1521-0111/12/8204-668–678$25.00
MOLECULAR PHARMACOLOGY
Vol. 82, No. 4
78709/3794320

Mol Pharmacol 82:668–678, 2012

D_2-Dopaminergic Receptor-Linked Pathways: Critical Regulators of CYP3A, CYP2C, and CYP2D[S]

Evangelos P. Daskalopoulos, Matti A. Lang, Marios Marselos, Foteini Malliou, and Maria Konstandi

Department of Pharmacology, School of Medicine, University of Ioannina, Ioannina, Greece (E.P.D., M.M., F.M., M.K.); National Research Centre for Environmental Toxicology, University of Queensland, Coopers Plains, Australia (M.A.L.); and Department of Biochemistry, University of Uppsala, Uppsala, Sweden (M.A.L.)

Received March 9, 2012; accepted July 6, 2012

ABSTRACT

Various hormonal and monoaminergic systems play determinant roles in the regulation of several cytochromes P450 (P450s) in the liver. Growth hormone (GH), prolactin, and insulin are involved in P450 regulation, and their release is under dopaminergic control. This study focused on the role of D_2-dopaminergic systems in the regulation of the major drug-metabolizing P450s, i.e., CYP3A, CYP2C, and CYP2D. Blockade of D_2-dopaminergic receptors with either sulpiride (SULP) or 4-(4-chlorophenyl)-1-(1*H*-indol-3-ylmethyl)piperidin-4-ol (L-741,626) markedly down-regulated CYP3A1/2, CYP2C11, and CYP2D1 expression in rat liver. This suppressive effect appeared to be mediated by the insulin/phosphatidylinositol 3-kinase/Akt/FOXO1 signaling pathway. Furthermore, inactivation of the GH/STAT5b signaling pathway appeared to play a role in D_2-dopaminergic receptor-mediated down-regulating effects on these P450s. SULP suppressed plasma GH levels, with subsequently reduced activation of STAT5b, which is the major GH pulse-activated transcription factor and has up-regulating effects on various P450s in hepatic tissue. Levels of prolactin, which exerts down-regulating control on P450s, were increased by SULP, which may contribute to SULP-mediated effects. Finally, it appears that SULP-induced inactivation of the cAMP/protein kinase A/cAMP-response element-binding protein signaling pathway, which is a critical regulator of pregnane X receptor and hepatocyte nuclear factor 1α, and inactivation of the c-Jun N-terminal kinase contribute to SULP-induced down-regulation of the aforementioned P450s. Taken together, the present data provide evidence that drugs acting as D_2-dopaminergic receptor antagonists might interfere with several major signaling pathways involved in the regulation of CYP3A, CYP2C, and CYP2D, which are critical enzymes in drug metabolism, thus affecting the effectiveness of the majority of prescribed drugs and the toxicity and carcinogenic potency of a plethora of toxicants and carcinogens.

Introduction

Cytochromes P450 (P450s) play major roles in the hepatic metabolism of xenobiotics such as drugs, carcinogens, and toxic agents. Metabolism can activate prodrugs to pharmacologically more-active metabolites, inactivate pharmacologically active compounds, or activate precarcinogens, thus triggering the formation of several types of tumors in humans (Gonzalez and Gelboin, 1994; Ingelman-Sundberg, 2004). P450s also catalyze the biotransformation of endogenous compounds, such as steroids and fatty acids (Spatzenegger and Jaeger, 1995; Guengerich, 2003). Inhibition of P450 isoforms that metabolize clinically used drugs may lead to increased levels of drug substrate in the body and may cause

This work was supported by the European Social Fund and Greek Ministry of Development Reinforcement Programme of Human Research Manpower [Grant 03EΔ/957] and by the European Union European Regional Development Fund and Greek national funds through the Thessaly-Mainland Greece and Epirus 2007–2013 Operational Program of the National Strategic Reference Framework [Grant 346985/80753].

This work was previously presented in part in the following publication: Daskalopoulos EP (2012) *Investigation of the Role of the Adrenergic and Dopaminergic Systems in the Regulation of the Cytochromes CYP3A1/2, CYP2C11 and CYP2D1/2*. Doctoral dissertation, University of Ioannina, Ioannina, Greece.

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.

http://dx.doi.org/10.1124/mol.112.078709.

[S] The online version of this article (available at http://molpharm.aspetjournals.org) contains supplemental material.

ABBREVIATIONS: P450, cytochrome P450; ACN, acetonitrile; CAR, constitutive androstane receptor; HPLC, high-performance liquid chromatography; PKA, protein kinase A; PCR, polymerase chain reaction; RIA, radioimmunoassay; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; CREB, cAMP-response element-binding protein; GH, growth hormone; HNF, hepatocyte nuclear factor; INS, insulin; JNK, c-Jun N-terminal kinase; PI3K, phosphatidylinositol 3-kinase; PRL, prolactin; PXR, pregnane X receptor; RXR, retinoic X receptor; SULP, sulpiride; WORT, wortmannin; H-89, 5-isoquinolinesulfonamide; SP600125, anthra[1,9-cd]pyrazol-6(2*H*)-one; L-741,626, 4-(4-chlorophenyl)-1-(1*H*-indol-3-ylmethyl)piperidin-4-ol.

adverse effects, especially when drugs with low therapeutic indices are used (Spatzenegger and Jaeger, 1995). Conversely, up-regulation of critical enzymes may accelerate the metabolism of drug substrate, thus leading to reduced pharmacological outcomes for the drug (Konstandi et al., 2005; Daskalopoulos et al., 2012). It is important, particularly for multidrug therapies, to be aware of the possibilities of drug-drug interactions that might lead to reduced or increased drug efficacy, severe toxicity, and/or tumorigenesis (Gonzalez and Gelboin, 1994; Guengerich, 2003; Konstandi et al., 2004; Pelkonen et al., 2008).

Some of the most important P450 enzymes for hepatic phase I drug metabolism are members of the CYP3A, CYP2C, and CYP2D subfamilies (Ingelman-Sundberg, 2004). These cytochromes are responsible for the biotransformation of more than 90% of the most widely prescribed drugs (Guengerich, 2003).

Central and peripheral catecholaminergic systems have been determined to regulate P450 expression (Konstandi et al., 1998, 2004; Rendic and Guengerich, 2010; Daskalopoulos et al., 2012). In addition, previous studies clearly indicated critical roles for hormones, such as growth hormone (GH) (Waxman and O'Connor, 2006), thyroid hormones (Takahashi et al., 2010), and insulin (Kim and Novak, 2007), in the regulation of various hepatic P450s (Daskalopoulos et al., 2012).

Diabetic rats display increased hepatic CYP2A1, CYP2C6/7, CYP2E1, CYP3A2, and CYP4A3 expression, which can be restored to normal levels with insulin administration (Shimojo et al., 1993). Insulin exerts many of its effects through the PI3K/Akt signaling pathway, which regulates the expression of several genes, including various P450 isoforms (Kim and Novak, 2007). Among upstream signaling factors regulating the release of insulin in response to increased plasma glucose levels, dopamine plays a significant role; stimulation of D_2-dopaminergic receptors in pancreatic β-cells suppresses insulin release (Rubí et al., 2005). In accordance with this finding and other earlier studies, we showed that blockade of D_2-dopaminergic receptors stimulated insulin release and markedly down-regulated CYP2E1 expression (Konstandi et al., 2008). It is possible that the insulin/PI3K/Akt signaling pathway plays a determinant role in this regulation (Woodcroft et al., 2002).

A broad spectrum of drugs prescribed to treat a variety of disease states, including schizophrenia, bipolar disorder, depression, and Parkinson's disease, exert their effects mainly through D_2-dopaminergic receptors and related signaling pathways (Beaulieu and Gainetdinov, 2011). Given its central role in the regulation of insulin release, it is conceivable that drugs acting through this pathway could alter the expression of various drug-metabolizing P450 enzymes, thus affecting the pharmacodynamic characteristics and toxicity of drug substrates during pharmacotherapy (Gonzalez and Yu, 2006).

The aim of this study was to assess the role of D_2-dopaminergic receptor-linked signaling pathways in the regulation of the P450s CYP3A, CYP2C, and CYP2D. For this purpose, rats were treated with the D_2-dopaminergic receptor antagonist sulpiride (Konstandi et al., 2008), a typical antipsychotic drug that is used mainly for the treatment of psychosis associated with schizophrenia or major depressive disorder (Maitre et al., 1994). The direct effects of sulpiride on hepatocytes were assessed in vitro with primary hepatocyte cultures. Blockade of the D_2-dopaminergic receptor signaling pathways caused strong reductions in the expression of CYP3A, CYP2C, and CYP2D. Given the fact that the vast majority of prescribed drugs are metabolized by these P450s, their down-regulation might lead to strongly increased blood levels of drug substrates during multidrug therapy and to drug-drug interactions with potentially detrimental side effects.

Materials and Methods

Animals

All experimental animals used in this study (adult male Wistar Kuo/Io/rr rats, 2–3 months of age) were inbred in the animal facility of the University of Ioannina (Ioannina, Greece) and were housed in groups of three or four in plastic cages, with standard rodent chow and water available ad libitum. The animals were maintained on a 12-h light/dark cycle (lights on at 6:00 AM) and were adapted to handling over a period of 5 to 7 days before the experiment. All procedures involving animals were reviewed and approved by the local ethics committee, and they conformed to the European Commission ethical standards for the care and use of laboratory animals (Directive 86/609-EEC).

In Vivo Assessment of Roles of D_2-Dopaminergic Receptor-Related Pathways in Regulation of Hepatic Drug Metabolism

Control animals (group I) received normal saline solution administered subcutaneously, twice daily, for 4 consecutive days. Animals in group II received sulpiride (SULP) (2 μg/kg b.wt.; Sigma-Aldrich, St. Louis, MO) administered subcutaneously, twice daily, for 4 consecutive days. Rats in group III received the highly selective D_2-dopaminergic receptor antagonist 4-(4-chlorophenyl)-1-(1*H*-indol-3-ylmethyl)piperidin-4-ol (L-741,626) (PubChem SID 50104688; 1.5 mg/kg b.wt.; Sigma-Aldrich) administered intraperitoneally, twice daily, for 4 consecutive days. All animals were euthanized 1 h after the last injection.

Isolation of Microsomes

Microsomal fractions were prepared from liver samples, which were homogenized in ice-cold homogenization buffer (0.15 M KCl, 10 mM K_2EDTA, 1 mM dithiothreitol, pH 7.4). The homogenates were centrifuged for 20 min at 14,075*g* (4°C). The upper phase was transferred into new vials and centrifuged for 60 min at 96,552*g* (4°C). The microsomal pellet was resuspended in ice-cold homogenization buffer, homogenized, and centrifuged for 45 min at 96,552*g* (4°C). The washed microsomal pellet was resuspended in ice-cold storage buffer (K_2HPO_4/KH_2PO_4, pH 7.4, 1 mM K_2EDTA, 0.1 mM dithiothreitol, 20% glycerol) and stored at −80°C until used in assays (Lang et al., 1981).

Primary Hepatocyte Cultures

Primary hepatocytes were isolated and used in cultures according to the method described by Klaunig et al. (1981). In brief, primary hepatocytes were isolated from rats (250–300 g) by using a two-step collagenase perfusion method. The hepatocytes were suspended in Williams' medium E (Invitrogen, Carlsbad, CA) containing 1% L-glutamine and 1% penicillin/streptomycin. The cells were counted in a Neubauer cell chamber and were plated at a density of 1×10^5 cells per well in collagen type I-coated dishes (diameter, 3.8 cm; BioCoat cell environment; BD Biosciences, San Jose, CA). The viability of the isolated hepatocytes was checked through trypan blue dye (0.4%) exclusion, and only cell preparations with viability rates of more than 85% just before plating were used. Hepatocytes were cultured at 37°C for 24 h in an atmosphere of humidified 5% CO_2, which allowed them to adhere to the wells. Time- and dose-response experiments were initiated 24 h later. Primary hepatocyte cultures were

treated with either SULP (1–25 μM) or insulin (INS), at different doses ranging from 1 to 100 μM, in combination with wortmannin, an inhibitor of the PI3K/Akt signaling pathway. The cAMP/PKA inhibitor 5-isoquinolinesulfonamide (H-89) (10 μM, 24 h) and the JNK inhibitor anthra[1,9-*cd*]pyrazol-6(2*H*)-one (SP600125) (10 μM, 24 h) were added to the primary hepatocyte cultures 30 min before SULP. Time-response experiments were conducted with drug treatments of primary hepatocytes ranging between 4 and 36 h.

Enzyme Activity Assays

Bufuralol 1′-Hydroxylation. Bufuralol 1′-hydroxylation reflects CYP2D activity (Matsunaga et al., 1990). Liver microsomal protein (40 μg) was preincubated at 37°C for 5 min in a 200-μl reaction mixture containing potassium phosphate (100 mM, pH 7.4), in the presence of 50 μM bufuralol substrate. The reaction was initiated with NADPH (0.5 mM) at 37°C and lasted 7.5 min; it was terminated with 20 μl of perchloric acid (60%). After centrifugation for 10 min at 14,075*g*, the concentration of 1′-hydroxybufuralol (the main metabolite of bufuralol) in the supernatant (100 μl) was determined through HPLC, with fluorescence detection at 252 and 302 nm. A reverse-phase Luna C_{18} column (5 μm, 150 × 3 mm; Phenomenex, Torrance, CA) was used. The mobile phase contained a 30% acetonitrile (ACN)/70% perchlorate buffer (20 mM, pH 2.5) mixture, and the sample was eluted with a flow rate of 1 ml/min for 15 min.

The positive control reaction for bufuralol 1′-hydroxylation was performed by using recombinant rat CYP2D1 and CYP2D2 plus P450 reductase BD Supersomes (BD Gentest, Woburn, MA). Approximately 200 μl of potassium phosphate buffer (0.1 M, pH 7.4) containing the substrate bufuralol (50 μM) were preincubated at 37°C for 2 min. Recombinant P450 (50 μM) and NADPH (1 mM) were then added, and the mixture was incubated at 37°C for 30 min. The reaction was terminated with 20 μl of ACN; the samples were left undisturbed on wet ice for 10 min and then were injected into the HPLC system for analysis.

Testosterone Hydroxylation. CYP3A1/2, CYP2C11, and CYP2A1 catalyze testosterone hydroxylation in rat liver. The activities studied were CYP3A1/2-dependent 6β-testosterone hydroxylation and CYP2C11-dependent 2α- and 16α-testosterone hydroxylation (Murray et al., 2001). Liver microsomal proteins (1 mg) were incubated at 37°C for 7.5 min in 500 μl of a mixture containing potassium phosphate (50 mM, pH 7.4), in the presence of 200 μM testosterone (the substrate was diluted in 5 μl of ACN). The reaction was initiated with NADPH (1 mM) and was terminated through the addition of 2 ml of a chloroform/methanol (2:1) mixture at 4°C. All incubations were performed in conditions under which linearity with time was established. After extraction, centrifugation, and separation, the organic phase was removed and evaporated under nitrogen. The residues from evaporation were dissolved in 200 μl of ACN, and aliquots of 100 μl were injected into the HPLC system. Testosterone metabolites were resolved on a reverse-phase Zorbax C_{18} column (5 μm, 150 × 4.6 mm; Agilent Technologies, Santa Clara, CA), with elution for 30 min with mobile phase A, containing 10% ACN and 0.5% acetic acid (flow rate, 0.8 ml/min), followed by elution for 35 min with mobile phase B, containing 36% ACN and 0.5% acetic acid.

For the positive control for 6β-testosterone hydroxylation, rat CYP3A1 and CYP3A2 Supersomes containing P450 reductase and cytochrome b_5 (BD Gentest) were used. For the positive control for 16α-testosterone hydroxylation, rat CYP2C11 plus P450 reductase plus cytochrome b_5 Supersomes were used (BD Gentest). As described above, approximately 200 μl of potassium phosphate buffer (0.1 M, pH 7.4) containing the substrate testosterone (5 μM) were preincubated at 37°C for 2 min. The recombinant P450 (50 μM) and NADPH (1 mM) were then added, and the mixture was incubated at 37°C for 30 min. The reaction was terminated with 20 μl of ACN, the samples were left undisturbed on wet ice for 10 min, and the samples were stored at −20°C for 24 h before analysis.

Ketoconazole (Sigma-Aldrich), a CYP3A inhibitor, was used for the negative control for CYP3A-dependent 6β-testosterone hydroxylation. Sulfaphenazole (Sigma-Aldrich), a CYP2C11 inhibitor, was used for the negative control for CYP2C-dependent 2α- and16α-testosterone hydroxylation. In brief, 200 μl of potassium phosphate buffer (0.1 M, pH 7.4) containing testosterone as substrate (5 μM), the P450 inhibitor (1 μM), and microsomes (0.1 mg) were preincubated at 37°C for 2 min. NADPH (1 mM) was added, and the mixture was incubated at 37°C for 30 min (when sulfaphenazole was used as the inhibitor) or 5 min (when ketoconazole was used as the inhibitor). The reaction was terminated with 200 μl of ACN (sulfaphenazole) or 200 μl of methanol (ketoconazole), the samples were kept on wet ice for 5 min, and then the samples were centrifuged for 15 min at 100,000*g* (4°C). The supernatant was collected and maintained at −20°C for 24 h. The following day, the samples were centrifuged for 10 min at 10,000*g* (4°C), for protein precipitation, and were analyzed through HPLC.

Western Blot Analyses

Immunoblot analysis of P450, STAT5b, and FOXO1 apoprotein levels was performed by using microsomes and nuclear extracts and cytosol from liver samples, respectively. For the preparation of nuclear extracts and cytosol, a nuclear and cytosolic extraction kit (Thermo Fisher Scientific, Waltham, MA) was used. The levels of phosphorylated CREB, JNK, Akt, and p70S6K were determined through Western blot analysis of total cellular proteins, which were extracted from the liver by using RIPA buffer supplemented with protease inhibitors, phenylmethylsulfonyl fluoride (10 μM), β-glycerophosphate (50 μM), and NaF (50 μM). Protein concentrations were determined with the bovine serum albumin assay (Thermo Fisher Scientific). Proteins were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting with the following antibodies: rat polyclonal CYP3A1-, CYP3A2-, CYP2C11-, and CYP2D1-specific IgGs, mouse monoclonal total STAT5a/b-specific

TABLE 1
Oligonucleotide sequences used as primers for quantitation of gene mRNA levels through quantitative PCR assays

Gene	Primer Sequence
CYP3A1	
Forward	5′-GGAAATTCGATGTGGAGTGC-3′
Reverse	5′-AGGTTTGCCTTTCTCTTGCC-3′
CYP3A2	
Forward	5′-GTCAAACGCCTGTGTTTGCC-3′
Reverse	5′-ATCAGGGTGAGTGGCCAGGA-3′
CYP2D1	
Forward	5′-TGGACCTCAGTAACATGCCA-3′
Reverse	5′-GATGCAAGGATCACACCTTG-3′
CYP2D2	
Forward	5′-GGTGGACTTTGAGAACATGC-3′
Reverse	5′-TTGCATCTCTGCTAGGAAGG-3′
CYP2C11	
Forward	5′-AGGACATCGGCCAATCAA-3′
Reverse	5′-GGGTAAACTCAGACTGCGGA-3′
CAR	
Forward	5′-CAGGCCTCCGGCCTACCTGT-3′
Reverse	5′-CCCTACCCACTCCCTGCCCC-3′
PXR	
Forward	5′-GAGCTCTGGGCAGAAACATC-3′
Reverse	5′-ACACGGCAGATTTGAAGACC-3′
RXR	
Forward	5′-TCAATGGCGTCCTCAAGGTTC-3′
Reverse	5′-TGTCACGGCAGGTGTAGGTCAG-3′
HNF1α	
Forward	5′-GGGAAGACTTCGCGCCACCC-3′
Reverse	5′-CCTCTCGCTGCTTGCGGACG-3′
HNF4α	
Forward	5′-GCCCAAAAACATGCGCTGAG-3′
Reverse	5′-GCAGATGGTTGTCCTTTAGG-3′
eNOS	
Forward	5′-CTGCTGCCCGAGATATCTTC-3′
Reverse	5′-CAGGTACTGCAGTCCCTCCT-3′
iNOS	
Forward	5′-CGTGTGCCTGCTGCCTTCCTGCTGT-3′
Reverse	5′-GTAATCCTCAACCTGCTCCTCACTC-3′

IgG (Santa Cruz Biotechnology, Santa Cruz, CA), and rabbit monoclonal phosphorylated (Tyr694) STAT5b-specific IgG (Cell Signaling Technology, Danvers, MA). Rabbit polyclonal phosphorylated (Ser133) CREB-1-specific IgG, rabbit polyclonal total JNK2-specific IgG, mouse monoclonal phosphorylated (Thr183 and Tyr185) JNK-specific IgG (Santa Cruz Biotechnology), rabbit polyclonal phosphorylated (Thr389) p70S6K- and total p70S6K-specific IgGs (Cell Signaling Technology), rabbit polyclonal phosphorylated (Ser256) FOXO1- and total FOXO1-specific IgGs (Santa Cruz Biotechnology), and rabbit polyclonal phosphorylated (Ser473) Akt- and total Akt-specific IgGs (Santa Cruz Biotechnology) were also used. Secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology) were used, and proteins were detected by using an enhanced chemiluminescence detection kit (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). Immunoblotting with glyceraldehyde 3-phosphate dehydrogenase- or β-actin-specific antibody (Santa Cruz Biotechnology) and anti-mouse IgG horseradish peroxidase-conjugated secondary antibody was used as a loading control.

Quantitative Real-Time Polymerase Chain Reaction Assays

For the isolation of total RNA from liver tissue and primary hepatocytes, the TRIzol reagent (Invitrogen) was used according to the manufacturer's protocol. The concentration of total RNA was determined with a spectrophotometric method. Quantitative, real-time, reverse transcription-polymerase chain reaction (PCR) assays were performed with cDNA generated from 1 μg of total RNA with a SuperScript II reverse transcriptase kit (Invitrogen). The sequences for the forward and reverse gene-specific primers used are shown in Table 1. SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) was used for the real-time reactions, which were performed by using a C1000 Touch thermal cycler with a real-time detection system (Bio-Rad Laboratories, Hercules, CA). Relative mRNA expression levels were normalized to β-actin levels (QuantiTect primer assay; QIAGEN, Valencia, CA), and values were quantified by using the comparative threshold cycle method.

Hormone Level Determinations

Serum corticosterone concentrations were measured by using a corticosterone RIA kit (Coat-A-Count kit; Diagnostic Products, Los Angeles, CA). The detection limit was approximately 5.7 ng/ml, and the intra-assay coefficient of variation was 4.0%. GH serum levels were assessed with a rat growth hormone RIA kit (Millipore Corp., Billerica, MA). The detection limit was 0.5 ng/ml, and the intra-assay coefficient of variation was 10%. PRL serum levels were measured with a rat prolactin RIA kit (MP Biomedicals, Solon, OH), and the detection limit was 0.5 ng/ml. Serum thyroid hormone concentrations were measured with triiodothyronine, thyroxin, and thyroid-stimulating hormone kits (Dynatest, Brahms, Germany); the normal ranges were 80 to 200 ng/dl for triiodothyronine levels, 4.5 to 12 μg/dl for thyroxin levels, and 0.4 to 4 mg/ml for thyroid-stimulating hormone levels. Insulin levels were measured with a rat enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden). The detection limit was 3.3 ng/ml, and the intra-assay coefficient of variation was 3.1%. Blood glucose levels were measured with a commercially available kit (Merck, Darmstadt, Germany), by using the glucose oxidase technique (Trinder, 1969).

Statistical Analyses

Data were analyzed by using one-way analysis of variance followed by multiple comparisons with Bonferroni's and Tukey's least honest significant difference methods, and results are presented as mean ± S.E. In all cases, p values of ≤0.05 were considered significant.

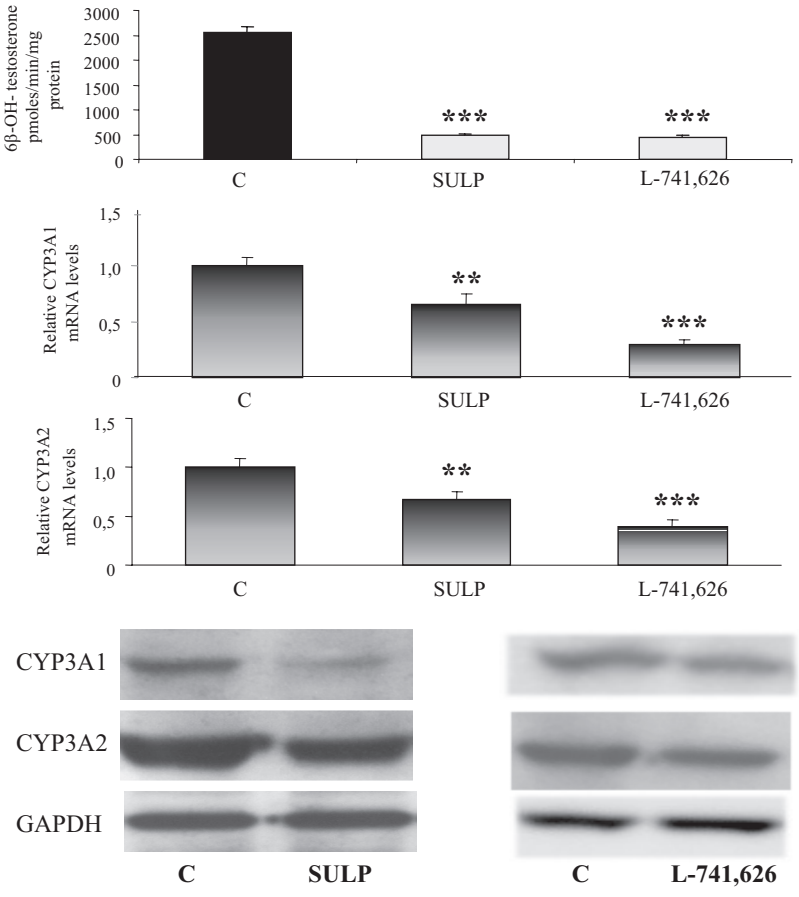


Fig. 1. D_2-dopaminergic receptor-mediated regulation of hepatic CYP3A1/2. Assessments of the effects of the D_2-dopaminergic receptor antagonists SULP and L-741,626 on CYP3A1/2-catalyzed 6β-testosterone hydroxylation levels were performed by using HPLC, on CYP3A1/2 apoprotein levels by using Western blotting, and on relative CYP3A1 and CYP3A2 mRNA expression levels by using quantitative PCR assays. Comparisons were between control (C) and drug-treated rats. Values are expressed as mean ± S.E. **, $p < 0.01$; ***, $p < 0.001$.

Results

Involvement of D_2-Dopaminergic Receptor-Linked Pathways in CYP3A Regulation. In vivo administration of the D_2-dopaminergic receptor antagonist SULP resulted in down-regulation of hepatic CYP3A1/2 expression. CYP3A-dependent 6β-testosterone hydroxylation and CYP3A1/2 apoprotein and mRNA levels were detected at markedly lower levels in SULP-treated rats, compared with control rats (Fig. 1). The highly selective D_2-dopaminergic receptor antagonist L-741,626 also repressed CYP3A1/2 expression at the mRNA, apoprotein, and activity levels (Fig. 1).

In contrast, in vitro experiments using primary hepatocyte cultures demonstrated that D_2-dopaminergic receptor blockade with SULP markedly increased CYP3A1 and CYP3A2 mRNA levels (Fig. 2A). This increase was not mediated by the JNK-, cAMP/PKA-, or PI3K-related pathways, inasmuch as neither the JNK inhibitor SP600125, the PKA inhibitor H-89, nor the PI3K inhibitor wortmannin (WORT) prevented it (Fig. 2A). Incubation of primary hepatocytes with INS strongly suppressed CYP3A1/2 expression, and this suppressive effect was completely reversed by WORT (Fig. 2B).

Involvement of D_2-Dopaminergic Receptor-Linked Pathways in CYP2C Regulation. In vivo administration of SULP markedly suppressed CYP2C11 expression in the liver of rats, as assessed at the enzyme activity level (2α- and 16α-testosterone hydroxylation) and the apoprotein and mRNA levels (Fig. 3). L-741,626 also repressed CYP2C11 expression at the mRNA, apoprotein, and 2α- and 16α-testosterone hydroxylation levels (Fig. 3). However, SULP significantly increased CYP2C11 expression in vitro (Fig. 2A), an effect that was not prevented by SP600125, H-89, or WORT (Fig. 2A). As in the case of CYP3A, treatment of primary hepatocytes with INS markedly decreased CYP2C11 mRNA transcript levels, an effect that was completely blocked by WORT (Fig. 2B).

Involvement of D_2-Dopaminergic Receptor-Linked Pathways in CYP2D Regulation. Blockade of D_2-dopaminergic receptors with SULP markedly decreased CYP2D-dependent 1′-bufuralol hydroxylation and CYP2D1 mRNA transcript and apoprotein levels, compared with control values (Fig. 4). L-741,626 also repressed CYP2D1/2 expression (Fig. 4). Similar to findings for CYP3A and CYP2C, treatment of primary hepatocytes with SULP resulted in up-regulation of CYP2D1 and CYP2D2 (Fig. 2A), and this up-regulation was not prevented by SP600125, H-89, or WORT (Fig. 2A). In contrast, INS strongly suppressed CYP2D1 and CYP2D2 expression, an effect that was completely blocked by WORT (Fig. 2B).

Positive and Negative Control of P450-Dependent Activities. Incubation of liver microsomes with ketoconazole markedly suppressed CYP3A-catalyzed 6β-testosterone hydroxylation, compared with that in microsomes incubated with vehicle (Supplemental Fig. 1A). Sulfaphenazole also significantly suppressed 2α- and 16α-testosterone hydroxylation (Supplemental Fig. 1, B and C). In contrast, incubation of liver microsomes with SULP did not modify these activities (Supplemental Fig. 1, A–C).

Incubation of recombinant CYP3A1 with SULP increased 6β-testosterone hydroxylation levels, whereas SULP had no

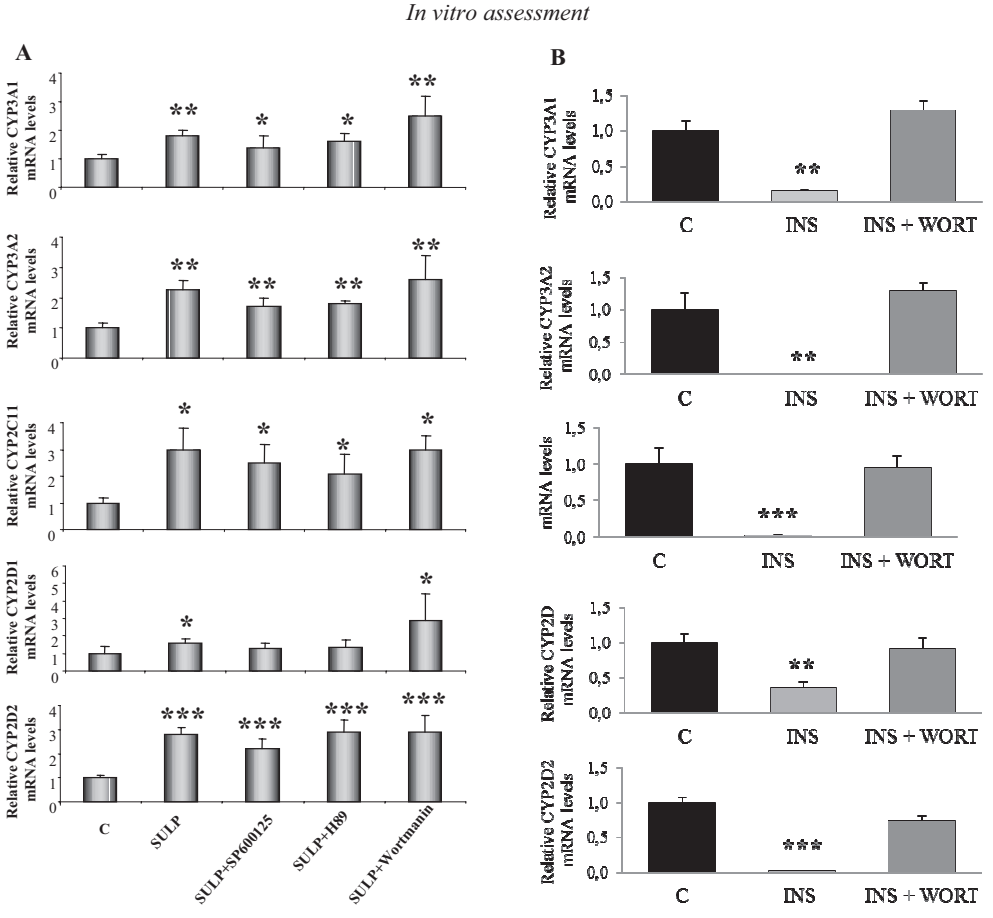


Fig. 2. In vitro assessment of the roles of the D_2-dopaminergic receptor and insulin/PI3K/Akt pathways in the regulation of hepatic CYP3A1/2, CYP2C, and CYP2D1/2. A, assessment of the effects of SULP on CYP3A1, CYP3A2, CYP2C11, CYP2D1, and CYP2D2 mRNA levels in primary hepatocytes. The roles of the JNK-, cAMP/PKA-, and PI3K-related signaling pathways in SULP-induced P450 regulation were assessed by using the following inhibitors before SULP: SP600125 (10 μM, 24 h), H-89 (10 μM, 24 h), and WORT (1 μM, 24 h), respectively. B, assessment of the effects of INS on CYP3A1, CYP3A2, CYP2C11, CYP2D1, and CYP2D2 mRNA levels in primary hepatocytes. Primary hepatocytes were treated with 1 μM INS for 24 h, alone or in combination with the PI3K inhibitor WORT (1 μM, 24 h). Comparisons were between dimethylsulfoxide-treated [control (C)] and drug-treated cells. Values are expressed as mean ± S.E. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

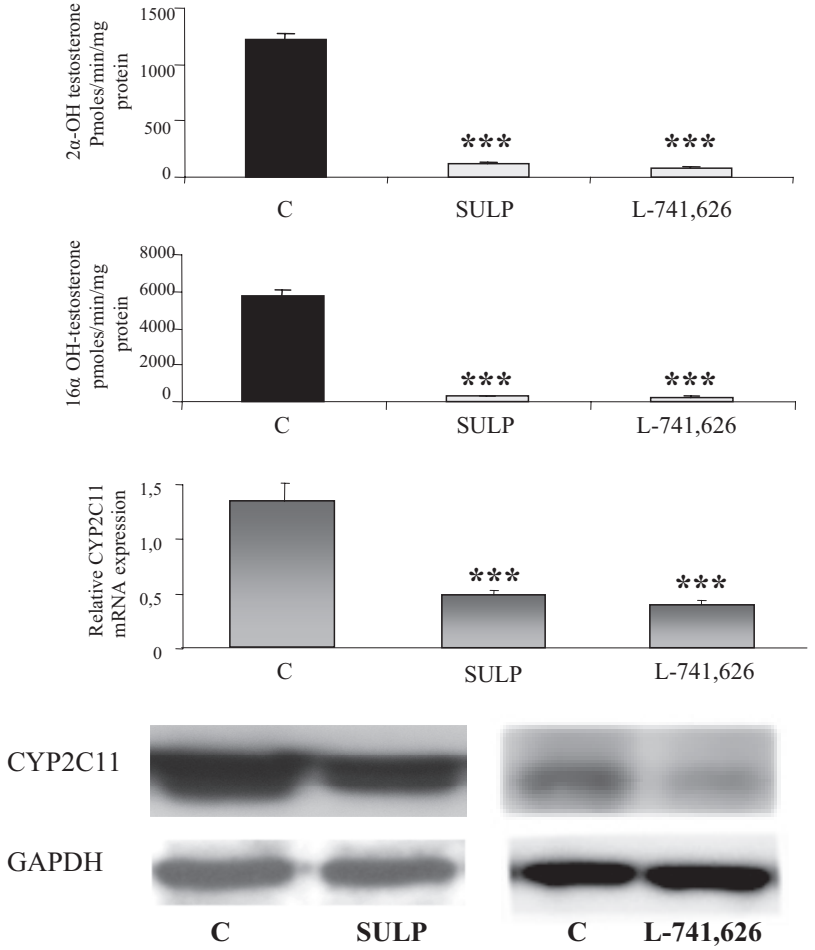


Fig. 3. D_2-dopaminergic receptor-mediated regulation of hepatic CYP2C11. Assessments of the effects of the D_2-dopaminergic receptor antagonists SULP and L-741,626 on CYP2C11-catalyzed 2α- and 16α-testosterone hydroxylation were performed by using HPLC, on CYP2C11 apoprotein levels by using Western blotting, and on relative CYP2C11 mRNA expression levels by using quantitative PCR assays. Comparisons were between control (C) and drug-treated rats. Values are expressed as mean ± S.E. ***, $p < 0.001$.

effect on recombinant CYP3A2-catalyzed activity (Supplemental Fig. 1D). The D_2-dopaminergic receptor antagonist markedly repressed recombinant CYP2C11-catalyzed 2α- and 16α-testosterone hydroxylation (Supplemental Fig. 1, E and F), whereas SULP had no effect on recombinant CYP2D1- or recombinant CYP2D2-catalyzed bufuralol 1′-hydroxylation (Supplemental Fig. 1, G and H).

Assessment of Effects of D_2-Dopaminergic Receptor Blockade on Hormone Levels. Treatment of rats with SULP strongly suppressed plasma GH, triiodothyronine, thyroxin, and corticosterone concentrations (Table 2). In contrast, SULP markedly increased plasma PRL levels (Table 2). It is of importance to this study that the drug also increased plasma insulin levels, which was followed by reduced plasma glucose concentrations (Table 2).

Assessment of D_2-Dopaminergic Receptor-Mediated Effects on PI3K/Akt/FOXO1 Pathway. The D_2-dopaminergic receptor antagonist SULP markedly increased the phosphorylation of Akt in the liver of rats (Fig. 5A). The phosphorylation of p70S6K, a downstream element in the PI3K/Akt pathway, was also strongly increased after SULP treatment (Fig. 5A). SULP strongly decreased the levels of phosphorylated FOXO1 in the nucleus of hepatocytes (Fig. 5B), whereas levels were markedly increased in the cytosol (Fig. 5C). It should be noted that SULP upregulated eNOS and iNOS (mean ± S.E.: iNOS, control versus SULP, 1.0 ± 0.13- versus 1.8 ± 0.22-fold induction, $n = 10$, $p < 0.01$; eNOS, 1.0 ± 0.12- versus 2.0 ± 0.14-fold induction, $n = 10$, $p < 0.01$). L-741,626 had effects similar to those of SULP on the PI3K/Akt/FOXO1 signal transduction pathway (Fig. 6).

Assessment of D_2-Dopaminergic Receptor-Mediated Effects on STAT5b Activation. It is well established that GH and the GH pulse-activated transcription factor STAT5b (Holloway et al., 2006) play major roles in the regulation of several P450s (Waxman and Holloway, 2009). Treatment of rats with SULP strongly suppressed STAT5b activation, as assessed at the nuclear and cytosolic STAT5b phosphorylation (Tyr694) levels (Fig. 5, B and C). Similarly, L-741,626 markedly reduced STAT5b activation in the nucleus and cytoplasm, compared with control values (Fig. 6, B and C).

Assessment of D_2-Dopaminergic Receptor-Mediated Effects on Nuclear Transcription Factors Involved in P450 Regulation. Constitutive androstane receptor (CAR), pregnane X receptor (PXR), retinoic X receptor α (RXRα), and HNF1α mRNA levels were detected at markedly lower levels in the livers of SULP- and L-741,626-treated rats, compared with control rats (Fig. 7, A–D). No significant change in hepatic HNF4α expression was observed after SULP treatment, whereas L-741,626 suppressed expression (Fig. 7E). CREB phosphorylation, a downstream element in the cAMP/PKA signaling pathway, and JNK phosphorylation were suppressed by both SULP and L-741,626 (Figs. 5A and 6A).

In vitro experiments using primary hepatocyte cultures showed that treatment of the cells with SULP increased CAR mRNA transcript levels. This increase was profoundly medi-

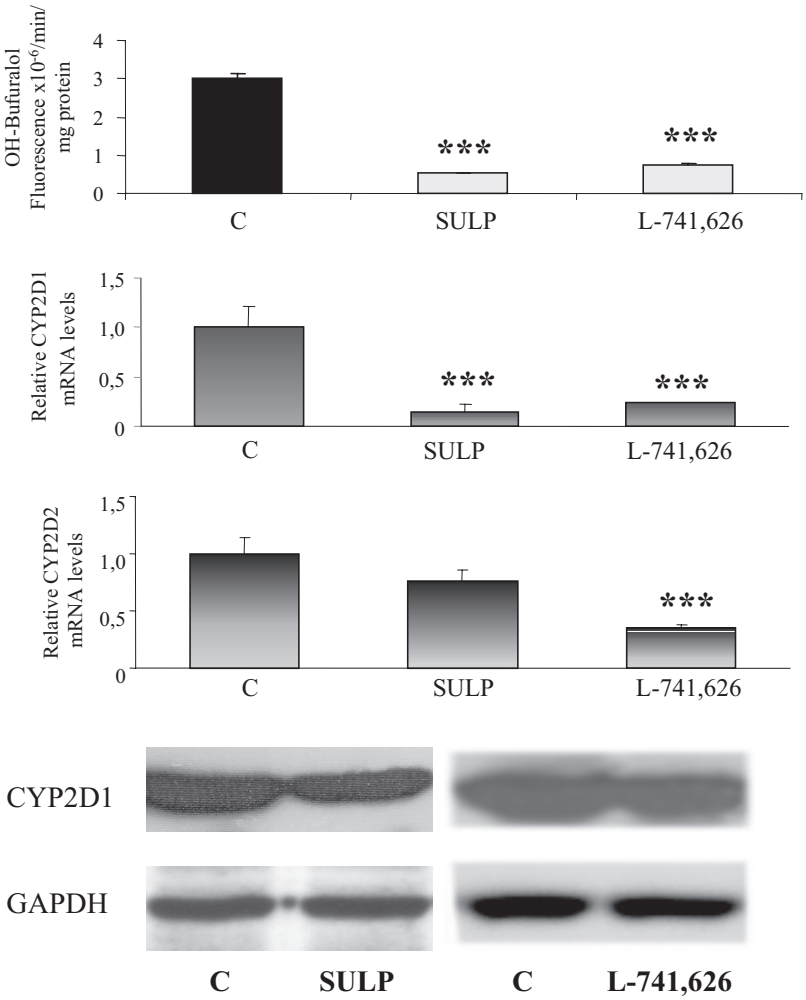


Fig. 4. D_2-dopaminergic receptor-mediated regulation of hepatic CYP2D1/2. Assessments of the effects of the D_2-dopaminergic receptor antagonists SULP and L-741,626 on CYP2D1/2-catalyzed 1′-bufuralol hydroxylation were performed by using HPLC, on CYP2D1 apoprotein levels by using Western blotting, and on relative CYP2D1 and CYP2D2 mRNA expression levels by using quantitative PCR assays. Comparisons were between control (C) and drug-treated rats. Values are expressed as mean ± S.E. ***, $p < 0.001$.

TABLE 2

Assessment of SULP-induced alterations in plasma hormone and glucose levels

Values are expressed as mean ± S.E. (n = 12).

	Control	SULP
Insulin, pg/ml	0.4 ± 0.02	1.3 ± 0.18***
Glucose, mg/dl	8.0 ± 0.25	7.2 ± 0.10*
GH, ng/ml	107.95 ± 2.99	29.76 ± 9.28***
Triiodothyronine, ng/dl	111.31 ± 5.80	66.86 ± 4.80***
Thyroxin, μg/dl	2.59 ± 0.10	1.40 ± 0.17***
Thyroid-stimulating hormone, ng/ml	1.66 ± 0.06	1.57 ± 0.08
Corticosterone, mg/ml	166.3 ± 12.4	73.3 ± 9.5**
PRL, ng/ml	35.01 ± 5.80	177.78 ± 11.89***

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

ated by the JNK-, cAMP/PKA-, and PI3K-linked signaling pathways, inasmuch as it was prevented by the corresponding inhibitors (i.e., SP600125, H-89, and WORT) (Fig. 7A). RXRα mRNA levels also were increased after treatment of the hepatocytes with SULP, and this increase was mediated mainly by the JNK-linked pathway (Fig. 7C). PXR, HNF1α, and HNF4α mRNA expression levels were not affected by direct exposure of hepatocytes to SULP (Fig. 7, B, D, and E).

Discussion

An increasing body of evidence suggests that central and peripheral catecholaminergic systems play an important role in the regulation of drug-metabolizing enzymes, including the P450s (Konstandi et al., 2004, 2005, 2006; Daskalopoulos et al., 2012). In accordance with this, the data in the present study clearly indicated a critical role for the dopaminergic pathways in P450 regulation. The investigation focused on the role of the D_2-dopaminergic receptor-related pathways, targets of many therapeutically important drugs prescribed for treatment of several neurodegenerative and psychopathological disorders, including Parkinson's disease, depression, and psychosis (Craig and Lin, 1981; Bonci and Hopf, 2005; Kabbani et al., 2012). These drugs, acting as either D_2-dopaminergic receptor agonists or antagonists, directly affect the dopamine receptor system by mimicking, blocking, or altering the sensitivity to dopamine, thus altering the functional output of dopaminergic systems (Cooper et al., 1996).

Pharmacological blockade of D_2-dopaminergic receptors using SULP, a selective D_2 receptor antagonist and typical antipsychotic drug, resulted in strong down-regulation of CYP3A1/2, CYP2C11, and CYP2D1 (but not CYP2D2). The involvement of D_2-dopaminergic receptors in the aforementioned P450 regulation was confirmed by using the highly selective D_2-dopaminergic receptor antagonist L-741,626. The SULP-induced down-regulation appeared to be indirect, inasmuch as treatment of primary hepatocytes with SULP had an opposite effect. We hypothesize that the SULP-induced down-regulation of these P450s is the outcome of com-

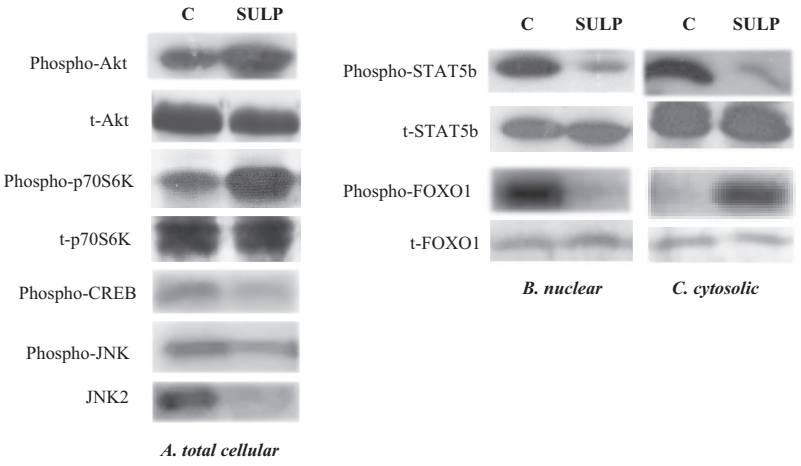


Fig. 5. In vivo assessments of the effects of SULP on signal transduction. A, Western blot showing SULP-induced activation of Akt and p70S6K and inactivation of CREB and JNK in total cellular proteins. B and C, Western blots showing nuclear (B) and cytosolic (C) STAT5b and FOXO1 phosphorylation after treatment with SULP. Lanes C, control.

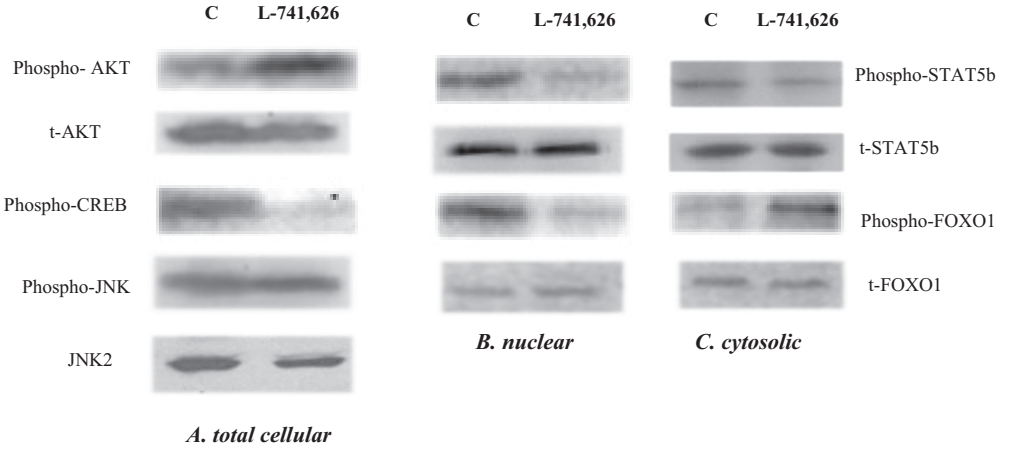


Fig. 6. In vivo assessments of the effects of L-741,626 on signal transduction. A, Western blot showing L-741,626-induced activation of Akt and inactivation of CREB and JNK in total cellular proteins. B and C, Western blots showing nuclear (B) and cytosolic (C) STAT5b and FOXO1 phosphorylation after treatment with L-741,626. Lanes C, control.

bined effects of the drug on central and peripheral aminergic and hormonal systems, which overrides the drug's direct up-regulating effect on hepatocytes. In terms of CYP2C11, it is of interest to note that in vitro experiments with recombinant CYP2C11 revealed a direct down-regulating effect of the drug on this P450.

The role of insulin in D_2 receptor-mediated P450 down-regulation appears to be crucial. This hypothesis is based on the notion that release of insulin, which exerts negative regulatory control on several P450s (Yoshida et al., 1996; Woodcroft and Novak, 1997, 1999) is under dopaminergic control through pancreatic β-cell D_2 receptors (Rubí et al., 2005) (Scheme 1).

We showed previously that blockade of D_2-dopaminergic receptors stimulated the release of insulin (Konstandi et al., 2008). The current in vitro experiments with primary hepatocytes clearly indicated that insulin down-regulated CYP3A1/2, CYP2C11, and CYP2D1 through the PI3K/Akt signaling pathway, inasmuch as pretreatment of hepatocytes with wortmannin, a PI3K inhibitor, completely reversed the suppressive effect of insulin. Further investigations indicated that treatment of rats with SULP increased plasma insulin levels, followed by activation of the PI3K/Akt signaling pathway, an event associated with phosphorylation of FOXO1 in the nucleus, subsequent translocation of FOXO1 into the cytoplasm, and termination of P450 transcription (Kodama et al., 2004; Kim and Novak, 2007). The Akt/p70S6K pathway does not seem to contribute to the SULP-induced suppressive effects on the aforementioned P450s, because activation of p70S6K is connected with increased gene transcription (Kim and Novak, 2007). The activation of JNK was markedly weaker in the liver of SULP-treated rats. Taking into account the accumulating evidence that Akt- and JNK-linked pathways have opposing effects on FOXO, with Akt preventing FOXO nuclear localization and inhibiting its activity but JNK increasing FOXO activity by promoting import into the nucleus (Brunet et al., 1999; Essers et al., 2004; Wang et al., 2005), we hypothesize that the SULP-induced down-regulating effects on P450s involving FOXO1 are the result of the drug's combined effects on the Akt and JNK signaling pathways.

FOXO1 is part of a complex cross-talk mechanism that includes the nuclear receptors CAR and PXR (Kodama et al., 2004). These nuclear receptors, along with RXR, HNF1α, and HNF4α (Liddle et al., 1998; Wiwi and Waxman, 2004), are known to regulate the most important hepatic P450 genes, including members of the CYP3A and CYP2C subfamilies (Dvorak and Pavek, 2010). SULP also down-regulated the expression of CAR, PXR, RXRα, and HNF1α, which indicates that the drug's suppressive effects on CYP3A1/2 and CYP2C11 might be mediated by these nuclear factors.

Moreover, SULP up-regulated both eNOS and iNOS in the liver. It is well established that both of these enzymes exert negative regulatory control on several P450s (Hara and Adachi, 2002). Possible contributions of eNOS and iNOS to the down-regulation of CYP3A, CYP2C, and CYP2D cannot be

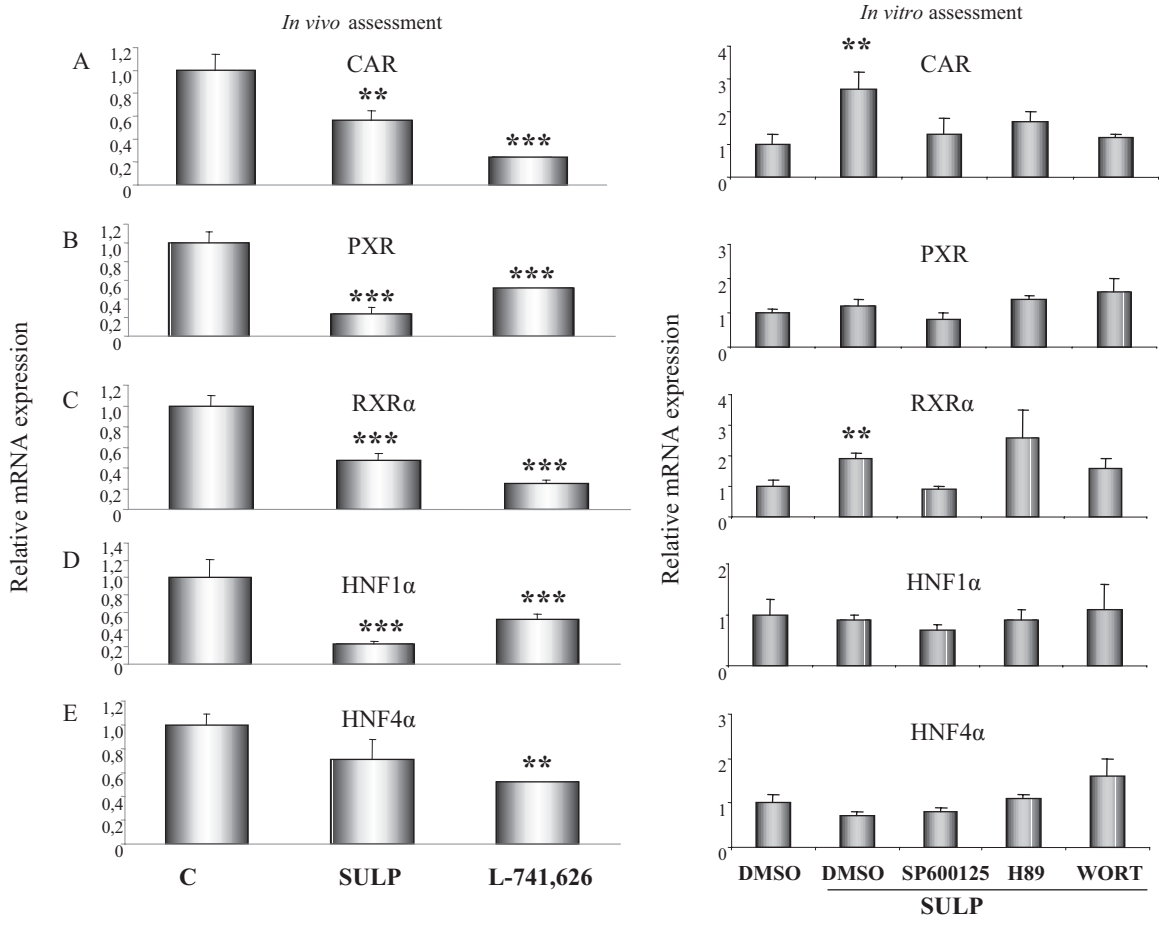
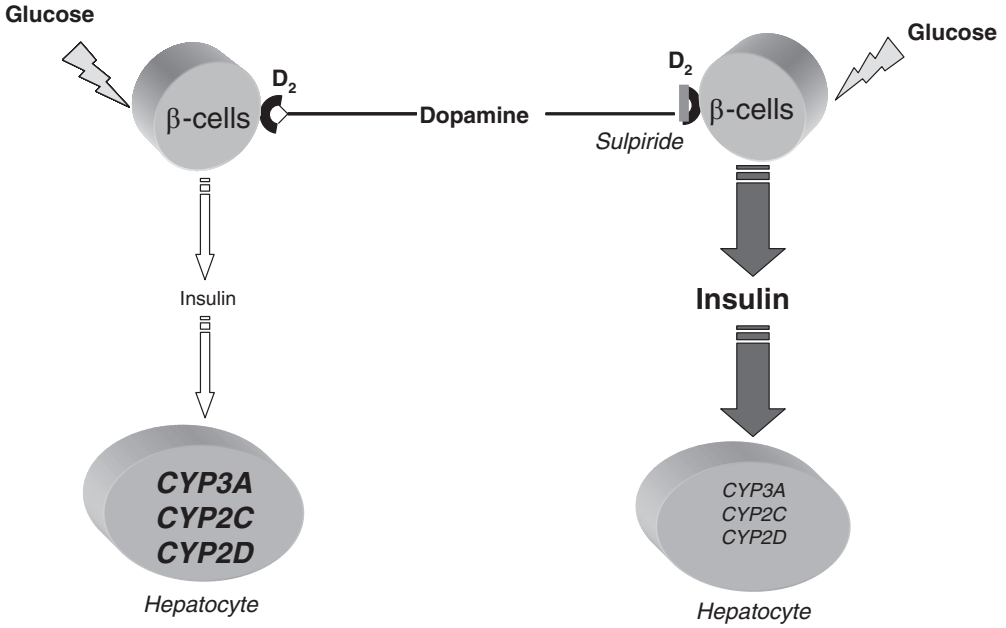


Fig. 7. In vivo and in vitro assessment of the role of D_2-dopaminergic receptor-linked pathways in the regulation of nuclear transcription factors critical for P450 regulation. SULP and L-741,626 (selective D_2-dopaminergic receptor antagonist) effects on CAR (A), PXR (B), RXRα (C), HNF1α (D), and HNF4α (E) expression levels (at relative mRNA levels) were determined by using quantitative PCR assays. Comparisons were between control (C) and drug-treated rats. For the in vitro assessment, primary hepatocytes were treated with dimethylsulfoxide (DMSO) (control) or SULP, alone or in combination with SP600125, H-89, or WORT (inhibitors of JNK, PKA, and PI3K signaling pathways, respectively). Values are expressed as mean ± S.E. **, $p < 0.01$; ***, $p < 0.001$.



Scheme 1. Insulin release from pancreatic β-cells in response to increased plasma glucose levels, is under dopaminergic control. By stimulating D_2-dopaminergic receptors expressed in β-cells, dopamine restricts the release of insulin. The sulpiride-induced blockade of D_2 receptors prevents the restricting effect of dopamine on insulin release, which results in repression of the expression of various genes, including P450s.

excluded. Finally, SULP-induced suppression of hepatic CREB phosphorylation may contribute to down-regulation of the aforementioned P450s, because it is well established that the cAMP/PKA/CREB signaling pathway controls the expression of PXR and HNF1α (Soutoglou et al., 2000; Kodama et al., 2007).

It is possible that both GH and PRL play roles in the down-regulation of these P450s by SULP. SULP reduced plasma GH levels, with subsequent reduced phosphorylation of STAT5b, which is the major GH pulse-activated transcription factor and is involved in the regulation of various P450s in the liver (Waxman and O'Connor, 2006; Waxman and Holloway, 2009). In contrast, PRL levels were increased by SULP, and it is well established that PRL exerts down-regulating control on several P450 isoforms (Yamazoe et al., 1987; Fitzgerald and Dinan, 2008).

The SULP-induced perturbation of the aforementioned signaling pathways might also be a result of off-target drug effects, including interactions of SULP with G protein-coupled receptors, a hypothesis that likely merits further investigation (Kristiansen, 2004; Theodoropoulou et al., 2008). In conclusion, the present data indicate several possible mechanisms that might play roles in the down-regulation of CYP3A, CYP2C, and CYP2D by SULP. It is apparent that the in vivo effects of SULP on P450s represent the result of the drug's combined effects mainly on the GH/STAT5b, PRL, cAMP/PKA/CREB, PI3K/Akt/FOXO1, JNK, and eNOS/iNOS signaling pathways. The use of the highly selective D_2-dopaminergic receptor antagonist L-741,626 confirmed the involvement of D_2 receptor-linked pathways in the modulation of the aforementioned signaling pathways, which mediate the down-regulation of CYP3A, CYP2C, and CYP2D. However, more-detailed studies are required to determine their respective roles in down-regulation.

On the basis of the data described above and previous reports (Konstandi et al., 2008), a question arises regarding the extent to which pharmacotherapy with D_2-dopaminergic receptor antagonists or agonists may influence the expression of CYP3A, CYP2C, and CYP2D and thus the metabolism of the majority of prescribed drugs. The possibility that drugs acting as D_2-dopaminergic receptor antagonists or agonists might modify the function of major signal transduction pathways involved in the regulation of P450s with crucial importance in the metabolism of numerous prescribed drugs, toxicants, and carcinogens, thus drastically affecting the outcomes of pharmacotherapy, drug toxicity, and carcinogenicity, leads to some very interesting prospects. For instance, several previous studies reported lower relative risks of cancer incidence among patients with psychosis who were receiving drug therapy, usually including D_2-dopaminergic receptor antagonists (Rassidakis et al., 1973; Rice, 1979; Craig and Lin, 1981; Mortensen, 1989). Could this finding be attributed to reduced expression of CYP3A, CYP2C, and CYP2D? Or should the strong SULP-mediated down-regulation of CYP2D be taken into account in assessments of the effectiveness of pharmacotherapy and possible drug-drug interactions among patients who receive antidepressant or antipsychotic therapy, given the fact that the majority of these drugs are metabolized by CYP2D (Wójcikowski and Daniel, 2009)? Furthermore, it is worth noting that the present findings shed more light on the regulation of CYP2D, which has been considered to be resistant to direct hormonal regulation and is known as "uninducible" P450.

Acknowledgments

We thank Drs. Ronald Wolf and Susumu Imaoka for their kind offer of the rat CYP3A1-, CYP3A2-, CYP2C11-, and CYP2D1-specific antibodies. We thank Dr. Mats Heinderstadt for his valuable support in the HPLC-based determination of P450-dependent activities. We are thankful to Olga Tsoumani for her valuable technical assistance.

Authorship Contributions

Participated in research design: Konstandi.

Conducted experiments: Daskalopoulos, Malliou, and Konstandi.

Contributed new reagents or analytic tools: Lang, Marselos, and Konstandi.

Performed data analysis: Daskalopoulos and Konstandi.

Wrote or contributed to the writing of the manuscript: Daskalopoulos, Lang, Marselos, and Konstandi.

References

Beaulieu JM and Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* **63:**182–217.

Bonci A and Hopf FW (2005) The dopamine D2 receptor: new surprises from an old friend. *Neuron* **47:**335–338.

Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, and Greenberg ME (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96:**857–868.

Cooper J, Bloom F, and Roth R (1996) Norepinephrine and epinephrine, in *The Biochemical Basis of Neuropharmacology* (House J ed) 7th ed, pp 226–292, Oxford University Press, New York.

Craig TJ and Lin SP (1981) Mortality among psychiatric inpatients: age-adjusted comparison of populations before and after psychotropic drug era. *Arch Gen Psychiatry* **38:**935–938.

Daskalopoulos EP, Malliou F, Rentesi G, Marselos M, Lang MA, and Konstandi M (2012) Stress is a critical player in CYP3A, CYP2C, and CYP2D regulation: role of adrenergic receptor signaling pathways. *Am J Physiol Endocrinol Metab* **303:**E40–E54.

Dvorak Z and Pavek P (2010) Regulation of drug-metabolizing cytochrome P450 enzymes by glucocorticoids. *Drug Metab Rev* **42:**621–635.

Essers MA, Weijzen S, de Vries-Smits AM, Saarloos I, de Ruiter ND, Bos JL, and Burgering BM (2004) FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J* **23:**4802–4812.

Fitzgerald P and Dinan TG (2008) Prolactin and dopamine: what is the connection? A review article. *J Psychopharmacol* **22 (2 Suppl):**12–19.

Gonzalez FJ and Gelboin HV (1994) Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. *Drug Metab Rev* **26:**165–183.

Gonzalez FJ and Yu AM (2006) Cytochrome P450 and xenobiotic receptor humanized mice. *Annu Rev Pharmacol Toxicol* **46:**41–64.

Guengerich FP (2003) Cytochromes P450, drugs, and diseases. *Mol Interv* **3:**194–204.

Hara H and Adachi T (2002) Contribution of hepatocyte nuclear factor-4 to down-regulation of *CYP2D6* gene expression by nitric oxide. *Mol Pharmacol* **61:**194–200.

Holloway MG, Laz EV, and Waxman DJ (2006) Codependence of growth hormone-responsive, sexually dimorphic hepatic gene expression on signal transducer and activator of transcription 5b and hepatic nuclear factor 4α. *Mol Endocrinol* **20:**647–660.

Ingelman-Sundberg M (2004) Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol* **369:**89–104.

Kabbani N, Woll MP, Nordman JC, and Levenson R (2012) Dopamine receptor interacting proteins: targeting neuronal calcium sensor-1/D2 dopamine receptor interaction for antipsychotic drug development. *Curr Drug Targets* **13:**72–79.

Kim SK and Novak RF (2007) The role of intracellular signaling in insulin-mediated regulation of drug metabolizing enzyme gene and protein expression. *Pharmacol Ther* **113:**88–120.

Klaunig JE, Goldblatt PJ, Hinton DE, Lipsky MM, Chacko J, and Trump BF (1981) Mouse liver cell culture. I. Hepatocyte isolation. *In Vitro* **17:**913–925.

Kodama S, Koike C, Negishi M, and Yamamoto Y (2004) Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Mol Cell Biol* **24:**7931–7940.

Kodama S, Moore R, Yamamoto Y, and Negishi M (2007) Human nuclear pregnane X receptor cross-talk with CREB to repress cAMP activation of the glucose-6-phosphatase gene. *Biochem J* **407:**373–381.

Konstandi M, Harkitis P, Kostakis D, Marselos M, Johnson EO, and Lang MA (2008) D2-receptor-linked signaling pathways regulate the expression of hepatic CYP2E1. *Life Sci* **82:**1–10.

Konstandi M, Johnson EO, Marselos M, Kostakis D, Fotopoulos A, and Lang MA (2004) Stress-mediated modulation of B(α)P-induced hepatic CYP1A1: role of catecholamines. *Chem Biol Interact* **147:**65–77.

Konstandi M, Kostakis D, Harkitis P, Johnson EO, Marselos M, Adamidis K, and Lang MA (2006) Benzo(α)pyrene-induced up-regulation of *CYP1A2* gene expression: role of adrenoceptor-linked signaling pathways. *Life Sci* **79:**331–341.

Konstandi M, Kostakis D, Harkitis P, Marselos M, Johnson EO, Adamidis K, and Lang MA (2005) Role of adrenoceptor-linked signaling pathways in the regulation of *CYP1A1* gene expression. *Biochem Pharmacol* **69:**277–287.
Konstandi M, Marselos M, Radon-Camus AM, Johnson E, and Lang MA (1998) The role of stress in the regulation of drug metabolizing enzymes in mice. *Eur J Drug Metab Pharmacokinet* **23:**483–490.
Kristiansen K (2004) Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modelling and mutagenesis approaches to receptor structure and function. *Pharmacol Ther* **103:**21–80.
Lang MA, Gielen JE, and Nebert DW (1981) Genetic evidence for many unique liver microsomal P-450-mediated monooxygenase activities in heterogeneic stock mice. *J Biol Chem* **256:**12068–12075.
Liddle C, Goodwin BJ, George J, Tapner M, and Farrell GC (1998) Separate and interactive regulation of cytochrome P450 3A4 by triiodothyronine, dexamethasone, and growth hormone in cultured hepatocytes. *J Clin Endocrinol Metab* **83:**2411–2416.
Maitre M, Ratomponirina C, Gobaille S, Hodé Y, and Hechler V (1994) Displacement of [^{3}H]γ-hydroxybutyrate binding by benzamide neuroleptics and prochlorperazine but not by other antipsychotics. *Eur J Pharmacol* **256:**211–214.
Matsunaga E, Zeugin T, Zanger UM, Aoyama T, Meyer UA, and Gonzalez FJ (1990) Sequence requirements for cytochrome P-450IID1 catalytic activity: a single amino acid change (Ile380Phe) specifically decreases V_{max} of the enzyme for bufuralol but not debrisoquine hydroxylation. *J Biol Chem* **265:**17197–17201.
Mortensen PB (1989) The incidence of cancer in schizophrenic patients. *J Epidemiol Community Health* **43:**43–47.
Murray M, Sefton RM, Croft KD, and Butler AM (2001) Differential regulation of endobiotic-oxidizing cytochromes P450 in vitamin A-deficient male rat liver. *Br J Pharmacol* **134:**1487–1497.
Pelkonen O, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J, and Raunio H (2008) Inhibition and induction of human cytochrome P450 enzymes: current status. *Arch Toxicol* **82:**667–715.
Rassidakis NC, Kelepouris M, Goulis K, and Karaiossefidis K (1973) On the incidence of malignancy among schizophrenic patients. *Agressologie* **14:**269–273.
Rendic S and Guengerich FP (2010) Update information on drug metabolism systems–2009, part II: summary of information on the effects of diseases and environmental factors on human cytochrome P450 (CYP) enzymes and transporters. *Curr Drug Metab* **11:**4–84.
Rice D (1979) No lung cancer in schizophrenics? *Br J Psychiatry* **134:**128.
Rubí B, Ljubicic S, Pournourmohammadi S, Carobbio S, Armanet M, Bartley C, and Maechler P (2005) Dopamine D2-like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion. *J Biol Chem* **280:**36824–36832.
Shimojo N, Ishizaki T, Imaoka S, Funae Y, Fujii S, and Okuda K (1993) Changes in amounts of cytochrome P450 isozymes and levels of catalytic activities in hepatic and renal microsomes of rats with streptozocin-induced diabetes. *Biochem Pharmacol* **46:**621–627.
Soutoglou E, Papafotiou G, Katrakili N, and Talianidis I (2000) Transcriptional activation by hepatocyte nuclear factor-1 requires synergism between multiple coactivator proteins. *J Biol Chem* **275:**12515–12520.
Spatzenegger M and Jaeger W (1995) Clinical importance of hepatic cytochrome P450 in drug metabolism. *Drug Metab Rev* **27:**397–417.
Takahashi N, Inui N, Morita H, Takeuchi K, Uchida S, Watanabe H, and Nakamura H (2010) Effect of thyroid hormone on the activity of CYP3A enzyme in humans. *J Clin Pharmacol* **50:**88–93.
Theodoropoulou MC, Bagos PG, Spyropoulos IC, and Hamodrakas SJ (2008) gpDB: a database of GPCRs, G-proteins, effectors and their interactions. *Bioinformatics* **24:**1471–1472.
Trinder P (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol* **22:**158–161.
Wang MC, Bohmann D, and Jasper H (2005) JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* **121:**115–125.
Waxman DJ and Holloway MG (2009) Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol Pharmacol* **76:**215–228.
Waxman DJ and O'Connor C (2006) Growth hormone regulation of sex-dependent liver gene expression. *Mol Endocrinol* **20:**2613–2629.
Wiwi CA and Waxman DJ (2004) Role of hepatocyte nuclear factors in growth hormone-regulated, sexually dimorphic expression of liver cytochromes P450. *Growth Factors* **22:**79–88.
Wójcikowski J and Daniel WA (2009) The brain dopaminergic system as an important center regulating liver cytochrome P450 in the rat. *Expert Opin Drug Metab Toxicol* **5:**631–645.
Woodcroft KJ, Hafner MS, and Novak RF (2002) Insulin signaling in the transcriptional and posttranscriptional regulation of CYP2E1 expression. *Hepatology* **35:**263–273.
Woodcroft KJ and Novak RF (1997) Insulin effects on CYP2E1, 2B, 3A, and 4A expression in primary cultured rat hepatocytes. *Chem Biol Interact* **107:**75–91.
Woodcroft KJ and Novak RF (1999) Insulin differentially affects xenobiotic-enhanced, cytochrome P-450 (CYP)2E1, CYP2B, CYP3A, and CYP4A expression in primary cultured rat hepatocytes. *J Pharmacol Exp Ther* **289:**1121–1127.
Yamazoe Y, Shimada M, Murayama N, and Kato R (1987) Suppression of levels of phenobarbital-inducible rat liver cytochrome P-450 by pituitary hormone. *J Biol Chem* **262:**7423–7428.
Yoshida Y, Kimura N, Oda H, and Kakinuma A (1996) Insulin suppresses the induction of *CYP2B1* and *CYP2B2* gene expression by phenobarbital in adult rat cultured hepatocytes. *Biochem Biophys Res Commun* **229:**182–188.

Address correspondence to: Dr. Maria Konstandi, Department of Pharmacology, School of Medicine, University of Ioannina, GR-451 10, Ioannina, Greece. E-mail: mkonstan@cc.uoi.gr