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Journal of Nutritional Biochemistry 15 (2004) 548–553

THE JOURNAL OF Nutritional Biochemistry

Dietary selenium supplementation prolongs pentobarbital induced hypnosis

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Abstract

The present studies characterized the influence of dietary selenium (Na_2SeO_3) on the duration of pentobarbital (PB) induced hypnosis (sleep) in the rat. Rats were fed semipurified diets varying from 0.01 to 2.0 mg Se/kg for up to 4 weeks. Consumption of diets containing 1.0 and 2.0 mg Se/kg significantly prolonged PB induced hypnosis. Hepatic selenium, but not hepatic glutathione peroxidase activity, correlated with the length of PB induced hypnosis. The prolongation of hypnosis caused by diets containing 1.0 mg Se/kg was substantially reduced or eliminated by repeated exposure to PB. Although single exposure to increasing quantities of PB (60–100 mg/kg body weight) led to a progressive increase in sleep duration, the proportional increase caused by supplemental selenium (2.0 vs 0.1 μg Se/g) remained relatively constant (~25%). Increasing maturity was inversely related to the duration of PB induced hypnosis, regardless of dietary selenium provided. Consumption of the 2.0 mg Se/kg diet prolonged PB induced hypnosis to a greater degree in immature than in mature rats ($P < 0.05$). Consumption of the selenium enriched diet (2 μg Se/g) resulted in an increase in cytochrome 2B, but had no effect on cytochrome 1A compared to controls (0.1 μg Se/g). Pretreatment of rats with P_{450} enzymes activators (i.e., PB, Aroclor 1254, or 3-methylcholanthrene) shortened the duration of PB induced sleep and masked the effects of dietary selenium. The current studies document that dietary selenium can influence the response to pentobarbital induced hypnosis and likely relates to changes in drug detoxification enzymes.

Keywords: Selenium; Hypnosis; Cytochrome; Pentobarbital; Glutathione peroxidase

1. Introduction

The microsomal cytochrome P_{450} superfamily of related isoenzymes is recognized to catalyze the oxidative metabolism of a wide variety of endogenous and foreign compounds [1]. Literally hundreds of natural and synthetic modifiers of the production and activity of these mixed function oxidases (MFOs) and associated components are known to occur. Enhanced liver metabolism and conjugation of pentobarbital (PB) is accompanied by a truncation of the duration of hypnosis caused by this drug. The aliphatic hydroxylation by cytochrome P_{450} dependent monooxygenases, mainly the CYP2B isoform, is the key to biotransformation of pentobarbital and to termination of its biological effects.

Several dietary factors are recognized to influence pentobarbital induced sleep time. For example, increased polyunsaturated fatty acid consumption in the rat is recognized to stimulate liver MFO activity and to decrease pentobarbital induced hypnosis [2]. Providing a diet enriched in α-tocopherol not only lowers lipid peroxidation but also enhances hepatic cytochrome pentoxyresulfin O-dealkylase (PROD) activity in phenobarbital treated rats compared to those fed a diet low in vitamin E [3]. Exposure to compounds such as perfluorochemicals (i.e., perfluorodecalin and perflourooctylbromide) prolong pentobarbital induced sleep, presumably by suppressing MFO activity [4]. Consumption of furoquinolines present in alkaloid fraction of leaves from the *Helietta apiculata* tree has also been observed to prolong pentobarbital induced sleep in the rat. Animals treated with this alkaloid extract have reduced microsomal protein and decreased activity of aminopyrine-N-demethylation and 3,4-benzpyrene hydroxylation [5]. Exposure to herbicides such as 3-amino-1,2,4,-triazol (3-AT) can not only

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0955-2863/04/$ – see front matter
doi:10.1016/j.nutbio.2004.04.002

block the induction of cytochrome P_{450} by pentobarbital but also inhibit the synthesis of heme [6].

Variation in the intake of several minerals can also influence MFO activity. For example, zinc deficiency is recognized to decrease total concentration of microsomal cytochrome P_{450} [7]. Selenium deficient rats treated with phenobarbital exhibited an increased cellular heme pool, possibly because of enhanced rate of cytochrome P_{450} degradation [8]. Selenium depletion is also recognized to impair cytochrome P_{450} induction caused by barbiturates in the rat [9]. Schnell and Early [10] noted that giving sodium selenite intraperitoneally (2.4 mg/kg body weight [BW]) increased hepatic MFO ethylmorphine demethylase and aniline hydroxylase activity. Accompanying these increases was a prolongation of hypnosis in rats given pentobarbital [11]. Nevertheless, it remains to be determined whether dietary selenium at more physiological exposures can lead to similar changes in drug induced hypnosis. This uncertainty serves as the primary aim of the current investigation. Several variables were examined including the influence of variation in selenium intake, quantity and duration of Pentobarbital provided, age/maturity of the rat model, and agents known to modify cytochrome P_{450} activity.

2. Methods and materials

All experiments were performed with male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN). Rats were housed separately in stainless steel cages in a room with controlled temperature (22°C) and lighting (12-hour light–dark cycle). Rats had free access to food and water. The basal diet contained the following: 37% saccharose, 31.5% corn starch, 15% casein, 10% corn oil, 2% cellulose, 3.5% AIN-76 mineral mixture, and 1% of AIN-76 vitamin. The basal diet was found to contain 0.011 μg Se/kg according to the method of McCarthy et al. [12]. The amount of supplemental selenium was experimentally manipulated up to 2.0 μg/g using sodium selenite.

Liver selenium and glutathione peroxidase (GPx) activity were determined by the methods of McCarthy et al. [12] and of Paglia and Valentine [13], respectively. Hydrogen peroxide was used as the substrate for GPx. One unit of GPx activity was defined as 1 mmole of NADPH oxidation per minute per mg protein. The concentration of cytochrome P_{450} was determined in liver microsomal fractions by the method of Omura and Sato [14]. Liver protein was estimated using the Lowry method.

In the first experiment 56 rats weighing initially 106 ± 1 g were randomly assigned to a diet containing 0.01, 0.5, 1.0, or 2.0 μg Se per g. After 4 weeks of experimental feeding all rats were given an intraperitoneal injection of pentobarbital (Nembutal sodium solution, Abbott Laboratories, West Chicago, IL) (60 mg/kg BW) for 3 successive days. Sleep duration was determined after each PB treatment. The sleep time was defined as the time necessary to regain the righting reflex after PB treatment. Return of this reflex was defined by the ability to place the forefeet for standing. Rats were killed by spinal cord displacement immediately after righting occurred. At the termination of each study liver from each rat was perfused in situ with ice cold 1.15% KCl solution, excised, blotted dry, and weighed. Se content and GPx activity were determined in liver homogenate (20%) prepared in 1.15% KCl.

The second study examined the influence of increased PB exposure on the duration of hypnosis in male rats, (n = 42) initially weighting 110 ± 1 g and fed a diet containing either 0.1 or 2.0 μg Se/g. In this study rats were treated with 60, 80, or 100 mg PB/kg BW. Experiment three evaluated BW (age) as a variable influencing PB induced hypnosis. In this study, 42 rats weighing 53 ± 2, 103 ± 3, and 204 ± 5 g were fed a diet containing 0.1 or 2.0 μg Se/g for 2 weeks. The duration of hypnosis was estimated after single PB treatment (70 mg PB/kg BW). In the fourth study, various cytochrome P_{450} modifiers were examined for their ability to influence the response to dietary selenium. In this study, 70 rats initially weighing 74 ± 2 g were fed a diet containing 0.1 or 2.0 μg Se/g for 2 weeks. Sixteen hours before evaluating PB induced hypnosis (70 mg PB/kg BW), rats in each treatment were given an intraperitoneal injection with NaCl (90 mg/kg BW control treatment), pentobarbital (70 mg/kg BW), Aroclor 1254 (500 mg/kg BW), 3-methylcholanthrene (20 mg/kg BW), or 3-amino-1,2,4-triazol (3 g/kg BW). The amount of cytochrome CYP1A and CYP2B in the liver of these rats was determined. Data are expressed as nmol cytochrome per mg of protein.

All data were evaluated statistically by analysis of variance and the application of a least significant difference test for mean comparisons. Mean differences were considered significant at $P < 0.05$.

3. Results

In experiment 1, increased dietary selenium intake did not consistently affect BW gain (16.3 ± 4.9, 20.8 ± 4.6, 22.1 ± 7.4, and 15.1 ± 3.8 g/week for rats fed 0.01, 0.5, 1.0, or 2.0 mg Se/kg, respectively). However, rats consuming the diet containing 2.0 μg Se/g gained significantly less than those fed 1.0 mg/kg during the 4 wk of feeding. Liver GPx activities were significantly elevated with increasing intake of selenium (0.046 ± 0.013, 1.011 ± 0.054, 1.250 ± 0.034, and 1.04 ± 0.132 units, for rats fed 0.01, 0.5, 1.0 and 2.0 mg Se/kg, respectively). Liver Se concentrations in rats were 0.175 ± 0.012, 1.664 ± 0.081, 2.354 ± 0.084, and 3.551 ± 0.144 ng/mg protein, respectively. Each intake was associated with a progressively higher liver selenium content ($P <$ 0.05). In experiment 1, PB induced hypnosis was significantly increased in rats receiving diets containing 1.0 and 2.0 μg Se/g compared to those fed 0.1 μg/g (Fig. 1). Repeated PB exposure was associated with a decrease in

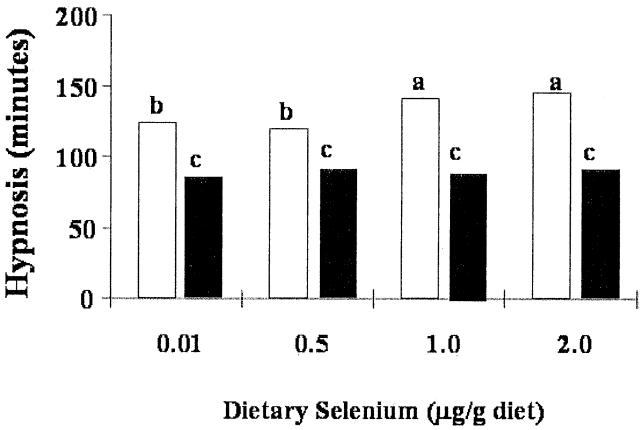

Fig. 1. The influence of feeding dietary selenium (0.01, 0.5, 1.0, and 2.0 μg/g) on pentobarbital (PB) induced hypnosis in male rats. All rats were given an i.p. injection of PB (60 mg/kg body weight) to induce hypnosis. Open bars are after 1 day of PB treatment and solid bars are after 3 successive days of PB treatment. Values are means of seven rats per treatment. Bars not sharing a common superscript letter are statistically different ($P < 0.05$).

hypnosis (day 3 vs day 1) and elimination of prolongation of sleep resulting from exaggerated selenium intake.

In experiment 2, consumption of the diet containing 2.0 μg Se/g diet prolonged hypnosis compared to that in rats fed 0.1 μg Se/g, regardless of the quantity of PB provided (Fig. 2). Again, PB induced hypnosis differences among dietary Se treatments disappeared by day 3 of PB treatment.

Experiment 3 examined the influence of age and dietary

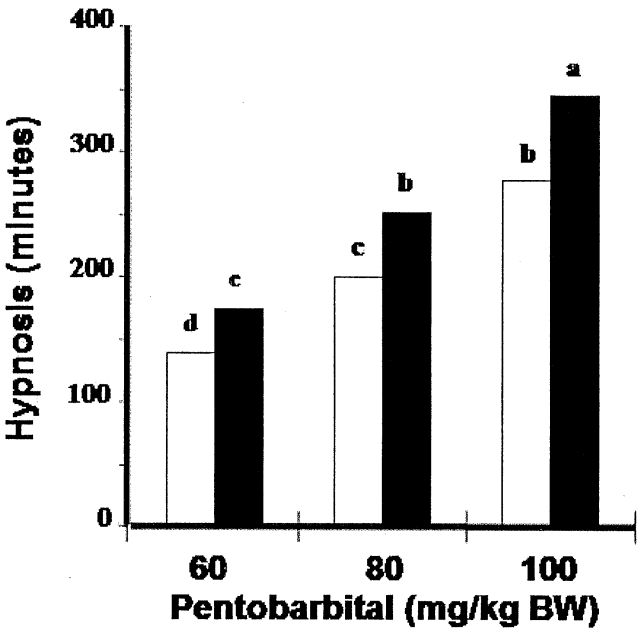

Fig. 2. Interaction between pentobarbital (PB) treatment (60, 80, and 100 mg/kg body weight [BW]) and dietary selenium (0.1 and 2.0 μg/g) on duration of hypnosis in rats. PB was administered for 1 or 3 successive days. Values are means for seven rats. Bars not sharing a common superscript letter are statistically different ($P < 0.05$).

Fig. 3. Influence of body weight (BW) on pentobarbital (PB) induced hypnosis in rats fed a diet containing 0.1 or 2.0 mg μg/g. PB was administered i.p. (70 mg/kg BW).

Se on hepatic Se, activity of GPx, and duration of PB (70 mg/kg BW) induced hypnosis. In rats weighing 200 g the GPx activity in liver was 0.678 ± 0.134 U/mg protein (0.1 μg/g) and 0.917 ± 0.116 (2.0 μg/g), 0.759 ± 0.101 and 0.944 ± 0.107 in the rats weighing 100 g, and 0.760 ± 0.075 and 0.881 ± 0.088 in those weighing 50 g, respectively. The Se concentration in the liver of largest rats was 1.17 ± 0.16 ng/mg protein (0.1 Se μg/g) and 2.97 ± 0.39 (2.0 μg Se /g), in the medium-sized rats 1.60 ± 0.06 and 3.69 ± 0.408, and in smallest rats 1.741 ± 0.275 and 4.88 ± 0.357, respectively. The youngest (50 g) rats slept longer compared to the oldest (200 g) rats (Fig. 3). Selenium supplementation caused a significant prolongation in hypnosis 164.7 ± 45.7 vs 201.9 ± 65.6 minutes. However the response in the oldest (200 g) rats was not statistically different. A highly significant negative correlation between hypnosis and BW was observed ($R^2 = -0.80$, $P < 0.0001$) (Fig. 4). No significant correlation was observed between hepatic GPx and hypnosis ($R^2 = 0.48$). Duration of sleeping time was positively related to hepatic Se concentration. This correlation was significant in all groups of animals and for all rats $R^2 = 0.66$, $P < 0.0001$. A stronger relationship was observed in younger rats ($R^2 = 0.79$) than in older ones ($R^2 = 0.54$) (Fig. 4).

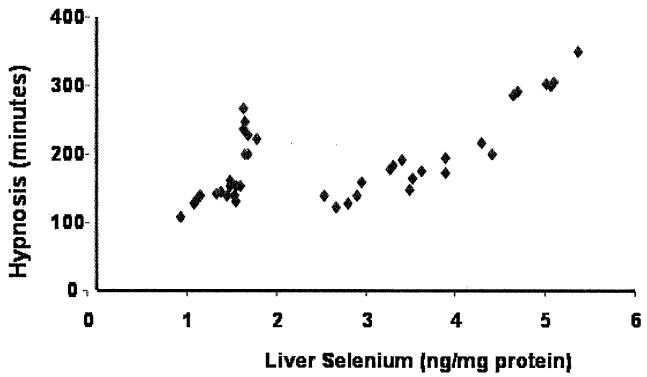

Fig. 4. Correlation between liver selenium concentration and pentobarbital (PB) induced hypnosis in rats. Data are from rats ranging in final weight from 100 to 230 g and receiving either 0.1 or 2.0 mg Se/kg diet. All rats were given an i.p. injection of PB (70 mg/kg body weight).

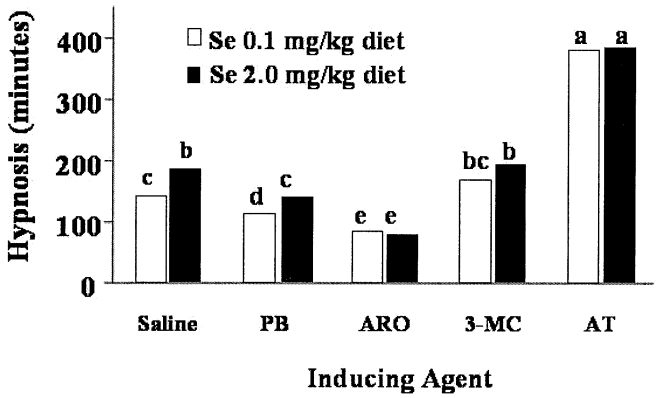

Fig. 5. The influence of cytochrome P-450 modifiers (pentobarbital [PB], Aroclor 1254 [ARO], 3-methylcholanthrene, and 3-amino-1,2,4-triazole [AT]) on the sleep time of rats followed i.p. injection of pentobarbital (PB) (70 mg/kg body weight [BW]). Rats were given 0.1 or 2.0 mg Se/kg diet. Means are given for seven rats. Bars with different superscript letters differ significantly at level of $P = 0.05$.

In experiment 4, pretreatment with cytochrome P_{450} activators (PB, ARO, 3-MC) or the inhibitor (3-AT) 16 hours before use of PB markedly influenced the duration of PB induced sleep time (Fig. 5). Treatment with 3-AT resulted in the greatest duration of hypnosis regardless of selenium intake. Pretreatment with ARO significantly shortened PB induced hypnosis. Treatment with the cytochrome modifiers eliminated differences between Se-adequate (0.1 μg Se/g) and Se-supplemented (2.0 μg Se/g) rats (Table 1). Use of PB or the polychlorinated biphenyls mixture (Aroclor 1254) increased CYP2B without changing CYP1A. Rats given 3-MC exhibited the highest CYP1A activity and only slightly higher CYP2B activity compared to saline treated controls. The use of 3-AT significantly decreased hepatic CYP2B. Rats fed the 2.0 μg Se/g diet had the same concentration of CYP1A but higher concentration of CYP2B than those provided 0.1 μg Se/g and given NaCl. The effects of selenium supplementation were not significant in rats given with any of the cytochrome modifiers.

4. Discussion

The present studies demonstrate that dietary selenium can significantly influence PB induced hypnosis in the rat. Animals fed enriched diets containing 1 or 2 μg Se/g had a prolongation of PB induced sleep. This effect is consistent with observation by Schnell and Early [11] that showed that an acute selenite injection enhanced hypnosis in rats treated with phenobarbital. Elevating dietary selenium to 1 μg/g or more was required to increase PB induced hypnosis. The expansion of hypnotic effect of pentobarbital was observed only after the first day of barbiturate treatment. As the effect of selenium disappeared with multiple PB treatments, the response likely points to a shift in drug detoxification.

The mode of action of barbiturates is recognized to be complex and can involve a host of confounding factors. Pentobarbital is known to have a relatively low partition coefficient and thus enters the brain rather slowly and before binding to GABAA receptors. Multiple uses of this barbiturate are recognized to cause tolerance and dependence, which has been found to correlate in the brain with flunitrazepam binding sites [15], adenylate cyclase and protein kinase activity [16] and NMDA receptor up-regulation [17,18]. Thus the variation in sleep time with increasing exposure to PB may not only reflect induction of cytochrome P_{450} metabolism but also fluctuations in brain receptors number and/or their affinity.

The half-life of PB is determined mainly by inducible cytochrome P_{450} activity in the liver. In the present studies, pretreatment of rats with PB 16 hours before estimating sleep cause a significant increase of CYP2B without changing CYP1A (Table 1). Roe at al. [19] reported that rats given phenobarbital increased the binding to putative AP-1 site in CYP2B2 promoter region. Ganem and Jefcoate [20] have shown that phenobarbital induction as evident by increased mRNA of CYP2B1 and CYP2B2 was also linked with thyroid hormone suppression. In the present studies the decrease in hypnosis was connected with increased hepatic CYP2B (Table 1).

In the current studies a relationship between the hepatic Se concentration and PB induced hypnosis was observed in selenium adequacy (0.1 μg/g) and after Se supplementation (2.0 μg/g) (Fig. 4). Increased dietary selenium given to male rats increase CYP2B in untreated rats and in rats given a single PB treatment (Table 1) compared to those provided a selenium-adequate diet (0.1 μg/g). In rats pretreated with PB, ARO, 3-MC, or 3-AT, the difference caused by selenium supplementation disappeared.

We observed that PB induced hypnosis was highly dependent on the age (weight) of the rat examined (Fig. 3). Hypnosis in the youngest rats (42 days) was about twice as long as that occurring in the oldest (80 days) rats. Consistent with this observation, Agrawal and Shapiro [21] found that cytochrome P_{450} was expressed in both young (65 days) and adult (150 days) rats, but that the phenobarbital induction was about 4 times higher in the adult animals. These authors suggested these differences in induction related to GH secretion patterns. However, using aged rats (>12 months of age), Groen at al. [22] reported that hexobarbital and phenobarbital induction of cytochrome P_{450} enzyme sensitivity to phenobarbital was significantly reduced in such rats compared to younger animals. The influence of dietary Se on prolongation of sleeping time induced by pentobarbital was much more efficient in young rats. Age appears to be an important factor influencing the response to PB and to selenium in rats.

The ability of Se to prolong PB induced hypnosis in rats is consistent with a depression in metabolism of foreign compounds. Selenium supplementation is known to modify the detoxification of several carcinogens, in-

Table 1
Influence of dietary selenium on PB, aroclor 1254, 3-methylocholantrene, and 3-amino-1,2,4-triazole–mediated changes in rat liver CYP1A (P_{450C}) and CYP2B (P_{450B}) content

	CYP1A (nmol/mg protein) Selenium (μg/g)		CYP2B (nmol/mg protein) Selenium (μg/g)	
Treatment	0.1	2.0	0.1	2.0
Control	$0.63 \pm 0.04^{b,c}$	0.66 ± 0.06^{b}	1.03 ± 0.04^{d}	$1.31 \pm 0.04^{d,*}$
NaCl	0.66 ± 0.04^{c}	0.64 ± 0.04^{b}	1.09 ± 0.07^{d}	$1.31 \pm 0.07^{d,*}$
PB	$0.59 \pm 0.13^{b,c}$	0.62 ± 0.05^{b}	1.61 ± 0.12^{b}	1.81 ± 0.15^{b}
Aroclor	0.64 ± 0.06^{c}	0.70 ± 0.04^{b}	2.51 ± 0.09^{a}	2.82 ± 0.31^{a}
3-MC	0.96 ± 0.06^{a}	1.08 ± 0.12^{a}	1.23 ± 0.16^{c}	1.50 ± 0.14^{c}
3-AT	0.53 ± 0.07^{c}	0.68 ± 0.06^{b}	0.68 ± 0.09^{e}	0.81 ± 0.07^{e}

Values are means ± SD for five rats per treatment. Means not sharing a common superscript letter differ ($P < 0.05$). In all rats except controls, PB (70 mg/kg BW) was administered 16 hours after treatment with the cytochrome modifiers. Control rats were treated with saline only.

cluding shifts in oxidation of 7,12-dimethylbenz(a)anthracene and 3,2′-dimethyl-4-aminobiphenyl [23,24]. The mechanism by which selenium likely brings about this effect is multifactorial, but P_{450} involvement seems a logical component [25]. Many chemicals, including carcinogens, are bioactivated to reactive intermediates by cytochrome P_{450} enzymes (phase I) and/or conjugated as glutathione or glucuronide metabolites (phase II). It is known that selenium may influence both phases of xenobiotic biotransformation. Changes in phase I and II enzymes may also explain the present hypnosis findings.

Overall, the current studies provide for the first time clear evidence that dietary selenium intake can influence PB induced hypnosis in rats. However, the response was found to be highly dependent on the duration of PB treatment and on several factors influencing the physiological state. More information is needed to determine whether variation in selenium intake influences the response in humans to a variety of foreign compounds, including those associated with sleep.

References

[1] Gonzalez FJ. The molecular biology of cytochrome P-450s. Pharmacol Rev 1988;40:243–88.

[2] Saito M, Oh-Hashi A, Kubota M, Nishide E, Yamaguchi M. Mixed function oxidases in response to different types of dietary lipids in rats. Br J Nutr 1990;63:249–57.

[3] Lii CK, Sung WC, Ko YJ, Chen HW. α-Tocopherol acetate supplementation enhances rat hepatic cytochrome PROD activity in the presence of phenobarbital induction. Nutr Cancer 1998;32:37–42.

[4] Lutz J, Krafft MP. Longitudinal studies on the interaction of perfluorochemicals with liver cytochromes P-450 by means of testing the rate of detoxification of pentobarbital. Adv Exp Med Biol 1997;411: 391–4.

[5] Golubkova TD, Heckler E, Rates SM, Henriques JA, Henriques AT. Inhibition of cytochrome P450-dependent monooxygenases by an alkaloid fraction from *Helietta apiculata* markedly potentiate the hypnotic action of pentobarbital. J Ethnopharmacol 1998; 60:141–8.

[6] Bhat KS, Padmanaban G. Cytochrome P-450 synthesis in vivo and in a cell-free system from rat liver. FEBS Lett 1978;89:337–40.

[7] Xu Z, Squires EJ, Bray TM. Effects of dietary zinc deficiency on the hepatic microsomal cytochrome P450 2B in rats. Can J Physiol Pharmacol 1994;72:211–6.

[8] Wrighton SA, Elswick B. Modulation of the induction of rat hepatic cytochromes P-450 by selenium deficiency. Biochem Pharmacol 1989;38:3767–71.

[9] Burk RF, Masters BS. Some effects of selenium deficiency on the hepatic microsomal cytochrome P-450 system in the rat. Arch Biochem Biophys 1975;170:124–31.

[10] Schnell RC, Early JL. Interaction between selenium and phenobarbital on drug response and hepatic microsomal enzyme activity in the male rat. Res Comm Chem Path Pharmacol 1981;32:181–4.

[11] Schnell RC, Early JL. Prolongation by selenium of pentobarbital hypnosis in male rats. Experientia 1983;39:176–7.

[12] Mc Carthy TP, Brodie B, Milner JA, Bevill RF. Improved method for selenium determination in biological samples by gas chromatography. J Chromatogr 1981;225:9–16.

[13] Paglia DE, Valentine WN. Studies on quantitative and qualitative characterization of ertythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–69.

[14] Omura T, Sato R. The carbon monooxide-binding pigment of liver microsomes. J Biol Chem 1964;239:2370–8.

[15] Pericic D, Strac DS, Jembrek MJ, Rajcan I. Prolonged exposure to gamma-aminobutyric acid up-regulates stably expressed recombinant alpha1 beta2 gamma2s GABAA receptors. Eur J Pharmacol 2003; 482:117–25.

[16] Oh S, Wellman SE, Ho IK. Changes in (3H) forskolin binding to adenylate cyclase and (3H)phorbol dibutyrate binding to protein kinase C in pentobarbital tolerant/dependent rats. Neurochem Res 1998; 23:463–7.

[17] Oh S, Hoshi K, Ho IK. Role of NMDA receptors in pentobarbital tolerance/dependence. Neurochem Res 1997;22:767–74.

[18] Tseng YT, Miyaoka T, Ho IK. Region-specific changes of GABAA receptors by tolerance to and dependence upon pentobarbital. Eur J Pharmacol 1993;36:23–30.

[19] Roe AL, Blouin RA, Howard G. In vivo phenobarbital treatment increases protein binding to a putative AP-1 site in CYP2B2 promoter. Biochem Biophys Res Commun 1996;228:110–4.

[20] Ganem LG, Jefcoate CR. Endocrine factors modulate the phenobarbital-mediated induction of cytochromes P 450 and phase II enzymes in a similar strain-dependant manner. Toxicol Appl Pharmacol 1998; 150:68–75.

[21] Agrawal AK, Shapiro BH. Phenobarbital induction of hepatic CYP2B1 and CYP2B2: pretranscriptional and post-transcriptional effects of gander, adult age and phenobarbital dose. Mol Pharmacol 1996;49:523–31.

[22] Groen K, Breimer DD, Jansen EJ, van Bezooijen CF. The influence of aging on the metabolism of simultaneously administered hexobar-

bital enantiomers and antipyryne before and after phenobarbital induction in male rats: longitudinal study. J Pharmacol Exp Ther 1994; 268:531–6.

[23] Schaffer E, Lui JZ, Milner JA. Garlic powder and allyl sulfur compounds enhance the ability of dietary selenite to inhibit 7,12-dimethylbenz(α)anthracene-induced mammary DNA adducts. Nutr Cancer 1997;27:162–8.

[24] Feng Y, Finley JW, Davis CD, Becker WK, Fretland AJ, Hein DW. Dietary selenium reduces the formation of aberrant crypts in rats administered 3,2′-dimethyl-4-aminobiphenyl. Toxicol Appl Pharmacol 1999;157:36–42.

[25] Pascoe GA, Sakai-Wong J, Soliven E, Correia MA. Regulation of intestinal cytochrome P-450 and heme by dietary nutrients. Biochem Pharmacol 1983;32:3027–35.