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Role of adrenoceptor-linked signaling pathways in the regulation of *CYP1A1 gene* expression

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Abstract

Alpha2-adrenoceptor agents as well as stress affect the activity of several hepatic monoxygenases including those related to CYP1A enzymes. This study was therefore designed to assess the role of central and/or peripheral catecholamines and, in particular, of adrenoceptors in the regulation of B(α)P-induced cytochrome CYP1A1 expression. In order to discriminate the role of central from that of peripheral catecholamines in the regulation of *CYP1A1* induction, the effect of central and peripheral catecholamine depletion using reserpine versus only peripheral catecholamine depletion using guanethidine was assessed. By using selected agonists and antagonists, the role of alpha and beta-adrenoceptors in the regulation of *CYP1A1* induction was evaluated. The results showed that the central catecholaminergic system has a negative regulatory effect on 7-ethoxyresorufin O-deethylase (EROD) inducibility by benzo(α)pyrene (B(α)P), and that this may be mediated via α1-, α2- and β-adrenoceptors. Specifically, stimulation of α2-adrenoceptors with dexmedetomidine and blockade of α1- or β-adrenoceptors with prazosin or propranolol respectively, resulted in a further increase of EROD inducibility. Adrenoceptors were found to be involved in the regulation of the *CYP1A1 gene* at mRNA level. Both, reduced noradrenaline release in central nervous system induced with dexmedetomidine and central catecholamine depletion, as well as blockade of central α1-adrenoceptors induced with prazosin, all were associated with up-regulation of *CYP1A1* expression. In contrast, stimulation of central beta-adrenoceptors with isoprenaline resulted in a down-regulation of *CYP1A1* expression. Our observations indicate that drugs, which stimulate or block adrenoceptors and catecholamine release may lead to complications in drug therapy and modulate the toxicity or carcinogenicity of drugs that are substrates for the CYP1A1.

Keywords: Catecholamines; Adrenoceptors; Benzo(α)pyrene; EROD; *CYP1A1 gene*; Rat

1. Introduction

Cytochrome P4501A1 (CYP1A1) is well known for its role in the bioactivation of environmental toxins and carcinogens such as aromatic amines and polycyclic aromatic hydrocarbons (PAHs) [1–5]. Induction and a high activity of the CYP1A1 have been associated with increased toxicity and cancer risk, when exposed to tobacco smoke or other environmental pollutants including these compounds [6].

A key activator of the *CYP1A1 gene* is the aromatic hydrocarbon (Ah) receptor, which acts as a ligand activated transcription factor interacting with xenobiotic responsive element(s) (XRE(s)) on the *CYP1A1* promoter [7]. The Ah receptor can be activated by several ligands including the PAHs, such as benzo(α)pyrene (B(α)P) [5]. Because the B(α)P and related substances act both as inducers of the *CYP1A1 gene* and as pre-carcinogenic substrates of the corresponding enzyme, they are considered a particularly important group of toxicants [4,6].

In addition to the Ah receptor, some other transacting factors may also modulate the expression of the *CYP1A1*.

Abbreviations: EROD, 7-ethoxyresorufin O-deethylase; CYP1A1, cytochrome P4501A1 gene; B(α)P, benzo(α)pyrene; PAHs, polycyclic aromatic hydrocarbons; Ah, aromatic hydrocarbon; NA, noradrenaline; XREs, xenobiotic responsive elements; RIA, radioimmunoassay

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Repeated restraint stress, an experimental model of psychological and physical stress [8,9], has been shown to affect the CYP1A1-dependent 7-ethoxyresorufin O-deethylase (EROD) activity in a complex manner [10,11]. The constitutive EROD of rat liver was suppressed under restraint stress [10], while the B(α)P-induced EROD was further enhanced [10,11]. Not much is known about the mechanisms by which stress affects EROD activity, although the majority of experimental evidence supports a central role of noradrenaline [8,12], which is released from either noradrenergic nerve terminals or adrenal medulla, and acts via binding to specific adrenergic receptors [13]. The stress response cascade involves several complex physiological changes, including changes in sensitivity and/or density of rat brain β-adrenoceptors following chronic stress. Although the control of adrenoceptors is complex, steroid hormones, such as corticosteroids, are now known to directly modulate target cell gene transcription [14].

In a previous study we showed that stimulation of α2-adrenoceptors with dexmedetomidine affects the activity of several hepatic monoxygenases including those related to CYP1A enzymes [11]. This could be of importance for drug toxicity and for drug–drug interactions as central and peripheral α2-adrenoceptors hold an important physiological role and are considered therapeutic targets for a variety of drugs that have applications in hypertension, angina pectoris, congestive heart failure, cardiac arrhythmia, asthma, depression, prostatic hypertrophy, and glaucoma [15,16].

The goal of this study was to find out, how central and/or peripheral catecholamines regulate the B(α)P-induced *CYP1A1* expression; in particular, the possible role of central and peripheral adrenoceptors in the modulation of *CYP1A1* induction was investigated. For this purpose, specific drugs were used to manipulate the levels of central and peripheral catecholamines in vivo, and selective agonists and antagonists of alpha and beta-adrenoceptors were used to modulate the adrenoceptor-dependent signaling pathways. In addition, the effects of peripheral adrenaline administration was assessed.

2. Materials and methods

2.1. Animals and study design

Adult male Wistar (Kuo/Io/rr rats, 2–3 months old) bred at Ioannina University Animal House, were used. Animals were housed in groups of five to six plastic cages under 12-h light/12-h dark cycle with lights on at 6.00 a.m. Standard rodent chow and tap water were provided ad libitum. All procedures conformed to the International European Ethical Standards (86/609-EEC) for the care and use of laboratory animals.

Drug administration paradigms are shown in Table 1. Dosage of adrenoceptor agonists and antagonists was chosen according to the literature to achieve sufficient stimulation or blockade of the adrenoceptors and consequent alterations in noradrenergic neurotransmission [15,17–19]. In addition, dosing regimes were such that all animals remained healthy throughout the experiment.

Verification of drug-induced modulation in noradrenaline (NA) levels was done by measuring NA in hypothalamus using ion-pairing reverse phase high performance liquid chromatography with electrochemical detection (HPLC), [20,21].

Reserpine (Sigma-Aldrich; 5 mg/kg, i.p.) and guanethidine (Sigma-Aldrich, 40 mg/kg, i.p.) were administered daily for four and five consecutive days, respectively. Adrenaline (Demo, 0.5 mg/kg, s.c.) was administered daily for four consecutive days. Adrenaline was administered in the optimal dose to avoid cardiovascular problems, in particular, heart cell necrosis [22].

Phenylephrine (Sigma-Aldrich, 2 mg/kg, i.p.) or prazosin (Sigma-Aldrich, 20 mg/kg, i.p.) were administered daily for four consecutive days. When both drugs were given, prazosin was administered 1 h before phenylephrine.

Dexmedetomidine (Orion Pharmaceuticals Farmos, 5 μg/kg, s.c.) or atipamezole (Orion Pharmaceuticals Farmos, 200 μg/kg, s.c.) were given daily for four consecutive days. The dose of dexometomidine (α2-agonist) was chosen at 5 μg/kg in order to avoid sedation of the animals and that of atipamezole (α2-antagonist) at 200 μg/kg, in order to maximally block the effect of dexmedetomidine [23]. In combined treatments, atipamezole was given 3 min before dexmedetomidine.

Isoprenaline (Isuprel, Abbott, 2 mg/kg, i.p.) or propranolol (Sigma-Aldrich, 10 mg/kg, i.p.) were administered daily for four consecutive days. When both drugs were given, isoprenaline was administered 10 min before propranolol.

Benzo(α)pyrene (Sigma-Aldrich, 10 mg/kg, i.p.) dissolved in olive oil (Minerva) was administered for three consecutive days (the last dose 24 h before sacrifice). Olive oil intraperitonally and normal saline subcutaneously were injected in controls for three and four consecutive days, respectively.

B(α)P administration started 12 h after reserpine in order to avoid the reserpine-induced massive release of catecholamines during the first 4 h. Thus, when B(α)P treatment started, central and peripheral catecholamine levels were strongly reduced [24]. Guanethidine administration started 24 h before B(α)P in order to achieve a permanent depletion of peripheral catecholamines during the administration of B(α)P [25].

Two hours after the last treatment, animals were sacrificed by decapitation and livers were dissected for isolation of microsomes and total RNA.

All reagents and chemicals used were of the highest quality grade available. [α-^{32}P]-dCTP was purchased from Amersham International Plc (Amersham) and the specific CYP1A1 cDNA was kindly donated by Dr Olavi Pelkonen (Oulu, Finland). The rat polyclonal CYP1A1 antibody was kindly donated by Dr Ronald Wolf (London, UK).

Table 1
Dosing regimens and treatment protocol to assess the role of catecholamines and adrenoceptors in the regulation of B(α)P-induced hepatic *CYP1A1* in rats

Treatment groups	Day								
1	2 (9:00–12:00 h)		3 (9:00–12:00 h)		4 (9:00–12:00 h)		5 (9:00–12:00 h)		
I	No treatment Guanethidine (9.00 a.m.) Reserpine (9.00 p.m.) No treatment	B(α)P or vehicle	Normal saline Guanethidine Reserpine Adrenaline	B(α)P or vehicle	Normal saline Guanethidine Reserpine Adrenaline	B(α)P or vehicle	Normal saline Guanethidine Reserpine Adrenaline	No treatment	Normal saline Guanethidine Normal saline Adrenaline
II		B(α)P or vehicle	Normal saline Phenylephrine Prazosin Phenylephrine + prazosin	B(α)P or vehicle	Normal saline Phenylephrine Prazosin Phenylephrine + prazosin	B(α)P or vehicle	Normal saline Phenylephrine Prazosin Phenylephrine + prazosin	No treatment	Normal saline Phenylephrine Prazosin Phenylephrine + prazosin
III		B(α)P or vehicle	Normal saline Dexmedetomidine Atipamezole Dexmedetomidine + atipamezole	B(α)P or vehicle	Normal saline Dexmedetomidine Atipamezole Dexmedetomidine + atipamezole	B(α)P or vehicle	Normal saline Dexmedetomidine Atipamezole Dexm edetomidine + Atipamezole	No treatment	Normal saline Dexmedetomidine Atipamezole Dexm edetomidine + atipamezole
IV		B(α)P or vehicle	Normal saline Isoprenaline propranolol Isoprenaline + propranolol	B(α)P or vehicle	Normal saline Isoprenaline Propranolol Isoprenaline + propranolol	B(α)P or vehicle	Normal saline Isoprenaline Propranolol Isoprenaline + propranolol	No treatment	Normal saline Isoprenaline Propranolol Isoprenaline + propranolol

On day 1, animals received either guanethidine (40 mg/kg, i.p.) in the morning or reserpine (5 mg/kg, i.p.) in the evening. In order to assess the involvement of central and/or peripheral catecholamines in the regulation of B(α)P-induced *CYP1A1*, animals were treated daily with B(α)P (10 mg/kg, i.p.) on days 2–4 followed by (1) normal saline, (2) reserpine, (3) guanethidine or (4) adrenaline (0.5 mg/kg, s.c.), as shown in the Table 1. The other three treatment groups followed a similar paradigm. In group II, the involvement of α1-adrenoceptors was assessed using an α1-agonist (phenylephrine (2 mg/kg, i.p.)) and/or an α1-antagonist (prazosin (20 mg/kg, i.p.)). In group III, the involvement of α2-adrenoceptors was evaluated using an α2-agonist (dexmedetomidine (5 μg/kg, s.c.)) and/or an α2-antagonist (atipamezole (200 μg/kg, s.c.)). In group IV, the involvement of beta-adrenoceptors was assessed using a β1/β2-agonist (isoprenaline (2 mg/kg, i.p.)) and/or a β1/β2-blocker (propranolol (10 mg/kg, i.p.)).

2.2. Assessment of EROD activity

Livers from individual rats were dissected and the microsomal fractions were prepared by differential centrifugation [26]. Microsomal protein content was determined by the method of Lowry et al. [27] and the 7-ethoxyresorufin O-deethylase (EROD) activity was measured fluorometrically using 7-ethoxyresorufin as substrate [28–33].

2.3. Western blot analysis

Microsomal proteins (10 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) for 35 min at 200 V. The proteins were then transferred by electroblotting to 0.2 μm nitrocellulose membrane. The membranes were blocked with 5% defatted milk and exposed first to a polyclonal antibody against rat CYP1A1 and then to a secondary anti-IgG antibody conjugated to horseradish peroxidase. The membranes were developed by chemiluminescence using the Phototope-HRP Detection Kit for Western blotting (Biolabs INC, New England) and exposed to film.

2.4. Northern blot analysis

Total cellular RNA was purified following the RNAzolTM B method described by Chomczynski (cited in Cinna/Biotex Bull. No 3). Forty micrograms of RNA was resolved by 1.2% denaturating formaldehyde–agarose gel electrophoresis followed by blotting onto nitrocellulose membranes. The RNA was fixed to the membrane by UV cross-linking (Crosslinker UV Stratalinker 1800, Stratagene). RNA bound on the nitrocellulose membrane was treated for hybridization with a specific rat CYP1A1 probe at +65 °C overnight. After hybridization, the filter was washed in 4% Na_2HPO_4, 1% SDS at +65 °C (2×15 min) and autoradiographed at −80 °C. A 1.0 kb cDNA fragment at *Eco*RI to *Hin*dIII site, which recognizes only CYP1A1 mRNA was used as a specific CYP1A1 probe and was cloned in pGEM-4Z vector (clone 15) [34]. The cDNA probe was radiolabeled with [α-^{32}P] dCTP by nick translation. The gels were photographed under UV to control for equal loading of RNA.

The films from Northern and Western blots were scanned at a resolution 300 using the HP Precisionscan Programme 3.1 (Hewlett Packard) and saved as files in the Adobe Photoshope 5.0. The density of lanes was analyzed using the Quantity One—4.4.1 Programme (BIO-RAD).

2.5. Plasma corticosterone determination

Plasma corticosterone concentrations were measured by RIA using the Coat-A-Count kit (Diagnostic Products Corporation, Los Angeles, USA). The detection limit was about 5.7 ng/ml and the intra-assay coefficient of variation was 4.0%.

2.6. Statistical analysis

The data is expressed as means ± S.E. and was analysed using one-way analysis of variance (ANOVA) followed by multiple comparisons with Bonferoni's and Tuckey's list honest significant difference methods. The significance level for all analyses was set at probability of less than or equal to 0.05.

3. Results

To elucidate the mechanisms by which catecholamines and adrenoceptors may modulate the inducibility of the *CYP1A1 gene*, the mRNA, protein and enzyme activity (EROD) levels were monitored after pharmacological manipulations.

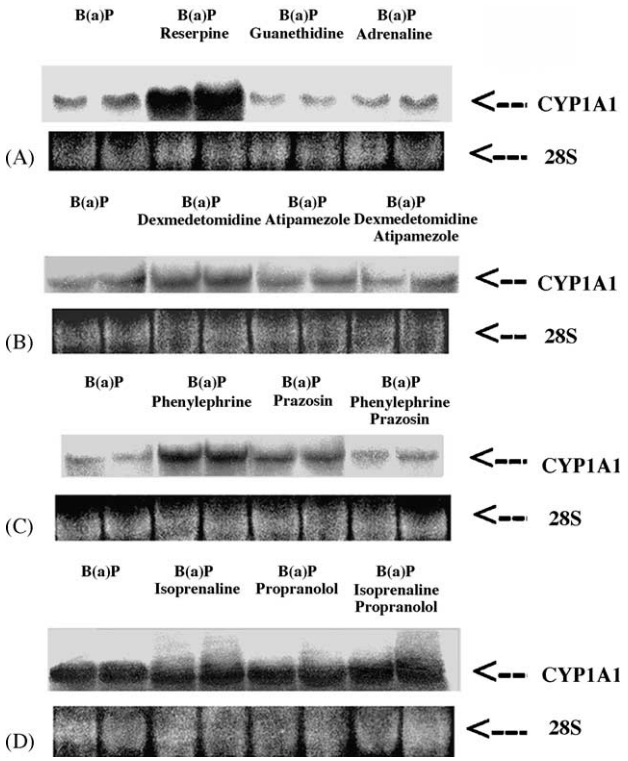


Fig. 1. Northern blot showing central and peripheral catecholamine involvement in the regulation of the B(α)P-induced hepatic *CYP1A1 gene* expression. Adrenoceptor involvement is also shown. Total RNA was isolated and the amount of CYP1A1 mRNA was determined using Northern blot analysis. CYP1A1 mRNA levels were standardized against 28S rRNA. Forty micrograms of total RNA from each sample were loaded in panel D, while 20 μg of total RNA in panels A–C. Two representative samples of five total animals per group are shown and every two lanes represent rats from the same treatment group. Panel A shows involvement of central and peripheral catecholamines in the regulation of *CYP1A1*; panel B depicts the involvement of α2-adrenoceptors; panel C shows the involvement of α1-adrenoceptors and panel D indicates the involvement of beta-adrenoceptors in the regulation of *CYP1A1*. The lower lanes in panels A–D show the corresponding ethidium bromide-stained RNA at 28S.

Table 2
Adrenoceptor-related changes in the expression of B(α)P-induced *CYP1A1*

Treatment	Western analysis (%)	Northern analysis (%)
Panel A		
B(α)P	100	100
B(α)P + reserpine	200**	400**
B(α)P + guanethidine	70*	35**
B(α)P + adrenaline	70*	55*
Panel B		
B(α)P	100	100
B(α)P + dexmedetomidine	200**	240**
B(α)p + atipamezole	100	100
B(α)P + dexmedetomidine + atipamezole	100	100
Panel C		
B(α)P	100	100
B(α)P + phenylephrine	230**	350**
B(α)p + prazosin	150*	200**
B(α)P + phenylephrine + prazosin	100	100
Panel D		
B(α)P	100	100
B(α)p + isoprenaline	100	100
B(α)P + propranolol	250**	100
B(α)P + isoprenaline + propranolol	150*	100

Semi-quantitative assessment of differentially expressed, B(α)P-induced CYP1A1 mRNA and protein levels as determined by Northern Blot and Western Blot analysis, respectively. All data was normalized against control levels indicated by "100". The column in the middle indicates percentage in CYP1A1 protein levels as compared to control; i.e. B(α)P treatment only. Right column indicates percentage in CYP1A1 mRNA levels, as compared to control. Indicative mean value ± S.E. of control after Western blot analysis is 119,003 ± 3610 pixels and 204,741 ± 8830 pixels after Northern blot analysis. Statistically significant changes in the expression of *CYP1A1* are shown as follows ($^{*}P < 0.05$ and $^{**}P < 0.001$). Analysis is based on data from five individuals in each treatment group. For further details, see Section 2.

3.1. Assessment of central versus peripheral catecholamine involvement in the modulation of the B(α)P-induced CYP1A1 expression

Reserpine-induced generalized catecholamine depletion potentiated the induction of *CYP1A1* by B(α)P, as evidenced by enhanced mRNA (Fig. 1A; Table 2A) and protein levels (Fig. 2A; Table 2A), as well as the EROD activity (Fig. 3).

In contrast, peripheral catecholamine depletion induced by guanethidine (central catecholamines remained intact) suppressed CYP1A1 mRNA (Fig. 1A; Table 2A) and to some extend, protein levels (Fig. 2A; Table 2A), while EROD activity was not modified (Fig. 3). Similarly, peripheral adrenaline administration suppressed CYP1A1 mRNA and protein levels (Figs 1Aand 2A, respectively; Table 2A), while EROD activity remained unaffected.

The results are summarized in Table 3, showing that a generalized catecholamine depletion is associated with a further increase of the B(α)P-induced *CYP1A1 gene* expression, whereas a selective, peripheral catecholamine depletion, where central catecholamines remain intact, has an opposite effect. The results indicate that central catecholamines down-regulate the B(α)P-induced *CYP1A1* expression, an effect which is prevented by peripheral catecholamines. It is noteworthy, however, that enrichment of peripheral catecholamines with adrenaline also suppressed the *CYP1A1* expression.

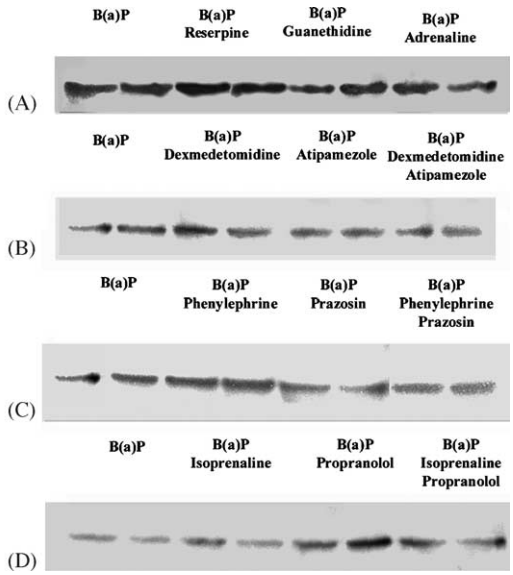


Fig. 2. Western blot showing central and/or peripheral catecholamine involvement in the modulation of CYP1A1 protein levels. Adrenoceptor involvement is also shown. Liver microsomal protein (10 μg) from each rat was resolved by SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose and subjected to immunoblotting using a polyclonal antibody to rat CYP1A1. Two representative samples of five total animals per group are shown and every two lanes represent rats from the same treatment group. Panel A shows involvement of central and peripheral catecholamines in the modulation of CYP1A1 protein; panel B depicts the involvement of α2-adrenoceptors; panel C shows the involvement of α1-adrenoceptors and panel D indicates the involvement of beta-adrenoceptors in the modulation of CYP1A1 protein levels.

3.2. Involvement of adrenoceptors in the modulation of B(α)P-induced CYP1A1 expression

3.2.1. Alpha2-adrenoceptors

Stimulation of α2-adrenoceptors by dexmedetomidine increased the CYP1A1 mRNA and protein levels as com-

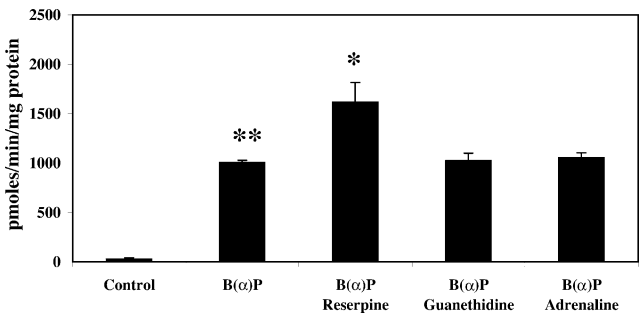


Fig. 3. B(α)P-induced hepatic EROD activity after pharmacological manipulations of central and peripheral catecholamines. EROD activity is expressed in pmoles/min/mg protein; five to six rats per group; all values are expressed as mean ± S.E.; $^{*}P < 0.005$; $^{**}P < 0.001$.

Table 3
The effect of catecholamines on B(α)P-induced *CYP1A1* regulation

	Treatment			
	B(α)P (total catecholamines intact[a])	B(α)P + reserpine (total catecholamines depleted[a])	B(α)P + guanethidine (peripheral catecholamines depleted-central catecholamines intact[a])	B(α)P + adrenaline (enrichment of peripheral catecholamines[a])
CYP1A1 mRNA	100	400**	35**	55*
CYP1A1 protein	100	200**	70*	70*
EROD	100	160#	100	100

The general evidence indicates that central catecholamines down-regulate B(α)P-induced CYP1A1. Comparisons were made between the B(α)P-induced state and after the pharmacological manipulations which altered central and/or peripheral catecholamines. All data is presented as percentage and was normalized against control levels indicated by ''100'', i.e. B(α)P treatment only. Indicative mean value + S.E. of control after Western blot analysis is 119,003 + 3610 pixels and 204,741 + 8830 pixels after Northern blot analysis. Indicative control mean value for B(α)P-induced EROD activity is 1150 pmol/min/g protein. Statistically significant changes in the regulation of CYP1A1 are shown as follows ($^{*}P < 0.05$, $^{\#}P < 0.005$ and $^{**}P < 0.001$).

[a] Catecholamine state.

pared to controls (Figs 1B and 2B; Table 2B). EROD activity was also increased (Fig. 4a). The increase was blocked by atipamezole, an α2-antagonist. Atipamezole, when administered alone, had no effect on *CYP1A1* expression (CYP1A1 mRNA and protein levels: Figs. 1B and 2B, respectively; Table 2B and EROD activity: Fig. 4a).

3.2.2. Alpha1-adrenoceptors

Phenylephrine, an α1-adrenoceptor agonist increased the CYP1A1 mRNA (Fig. 1C; Table 2C) and to a lesser extent the protein levels (Fig. 2C; Table 2C).

Blockade of α1-adrenoceptors with prazosin also led to an increase of both CYP1A1 mRNA and protein levels

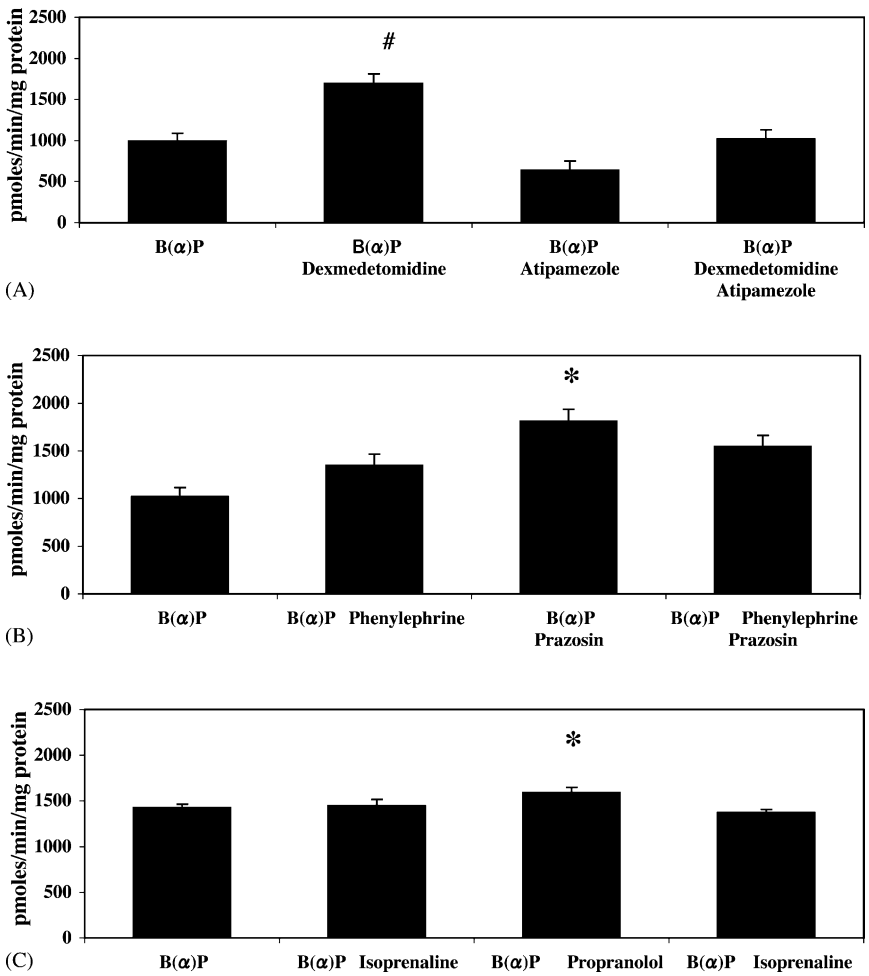


Fig. 4. B(α)P-induced hepatic EROD activity after pharmacological manipulations of adrenoceptors. EROD activity is expressed in pmoles/min/mg protein. Panel a depicts α2-adrenoceptor involvement as shown following dexmedetomidine and/or atipamezole administration. Panel b depicts α1-adrenoceptor involvement as shown following phenylephrine and/or prazosin administration; panel c depicts β-adrenoceptor involvement as shown following isoprenaline and/or propranolol administration; five to six rats per group; All values are expressed as mean ± S.E.; $^{*}P < 0.05$; $^{\#}P < 0.001$.

(Figs 1C and 2C; Table 2C). In this case, the increase was weaker compared to that induced by phenylephrine. EROD activity was also increased (Fig. 4b). This up-regulating effect was reversed by phenylephrine (Figs 1C and 2C; Table 2C and Fig. 4b).

The mechanisms that are involved in phenylephrine and prazosin-induced increases in *CYP1A1* expression are not known. It is hypothesized that phenylephrine's peripheral effect on hepatic plasma membrane α1-adrenoceptor signaling pathways predominates its central inhibiting effect. Accordingly, prazosin increased *CYP1A1* expression likely via blockade of central α1-adrenoceptors, an effect that may predominate the drug's peripheral α1-blocking effect.

3.2.3. Beta-adrenoceptors

Stimulation of beta-adrenoceptors with isoprenaline had no effect on CYP1A1 mRNA levels (Fig. 1D; Table 2D) the protein levels (Fig. 2D; Table 2D) and EROD activity (Fig. 4c).

In contrast, blockade of beta-adrenoceptors with propranolol increased the CYP1A1 protein levels (Fig. 2D; Table 2D) and EROD activity (Fig. 4c), while it had no effect on CYP1A1 mRNA levels (Fig. 1D, Table 2D). In general, manipulations of beta-adrenoceptors appear to have a relatively weak effect on B(α)P-induced *CYP1A1* expression.

In conclusion, it appears that adrenoceptors are involved in the regulation of B(α)P-induced *CYP1A1* expression. In particular, stimulation of α2-adrenoceptors and blockade of α1-adrenoceptors up-regulate the *CYP1A1* inducibility by B(α)P.

3.3. Verification of drug-induced modulation of noradrenaline in hypothalamus

Determination of noradrenaline levels in hypothalamus was carried out in order to verify that the chosen drug treatments had the anticipated effects. As expected, reserpine depleted noradrenaline in hypothalamus, while guanethidine did not (Table 4). Also peripheral adrenaline administration suppressed hypothalamic noradrenaline (Table 4). Dexmedetomidine-induced stimulation of presynaptic α2-adrenoceptors suppressed noradrenaline release in hypothalamus, while α2-blockade with atipamezole increased the noradrenaline release (Table 4). Phenylephrine (α1-agonist) suppressed noradrenaline, while prazosin (α1-blocker) had no effect (Table 4). Propranolol-induced beta-blockade suppressed hypothalamic noradrenaline, while isoprenaline (beta-agonist) had no significant effect (Table 4).

3.4. Assessment of drug effect on plasma corticosterone levels

Reserpine-induced generalized catecholamine depletion was followed by increased plasma corticosterone levels. Peripheral adrenaline administration also increased plasma corticosterone levels compared to controls, while all the other drugs used had no effect (Table 5).

Table 4
Alterations in noradrenaline levels in hypothalamus after pharmacological manipulations in catecholamines and adrenoceptors

Groups	Treatment	Noradrenaline in nmol/g tissue
I	Normal saline	4.5 ± 1.2
	Reserpine	0.2 ± 0.02[a]
	Guanethidine	3.4 ± 1.3
	Adrenaline	0.023 ± 0.005[b]
II	Normal Saline	2.9 ± 0.3
	Dexmedetomidine	1.9 ± 0.2[c]
	Atipamezole	3.6 ± 0.2[d]
	Dexmedetomidine + atipamezole	2.5 ± 0.3
III	Normal saline	3.4 ± 1.3
	Phenylephrine	0.8 ± 0.2[e]
	Prazosin	3.2 ± 1.7
	Phenylephrine + prazosin	2.8 ± 0.8
IV	Normal saline	4.5 ± 1.2
	Isoprenaline	3.3 ± 1.7
	Propranolol	0.13 ± 0.03[f]
	Isoprenaline + propranolol	3.2 ± 1.8

Hypothalamic noradrenaline levels in B(α)P-induced rats following various pharmacological manipulations of catecholamines and adrenoceptors. Data are presented as mean ± S.E. ($n = 5$–6).

[a] The noradrenaline levels are significantly lower in reserpine-treated rats compared to the corresponding control ($P < 0.001$).

[b] The noradrenaline levels are significantly lower in adrenaline-treated rats compared to the corresponding control ($P < 0.001$).

[c] The noradrenaline levels are significantly lower in dexmedetomidine-treated rats compared to the corresponding control ($P < 0.05$).

[d] The noradrenaline levels are significantly higher in atipamezole-treated rats compared to the corresponding control ($P < 0.05$).

[e] The noradrenaline levels are significantly lower in phenylephrine-treated rats compared to the corresponding control ($P < 0.05$).

[f] The noradrenaline levels are significantly lower in propranolol-treated rats compared to the corresponding control ($P < 0.001$).

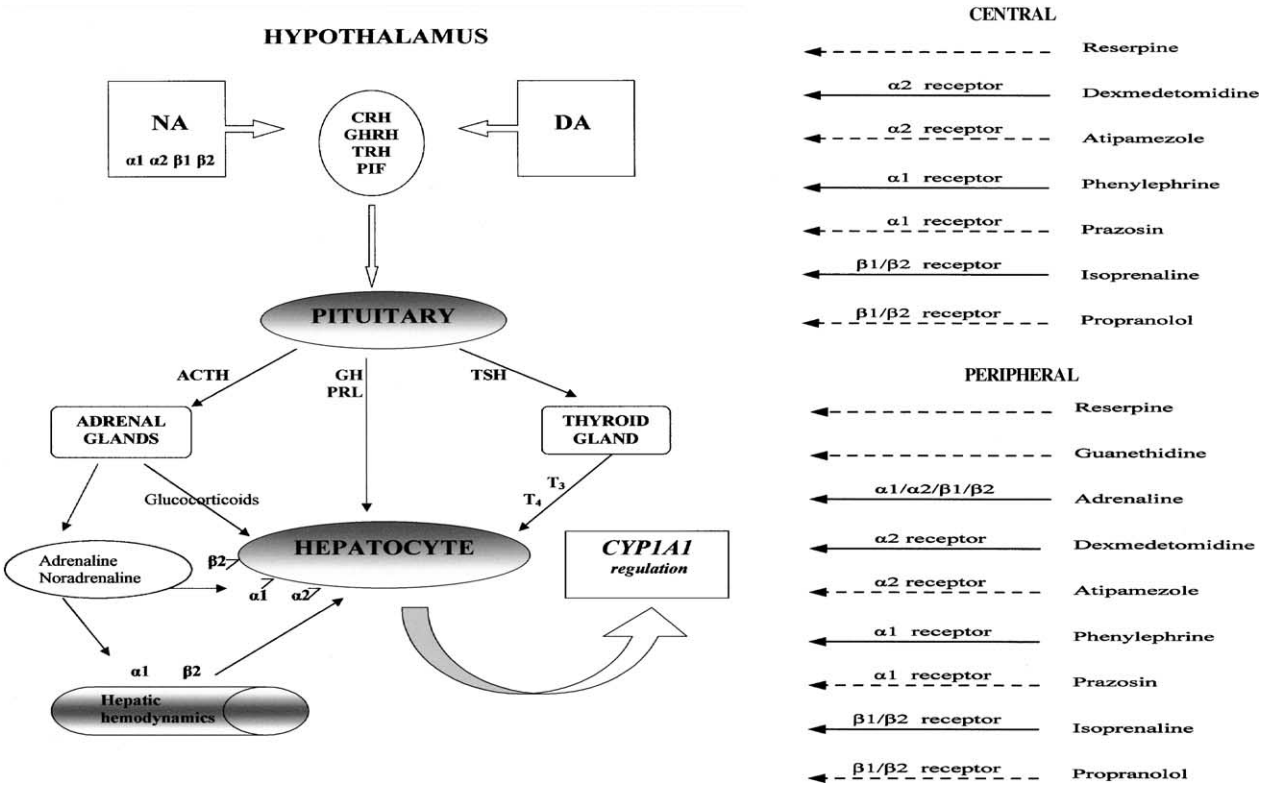
Table 5
Plasma corticosterone levels in B(α)P-induced rats after pharmacological manipulations of catecholamines and adrenoceptors

Treatment	Plasma corticosterone levels (ng/ml)
Oil	164 ± 6
Reserpine	319 ± 34[a]
Guanethidine	191 ± 38
Adrenaline	449 ± 21[b]
Dexmedetomidine	166 ± 16
Atipamezole	140 ± 21
Dexmedetomidine + atipamezole	148 ± 10
Phenylephrine	208 ± 23
Prazosin	189 ± 37
Phenylephrine + prazosin	239 ± 73
Isoprenaline	175 ± 39
Propranolol	166 ± 23
Isoprenaline + propranolol	153 ± 7

Data are presented as mean + S.E. (n:5-6).

[a] Plasma corticosterone levels are significantly higher in reserpine-treated rats compared to the corresponding controls ($P < 0.001$).

[b] Plasma corticosterone levels are significantly higher in adrenaline-treated rats compared to the corresponding controls ($P < 0.001$).



Scheme 1. Modified from Konstandi et al. [12]. Scheme indicates current knowledge about the neuroendocrine influence of the regulation of *CYP1A1 gene* expression. Arrows indicate positive (stimulatory) effects, while dotted arrows depict negative (inhibitory) effects. Pharmacologic drugs used to manipulate circulating catecholamine levels and adrenoceptors are shown on the right-hand side with indications of their site of action (e.g. specific adrenoceptor). NA: noradrenaline, DA: dopamine; CRH: corticotropin releasing hormone; GHRH: growth hormone releasing hormone; TRH: thyrotropin releasing hormone; TSH: thyrotropin stimulating hormone; PIF: prolactin inhibiting factor; GH: growth hormone; T3, T4: thyroid hormones; PRL: prolactin; ACTH: adrenocorticotropin hormone.

4. Discussion

This study showed that adrenoceptor-dependent signaling pathways may modulate the inducibility of hepatic *CYP1A1 gene* by the Ah-receptor ligand B(α)P. The central catecholaminergic system appears to have a negative modulatory role in the expression. Stimulation of central α2-adrenoceptor related signaling pathways seem to up-regulate the expression, whereas stimulation of central α1-adrenoceptor-dependent pathways reduce the expression. By comparison, the beta-adrenoceptor-dependent pathways appear to have a weak modulatory effect on the regulation. In addition to the Ah receptor, the study demonstrates that catecholamine-related signaling pathways may be involved in the regulation of the *CYP1A1 gene*. This is in agreement with our earlier findings that psychological and physical stress can affect *CYP1A1* expression [10,12].

Since stress alters catecholamine release from adrenal medulla and discrete brain subregions [8,10,35,36], we hypothesized that the stress effect on *CYP1A1* expression is mediated by catecholamines. These biogenic amines may be linked to *CYP1A1 gene* regulation pathways; directly or indirectly via various neuroendocrine systems, which play a major role in the regulation of the induction of CYP1A enzymes ([37]; Scheme 1).

To assess whether central and/or peripheral catecholamines are involved in the regulation of *CYP1A1* induction, central and/or peripheral catecholamine depletion was induced with reserpine [24,38]. Reserpine acts as a specific, irreversible inhibitor of the vesicular amine pump, inhibiting the uptake of catecholamines and ultimately resulting in depletion of catecholamine stores [38–40]. On the other hand, peripheral catecholamine depletion was achieved with guanethidine, which does not penetrate the blood brain barrier and thus causes only peripheral sympathectomy [25]. Peripheral catecholamine levels were also manipulated by adrenaline administration. Adrenaline does not penetrate blood–brain barrier and its action is therefore restricted in the periphery [22].

A variety of alpha and beta-adrenoceptor agonists and antagonists was used in the experiments, in order to assess the involvement of adrenoceptors in the regulation of *CYP1A1* induction. Atipamezole is a selective α2-AR antagonist, which penetrates rapidly into the brain and stimulates the release of central and plasma noradrenaline levels [23,41]. On the other hand, dexmedetomidine, a highly selective agonist of α2-adrenoceptors [42], suppresses brain and plasma noradrenaline levels in a dose-dependent manner [23,43,44]. Only subacute treatment with atipamezole or dexmedetomidine has been found to

result in a sufficient increase or inhibition of hypothalamic noradrenaline release, respectively [23,43,45].

Hypothalamic noradrenaline levels were determined in order to verify the effects of pharmacological manipulations. The hypothalamus is involved in the regulation of several hormones that are under noradrenergic control, some of which are known regulators of the induction of *CYP1A genes* ([37]; Scheme 1). It appears that the chosen pharmacological manipulations had the expected effects on noradrenaline levels [23,25,38,43–47].

Reserpine-induced generalized catecholamine reduction increased the expression of the *CYP1A1 gene* at mRNA, protein and enzyme activity levels. In contrast, the guanethidine-induced peripheral catecholamine depletion suppressed the *gene* expression. This suggests, that the central catecholaminergic system has a down-regulating effect on *CYP1A1* inducibility, which is prevented in the presence of peripheral catecholamines. It is noteworthy, however, that enrichment of peripheral catecholamines with adrenaline administration suppressed *CYP1A1 gene* expression. The mechanism underlying this observed discrepancy is not known. One possible explanation is that adrenaline by affecting the hypothalamo–pituitary–adrenal axis feedback loop [36] may have triggered the release of hormones that down-regulate the *CYP1A1* expression [12,37,48–50]. It is also evident that some up-regulating factors of the *CYP1A1* induction exist, which are under central catecholaminergic control.

Although, our studies do not allow us to definitively elucidate the mechanisms by which central catecholamines suppress the *CYP1A1* expression or how peripheral catecholamines eliminate this effect, the fact that the effect of catecholamines is seen on mRNA levels suggests a pre-translational mode of regulation. Furthermore, since stimulation of central pre-synaptic α2-adrenoceptors or blockade of α1- or β-adrenoceptor-related pathways, all led to a further increase of *CYP1A1* expression after B(α)P induction, it is suggested that central α1-, α2- and β-adrenoceptors have a role in the modulation of *CYP1A1* expression. One possibility is that central noradrenergic pathways via central adrenoceptors control the release of several hormones, such as growth hormone and/or thyroid hormones, known to play an inhibiting role in the regulation of B(α)P-induced *CYP1A genes* [12,37,48–50].

Glucocortoids are considered modulators of CYP1A induction by PAH-like compounds [51–54]. The data of the present study does not allow us to elucidate a potential role of glucocorticoids in catecholamine and adrenoceptor-mediated alteration of *CYP1A1* regulation. However, as the available evidence suggests that corticosteroids modulate β-adrenoceptor-AMP systems in brains of stressed animals [55,56], it is likely that the observed effects may be mediated, at least in part, by corticoisteroids. Future studies are required to clearly address this issue in this paradigm.

The alterations in CYP1A1 mRNA levels were not always consistent with the changes in B(α)P-induced EROD activity or with the levels of the corresponding protein. One possibility is that the different pharmacological manipulations may not only affect the transcription of the *gene* but may also have effects on the subsequent post-transcriptional steps along the *gene* expression pathway. In addition, EROD activity is not entirely specific for CYP1A1 but is partly catalysed by CYP1A2, which is regulated differently, at least up to some point, from the CYP1A1 [31–33].

In conclusion, the data of the present study showed that adrenoceptors and catecholamines are involved in the regulation of the B(α)P-induced *CYP1A1* expression. This suggests that drugs that can penetrate the blood–brain barrier and bind to central adrenoceptors or drugs that directly interact with adrenoceptors in the liver, and factors that challenge central and/or peripheral catecholaminergic systems may interfere with the regulation of the *CYP1A1* induction. This, in turn, may alter drug effectiveness or toxicity and carcinogenicity of CYP1A1 substrates [54]. Intriguingly, stress, by altering central and peripheral catecholamine levels may affect drug therapy and toxicity, modulating the expression of the *CYP1A1*. In this regard, the observed increase in the relative risk of lung cancer [57,58] and other types of cancer [59,60] in relation to chronic stress, may be related, in part, to stress-induced alterations in the expression of the carcinogen metabolizing *CYP1A*.

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