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Diabetes induces selective alterations in the expression of protein kinase C isoforms in hepatocytes

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Membrane and cytosol fractions from hepatocytes of both normal and streptozotocin-induced diabetic animals were probed with a panel of polyclonal anti-peptide antisera in order to identify protein kinase C (PKC) isoforms. Immunoreactive species were noted with antisera specific for α (~81 kDa), β-II (~82 kDA), ε (~95 kDa) and ζ (~79 kDa). In addition, a species migrating with an apparent size of ~94 kDa was also detected in cytosol fractions using an antiserum specific for PKC-α. Each of these species was specifically displaced when the PKC-isoform specific peptide was included in the immunodetection system. No immunoreactive species consistent with the presence of the β-I, γ, δ and η isoforms of protein kinase C was observed. Induction of diabetes using streptozotocin invoked selective alterations in the expression of PKC isoforms which were reversed upon insulin therapy. In the cytosol fraction, marked increases of ~3-fold occurred in levels of the β-II isoform and the ~90 kDa (upper) form of PKC-α, with no apparent/little change in the levels of the ~81 kDa (lower) form of PKC-α and those of PKC-ζ. Diabetes induction also appeared to have elicited the translocation of PKC-β-II and the ~81 kDa (lower) form of PKC-α to the membrane fraction where immunoreactivity for these species was now apparent. The level of PKC-ε, which was noted only in membrane fractions, was also increased upon induction of diabetes. It is suggested that the selective alterations in the expression of PKC isoforms occurring upon streptozotocin-induced diabetes may lead to altered cellular functioning and underly defects in inhibitory G-protein functioning and insulin action which characterise this animal model of diabetes.

Protein kinase C; Isoform; Diabetes; Streptozotocin; Insulin; Liver; Hepatocyte; Phosphorylation

1. INTRODUCTION

Protein Kinase C (PKC) was first discovered in 1977 as an undefined protein kinase which was activated by limited proteolysis with the neutral protease calpain [1]. Its ability to be activated by tumour-promoting phorbol esters and by diacyl glycerol (DAG), produced through receptor-stimulated phospholipid metabolism, has led to the realisation that this enzyme plays a major role in signal transduction events [1–4].

Molecular cloning and enzymological analysis have now identified a number of structurally related protein kinase C isozymes which include the Ca^{2+}-regulated species α, βI, βII, γ, and those that lack such a regulatory domain, namely δ, ε, η, ζ and L-forms [5–8]. There is considerable interest in the possibility that individual isoforms may have distinct functional roles in the cell. These may be reflected by differences in their degree of activation, target protein specificity, susceptibility to activation by DAG species with different acyl chains, sensitivity to Ca^{2+} and arachidonate and also regulation by phosphorylation [1,3,5,6,9,10]. There is also some data indicating the selective expression of protein kinase C isoforms, with PKC-α apparently being expressed ubiquitously in mammalian cells, βII being found in most tissues and with other isoforms showing much more restricted cellular distributions [1,5,6,11,12]. That there are differences in the distribution between membrane and cytosol fractions of the various isoforms and that some species can undergo translocation as a result of ligand or phorbol ester challenge [1–4] again indicates that specific functions may be attributable to members of this enzyme family.

There is now considerable evidence for the occurrence of 'cross-talk' between different signalling pathways [13,14]. This certainly occurs in hepatocytes, which provides a good example of the modulation of adenylyl cyclase and cyclic AMP metabolism by lipid signalling pathways exerting actions through PKC [13–17]. The desensitization of glucagon's ability to stimulate adenylyl cyclase takes the form of an uncoupling of the receptor from G_s which is mediated through the activation of protein kinase C and is presumed to result from the phosphorylation of the glucagon receptor [18].

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Abbreviations: PKC, Protein kinase C; G-protein, guanine nucleotide regulatory (binding) protein; G_s, stimulatory G-protein acting on adenylate cyclase; G_i, inhibitory G-protein acting on adenylate cyclase; DAG, diacyl glycerol.

Such cAMP-independent actions of glucagon appear to be attributable to its ability to activate lipid signalling pathways leading to the production of DAG [19–22], elevation of Ca^{2+} levels and the activation of PKC [22,23]. The recent molecular cloning of the glucagon receptor indicates that a single receptor, able to couple to more than one signalling system, is responsible for these actions [24]. As well as this, activation of protein kinase C also causes the loss of 'tonic' GTP-mediated inhibition of adenylyl cyclase through G_i [17]. This apparently physiological 'crosstalk' mechanism appears, however, to be inappropriately activated in streptozotocin-induced diabetes [25,26] where we have noted the loss of such 'tonic' GTP-elicited inhibition of adenylyl cyclase [25,26]. This appears to be due in part to the reduced expression of the inhibitory G-protein α-G_i-2 but also to the increased phosphorylation, through the action of PKC, of α-G_i-2 itself [18,26].

Streptozotocin-induced diabetes has recently been shown [27] to lead to increased levels of the β-II isoform in the membrane, but not the cytosol fraction, of rat aorta and heart, whilst not affecting the expression of the α isoform. It is possible that abnormalities in PKC expression might occur in hepatocytes from streptozotocin-induced diabetes as reflecting the altered functioning of this enzyme activity deduced from analyses done on G_i function and phosphorylation [25,26]. We here describe the use of a panel of sub-type specific antisera to probe alterations in the expression and distribution, between membrane and cytosol compartments, of various protein kinase C isoforms.

2. MATERIALS AND METHODS

2.1. *Materials*

Streptozotocin, 1,2-dioleoylglycerol, histone H1 (type IIIS), aprotinin were from Sigma, Poole, Dorset, UK. Leupeptin was from Peptide Synthesis Inc., via Scientific Research Associates, London, UK. Phosphatidylserine was from Lipid Products, London, UK. Collagenase and Diabur-Test 5000 was from Boehringer Mannheim Biochemicals, UK. Dextrostix for blood glucose determination was from Ames Division, Miles Ltd., Slough, UK. [γ-^{32}P]ATP, ECL detection kit and ECL-hyperpaper were from Amersham International, Amersham, Bucks, UK. Bradford reagent was from Bio-Rad. HRP-antirabbit antibody and donkey serum were from Scottish Antibody Production Unit, Scotland. Lentard MC porcine/bovine insulin was from Novo Laboratories Ltd., Basingstoke, UK. All other reagents were at least AnalaR grade, from Sigma, Boehringer Mannheim Biochemicals.

The protein kinase C isozyme-specific antisera employed were as described previously by one of us [28]. These were raised using peptides corresponding to unique sequences within the different isozymes α, βI, βII, γ, ϵ and ζ. Their specificity has been reported on previously [28]. Results were also confirmed using a new panel of antisera (prefix GUCSG-; results not shown). The antisera codes were for α-PKC ((C)-PQFVHPILQSAV, V5 domain) [28] and also antiserum GUCSG-1986α (amino acids 317–329; VISPSEDRRQPSC); βII-PKC(β1-), with antiserum v5β1 (C-SEFLKPEVKS) [28] and also antiserum GUCSG-1942βII (amino acids 660-673;C-SFVNSEFLKPEVKS); βI-PKC(β2-), with antiserum v5β2 [28] (C-NPEFVINV) and also antiserum GUCSG-647βI (amino acids; C-RDKRDTSNFDKEFT); γ-PKC, with antiserum v5γ (C-PDARSPISPTPVPVM) [28] and also antiserum GUCSG-1726γ (amino acids 306-318; C-NYPLELYERVRTG); ε-PKC, with antiserum ε (C-NQEEFKGFSYFGEDLMP) [29] and also antiserum GUCSG-637ε (amino acids 721–737; C-NQEEFKGFSYFGEDLMP); ζ-PKC, with antiserum ζ [30] and also antiserum GUCSG-1985ζ (amino acids 577-592; C-GFEYINPLLLSAEESV); and an antiserum against the C-terminal decepeptide of protein kinase C δ (C-VNPKYEQFLE) called antiserum-δ [31] and with antiserum η (C-QDEFRNFSYVSPELQ) [32]. We also employed the MC5 monoclonal antibody which recognises the α, β-II, β-II and γ forms of protein kinase C [33].

2.2. *Induction of experimental diabetic rats*

The induction of diabetes (single dose of streptozotocin, 80 mg/kg body wt. in sodium citrate pH 4.5, i.p.) into male Sprague–Dawley rats (180–200 × *g*) with assessment of glucose levels in urine and blood, was done as described before in detail by one of us [25]. In some instances the diabetic animals were subdivided into insulin-treated or untreated group with, as before [25], insulin-treated animals received a daily subcutaneous injection of 10 I.U. of Lentard porcine insulin for 7 days, beginning 4 days after streptozotocin administration.

2.3. *Preparation of hepatocytes*

Hepatocytes were prepared from fed male Sprague–Dawley rats (200–250 × *g*) essentially as previously described by us [15,23], using a procedure based upon that of Berry and Friend [34], except that 0.75 mM $CaCl_2$ was added to the collagenase-containing buffer. After isolation, the cells were washed twice in Hanks balanced salt solution and then resuspended in 10 vol. of Hanks buffer supplemented with 1% (w/v) dialysed fatty acid free BSA. 50 ml of the resulted suspension was pre-incubated at 37°C (gassed with O_2/CO_2, 19:1) for 30 min before any experimental manipulation.

2.4. *Preparation of hepatocyte extracts and whole liver extracts*

Hepatocytes were sedimented and further washed twice in 10 vol. of ice-cold PBS and resuspended in 10 ml of homogenisation buffer (20 mM Tris-HCl, 2 mM EDTA, 2 mM EGTA, 6 mM β-mercaptoethanol, 20 μg/ml leupeptin, 4 μg/ml aprotinin, pH 7.4). All procedures were carried out at 4°C from this point unless otherwise stated. Cells were then lysed by either employing a very brief period of sonication on ice with a micro-probe attachment for three 10 s bursts on a Dawes sonicator at 80 W [23] or by using the gentle N_2-pressurisation and homogenisation procedure developed by us previously [15]. The resulting homogenates (15 ml) were centrifuged at 1,500 × *g* at 4°C for 10 min to remove unbroken cells. The pellets were discarded and the supernatant was centrifuged at 100,000 × g_{av} at 4°C for 30 min on a Beckman L-8 ultracentrifuge with a Ti60 rotor. The resultant supernatant, referred as the cytosolic extract, was collected, aliquotted and stored at −80°C until use. The pellet was resuspended in 8 ml of homogenisation buffer containing 1% (v/v) Nonidet P-40 and left to extract for 30 min. The mixture was then centrifuged at 100,000 × *g* at 4°C for 30 min and the supernatant is referred as the membrane extract and was stored at −80°C. The protein concentration was determined by the method of Bradford [35] using dialysed fatty acid free BSA as standard.

When cytosolic and membrane extracts were prepared from whole liver, the following procedure was used in order to minimize contamination with protein from blood and blood cells. Sprague–Dawley rats were anesthetised by intraperitoneal injection of 0.3 ml Sagatal. When the animal was unconscious, 0.2 ml of heparin solution (1 mg/ml) was infused into the tail vein. The vena cava was cannulated and led to waste. The liver was perfused via the portal vein with ice-cold PBS, supplemented with 2 mM EGTA and 2 mM EDTA ('PEE' buffer), until the liver was clear of blood and attained ice-cold temperature. All procedures were carried out at 4°C from this point unless otherwise stated. The liver was excised into PEE buffer, cut into small pieces and rinsed with 20 ml homogenisation buffer. The tissue was resuspended in 40 ml of homogenisation buffer and homogenised with 15 strokes in a Dounce homogenizer. The resultant homogenate was treated as described above for hepatocyte homogenates.

2.5. *Immunoblotting of protein kinase C isozymes*

Cytosolic extracts (200 μg of protein) and membrane extracts (200 μg of protein) were separated by SDS/PAGE (7.5% gels) after being solubilised and boiled in Laemmli buffer [36]. Western blotting was performed essentially as described before by us (see e.g. [37]) using a 2 h transfer time onto nitrocellulose paper in blotting buffer (0.005% SDS, 5 g/l Tris base and 14.4 g/l glycine). The blots were blocked with 4% (w/v) skimmed milk, 2% (v/v) donkey serum in PBS, 0.05% NP-40 and 20%-methanol for 2 h, followed by an incubation with the designated isozyme specific antiserum (appropriately diluted in 0.02% thimerosal) overnight. The blots were rinsed four times for 15 min each with PBS/0.05% Nonidet P-40 and then probed with HRP-donkey anti-rabbit antiserum (1:250 in blocking solution) for 2 h, followed by four rinses in PBS/005% Nonidet P-40, each for 15 min. The blots were finally rinsed in PBS and immuno-detection was carried out using an enhanced chemilluminescence kit from Amersham International (ECL kit). The procedure was carried out as described as per the manufacturers instructions and immunoreactivity visualised using ECL-hyperpaper exposed for 2–30 min. The images on hyperpaper were quantitated by densitometry on a Shimadzu CS-9000 scanning densitometer.

In experiments where synthetic C-terminal peptides were used to compete with protein kinase C for binding to the isozyme-specific antisera, these (stock concentration of 1 mg/ml) were pre-mixed at a ratio of 1:1 with the antisera and then incubated for 1 h before being diluted into the blocking solution.

3. RESULTS AND DISCUSSION

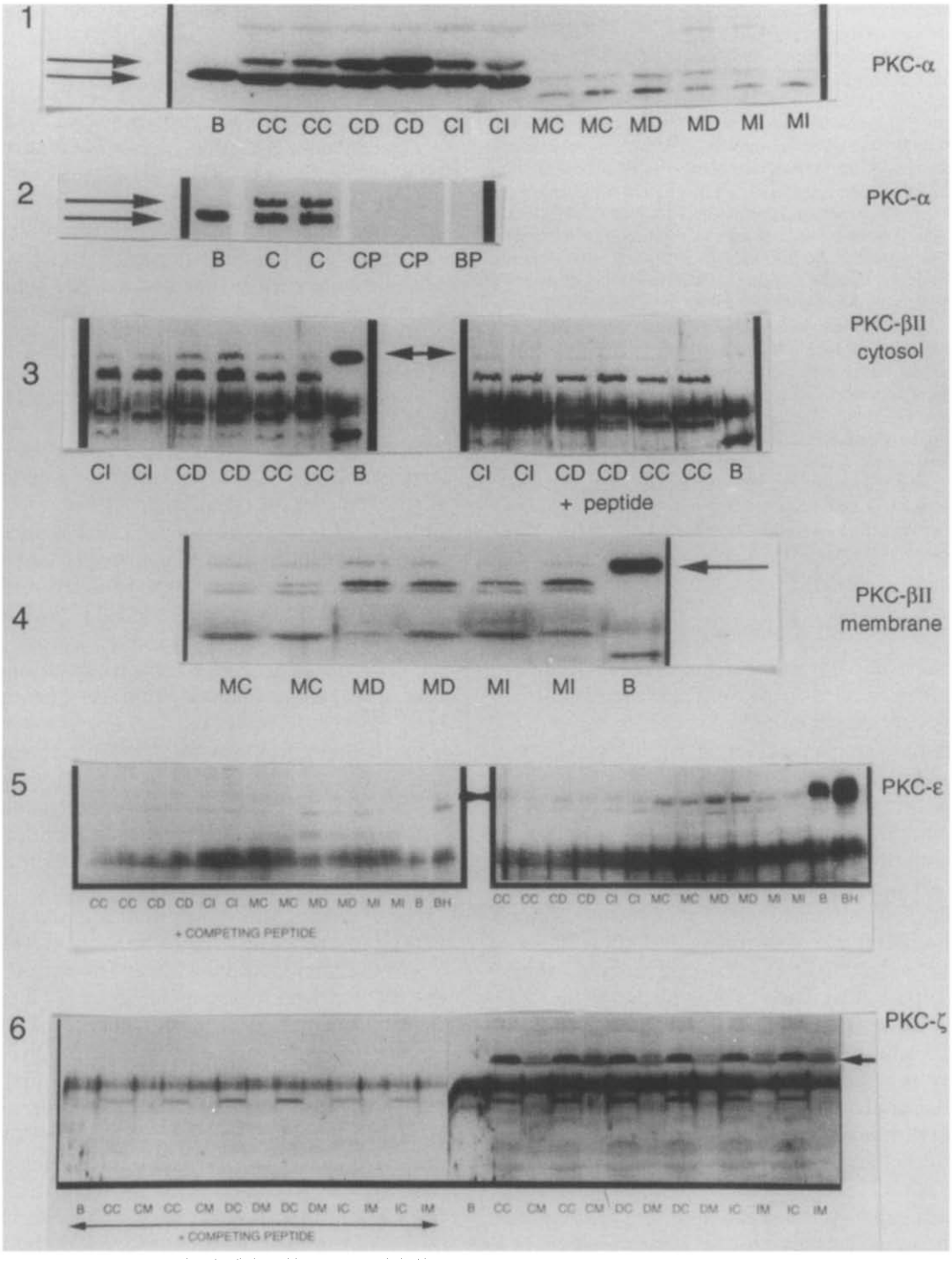
In hepatocytes of streptozotocin-induced diabetic rats [25,26] there is a loss of G_i function which appears to result from both a reduction in the expression of G_i-2 and also its increased phosphorylation by protein kinase C. Treatment of a variety of different cell types with tumour promoting phorbol esters has been shown to lead to the loss of guanine nucleotide-elicited, G_i-mediated inhibition of adenylate cyclase activity [13,37]. Similar observations of crippled G_i function have also been seen in cells challenged with ligands which stimulate phospholipid metabolism or with DAG [13], suggesting that it is the activation of protein kinase C which provides the molecular basis of this phenomenon. Indeed, studies on hepatocytes [14,18,26,38], platelets [39–41] and U973 cells [42] have indicated that the α-subunit of G_i-2 may provide a target for phosphorylation of G_i-2. And, certainly, in hepatocytes [14,18] the loss of guanine nucleotide-mediated inhibition of adenylate cyclase parallels the phosphorylation of α-G_i-2. Consistent with such observations are those showing that treatment of either purified G_i [43] or membranes [18,25] with purified brain protein kinase C preparations led to the phosphorylation of α-G_i-2.

Such observations of loss of tonic GTP-elicited G_i function suggest that there may be aberrations at the level of PKC in hepatocytes of streptozotocin-induced diabetic rats. In order to assess whether changes in expression ensued as a result of the induction of diabetes, we utilised immunoblotting techniques to detect PKC in cytosol and membrane fractions from hepatocytes of normal rats, those made diabetic with streptozotocin and diabetic rats subjected to insulin therapy in order to normalise their hypoinsulinaemia and hyperglycaemia. Initial experiments were done using the monoclonal antibody MC5 to detect PKC. This antibody is non-selective as regards the major PKC isoforms [33] and was used here merely as a general indicator of changes in expression occurring in PKC levels. We have previously noted [23] that this antibody detects a single immunoreactive species of ~84 kDa in both the cytosol and membrane fraction of hepatocytes. In the present study we noted that diabetes induction elevated levels of detected PKC immunoreactivity around 2-fold (105 ± 12% and 93 ± 11% increase over control, respectively) in the cytosol and membrane fractions (means ± S.D., $n = 4$ separate experiments using different aminals; data not shown).

In order, however, to attribute changes to specific PKC isoforms we used a previously characterised (see section 2) panel of polyclonal antisera specific for the α, β-I, β-II, γ, δ, ε, η and ζ isoforms. Analysis of membrane and cytosol fractions from hepatocytes of both normal and streptozotocin-induced diabetic animals failed to identify immunoreactive species consistent with the presence of the β-I, γ, δ and η isoforms of PKC ($n = 5$ separate experiments using different cell preparations). However, immunoreactive species were noted with antisera for α, β-II, ε and ζ which co-migrated with that detected in a purified brain PKC preparation (Fig. 1).

Intriguingly, however, analysis of the cytosol fraction with antisera for PKC-α led us to observe an additional immunoreactive species which migrated more slowly, with an apparently higher molecular size of ~90 kDa, compared to that of the 81 kDa species found in both membrane and cytosol fractions (Sections 1 and 2, Fig. 1). Both these species which were detected with the anti-PKC-α antisera appeared to be authentic PKC-α entities in that their detection was specifically ablated when the PKC-α peptide was included in the immunodetection (blotting) assay. The ~81 kDa species is of a similar size to that reported by others for PKC-α purified from brain (see e.g. Marais and Parker [28]), although that, in itself, is larger than the molecular size of 76.8 kDa that can be predicted from the sequence deduced from cDNA cloning [44]. It has been noted, however, that PKC-α and other isoforms are subject to posttransational modification which can lead to marked alterations in their apparent molecular size upon SDS-PAGE (see e.g. [10]) which may explain the apparent presence of multiple forms of protein kinase C-α seen here and by others previously [10].

We also observed marked differences in the distribution of the various PKC isoforms between membrane and cytosol fractions. Thus the more slowly migrating form of PKC-α appeared only to occur in the cytosol fraction as did the majority of the ~81 kDa form of PKC-α (Section 1, Fig. 1; Table I). Similarly, the β-II isoform was essentially only detected in the cytosol fraction (Sections 3 and 4, Fig.1: Table I), where it migrated



as a species of around 82 kDa. This size is similar to that observed for the purified isoform from brain (see e.g. [28]) but larger than the molecular size of ~77 kDa predicted from the cDNA sequence [44].

In contrast to the location of the β-II isoform, PKC-ε was only detected in the membrane fraction (Sections 4 and 5 of Fig. 1; Table I) where it was found as species of ~95 kDa. This is of a similar size (~89 kDa) to that noted by Schaap et al. [29] for the brain enzyme expressed in COS cells.

PKC-ζ was apparently equally distributed, as a ~79 kDa species, between both membrane and cytosol fractions (Fig. 1). Its size compares with a value of ~80 kDa reported for the enzyme detected using immunoblotting

Fig. 1. Identification of PKC isoforms in hepatocyte membrane and cytosol fractions. Membranes and cytosol fractions of hepatocytes from normal animals, streptozotocin-diabetic animals and insulin-treated streptozotocin diabetic animals were subjected to SDS-PAGE and subsequently immunoblotted with isoform-specific PKC antisera. The gels shown are typical of experiments done at least four times with cells made from different animals. In all sections the track labelled 'B' refers to a purified PKC preparation made from brain. Section 1 shows PKC-α antiserum used to analyse membrane (MC, MD, MI) and cytosol (CC, CD, CI) fractions from normal (MC, CC), streptozotocin-diabetic (MD, CD) and insulin-treated streptozotocin diabetic (MI, CI) fractions. Note the co-migration of an immunoreactive species of ~84 kDa (lower arrow) in hepatocyte samples with that seen in a purified protein kinase C preparation from brain (B). A more slowly migrating species of ~90 kDa (upper arrowed band) is also identifiable. In section II the use of a competing peptide (CP, BP) is shown, which specifically blocks interaction of the antiserum with PKC-α species in brain (B) and both the ~84 (lower arrow) and ~90 kDa (upper arrow) species identifiable in hepatocyte cytosol (C) preparations. Sections 3 and 4 show the use of PKC-βII antiserum to analyse cytosol (CC, CD, CI) and membrane (MC, MD, MI) fractions from normal (MC, CC), streptozotocin-diabetic (MD, CD) and insulin-treated streptozotocin diabetic (MI, CI) fractions. The ~82 kDa species (arrowed) which co-migrates with that detected in a purified PKC preparation from brain (B) could be specifically displaced by the competing peptide (Section 2). Section 5 shows the use of a PKC-ε specific antiserum to analyse both membrane (MC, MD, MI) and cytosol (CC, CD, CI) fractions from normal (MC, CC), streptozotocin-diabetic (MD, CD) and insulin-treated streptozotocin diabetic (MI, CI) fractions. A ~95 kDa species is noted in both membrane fractions and the PKC brain (B) preparation which is displaced specifically by the appropriate competing peptide (arrowed). Section 6 shows data labelled as per section 5 but using anti-sera specific for PKC-ζ. In this instance immunoreactivity is observed in both cytosol and membrane preparations with an apparent size of ~79 kDa (arrowed).

←

techniques applied to a variety of cell lines [30]. This value is, however, greater than that predicted (~67 kDa) from the cDNA sequence [45].

Table I

Distribution of PKC isoforms between membrane and cytosol compartments of hepatocytes. Membrane and cytosol samples isolated from rat hepatocytes were analysed by SDS-PAGE with subsequent immunoblotting using PKC isoform-specific antisera as described in Section 2. Scanning densitometry of immunoreactive bands allowed for the estimation of comparative changes in the levels of PKC isoforms occurring upon induction of diabetes using streptozotocin and in insulin-treated diabetic animals. The '%-age change' indicates the percentage increase/decrease (±) in PKC isoform levels compared to that seen in the control animals (e.g. a 100% change is equivalent to a doubling or 2-fold change in the level). Errors are ± S.D. for at least 4 separate experiments using cell preparations from different animals. The 'upper' and 'lower' species of PKC-α refer to the ~94 kDa and ~84 kDa species, respectively (see Fig. 1 Section 1). 'n.a.' is not applicable as no immunoreactive species was apparent even under conditions where the applied sample protein level was doubled. 'dt' is detectable in the diabetic state but not in the control state and thus no meaningful relative change can be calculated. 'n.c.' is no change in levels (< 10% difference). *Significant at $P < 0.005$; **significant at $P < 0.01$; ***not significant, Students t-test.

Isoform	% Change in PKC isoform levels occurring upon induction of diabetes			
	Membrane		Cytosol	
	Diabetic	Insulin-treated	Diabetic	Insulin-treated
PKC-α (upper; ~90 kDa)	n.a.	n.a.	160 ± 78*	19 ± 8***
PKC-α (lower; ~81 kDa)	dt (seen in diabetes only)	n.a.	20 ± 9***	5 ± 11***
PKC-β-II	dt (seen in diabetes only)	n.a.	220 ± 90*	35 ± 15***
PKC-ε	67 ± 14**	−48 ± 6**	n.a.	n.a.
PKC-ζ	nc (< 10% change)	nc (< 10% change)	nc (< 10% change)	nc (< 10% change)

The induction of diabetes using streptozotocin had a profound effect upon the levels of expression and the distribution of PKC isoforms. Subjecting the diabetic animals to insulin therapy, however, indicated that such changes were reversible (Fig. 1; Table I), which militates against the effect of streptozotocin being a non-specific action of the compound acting on hepatic tissue but, rather, suggests that such changes were due to the diabetic condition itself. For PKC-α, the induction of diabetes now allowed for the detection of the 81 kDa species in the membrane fraction as well as eliciting a small increase in that found in the cytosol (Fig. 1 Section 1; Table I). Most dramatic, however, was the large increase (~2-fold) in the amount of the 90 kDa species whose location appeared exclusive to the cytosolic fraction (Fig. 1 Section 1; Table I). Such data imply an overall increase in the expression of PKC-α, in both membrane and cytosol fractions, coupled with the translocation of a portion of the ~81 kDa species to the membrane fraction.

Streptozotocin-induced diabetes elicited a marked increase in the amount of cytosolic PDC-βII. This was also accompanied by our ability to detect immunoreactive PKC-βII in membranes from the hepatocyte of diabetic rats, implying that translocation of PKC-βII from the cytosol had occurred (Fig. 1 Sections 3 and 4; Table I).

The PKC-ε isoform seemed to be exclusively associated with the membrane fraction where diabetes induction caused an increase in its level (Fig. 1 Section 5; Table I). Interestingly, however, we noted that in hepatocytes from diabetic rats subjected to insulin therapy then levels of the PKC-ε isoform were actually decreased to values which were below those found in the control, untreated animals (Fig. 1 Section 5).

In contrast to the marked, reversible changes in the expression of PKC-α, -βII and ε, which were induced by diabetes, we failed to observe any change in the level of expression of PKC-ζ found in either membrane or cytosol fractions. Neither did treatment of the diabetic

animals with insulin alter the levels of this PKC isoform (Fig. 1 Section 6; Table I).

The increase in the levels and altered distribution between membrane and cytosol compartments of certain protein kinase C isoforms may thus provide an explanation for the aberrant increase in the level of protein kinase C-mediated phosphorylation of G_i-2 seen in the hepatocytes of streptozotocin-induced diabetic rats [26]. As G_i-2 is found to be partly phosphorylated under resting (basal) conditions in hepatocytes from normal animals [14,26,38] then it is possible that by merely effecting an increase in the amount of the PKC isoform responsible for phosphorylating this G-protein, then such an action could lead to an increase in the level of phosphorylation of G_i-2. The trigger influencing alterations in PKC expression remains to be identified. However, elevated levels of both glucagon and vasopressin characterise streptozotocin-diabetic rats [46], both of which serve to increase hepatocyte DAG levels and can activate both membrane and cytosolic protein kinase C activity [23]. Thus such conditions of chronic stimulation may of themselves lead to alterations in PKC expression and intracellular distribution.

Undoubtedly such changes in PKC expression are likely to have widespread effects, some of which may in themselves affect the action of insulin on target tissues. For the insulin receptor itself is known to provide a substrate for this enzyme [47–50] with such modifications being implicated as attenuating at least certain of the functions of the receptor and thus exerting a selective effect on its different signalling actions (Houslay [48]). It may be then that aberrant expression of specific PKC isoforms can alter cell signalling pathways leading to (selective) hormone resistant states. Such a reversible phenomenon may underly facets of insulin-resistance seen not only in streptozotocin-induced diabetes but in human type-I and type-II diabetes, prompting isoform-specific analyses of protein kinase C expression and distribution in such conditions.

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REFERENCES

[1] Nishizuka, Y. (1988) Nature 334, 661–665.
[2] Shears, S.B. (1990) Cell. Signal. 1, 125–134.
[3] Sando, J.J., Maurer, M.C., Bolen, E.J. and Grisham, C.M. (1992) Cell. Signal. 4, 595–611.
[4] Cooke, S.J. and Wakelam, M.J.O. (1992) Cell. Signal. 3, 273–282.
[5] Nishizuka, Y. (1989) Cancer 63, 1892–1903.
[6] Stabel, S. and Parker, P.J. (1991) Pharmacol. Ther. 51, 71–95.
[7] Nixon, J.S., Bishop, J., Bradshaw, D., Davis, P.D., Hill, C.H., Elliott, L.H., Kumar, H., Lawton, G., Lewis, E.J., Mulqueen, M., Westmacott, D., Wadsworth, J. and Wilkinson, S.E. (1992) Biochem. Soc. Trans. 20, 419–434.
[8] Parker, P.J., Cook, P.P., Olivier, A.R., Pears, C., Ways, D.K. and Webster, C. (1992) Biochem. Soc. Trans. 20, 415–418.
[9] Murakami, K., Chan, S.Y. and Routtenber, A. (1986) J. Biol. Chem. 261, 15424–15429.
[10] Pears, C., Stabel, S., Cazaubon, S. and Parker, P.J. (1992) Biochem. J. 283, 515–518.
[11] Ono, Y. and Kikkawa, U. (1987) Trends. Biochem. Sci. 12, 421–423.
[12] Dreher, M.L. and Hanley, M.R. (1988) Trends Pharmacol. Sci. 9, 114–115.
[13] Houslay, M.D. (1991) Eur. J. Biochem. 195, 9–27.
[14] Houslay, M.D. (1991) Cell. Signal. 3, 1–9.
[15] Heyworth, C.M. and Houslay, M.D. (1983) Biochem. J. 214, 93–98.
[16] Murphy, G.J., Hruby, V.J., Trivedi, D., Wakelam, M.J.O. and Houslay, M.D. (1987) Biochem. J. 243, 39–46.
[17] Murphy, G.J., Gawler, D., Milligan, G., Wakelam, M.J.O., Pyne, N.J. and Houslay, M.D. (1989) Biochem. J. 259, 191–197.
[18] Bushfield, M., Murphy, G.J., Lavan, B.E., Parker, P.J., Hruby, V.J., Milligan, G. and Houslay, M.D. (1990) Biochem. J. 268, 449–457.
[19] Bocckino, S.B., Blackmore, P.F. and Exton, J.H. (1985) J. Biol. Chem. 260, 14201–14207.
[20] Blackmore, P. and Exton, J.H. (1986) J. Biol. Chem. 261, 11056–11063.
[21] Wakelam, M.J.O., Murphy, G.J., Hruby, V.J. and Houslay, M.D. (1986) Nature 323, 68–71.
[22] Pittner, R.A. and Fain, J.N. (1991) Biochem. J. 277, 371–378.
[23] Tang, E.K.Y. and Houslay, M.D. (1992) Biochem. J. 283, 341–346.
[24] Jelinek, L.J., Lok, S., Rosenberg, G.B., Smith, R.A., Grant, F.J., Biggs, S., Bensch, P.A., Kuijper, J.L., Sheppard, P.O., Sprecher, C.A., O'Hara, P.J., Foster, D., Walker, K.M., Chen, L.H.J., McKernan, P.A. and Kindsvogel, W. (1983) Science 259, 1614–1616.
[25] Gawler, D., Milligan, G., Spiegel, A.M., Unson, C.G. and Houslay, M.D. (1987) Nature 327, 229–232.
[26] Bushfield, M., Griffiths, S.L., Murphy, G.J., Pyne, N.J., Knowler, J.T., Milligan, G., Parker, P.J., Mollner, S. and Houslay, M.D. (1990) Biochem. J. 271, 365–372.
[27] Inoguchi, T., Battan, R., Handler, E., Sportsman, J.R., Heath, W. and King, G.L. (1992) Proc. Natl. Acad. Sci. USA 89, 11059–11063.
[28] Marais, R.M. and Parker, P.J. (1989) Eur. J. Biochem. 182, 129–137.
[29] Schaap, Parker, P.J., Bristol, A., Kriz, R. and Knopf, J. (1989) FEBS Lett. 243, 351–357.
[30] Ways, D.K., Cook, P.P., Webster, C. and Parker, P.J. (1992) J. Biol. Chem. 267, 4799–4805.
[31] Dekker, L.V., Parker, P.J. and McIntyre, P. (1992) FEBS Lett. 312, 195–199.
[32] Young, S., Rothbard, J. and Parker, P.J. (1989) Eur. J. Biochem. 173, 247–252.
[33] Berry, M.N. and Friend, D.S. (1969) J. Cell Biol. 43, 506–514.
[34] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[35] Laemmli, U.K. (1970) Nature 227, 680–685.
[36] Bell, J.D. and Brunton, L.L. (1986) J. Biol. Chem. 261, 12036–12041.
[37] Livingstone, C., McLellan, A.R., McGregor, M.A., Wilson, A., Connell, J.M.C., Small, M., Milligan, G., Paterson, K.R. and Houslay, M.D. (1991) Biochim. Biophys. Acta 1096, 127–133.
[38] Rothenberg, P.L. and Kahn, R. (1988) J. Biol. Chem. 263, 15546–15552.
[39] Halenda, S.P., Volpi, M., Zavoica, G.B., Sha'afi, R.I. and Feinstein, M.B. (1986) FEBS Lett, 204, 341–346.
[40] Crouch, M.F. and Lapetina, E.G. (1988) J. Biol. Chem. 263, 3363–3371.
[41] Yatomi, Y., Arata, Y., Tada, S., Kume, S. and Ui, M. (1992) Eur. J. Biochem. 205, 1003–1009.
[42] Daniel-Issakani, S., Spiegel, A.M. and Strulovic, B. (1989) J. Biol. Chem. 264, 20240–20247.
[43] Katada, T., Gilman, A.G., Watanabe, Y., Bauer, S. and Jakobs, K.H. (1985) Eur. J. Biochem. 151, 431–437.

[44] Parker, P.J., Coussens, L., Totty, N., Rhee, L., Young, S., Chen, E., Stabel, S., Waterfield, M.D. and Ullrich, A. (1986) Science 233, 853–859.
[45] Ono, Y., Fuji, T., Ogita, K., Kikkawa, U., Igarashi, K. and Nishizuka, Y. (1989) Proc. Natl. Acad. Sci. USA 86, 3099–3103.
[46] Brooks, D.P., Nutting, D.F., Crofton, J.T. and Sharpe, L. (1989) Diabetes 38, 54–57.
[47] Takayama, S., White, M.F. and Kahn, C.R. (1988) J. Biol. Chem. 263, 3440–3447.
[48] Houslay, M.D. (1989) Trends Endocrinol. Metab. 1, 83–89.
[49] Koshio, O., Akanuma, Y. and Kasuga, M. (1989) FEBS Lett. 254, 22–24.
[50] Lewis, R.E., Cao, L.K., Perregaux, D. and Czech, M.P. (1989) Biochemistry 29, 1807–1813.