Protein Kinase C Isozymes in Skeletal Muscles During the Early Stage of Genetic and Streptozocin Diabetes (44308)

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Abstract. **This study examined the changes in PKC isozyme activity, content, and cellular distribution in rat gastrocnemius and soleus muscles prior to any evidence of neural degeneration or impaired skeletal muscle function, during the onset of streptozocin-induced (STZ) and genetic diabetes mellitus (DM). PKC activity was increased more in the particulate than in the soluble fractions of the soleus and gastrocnemius muscles obtained from rats treated with STZ and the gastrocnemius muscle obtained from BB-Wor diabetic rats (D rats). The predominant constitutive PKC isozymes in the skeletal muscles obtained from the STZ-treated and D rats were PKCα >> PKCε > PKCδ as determined by Western immunoblot assay. The content of each PKC isozyme did not differ between the soleus and gastrocnemius muscles of the control Sprague-Dawley rats for the STZ-treated rats and the BB Wor diabetic resistant (DR) rats. Moreover, the PKC isozyme content did not differ in the soluble fraction of D or STZ rats when compared to their corresponding control animals. PKCδ increased more than PKCα or PKCε in the particulate fraction of gastrocnemius and soleus muscles when obtained from either D or STZ rats. Since similar changes in skeletal muscle PKC isozyme profiles occurred independent of the duration of the diabetes and thereby the degree of nerve degeneration, insulin resistance, and the model of DM tested, we conclude that changes in skeletal muscle PKC precede the skeletal muscle myopathy of DM.** [P.S.E.B.M. 1998, Vol 218]

Skeletal muscle myopathy is a common manifestation of established diabetes mellitus, and symptoms occur in approximately 10% of all diabetic patients (1, 2). Included among the biochemical and metabolic defects associated with the skeletal muscle myopathy are skeletal muscle weakness, impaired calcium-activated actinomyosin ATPase activity, impaired use of glycogen stores and glucose, and a decrease in the number of glucose transporters (GLUT-4) in skeletal muscle membranes (1–5). Hyperglycemia upregulates diacylglycerol (DAG), which may be an important mediator of the progression of skeletal muscle from the normal state to that of a myopathic musculature in type 1 and type 2 diabetes mellitus by virtue of its ability to upregulate protein kinase C (PKC) activity and the sequelae in muscle function that arises (6–10).

Protein kinase C is an important modulator of skeletal muscle function. Skeletal muscle uses both stored glycogen and circulating glucose as energy sources for maintenance of cellular integrity and function. PKC can phosphorylate glycogen synthase either directly or through other protein kinases to render it inactive. The uptake of glucose by skeletal muscle is in part insulin dependent. PKC can also phosphorylate the serine residues of the insulin receptor causing their downregulation or inactivation thereby impairing glucose transport (11). Thus, if elevated in skeletal muscle, PKC may contribute to the insulin resistance of skeletal muscle in diabetes mellitus (8, 11, 12). PKC is not only a modulator of tissue sensitivity to insulin but also a mediator of the phosphorylation and dephosphorylation of enzymatic and regulatory protein substrates involved in the modulation

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of skeletal muscle metabolism, contractility, gene expression, and growth (11–17). PKC-mediated phosphorylation of troponin T, troponin I, troponin-tropomyosin complex, and troponin-C-protein in isolated cardiac and skeletal muscle cells is associated with inhibition of calcium-activated myofibrillar actomyosin MgATPase activity and inhibition of contractility (11–17). PKCδ, which can increase calcium influx through L-type calcium channels in myocardial cells (18, 19), may also increase the influx of calcium ion into skeletal muscle which, in excess, may contribute to calcium overload of the mitochondria and impaired skeletal muscle metabolism and function (11–20). Under normal conditions, the effects of PKC are usually transient since sustained activation of PKC usually does not occur due to downregulation of PKC activity with maintained stimulation by diacylglycerol (DAG) or its phorbol ester analogs (12–16). However, if changes in PKC are causal to the development of diabetic myopathy, we hypothesize that alterations in PKC should be present early in the development of diabetic myopathy (i.e., at a time when functional abnormalities would be minimal or absent).

We tested this hypothesis in two rat models of diabetes. The BB/Wor diabetic rat represents a model of genetic, type 1 diabetes in which all rats become diabetic within 49 days after birth and insulin is absolutely required for survival of the rat (4). In this study we used BB/Wor diabetic rats that had diabetes of 4–6 weeks duration (D rats) and their age matched diabetic resistant (DR) rats. Insulin-treated D rats that have been diabetic for only 4 to 6 weeks do not exhibit any signs of neural degeneration (4). The streptozocin-induced diabetic rat represents a model of type 2 or adult onset diabetes. The severity of the diabetes and the dependence of the survival of the rat on endogenous insulin reserves, in contrast to exogenously administered insulin, can be manipulated by the amount of streptozocin given to induce the diabetic state. For this study the rats were made diabetic by a single injection of streptozocin (STZ-treated rats), and both they and their euglycemic control Sprague-Dawley rats (NR) were monitored for the next 18 weeks during the onset and progression of the diabetes. STZ-treated rats exhibit neural degeneration within 18 weeks of STZ treatment (1, 2, 4, 5, 21). Thus, we utilized two different models of diabetes that displayed differences in the origin of the diabetes (genetic versus STZ-induced), the duration of the hyperglycemia (4–6 weeks vs 18 weeks), and the necessity for using insulin to control the diabetes in the BB/Wor D rat. Nevertheless, all the diabetic rats exhibited the classical symptoms of polydipsia and polyuria and had elevated fasting blood glucose levels that exceeded 350 mg/dl. Thus, the data are consistent with the conclusion that the rats were diabetic but that the rats used represented models of DM that reflected both the absence (D rats) and presence (STZ rats) of neural impairment of skeletal muscle function. Finally, this study evaluated the changes in PKC in the gastrocnemius and soleus muscles, representing fast- and slow-responding skeletal muscle fibers. This paradigm was chosen for study since differences exist in the insulin sensitivity, distribution of glucose transporters, and severity of neural impairment with diabetes. If the hypothesis proposed were correct, then we would expect changes in PKC to occur almost equally in both types of muscle.

Materials and Methods

General Experimental Protocol. Three-month-old male BB/Wor D rats (diabetic for 30–41 days) (NIH-NIDDK breeding facility, Univ. of Massachusetts, Boston, MA) were maintained on daily subcutaneous (sc) injections of 0.6–3.0 U/rat of a long-acting (once/day) protamine, zinc, and insulin suspension (Protamine, Zinc and Iletin I, Eli Lilly, Inc., Indianapolis, IN) prior to their feeding period based on their urinary excretion of glucose from the first appearance of glucosuria. Daily injections of insulin were necessary since the D rats, a model for genetic IDM (14), die within a few days without insulin maintenance. Age- and sex-matched DR rats obtained from the same facility were given equivalent sc injections of vehicle. Male, Sprague-Dawley rats (275–325 g) (n = 5/gp) were given a single injection of either STZ (45 mg/kg, iv; Sigma Chemical Co., St. Louis, MO) in citrate buffer (20 m*M*, pH 4.5) or citrate buffer *via* the tail vein (NR). The rats were maintained for 18 weeks following administration of STZ or buffer. All animals were housed on a 12-hr light-dark cycle with access to water and standard rat chow *ad libitum*. Six-hour fasting glucose levels were measured in tail-vein blood by a modified glucose oxidase method using an Accucheck III monitor (Boehringer Mannheim, Indianapolis, IN). Glycosylated hemoglobin levels were also measured with the method of Yue *et al.* (22). All animals were then anesthetized with a solution of ketamine-xylazine (50 mg/kg ketamine, 4 mg/kg xylazine, im), and their gastrocnemius muscles (D and DR rats) or gastrocnemius and soleus muscles (NR and STZ rats) were isolated, removed, frozen in liquid nitrogen, and assayed for PKC activity or for PKC isozyme content in the soluble (cytoplasmic) and particulate (membrane) fractions, as described previously in detail (23, 24). Our inability to evaluate the glycosylated hemoglobin and soleus muscles from the BB/Wor D and DR rats resulted from the loss of the samples due to mechanical failure of the −120°C freezer during the holiday school closure.

Measurement of PKC Activity. Gastrocnemius and soleus muscles were removed rapidly from the D, DR, NR, and STZ rats, washed in ice-cold buffered saline (PBS) to remove residual blood, and then frozen in liquid nitrogen. Portions of the frozen muscles were quantitatively pulverized and then placed in homogenization buffer I (20 m*M* TRIS-HCl pH 7.5, 0.25 *M* sucrose, 2 m*M* EDTA, 2 m*M* EGTA, 0.02% leupeptin, 1 m*M* PMSF) and homogenized in a cold room with a Polytron set at 7 for 20 sec, followed by homogenization for 60 strokes using a Dounce homogenizer (Fisher Scientific, Harrahan, LA). An aliquot of this homogenate was assayed for total PKC activity (see below). The homogenates were centrifuged (17,500*g* for 30 min at 4°C).

The resultant supernatants were then centrifuged at 37,000*g* (1 hr at 4°C) to isolate the mitochondria, and the resulting supernatant was then centrifuged at 100,000*g* at 4°C, and the particulate (membrane) and soluble fractions (cytosol) obtained. The pellets were washed and resuspended in buffer II (20 m*M* TRIS-HCl pH 7.5, 2 m*M* EDTA, 2 m*M* EGTA, 0.02% leupeptin, 1 m*M* PMSF and 0.1% Triton-X 100) and again homogenized. The homogenates were solubilized in buffer II without Triton-X 100. After a 45-min incubation period at 4°C, the soluble fraction was obtained by ultracentrifugation at 100,000*g* for 30 min at 4°C, and the pellet was retained as the particulate or membrane fraction. The membrane fraction was resolubilized in Buffer II without the Triton-X 100. Both membrane and soluble fractions were passed through 0.5-ml DEAE columns (Pharmacia, Inc, Gaithersburg, MD) that were washed twice with buffer II (2 ml), and then eluted with 0.4 ml of phosphate buffer containing 200 m*M* NaCl (23, 24). Specific PKC activity was determined by measurement of the phosphorylation of the specific substrate octapeptide (RKRTLRRL) in the presence of phosphatidyl serine (PS), calcium ion, and DAG using [γ^{32}-P]-ATP (Amersham Searle, Waltham, MA) (23, 24). The protein content of the fractions was measured by the bicinchoninic acid method (25) as described previously in detail (23, 24, 26). The enzymatic data were expressed as pmol/mg protein/min after comparison to a standard curve and corrected for the nonspecific kinase activity obtained in the absence of calcium, PS, and DAG (23, 24). The particulate PKC activities of the gastrocnemius muscles obtained from each individual D, DR, NR, and STZ-treated rats, or the soleus muscle from the NR and STZ-treated rats, were regressed against their individual fasting plasma glucose concentrations obtained on the day of the terminal experiment. The regression equation and correlation coefficient were determined with a computer best fit equation utilizing Sigma-Stat for Windows V2.01 (Jandel Scientific, San Rafael, CA).

Western Blot Analyses of PKC Isozymes. Western blot analyses were performed as described previously for the assay for PKC (23, 24). PKC isozymes were determined in 50-μg samples of protein from the crude homogenate, cytosolic, and particulate fractions. The antibody-antigen complex was detected by the enhanced chemiluminescence method, according to the manufacturer's instructions (Amersham-Searle, Arlington Heights, IL). Exposure times of immunoblots to Hyperfilm were 5 min and were identical for samples obtained from NR, STZ, DR, and D rats. All antibodies were obtained from Gibco, Inc. (Gaithersburg, MD). PKC isozyme standards were obtained from Transduction Labs (Louisville, KY). The selectivity and specificity of the Gibco anti-PKC antibodies against the specific isozymes of PKC have been established and the absence of cross reactivity validated (24). Moreover, semiquantitation was made for each PKC isozyme relative to each other since standard curves for each concentration of isozyme was determined using PKC isozyme standards in tissue protein homogenates (24). Data are expressed in relative grey scale units that reflect the optical density of the x-ray film.

Statistical Analysis of Data. Each experiment contained four to six animals per treatment group. Data were analyzed with analyses of variance (ANOVA) or multiple analyses of variance and means compared with Newman-Kuehls test; $P < 0.05$ was accepted for statistical differences between and among means.

Results

Body Weights and Blood Glucose Concentrations. Table I summarizes the body weights, fasting blood glucose levels, and glycosylated hemoglobin values of the 3-month-old BB/Wor D and DR rats and the Sprague-Dawley rats at 3 months of age and after 18 weeks of diabetes. The mean (± SD) body weights of the DR and D rats did not differ from each other after 4 weeks of diabetes ($P > 0.35$). In contrast, the STZ rats failed to gain weight as rapidly as the NR rats over the 18 weeks following treatment with STZ. The fasting blood glucose levels of the D rats compared to the DR rats clearly indicated the presence of hyperglycemia despite the daily administration of insulin (Table I). The fasting blood glucose levels of the STZ-

Table I. Body Weights and Characteristics of Diabetic and Control Rats

Parameter	BB/Wor D	BB/Wor DR	STZ	NR
Initial body weight (g)	371 ± 28	391 ± 31	296 ± 32	301 ± 20
Final body weight (g)	NA	NA	332 ± 36*	581 ± 48
Blood glucose (mg/dl)				
Initial	368 ± 67*	155 ± 16	168 ± 23	171 ± 23
Final	NA	NA	462 ± 82*	181 ± 11
Glycosylated Hb (%)	NT	NT	13.9 ± 1.5*	5.9 ± 1.3
Insulin required	YES	NO	NO	NO
Polydypsia	YES	YES	YES	YES
Polyphagia	YES	YES	YES	YES
Polyuria	YES	YES	YES	YES

Note. The initial body weights and blood glucose were measured in 3-month-old BB/Wor D and DR rats and 3-month-old Sprague-Dawley rats prior to injection of STZ (45 mg/kg) or citrate. The final body weights, blood glucose, and glycosylated hemoglobin were obtained 18 weeks after administration of STZ or citrate. An asterisk (*) indicates that the responses of the D and STZ rats differ ($P < 0.05$) from their euglycemic controls ($P < 0.05$). NA, not applicable; NT, not tested. Each value is the mean ± SD from 5 rats/gp.

treated and NR rats did not differ prior to administration of STZ. Fasting blood glucose levels of the STZ-treated rats were greater than 400 mg/dl 4 days after administration of STZ and remained relatively stable at these values over the next 18 weeks after administration of STZ (Table I). The glycosylated hemoglobin levels of the STZ-treated and NR rats paralleled the severity of the STZ-induced hyperglycemia (Table I). Despite the elevated fasting glucose levels of the STZ rats and the presence of polydypsia, polyuria, and polyphagia, insulin injections were not required for survival of the STZ-treated rats during the entire 18-week period of study.

PKC Activity. The total and particulate PKC activity of the gastrocnemius muscle obtained from D rats was significantly greater ($P < 0.05$) than the total and particulate PKC activity of the gastrocnemius muscle obtained from DR rats (Fig. 1). Despite the difference in the ages of the rats, the PKC activity of the soluble and particulate fractions of the gastrocnemius muscles obtained from the Brattleboro DR rats and Sprague-Dawley NR rats did not differ. The soluble PKC activity of the soleus muscle obtained from NR rats also did not differ from that of gastrocnemius muscle from either DR or NR rats. However, the particulate PKC activity of the slow soleus muscle obtained from the NR rat was less ($P < 0.05$) than that of the more rapid gastrocnemius muscle of the NR rat (Fig. 1). In contrast to these findings, both the soluble and the particulate PKC activity of the gastrocnemius and soleus muscles obtained from the STZ-treated rats were significantly elevated ($P < 0.05$) when compared to the PKC activities obtained from the corresponding fractions of the gastrocnemius and soleus muscles of NR rats (Fig. 1). Also, the absolute PKC activity of the gastrocnemius muscles was greater than that of the soleus muscles obtained from the STZ-treated rats (Fig. 1).

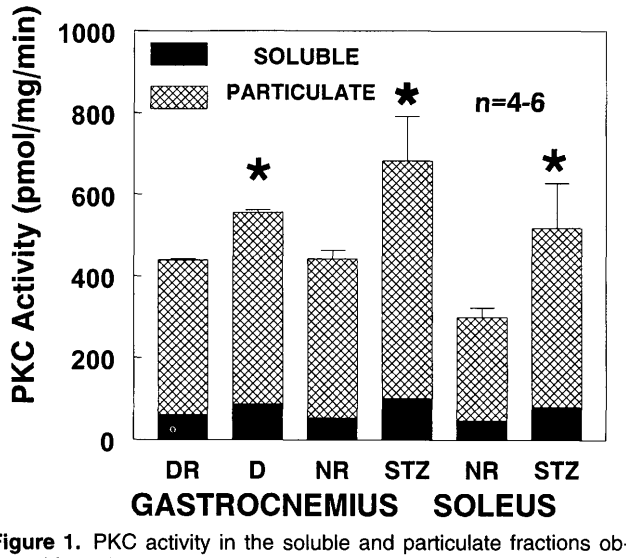

Figure 1. PKC activity in the soluble and particulate fractions obtained from the gastrocnemius muscle of the BB/Wor D and DR rats and from the gastrocnemius and soleus muscles of Sprague-Dawley STZ (45 mg/kg, iv)-treated and citrate buffer-treated (NR) rats (n = 4–6/gp). Each value is mean ± SD.* denotes $P < 0.05$ from DR and NR rats.

However, the absolute increase of particulate PKC activity (pmoles/mg protein/min) produced by STZ did not differ between the gastrocnemius and soleus muscles ($P > 0.27$) (Fig. 1).

Relationship Between Plasma Glucose and Gastrocnemius PKC Activity. When the particulate PKC activity of the gastrocnemius muscles obtained from the individual D, DR, NR, and STZ-treated rats was regressed against the individual plasma glucose concentrations, a linear relationship was evident (Fig. 2) that was described by the equation $y = 0.8575x + 299$ ($r^2 = 0.91$), where y and x were the muscle PKC and plasma glucose concentrations, and r^2 was the correlation coefficient, respectively. Similarly, when the soleus muscle PKC activity was regressed against the plasma glucose concentration of the NR and STZ-treated rats, a second linear relationship was obtained that was characterized by the equation $y = 0.8427x + 216$ ($r^2 = 0.86$), respectively (Fig. 2, top). A

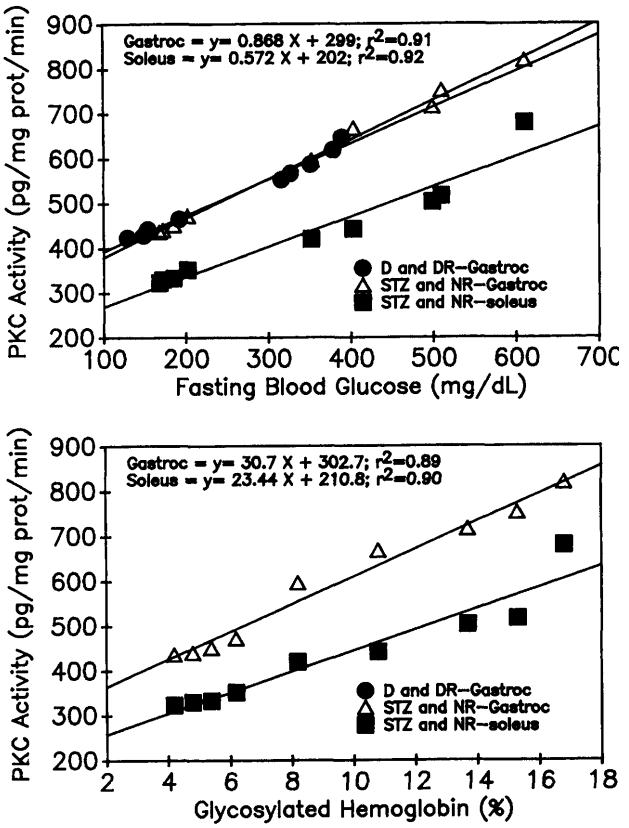

Figure 2. (Top panel) Relationship between fasting blood glucose concentration on the morning of the experiment and the particulate PKC activity of the gastrocnemius muscle from D and DR rats (closed circles) and STZ-treated and NR rats (open circles) and the PKC activity of the soleus muscle from STZ-treated and NR rats (closed boxes). The computer-drawn regression parameters for the gastrocnemius muscles from the BB/Wor and STZ diabetic rats did not differ and were combined to provide the equation and correlation coefficient shown within the diagram. (Bottom Panel) Relationship between fasting glycosylated hemoglobin concentration on the morning of the experiment and the particulate PKC activity of the gastrocnemius muscle (open circles) and the soleus muscle (closed boxes) from STZ-treated and NR rats. The computer-drawn regression parameters provided the equation and correlation coefficient shown within the diagram.

similar relationship was evident in the muscles of the NR and STZ-treated rats when the PKC activity was regressed against the glycosylated hemoglobin (Fig. 2, bottom).

PKC Isozymes in Normal and Diabetic Rat Skeletal Muscle. Each of the PKC-isozyme specific antibodies elicited a signal when reacted with authentic PKC isozyme standards and with the brain extract at a MW corresponding to the authentic PKC isozyme. The absence of cross reactivity between the antibodies for PKCα, PKCβ2, PKCδ, PKCε, PKCζ, PKCγ or PKCθ and each of the PKC isozymes has been documented previously (data not shown, 24). PKCα and small amounts of PKCζ were the only constitutive PKC isozymes present in the soluble fraction of the gastrocnemius muscle obtained from the DR and D rats whereas PKCα >> PKCδ >> PKCε in the particulate fraction obtained from these rats (Fig. 3). The relative units of gel density of each of the isozymes was greater in the gastrocnemius muscle obtained from the D rat when compared to the DR rat. However, PKCα increased by 327% ($P < 0.05$), whereas that of PKCδ increased by 87% ($P < 0.05$), and PKCε increased by 92% ($P < 0.05$) (Fig. 3).

The PKC isozymes detectable in the particulate fraction (membrane and nuclei) of the soleus and gastrocnemius muscles obtained from the NR and STZ-treated rats (Fig. 4) were also PKCα >> PKCε = PKCδ whereas the PKC isozymes detectable in the cytosol were PKCα >>> PKCζ. PKCζ was only detectable in the cytoplasm of the unstimulated skeletal muscles (Fig. 4). PKCβ, PKCγ, and PKCϕ, which are detectable in rat diaphragm (27), were absent in the soluble and particulate fractions of each of the replicates of gastrocnemius and soleus muscles obtained from the STZ-treated and NR Sprague-Dawley rats. The gel density

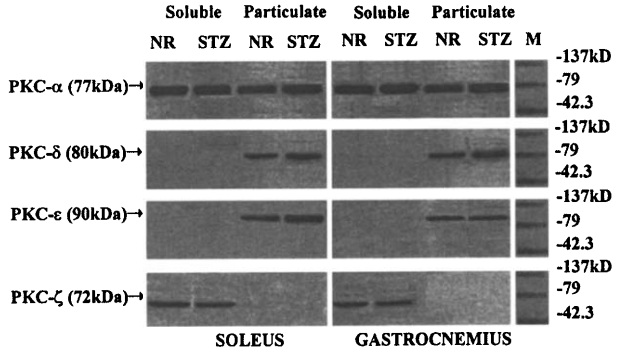

Figure 4. Representative Western blot of PKC isozymes in the soluble and particulate fractions of the gastrocnemius muscle (left panel) and soleus muscle (right panel) obtained from the STZ-treated and NR rat. This gel is representative of each of the five individual experiments. M = kilodalton marker.

of PKCα or PKCζ in the soluble fractions of both the soleus and gastrocnemius muscles did not differ between the NR and STZ-treated rats (Figs. 4 and 5). However PKCα increased ($P < 0.05$) by 31% and 34% respectively in the particulate fractions of the gastrocnemius and soleus muscles obtained from the STZ-treated rats when compared to the PKCα content of the particulate fraction of the corresponding muscles obtained from the NR rats. In contrast, the content of PKCδ isozyme increased significantly more (197%) in the particulate fraction of the gastrocnemius muscle obtained from the STZ-treated rats when compared to the 57% increase in this PKC isozyme in the particulate fraction of the soleus muscle obtained from STZ-treated rats (Figs. 4 and 5). Finally, PKCε increased by 34% and 72% in the particulate fractions of the gastrocnemius and soleus

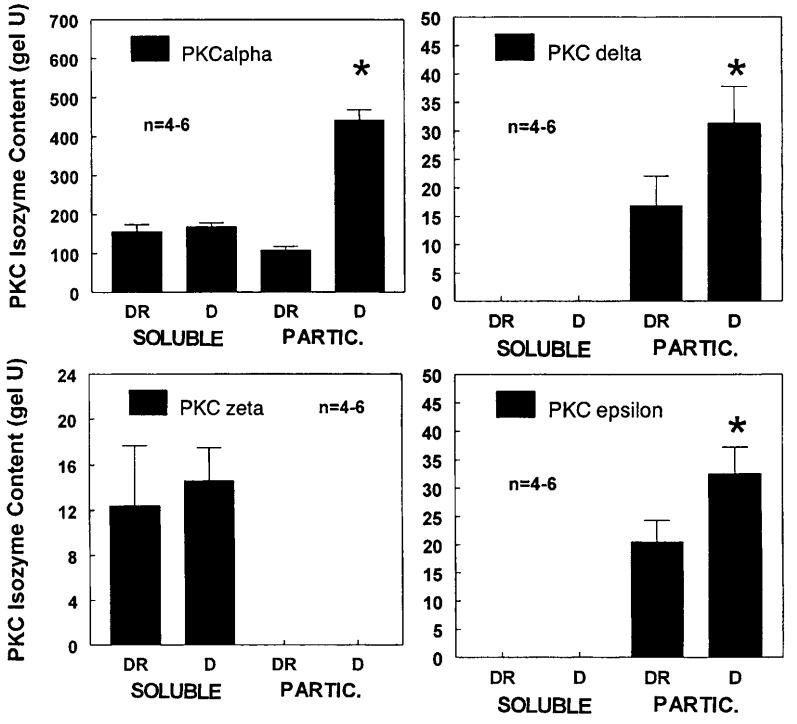

Figure 3. PKC isozyme protein content (gel density units/50 μg of protein) in the soluble and particulate fractions of the gastrocnemius muscle obtained from D and DR rats. Each value is the mean and SD from four or five rats. An asterisk denotes that the responses differ from DR ($P < 0.05$).

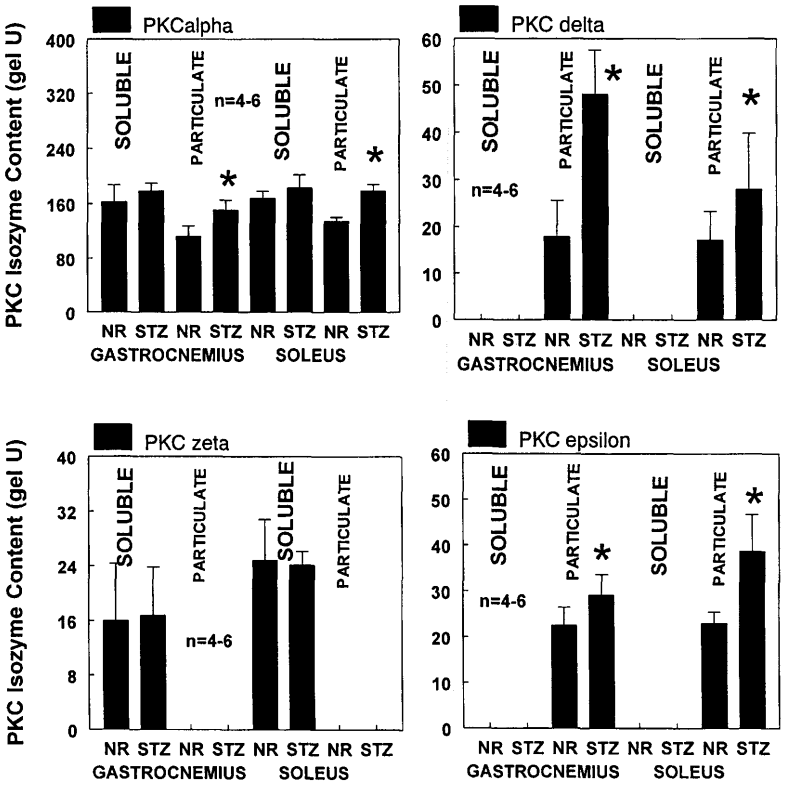

Figure 5. PKC isozyme protein content (gel density units/50 μg of protein) in the soluble and particulate fractions of the gastrocnemius and soleus muscles obtained from STZ-treated and NR rats. Each value is the mean and SD from four or five rats. An asterisk denotes that the responses differ from NR ($P < 0.05$).

muscles, respectively, when obtained from STZ-treated rats compared to that of the NR rats (Figs. 4 and 5).

Discussion

The results of these studies demonstrate that skeletal muscle PKC activity and the content of both calcium-dependent and calcium-independent PKC isozymes were increased in the fast and slow skeletal muscle from rats with genetic and streptozocin-induced diabetes mellitus. Moreover, the increase in PKC activity was related to the concentration of plasma glucose and apparently did not reflect differences in the duration of the hyperglycemia. The relative increases in the PKC activity and PKC isozyme content, as well as the distribution of PKC between the membrane and the cytosol, supports the contention that these measurements reflect the PKC activity and content of the skeletal muscle rather than the PKC activity and content of the degenerating nerve terminals of these muscles (28, 29). Thus, maintained increases in skeletal muscle basal PKC activity and particulate isozyme content accompany the development of both of these experimental models of type 1 and type 2 diabetes mellitus.

Differences existed in the origin of the diabetes (genetic versus STZ-induced), the duration of the hyperglycemia (4–6 weeks vs 18 weeks), and the necessity for use of insulin in the genetic model of DM. Nevertheless, all STZ and D rats exhibited classical symptoms of diabetes (polydipsia, polyuria, polyphagia, and elevated fasting blood glucose levels that exceeded 350 mg/dl). Since STZ-treated rats exhibit neural degeneration within 18 weeks of STZ treatment (1, 2, 5, 21, 29, 30) whereas young, insulin-treated D rats of 4 weeks duration do not exhibit any signs of neural degeneration (4), the rats used represent models of diabetes mellitus that reflect both the absence and presence of neural impairment of skeletal muscle function.

Total basal PKC activity and the relative activity of the soluble and particulate fractions obtained from gastrocnemius muscles of the DR and NR rats did not differ despite the difference in the age of the rats from which the muscles were obtained. Thus, it is also unlikely that the different PKC activities of the gastrocnemius muscles obtained from the D and STZ-treated rats reflected their different ages. It is also unlikely that the increase in PKC activity in the muscle of the D rat when compared to the DR rat reflected the administration of insulin to the D rats. Although acute administration of insulin increases protein and tyrosine kinase activities and proteolysis (31, 32), maintained administration of insulin and sustained action of PKC should result in down regulation of PKC activity (11, 13–15). However, despite the administration of insulin to D rats, plasma glucose concentrations remained elevated in D and STZ-treated rats for 4–6 and 18 weeks, respectively, compared to the DR and NR rats. Speculatively, this should have maintained the stimulation by DAG of PKC that should have downregulated PKC activity in the gastrocnemius muscle (8, 11, 13–15). Thus, the elevated PKC activity of the gastrocnemius and soleus muscles were inappropriately elevated or failed to downregulate in the face of maintained stimulation. However, when the particulate PKC activity of gastrocnemius and soleus muscles obtained from the indi-

vidual rats was regressed against the individual plasma glucose concentrations, a correlative, linear relationship was found. These findings lead us to conclude that the increased PKC activity in the gastrocnemius and soleus muscles of the D and STZ-treated rats with DM reflects the level of, and probably the duration of, the hyperglycemia. Moreover, the inability of PKC to downregulate within the skeletal muscle despite a maintained stimulus for elevated PKC suggests a potential defect in the negative feedback regulation of PKC in the genetic and STZ-induced models of DM. Since the increase in PKC activity in the muscles obtained from the BB Wor D rat occurred within 30–41 days of the onset of genetic diabetes at a time when impaired neural innervation to skeletal muscle and skeletal muscle dysfunction were not yet present, we also postulate that changes in skeletal muscle PKC activity in DM may precede the onset of skeletal muscle myopathy and speculatively may contribute to the myopathy (1–5, 30–33). Alternatively, since glucose transport in skeletal muscle can be stimulated not only by insulin but also by activation of PKC in muscle and other insulin-responsive cells, it is also possible that the increased PKC reflects a physiologic regulatory mechanism to upregulate glucose transport in skeletal muscle to compensate for diabetes-induced decreases in insulin and glut-4 receptors (4, 30, 34–37). Further studies are required to test this speculation.

The distribution of PKC isozymes PKCα in the cytosol and particulate fractions and PKCε and PKCζ only in the membrane and soluble fractions in the basal state of the muscles from the normal and diabetic rats is consistent with the distribution found in the heart and alveolar macrophages of the BB Wor and Sprague-Dawley rats (23, 24). However, the finding of PKCδ only in the membrane fraction differs from that of PKCδ in the myocardium and alveolar macrophage in which this PKC isozyme is present in both fractions. This finding suggests that the isozyme of PKC is compartmentalized differently in skeletal muscle than in other tissues. Moreover, since little reserve of soluble PKC is evident and the increased PKC content was found primarily in the particulate fraction of the skeletal muscle from D and STZ-treated rats, the data suggest that the increased PKC activity probably reflected activation of a greater amount of PKC enzyme rather than changes in the kinetic properties of the PKC isozymes (11, 13–15). This suggests that increased transcription or translation of PKC isozymes occurred during the development of diabetes mellitus or that degradation of the PKC isozymes was impaired in both experimental or STZ-induced DM (11, 13–15). Further studies are required to investigate these potential mechanisms.

It has been demonstrated that two species of the PKCα isozyme may exist in skeletal muscle during elution with hydroxyapatite chromatography (38). These species of PKCα are sensitive to anti-PKCα antibodies and demonstrate identical requirements for Ca^{2+} ion and lipid activation. Moreover, PKCα1 and PKCα2 phosphorylate equally well the modified conventional PKC pseudo substrate peptide (19–31, Ser-25) (38). However PKCα1 phosphorylates peptides derived from glycogen synthase to a much greater extent than does PKCα2. Similarly, PKCα1 and PKCα2 produce different protein phosphorylation patterns (38). It is unknown whether diabetes preferentially increases one or both species of PKCα. Nevertheless, the data presented clearly demonstrate that PKC activity and the amount and distribution of PKC isozymes in the particulate fraction of the gastrocnemius and/or soleus muscles increase in the early stage of genetic DM in the absence of neural damage equally as well as in the muscles of the STZ-treated rats in which hyperglycemia was present for 18 weeks (4, 21).

Skeletal muscle weakness and dysfunction and their associated sensory, motor, or autonomic symptoms, common manifestations associated with diabetes mellitus, have been attributed primarily to impaired peripheral neural function (1–5, 8, 21). In fact it has been suggested that the muscle weakness and impaired exercise tolerance of patients with DM results essentially from diabetic neuropathy, even when only minor changes in nerve conduction are evident (39). Skeletal muscle dysfunction in DM has been described almost entirely as neural in origin resulting from the reductions in motor and sensory nerve conduction velocity, impaired axonal transport, and degeneration of the nerve terminals in the end plate region of the neuromuscular junction in the nerves of both diabetic animals and man (1–5, 8, 21). One current hypothesis suggests that the elevated glucose levels of diabetes accumulate as sorbitol in neurons with a resultant increased osmolarity and paranodal swelling that may lead to axonal damage and/or impaired axoplasmic flow associated with eventual neuronal damage. Diabetic neuropathy ultimately results in some progressive degeneration of autonomic and somatic nerves innervating skeletal muscle (1, 2, 5, 8, 21, 39). Neuronal degeneration in the region of the end plate of skeletal muscle, which occurs later in the progression of DM, upregulates the receptors in the neuromuscular junction endplate region. That upregulation increases phosphoinositide turnover, further elevating the concentration of DAG in skeletal muscle already altered by prolonged hyperglycemia (1, 5, 7, 9, 21). However, complete neural denervation of rat diaphragm skeletal muscle only increases the PKCα isozyme content by 80% from control values and decreases atypical PKC isozymes in the end-plate region (30). Skeletal muscle function resulting from neuropathy is not present in BB Wor rats with 4–6 weeks of hyperglycemia (4). STZ-treated rats of 18-week duration exhibit changes in skeletal muscle function but only have mild disturbances in neural conduction velocity (1, 5, 21). Therefore, it is unlikely that the changes in skeletal muscle PKC reported herein are secondary to neural degeneration. The data support the speculation that elevated PKC may contribute to impaired skeletal muscle function during the progression of DM, independent of those changes arising from diabetic peripheral neuropathy. Further studies are required to test this postulate.

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