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Supplementary Information for

The Glucocorticoid Receptor Agonistic Modulators CpdX and CpdX-D3 do not generate the debilitating effects of synthetic Glucocorticoids

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Supplementary Information Text

SI Materials and Methods

Materials

Dexamethasone (Dex), estradiol, progesterone, tamoxifen and Glucose were from Sigma Aldrich. Insulin (Humulin R; Eli Lilly) was from Eurobio ingen. Pan AKT (C67E7), pAKT S473 (9271L), IRS-1 (D23G12) and pIRS-1 S318 (D51C3) antibodies were from Cell Signaling. pLuc-TK-2xPRE plasmid was from Addgene.

Histology

Mouse samples were fixed overnight at 4°C in 4% paraformaldehyde and embedded in paraffin. Sections (5µm) were stained with hematoxylin and eosin.
Liver lipid deposition was revealed by 5% Red oil staining of frozen sections.

RNA extraction and quantitative RT-PCR

Total RNA was extracted from mouse dorsal skin, tibia, adrenal gland or liver with Trizol reagent (Invitrogen) according to a standard protocol. RNA was reverse-transcribed using random oligonucleotide hexamers and amplified by means of quantitative PCR with a LightCycler 480 (Roche Diagnostics) and the SYBR Green kit (Roche), according to manufacturer's instructions. Relative RNA levels were calculated with hypoxanthine phosphoribosyl-transferase (HPRT) as an internal control. For analysis of each set of gene expression, an arbitrary value of 1 was given to the sample exhibiting the highest level, and the remaining samples were plotted relative to this value. PCR primers are available upon request.

Micro-CT analysis

From each animal included in the experiments, one femur and the ipsilateral tibia were dissected and preserved in 70% ethanol. The FX Quantum micro-CT scanner (Perkin Elmer) was used to perform measurements at the distal femur and midshaft tibia. All scans

were performed with an isotropic voxel size of 10 µm, 160 µA tube current and 90 kV tube voltage. Gray scale images were initially pre-processed using ImageJ software. Morphological 3D measurements were further performed using the CTAn software (Bruker). Cortical bone parameters, which were measured in the tibia midshaft included, measures of cortical thickness, bone area fraction, total area, bone area and marrow area. The region of interest was selected from below the distal tibial crest and continued for 20 slices toward the proximal end of the tibia. Trabecular bone parameters were measured in the distal metaphysis of the femurs. They included bone volume fraction, trabecular thickness, trabecular number and trabecular separation. The region of interest was selected from below the distal growth plate where the epiphyseal cap structure completely disappears, and continued for 100 slices toward the proximal end of the femur.

Determination of plasmatic parameters

Blood was collected at 10 a.m. and 6 p.m. by retro-orbital puncture in lithium-heparin coated vials. The plasmatic levels of corticosterone, insulin, total cholesterol and bile acids were determined.

Intraperitoneal glucose tolerance test (IPGTT)

Mice were fasted for 14 hours before test. Mouse blood glucose concentration was measured at 10 a.m. before (T_0) and during two hours (T_{120}) after glucose i.p. injection (2 mg/kg body weight).

Intraperitoneal insulin tolerance test (IPITT)

Mice were fasted for 6 hours before test. The blood glucose concentration was measured both before (T0) and during a one-hour period after insulin i.p. injection (0.75 U/kg body weight).

Western Blot

Liver samples were harvested and lysed in the RIPA buffer (20 mM Tris pH 8, 150 mM NaCl, 10% glycerol, 1% NP-40 and 2 mM EDTA). Antibodies from Cell Signaling were

used to assess by Western-Blotting the relative level of p-IRS1 (S318), IRS-1, p-AKT (S473) and AKT.

Cell Culture Conditions for Luciferase assays

Cos-1 monkey kidney fibroblast-like cells (CCL-70, *ATCC*) were maintained in DMEM (1 g/l glucose) medium containing 5% fetal calf serum (FCS) and gentamycin. For luciferase reporter assays, Cos-1 cells seeded on 24-well tissue culture plates overnight were transfected at 60% confluency with 100 ng pCMV β galactosidase, 200 ng luciferase reporter (pGL3-17mer-ERE or pLuc-TK-2xPRE) and 500 ng expression vector (pSG5-ER or pSG5-PR) into each well. 6hr post-transfection, medium was changed and cells were maintained in medium containing charcoal-treated FCS. 24hr post-transfection, chemicals were added, as indicated, for 6hr. Luciferase assay was carried out as instructed (Promega). Normalized values are reported as the mean ± SEM; each value originated from at least three individual transfections with assays performed in triplicate.

Table S1: Primers used for Q-RT PCR analyses

mHPRT F	5’ GTTGGATACAGGCCAGACTTTGTTG
mHPRT R	5’ GATTCAACTTGCGCTCATCTTAGGC
mKindlin 1 F	5’ TGGAGAGCAGCAGACAGAGA
mKindlin 1 R	5’ AGGGGTGAAGAGAAGGTTGG
mREDD1 F	5’ TAGTGCCCACCTTTCAGTTG
mREDD1 R	5’ GTCAGGGACTGGCTGTAACC
mWNT16 F	5’ GAGCTGTGCAAGAGGAAACC
mWNT16 R	5’ GAATGCTGTCTCCTTGGTGC
mCyp11b2 F	5’ GCTGGCTGGAGAGGAAAAG
mCyp11b2 R	5’ GCTGGGCATCAAAACAAAG
mFASN F	5’ GCCCTTGACCTTCTGGTGT
mFASN R	5’ AGGCTCTCCCACTCCTCCT
mSCD1 F	5’ AGAGTCAGGAGGGCAGGTTT
mSCD1 R	5’ CAGTGATGGTGGTGGTGGT