DHEA improves glucose uptake via activations of protein kinase C and phosphatidylinositol 3-kinase


DHEA improves glucose uptake via activations of protein kinase C and phosphatidylinositol 3-kinase. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E196–E204, 1999.—We have examined the effect of adrenal androgen, dehydroepiandrosterone (DHEA), on glucose uptake, phosphatidylinositol (PI) 3-kinase, and protein kinase C (PKC) activity in rat adipocytes. DHEA (1 µM) provoked a twofold increase in 2-[3H]deoxyglucose (2-DG) uptake for 30 min. Pretreatment with DHEA increased insulin-induced 2-[3H]DG uptake without alterations of insulin specific binding and autophosphorylation of insulin receptor. DHEA also stimulated PI 3-kinase activity. 125I-DHEA bound to purified PKC containing PKC-α, -β, and -γ. DHEA provoked the translocation of PKC-β and -γ from the cytosol to the membrane in rat adipocytes. These results suggest that DHEA stimulates both PI 3-kinase and PKCs and subsequently stimulates glucose uptake. Moreover, to clarify the in vivo effect of DHEA on Goto-Kakizaki (GK) and Otsuka Long-Evans fatty (OLETF) rats, animal models of non-insulin-dependent diabetes mellitus (NIDDM) were treated with 0.4% DHEA for 2 wk. Insulin- and 12-0-tetradecanoyl phorbol-13-acetate-induced 2-[3H]DG uptake of adipocytes were significantly increased, but there was no significant increase in the soleus muscles in DHEA-treated GK/Wistar or OLETF/Leto rats when compared with untreated GK/Wistar or OLETF/Leto rats. These results indicate that in vivo DHEA treatment can result in increased insulin-induced glucose uptake in two different NIDDM rat models.

Dehydroepiandrosterone; non-insulin-dependent diabetes mellitus; 2-deoxyglucose

**Materials and Methods**

Materials. Pork insulin was obtained from Novo (Copenhagen, Denmark). 2-1,2-[3H]DG was purchased from New England Nuclear (Boston, MA). Phenylmethylsulfonyl fluoride (PMSF), leupeptin, 12-0-tetradecanoyl phorbol-13-acetate (TPA), BSA, phosphatidylserine, diolein, histone, (type III-S), D-glucose, ATP, dexamethasone, prednisolone, and DHEA were purchased from Sigma (St. Louis, MO). Silicone oil was obtained from Alkem Chemical (Milwaukee, WI). [γ-32P]ATP (3,000 Ci/mmol), 2-[3H]DG (50 Ci/mmol), L-[1-14C]glucose (47 mCi/mmol), 125I-Dexamethasone (50 Ci/mmol), and 1,2-[3H]DHEA (60 Ci/mmol) were purchased from New England Nuclear (Tokyo, Japan). RU-38486 (17β hydroxy-11β,4-dimethylaminophenyl-17α-propynyl estradiol trihydrate) was generously donated by Roussel-Uclaf Research Center. All other chemicals were of reagent grade or better. Polypropylene plastic tubes were used in each experiment, unless otherwise stated.

Adipocyte experiments. Male Wistar and GK rats, a non-insulin-dependent diabetes mellitus (NIDDM) animal model (8, 9) weighing 150–200 g (8 wk of age), and Long-Evans Tokushima (LET) rats (16 wk of age; see Refs. 16, 21) were fed with CE2 (Japan Clea, Tokyo, Japan) ad libitum, treated with CE2 powder containing 0.4% DHEA for 2 wk, and then killed by decapitation. As shown in Table 1, in vivo treatment with 0.4% DHEA for 2 wk resulted in significant increases in plasma DHEA concentration in GK/Wistar and OLETF/LET rats. Isolated adipocytes were obtained by collagenase digestion of rat epididymal fat pads (26, 29) in Krebs-Ringer phosphate (KRP) buffer (pH 7.4) containing 127 mM NaCl, 12.3 mM NaH2PO4, 5.1 mM KCl, 1.3 mM MgSO4, 1.4 mM CaCl2, 3% BSA, and 2.5 mM glucose. Adipocytes were washed twice with medium, preincubated at 37°C in glucose-free KRP buffer containing 1% BSA for 30 min, and then incubated with or without (control) 10−6 M DHEA.
were determined by measurement of radioactivity of 25-ml Erlenmeyer flasks under 95% O₂-5% CO₂ in 5 ml and one stimulated sample. Soleus muscles were incubated in and muscle tension was maintained by ligatures attached to the solei were washed and incubated for 30 min in glucose-

Table 1. DHEA concentration in GK/ Wistar and OLETF/LETO rats after treatment with 0.4% DHEA for 2 wk

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<th>DHEA Concentration, nM</th>
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<td>GK</td>
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Values are means ± SE; n, no. of rats. DHEA, dehydroepiandrosterone; GK, Goto-Kakizaki; OLETF, Otsuka Long-Evans fatty; LETO, Long-Evans Tokushima. *P < 0.01 vs. untreated values by ANOVA.

(dissolved in <0.01% ethanol) for 60 min, followed by incubation with 10 nM insulin for 30 min. There was no effect of 0.01% ethanol, used for the control, on insulin-induced glucose uptake in rat adipocytes. 2-[³H]DG (0.08 µCi) and unlabeled 2-DG (0.05 mM) were then added to 300 µl of a 10% (vol/vol) adipocyte suspension, and uptake of 2-[³H]DG was measured over 1 min. Corrections for trapped buffer or non-carrier-mediated uptake were determined by measuring uptake in the presence of 70 µM cytochalasin, which reduced control and agonist-stimulated values (14).

PKC experiments. Adipocytes were incubated with or without (control, 0 min) 1 µM DHEA for 5, 10, and 20 min. Reactions were terminated by the addition of 20 mM Tris-HCl buffer (pH 7.5) containing 0.25 M sucrose, 1.2 mM EGTA, 0.1 mM PMSF, 20 µg/ml leupeptin, and 20 mM 2-mercaptoethanol (buffer I), washed two times, and homogenized in buffer I. The homogenates were centrifuged for 60 min at 105,000 g to obtain the cytosol and membrane fractions. The latter was homogenized in buffer I containing 5 mM EGTA, 2 mM EDTA, and 1% Triton X-100. Activation of PKC in rat adipocytes was assayed by changes in the subcellular distribution of immunoreactive PKC with methods described previously (14, 15). Equal amounts of cytosol or membrane-associated fraction were prepared, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose, and incubated first with polyclonal antiserum raised to synthetic peptide to PKC-ζ and -γ (GIBCO) and second with goat anti-rabbit globulin complexed to alkaline phosphatase (Sigma). This immunoblotting method detected a single major immunoreactive band that comigrated on SDS-PAGE and blotted identically with 80-kDa (PKC-β) and 73-kDa (PKC-ζ) synthetic peptides.

Soleus muscle experiments. Soleus muscles were excised, and muscle tension was maintained by ligatures attached to the tendons. The two solei from each rat provided one control and one stimulated sample. Soleus muscles were incubated in 25-ml Erlenmeyer flasks under 95% O₂-5% CO₂ in 5 ml Krebs-Ringer bicarbonate (KRB) buffer (119 mM NaCl, 4.8 mM KCl, 1 mM KH₂PO₄, 1.2 mM MgSO₄, 1 mM CaCl₂, 24 mM NaHCO₃, 12 mM HEPES, 0.1% BSA, and 2 mM sodium pyruvate). For studies of glucose transport, after an initial 30-min incubation in KRB buffer containing 5 mM glucose, the solei were washed and incubated for 30 min in glucose-free KRB for 30 min with or without insulin. 2-[³H]DG (1 µCi), L-[¹³C]glucose (0.1 µCi), and 0.1 mM unlabeled 2-DG were then added, and incubation was continued for 10 min. After incubation, tissue was removed, rapidly rinsed in isotopically-free medium, blotted, weighed, homogenized in 5% trichloroacetic acid, and counted simultaneously for ³H and ¹³C. Corrections for 2-[³H]DG in tissue samples unrelated to specific transport were determined by measurement of radioactivity of L-[¹³C]glucose. As reported previously (15), uptake of 2-[³H]DG was linear for at least 30 min.

DHEA binding study. For [³H]DHEA specific binding to purified PKC from rat brain, Mono Q column-purified PKC was obtained using an HPLC system as described previously (14, 15), and then the collected samples were concentrated fivefold in buffer I containing 5 mM EGTA, 2 mM EDTA, and 5% glycerol. Moreover, purified PKC (BIOML Research Laboratories), 50 nM [³H]DHEA, 1,000-fold DHEA, and 100-fold RU-38466 were incubated for 24 h at 4°C with and without 0.5 mM Ca²⁺ and were separated by the addition of charcoal dextran. Radioactivity in the supernatant was measured with a liquid scintillation counter. [³H]DHEA specific binding to free adipocytes was carried out as described previously (34). Briefly, free adipocytes (80 µl) and 50 nM [³H]DHEA 1,000-fold unlabeled DHEA in the presence or absence of 100-fold RU-38466 were incubated for 60 min at 37°C, separated by passage through Whatman filter paper (25 mm), and rinsed several times with an excess of 0.9% saline. The radioactivity in this paper was counted with a liquid scintillation counter.

PI 3-kinase assay. Isolated adipocytes were treated with or without insulin (10 nM) for 0, 1, 5, 10, and 60 min at 37°C, lysed in buffer containing 1% (vol/vol) Nonidet P-40 (NP-40; see Ref. 11), and immunoprecipitated with an anti-phosphotyrosine antibody (19) and protein A-agarose. The immunoprecipitates were washed and subjected to the PI 3-kinase assay as described elsewhere (11). Briefly, cells were treated with 10 nM insulin, 1 µM TPA, or 1 µM DHEA for the indicated periods, lysed in the buffer (20 mM Tris-HCl, pH 7.4, 1% NP-40, 0.5 mM EDTA, 5% glycerol, 1 mM orthovanadate, and 20 mM p-aminophenylmethylene sulfonyl fluoride hydrochloride), and sonicated. The homogenates were centrifuged at 15,000 rpm for 20 min, and the indicated supernatants were incubated with 4 µg of anti-phosphotyrosine antibody for 2 h at 4°C. The immunocomplexes were precipitated with 40 µl of protein A-agarose. The immunoprecipitates were subjected to the PI 3-kinase assay.
3-kinase assay in a 50-µl reaction mixture containing 20 mM Tris·HCl, pH 7.4, 100 mM NaCl, 10 mM MgCl₂, 0.5 mM EGTA, 100 µM PI, 100 µM phosphatidylyserine, and 10 µM \( [\gamma^{32P}]ATP \) (0.1 µCi/µl). After 10 min at 30°C, the reaction was stopped by adding 200 µl of 1 M HCl and 80 µl of chloroform-methanol (1:1, vol/vol). A 30-µl portion of the lower layer was spotted on a Silica Gel 60 plate (Merck) and was developed in chloroform-methanol-25% NH₄Cl-water (43:38:5:7, vol/vol/vol/vol). The radioactive PI phosphate spot was detected by autoradiography, and scrapes from the plate were counted by liquid scintillation counting.

Immunoblot. Immunoprecipitate (500 µg protein) with an anti-phosphotyrosine antibody (5 µg) or anti-insulin receptor antibody (5 µg; Upstate Biotechnology, Lake Placid, NY) was separated by SDS-PAGE and transferred to nitrocellulose paper. The paper was blocked with 3% gelatin Tris-buffered saline and was incubated with an anti-p85 PI 3-kinase antibody or anti-phosphotyrosine antibody (1:1,000 dilution; Transduction Laboratories, Lexington, KY) for 4–5 days. Protein bands were located with an enhanced chemiluminescence system (Amersham, Tokyo, Japan). Immunoblots were quantified by laser scanning densitometry.

Insulin binding studies. Isolated adipocytes were suspended in KRP buffer, incubated for 60 min with or without (control) 1 µM DHEA, and then incubated with \([125I]\)insulin. After incubation, the cells were washed with ice-cold KRP buffer and the radioactivity was measured. Data are plotted by Scatchard analysis as the mean of triplicate determinations (A). Immunoprecipitates with antibody against insulin receptor were separated by SDS-PAGE. Immunoblot analysis of 100 nM insulin-induced 95-kDa tyrosine autophosphorylation of insulin receptor was performed in DHEA-treated cells for 60 min and control cells. Representative experiment is shown (B), and data by densitometric analysis are plotted as the means ± SE of 3 separate experiments (C). B/F, bound-to-free ratio; C, control.
(2,000 Ci/mmol; Amersham) and unlabeled insulin (1–1,000 nM) in plastic tubes at 25°C in a shaking water bath for 60 min as described previously (17). Incubations were terminated by removing 300-µl aliquots from the cells in plastic microfuge tubes to which 100 µl silicone oil had been added. The cells were then separated by cutting a plastic tube just over the silicone oil layer, and the radioactivity was determined. All studies were performed in triplicate.

Incorporation of 3H in diacylglycerol. Adipocytes were incubated for 30 min in 0.5 ml KRP buffer. [3H]glycerol (10 µCi) was then added, and, after prelabeling adipocytes for 15 min, a vehicle (control) or 10 nM insulin was added after pretreatment with 1 µM DHEA for 60 min. Incubation was continued for 2, 5, 10, and 20 min. Reactions were stopped by the addition of methanol (final concentration, 50%). Samples were transferred to glass tubes. Chloroform (2 vol) was added, and extraction of the lipids was performed as described previously (6, 12).

Statistical analysis. Statistical comparisons were performed using ANOVA followed by Fisher’s protected least significant difference (PLSD) test. Unless otherwise stated, all data are expressed as the means ± SE.

RESULTS

Effect of DHEA on insulin-induced 2-[3H]DG uptake. DHEA (1 µM) alone stimulated 2-[3H]DG uptake by 150–200% from the basal level within 30 min (P < 0.05 by ANOVA with Fisher’s PLSD) but did not stimulate androstenedione, ethiocholanolone, or testosterone (Fig. 1). Maximal effect of DHEA on glucose uptake was observed at 1 µM (data not shown). Pretreatment with 1 µM DHEA resulted in a significant increase (P < 0.01 by ANOVA with Fisher’s PLSD) in insulin-induced 2-[3H]DG uptake. Maximal effect of DHEA on insulin-induced 2-[3H]DG uptake was observed at 10^(-8) M (Fig. 2).

Effect of DHEA on insulin specific binding to its receptor and 95-kDa autophosphorylation of insulin receptor. To clarify the effect of DHEA on insulin specific binding activity and insulin receptor autophosphorylation activity, which is the first insulin signaling step, we examined [125I]insulin specific binding to the
receptor of adipocytes and 95-kDa autophosphorylation of the insulin receptor β-subunit. As shown in Fig. 3, there was no significant difference in [125I]insulin specific binding to the receptor of adipocytes (control cells bound 2.9 ± 0.2% of [125I]insulin/tube, cells treated with DHEA bound 2.8 ± 0.3% of [125I]insulin/tube; Fig. 3A), and there was also no significant difference in 95-kDa insulin receptor autophosphorylation (Fig. 3B) between treatment with and without 1 µM DHEA. Densitometric analysis indicated that there was no difference in insulin-induced 95-kDa autophosphorylation between DHEA-treated and untreated adipocytes (925 ± 37 vs. 918 ± 46%; Fig. 3C).

DHEA-induced PI 3-kinase activation. Moreover, to clarify the effect of DHEA on PI 3-kinase, which binds to tyrosine-phosphorylated IRS-1 via the SH2 domain of PI 3-kinase, downstream of the insulin signaling pathway, we examined whether DHEA stimulates PI 3-kinase in rat adipocytes. When adipocytes were incubated with 1 µM DHEA, unexpectedly, enzyme activity (Fig. 4A) and the p85 subunit of PI 3-kinase (Fig. 4B), after immunoprecipitation with anti-phosphotyrosine antibody, increased for 5 and 10 min, similar to 100 nM insulin-induced PI 3-kinase activation (Fig. 4A, left).

Effect of DHEA on diacylglycerol production. To resolve the DHEA-stimulated glucose uptake mechanism, we focused on diacylglycerol-PKC signaling. When adipocytes were labeled with [3H]glycerol, 1 µM DHEA alone stimulated diacylglycerol production to 150% from basal levels (Fig. 5A). Insulin-stimulated incorporation of [3H]glycerol in [3H]diacylglycerol for 2 and 20 min was markedly enhanced almost twofold by pretreatment with 1 µM DHEA for 60 min (Fig. 5B).

DHEA-induced PKC translocation. DHEA stimulated both PI 3-kinase and diacylglycerol production as shown in Figs. 4 and 5. We examined whether DHEA activates PKC-ζ, which is thought to be downstream of PI 3-kinase (23), and PKC-β. Isolated adipocytes were treated with or without (control: 0 min) 1 µM DHEA for 5, 10, and 20 min. Cytosolic PKC-β and -ζ immunoreactivities gradually decreased, and membrane-associated PKC-β and -ζ immunoreactivity was inversely increased (Fig. 6). Dexamethasone induced PKC-β and -ζ translocations from the cytosol to the membrane, as shown previously (18).

[3H]DHEA binding to PKC purified from rat brain. When 10 ng purified conventional PKC (PKC-α, -β, and -γ) activity was measured using histone III-s as the substrate in the presence of 0.001–1 mM Ca²⁺, phosphatidylinerse, and diolein, the most effective Ca²⁺ concentration was 0.5 mM. On the other hand, in the presence of 40 µg/ml phosphatidylinerse, protein kinase activity was most activated by the addition of 1 µM DHEA in 0.5 mM Ca²⁺ (Fig. 7). Immunoprecipitate with anti-PKC-ζ antibody was also activated in the presence of phosphatidylinerse/diolein (data not shown). In addition to the above results, we examined [3H]DHEA specific binding to conventional PKC and atypical PKC. [3H]DHEA specific binding to conventional PKC was found to be as shown in Fig. 8. Glucocorticoid receptor antagonist RU-38486 did not affect [3H]DHEA binding to PKC in vitro. [3H]DHEA specific binding to PKC-ζ was also observed to a similar extent (data not shown).

Effect of DHEA treatment in vivo on insulin- or TPA-induced glucose uptake in adipocytes of GK/Wistar and OLETF/LETO rats. At 10 wk of age, 10 nM insulin- or 1 µM TPA-induced [2-3H]DG uptake in adipocytes of GK rats decreased by only 20% compared with Wistar rats. There were no significant differences in body weight (DHEA-treated vs. untreated Wistar rats).
rains, 270 ± 7 vs. 285 ± 6 g; DHEA-treated vs. untreated GK rats, 252 ± 6 vs. 211 ± 11 g), plasma glucose level (DHEA-treated vs. untreated Wistar rats, 128 ± 14 vs. 130 ± 15 mg/dl; DHEA-treated vs. untreated GK rats, 211 ± 33 vs. 234 ± 14 mg/dl), or plasma insulin level (DHEA-treated vs. untreated Wistar rats, 83 ± 16 vs. 84 ± 18 µM; DHEA-treated vs. untreated GK rats, 79 ± 11 vs. 78 ± 12 µM) in Wistar and GK rats after in vivo treatment with 0.4% DHEA. In the 2-wk treatment with 0.4% DHEA in vivo, 10 nM insulin- or 1 µM TPA-induced 2-[3H]DG uptake was significantly increased by 50–100%, but basal 2-[3H]DG uptake was not increased compared with untreated GK and Wistar rats at 10 wk of age. However, DHEA-induced 2-[3H]DG uptakes in adipocytes of DHEA-treated GK and Wistar rats were not significantly increased compared with those of untreated GK and Wistar rats (Fig. 9A). It should be noted that glucose uptake in adipocytes of GK rats was not markedly different from that in Wistar control rats. Therefore, we selected another NIDDM animal model, the OLETF rat, and examined the effect of in vivo DHEA treatment on insulin-, TPA-, or DHEA-induced 2-[3H]DG uptake. At 10 wk of age, 10 nM insulin- and 1 µM TPA-induced 2-[3H]DG uptake in adipocytes of OLETF rats decreased by 45 and 40%, respectively, when compared with LETO rats (control). In the 2-wk treatment with 0.4% DHEA in vivo, there were no significant differences in body weight (DHEA-treated vs. untreated LETO rats, 386 ± 41 vs. 420 ± 45 g; DHEA-treated vs. untreated OLETF rats, 454 ± 55 vs. 517 ± 58 g) or plasma insulin level (DHEA-treated vs. untreated LETO rats, 182 ± 43 vs. 185 ± 42 µM; DHEA-treated vs. untreated OLETF rats, 257 ± 38 vs. 252 ± 37 µM), but there was a significant difference in plasma glucose of OLETF rats (DHEA-treated vs. untreated LETO rats, 112 ± 25 vs. 124 ± 20 mg/dl; DHEA-treated vs. untreated OLETF rats, 144 ± 19 vs. 201 ± 21 mg/dl; P < 0.05 by ANOVA with Fisher’s PLSD). After in vivo treatment with DHEA, insulin and TPA-induced glucose uptakes were significantly increased by 50–100% when compared with untreated OLETF rats. In the 2-wk treatment with 0.4% DHEA in vivo, no significant differences in body weight (DHEA-treated vs. untreated LETO rats, 386 ± 41 vs. 420 ± 45 g; DHEA-treated vs. untreated OLETF rats, 454 ± 55 vs. 517 ± 58 g) or plasma insulin level (DHEA-treated vs. untreated LETO rats, 182 ± 43 vs. 185 ± 42 µM; DHEA-treated vs. untreated OLETF rats, 257 ± 38 vs. 252 ± 37 µM) were observed. However, DHEA-induced 2-[3H]DG uptake in soleus muscles of DHEA-treated OLETF/LETO rats was not significantly different from those in untreated OLETF/LETO rats.
DISCUSSION

The present findings indicate that DHEA alone stimulates glucose uptake through diacylglycerol-PKC and PI 3-kinase signaling pathways. It has been reported that the glucose uptake mechanism is mediated by an IRS-1-PI 3-kinase pathway (30) and a diacylglycerol-PKC pathway (15). Recently, the PI 3-kinase inhibitor wortmannin was shown to decrease insulin-induced glucose uptake in rat adipocytes (25). Therefore, PI 3-kinase plays a pivotal role in the regulation of insulin. On the other hand, insulin and phorbol esters increase membrane-associated PKC via increases in diacylglycerol probably via phospholipase C and phospholipase D activation (31). Moreover, atypical PKC-ζ, which is a diacylglycerol-insensitive PKC, operates downstream of PI 3-kinase (23). In addition to the PI 3-kinase-PKC-ζ pathway, PKC-ζ may be a candidate for insulin-induced glucose transport in insulin-sensitive tissue (33). On the basis of insulin signaling, we have found that DHEA alone provokes diacylglycerol production by phospholipase C or phospholipase D activation in rat adipocytes. These results suggest the following mechanisms. First, DHEA binds to a specific membrane receptor. Second, DHEA binds to PKC, leading to subsequent stimulation of phospholipase D. The first suggestion has already been reported as follows (22, 24). Meikle et al. (22) have reported that a high-affinity DHEA specific binding site can be found in the cytosol and nuclear fraction in mouse T lymphocytes. The second suggestion is based on the time course of DHEA-induced diacylglycerol production as shown in Fig. 5. DHEA binds to conventional PKC and atypical PKC-ζ such as dexamethasone, as previously reported (17), and activates conventional PKC in the presence of Ca²⁺ and atypical PKC-ζ in vivo and in vitro as shown in Figs. 6–8. Finally, these diacylglycerol-sensitive PKC may possibly act as a phospholipase D activator as previously described (13, 32).

Conversely, DHEA stimulates PI 3-kinase through binding to an unknown tyrosine phosphorylated pro-
tein unlike insulin as shown in Fig. 4. It should be reasonable to stimulate glucose transport via activation of PKC-ζ by DHEA-induced PI 3-kinase activation. In contrast, dexamethasone does not stimulate glucose uptake, probably due to inhibition of glucose transporter translocation. In fact, a previous report has indicated that dexamethasone suppresses GLUT-4 translocation in rat adipocytes (4). As shown in Fig. 9, in vivo treatment with DHEA results in an increase in insulin-stimulated glucose uptake. The plasma DHEA concentration reaches 10^{-8} to 10^{-7} M in 0.4% DHEA-treated rats as shown in Table 1. This concentration of insulin- and TPA-induced 2-[3H]DG uptake in adipocytes results in a significant increase in insulin-stimulated glucose uptake. The plasma DHEA concentration reaches 10^{-7} M in 0.4% DHEA-treated rats. This concentration of insulin-stimulated glucose uptake is indicated in Fig. 2. Therefore, DHEA actually decreases insulin resistance in GK and OLETF rats, NIDDM animal models, without affecting body weight, glucose, or insulin levels. However, we have to consider that there is much less DHEA in rodents than in humans (3). Therefore, it is possible that the effect of in vivo treatment with DHEA may alter insulin resistance dramatically in OLETF rats. On the other hand, it should be noted that insulin- and TPA-induced glucose uptake in soleus muscles of OLETF/LETO rats is not altered by in vivo DHEA treatment for 2 wk. This result may be due to a short period of in vivo DHEA treatment and a weak effect of DHEA on skeletal muscle. Accordingly, it is suggested that DHEA mainly affects adipose tissue as previously reported (10). Further studies will be required on the effect of in vivo treatment with DHEA on diabetic patients.

In conclusion, first, DHEA alone stimulates glucose uptake in rat adipocytes. Second, DHEA stimulates the production of diacylglycerol. Third, pretreatment with DHEA enhances insulin-induced diacylglycerol production. Fourth, DHEA stimulates PI 3-kinase activity, conventional PKC, and atypical PKC. Fifth, specific DHEA binding activity to PKC can be found. Sixth, histone phosphorylation activity increases with the addition of DHEA in the presence and absence of Ca^{2+}. Seventh, treatment with 0.4% DHEA in vivo for 2 wk in GK and OLETF rats results in a significant increase in insulin- and TPA-induced 2-[3H]DG uptake in adipocytes. Finally, DHEA may be useful in overcoming insulin resistance in NIDDM.

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