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Down-regulation of astroglial CYP2C, glucocorticoid receptor and constitutive androstane receptor genes in response to cocaine in human U373 MG astrocytoma cells

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Abstract

Psychostimulant drugs abuse is associated with an increased risk of stroke. Cytochromes P450 (CYP), especially the astrocytic members of the CYP2C subfamily may play an important role in the modulation of cerebrovascular functions, by generating vasodilatator metabolites from arachidonic acid (AA). Our study examined the regulation of CYP2C genes in response to cocaine or amphetamine in the human astrocyte-like U373 MG cells, using reverse transcription-polymerase chain reaction (RT-PCR) and western-blot analysis. A treatment for 48 h with increasing concentrations of cocaine caused a significant down-regulation of CYP2C8 and CYP2C9 genes and decreased the protein level. These effects were not observed with amphetamine. One mechanism of the CYP2C mRNA regulation implicates various specific receptors including glucocorticoid receptor (GR) and constitutive androstane receptor (CAR). Effects of cocaine on CYP2C were accompanied by a decrease in the GR and CAR gene expression suggesting that these nuclear receptors could be involved in the CYP2C repression by cocaine in the U373 MG cell line. These findings represent a possible molecular mechanism involved in the cerebrovascular risk associated with cocaine abuse.

Keywords: U373 MG astrocytoma; Cocaine; CYP2C; Constitutive androstane receptor; Glucocorticoid receptor

1. Introduction

Psychostimulant drugs like cocaine or amphetamine are known to produce cardio- and cerebrovascular complications. A high frequency of brain perfusion defects and an increased risk of stroke have been observed in psychostimulant users (Billman, 1995; Petitti et al., 1998; Perez et al., 1999).

Abbreviations: CAR, constitutive androstane receptor; CNS, central nervous system; CYP, cytochrome P450; EETs, epoxyeicosatriénoïdes; PCR, polymerase chain reaction; PXR, pregnane X receptor; RT, reverse transcriptase

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Several mechanisms may be responsible for these cerebrovascular complications, like vasoconstriction and thrombosis in cerebral vessels (Konzen et al., 1995). Cocaine induces cerebral vasospasm by a rapid elevation of intracellular calcium in vascular smooth muscle cells (Zhang et al., 1996) and produces an immediate and brief period of vasoconstriction in the large arteries of the brain (Herning et al., 1999). In long-term users of cocaine, endothelium-dependent vasorelaxation is impaired (Havranek et al., 1996). The physiopathology of stroke associated with the use of amphetamines may be either hemorrhagic or ischemic, in relation with elevated blood pressure leading to vessel rupture. Direct vascular toxicity has also been postulated as an etiological factor (Perez et al., 1999).

Cytochromes P450 (CYP)-derived arachidonic acid (AA) metabolites play a role in the modulation of cerebrovascular physiology and pathology (Capdevila et al., 2002). CYP2C is a crucial enzyme subfamily in the vascular function because CYP2C8 and CYP2C9 isoforms leads to the formation of epoxyeicosatrienoic acids (EETs) from AA. These metabolites exhibit a relaxant activity on bovine coronary or rabbit pulmonary arteries and CYP2C8 has even been described as an endothelial-derived hyperpolarizing factor (EDHF) synthase (reviewed by Roman, 2002). In the brain, EETs are produced by the astroglial cytochrome P450 epoxygenase activity and regulate cerebral blood flow (Amruthesh et al., 1993; Harder et al., 2002).

Alkayed et al. (1996) confirm this important role for astrocytes in the control of the cerebral microcirculation, showing that CYP2C11 mRNA is expressed in rat astrocytes and may be responsible for astrocytes epoxygenase activity (Alkayed et al., 1996). One mechanism involved in these cerebrovascular injuries would appear to be a CYP2C modulation in astrocytic cells. Several authors suggest that the mechanism of CYP2C mRNA up-regulation implicates at least three specific receptors: the glucocorticoid receptor (GR), the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) (reviewed by Pascussi et al., 2003a). CAR and PXR are also implicated in CYPs repression. In mouse liver, Beigneux et al. (2002) showed a concomitant decrease in PXR, CAR, cyp2b and cyp3a mRNA expressions in response to inflammation. It was known that GR is expressed in human brain (Joels and de Kloet, 1994), CAR is only reported as primarily expressed in the liver and to a lesser extent in the intestinal epithelium and the stomach (Drocourt et al., 2002; Wei et al., 2002) and PXR is abundantly and selectively expressed in the liver and intestine (Moore and Kliewer, 2000).

In the present work, we detected the total CYP2C, CYP2C8, CYP2C9, GR and CAR mRNA in the astrocyte-like U373 MG cells and we demonstrated a repressor effect of cocaine on these genes.

2. Materials and methods

2.1. Materials

All chemicals were obtained from Sigma (Dorset, UK) excepted Taq polymerase from Qbiogene (Illkirch, France), RNA isolation, oligo kit and reverse transcriptase from Promega (Madison, WI, USA), primers from Sigma Genosis (Cambridge, UK). Cocaine hydrochloride or D-amphetamine sulfate were obtained with the appropriate permission of the French authorities (AFSSAPS, France). Antibodies directed against CYP2C8 and CYP2C9 were provided by Serotec (Oxford, UK) and Gentest (Woburn, MA, USA), respectively.

2.2. Cell culture and treatments

The human astrocytoma cell line U373 MG was purchased from the American Type Cell Collection (ATCC, Rockville, MD). Cells were maintained in DMEM medium supplemented with 10% (v/v) foetal calf serum, penicillin (10 U/ml), streptomycin (0.1 mg/ml) and amphotericin B (25 μg/ml) in an atmospheric mixture of 5% CO_2 and 95% air at 37 °C. The medium was replaced by a serum-free medium and cells were treated during 24 h, 48 h or 72 h with increasing concentrations (1, 10, 100 μM) of cocaine hydrochloride or D-amphetamine sulfate. Treatments were renewed every 24 h.

2.3. Cytotoxicity

After the different treatments, the cytotoxicity was examined by measuring mitochondrial dehydrogenase activity with a spectrophotometric method using MTT. The absorbance was measured in a Power wave (Bio-Tech) at 540 and 690 nm, the latter being substracted