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Atypical β-adrenergic effects on insulin signaling and action in β_3-adrenoceptor-deficient brown adipocytes

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Jost, Petra, Mathias Fasshauer, C. Ronald Kahn, Manuel Benito, Marco Meyer, Volker Ott, Bradford B. Lowell, H. Harald Klein, and Johannes Klein. Atypical β-adrenergic effects on insulin signaling and action in β_3-adrenoceptor-deficient brown adipocytes. *Am J Physiol Endocrinol Metab* 283: E146–E153, 2002. First published March 5, 2002; 10.1152/ajpendo.00531.2001.—Cross talk between adrenergic and insulin signaling systems may represent a fundamental molecular basis of insulin resistance. We have characterized a newly established β_3-adrenoceptor-deficient (β_3-KO) brown adipocyte cell line and have used it to selectively investigate the potential role of novel-state and typical β-adrenoceptors (β-AR) on insulin signaling and action. The novel-state β_1-AR agonist CGP-12177 strongly induced uncoupling protein-1 in β_3-KO brown adipocytes as opposed to the β_3-selective agonist CL-316,243. Furthermore, CGP-12177 potently reduced insulin-induced glucose uptake and glycogen synthesis. Neither the selective β_1- and β_2-antagonists metoprolol and ICI-118,551 nor the nonselective antagonist propranolol blocked these effects. The classical β_1-AR agonist dobutamine and the β_2-AR agonist clenbuterol also considerably diminished insulin-induced glucose uptake. In contrast to CGP-12177 treatment, these negative effects were completely abrogated by metoprolol and ICI-118,551. Stimulation with CGP-12177 did not impair insulin receptor kinase activity but decreased insulin receptor substrate-1 binding to phosphatidylinositol (PI) 3-kinase and activation of protein kinase B. Thus the present study characterizes a novel cell system to selectively analyze molecular and functional interactions between novel and classical β-adrenoceptor types with insulin action. Furthermore, it indicates insulin receptor-independent, but PI 3-kinase-dependent, potent negative effects of the novel β_1-adrenoceptor state on diverse biological end points of insulin action.

β-adrenoceptor; adipose tissue; insulin resistance; CGP-12177

INTERACTION BETWEEN THE SYMPATHETIC nervous system and insulin plays an important role in the pathogenesis of obesity, insulin resistance, and their related cardiovascular complications (24). The β_3-adrenergic receptor (β_3-AR) is predominantly expressed in brown and white adipose tissue and represents a potential target for antiobesity drug therapy (25, 26). Selective activation of this adrenoceptor results in an increase in lipolysis and energy expenditure. Regulation of energy balance in rodents is mediated by activation of uncoupling protein-1 (UCP-1), which dissipates energy stored in the proton gradient across the inner mitochondrial membrane, thereby uncoupling mitochondrial respiration from ATP production (19, 20). Previous studies in brown adipocytes have shown that insulin inhibits β_3-adrenergic stimulation of UCP-1 (15) and that β_3-adrenergic stimulation leads to decreased activation of the insulin-signaling system (16). These findings indicate a functionally relevant interplay between adrenergic and insulin-signaling systems.

From a variety of studies using the aryloxypropanolamine CGP-12177 in both β_3-knockout (β_3-KO) and wild-type mice, the concept of a novel atypical adrenergic receptor, initially dubbed β_4-AR, in adipose tissue (3, 6, 23), heart (12), and gut (21) has been developed. CGP-12177-induced effects that can be neither mimicked by β_3-AR-selective phenethanolamine agonists nor abolished by β_1- and β_2-AR blockade indicated a novel atypical β-AR (11). However, most recent studies using recombinant β-AR subtypes (17, 18, 22) and knockout mice (9) have demonstrated that the β_4-AR is a novel state of the β_1-AR (7, 17). Thus Konkar et al. (17) found that CGP-12177-mediated activation of brown fat adenylyl cyclase in β_3-KO mice occurs through a receptor that is pharmacologically identical to recombinant β_1-AR. This receptor phenotype is absent in β_1-KO mice, indicating that the observed agonist effects of CGP-12177 are mediated by the β_1-AR. Furthermore, studies in β_1- and β_1-/β_2-KO mice showed an obligatory role of the β_1-AR for putative β_4-AR effects (9). CGP-12177-induced effects have been shown to be

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coupled to activation of the G_s protein-adenylyl cyclase system (10). In brown adipose tissue, CGP-mediated induction of oxygen consumption has been shown (8, 23). All of these effects can be completely accounted for by the notion of an atypical state of the β_1-AR.

To further dissect molecular and functional aspects of this atypical receptor state from the atypical β_3-AR, we have created a brown adipocyte cell line from β_3-KO mice. In the present report, we characterize this novel cell line and use it to study the novel CGP-12177-activated receptor state and the classical β_1- and β_2-ARs as potential candidates for the induction of insulin resistance. We demonstrate a multidirectional inhibitory role in cross talk with insulin.

MATERIALS AND METHODS

Materials. The partial β_3-receptor agonist CGP-12177 was purchased from Tocris Cookson (Bristol, UK), and the selective β_3-receptor agonist CL-316,243 was a generous gift of Dr. Kurt Steiner (Wyeth-Ayerst Research, Princeton, NJ). Adenosine deaminase was obtained from Roche Molecular Biochemicals (Mannheim, Germany). Antibodies used for immunoprecipitation and immunoblotting included anti-insulin receptor substrate (IRS)-1, anti-IRS-2, and anti-phosphatidylinositol (PI) 3-kinase p85 (Upstate Biotechnology, Lake Placid, NY), anti-β_3-AR and anti-UCP-1 (Alpha Diagnostic International, San Antonio, TX), anti-phosphospecific Ser^{473}-PKB, anti-phosphospecific mitogen-activated protein (MAP) kinase (New England Biolabs, Beverly, MA), and anti-phosphotyrosine (Transduction Laboratories, Lexington, KY). Peroxidase-coupled antibodies came from Dako (Glostrup, Denmark); recombinant IRS-1 was from Upstate Biotechnology; ^{125}I-labeled Tyr-A^{14}-monoiodoinsulin was purchased from Amersham-Pharmacia (Freiburg, Germany); and [γ-^{32}P]ATP, 2-deoxy-[^{3}H]glucose, and D-[1-^{14}C]glucose were from NEN Life Technologies (Dreieich, Germany). Protein G-Sepharose was from ImmunoPure (Pierce, Rockford, IL), and nitrocellulose was from Schleicher & Schuell, (Dassel, Germany). All other materials were from Sigma (Deisenhofen, Germany).

Cell isolation and culture. Brown adipocytes and their precursor cells were isolated from newborn β_3-AR KO mice by collagenase digestion as described previously (16). Briefly, preadipocytes were immortalized by infection with the retroviral vector pBabe, encoding SV40 T antigen (kindly provided by J. DeCaprio, Dana Farber Cancer Institute, Boston, MA) and selected with puromycin (1 μg/ml). Preadipocytes were grown to confluence in culture medium supplemented with 20 nM insulin and 1 nM triiodothyronine (T_3) (differentiation medium). Adipocyte differentiation was induced by treating confluent cells for 24 h in differentiation medium further supplemented with 0.5 mM isobutylmethylxanthine, 0.5 μM dexamethasone, and 0.125 mM indomethacin. After this induction period (24 h), cells were changed back to differentiation medium for 4–5 days until exhibiting a fully differentiated phenotype with massive accumulation of multilocular fat droplets. The different stimulation experiments were carried out after the cells were starved in serum-free medium for 48 h and pretreatment with adenosine deaminase (ADA; 2 U/ml).

Western blot analysis. Western blotting was performed as previously described (4). Briefly, after the stimulation period, cells were washed twice with ice-cold PBS and harvested in lysis buffer (in mM: 50 HEPES, 137 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 $Na_4P_2O_7$, 10 NaF, 2 EDTA, and 2 vanadate, and 10% glycerol, 1% Igepal CA-630, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 2 mM phenylmethylsulfonyl fluoride, pH 7.4). Lysates were clarified by centrifugation at 12,000 rpm for 10 min at 4°C. Protein content was determined by the method of Bradford (1). Equal amounts of protein (100 and 500 μg, respectively) were either solubilized directly in Laemmli sample buffer or immunoprecipitated for ≥2 h at 4°C with the indicated antibodies. Immunocomplexes were collected by adding 50 μl of protein G-Sepharose for 2 h at 4°C, washed in lysis buffer, and solubilized in Laemmli sample buffer. Proteins were resolved by SDS-PAGE, transferred to nitrocellulose membranes, blocked for 1 h, and immunoblotted with the appropriate antibodies for 2 h. Specifically bound primary antibodies were detected with peroxidase-coupled secondary antibody and enhanced chemiluminescence.

Insulin receptor kinase and binding activities. Insulin receptor binding and kinase activity were measured essentially as described (13). Briefly, 40 μl of cell lysate were added to microwells coated with anti-insulin receptor antibodies for 16 h at 4°C. Wells were washed, and receptor-mediated ^{32}P incorporation into recombinant IRS-1 was measured at a concentration of 240 nmol/l [γ-^{32}P]ATP and 2.3 μg/ml recombinant IRS-1. Insulin receptor kinase activity was expressed as attomoles ^{32}P incorporated into IRS-1 per minute per femtomole insulin-binding capacity (14). Furthermore, [^{125}I]Tyr-A^{14}-monoiodoinsulin binding to immobilized insulin receptors was also measured in the wells as described. Insulin-binding capacity was defined as the amount of specifically bound insulin at a concentration of 8.7 nmol/l (14).

Glucose uptake assay. Cells were assayed for glucose uptake essentially as described (5). After a serum-free starvation period of 48 h and pretreatment with ADA (2 U/ml), fully differentiated monolayers of brown adipocytes were treated with insulin (100 nM) for 30 min. At the end of the stimulation period, cells were exposed to 50 μl of 2-deoxy-[^{3}H]glucose (0.5 μCi/ml final concentration) for 4 min, and the incorporated radioactivity was determined by liquid scintillation counting. Nonspecific uptake was measured in the presence of 10 μM cytochalasin B and subtracted from all measured values.

Glycogen synthesis assay. Fully differentiated monolayers of brown adipocytes were starved in serum-free medium for 48 h, washed two times with Krebs-Ringer-HEPES buffer, and then pretreated with ADA (2 U/ml). After the pretreatment period with the indicated agonists and antagonists, insulin (100 nM) was added for 30 min. Cells were exposed for 1 h to 50 μl of D-[1-^{14}C]glucose (0.2 μCi/ml final concentration) and then washed two times with ice-cold PBS. After an incubation with 30% KOH for 15 min at 37°C, lysates were transferred to new vials prefilled with 50 μl of glycogen (40 mg/ml), and samples were boiled for 30 min. After addition of 95% ethanol, samples were centrifuged for 5 min at 13,000 rpm. The supernatant was removed and the pellet redissolved in 500 μl of distilled water. The incorporated radioactivity was determined by liquid scintillation counting.

Statistical analysis. Results are indicated as means ± SE. Unpaired Student's *t*-tests were used for analysis of differences between various cell treatments. P values <0.05 are considered significant and <0.01 highly significant.

RESULTS

β_3-KO adipocytes can be fully differentiated and are insulin sensitive. As expected, immunoblot analyses failed to detect the β_3-AR in brown preadipocytes derived from the β_3-KO mouse (Fig. 1*A*). By use of a differentiation protocol with triiodothyronine, insulin,

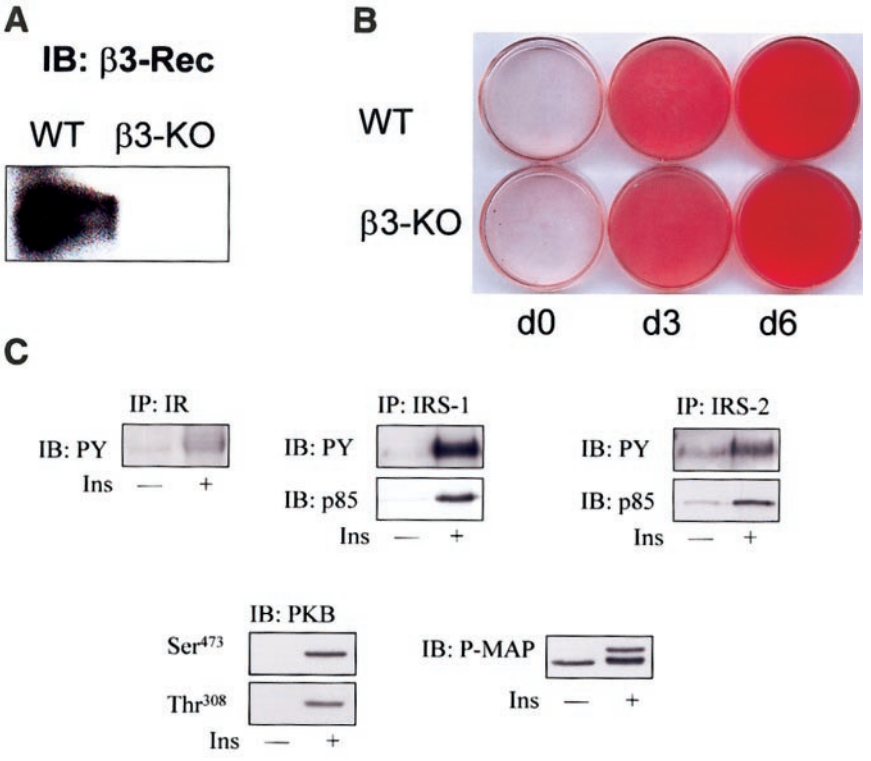

Fig. 1. β_3-Adrenergic receptor (β_3-AR)-deficient brown adipocytes differentiate like wild-type (WT) control cells and are insulin sensitive. β_3-knockout (KO) adipocytes were prepared and cultured as described in MATERIALS AND METHODS. Immunoblots using a mouse β_3-AR-specific antibody (β_3-Rec) were prepared from lysates of β_3-KO and WT control cells differentiated for 6 days after induction with dexamethasone, indomethacin, and isobutylmethylxanthine (*A*). At the indicated days of differentiation (d), cells were stained with Oil red O (*B*). After serum starvation for 48 h, cells were stimulated with insulin (Ins; 100 nM) for 5 min. Tyrosine phosphorylation (PY) of the insulin receptor (IR) and IR substrate (IRS)-1/2, IRS-1/2 binding to the regulatory subunit p85 of phosphatidylinositol (PI) 3-kinase (p85), and the phosphorylation of Ser^{473}/Thr^{308}-protein kinase B (PKB) and of mitogen-activated protein (MAP) kinase (P-MAP) were measured by immunoprecipitation (IP) and -blotting (IB) with the indicated antibodies. A representative blot of ≥2 independent experiments is shown (*C*).

dexamethasone, indomethacin, and isobutylmethylxanthine, both wild-type and β_3-KO cells massively accumulated fat in typical multilocular droplets over a period of 10 days, which was demonstrated by the fat-specific Oil red O stain (Fig. 1*B*). No major difference was noted between wild-type and β_3-KO cells with regard to kinetics and extent of maximal differentiation. Insulin stimulation of fully differentiated β_3-KO cells demonstrated a remarkable increase in tyrosine phosphorylation of the insulin receptor, IRS-1, and IRS-2 (Fig. 1*C*). Insulin also induced a robust augmentation in binding of the regulatory PI 3-kinase subunit p85 to IRS-1 and IRS-2 (Fig. 1*C*). Finally, a strong insulin-induced activation of the major downstream signaling intermediates PKB and MAP kinase was found in differentiated β_3-KO cells, thus demonstrating a high level of insulin sensitivity in these cells (Fig. 1*C*).

CGP-12177 induces UCP-1 protein expression in β_3-KO adipocytes. Fully differentiated β_3-AR-deficient brown adipocytes were stimulated with 10 μM partial β_3-AR agonist CGP-12177 for 18 h. Western blot analysis with a UCP-1-specific antibody showed a fivefold increase of UCP-1 protein levels (Fig. 2). In contrast, the β_3-selective agonist CL-316,243 had no effect on basal UCP-1 protein expression (Fig. 2). The observed CGP-12177 effect was dose dependent, with small increases detectable at concentrations as low as 1 nM (Fig. 2).

CGP-12177 reduces insulin-induced glucose uptake and glycogen synthesis independently of β_1- and β_2-ARs. Glucose uptake and glycogen synthesis represent two important functional end points of the insulin-signaling cascade. Insulin treatment (100 nM) stimulated a 6.5-fold increase in basal glucose uptake and an 11-fold increase in glycogen synthesis in differentiated β_3-KO adipocytes (Fig. 3, *A* and *B*). When cells were pretreated with CGP-12177 at a concentration of 1 μM 30 min before insulin stimulation, the insulin-induced glucose uptake was reduced by 65% ($P < 0.01$; Fig. 3*A*). Similarly, insulin-induced glycogen synthesis was decreased by 50% ($P < 0.01$; Fig. 3*B*). These effects were dose responsive, with decreases already detectable at a CGP-12177 concentration of 10 nM (Fig. 3, *A* and *B*).

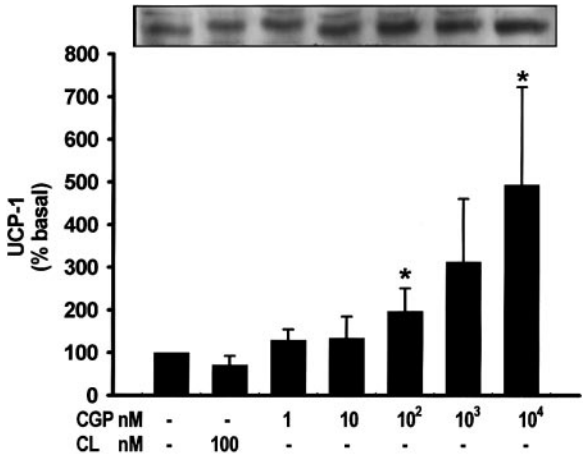

Fig. 2. Uncoupling protein-1 (UCP-1) protein expression is induced by CGP-12177 in β_3-KO brown adipocytes. Cells were stimulated with CL-316,243 (CL; 100 nM) or CGP-12177 (CGP; 1–10,000 nM) for 18 h. Whole cell lysates were immunoblotted with a UCP-1-specific antibody as described in MATERIALS AND METHODS. A representative blot and the statistical analysis of 3 independent experiments (means ± SE) are shown. *Statistical significance, $P < 0.05$.

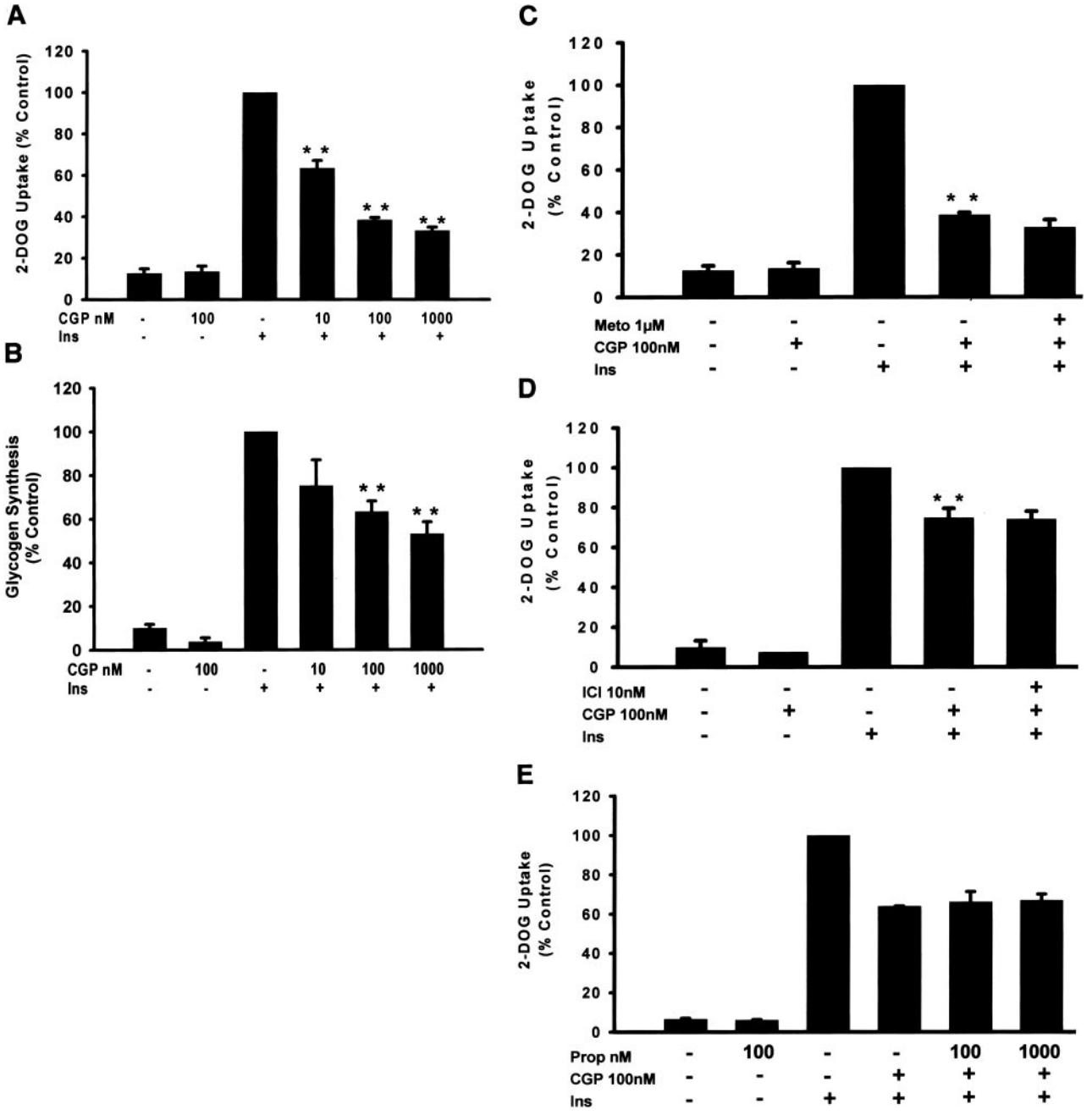

Fig. 3. Inhibition of insulin-induced 2-deoxyglucose (2-DOG) uptake and glycogen synthesis by pretreatment with CGP-12177. Brown adipocytes were pretreated for 30 min with the indicated concentrations of CGP in the absence (*A* and *B*) or presence of the indicated concentrations of metoprolol (Meto; *C*), ICI-118,551 (ICI; *D*), and propranolol (Prop; *E*) before Ins (100 nM) was added for another 30 min. 2-DOG uptake (*A, C, D, E*) and glycogen synthesis (*B*) were assayed as described in MATERIALS AND METHODS. All experiments were carried out in the presence of adenosine deaminase (ADA; 2 U/ml). 2-DOG uptake and glycogen synthesis are presented as %maximal response obtained with 100 nM insulin (100%). The statistical analysis of 3–4 independent experiments (except for *E*, $n = 2$) (means ± SE) is shown. **High statistical significance, $P < 0.01$.

To exclude the possibility that the observed negative CGP-12177 effects on insulin-induced glucose uptake in β_3-KO adipocytes are mediated via the classical β_1- or β_2-ARs, cells were pretreated with selective antagonists for 30 min before CGP-12177 stimulation (100 nM). Neither the selective β_1-antagonist metoprolol (1 μM) nor the selective β_2-antagonist ICI-118,551 (10 nM) could block the negative effect of CGP-12177 (Fig. 3, *C* and *D*). Additionally, pretreatment with the nonselective β-AR antagonist propranolol at concentrations of 100 nM and 1 μM did not alter the observed inhibitory effects of CGP-12177 (Fig. 3*E*).

Dobutamine and clenbuterol inhibit insulin-induced glucose uptake via β_1- and β_2-ARs. Furthermore, we determined whether activation of the classical β_1- and β_2-AR also inhibits insulin-induced glucose uptake.

When β_3-KO adipocytes were pretreated for 30 min with the β_1-AR agonist dobutamine (1 μM) or the β_2-AR agonist clenbuterol (10 nM), insulin-induced glucose uptake was diminished by maximally 70% and 85%, respectively ($P < 0.01$; Fig. 4, *A* and *B*). As expected, and in contrast to CGP-12177 treatment, the β_1-AR-mediated negative effects of dobutamine were diminished by pretreatment of cells with the selective β_1-antagonist metoprolol in a dose-dependent manner and could be almost completely abolished at metoprolol concentrations of 1 μM ($P < 0.01$; Fig. 4*C*). Similarly, the β_2-AR-mediated effects could be fully blocked by pretreatment with the selective β_2-antagonist ICI-118,551 at concentrations as low as 10 nM (Fig. 4*D*).

CGP-12177 impairs insulin-stimulated binding of IRS-1 and IRS-2 to PI 3-kinase and PKB activation. Because the observed reductions in insulin-induced glucose uptake and glycogen synthesis by CGP-12177 stimulation might be caused by alterations at a proximal level of insulin signaling, we first measured the activity of the insulin receptor kinase toward IRS-1. Insulin stimulation induced an 11-fold increase in receptor kinase activity (Fig. 5*A*). However, this increase was not altered by CGP-12177 pretreatment (Fig. 5*A*). Furthermore, insulin binding to its receptor showed no difference between CGP-12177-treated and nontreated cells (data not shown). To investigate whether CGP-12177 treatment impairs the insulin-signaling cascade distal from the insulin receptor, tyrosine phosphorylation of IRS-1 and IRS-2, IRS-1/2 binding to the p85 regulatory subunit of PI 3-kinase and the phosphorylation of Ser^{473}-PKB and MAP kinase were measured. CGP-12177 pretreatment tended to diminish insulin-induced tyrosine phosphorylation of IRS-1 and IRS-2 by 10%, but this change did not reach the level of statistical significance (data not shown). Furthermore, insulin-induced binding of p85 to IRS-1 and IRS-2 was reduced by ~25 and 35%, respectively, after CGP-12177 (1 μM) pretreatment ($P < 0.05$; Fig. 5, *B* and *C*). Moreover, CGP-12177 treatment led to a small but highly significant decrease in insulin-mediated Ser^{473}-PKB phosphorylation by 20% ($P < 0.01$; Fig. 5*D*),

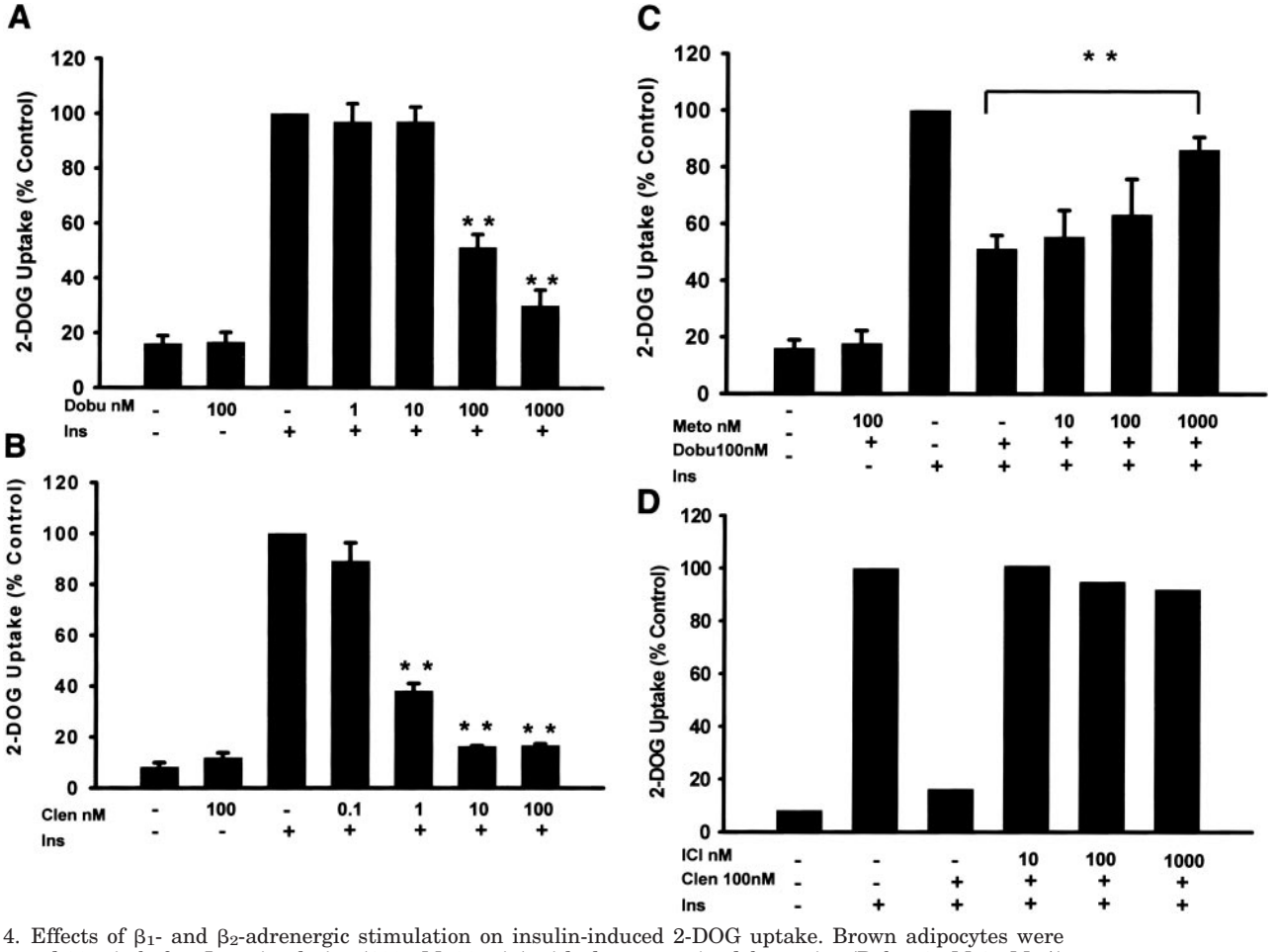

Fig. 4. Effects of β_1- and β_2-adrenergic stimulation on insulin-induced 2-DOG uptake. Brown adipocytes were pretreated 30 min before Ins stimulation (100 nM, 30 min) with the β_1-agonist dobutamine (Dobu; 1 nM-1 μM; *A*) and the β_2-agonist clenbuterol (Clen; 100 pM-100 nM; *B*) in the presence or absence of the β_1-antagonist Meto (10 nM–1 μM; *C*) and the β_2-antagonist ICI (10 nM-1 μM; *D*), respectively. All experiments were carried out in the presence of ADA (2 U/ml). 2-DOG uptake is presented as %maximal response obtained with 100 nM Ins (100%). The statistical analysis of 3–4 independent experiments (except for *D*, $n = 1$) (means ± SE) is shown. **High statistical significance, $P < 0.01$.

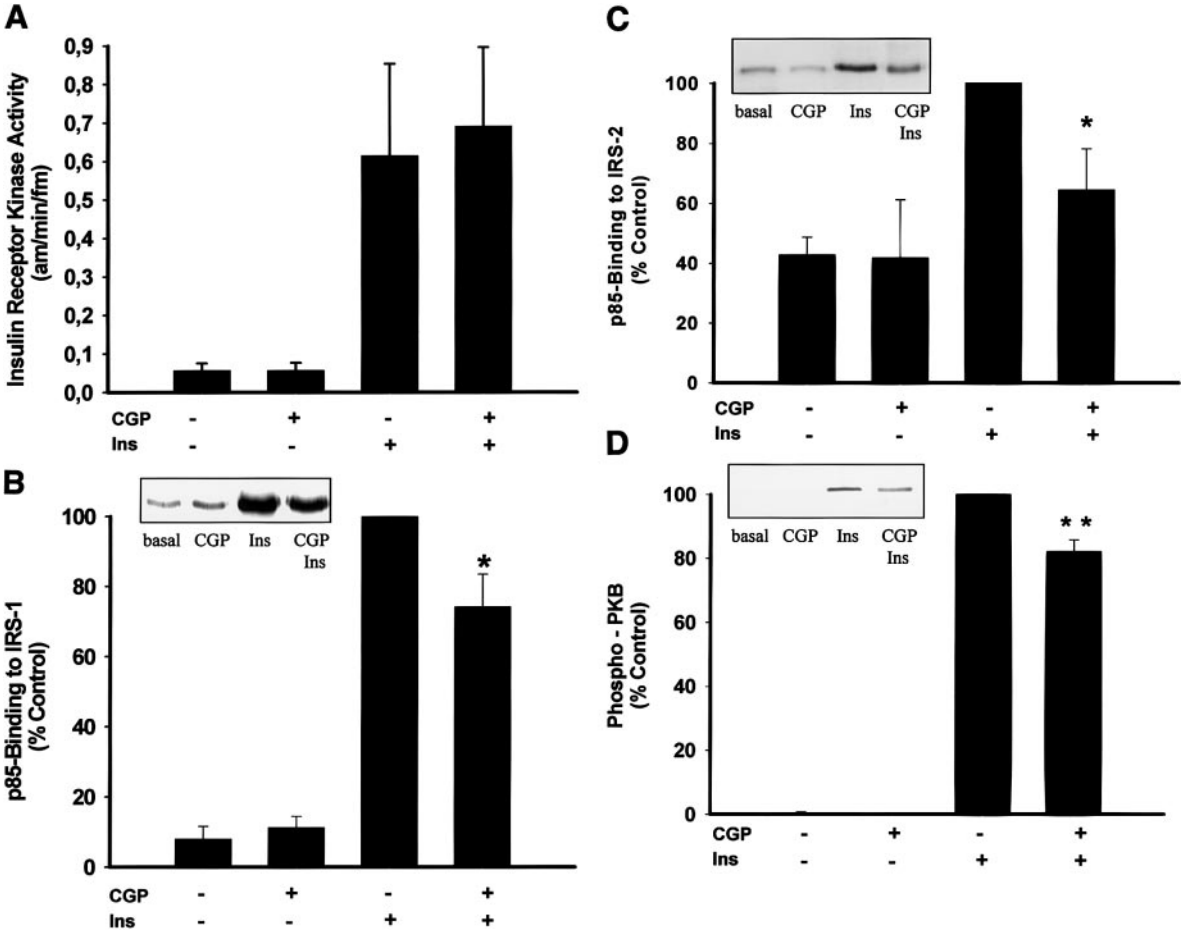

Fig. 5. Effects of CGP on the Ins signaling cascade. Brown adipocytes were pretreated 30 min before Ins stimulation (100 nM, 5 min) with CGP (1 μM). Ins receptor kinase activity was assayed as described in MATERIALS AND METHODS (*A*). IRS-1/2 binding to the p85 regulatory subunit of PI 3-kinase (*B* and *C*) and the phosphorylation of Ser^{473}-PKB (*D*) were measured by immunoprecipitation and -blotting with the indicated antibodies. All experiments were carried out in the presence of ADA (2 U/ml). Results of *B, C,* and *D* are presented as %maximal response obtained with 100 nM Ins (100%). The statistical analysis of 3–4 independent experiments (means ± SE) is shown. *Statistical significance, $P < 0.05$; **high statistical significance, $P < 0.01$.

whereas the phosphorylation of MAP kinase was not impaired (data not shown).

DISCUSSION

In this study, we characterize a newly established brown adipocyte line derived from the β_3-AR knockout mouse and use it to dissect a potent inhibitory role of the atypical β_1-AR state and the classical β_1- and β_2-AR in the interplay with insulin signaling and action.

We have previously described an adipocyte cell system that allows for the generation of cell lines from a single, late fetal or newborn mouse from a broad variety of different mouse models, including those with perinatal mortality (4, 5, 15, 16). We have now used the same approach to establish SV40T-immortalized brown adipocytes from the β_3-AR knockout mouse (27). As we demonstrate here, immortalized β_3-KO brown adipocytes differentiate normally and are highly insulin sensitive. Therefore, the β_3-KO adipocytes provide an ideal tool for separating biological effects of the novel CGP-12177-activated β_1-AR state from β_3-adrenergic effects and for characterizing its role in cross talk with insulin.

As expected, the highly selective β_3-AR agonist CL-316,243 fails to induce UCP-1 protein expression in the β_3-AR-deficient cell line, confirming the absence of the β_3-AR (27). The aryloxypropanolamine CGP-12177 has been used in previous studies to infer the existence of a novel atypical β-AR. Although the actions of CGP-12177 at the respective receptor site are incompletely understood, a number of studies provide compelling evidence for the notion of a novel state of the β_1-AR mediating this compound's effects (17, 18, 22). We show that, in contrast to CL-316,243, CGP-12177 induces UCP-1 protein expression in a dose-dependent manner in the β_3-KO cells. This most likely provides the molecular basis for previous findings demonstrating an increase in oxygen consumption in adipose tissue of β_3-KO mice after CGP-12177 treatment (8, 23).

Recently, we have delineated cross talk between the sympathetic nervous system and the insulin signaling

cascade. Thus, on the one hand, β_3-adrenergic stimulation with the selective β_3-AR phenethanolamine agonist CL-316,243 inhibits the insulin signaling cascade on several levels and results in a reduction of insulin-induced glucose uptake (16). On the other hand, insulin impacts on β-adrenergic regulation of UCP-1 expression (15). This cross talk between adrenergic and insulin signaling systems may play an important role in the pathogenesis of insulin resistance and the insulin resistance syndrome. We now compared effects of CGP-12177 on important biological end points of insulin action to those of classical β_1- and β_2-AR agonists and antagonists. When cells were pretreated with the β_1-AR agonist dobutamine, insulin-induced glucose uptake was almost completely abolished. These negative effects could be blocked by pretreatment with the classical selective β_1-AR antagonist metoprolol, indicating that the observed inhibition is mediated via the β_1-AR. In parallel, β_2-adrenergic stimulation with clenbuterol dramatically decreased insulin-induced glucose uptake, and this inhibition could be completely abolished by pretreatment with the selective β_2-AR antagonist ICI-118,551. The negative CGP-12177 effects on insulin action were similar in their extent compared with β_1- and β_2-AR agonists, but in contrast to these classical β-ARs, the CGP-12177-mediated inhibition of insulin action in the β_3-KO adipocytes could be blocked neither by selective β_1- and β_2-antagonists nor by nonselective β-blockade, thus fulfilling the functional criteria for an atypical β-adrenoceptor, i.e., *1*) stimulation by CGP-12177, *2*) resistance to β-blockade, and *3*) lack of activation by β_3-selective agonists like CL-316,243. This can be taken to indicate a concurrent negative role of both the novel β_1-AR state and the classical β_1- and β_2-ARs in controlling insulin action with respect to both glucose uptake and glycogen synthesis. Our study characterizes for the first time the role of this novel atypical receptor state and of the classical β_1- and β_2-AR in the interplay with insulin action in brown adipocytes. It has to be noted, however, that actions of CGP-12177 at the β_1-AR site are incompletely understood. Due to the present lack of detailed information on the molecular properties of the novel β_1-AR state, conclusions as to its effects using this compound remain indirect.

The observed reduction of insulin-induced glucose uptake by conventional β_1-, β_2-, and the novel atypical β_1-receptor state is similar to the one seen after β_3-adrenergic stimulation in brown adipocytes from wild-type mice (16). In line with these previous findings, it appears safe to assume a signaling pathway from a stimulatory β-adrenergic G protein subunit that activates adenylyl cyclase and protein kinase A to exert the negative effect on both glucose uptake and, as shown here for the first time, glycogen synthesis. Konkar et al. (17) found that CGP-12177 activated brown fat adenylyl cyclase in β_3-KO mice. Furthermore, increases of intracellular cAMP levels, enhanced activity of cAMP-dependent protein kinase, and potentiation of effects by the phosphodiesterase inhibitor isobutylmethylxanthine have been demonstrated for stimulation with CGP-12177 (10). Yet, interestingly, in our study, the insulin receptor kinase activity and the insulin-binding capacity were not impaired after CGP-12177 stimulation, whereas p85-binding to IRS-1 and IRS-2 was diminished. These findings may indicate a negative regulation of the novel β_1-AR state on an insulin receptor-independent proximal level of the insulin signaling cascade. This appears to be in contrast to the β_3-AR, where reduced tyrosine phosphorylation of IRS-1/2 and the associated PI 3-kinase activity were correlated with decreased phosphorylation of the insulin receptor (16). Thus the novel atypical adrenoceptor may employ different signaling pathways to interact with insulin action. Potential signaling candidates could be protein kinase C- and p38 MAP kinase-dependent pathways. In line with this concept, qualitatively different effects of different conformational β_1-AR states in mouse cardiac muscle have been suggested (9). In this context, it should also be mentioned that switching from G_s to G_i protein coupling of receptors, depending on specific receptor phosphorylation sites, has been described for the β_2-AR (2). This could well be another intriguing signaling mechanism of the novel β_1-AR state. Nevertheless, similar to the β_3-AR, a relatively small inhibition of PKB, a key element for metabolic insulin signaling, by the novel-state β_1-AR appears to be sufficient to cause a considerable inhibition of glucose uptake and glycogen synthesis.

Taken together, the newly established β_3-KO brown adipocyte cell line provides an ideal tool for investigating adrenoceptor-insulin cross talk and its impact on adipose tissue insulin resistance. The novel state β_1-AR in β_3-KO brown adipocytes increases UCP-1 protein expression and reduces insulin-induced glucose uptake and glycogen synthesis by postreceptor alterations of proximal insulin signaling elements. Stimulation of classical β_1- and β_2-AR results in the same negative effects on insulin action. These interactions may play a role in the development of the insulin resistance syndrome.

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