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RESEARCH ARTICLE

Alterations in Cerebral Xenobiotic-Metabolizing Enzymes in Iodine and/or Selenium Deficient Rats

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Alterations in Cerebral Xenobiotic-Metabolizing Enzymes in Iodine and/or Selenium Deficient Rats

Summary

The aim of this study was to investigate the effects of iodine and/or selenium deficiency on cerebral enzyme levels/activities responsible for xenobiotic metabolism on rats. Three-week old male Wistar rats were used and feeding period was 7-weeks. Selenium deficiency was introduced by a diet containing <0.005 mg/kg selenium, and iodine deficiency was produced by sodium perchlorate containing drinking water. The levels of total microsomal cytochrome P450 (CYP450) and cytochrome b5 (CYP b5) as well as the activities of microsomal NADPH-cytochrome P450 reductase (P450R), aniline hydroxylase (CYP2E1), and cytosolic glutathione S-transferase (GST) were determined. Significant decreases in CYP2E1 activity of iodine deficient, and combined iodine/selenium deficient groups, and marked increase in P450R activity of selenium deficient animals were observed. The generated data suggested that cerebral xenobiotic metabolizing system was affected by the iodine and/or selenium deficiency states which might alter the pharmacological response of central nervous system acting drugs and other xenobiotics in rats.

Key Words: *Iodine deficiency, selenium deficiency, combined iodine and selenium deficiency, thyroid hormones, xenobiotic metabolizing enzymes, cytochrome P450 enzymes*

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İyot ve/veya Selenium Eksikliği Olan Sıçanlarda Serebral Ksenobiyotik Metabolize Edici Enzim Aktivitelerinin Belirlenmesi

Özet

Bu çalışmanın amacı sıçanlarda iyot ve/veya selenyum eksikliğinin serebral ksenobiyotik metabolize edici enzim aktiviteleri üzerine etkilerinin araştırılmasıdır. Üç haftalık erkek Wistar sıçanlar <0.005 mg/kg selenyum içeren diyetle beslenmiştir ve iyot eksikliği içme suyuna sodyum perklorat eklenerek oluşturulmuştur. Total mikrozomal sitokrom 450 (CYP450) ve sitokrom b5 düzeyleri, mikrozomal NADPH-sitokrom P450 redüktaz (P450R), anilin hidroksilaz (CYP2E1) ve sitozolik glutatyon S-transferaz (GST) aktiviteleri belirlenmiştir. Iyot eksikliği olan ve kombine iyot/selenyum eksikliği olan gruplarda CYP2E1 aktivitesinin belirgin bir şekilde azaldığı ve selenyum eksikliği olan hayvanlarda P450R aktivitesinin belirgin şekilde arttığı gözlenmiştir. Elde edilen veriler serebral ksenobiyotik metabolize edici sistemin iyot ve/veya selenyum eksikliğinden etkilendiğini ve bunun da sıçanlarda santral sinir sistemini etkileyen ilaçlar ve diğer ksenobiyotiklere farmakolojik cevabı değiştirebileceğini belirtmektedir.

Anahtar Kelimeler: *İyot eksikliği, selenyum eksikliği, kombine iyot ve selenyum eksikliği, tiroid hormonları, ksenobiyotik metabolize edici enzimler, sitokrom P450 enzimleri*

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INTRODUCTION

Thyroid hormones are essential for the basal metabolism and for the proper development and differentiation of all cells of the human body (1,2). Normal levels of thyroid hormones are vital for the development of the fetal and neonatal brain, and the association between thyroid hormone status and neuro-developmental disorders has been well-established (3). Iodine deficiency is regarded as the most important cause of neurobiological disorders and mental retardation in children throughout the globe and more than one billion people are at the risk of iodine deficiency (4-6). Moreover, maternal thyroid status is of particular importance in fetal brain development (7).

Besides iodine, structure and function of thyroid hormones require adequate availability of selenium, because the isozymes of iodothyronine 5-deiodinases, the enzymes responsible for T_3 production, are selenoenzymes. Therefore, these two essential trace elements affect homeostasis of thyroid hormone-dependent metabolic pathways, and deficiency of iodine and/or selenium has important impact on the health of large populations (4,8). Like iodine, selenium is inadequately available for man and livestock in several parts of the world and its dietary intake is relatively low (9).

Although drug metabolizing cytochrome P450 (CYPs) enzymes are mainly expressed in the liver, multiple forms of CYPs also exist in extrahepatic tissues (10). The functions of CYPs are under the control of several liver-enriched transcription factors and nuclear receptors among which nuclear thyroid hormone receptors exist (11,12). Clinical studies have suggested that in several disease states, expression of oxidative drug metabolizing enzymes are affected at transcriptional or translational level in association with altered circulating concentrations of iodothyronines (13,14). Some CYP450 isoforms are known to exist in brain and show regional and cellular specificity while some forms are identical to their liver specific forms (15,16). Brain CYP450 isoforms they are active and inducible (17,18). Though the CYP450 content is low, bioactivation of drugs or certain chemicals in brain is of considerable importance as brain has a limited capacity of regeneration (19). Furthermore, a recent *in vivo* study has showed that localized metabolism in the brain by certain CYP450s can alter pharmacological effect (20).

Selenium deficiency was shown to increase the total level of CYP450s in liver due to an increase in microsomal antioxidant heme oxygenase (MHO) activity (21,22). Thyroid hormones have also been shown to affect MHO activity and T_3 has been found to affect specific rat liver mRNAs among which CYP450 mRNAs are present (23-25). However, there is no study showing whether these effects on hepatic CYP450 system are also observed in cerebral CYP450s. Moreover, electron donors of P450 system, namely CYP b5 and NADPH-cytochrome P450 reductase (P450R) should also be considered, as the changes in their levels/activities might cause alterations in the activity and rate of CYP-mediated Phase I reactions (26,27). Thyroid hormones were shown to induce P450R activity in liver and extrahepatic tissues (28,29). As CYP b5 is a dynamic protein responding to internal and external factors, it is also affected by the circulating levels of thyroid hormones (27,30). However, there is limited number of studies showing the effects of iodine and/or selenium deficiency on P450R and CYP b5 (22,30).

Glutathione S-transferases (GSTs) are regulated by various hormones including sex hormones, growth hormone and thyroid hormones. T_3 and T_4 reduce the activity of GSTs in hepatocyte cultures (31). However, there are conflicting results in the literature about the effect of selenium deficiency on GST activity and there is no study showing whether cerebral GST levels are affected by different deficiency states (32,33).

The aim of this study was to investigate the effects of iodine and/or selenium deficiency on the levels and/or activities of cerebral enzymes responsible for detoxification or bioactivation of drugs and other xenobiotics on rats.

MATERIALS and METHODS

Chemicals

All chemicals were from Sigma-Aldrich (St.Louis, MO, USA) or Merck (Darmstad, Germany). Selenium deficient diet (<0.005 mg selenium/kg) was supplied by Scientific Animal Food and Engineering (SAFE) Laboratories (Augy, France).

Animals and diets

Male Wistar rats, 3-weeks old, supplied from Hacettepe University Experimental Animals Laboratory, were used in the experiments. The animals were housed as a group in plastic cages with stainless-steel grid tops and the cages were placed in a room with controlled temperature (23°C), humidity (50%) and a 12-hour light-dark cycle. There were 8 animals in each group. Body weights (bw) were monitored weekly. Feeding period was 7 weeks. The animals were treated humanely and with regard for alleviation of suffering, and the study was approved by Hacettepe University Ethical Committee.

Experimental groups

Experimental groups were: (1) Control Group (C), was fed regular rat chow (~0.15 mg/kg selenium) and drinking water; (2) Iodine deficient group (ID) was fed the same regular rat chow and received 1% sodium perchlorate containing drinking water; (3) Selenium deficient group (SeD) was fed selenium-deficient diet containing <0.005 mg of selenium/kg and received normal drinking water; (4) Iodine and selenium-deficient group (ISeD) received selenium-deficient diet (<0.005 mg selenium/kg) and 1% sodium perchlorate containing drinking water.

At the end of feeding period, overnight fasted animals were weighed and then decapitated under i.p. thiopental anesthesia. Brains were rapidly removed, weighed and frozen at -80°C until the preparation of cytosolic and microsomal fractions.

Preparation of cytosolic and microsomal fractions

The homogenization of brain was carried out in a Teflon-glass homogenizer in a volume of potassium chloride-Tris buffer (0.154 M potassium chloride and 50 mM Tris HCl, pH 7.4) to obtain 3 g/ml brain homogenate. Following centrifugation at 2500 x *g* for 10 min, separated supernatant was further centrifuged at 10000 x *g* for 10 min. The latter supernatant was centrifuged at 105000 x *g* for 60 min for 2 times and cytosolic supernatant was collected and used for the measurement of GST activity. The microsomal pellet was re-suspended in a buffer containing Tris- EDTA-sucrose (20 mM Tris, 5 mM EDTA and 0.25 M sucrose; pH 7.4; 1 g brain tissue/ml).

Measured parameters and methods

CYP450 and CYP b5 levels

CYP450 and CYP b5 contents in cerebral microsomes were determined according to the method of Omura and Sato with extinction coefficients of 91 $mM^{-1}cm^{-1}$ (between 490 and 450 nm) and 185 $mM^{-1}cm^{-1}$ (between 424 and 409 nm), respectively (34).

NADPH-cytochrome reductase activity

P450R activity was measured at 550 nm and 37°C by monitoring the reduction of cytochrome c in the presence of NADPH (35).

Xenobiotic metabolizing enzyme activities

Microsomal aniline hydroxylase (CYP2E1) activity was determined by measuring p-aminophenol production according to the method of Imai *et al.* (36). Cytosolic GST activity was determined using 1-chloro-2,4 dinitrobenzene as a substrate, according to the method of Habig et al. (37).

Protein determination

Protein concentrations were determined by the standard method of Lowry et al. (38).

Statistical analysis

All results were expressed as mean ±SEM. Data processing was carried out using a Statistical Package for Social Sciences Program (SPSS) 17.0 for windows packed program. Comparison between the groups was done by t-test. The p values <0.05 were considered significant.

Results

Body, brain and relative brain weights

As shown in the Table 1, after a 7- week of treatment period, iodine deficiency caused a slight (~10%, $p<0.05$) decrease in bw of the animals; while selenium deficiency, and combined iodine and selenium deficiency caused marked decreases (41% in SeD and 25% in ISeD, $p<0.05$) compared to control group. Relative brain weights were found to increase significantly in ID, SeD and ISeD groups (12%, 50%, 21%, respectively, $p<0.05$) compared to control animals.

CYP450 and CYP b5 levels and P450R activity

As shown in Table 2, iodine and/or selenium deficiency did not affect cerebral total CYP450 content

Table 1. Body weight, brain weight and relative brain weight in the study groups.

	Body Weight (g)	Brain Weight (g)	Relative Brain Weight (g/ 100 g bw)
C	239.80 ±5.7[a]	2.00 ±0.02[a]	0.84 ±0.02[a]
ID	215.40 ±8.3[b]	2.01 ±0.04[a]	0.94 ±0.03[b]
SeD	141.78 ±4.8[c]	1.76 ±0.03[b]	1.25 ±0.04[c]
ISeD	179.3 ±4.7[d]	1.92 ±0.02[a]	1.01 ±0.02[b]

Experimental groups: Control group (C); Iodine deficient group (ID),; Selenium deficient group (SeD); Iodine and selenium-deficient group (ISeD)
Values are given as mean ±SEM of duplicate measurements.
[a,b,c]Means within each column that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

and CYP b5 levels. However, selenium deficiency caused a significant increase of 72% ($p<0.05$) in P450R activity in comparison to control group.

Xenobiotic-metabolizing enzyme activities

CYP2E1 activity did not change in SeD group, but decreased markedly in ID and ISeD rats (47%, 30%, respectively, $p<0.05$) vs. control. Cytosolic GST activity was not found to change significantly in any of the groups.

Discussion

A variety of CYPs, including CYP2B, CYP2D, CYP2E1, CYP3A, and CYP4, have been detected in the brains of different species, including mouse, rat, dog, monkey and humans (39,40). Total CYP levels in the brain are low, therefore until recently, it was postulated that CYP-mediated metabolism in the brain does not substantially influence systemic metabolite levels, and is not sufficient to alter the resulting drug effects (41). However, distribution of CYP450s in the brain is heterogeneous, expression levels vary among different brain regions (42). Besides, localization of brain CYP450s in specific regions and in different cell types enables a potentially considerable effect on local metabolism of certain exogenous substances, including toxins and endogenous substances (i.e. neurotransmitters and neurosteroids) in certain brain microenvironments and in the whole brain (43). In specific neurons, the levels/activities of certain CYPs may also be comparable to, or even higher than, levels in hepatocytes (41). In addition, a recent study has provided strong evidence supporting a role for local drug metabolism by brain CYPs in altering the pharmacological actions of drugs (20). The authors showed that selective manipulation of brain CYP2B,

Table 2. The CYP450 and CYP B5 levels; P450R, CYP2E1 and GST activities in the study groups.

	CYP450 (nmol/mg protein)	CYP b5 (nmol/mg protein)	P450R (nmol/mg protein/min)	CYP2E1 (pmol/mg protein/min)	GST (nmol/mg protein/min)
C	0.151 ±0.010[a]	0.135 ±0.001[a]	3.182 ±0.229[a]	0.292 ±0.020[a]	0.030 ±0.003[ab]
ID	0.150 ±0.014[a]	0.148 ±0.010[a]	3.332 ±0.389[a]	0.154 ±0.016[b]	0.028 ±0.003[a]
SeD	0.155 ±0.013[a]	0.131 ±0.014[a]	5.479 ±1.252[b]	0.261 ±0.011[a]	0.035 ±0.0005[b]
ISeD	0.151 ±0.001[a]	0.129 ±0.013[a]	3.648 ±0.601[a]	0.205 ±0.007[c]	0.035 ±0.0006[b]

Experimental groups: Control group (C); Iodine deficient group (ID); Selenium deficient group (SeD); Iodine and selenium-deficient group (ISeD).
Values are given as mean ±SEM of triplicate measurements.
[a,b,c]Means within each column that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

by using CYP2B mechanism-based inhibitors, altered the effects (sleep response) of anesthetic agent propofol, and demonstrated that propofol levels in brain, but not in plasma, increased and no effect was observed on hepatic enzyme activity (20).

Brain CYPs are regulated by transcriptional, post-transcriptional and post-translational mechanisms. In general, molecular mechanisms underlying the regulation of brain CYP expression are poorly understood. However, thyroid hormones are known to be involved in the regulation of CYP450 isozymes and phase II enzymes (30,41). In the present study, there was no alteration in the cerebral drug metabolizing enzymes of ID group except for a marked (47%) decrease in CYP2E1 activity. The alteration in cerebral CYP2E1 in iodine deficiency was more significant than the change (30%) observed in livers of the same animals (30). On the other hand, in our previous study we have shown that pulmonary CYP450 levels (24%) and P450R (33%) activity decreased significantly in ID group while CYP b5 levels increased markedly (33%) vs. control. CYP2E1 (21%) and GST (59%) activities significantly decreased in ID animals vs. control. However, both EROD (24%) and PROD (11%) activities increased markedly in ID group compared to control animals (44).

The effects of iodine deficiency on the other hepatic enzymes, however, were quite different than brain suggesting an organ specific effect (22). CYP2E1 has a ubiquitous role in the biotransformation and activation of several solvent carcinogens such as *N*-nitrosamines, benzene, styrene, carbon tetrachloride, ethylene glycol, several anesthetics (i.e. halothane, isoflurane, enflurane), as well as acetaminophen, dapsone, and theophylline (45). CYP2E1 has also an important role in alcohol metabolism (46). Our results suggested that iodine deficiency might lead to decreases in the metabolism of several drugs and other xenobiotics that undergo biotransformation by this particular enzyme in the brain. On the other hand, there is no data available in literature concerning the effect of iodine plus selenium deficiency on cerebral xenobiotic metabolizing enzymes. The generated data in the present study indicated that in rats, combined deficiency state cause a significant decrease in CYP2E1 activity (30%). Both iodine and selenium deficiency may be responsible for this effect, however, iodine deficiency seems to have a pronounced role in this phenomenon as no significant alteration was observed in selenium deficient group.

The primary function of several forms of cellular selenoproteins is to modulate the intracellular redox equilibrium. Thus, selenium has a critical importance for the cellular antioxidant defense, and its role in the detoxification of several carcinogens has been suggested (46,47). Selenium deficiency was shown to cause alterations in the activity of some glutathione requiring enzymes (48), and existing data indicate significant alterations in hepatic xenobiotic metabolism in selenium deficient animals (30,47). However, some of the reported data are conflicting possibly due to the differences in study designs with respect to feeding period, dietary selenium levels and species of animals used. Although there was no effect of selenium status on the contents of P450 and CYP b5 in earlier studies (21,22), as we reported recently, significant inductions in total CYP450 and CYP b5 contents (~50%, and ~60%, respectively) along with significant alterations in the activities of other enzymes were observed in liver of the same animals (30). In the present study, however, the only significant alteration observed in cerebral enzymes of SeD rats was a marked increase (72%) in P450R activity. Thus, these results showed that the effect of selenium deficiency on xenobiotic metabolizing enzyme activities is rather organ-spesific.

Although limited number of enzymes was examined in the present study, generated data suggested that cerebral xenobiotic metabolizing system is affected by the iodine and/or selenium deficiency state in rats. The alterations caused by these deficiencies in brain might alter the pharmacological response of central nervous system (CNS) acting drugs by altering mainly their CYP450-mediated local metabolism. Further elaboration of impact of such changes on the localized CNS metabolism of centrally active drugs, neurotoxins, as well as endogenous neuro-substrates, such as serotonin and dopamine and, thus, on the normal brain function is needed and will provide valuable information in this context.

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Conflict of interest
The authors declare no conflicts of interest.

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