

The phosphatidylinositol/AKT/atypical PKC pathway is involved in the improved insulin sensitivity by DHEA in muscle and liver of rats in vivo

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Received 21 January 2004; accepted 14 June 2004

Abstract

DHEA improves insulin sensitivity and has anti-obesity effect in animal models and men. However, the molecular mechanisms by which DHEA improves insulin action have not been clearly understood. In the present study, we examined the protein levels and phosphorylation state of insulin receptor (IR), IRS-1 and IRS-2, the association between IRSs and PI3K and SHP2, the insulin-induced IRSs associated PI 3-kinase activities, and the phosphorylation status of AKT and atypical PKC ζ/λ in the liver and the muscle of 6 month-old Wistar rats treated with DHEA. There was no change in IR, IRS-1 and IRS-2 protein levels in both tissues of treated rats analysed by immunoblotting. On the other hand, insulin-induced IRS-1 tyrosine phosphorylation was increased in both tissues while IRS-2 tyrosyl phosphorylation was increased in liver of DHEA treated group. The PI3-kinase/AKT pathway was increased in the liver and the PI3K/atypical PKC ζ/λ pathway was increased in the muscle of DHEA treated rats. These data indicate that these regulations of early steps of insulin action may play a role in the intracellular mechanism for the improved insulin sensitivity observed in this animal model.

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Keywords: Insulin action; DHEA; IRS-1; IRS-2

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Introduction

The insulin action has a critical role in the regulation of glucose homeostasis. Our understanding of the molecular mechanisms of the insulin action has increased as evidence of the complex signalling pathways and multiple isoforms of key signalling molecules has accumulated. The early steps of insulin action involve binding of the hormone to a specific cell surface receptor, which leads to autophosphorylation and activation of an intrinsic tyrosine kinase of the insulin receptor β -subunit (Cheatham and Khan, 1995; Myers and White, 1996). This tyrosine kinase subsequently phosphorylates proteins known as insulin receptor substrates (IRS-1 and IRS-2) (Sun et al., 1991, 1995; Myers and White, 1996). IRS proteins mediate the pleiotropic effects of insulin such as glucose transport, protein metabolism, and the control of cell growth and survival. After stimulation by insulin, IRS-1 and IRS-2 associate with several proteins, including phosphatidylinositol (PI) 3-kinase, phosphotyrosine phosphatase SHP2, and adapter molecules as Nck, GRB2, and Fyn (Backer et al., 1992; Hadari et al., 1992; Myers and White, 1996).

The enzyme PI3-kinase has been shown to play a critical role in many metabolic effects of insulin, including stimulation of glucose transport, activation of glycogen synthase, and inhibition of phosphoenol pyruvate carboxykinase (PEPCK), the key enzyme of gluconeogenesis (Cheatham et al., 1994).

Insulin resistance is a classic feature of type 2 non-insulin-dependent diabetes mellitus (NIDDM) that is often associated with glucose intolerance, hypertension, dyslipidemia, arteriosclerosis, obesity and cardiovascular disease both in human and animal models, including aged Wistar rats (Kimura et al., 1998; Gotarredona et al., 1998). Insulin resistance may be characterized by a compensatory raise in circulating insulin concentration or hyperinsulinism (Gotarredona et al., 1998).

We have previously demonstrated changes in the early steps of insulin action in liver and muscle of old (obese) Wistar rats that may be important in the altered glucose metabolism observed in aging (obese) rats (Carvalho et al., 1996). Another feature in aging is the reduced serum concentration of dehydroepiandrosterone (DHEA) and its sulphate (DHEA-S), the most abundant adrenal steroids secreted by the adrenal cortex in response to adrenocorticotrophin (ACTH) (Mazza et al., 1999). Although the physiological role of DHEA is still unknown (Mukasa et al., 1998), the decreased DHEA serum level is associated to increased incidence of atherosclerosis, obesity, and type 2 diabetes mellitus (Nestler et al., 1989; Barrett-Connor et al., 1986; Beer et al., 1993a; Mazza et al., 1999).

Despite the reduced production of DHEA in rats, it has an anti-obesity effect and reduces the hyperglycemia, hyperphagia, hyperlipidemia in experimental animals (Richards et al., 2000; Yen et al., 1977; Mukasa et al., 1998; Coleman et al., 1982; Clearly et al., 1984) while the pharmacological amelioration of insulin resistance raises plasma DHEA and DHEA-S concentrations in humans (Beer et al., 1993a,b).

Clearly, there are evidences from different sources demonstrating that DHEA may improve insulin sensitivity (Yen et al., 1977; Richards et al., 2000; Nakashima et al., 1995a,b; Mukasa et al., 1998; Kimura et al., 1998; Coleman et al., 1982). However, the mechanisms underlying these effects are not fully understood. We show here that a single DHEA injection led to increased insulin-induced IRS-1, IRS-2, AKT and atypical PKC ζ/λ phosphorylation in a tissue-specific manner in intact rats.

Materials and methods

Materials

The reagents and apparatus for SDS-PAGE and immunoblotting were obtained from Bio-Rad (Richmond, CA). Human biosynthetic DHEA, tris, phenylmethylsulfonylfluoride (PMSF), aprotinin and dithiothreitol were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium amobarbital and human recombinant insulin (Humulin R) were purchased from Eli Lilly Co. (Indianapolis, IN). Nitrocellulose (0.45 μ m), protein A-Sepharose 6 MB, and enhanced chemiluminescence's reagents kit were purchased from Pharmacia (Uppsala, Sweden). Male Wistar rats were from the Institute of Biomedical Sciences Animal Breeding Centre. Anti-PI 3-kinase (p85) antibody was from Upstate Biotechnology (Lake Placid, NY). Anti-IRS-1, anti-IRS2, antiphosphotyrosine, anti-SHP2 and anti-insulin receptor antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-AKT and anti-phospho-PKC ζ/λ were from Cell Signalling (Beverly, MA). [32 P]ATP was from Amersham (Buckinghamshire, UK). Rat liver phosphatidylinositol was from Avanti Polar Lipids (Alabaster, AL, USA).

Animals

Male 24-weeks old Wistar rats were divided into two groups and the studies were performed in parallel using control and treated rats. The rats were provided with standard rodent chow and water ad libitum. DHEA was injected subcutaneously (10mg/kg body weight) diluted in mineral oil 1 week before experiment. The control group received equal volume of vehicle. Food was withdrawn 12–14h before experiments. The Institute of Biomedical Sciences Animal Experimental Committee guaranteed ethics approval.

Methods

Rats were anaesthetized with sodium amobarbital (15mg/kg BW, ip) and used in experiments 15–20 min later, i.e. as soon as anaesthesia was assured by loss of pedal and corneal reflexes. The abdominal cavity was opened, the portal vein was exposed and 0.5 mL normal saline (0.9%NaCl) with or without 10^{-5} M insulin was injected. After 30 seconds, a liver fragment was removed, minced coarsely, and homogenized immediately in approximately 10 vol of extraction solubilization buffer in ice-cold bath using a Polytron-Aggregate (Luzern, Switzerland) operated at maximum speed for 10s. Ninety seconds after injection, hind limb muscle gastrocnemius was quickly excised and homogenized as described for liver. The extracts were centrifuged at 15,000 rpm at 4°C in Eppendorf 5804-R (Hamburg, Germany) for 20min to remove insoluble material; the supernatant was then used for the assay.

Protein analysis by immunoblotting

The supernatant was either used for immunoprecipitation with anti-IR, anti-IRS-1, anti-IRS-2 and protein A-Sepharose 6MB or treated as whole extract tissues with Laemmli sample buffer treatment and electrophoresis in SDS-PAGE as described elsewhere (Carvalho et al., 1996, 1997). For whole tissue

extracts, similar sized aliquots (200 µg protein) were subjected to SDS-PAGE and immunoblotted with anti-phospho-AKT or anti-phospho-PKC antibodies. Electrotransfer of proteins from gel into nitrocellulose was performed for 90 min at 120V (constant). In order to reduce nonspecific protein binding to the nitrocellulose, the filter was pre-incubated overnight at 4°C in blocking buffer (5% nonfat dry milk, 10 mM Tris, 150 mM NaCl and 0.02% Tween 20). The nitrocellulose blots were incubated for 4 h at 22°C with specific antibodies described in figure legends diluted in blocking buffer (3% nonfat dry milk). Commercial enhanced chemiluminescence's reagents were used to visualize the autoradiogram, which was later exposed to photographic film. Quantitative analysis of blots was done using Scion Image software.

PI 3-kinase activity

Aliquots of supernatants containing equal amounts of proteins were incubated overnight at 4°C using antibodies against IRS-1 or IRS-2. The immunocomplexes were precipitated with a 50% solution of Protein A-Sepharose 6MB. In vitro PI 3-kinase assays were performed as previously described (Folli et al., 1992). The ³²P-labelled 3-P-phosphatidylinositol was detected by conventional autoradiography and quantified by densitometry using the Scion Image software.

Other

DHEA levels were determined through a commercial radioimmunoassay kit (Texas, USA). In order to investigate the glucose disappearance rate (K_{itt}), twenty animals from control and DHEA treated groups underwent an insulin tolerance test. Twenty µL of blood were collected from the tail of anaesthetized rats at 0 (basal), 4, 8, 12 and 16 min after 10^{-5} M insulin infusion into the portal vein. The blood glucose level was measured by glucose oxidase using a commercial kit from Labtest (Guarulhos, Brazil). The K_{itt} was calculated from the formula $0.693/t_{1/2}$. The glucose $t_{1/2}$ was calculated from the slope of the least square analysis of blood glucose concentration during linear phase of decline (Bonora et al., 1989).

Statistical analysis

Experiments were always performed by studying control and DHEA treated groups in parallel. For comparisons, the unpaired Student's *t* test was used. The level of significance was set at $P < 0.05$.

Results

Body weight, serum DHEA levels, and glucose disappearance rate (K_{itt})

Table 1 summarizes the body weight, serum DHEA levels and glucose disappearance rate during an intravenous insulin tolerance test (K_{itt}) in control and DHEA treated rats at the seventh day after the steroid subcutaneous injection. The administration of one single DHEA dose resulted in 4.1 fold-increased in serum DHEA level and improved insulin sensitivity detected by increased K_{itt} . After the

Table 1
Characteristics of the rats studied

Groups	Body weight (g)	DHEA (ng/ml)	K _{itt} (%/min)
control	365 ± 7 (33)	7.4 ± 1.5 (4)	2.2 ± 0.4 (10)
DHEA	365 ± 8 (35)	31 ± 6.1* (5)	4.6 ± 0.8* (10)

The data are represented as the mean ± S.E.M. of the number of animals shown in parentheses.

* Significant differences at least at $P < 0.05$.

first day from the subcutaneous injection there was a similar increase in DHEA serum level detected at the seventh day (data not shown).

Effect of DHEA on insulin receptor, IRS-1, IRS-2, AKT, PI 3-kinase associated activity and PKC ζ /λ in the muscle of rats

The protein level and the phosphorylation status of insulin receptor of 6 month-old rats were similar in both DHEA-treated and control rats as demonstrated in Fig. 1A and B. The protein level of IRS-1 detected by using a specific antibody against IRS-1 revealed no effect of DHEA treatment in the muscle of rats treated with DHEA (Fig. 2A). Despite any change in IRS-1 protein level, the insulin-induced IRS-1 tyrosine phosphorylation was increased to $185 \pm 37\%$ ($P < 0.05$) in the muscle of 6 month-old rats (Fig. 2B). Incubation of the same blots with antibodies against the 85-kDa subunit of PI 3-kinase in order to assess PI 3-kinase association with IRS-1 showed no difference from that observed in the control group in the basal state (before insulin stimulation) but an increase of $178 \pm 21\%$, $P < 0.05$ (Fig. 2C), was detected after insulin injection. Moreover, the insulin-induced IRS-1 associated PI 3-kinase activity raised by $88 \pm 10\%$, $P < 0.05$ (Fig. 2I) in DHEA treated rats. The

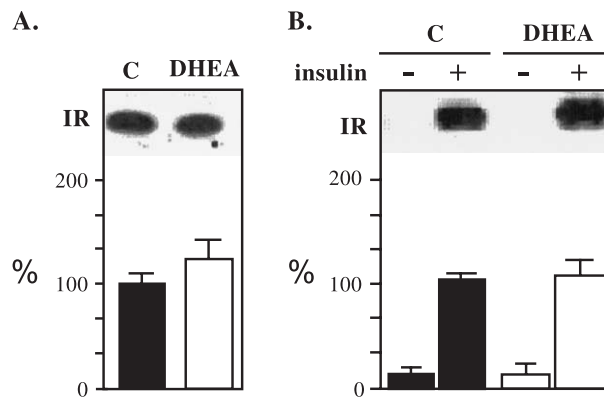
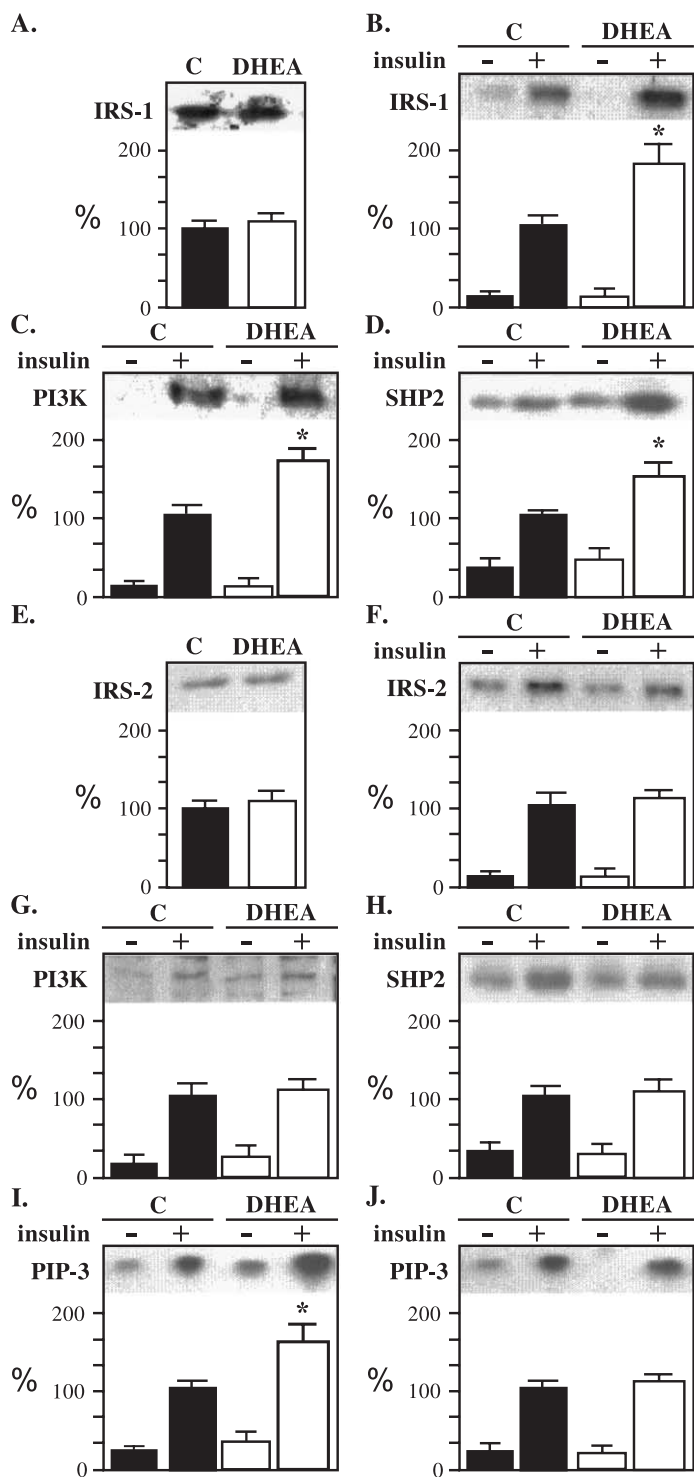


Fig. 1. Representative immunoblots showing the effect of DHEA treatment on insulin receptor protein level and autophosphorylation in the skeletal muscle of rats. Briefly, rats were anaesthetized, and the abdominal wall was incised to expose the viscera. Normal saline (–) or 60 µg of insulin (+) was injected into a portal vein as a bolus, and 90 s later, gastrocnemius muscle was excised and homogenized in extraction buffer kept on ice; after centrifugation, aliquots from the supernatant, containing 2 mg total protein, were immunoprecipitated with anti-insulin-receptor (IR) and immunoblotted with anti-IR (A) and antiphosphotyrosine (B) antibodies. Scanning densitometry was performed on autoradiograms from six experiments similar to experiments shown here (12 rats from each group were used). The values are expressed as the mean ± SEM.



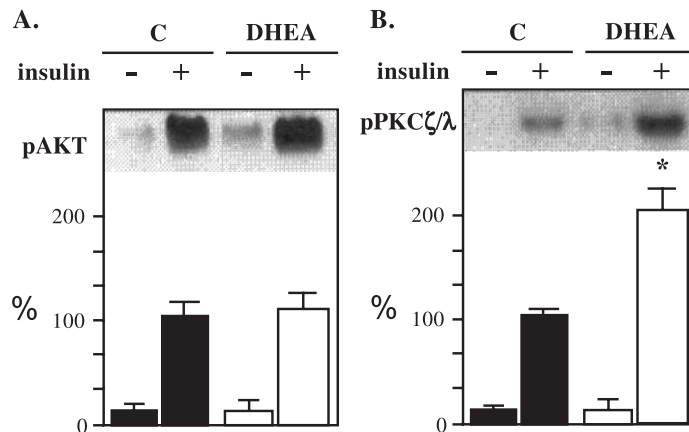


Fig. 3. Representative immunoblots showing the effect of DHEA treatment on AKT and PKC ζ/λ in the skeletal muscle of rats. Muscle samples from Control (C) and DHEA-treated rats (DHEA) were treated as whole extract tissue and aliquots with 200 μ g of protein were resolved in 8% SDS-PAGE. The nitrocellulose membranes were incubated either with anti-phospho-serine-AKT (A) or with anti-phospho-PKC ζ/λ (B). The values are expressed as the mean \pm SEM of the scanning densitometry of four experiments (8 rats from each group were used). * $P < 0.05$, control (+) \times DHEA (+).

same approach was used to analyse the insulin-induced association between IRS-1/SHP-2. This last association was also increased to $147 \pm 12\%$, $P < 0.05$, after insulin infusion in the muscle of rats treated with DHEA (Fig. 2D).

There was no change in the protein levels (Fig. 2E) and insulin-induced tyrosyl phosphorylation (Fig. 2F) of IRS-2 in the muscle of DHEA treated rats when compared to control rats. Following insulin stimulation, the phosphorylated IRS-2 has docking sites for PI 3-kinase and SHP2 association. We detected no difference in the associations between IRS-2/PI3-kinase (Fig. 2G) and IRS-2/SHP-2 (Fig. 2H) in the muscle of DHEA treated and control rats as determined by immunoblotting with specific antibodies against these substrates. Moreover basal and insulin-induced IRS-2 associated PI 3-kinase activity was not affected by DHEA in muscle (Fig. 2J).

Downstream steps of PI 3-kinase activation involve AKT and atypical PKC ζ/λ phosphorylation/activation. Insulin-induced AKT serine phosphorylation was similar in controls and in DHEA treated rats (Fig. 3A). However, DHEA treatment led to insulin-induced increase in PKC ζ/λ phosphorylation to $226 \pm 14\%$, $P < 0.05$, compared to control rats (Fig. 3B).

Fig. 2. Representative immunoblots showing the effect of DHEA treatment on early steps of insulin action, after insulin receptor autophosphorylation, in the skeletal muscle of rats. Muscle samples from Control (C) and DHEA-treated rats (DHEA), containing 2 mg total protein, were used for immunoprecipitation with anti-IRS-1 antibody (A–D) or anti-IRS-2 antibody (E–H). To analyse the protein levels the blots were incubated with anti-IRS-1, 1:400 (A) or with anti-IRS-2, 1:400 (E). To identify the tyrosyl phosphorylation level before (–) and after insulin injection (+) the blots were incubated with antiphosphotyrosine antibodies, 1:400 (B and F). The association of IRS-1/PI 3-kinase and IRS-2/PI 3-kinase were visualized by incubating the blots with anti-p85-PI 3-kinase, 1:2000 (C and G). The association of IRS-1/SHP2 and IRS-2/SHP2, the blots were incubated with anti-SHP2 antibodies, 1:400 (D and H). The insulin-induced IRS-1 and IRS-2 associated PI 3-kinase activities were also analysed (I and J). The values are expressed as the mean \pm SEM of the scanning densitometry of eight experiments (for immunoblotting assays 16 rats from each group were used, for PI 3-kinase activities assays 4 rats from each group were used). * $P < 0.05$ control (+) \times DHEA (+).

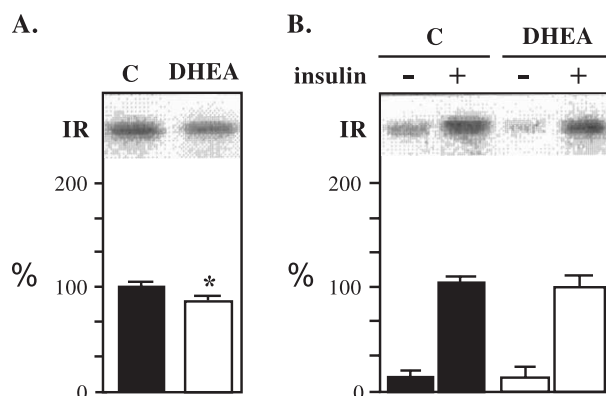


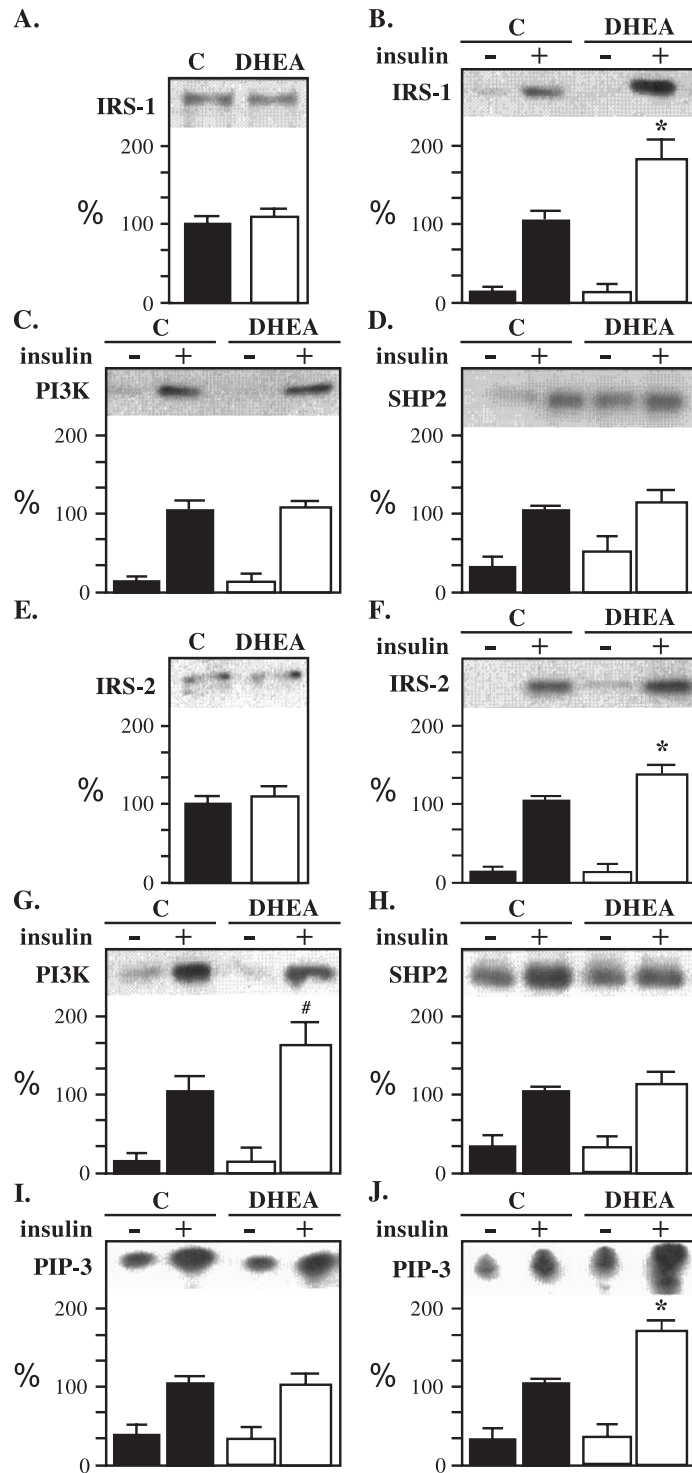
Fig. 4. Representative immunoblots showing the effect of DHEA treatment on insulin receptor protein level and autophosphorylation in the liver of rats. Briefly, rats were anaesthetized, and the abdominal wall was incised to expose the viscera. Normal saline (–) or 60 µg of insulin (+) was injected into a portal vein as a bolus, and 30 s later, liver fragment was excised and homogenized in extraction buffer kept on ice; after centrifugation, aliquots from the supernatant, containing 2 mg total protein, were immunoprecipitated with anti-IR and immunoblotted with anti-insulin-receptor (A) and antiphosphotyrosine (B). Scanning densitometry was performed on autoradiograms from six experiments similar to experiments shown here (12 rats from each group were used). The values are expressed as the mean \pm SEM. * $P < 0.05$, control \times DHEA-treated.

Effect of DHEA on insulin receptor, IRS-1, IRS-2, PI 3-kinase associated activity and AKT in the liver of rats

DHEA treatment led to significant decrease in the insulin receptor protein level when compared to control ($72 \pm 7\%$ vs. $100 \pm 9\%$, $P < 0.05$, respectively) (Fig. 4A). However, insulin-induced insulin receptor autophosphorylation was similar in control and experimental groups (Fig. 4B).

The IRS-1 protein levels in the DHEA treated group were similar to the control (Fig. 5A), but after stimulation with insulin, there was a significant increase to $200 \pm 43\%$, $P < 0.05$, in the IRS-1 tyrosyl phosphorylation (Fig. 5B). The IRS-1/PI 3-kinase association (Fig. 5C), the PI 3-kinase-IRS-1-associated activity (Fig. 5I) and the IRS-1/SHP2 (Fig. 5D) association were similar in the liver of both DHEA treated and control groups. The immunoblotting with anti-IRS-2 antibody showed no change in the IRS-2 protein level after DHEA treatment (Fig. 5E). Moreover, the IRS-2 phosphorylation induced by insulin was greater in rat liver of DHEA treated rats when compared to controls, to $158 \pm 3\%$, $P < 0.05$ (Fig. 5F). The insulin-induced IRS-2/PI 3-kinase association was increased by $92\% \pm 54\%$, $P < 0.05$.

Fig. 5. Representative immunoblots showing the effect of DHEA treatment on early steps of insulin action, after insulin receptor autophosphorylation, in the liver of rats. Liver samples from Control (C) and DHEA-treated rats (DHEA), containing 2 mg total protein, were used for immunoprecipitation with anti-IRS-1 antibody (A–D) or anti-IRS-2 antibody (E–H). To analyse the protein levels the blots were incubated with anti-IRS-1, 1:400 (A) or with anti-IRS-2, 1:400 (E). To identify the tyrosyl phosphorylation level before (–) and after insulin injection (+) the blots were incubated with antiphosphotyrosine antibodies, 1:400 (B and F). The association of IRS-1/PI 3-kinase and IRS-2/PI 3-kinase were visualized by incubating the blots with anti-p85-PI 3-kinase, 1:2000 (C and G). The association of IRS-1/SHP2 and IRS-2/SHP2, the blots were incubated with anti-SHP2 antibodies, 1:400 (D and H). The insulin-induced IRS-1 and IRS-2 associated PI 3-kinase activities were also analysed (I and J). The values are expressed as the mean \pm SEM of the scanning densitometry of eight experiments (for immunoblotting assays 16 rats from each group were used, for PI 3-kinase activities assays 4 rats from each group were used). * $P < 0.05$ control (+) \times DHEA (+).



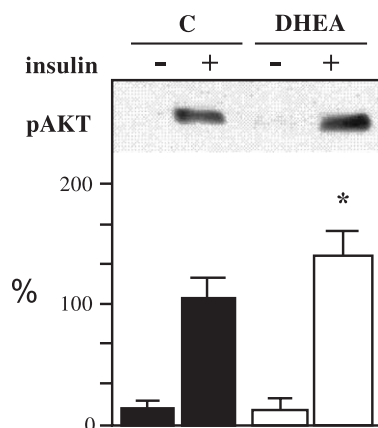


Fig. 6. Representative immunoblots showing the effect of DHEA treatment on AKT in the liver of rats. Liver samples from Control (C) and DHEA-treated rats (DHEA) were treated as whole extract tissue and aliquots with 200 μ g of protein were resolved in 8% SDS-PAGE. The nitrocellulose membrane was incubated with anti-phospho-serine-AKT. The values are expressed as the mean \pm SEM of the scanning densitometry of four experiments (8 rats from each group were used). * $P < 0.05$, control (+) \times DHEA (+).

0.05, while the insulin-induced IRS-2 associated PI 3-kinase activity was increased by $72 \pm 18\%$ in the DHEA treated rats (Figs. 5G and 5J). The IRS-2/SHP2 association was similar to the observed in the liver of control animals (Fig. 5H).

The phosphorylation status of AKT was increased in the DHEA treated rats after insulin stimulus to $137\% \pm 5\%$, $P < 0.05$ (Fig. 6).

Discussion

In humans and animal models of insulin resistance, DHEA increases sensitivity to insulin (Kimura et al., 1998). Our results demonstrated that a single subcutaneous dose of DHEA induced an enhanced in this steroid hormone serum level from the first day after the treatment until the seventh day when an improvement in insulin sensitivity shown by an increase in the glucose disappearance rate during the 15-min insulin tolerance test, in 6-months-old rats was detected. In this study, we investigated the role of insulin receptor substrates 1 and 2 and their association to PI3-kinase and to SHP2, the insulin-induced IRSs associated PI3-kinase activity and the phosphorylation status of AKT and atypical PKC in liver and muscle of 6 month-old rats treated with DHEA one week prior to the experiments.

Studies from distinct sources using knockout mice identified tissue-specific roles for each one of these proteins. The studies in IRS-1 $-/-$ mice demonstrated a potential role of IRS-1 in the growth-promoting function of insulin and IGFs, and also a compensatory hyperinsulinemia caused by β cell hyperplasia (Araki et al., 1994; Tamemoto et al., 1994; Yamauchi et al., 1996). It also demonstrated that IRS-1 can mediate the insulin action in the muscle for metabolic biologic effects (Tamemoto et al., 1994; Araki et al., 1994). On the other hand, the targeted disruption of the IRS-2 gene revealed an important role for IRS-2 in the development of insulin resistance without compensatory hyperinsulinemia leading to diabetes phenotype (Whiters et al., 1998, 1999). The IRS-2 knockout mice had defective PI 3-kinase activation in liver, being this major factor underlying defective glucose metabolism (Kubota et al., 2000).

Previs et al., 2000, using hyperinsulinemic-euglycemic clamp showed that, in vivo, IRS-1 seems to have its major role in muscle alone whereas IRS-2 mediates insulin action in liver, and muscle.

In this regard, the detected improvement of insulin-induced IRS-1 associated PI 3-kinase activity in muscle and IRS-2 associated PI 3-kinase activity in liver may play a role in DHEA enhanced insulin sensitivity. The implications of these regulations to glucose metabolism are potentially interesting.

PI 3-kinase is the best-studied signaling molecule activated by IRS-1. It plays an important role for the metabolic effects of insulin, including glucose transport, activation of glycogen synthase, and inhibition of PEPCK, the key enzyme of gluconeogenesis (Cheatham and Khan, 1995). Evidences from distinct approaches have demonstrated that AKT, a serine-threonine kinase with a pleckstrin homology domain and atypical protein kinase C (PKC ζ/λ) are functionally located downstream of PI 3-kinase (Alessi and Cohen, 1998; Standaert et al., 1997). Both proteins are phosphorylated and activated by upstream regulators. Our study shows an increase in insulin-induced AKT phosphorylation levels in liver with a concomitant increase in PKC ζ/λ phosphorylation in muscle of DHEA-treated rats. Since AKT and PKC ζ/λ phosphorylation are closely related to both kinase activities, we can suggest that AKT and PKC activity induced by insulin are probably increased in liver and muscle, respectively, of DHEA treated rats.

The molecular mechanism within these regulations may involve the activity or action of factors that are upstream AKT and PKC ζ/λ , i.e. PI 3-kinase, which, in turn, increased AKT or PKC ζ/λ phosphorylation; however, in this scenario, since increased insulin-stimulated activation of AKT and PKC ζ/λ were tissue-specific, it would be necessary to postulate that one kinase is less effectively activated at similar levels of PI 3-kinase activation, or there are separate pools of upstream signaling factors, including PI 3-kinase and PDK-1, that regulate PKC ζ/λ and AKT. As another alternative, it is possible that the improved insulin sensitivity by DHEA treatment, may be consequence of a reduction in the activity of a factor that negatively modulates PKC ζ/λ or AKT (e.g. by dephosphorylation of the activation loop and/or autophosphorylation sites, or by enhanced of the catalytic or substrate-binding sites of both PKC ζ/λ or AKT in addition to, PI 3-kinase and PDK-1 activation). Further studies are needed to more fully define the mechanism of DHEA enhanced insulin-stimulated PKC ζ/λ activation in muscle and AKT activation in liver of rats. Since AKT activation plays important role in glycogen synthesis in liver, and PKC ζ/λ activation may modulate GLUT4 translocation and glucose transport in muscle (reviewed by Farese, 2001), we can suggest that the increases in these activations may contribute to the molecular mechanism by which DHEA improves insulin action.

In addition to PI3-kinase, tyrosine phosphorylated motifs in the IRS proteins bind to the SH2 domains in several small adapter proteins, including SHP2. The association of IRS-1/SHP2 has been implicated in insulin-induced transcription of immediate early gene such as GLUT-1, which is crucial for basal glucose transport in muscle and adipocytes (Hausdorff et al., 1995). Our data of increased insulin-induced IRS-1/SHP2 in muscle of DHEA-treated rats are in agreement with others studies using cell culture, which showed increases in the expression of GLUT-1 and GLUT-4 by DHEA (Nakashima et al., 1995a,b; De Pergola, 2000).

Despite the fact that steroid hormones bind specific intracellular receptors, there is not an isolated intracellular receptor for DHEA. In contrast, it was recently identified a plasma membrane DHEA receptor that is functionally coupled to G-protein of G $\alpha_{i2,3}$ subtypes (Liu and Dillon, 2002). In this regard, one possibility by which DHEA treatment could improve IR signaling pathway could involve the modulation of specific phosphotyrosine phosphatases that respond to the intracellular cAMP levels. This

possibility is supported by recent evidence obtained from transgenic mice, which over express protein $G\alpha_i$. The $G\alpha_i$ -transgenic mice have an enhanced insulin-induced IR and IRS-1 tyrosine phosphorylation mediated by the suppression of protein-tyrosine phosphatase-1B activity and expression (Tao et al., 2001). However, further studies will be required to confirm this hypothesis.

In short, this study has shown that, following treatment with DHEA, there an increase in insulin sensitivity, accompanied by an increase in insulin-induced IRS-1/PI3K/AKT pathway in liver and in insulin-induced IRS-1/PI3K/ PKC ζ / λ pathway in muscle of 6 months-old rats. These data suggest that DHEA induces tissue-specific modulation of early steps in insulin pathway pointing to molecular mechanisms by which this drug improves insulin action.

Acknowledgements

The authors thank Luiz Janeri, José Luiz dos Santos, and Luciene M. Ribeiro (Teca) for their technical assistance, and Dr. Luiz S. Menna-Barreto for helpful comments during preparation of the manuscript. This work was supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and CNPq.

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