies in human platelets from diabetic subjects (Bastyr *et al.*, 1989) and glomeruli of streptozotocin-diabetic rats (Craven & DeRubertis, 1989) have in fact noted a decrease in polyphosphoinositide turnover, 1,2-diacylglycerol levels were found to be elevated, not lowered, in these latter cells (Craven & DeRubertis, 1989). Moreover, in diabetic-rat glomeruli PKC was found to be activated (Craven & DeRubertis, 1989). Based on their findings, Craven & DeRubertis (1989) suggested that the alterations in rat glomerular PKC may be involved in renal hypertrophy and hyperplasia and increases in the glomerular filtration rate which are characteristic of early diabetes.

As noted earlier, experimental diabetes also leads to a number of abnormalities in the small intestine, including an increase in the mucosal mass of this organ. To date, however, the effects of the streptozotocin-induced diabetic state on phosphoinositide turnover, 1,2-diacylglycerol mass and production, and PKC total activity and cellular distribution have not been studied in the rat small intestine. It was therefore of interest to examine and compare these biochemical parameters in the small intestines of diabetic, control and insulin-treated diabetic animals. The results of these studies and discussion of their physiological significance serve as the basis for the present paper.

MATERIALS AND METHODS

Materials

Streptozotocin, histone (type IIIS), phosphatidylserine, leupeptin, phenylmethanesulphonyl fluoride, phosphatidyl-

Abbreviations used: PKC, protein kinase C; KRB, Krebs–Ringer bicarbonate buffer; KRBG, Krebs–Ringer bicarbonate buffer containing glucose; IP, inositol 1-monophosphate; IP_2 , inositol 1,4-bisphosphate; IP_3 , inositol 1,4,5-trisphosphate; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2 , phosphatidylinositol 4,5-bisphosphate.

* To whom correspondence including reprint requests should be sent, at Department of Medicine, Box 400, 5841 S. Maryland Avenue, Chicago, IL 60637, U.S.A.

inositol standards, ceramide (type III), DEAE-cellulose, Mops buffer salt, LiCl and ATP (Na salt) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). AG-I $\times$ 8 (HCOO⁻) pre-filled chromatography columns were obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). *sn*-1,2-Diacylglycerol kinase was purchased from Lipidex (Westfield, NJ, U.S.A.), octyl β -D-glucoside from Boehringer Mannheim Biochemicals (Indianapolis, IN, U.S.A.) and cardiolipin and dioleoylglycerol from Avanti Polar Lipids (Pelham, AL, U.S.A.). Phosphatidic acid was obtained from Serdary Research Laboratories (Ontario, Canada). [γ -³²P]ATP, [¹⁴C]glucose, [³H]arachidonate, [³H]-phosphatidylinositol compounds and [³H]inositol phosphate compounds were obtained from New England Nuclear (Boston, MA, U.S.A.). *myo*-[³H]inositol with PT6-271 was purchased from Amersham (Arlington Heights, IL, U.S.A.).

Induction of experimental diabetes

Male Lewis rats (200–250 g; Charles River, Wilmington, MA, U.S.A.) were randomly divided into two groups. Diabetes mellitus was induced in one of the groups by the administration of a single dose of streptozotocin (50 mg/kg body wt. in 0.9% NaCl; intraperitoneal injection), whereas the other group (controls) received the vehicle alone. The diabetic rats were further subdivided into insulin-treated or non-treated groups. Insulin-treated diabetic rats received 4–5 units of Iletin II pig insulin per day (Eli Lilly & Co., Indianapolis, IN, U.S.A.) from subcutaneously implanted osmotic minipumps (2001–2002; Alzet Corp., Palo Alto, CA, U.S.A.) for 5 days, beginning 7 days after streptozotocin administration (Patel, 1983). The streptozotocin-treated rats were periodically analysed for glycosuria and were eliminated from the study if they did not show at least 1% glycosuria at all times. Animals that were ketotic were also removed from the study. Plasma glucose levels were monitored as described (Brasitus & Dudeja, 1985). Blood was routinely drawn from the tail vein to monitor the blood glucose levels with Glucoscan 2000 (Lifescan, Mount View, CA, U.S.A.). Only those insulin-treated rats in which the blood glucose levels stabilized at 120 ± 20 mg/dl for at least 3 days before they were killed were used in the present experiments.

Isolation of enterocytes

Control, diabetic and insulin-treated diabetic rats were starved for 16–18 h, but with water provided *ad libitum*, before being killed by cervical dislocation. The distal one-half of the intestines were then cleaned with ice-cold 0.5% NaCl containing 0.5 mM-dithiothreitol, and intestinal wet weights were determined. Enterocytes along the entire crypt–villus axis from each group were harvested using a technique which combined chelation of divalent cations with mild mechanical dissociation as described previously (Weiser, 1973).

Partial purification of PKC

The isolated enterocytes (approx. 5 mg of protein) were washed twice with Krebs–Ringer bicarbonate buffer containing 180 mg of glucose/100 ml (KRBG) and then homogenized in 5 ml of buffer containing 20 mM-Tris/HCl, pH 7.5, 0.5 mM-EGTA, 2 mM-EDTA, 2.0 mM-phenylmethanesulphonyl fluoride, 0.5 mM-benzimidazole, 5.0 mM-2-mercaptoethanol and 10 mg of leupeptin/l (Kraft *et al.*, 1982). The homogenate was then centrifuged at 100 000 *g* for 60 min; the supernatant (S₁; cytosolic fraction) was saved, and the pellet was resuspended in the homogenizing buffer containing 0.3% Triton X-100. After 1 h on ice this suspension was centrifuged at 100 000 *g* for 1 h and the supernatant (S₂) was saved as the solubilized membrane fraction. Both fractions were then applied to DEAE-cellulose columns (0.8 cm $\times$ 4 cm) and washed with the homogenizing buffer con-

taining 0.02 M-NaCl. Partially purified PKC was then eluted in 0.08 M-NaCl as previously described (Wali *et al.*, 1990).

Assay of PKC

PKC activity was determined in the DEAE-cellulose-purified fractions by a histone phosphorylation assay as described previously (Kraft *et al.*, 1982). The enzyme was incubated for 3 min at 30 °C in a reaction mixture containing 20 mM-Tris/HCl (pH 7.2), 10 mM-MgCl₂, 400 μ g of histone (type IIIS)/ml, 1.83 mM-CaCl₂ and 50 μ M-[γ -³²P]ATP (1 μ Ci) with and without phosphatidylserine (80 μ g/ml). At the end of the reaction, the products were blotted on to 2.5 cm $\times$ 2.5 cm phosphocellulose paper (no. P81; Whatman, Clifton, NY, U.S.A.) that had been prewashed in 10% trichloroacetic acid/2 mM-NaH₂PO₄. The papers were sequentially washed in 10% trichloroacetic acid, ethanol and ether and finally air-dried before Cerenkov counting. PKC activity was calculated from the difference in phosphorylation in the presence and absence of phosphatidylserine and was expressed as pmol of ³²P/min per mg of protein.

Determination of labelled diacylglycerol production

The incorporation of radiolabelled arachidonate or glucose into diacylglycerol was assessed by incubating the enterocytes with either 20 μ Ci of [³H]arachidonate (76.0 Ci/mmol) or 5 μ Ci of [¹⁴C]glucose (340 mCi/mmol) in KRB (flushed with 95% O₂/CO₂, 19:1) containing 5 mM-glucose at 37 °C for 30 min. After labelling, the cells were washed twice with KRB containing 1% fatty acid-free BSA. For extraction, 5 ml of ice-cold chloroform/methanol (1:2, v/v) was added, followed by chloroform and water to obtain a final ratio of chloroform/methanol/water of 2:2:1 (by vol.) (Bligh & Dyer, 1959; Peter-Riesch *et al.*, 1988). After 30 min on ice, samples were centrifuged at 2000 *g* for 5 min and the aqueous layer was discarded. After two washes with chloroform/water (1:1, v/v), the lipid extract was dried under N₂ and reconstituted in chloroform/methanol/water (75:25:2, by vol.) before spotting on to pre-absorbent silica gel G plates (Analtech, Newark, DE, U.S.A.). The plates were developed in benzene/ether/ethanol/acetic acid (25:20:1:0.1, by vol.) (Banschbach *et al.*, 1974) and diacylglycerol and other neutral lipids were identified by comparison with co-migrating authentic standards and visualized by exposure to iodine vapour.

Quantification of 1,2-diacylglycerol mass

For quantifying 1,2-diacylglycerol mass, total cellular lipids from epithelial cells were extracted in chloroform/methanol (2:1, v/v) as described by Folch *et al.* (1957). Same-day portions of the lipid extract were assayed either for phosphorus by the procedure of Bartlett (1959) or for 1,2-diacylglycerol mass by the procedure of Preiss *et al.* (1986) using bacterial diacylglycerol kinase. For each assay, diacylglycerol kinase (11 munits in 4 μ l) was combined with 50 μ l of reaction buffer (100 mM-Mops, pH 6.6, 100 mM-NaCl, 25 mM-MgCl₂ and 2 mM-EGTA), 2 μ l of 100 mM-dithiothreitol and 10 μ l of 10 mM-[γ -³²P]ATP (5.0×10^4 c.p.m./nmol), and incubated at 25 °C for 30 min as described previously (Wright *et al.*, 1988). Measured portions of lipid extract or diacylglycerol standards were dried, resuspended in a 20 μ l of detergent solution containing 7.5% octyl β -D-glucoside, 5 mM-cardiolipin and 1 mM-diethylenetriaminepentaacetic acid, vortex-mixed, sonicated and incubated with 80 μ l of the enzyme/ATP mixture for 30 min at 25 °C (Preiss *et al.*, 1986). The reaction was terminated by the addition of 1.67 ml of chloroform/methanol/12 M-HCl (66:33:1, by vol.) followed by 1.67 ml of methanol/water/chloroform (48:47:3, by vol.) as described by MacDonald *et al.* (1988); the incubation mixture

was then vortex-mixed and centrifuged for 10 min at 1000 *g*. After removal of the upper phase, the lower phase was re-extracted with methanol/water/chloroform (48:47:3, by vol.) and centrifuged as above. The radiolabelled phosphatidic acid in the clear lower phase was assayed directly by scintillation counting of a 0.5 ml sample. Standard curves were generated from the assay of known amounts of 1,2-diacylglycerol and data are expressed as nmol of 1,2-diacylglycerol/100 nmol of lipid phosphorus (mol %) (MacDonald *et al.*, 1988). Since ceramide is also phosphorylated by diacylglycerol kinase, in certain experiments the chloroform extract was dried and t.l.c. was performed as described by Priess *et al.* (1986). Phosphorylated ceramide constituted about 20% of the total phosphorylated products in the cell extract, with no difference between control, diabetic and insulin-treated diabetic groups.

Isolation of intact small intestinal epithelium

For studies of phosphatidylinositol metabolism, intact rat small intestinal epithelial preparations were used [as described by Bjerknes & Cheung (1981)] instead of isolated enterocytes, since the preparations had to be incubated with *myo*-[³H]inositol for 2 h in order to ensure that sufficient radioactivity was incorporated into the different fractions (Craven *et al.*, 1986). Briefly, after anaesthesia with pentobarbital (50 mg/kg intraperitoneal), the distal portion of the small intestine was flushed with 60 ml of Ca²⁺/Mg²⁺-free Hank's balanced salt solution at 37 °C. The left ventricle was perfused with 100 ml of 30 mM-EDTA in Ca²⁺/Mg²⁺-free Hank's solution, flushed with O₂/CO₂ (19:1) at 37 °C, at a flow rate of 20 ml/min for 3–4 min. The distal small intestine was then excised, gently everted and slid over a 5 ml pipette attached to a rheostat-controlled motor. The epithelium was removed by 5 s bursts of rotation at 1500 rev./min and collected in cold Ca²⁺/Mg²⁺-free Hank's buffer, pH 7.5. The epithelium was then centrifuged at 500 *g*, washed and resuspended in buffer containing 180 mg of glucose/100 ml (KRBG).

Determination of labelled inositol phosphates and phosphoinositides

The intestinal epithelia (approx. 5 mg of protein) from control, diabetic and insulin-treated diabetic rats were incubated for 2 h at 37 °C in 2 ml of KRBG containing 20 µCi of *myo*-[³H]inositol (112.2 Ci/mmol). The epithelium was centrifuged at 500 *g* for 10 min at 4 °C and washed three times with cold KRBG. The preparations were then resuspended in 2 ml of 20 mM-Hepes/Tris buffer, pH 7.2, containing 25 mM-β-glycerophosphate, 0.1 M-KCl, 5 mM-β-mercaptoethanol, 5 mM-MgCl₂, 0.02% trypsin inhibitor and 10 mM-LiCl. The suspensions were incubated for 20 min at 37 °C, then 0.67 ml of 10% HClO₄ was added and this mixture was allowed to stand on ice for 15 min. After centrifugation at 500 *g*, the supernatant was neutralized with 20 mM-Hepes/Tris buffer (pH 8.0) before determination of inositol phosphates. The pellet was mixed with 0.5 ml of chloroform/methanol/12 M-HCl (200:100:0.75, by vol.) and the extracted phosphoinositides were saved for later analyses. Separation of different species of inositol phosphates was achieved by anion-exchange chromatography on 0.3 ml AG-I × 8 (HCOO⁻) columns (200–400 mesh) based on the method of Downes *et al.* (1982). After loading, the columns were eluted serially with 1.5 ml each of (i) water, (ii) 0.1 M-formic acid/0.2 M-ammonium formate, (iii) 0.1 M-formic acid/0.4 M-ammonium formate, and (iv) 0.1 M-formic acid/1 M-ammonium formate. Inositol 1-monophosphate (IP), inositol 1,4-bisphosphate (IP₂) and inositol 1,4,5-trisphosphate (IP₃) were eluted in the second, third and fourth fractions respectively. Radioactivity was assayed by scintillation counting. For the separation and quantification

of phosphoinositides, 0.2 ml of the acidified pellet extract was treated with 1.5 ml of chloroform/methanol (2:1, v/v), vortex-mixed and centrifuged. The upper phase was discarded and the lower phase was washed twice with 0.75 ml of methanol/0.6 M-HCl (1:1, v/v) and then separated on 1% potassium oxalate-impregnated silica gel G chromatography plates (Analtech). Unlabelled phosphoinositides were added as carriers. The plates were developed in chloroform/acetone/methanol/glacial acetic acid/water (40:15:13:12:7, by vol.) (VanDongen *et al.*, 1985). The labelled phosphoinositides including phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP), and phosphatidylinositol 4-5-bisphosphate (PIP₂) were identified by comparison with phosphoinositide standards and quantified by scraping the silica gel from the plates and counting the radioactivity in a liquid scintillation counter. Routinely, more than 90% of radioactivity added to the thin-layer plates was recovered.

RESULTS

Animal groups

Body weights (initial and final), intestinal wet weights and blood glucose values of control rats and streptozotocin-treated rats (with and without insulin treatment) are given in Table 1. Unlike control rats, diabetic rats failed to gain weight and actually lost body weight after induction of diabetes by streptozotocin. Administration of insulin to diabetic rats for 5 days was found to partially correct this weight loss in these animals. Wet weights of the distal intestines from the diabetic animals, however, were found to be significantly greater than those of their control counterparts, and this parameter was corrected by insulin treatment for 5 days. Blood glucose values for diabetic rats were also found to be significantly greater than those for control animals as well as to which insulin was administered (Table 1).

Effect of diabetes on PKC activity in the small intestine

As shown in Table 2, the total activity of intestinal PKC was significantly lower in diabetic rats compared with their control counterparts. This was due to an approx. 60% decrease of PKC activity in the cytosolic fraction of these preparations, with only a slight increase (~10%) in membrane-bound PKC activity in diabetic animals. As a result of these alterations in activity, the percentage distribution of PKC in the membrane fraction was significantly elevated in the intestines of these rats compared with control values. Moreover, insulin administration for 5 days to diabetic animals restored both the total activity and the percentage distribution of intestinal PKC to control values.

Effect of diabetes on phosphoinositides and inositol phosphates in the small intestine

Table 3 summarizes the data on polyphosphoinositol metabolism in the distal intestines of control, diabetic and insulin-

Table 1. Profile of animal groups

Values represent means ± S.E.M. of *n* separate animals analysed in each group. **P* < 0.05 compared with control group.

Group	<i>n</i>	Initial body wt. (g)	Final body wt. (g)	Intestinal wet wt. (g)	Blood glucose (mg/dl)
Control	52	227.3 ± 4.5	282.0 ± 4.7	5.1 ± 0.3	98.8 ± 9.3
Diabetic	44	228.7 ± 3.8	208.5 ± 7.5*	8.9 ± 0.6*	390.7 ± 11.0*
Diabetic + insulin	40	210.2 ± 6.8*	248.2 ± 5.4*	5.4 ± 0.9	119.3 ± 18.2

Table 2. Effect of diabetes on small intestinal PKC cellular distribution and total activity

Values represent means $\pm$ S.E.M. of n separate preparations analysed in each group. Values in parentheses refer to percentage of total activity. * $P < 0.05$ compared with control group.

Group	n	PKC activity (pmol/min per mg of protein)		
		Membrane-associated	Cytosol-associated	Total
Control	5	0.42 $\pm$ 0.05 (37.3 $\pm$ 2.5)	0.71 $\pm$ 0.07 (62.7 $\pm$ 2.5)	1.13 $\pm$ 0.06
Diabetes	4	0.48 $\pm$ 0.04 (61.8 $\pm$ 2.1)*	0.30 $\pm$ 0.06* (38.2 $\pm$ 2.1)*	0.78 $\pm$ 0.05*
Diabetes + insulin	4	0.49 $\pm$ 0.05 (42.0 $\pm$ 3.5)	0.65 $\pm$ 0.07 (58.0 $\pm$ 3.5)	1.14 $\pm$ 0.06

treated diabetic animals. The turnover of *myo*-[^3H]inositol-labelled PIP₂ was found to be significantly decreased in the intestines of diabetic animals compared with controls. Whereas insulin administration tended to decrease this value even further, the effect of this agent on these intestinal phosphoinositides and inositol phosphates was not found to be significant ($P > 0.05$).

Effect of diabetes on diacylglycerol mass and production in the small intestine

As shown in Table 4, intestinal diacylglycerol mass was found to be significantly increased in diabetic animals compared with their control counterparts. Insulin administration for 5 days to diabetic animals, moreover, led to further increases in diacylglycerol mass in these intestinal preparations.

To further define the mechanism(s) responsible for these alterations in diacylglycerol mass, the intestinal epithelia of all three groups of animals were labelled with either [^3H]arachidonate or [^{14}C]glucose to assess whether differences in the turnover of inositol-containing phospholipids or synthesis of 1,2-diacylglycerol *de novo* via phosphatidic acid respectively were responsible for these findings. In agreement with prior studies in our laboratory (Wali *et al.*, 1990), after incubation of [^3H]arachidonate for 30 min, phosphoinositides were the major class labelled in the intestines of all three groups of animals (results not shown). Moreover, no significant differences in diacylglycerol production were noted in the intestines of these groups, as assessed by [^3H]arachidonate labelling (results not shown).

In contrast with these findings, however, in studies using intestinal epithelium labelled with [^{14}C]glucose, diacylglycerol production was found to be significantly increased in diabetic

Table 4. Effects of diabetes on small intestinal 1,2-diacylglycerol mass and production using [^{14}C]glucose

Values represent means $\pm$ S.E.M. of three separate preparations analysed for each group. * $P < 0.05$ compared with control group; † $P < 0.05$ compared with diabetic group values.

Group	1,2-Diacylglycerol mass (mol %)	Incorporation of [^{14}C]glucose into 1,2-diacylglycerol (c.p.m./mg of protein)
Control	0.86 $\pm$ 0.04	289 $\pm$ 40
Diabetes	1.28 $\pm$ 0.03*	376 $\pm$ 28*
Diabetes + insulin	1.50 $\pm$ 0.12*†	500 $\pm$ 70*†

animals compared with their control counterparts (Table 4). Insulin administration further accentuated this increase in diacylglycerol production in the intestines of diabetic animals. These findings indicate that increases in the diacylglycerol mass seen in the intestines of diabetic animals in the presence and absence of insulin are, at least in part, secondary to activation of 1,2-diacylglycerol synthesis *de novo* (Chiarugi *et al.*, 1989; Craven & DeRubertis, 1989).

DISCUSSION

The present results demonstrate for the first time that the distal small intestines of streptozotocin-induced diabetic rats have alterations in polyphosphoinositide turnover, diacylglycerol mass and PKC total activity and cellular distribution compared with their control counterparts.

In agreement with the present observations in the diabetic rat intestine, previous studies with human platelets (Bastyr *et al.*, 1989), rat sciatic nerves (Clements & Stockard, 1980) and rat glomeruli (Craven & DeRubertis, 1989) have all shown that the diabetic state was associated with decreases in membrane polyphosphoinositide turnover. Based on earlier findings in other tissues of streptozotocin-diabetic rats (Clements & Stockard, 1980; Craven & DeRubertis, 1989), this decrease in phosphoinositide turnover was possibly a result of decreases in membrane inositol content.

Despite this decrease in intestinal polyphosphoinositide turnover, diabetic rats were found to possess an increase in intestinal 1,2-diacylglycerol mass. Similar results have been noted in the myocardium of diabetic rats, although no attempt was made to assess the possible source(s) of this lipid in these cells (Okumara *et al.*, 1988). In the present experiments, enhanced synthesis of 1,2-diacylglycerol *de novo*, but not enhanced phosphoinositide

Table 3. Effect of diabetes on small intestinal phosphoinositides and inositol phosphates

Values represent means $\pm$ S.E.M. for n separate preparations analysed in each group and are percentages of total d.p.m. incorporated into phosphoinositides and inositol phosphatases. * $P < 0.05$ compared with control group. No significant differences were noted between the diabetes and diabetes + insulin groups.

Group	n	Incorporation (%)					
		Phosphoinositides			Inositol phosphates		
		PI	PIP	PIP ₂	IP	IP ₂	IP ₃
Control	4	61.4 $\pm$ 3.7	7.1 $\pm$ 2.3	12.1 $\pm$ 1.0	11.2 $\pm$ 1.7	4.2 $\pm$ 0.7	4.0 $\pm$ 0.7
Diabetes	3	59.5 $\pm$ 2.8	12.1 $\pm$ 3.3	8.6 $\pm$ 1.1*	13.8 $\pm$ 1.5	3.1 $\pm$ 0.5	2.9 $\pm$ 0.3
Diabetes + insulin	3	60.5 $\pm$ 3.9	14.2 $\pm$ 3.8	7.9 $\pm$ 1.9*	12.9 $\pm$ 2.6	2.4 $\pm$ 0.7	2.1 $\pm$ 0.5

breakdown, was found to be responsible, at least in part, for the increase in 1,2-diacylglycerol mass in the intestine of diabetic animals. These results are in agreement with previous studies performed with rat diabetic glomeruli (Craven & DeRubertis, 1989). Whether other sources of 1,2-diacylglycerol, such as the breakdown of phosphatidylcholine and phosphatidylethanolamine (Huang, 1989), also contribute to its increased mass in the diabetic rat intestine must await further study.

In glomeruli from diabetic rats, total PKC activity was unchanged, whereas the percentage of enzyme activity associated with the membrane fraction was increased, i.e. PKC was 'activated' (Craven & DeRubertis, 1989). In contrast with these findings, and in agreement with previous studies performed with rat diabetic nerves (Clements & Stockard, 1980), intestinal PKC total activity was decreased due to a loss of soluble activity in diabetic animals compared with their control counterparts. Although the percentage of enzyme activity in the particulate fraction, an index of 'activation', was indeed increased in the diabetic intestine, the physiological consequences of such a change in the cellular distribution of PKC on cellular proliferation or other intestinal abnormalities are unclear at this time (Cooper *et al.*, 1987). Taken together, the results of the present and previous studies (Klip & Douen, 1989; Craven & DeRubertis, 1989) indicate that generalizations concerning PKC alterations in diabetic cells should be avoided.

Administration of insulin for 5 days to diabetic animals led to further increases in the intestinal mass of 1,2-diacylglycerol, a finding previously noted in diabetic rat myocardial cells (Okumara *et al.*, 1988). Further enhancement of synthesis of 1,2-diacylglycerol *de novo* via the glycolytic pathway in diabetic rats treated with insulin appeared to be at least partially responsible for this finding. In this regard, however, it should be noted that whereas insulin has not been generally shown to stimulate polyphosphoinositide turnover (Klip & Douen, 1989), recent studies have clearly demonstrated that insulin can produce increases in 1,2-diacylglycerol from the hydrolysis of membrane phosphoinositol glycan(s) (Saltiel *et al.*, 1986, 1987; Farese *et al.*, 1987; Fox *et al.*, 1987). It is therefore likely that the increase in intestinal 1,2-diacylglycerol mass induced by insulin was derived from several sources. Additional studies will be necessary to clarify this issue.

Insulin administration to diabetic rats restored total PKC to normal by increasing the cytosolic, but not the intestinal membrane-bound, enzymic activity. Interestingly, a number of previous studies in non-diabetic cells have also shown an increase in cytosolic PKC in response to this hormone (Klip & Douen, 1989). Although the mechanism(s) responsible for this action of insulin on intestinal cytosolic PKC remain enigmatic, as previously discussed by Cooper *et al.* (1987), it is possible that other factors such as phosphorylation, limited proteolysis, additional ligands, or endogenous activators or inhibitors might be responsible for this insulin effect.

In this regard, it should also be noted that several isoenzymes of PKC have been described that vary in their tissue distribution and cellular localization (Ramsdell *et al.*, 1986; Yoshida *et al.*, 1988; Huang, 1989). It is therefore possible that the changes noted in PKC total activity in the intestines of diabetic animals in the presence and absence of insulin may reflect differences in PKC isoenzymes. Regardless of the exact mechanism(s) involved in insulin's action on intestinal cytosolic PKC, however, the present results demonstrate that insulin-induced changes in the regulation of this enzyme in diabetic animals may be more complex than previously thought, at least in the small intestine.

In summary, the results of the present experiments demonstrate that the intestines of diabetic animals have alterations in polyphosphoinositide turnover, 1,2-diacylglycerol mass and PKC

activity. Insulin administration, moreover, corrected the PKC alterations, but not the polyphosphoinositide turnover abnormalities, and actually accentuated the 1,2-diacylglycerol abnormalities seen in the intestines of diabetic animals. These latter findings strongly suggest that the major pool of 1,2-diacylglycerol in the enterocyte (at least partially glucose-derived) fails to communicate with the major PKC pool, presumably as a consequence of differences in compartmentalization. Furthermore, the inability of insulin to normalize the changes in polyphosphoinositide turnover and 1,2-diacylglycerol level in diabetic animals suggests that other hormones, such as cortisol, parathyroid hormone and growth hormone, which have previously been shown to be abnormal in this diabetic model (Schedl *et al.*, 1978, 1988; El-Seifi *et al.*, 1987), may be important regulators of these components of signal transduction. Additional studies to further define the mechanism(s) involved in these processes as well as their possible relationships to the known structural and functional alterations in the intestine of diabetic animals will therefore clearly be of interest.

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