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Gene expression of drug metabolizing enzymes in adult and aged mouse liver: A modulation by immobilization stress

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Abstract

The role of stress in the regulation of enzymatic systems involved in the biotransformation of xenobiotics, as well as endogenous substrates in the liver was investigated using single immobilization stress as a model. Adult (3 months of age) and aged (26 months) C3H/a male mice were used. Cytochrome P450 1A1 and 1A2 (CYP1A1 and CYP1A2), glutathione *S*-transferase M1 (GSTM1), aryl hydrocarbon receptor (AHR), aryl hydrocarbon receptor nuclear translocator (ARNT) and catechol-*O*-methyltransferase (COMT) mRNA levels in the mouse liver were measured by a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) method. Excluding CYP1A1, experiments revealed significant differences in the expression of these genes between adult- and aged-control animals. The influence of stress on the expression of genes studied was shown to be higher in adult mice than in aged ones. Our results clearly demonstrate the lack of response or even the attenuation of gene expression in aged animals that may play an important role in age-related pathologies and diseases.

Keywords: Ageing; Immobilization stress; Drug metabolizing enzymes; Cytochrome P450 1A; Catechol-*O*-methyltransferase; Mouse liver

1. Introduction

Most multicellular organisms exhibit a progressive and irreversible physiological decline that characterises senescence, the molecular basis of which remains unknown. One of the postulated mechanisms includes epigenetic alterations that lead to altered gene expression patterns (Frolkis, 1993). Besides that, a number of key genes involved in neuronal stress reaction and signaling could be differentially expressed in the aged and normal liver, and may contribute to age-related pathology and diseases.

Changes in nervous and hormonal regulation have significance in age-dependent pathologies as well as in

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a number of alterations in cellular metabolism during stress (Frolkis, 1993). Growth hormone, sex steroids, glucocorticoids and thyroid hormones are considerably capable of modulating gene expression of hepatic biotransformation enzymes; in particular, the expression of cytochrome P450s (phase I enzymes), glutathione *S*-transferases (GSTs) and catechol-*O*-methyltransferase (COMT) (phase II enzymes), which play an extremely important role in the maintenance of a stationary level of endogenous ligands, such as estrogens and uroporphyrinogen (Moreno et al., 1980; Mugford and Kedderis, 1998; Srivastava and Waxman, 1993; Whalen and Boyer, 1998). These enzymes influence proliferation, differentiation, apoptosis or necrosis and also cellular homeostasis as well as neuro-humoral function by means of participating in the metabolism of a wide-spectrum of endogenous and exogenous compounds, including therapeutic agents and toxicants. Cytochrome P450s were shown to metabolize steroid hormones (Shou et al., 1997; Suchar et al., 1996; Waxman et al., 1991). Recent studies revealed a direct effect of stress on cytochrome P450 gene expression in the liver of mice and rats (Konstandi et al., 1998, 2000).

The expression of cytochrome P450 1A (CYP1A1 and CYP1A2) gene products is of particular interest because of the role these enzymes play in the activation of exogenous procarcinogens, including polycyclic aromatic hydrocarbons (PAH) and heterocyclic amines (Kadlubar, 1994; Nebert and McKinnon, 1994). Although parent foreign chemicals are often inert, their metabolism by CYP1A enzymes leads to highly reactive intermediates. These metabolites can form adducts with DNA and cause the mutagenic events responsible for tumour initiation (Daly et al., 1994; Ioannides and Parke, 1993). PAH, as many other procarcinogens, induce CYP1A, which is accompanied by an increase of both the mRNA level and the enzyme activity. In this case, the amount of active metabolite formed and the efficiency of its detoxification determine the toxic effect of a carcinogen. This result directly depends on the level of P450 1A isozyme induced, which activates the carcinogen involved.

In the majority of known cases, CYP1A1 and CYP1A2 induction is carried out through an Ah-receptor-dependent mechanism (Dogra et al., 1998; Gonzalez et al., 1993; Nebert et al., 2000). This mechanism involves direct participation by the aryl hydrocarbon receptor (AHR) and the aryl hydrocarbon receptor nuclear translocator (ARNT).

Glutathione *S*-transferase M1 (GSTM1), a μ class GST gene family member, is an important phase II enzyme also involved in the detoxification of many environmental carcinogens, including PAH (Alexandrie et al., 2000; Chen et al., 2002).

Catechol-*O*-methyltransferase is a ubiquitous enzyme that is crucial to the initial steps of metabolic transformation of carcinogenic catechols and catecholamines (Zhu, 2002). In mammals, COMT is distributed throughout various organs with the highest activities being found in the liver and kidney. The liver is considered as playing an important role in the removal of circulating catecholamines in many species. COMT and GSTM1 may play an important role in the pathophysiology of different human disorders, including estrogen-induced cancers (Mitrunen et al., 2002; Yager, 2000).

Recent studies showed that both psychological and physiological stress might modulate drug metabolizing enzymes (Konstandi et al., 1998). Rodents exposed to various stressful conditions show a decreased rate of oxidative metabolism of xenobiotics (Pollack et al., 1991), which could alter the toxicity of these compounds. Different stress models were demonstrated to vary in their effect on biotransformation enzymes. For example, restraint stress increased several enzyme activities in the liver of mice and decreased other ones, while mild unpredictable stress acted in a different, sometimes even in a contrasting way (Konstandi et al., 1998; Matamoros and Levine, 1996).

Up to now, many of the stressors such as handling, immobilization stress, anticipation of a painful stimulus and hypotensive hemorrhage are used in animal research. In our study, single immobilization stress was used as a model (Kvetnansky and Mikulaj, 1970). Hans Selye was the first researcher who used immobilization stress, which led in rats to the manifestation of his stress syndrome: adrenal hypertrophy, gastric ulceration and thymicolymphatic involution (Selye, 1936). Immobilization stress-induced patterns of activation of various stress-effector systems result from restraint, pain stress and changes in body temperature. This type of stress is well studied in connection with a lot of different endocrinal and neural aspects, and it was shown that exposure to 30–180 min of immobilization stress resulted in changes in mRNA level of a number of genes