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Increase in P-glycoprotein accompanied by activation of protein kinase Cα and NF-κB p65 in the livers of rats with streptozotocin-induced diabetes

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ABSTRACT

It is known that protein kinase C (PKC) signal transduction is enhanced in a diabetic state, and that PKC activator phorbol esters increase the gene expression of MDR1 in human tumor cells. To clarify the expression of the liver transporters under diabetic conditions and the roles of PKCα and the transcription factor NF-κB, we investigated the expression levels of Mdr1a, Mdr1b, Mdr2, Mrp2, Bcrp, Bsep, Oct1, Oat2, and Oat3 transporters, PKCα, IκB, and NF-κB in the liver of rats with STZ-induced hyperglycemia. A selective increase in the gene expression of Mdr1b was detected by RT-PCR. Western blotting with C219 antibody revealed an increase in P-glycoprotein. Although the mRNA level of PKCα was not affected, translocation of PKCα to the microsomal fraction was detected. NF-κB p65, IκBα and IκBβ mRNA levels were increased as was the level of nuclear NF-κB p65. From these findings, it was suggested that STZ-induced hyperglycemia caused the upregulation of Mdr1b P-gp expression through the activation of PKCα and NF-κB.

1. Introduction

Hepatocytes express various transporter proteins that take up and export xenobiotics and bile components. These transporters include the solute carrier family protein organic cation transporter 1 (Oct1), organic anion transporter (Oat) 2, and Oat3, and ATP-binding cassette (ABC) transporters, Mdr1a (Abcb1a), Mdr1b (Abcb1b), Mdr2 (Abcb4), Mrp2 (Abcc2), Bcrp (Abcg2), and Bsep (Abcb11). Mdr1a and Mdr1b are homologues of human MDR1 P-glycoprotein (P-gp) which transports xenobiotics such as doxorubicin and cyclosporin A [1]. The Mdr2 P-gp, a homologue of MDR3 in humans, transports phosphatidylcholine into bile. Mrp2 is a transporter of organic anions such as bilirubin glucuronides. Bcrp is known as a transporter of methotrexate, and Bsep is an export pump of bile acids.

Several reports have indicated that the expression of transporters was affected by various chemical substances and pathophysiological conditions [2–5]. In rats with streptozotocin (STZ)-induced diabetes, van Waarde et al. [6] showed that the Mdr2 gene expression and the protein level were increased in the liver and that the secretion of biliary phospholipids was enhanced. Human MDR1 gene expression is well known to be enhanced by various stimulants including antitumor drugs [7] and protein kinase C activator phorbol esters [8,9]. As for two rat Mdr1 genes, it has been reported that the Mdr1a gene lacked any response to STZ while the Mdr1b gene showed a massive increase in expression in both the liver and kidney after STZ treatment [10]. The different responses of these two Mdr1 genes have also been seen in LPS-induced endotoxemic rat liver [11] and in doxorubicin-treated rat astrocytes [12], in which only the Mdr1b gene was upregulated. Thus, the Mdr1b gene prefers to respond to external stimulations as in the case of the human MDR1 gene, although whether the P-gp level is affected under diabetic conditions has remained unclear.

It is widely recognized that the PKC signal transduction system is enhanced in the diabetic state [13]. PKC expression is persistently upregulated and this is recognized as an initial event leading to insulin resistance, cardiac disease, and nephropathy in diabetes [14]. It is also indicated that the activation of PKC is involved in the hyperglycemia-induced sustained activation of the transcription factor NF-κB [15,16]. PKC consists of a family of at least eleven isoforms, which are categorized into the classic (α, β1, β2, γ), the novel (δ, ε, η, θ, μ), and the typical (ζ, ι) [17,18]. Among these isoforms, PKCα activated in kidney cells, is indicated to mediate NF-κB's activation [19].

In the present study, to clarify the changes in the expression of the liver transporters under diabetic conditions and the role of PKC isoforms,

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Abbreviations: ABC, ATP-binding cassette; MDR, multidrug resistance; P-gp, P-glycoprotein; STZ, streptozotocin; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OCT, organic cation transporter; PKC, protein kinase C; NF-κB, nuclear factor κB; IκB, inhibitory κB; PCR, polymerase chain reaction

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Table 1
Primer sets for PCR

	Forward (5′–3′)	Reverse (5′–3′)	Annealing temperature	Product size	Ref
Mdr1a	GGGCCACATGATCAAGACGG	AGCGTCATTGGCAAGCCTGG	65	447	[2]
Mdr1b	ACAGAAACAGAGGATCGC	AGAGGCACCAGTGTCACT	55	352	[15]
Mdr2	ACAGAAACAGAGGATCGCCA	ATGCGTGCTTTCCAGCCA	55	386	[15]
Bsep	TGCTTATGGGAGGCGTAT	GGGCTGACAGCAAGAATC	55	565	[16]
Mrp2	GGCTGAGTGCTTGGAC	CTTCTGACGTCATCCTCAC	55	789	[17]
Bcrp	CAATGGGATCATGAAACCTG	GAGGCTGGTGAATGGAGAA	60	536	[18]
Oct1	GATCTTTATCCCGCATGAGC	TTCTGGGAATCCTCCAAGTG	60	478	[19]
Oat2	CGCTCAGAATTCTCCTCCAC	ACATCCAGCCACTCCAACTC	60	311	[20]
Oat3	TGAGAAGTGTCTCCGCTTCG	CTGTAGCCAGCGCCACTGAG	65	508	[21]
PKCα	GGAACTCAGGCAGAAGTTCG	CAGTTCCTCTGTTCCCTTCC	58	196	Original
IκBα	GACGAGGATTACGAGCAGAT	CCTGGTAGGTTACTCTGTTG	60	634	[22]
IκBβ	CCCAAGAGATGCCTCAGATA	GCCTTCATCTCTGTTGTCAC	65	520	[22]
NF-κB	AAGATCAATGGCTACACGGG	CCTCAATGTCTTCTTTCTGC	60	493	[23]
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTGGCTGTA	58	452	[24]

we investigated the levels of various transporters and PKCα in the liver of rats with STZ-induced hyperglycemia. The results indicate that the increased expression of Mdr1b P-gp is associated with the translocation of the PKCα isoenzyme to membrane. Together with evidence for the activation of NF-κB, we added a discussion about the system contributing to the up-regulation of Mdr1b P-gp in the diabetic rat liver.

2. Materials and methods

2.1. Animals

Male Wistar rats (9 weeks old, 280–300 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and housed in a controlled environment. The rats were kept on a commercial laboratory chow (CRF-1; Charles River Laboratories Japan, Inc., Yokohama, Japan) and had free access to tap water. The STZ-induced diabetes was established with a single intravenous injection of STZ (50 mg/kg body weight, Sigma) into the tail. The control rats were injected with the vehicle (sodium citrate buffer, pH4.5). On the 7th day after the injection, the increased urinary excretion of glucose was checked with a commercial urine dipstick (Wako Pure Chemicals, Japan) and the next day the rats were killed after starvation for 24 h. Blood was collected from the aorta abdominalis under anesthesia with an intraperitoneal injection of pentobarbital (40 mg/kg) and the liver was excised after perfusion with a 0.9% NaCl solution from the portal vein. Small pieces of the liver were immersed into Trizol (Invitrogen Japan K.K.) for the isolation of total RNA, and other parts of the tissue were frozen on dry ice and stored at −80 °C until further use. All experiments were performed according to the institutional guidelines for the care and use of laboratory animals in research.

2.2. Gene expression studies

Total RNA was isolated using Trizol reagent according to the manufacturer's instructions. Then, cDNA was prepared by incubation of 0.5–1.0 μg of the RNA with random primers (12.5 ng, Invitrogen), RNase inhibitor (RNaseOUT, 20 U, Invitrogen), 0.5 mM deoxynucleotides (dNTPs, Promega, WI), and 100 U of RNA reverse transcriptase (ReverTra Ace, TOYOBO, Japan) in 20 μL of reaction buffer according to the ReverTra Ace data sheet. Following inactivation of the enzyme by incubation at 99 °C for 5 min, polymerase chain reaction (PCR) was carried out with 0.625 U of G-Taq Taq DNA polymerase (Hokkaido System Science Co., Japan) in 25 μL of PCR solution (1 μL of cDNA solution, 0.4 μM primers, 0.2 mM dNTPs, and 2.5 μL of 10× PCR buffer) in a Thermal Cycler PxE (Thermo Fisher Scientific, MA). PCR primers (Table 1) were synthesized by Hokkaido System Science. The PCR products (10 μL) were electrophoresed on a 1.5% agarose gel and visualized with ultraviolet light after immersion in an ethidium bromide solution (1 μg/mL) for 15 min. Images were taken with a digital camera C-3030 zoom (Olympus, Japan) equipped with a BPB-60 filter (Fujifilm Japan). Densitometric analysis was performed using Scion Image for Windows supplied by the Scion Corporation.

2.3. Western blot analysis of PKCα and P-gp

Small pieces of the liver were homogenized with a Potter–Elvehjem homogenizer in 5 vol of Buffer A containing 10 mM Tris–HCl (pH7.5), 0.1 mM phenylmethylsulfonylfluoride (Sigma), and 1 mM dithiothreitol (Sigma). The homogenates were centrifuged at 800 ×g for 10 min at 4 °C and then the supernatant was centrifuged at 15,000 ×g for 30 min at 4 °C. The 15,000 ×g supernatant was further centrifuged at 100,000 ×g for 30 min at 4 °C to obtain a cytosolic fraction (supernatant) and a microsomal fraction (pellet). The pellet was re-suspended in a small volume of Buffer A. These fractions were used to detect PKC isoforms. The 15,000 ×g pellet was solubilized in Buffer A with 1% Triton X100 and centrifuged at 15,000 ×g for 30 min at 4 °C. The resultant supernatant (Fraction PM) was used for the detection of P-glycoprotein.

The protein concentration was determined by using a DC protein assay kit (Bio-Rad Laboratories, Inc.).

2.3.1. Detection of PKCα

The cytosolic and microsomal fractions were used for the detection of PKCα. Each fraction (20–100 μg protein) was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE, 10% gel), and the separated proteins were transferred onto an Immobilon-P transfer membrane (Nihon Millipore, Japan). After blocking with skim milk, the membrane was treated with an isoform-specific antibody for PKCα (Santa Cruz Biotechnology, Inc., CA) followed by horseradish-labeled goat anti-mouse IgG_1 antibody (Santa Cruz Biotechnology, Inc.), and the specific immunoreactive band was detected using an ECL-detection kit (Amersham Japan). Densitometric analysis was performed using Scion Image for Windows.

2.3.2. Detection of P-gp

As was done for the detection of PKC isoforms, Fraction PM was isolated by SDS-PAGE (8% gel) and then P-gp was detected with the C219 anti-MDR1 P-gp mouse monoclonal antibody (Centocor, Inc., Malvern, PA) as a primary antibody and horseradish-labeled goat anti-mouse IgG_{2a} antibody (Santa Cruz Biotechnology, Inc.) as a secondary antibody. The level of P-gp was detected and evaluated as described above.

2.4. Western blot analysis of NF-κB p65

Cytoplasmic and nuclear fractions were prepared according to the report by Kiemer et al. [20]. Each fraction (75–100 μg protein) was isolated by SDS-PAGE (10% gel), and then NF-κB p65 was detected with anti-NF-κB p65 antibody (Cell Signaling Technology, Inc., MA) as a primary antibody and horseradish-labeled goat anti-rabbit IgG antibody (Cell Signaling Technology, Inc.) as a secondary antibody. The level of NF-κB p65 was detected and evaluated as described above.

2.5. Statistical analysis

Data are shown as means ± S.D. for five animals. Statistical analyses were performed using Student's *t*-test.

3. Results

3.1. Phenotype of the control and STZ-induced diabetic rats

On the 8th day after the injection of STZ or vehicle, diabetic rats had a significantly lower body weight than the control rats (Table 2). As the liver weight was not affected by the injection of STZ, the liver weight/body weight ratio was significantly increased. The average fast blood glucose level of STZ-treated rats was 310 mg/dL compared to

Table 2
Comparison of body weight, liver weight, and plasma glucose level

Group	Body weight (g)	Liver weight (g)	Ratio of liver to body weight (%)	Plasma glucose (mg/dl)
Control	309 ± 4.2	7.7 ± 0.4	2.5 ± 0.1	128 ± 11
STZ	265 ± 15**	8.1 ± 0.6	3.0 ± 0.1**	310 ± 93**

**$p < 0.01$ vs. control.

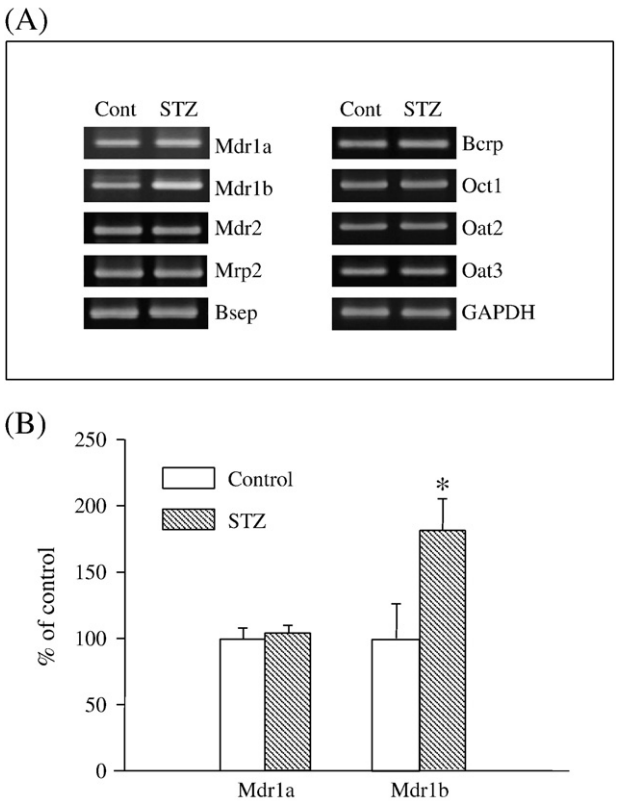

Fig. 1. The expression levels of hepatic transporter genes in rats with STZ-induced diabetes. The expression of transporter genes was investigated by semi-quantitative RT-PCR methods using optimal concentrations of total RNA and primer sets shown in Table 1. The photograph (A) is typical of each group. Column graph data are given relative to the level of GAPDH and expressed as means±S.D. for five animals. *P<0.05, compared with the control.

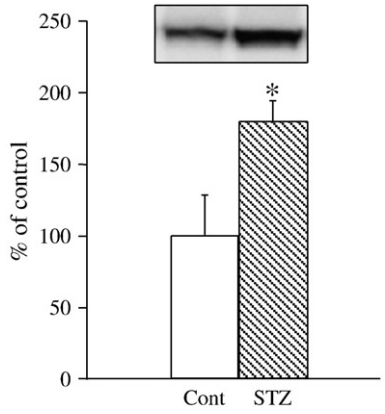

Fig. 2. The increase of hepatic P-gp levels in rats with STZ-induced diabetes. A liver membrane fraction was subjected to a Western blot analysis of P-gp as described in Materials and methods. Column graph data are means±S.D. for five animals. *P<0.05, compared with the control. The photograph (A) is typical of each group.

128 mg/dL in control rats. Then, the STZ-treated rats were used as the animal model of diabetes.

3.2. Effects on hepatic drug transporters

As shown in Fig. 1(A, B), the semi-quantitative PCR-based analysis of nine transporters revealed the upregulation (2-fold) of Mdr1b mRNA expression in the liver of the diabetic rats. By contrast, Mdr1a was not affected by the STZ-treatment as shown previously [10]. To determine whether the increase in Mdr1b mRNA affects the P-gp level, we conducted a Western blot analysis. As shown in Fig. 2, the expression of P-gp was significantly upregulated (2-fold) in the diabetic rat liver. These results indicate that the increase in Mdr1b mRNA level caused by STZ-treatment is large enough to raise the liver concentration of P-gp. Although the mRNA levels of Mdr2, Mrp2, Bcrp, Bsep, Oct1, Oat2, and Oat3 were investigated as shown in Fig. 1(A), no detectable changes were observed. These results indicate that Mdr1b is most sensitive to hyperglycemic conditions among these apical and basolateral transporters in the liver.

3.3. Effects on PKC expression

PKC is a stimulating factor of P-gp expression [8,9] and is activated in hyperglycemic conditions [13,14,21]. Moreover, PKCα and PKCβ2 isoforms have been indicated to be increased in STZ-induced diabetes [22]. However, there are conflicting reports that PKCα levels were lower in the hepatocytes of diabetic rats than normal rats [23] and that the approximately 80 kDa PKCα was not increased in the cytosolic or membrane fraction in the diabetic rats while the 90 kDa protein was [24]. Then, we investigated the mRNA and protein levels of PKCα as shown in Fig. 3. The results showed that PKCα mRNA levels were not affected in the STZ-induced diabetic model, while the Western blot analysis revealed that the 80 kDa PKCα protein in the microsomal fraction was significantly increased while in the cytosolic fraction, the PKCα protein level was not affected. These results indicated that the production of PKCα protein was not induced and most of the protein persistently translocated to the membrane in STZ-induced diabetes.

3.4. Effects on IκB and NF-κB expression

NF-κB, a major transcription factor of the MDR1 gene [25,26], has been indicated to be persistently activated in diabetic state, and the

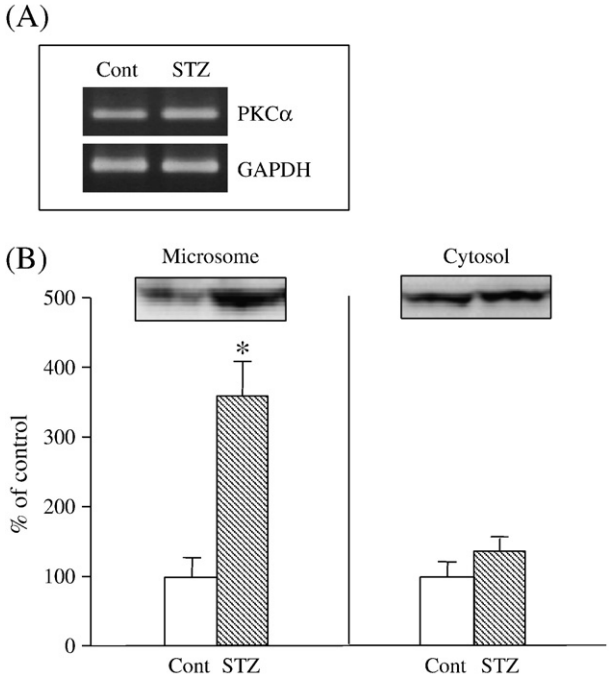

Fig. 3. Expression and translocation of PKCα in the liver of rats with STZ-induced diabetes. (A) The PKCα mRNA was examined by semi-quantitative RT-PCR using optimal concentrations of total RNA and primer sets shown in Table 1. (B) Western blot analysis of PKCα. The microsomal and cytosolic fractions of the liver were prepared as described in Materials and methods. The photograph is typical of each group. Column graph data are means±S.D. for five animals. *P<0.05, compared with the control.

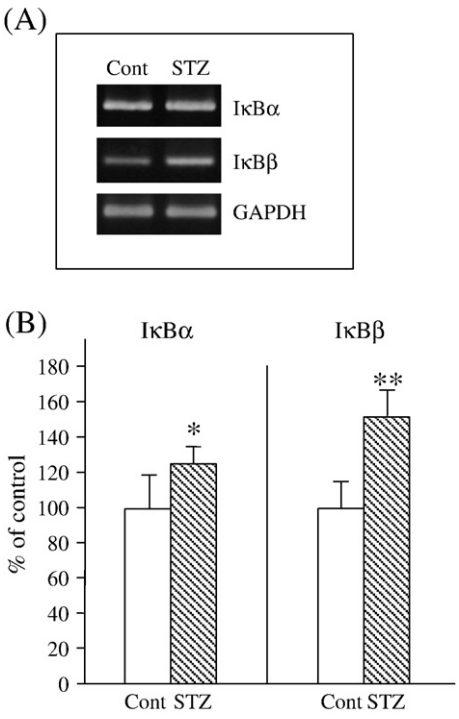

Fig. 4. Upregulation of the expression of IκB genes in the liver of rats with STZ-induced diabetes. The expression of IκBα and IκBβ was examined by semi-quantitative RT-PCR using optimal concentrations of total RNA and primer sets shown in Table 1. The photograph (A) is typical of each group. Column graph data are given relative to the level of GAPDH and expressed as means ± S.D. for five animals. *$P<0.05$, **$P<0.01$, compared with the control.

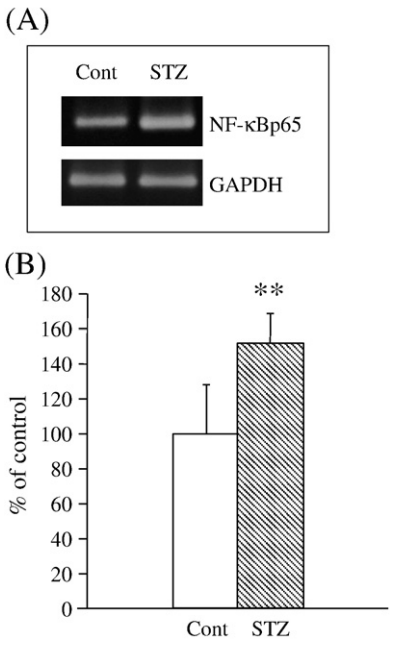

Fig. 5. Upregulation of the expression of the NF-κB p65 gene in the liver of rats with STZ-induced diabetes. The expression of the gene was examined by semi-quantitative RT-PCR using optimal concentrations of total RNA and primer sets shown in Table 1. The photograph (A) is typical of each group. Column graph data are given relative to the level of GAPDH and expressed as means ± S.D. for five animals. **$P<0.01$, compared with the control.

activation of NF-κB is suggested to be associated with the de novo synthesis of NF-κB p65 in the presence of newly synthesized IκBα and IκBβ [16]. Furthermore, it has been indicated that NF-κB was activated by PKCα [27]. Then we investigated the mRNA levels of NF-κB p65, IκBα and IκBβ in STZ-treated rats compared with the control rats. As shown in Fig. 4, the NF-κB p65 mRNA level was increased by 50% and IκBα and IκBβ mRNA levels were increased 50% and 100% respectively in the STZ-exposed rat liver. Next, the nuclear translocation of NF-κB p65 was investigated by Western blotting. As shown in Fig. 5, we detected increased nuclear translocation of p65 in STZ-treated rats and a slight increase in cytoplasmic p65 levels.

4. Discussion

In the present study, we demonstrated an increase in P-gp among nine hepatic canalicular and basolateral drug transporter genes investigated, and the translocation of PKCα to the membrane and of NF-κB p65 to the nucleus, in the liver of rats with STZ-induced diabetes. Although P-gp is encoded by the Mdr1a and Mdr1b genes in rats, only the Mdr1b gene expression was upregulated and contributed to the increase in P-gp.

As for the level of P-gp under diabetic conditions, Tramonti et al. [28] reported that, while the renal tubular Mdr1a and Mdr1b genes were upregulated in their expression, P-gp levels were not affected in diabetic rats. Contrary to this report, Liu et al. [29] showed that P-gp levels in the brain were lower in diabetic rodents than control animals. Thus the present study clearly showed that the expression of liver P-gp responded to the diabetic state in a different manner. Considering that P-gp regulates the absorption, distribution, and disposition of a large number of medicines and that hepatic P-gp confers vectorial flow of various lipophilic compounds into the canalicular space, the increase in P-gp probably leads to the accelerated excretion of medicines or xenobiotics into bile in the diabetic state.

In the present study, concurrently with the upregulation of P-gp expression in the liver, the level of PKCα in the membrane fraction increased which generally means the activation of the enzyme. PKC-related upregulation of MDR1 gene expression has been observed in multidrug-resistant human tumor cells [8]. Generally, such resistant cells express high levels of the PKCα isoform, and it has been shown that PKCα contributed to the overexpression of P-gp [30,31]. Independently of P-gp research, isoform-specific increases in PKC have been indicated by several studies in the rats with STZ-induced diabetes Fig. 6. In renal and myocardial tissues, selective upregulation of the expression of the PKCα isoform has been shown [32], and in hepatocytes, increased levels of PKCα and -β2 isoforms and the

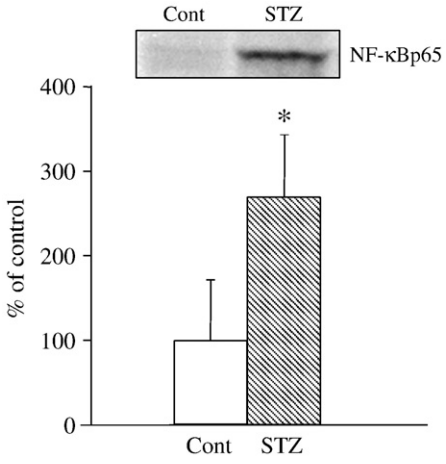

Fig. 6. Increase of nuclear NF-κB p65 in the liver of rats with STZ-induced diabetes. A liver nuclear fraction was subjected to a Western blot analysis of NF-κB p65 as described in Materials and methods. Column graph data are means ± S.D. for five animals. *$P<0.05$, compared with the control. The photograph (insert) is typical of each group.

translocation of PKCε have been noted [22]. Considering these findings, the present study is the first to suggest an association of PKCα with the upregulation of Mdr1b gene expression and P-gp level in an animal model of diabetes.

Van Waarde et al. [6] reported that STZ-induced advanced diabetes with high levels of serum bile acids induced Mdr2 gene expression in the liver. However, we found no change of Mdr2 gene expression in the present study. As the expression of the human MDR3 gene is downregulated by PKCβ in vitro [33], and that of both the MDR3 and Mdr2 genes can be upregulated by bile acids through the farnesoid X receptor [34,35], we may have to consider the influence of these factors and duration of disease on the expression of Mdr2 in models of diabetes that use rats.

As for the persistent upregulation of PKC expression in diabetes, it was indicated that it was generally accompanied by an increased diacylglycerol (DAG) level and was caused by hyperglycemia [14]. On the other hand, it is reported that hyperglycemia increased mitochondrial reactive oxygen species (ROS) production and also increased ROS-mediated PKC activation [36]. As it has been shown that PKC induced ROS production [37], PKC activation and ROS production may mutually contribute to the sustained activation of PKC in the diabetic state. Based on these reports, although further investigation is needed, DAG and ROS production contribute to the induction of P-gp expression and the increase in the level of PKCα in the membrane.

In the present study, we also indicated the activation of the transcription factor NF-κB by showing upregulation of the transcription and nuclear translocation of NF-κB p65 accompanied by increased IκB transcription. It has been shown that phorbol ester, an activator of PKC, induced the degradation of IκB and appearance of active NF-κB (p65) [38]. Furthermore, it has been shown that PKCα induced the activation of NF-κB [27] and that NF-κB is one of the major transcription factors of the human MDR1 gene [25,39] and rat Mdr1b gene [40]. Then, the activation of NF-κB in STZ-treated rats seems to contribute to the transduction of PKCα activity to upregulate the expression of P-gp.

IκB consists of IκBα and IκBβ subunits and forms a complex with NF-κB (a heterodimer of p50 and p65) in the cytosol and plays a major role in the regulation of NF-κB activity. Degradation of IκBα after phosphorylation by IKK is followed by the translocation of NF-κB p65 and also by the expression of IκB mRNA [41,42]. In calorie non-restricted aged rats, increases in the mRNA and protein levels of IκBα were found consistent with the upregulated nuclear translocation of NF-κB p65 and in correlation with age-related oxidative status [43,44]. These findings imply that in chronic pathophysiological conditions both the nuclear translocation of NF-κB and the expression of IκBα were persistently upregulated. On the other hand, IκBβ binds to NF-κB in place of IκBα and the complex translocates to the nucleus and it has been suggested that this translocation contributed to the sustained activation of NF-κB [45,46]. Considering the nuclear translocation of NF-κB p65 accompanied by increased IκBβ transcription in the present STZ-generated model of diabetes, hyperglycemia seems to cause persistent activation of NF-κB and to induce overexpression of hepatic P-gp.

In conclusion, we found a marked increase in the level of Mdr1b P-gp in the liver at a relatively early stage of STZ-induced diabetes. Thus we indicated that the distribution of xenobiotics is affected through the upregulation of P-gp expression in the diabetic state. Concomitantly with this upregulation, we also found activation of the PKCα and NF-κB transcription system. From these findings, it is strongly suggested that STZ-induced hyperglycemia caused the upregulation of mdr1b P-gp expression through the activation of PKCα and NF-κB.

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References

[1] L. Lothstein, S.I. Hsu, S.B. Horwitz, L.M. Greenberger, Alternate overexpression of two P-glycoprotein genes is associated with changes in multidrug resistance in a J774.2 cell line, J. Biol. Chem. 264 (1989) 16054–16058.
[2] H. Nakatsukasa, J.A. Silverman, T.W. Gant, R.P. Evarts, S.S. Thorgeirsson, Expression of multidrug resistance genes in rat liver during regeneration and after carbon tetrachloride intoxication, Hepatology 18 (1993) 1202–1207.
[3] C. Ziemann, A. Burkle, G.F. Kahl, K.I. Hirsch-Ernst, Reactive oxygen species participate in mdr1b mRNA and P-glycoprotein overexpression in primary rat hepatocyte cultures, Carcinogenesis 20 (1999) 407–414.
[4] E. Hinoshita, K. Taguchi, A. Inokuchi, T. Uchiumi, N. Kinukawa, M. Shimada, M. Tsuneyoshi, K. Sugimachi, M. Kuwano, Decreased expression of an ATP-binding cassette transporter, MRP2, in human livers with hepatitis C virus infection, J. Hepatol. 35 (2001) 765–773.
[5] L. Fouassier, M. Beaussier, E. Schiffer, C. Rey, V. Barbu, M. Mergey, D. Wendum, P. Callard, J.Y. Scoazec, E. Lasnier, B. Stieger, A. Lienhart, C. Housset, Hypoxia-induced changes in the expression of rat hepatobiliary transporter genes, Am. J. Physiol. Gastrointest. Liver Physiol. 293 (2007) G25–G35.
[6] W.M. van Waarde, H.J. Verkade, H. Wolters, R. Havinga, J. Baller, V. Bloks, M. Muller, P.J. Sauer, F. Kuipers, Differential effects of streptozotocin-induced diabetes on expression of hepatic ABC-transporters in rats, Gastroenterology 122 (2002) 1842–1852.
[7] V. Ling, Multidrug resistance: molecular mechanisms and clinical relevance, Cancer Chemother. Pharmacol. 40 (1997) S3–S8.
[8] P.M. Chaudhary, I.B. Roninson, Activation of MDR1 (P-glycoprotein) gene expression in human cells by protein kinase C agonists, Oncol. Res. 4 (1992) 281–290.
[9] M.T. Osborn, A. Berry, M.S. Ruberu, B. Ning, L.M. Bell, T.C. Chambers, Phorbol ester induced MDR1 expression in K562 cells occurs independently of mitogen-activated protein kinase signaling pathways, Oncogene 18 (1999) 5756–5764.
[10] J.M. Brady, N.J. Cherrington, D.P. Hartley, S.C. Buist, N. Li, C.D. Klaassen, Tissue distribution and chemical induction of multiple drug resistance genes in rats, Drug Metab. Dispos. 30 (2002) 838–844.
[11] T.A. Vos, G.J. Hooiveld, H. Koning, S. Childs, D.K. Meijer, H. Moshage, P.L. Jansen, M. Muller, Up-regulation of the multidrug resistance genes, Mrp1 and Mdr1b, and down-regulation of the organic anion transporter, Mrp2, and the bile salt transporter, Spgp, in endotoxemic rat liver, Hepatology 28 (1998) 1637–1644.
[12] C. Mercier, X. Decleves, C. Masseguin, P. Fragner, M. Tardy, F. Roux, J. Gabrion, J.M. Scherrmann, P-glycoprotein (ABCB1) but not multidrug resistance-associated protein 1 (ABCC1) is induced by doxorubicin in primary cultures of rat astrocytes, J. Neurochem. 87 (2003) 820–830.
[13] M.E. Cooper, F. Bonnet, M. Oldfield, K. Jandeleit-Dahm, Mechanisms of diabetic vasculopathy: an overview, Am. J. Hypertens. 14 (2001) 475–486.
[14] D. Koya, G.L. King, Protein kinase C activation and the development of diabetic complications, Diabetes 47 (1998) 859–866.
[15] G.M. Pieper, Riaz-ul-Haq, Activation of nuclear factor-kappaB in cultured endothelial cells by increased glucose concentration: prevention by calphostin C, J. Cardiovasc. Pharmacol. 30 (1997) 528–532.
[16] A. Bierhaus, S. Schiekofer, M. Schwaninger, M. Andrassy, P.M. Humpert, J. Chen, M. Hong, T. Luther, T. Henle, I. Kloting, M. Morcos, M. Hofmann, H. Tritschler, B. Weigle, M. Kasper, M. Smith, G. Perry, A.M. Schmidt, D.M. Stern, H.U. Haring, E. Schleicher, P.P. Nawroth, Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB, Diabetes 50 (2001) 2792–2808.
[17] Y. Nishizuka, Protein kinase C and lipid signaling for sustained cellular responses, FASEB J., 9 (1995) 484–496.
[18] S. Ohno, Y. Nishizuka, Protein kinase C isotype and their specific functions: prologue, J. Biochem. 132 (2002) 509–511.
[19] M. Banzi, G. Aguiari, V. Trimi, A. Mangolini, P. Pinton, R. Witzgall, R. Rizzuto, L. del Senno, Polycystin-1 promotes PKCalpha-mediated NF-kappaB activation in kidney cells, Biochem. Biophys. Res. Commun. 350 (2006) 257–262.
[20] A.K. Kiemer, A.M. Vollmar, M. Bilzer, T. Gerwig, A.L. Gerbes, Atrial natriuretic peptide reduces expression of TNF-alpha mRNA during reperfusion of the rat liver upon decreased activation of NF-kappaB and AP-1, J. Hepatol. 33 (2000) 236–246.
[21] P.A. Craven, F.R. DeRubertis, Protein kinase C is activated in glomeruli from streptozotocin diabetic rats. Possible mediation by glucose, J. Clin. Invest. 83 (1989) 1667–1675.
[22] E.Y. Tang, P.J. Parker, J. Beattie, M.D. Houslay, Diabetes induces selective alterations in the expression of protein kinase C isoforms in hepatocytes, FEBS Lett. 326 (1993) 117–123.
[23] F. Croquet, A. Bréhier, S. Gil, J. Davy, J. Féger, Five isoenzymes of protein kinase C are expressed in normal and STZ-diabetic rat hepatocytes: effect of phorbol 12-myristate 13-acetate, Biochim. Biophys. Acta 1315 (1996) 163–168.
[24] V. Nivet, P.J. Antoine, M. Amessou, G. Descamps, B. Desbuquois, J.P. Clot, D. Durand, Increased expression of liver PKC alpha in hypoinsulinemic diabetic rats: a post-translational effect, Mol. Cell. Endocrinol. 146 (1998) 177–185.
[25] M.M. Cornwell, The human multidrug resistance gene: sequences upstream and downstream of the initiation site influence transcription, Cell Growth Differ. 1 (1990) 607–615.
[26] M. Bentires-Alj, V. Barbu, M. Fillet, A. Chariot, B. Relic, N. Jacobs, J. Gielen, M.P. Merville, V. Bours, NF-kappaB transcription factor induces drug resistance through MDR1 expression in cancer cells, Oncogene 22 (2003) 90–97.
[27] S.B. Lin, L.C. Wu, S.L. Huang, H.L. Hsu, S.H. Hsieh, C.W. Chi, L.C. Au, In vitro and in vivo suppression of growth of rat liver epithelial tumor cells by antisense oligonucleotide against protein kinase C-alpha, J. Hepatol. 33 (2000) 601–608.

[28] G. Tramonti, P. Xie, E.I. Wallner, F.R. Danesh, Y.S. Kanwar, Expression and functional characteristics of tubular transporters: P-glycoprotein, PEPT1, and PEPT2 in renal mass reduction and diabetes, Am. J. Physiol. Renal Physiol. 291 (2006) F972–F980.
[29] H. Liu, X. Xu, Z. Yang, Y. Deng, X. Liu, L. Xie, Impaired function and expression of P-glycoprotein in blood-brain barrier of streptozotocin-induced diabetic rats, Brain Res. 1123 (2006) 245–252.
[30] G. Yu, S. Ahmad, A. Aquino, C.R. Fairchild, J.B. Trepel, S. Ohno, K. Suzuki, T. Tsuruo, K.H. Cowan, R.I. Glazer, Transfection with protein kinase C alpha confers increased multidrug resistance to MCF-7 cells expressing P-glycoprotein, Cancer Commun. 3 (1991) 181–189.
[31] S. Ahmad, R.I. Glazer, Expression of the antisense cDNA for protein kinase C alpha attenuates resistance in doxorubicin-resistant MCF-7 breast carcinoma cells, Mol. Pharmacol. 43 (1993) 858–862.
[32] N. Kang, G. Alexander, J.K. Park, C. Maasch, I. Buchwalow, F.C. Luft, H. Haller, Differential expression of protein kinase C isoforms in streptozotocin-induced diabetic rats, Kidney Intern. 56 (1999) 1737–1750.
[33] S. Suzuki, H. Hayashi, K. Takagi, T. Kondo, K. Takagi, J. Ueyama, S. Wakusawa, Protein kinase Cbeta isoform down-regulates the expression of MDR3 P-glycoprotein in human Chang liver cells, Biochim. Biophys. Acta 1760 (2006) 1552–1557.
[34] L. Huang, A. Zhao, J.L. Lew, T. Zhang, Y. Hrywna, J.R. Thompson, N. de Pedro, I. Royo, R.A. Blevins, F. Pelaez, S.D. Wright, J. Cui, Farnesoid X receptor activates transcription of the phospholipid pump MDR3, J. Biol. Chem. 278 (2003) 51085–51090.
[35] Y. Liu, J. Binz, M.J. Numerick, S. Dennis, G. Luo, B. Desai, K.I. MacKenzie, T.A. Mansfield, S.A. Kliewer, B. Goodwin, S.A. Jones, Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra- and extrahepatic cholestasis, J. Clin. Invest. 112 (2003) 1678–1687.
[36] T. Nishikawa, D. Edelstein, M. Brownlee, The missing link: a single unifying mechanism for diabetic complications, Kidney Inter., Suppl. 77 (2000) S26–S30.
[37] P. Pinton, A. Rimessi, S. Marchi, F. Orsini, E. Migliaccio, M. Giorgio, C. Contursi, S. Minucci, F. Mantovani, M.R. Wieckowski, G. Del Sal, P.G. Pelicci, R. Rizzuto, Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc, Science 315 (2007) 659–663.
[38] T. Henkel, T. Machleidt, I. Alkalay, M. Kronke, Y. Ben-Neriah, P.A. Baeuerle, Rapid proteolysis of I kappa B-alpha is necessary for activation of transcription factor NF-kappa B, Nature 365 (1993) 182–185.
[39] F. Thevenod, J.M. Friedmann, A.D. Katsen, I.A. Hauser, Up-regulation of multidrug resistance P-glycoprotein via nuclear factor-kappaB activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis, J. Biol. Chem. 275 (2000) 1887–1896.
[40] J.A. Silverman, H. Raunio, T.W. Gant, S.S. Thorgeirsson, Cloning and characterization of a member of the rat multidrug resistance (mdr) gene family, Gene 106 (1991) 229–236.
[41] K. Brown, S. Park, T. Kanno, G. Franzoso, U. Siebenlist, Mutual regulation of the transcriptional activator NF-kappa B and its inhibitor, I kappa B-alpha, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 2532–2536.
[42] S.C. Sun, P.A. Ganchi, D.W. Ballard, W.C. Greene, NF-kappa B controls expression of inhibitor I kappa B alpha: evidence for an inducible autoregulatory pathway, Science 259 (1993) 1912–1915.
[43] H.J. Kim, K.W. Kim, B.P. Yu, H.Y. Chung, The effect of age on cyclooxygenase-2 gene expression: NF-kappaB activation and IkappaBalpha degradation, Free Radic. Biol. Med. 28 (2000) 683–692.
[44] H.J. Kim, B.P. Yu, H.Y. Chung, Molecular exploration of age-related NF-kappaB/IKK downregulation by calorie restriction in rat kidney, Free Radic. Biol. Med. 32 (2002) 991–1005.
[45] J.E. Thompson, R.J. Phillips, H. Erdjument-Bromage, P. Tempst, S. Ghosh, I kappa B-beta regulates the persistent response in a biphasic activation of NF-kappa B, Cell 80 (1995) 573–582.
[46] H. Suyang, R. Phillips, I. Douglas, S. Ghosh, Role of unphosphorylated, newly synthesized I kappa B beta in persistent activation of NF-kappa B, Mol. Cell. Biol. 16 (1996) 5444–5449.