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Regional and cellular distribution of CYP2E1 in monkey brain and its induction by chronic nicotine

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Abstract

CYP2E1 is expressed in liver and extrahepatic tissues, including brain. It metabolizes ethanol and other drugs and toxins, such as acetaminophen, chlorzoxazone and tobacco-derived nitrosamines. Hepatic CYP2E1 is inducible by nicotine, and cigarette smoke accelerates chlorzoxazone metabolism. Smokers have higher levels of brain CYP2E1 expression than non-smokers, but the specific regions and cell types which have the higher expression differ from nicotine-induced rat brain. We therefore investigated the expression and distribution of brain CYP2E1 in a non-human primate, the African green monkey, and determined the effect of nicotine treatment. CYP2E1 levels varied among saline-treated monkey brain regions ($P < 0.01$) and expression was cell-type specific. Chronic nicotine treatment induced CYP2E1 expression in the frontal cortex (1.5-fold, $P < 0.05$) and cerebellum (1.5-fold, $P < 0.01$), specifically in cortical pyramidal neurons and cerebellar Purkinje cells but no change was seen in temporal cortex ($P = 0.20$), hippocampus ($P = 0.29$), putamen ($P = 0.26$) and thalamus ($P = 0.08$). CYP2E1 expression pattern in monkey brain following chronic nicotine treatment is similar to that in smokers, suggesting that nicotine may be the primary component in cigarette smoke that induces CYP2E1. Increased CYP2E1 in brain may contribute to oxidative stress and alter localized metabolism, and resulting pharmacology, of centrally acting drugs metabolized by CYP2E1.

Keywords: CYP2E1; Brain; Monkey; Nicotine; Smoker; Induction

1. Introduction

Cytochromes P450 (CYP) are mixed function oxidases that biotransform drugs, endogenous compounds, dietary constituents and environmental toxins (Lieber, 1999). These enzymes are expressed in the liver and also in other tissues, including lung, kidney and brain. Although CYP content in the central nervous system (CNS) is relatively low (Warner and Gustafsson, 1994), each CYP appears to be localized to specific regions and cell-types in the brain. It is unlikely that brain CYPs contribute substantially to overall clearance of xenobiotics but they may metabolize a variety of compounds increasing or decreasing their pharmacological effects within specific areas of the brain. Given their localized expression and sensitivity to environmental inducers, they may contribute to the observed inter-individual variation in response to centrally acting drugs, their side effects and toxicities (Miksys and Tyndale, 2002).

Cytochrome P450 2E1 (CYP2E1), an ethanol-metabolizing enzyme, is also involved in the metabolism of drugs such as acetaminophen, chlorozoxazone, halothane as well as endogenous substrates such as arachidonic acid, fatty acids, gluconeogenic precursors and estrogenic metabolites (Lieber, 1999; Ohe et al., 2000). It also bioactivates procarcinogens (e.g. tobacco-derived nitrosamines and benzene) and cytotoxins (e.g. carbon tetrachloride) to their reactive intermediates (Lieber, 1999). Expression of human CYP2E1 in mammalian cell lines makes cells highly susceptible to cytotoxicity and DNA damage by nitrosamines (Lin et al., 1998; Lees Murdock et al., 2004). CYP2E1 can also generate reactive oxygen species and substrate-derived radicals that can mediate lipid peroxidation, protein inactivation and DNA damage, leading

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to cellular injury especially in the presence of CYP2E1 inducers (Cederbaum et al., 2001).

In rat brain, CYP2E1 expression was seen in pyramidal cells of frontal cortex, pyramidal and polymorphic cell layer in hippocampus and glial cells in olfactory bulb and piriform cortex (Hansson et al., 1990; Howard et al., 2003). In a pilot study, human CYP2E1 expression was detected in the brains of nonalcoholic nonsmokers in granular cells of the dentate gyrus, pyramidal cells of hippocampus and pyramidal neurons of frontal cortex (Howard et al., 2003).

Cigarette smoking accelerates chlorzoxazone metabolism in humans, most likely by induction of hepatic CYP2E1 activity (Benowitz et al., 2003). Rat hepatic CYP2E1 is inducible by chronic low doses of nicotine, comparable to levels of nicotine in environmental tobacco smoke exposure in humans (Howard et al., 2001; Micu et al., 2003). In addition, cigarette smoke appears to increase CYP2E1 levels in human brain. Higher CYP2E1 immunoreactivity was seen in the Purkinje cells of cerebellum, granular cells of the dentate gyrus, pyramidal cells of CA2 and CA3 hippocampal regions and pyramidal neurons in the frontal cortex of alcoholic smokers (Howard et al., 2003). Chronic nicotine treatment increases CYP2E1 expression in rat brain in a regiospecific manner (Anandatheerthavarada et al., 1993; Howard et al., 2003); however, the pattern of distribution of nicotine-induced CYP2E1 in the rat differs from that found in human brains from smokers. The differences in CYP2E1 brain expression suggest a species (rodent versus primate) or inducer difference (nicotine versus smoking). This could be a result of differences in regulatory factors or variation in brain structure/function between rodents and primates. Whether induction of primate CYP2E1 occurs due to nicotine in cigarette smoke is not known.

Our first objective was to investigate the regional and cellular distribution of CYP2E1 in African green monkey brain from saline-treated animals in order to obtain information about the basal expression of CYP2E1 in primate brain. The second objective was to examine whether chronic in vivo nicotine treatment induces CYP2E1 in the monkey brain in a regiospecific manner and to compare the distribution pattern in monkey brain to that in rat and human brain.

Part of this work has been published previously in the form of an abstract (Joshi et al., 2005).

2. Materials and methods

2.1. Materials

Nicotine bitartrate was purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). The protein assay dye reagent concentrate was obtained from Bio-Rad (Hercules, CA, USA). Prestained molecular weight protein markers were purchased from MBI Fermentas (Flamborough, ON, Canada). Microsomes from CYP2E1-expressing lymphoblastoid cells and anti-human CYP2E1 antiserum used in immunocytochemistry were obtained from BD Gentest (Woburn, MA, USA). The rabbit anti-rat CYP2E1 polyclonal antibody used for Western blotting was generously provided by Magnus Ingelman-Sundberg (Department of Physiological Chemistry, Karolinska Institute, Stockholm, Sweden). Horseradish peroxidase-conjugated goat anti-rabbit antibody and Super-Signal West Pico and Femto chemiluminescence substrate was purchased from Pierce Biotechnology Inc. (Rockford, IL, USA). Biotinylated anti-goat antibody, ABC Elite kit and DAB kit were purchased from Vector Laboratories Inc. (Burlington, ON, Canada). BioTrace NT nitrocellulose membrane was from Pall Corp. (Pensacola, FL, USA) and autoradiography film was purchased from Ultident (St Laurent, PQ, Canada). All other reagents were obtained from standard commercial sources.

2.2. Animals

Male African green monkeys (*Cercopithecus aethiops*, vervet monkeys) were housed at Behavioural Sciences Foundation, St. Kitts. The animals were given Purina monkey chow supplemented with fresh fruit and vegetables, and water ad libitum. The experimental protocol was reviewed and approved by the Institutional Review Board of Caribbean Primates Ltd. and Behavioural Sciences Foundation. All procedures were conducted according to the guidelines of the Canadian Council on Animal Care.

2.3. Drug treatment

Monkeys ($n = 6$ per group) were injected subcutaneously twice daily with saline or nicotine, in the form of nicotine bitartrate in sterile saline adjusted to pH 7.4 as previously described (Schoedel et al., 2003). The nicotine-treated group were given nicotine at 0.05 mg/kg for 2 days followed by 0.15 mg/kg for 2 days and 0.3 mg/kg for 18 days. The total daily dose of nicotine was 0.6 mg/kg and is similar to the average daily amount received by a smoker [0.53 mg/kg of nicotine per day (Benowitz and Jacob, 1984)]. This dosing regime has been shown to decrease hepatic CYP2A6, as seen in smokers (Schoedel et al., 2003). On day 22, 6 h after the morning drug injection, monkeys were euthanized under ketamine anesthesia. Brains were rapidly removed and the halves separated. One half was frozen immediately in liquid nitrogen and the other half was fixed with 4% paraformaldehyde and stored at −80 °C.

2.4. Membrane preparation for Western blotting

Monkey brain regions were dissected by visual differentiation of the major brain regions based on atlases for the macaque (Martin and Bowden, 2000) and rhesus monkey (Snider and Lee, 1961). Whole membranes were prepared as previously described (Miksys et al., 2002). Briefly, membranes were prepared by homogenizing tissues in 100 mM Tris (pH 7.4) containing 0.1 mM EDTA, 0.1 mM DTT and 0.32 M sucrose, centrifuging at 3000 × *g* for 5 min to remove nuclei and cellular debris, followed by centrifugation at 110,000 × *g* for 90 min at 4 °C. Total membrane pellets were resuspended in 100 mM Tris (pH 7.4) containing 0.1 mM EDTA, 0.1 mM DTT, 1.15% w/v KCl and 20% v/v glycerol, aliquoted and stored at −80 °C. The protein content of each sample was assayed with a Bio-Rad Protein Assay Kit.

2.5. Western blotting

Membrane protein from each brain region of a saline-treated animal was serially diluted and used to construct a standard curve to determine the linear range of detection for the assay. Western blotting was performed as described earlier (Miksys et al., 2002). Membrane proteins were separated by SDS–PAGE (4% stacking and 10% separating), transferred overnight onto nitrocellulose membrane, blocked with 3% skim milk in Tris-buffered saline containing 0.05% Triton X-100, and probed with polyclonal rabbit anti-rat CYP2E1 antibody. Blots were then incubated with peroxidase-conjugated anti-rabbit antibody and developed using chemiluminescent detection. Lymphoblastoid-expressed CYP2E1 and monkey liver microsomes were used as internal standards for the detection of CYP2E1. Digital images of immunoblots were analyzed using MCID Elite software (Imaging Research Inc., St. Catherine's, ON, Canada). The density of each protein band was expressed as arbitrary density units after correcting for background.

2.6. Immunocytochemistry

Frozen sections from fixed monkey brain were used for CYP2E1 immunocytochemical analysis. Immunodetection was performed as described earlier