SELENIUM AND HEPATIC MICROSOMAL HEMOPROTEINS[1]

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SUMMARY

The microsomal share of liver homogenate ^{75}Se after injection of a tracer dose of $^{75}SeO_3^{2-}$ was three times greater in rats fed a selenium-deficient diet than in rats fed a selenium-adequate diet. Basal levels of microsomal cytochromes P-450 and b_5 were unaffected by selenium deficiency. However, induction of these cytochromes by phenobarbital was markedly impaired in selenium-deficient rats, whereas liver weight increase and NADPH cytochrome c reductase induction were not impaired. These data indicate that selenium is essential for phenobarbital induction of microsomal hemoproteins.

Although selenium is considered an essential element for many species, little is known of its specific biochemical function in animals except that it is a constituent of glutathione peroxidase (1). In this report we describe a selective incorporation of selenium into hepatic microsomes of selenium-deficient rats and impaired phenobarbital induction of cytochromes P-450 and b_5 in these animals. These data suggest that selenium may have an important role in hepatic microsomal function.

MATERIALS AND METHODS

From the time they were received male Holtzman rats[3] (weight 60-90 g)

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[3] In conducting the research described in this report the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

were fed a selenium-deficient Torula yeast diet (2). Two dietary groups were formed - one with added selenium (0.5 mg selenium/kg as Na_2SeO_3) and the other without selenium supplementation.

Subcellular distribution of ^{75}Se. Four rats from each dietary group fed the experimental diet for 14 months were each injected intraperitoneally with 200 μCi of $^{75}SeO_3^{2-}$ (2.2 μg selenium/kg). Animals were killed by exsanguination 7 days later. The livers were removed and 3 g of each was homogenized in 9 volumes of cold 0.25 M sucrose by 3 passes of a Teflon pestle at 2000 r.p.m. Subcellular fractions were separated by differential centrifugation -- nuclear fraction 600 x g for 10 min, mitochondrial fraction 14,000 x g for 10 min, microsomal fraction 105,000 x g for 1 hour -- and resuspended in 0.25 M sucrose. The ^{75}Se content of a 0.5 ml aliquot of each fraction was determined, and total ^{75}Se in each fraction was calculated.

Microsomal proteins and response to phenobarbital. Animals from each dietary group fed the experimental diet a minimum of 3 months were subdivided into two further groups. Within each dietary group some animals received a daily intraperitoneal injection of phenobarbital (75 mg/kg) for 4 days, while the other animals received only the vehicle 0.9% NaCl. All animals were killed by exsanguination 24 hours after the last injection. The livers were homogenized as above in 4 volumes of cold 0.25 M sucrose and centrifuged at 9000 x g for 35 min at 4° C. The supernatant was then centrifuged at 105,000 x g for 1 hour at 4° C, and the microsomal pellet was washed according to the method of Weihing et al.(3). The washed pelleted microsomes were frozen and held overnight. They were then resuspended in 0.1 M Tris-HCl, pH 7.4, to a concentration of 1.5 - 2.0 mg protein per ml. Cytochromes P-450 and b_5 were assayed by the method of Omura and Sato (4) using a Cary model 14 spectrophotometer with a 0 - 0.2 slide wire. NADPH cytochrome c reductase was measured by the method of Baron and Tephly (5) at 37° C. Not all determinations were performed on each microsomal sample. NADPH, NADH and cytochrome c were obtained from Sigma Chemical Company, while sodium dithionite and carbon monoxide were purchased from Matheson Scientific.

TABLE I. Subcellular distribution of ^{75}Se in livers of rats fed selenium-deficient (0 ppm) and selenium-adequate (0.5 ppm) diets

Subcellular Fraction	Percentage of Homogenate ^{75}Se[a]	
	0 ppm	0.5 ppm
Nuclear	24.8 ± 1.8	27.4 ± 5.7
Mitochondrial	19.3 ± 1.2	18.4 ± 2.8
Microsomal	23.1 ± 1.8	7.1 ± 0.9
Soluble	32.8 ± 1.2	47.1 ± 2.5
Total	91.3 ± 5.5	88.1 ± 4.9

[a] Mean ± S.D. from 4 rats

RESULTS

Subcellular distribution. As illustrated in Table I the hepatic subcellular distribution of ^{75}Se was influenced by the dietary selenium content. In selenium-deficient animals a greater proportion of hepatic ^{75}Se was found in the microsomal fraction (23.1% compared to 7.1%) while its incorporation into the soluble fraction was correspondingly reduced.

Microsomal response to phenobarbital. Basal levels of cytochromes P-450 and b_5, as well as NADPH cytochrome c reductase, were unaffected by the selenium content of the diet (Table II). Liver weight and NADPH cytochrome c reductase level increased in response to phenobarbital administration regardless of selenium status. However, microsomal cytochrome P-450 and cytochrome b_5 content failed to rise in response to phenobarbital in the selenium-deficient rats, while a clear response to such treatment was observed in the selenium-replete rats.

DISCUSSION

The observation that a greater than normal proportion of hepatic

TABLE II. Effect of dietary selenium concentration and phenobarbital administration on liver weight and microsomal P-450, cytochrome b_5, and NADPH cytochrome c reductase

Selenium added to diet	Phenobarbital	Liver Wt.[a] (g/100 g body wt.)	Cytochrome[a,b] P-450	Cytochrome[a,b] b_5	NADPH Cytochrome[a,c] c Reductase
0 ppm	-	3.18 ± 0.46(5)[d]	0.73 ± 0.15(5)	0.93 ± 0.10(3)	141 ± 12(4)[h]
	+	4.53 ± 0.71(6)[d]	0.92 ± 0.24(6)	1.01 ± 0.24(4)	262 ± 35(5)[h]
0.5 ppm	-	2.98 ± 0.28(6)[e]	0.84 ± 0.26(6)[f]	1.01 ± 0.31(4)[g]	136 ± 13(4)[k]
	+	4.42 ± 0.32(6)[e]	1.85 ± 0.42(6)[f]	1.58 ± 0.14(4)[g]	294 ± 15(4)[k]

[a] All values ± S.D. with No. of rats studied in parentheses
[b] nanomoles/mg protein
[c] nanomoles cytochrome c reduced/mg protein/min
[d-k] Pairs with same superscript different by t-test, $p < 0.05$

homogenate ^{75}Se was present in the microsomal fraction of selenium-deficient rat liver suggested that selenium might have an important role in hepatic microsomal function.

The data presented in Table II demonstrate that the normal response of cytochromes P-450 and b_5 to phenobarbital administration was severely impaired by selenium deficiency. However, although the inductive response of the hemoproteins was restricted, both liver weight and the flavoprotein NADPH cytochrome c reductase exhibited a normal response to phenobarbital in the selenium-deficient animals. Thus, selenium deficiency is apparently selectively associated with impaired microsomal hemoprotein response to phenobarbital.

How selenium facilitates microsomal hemoprotein induction by phenobarbital is unknown. In spite of the recent discovery of a selenium-containing hemoprotein in sheep skeletal muscle (6), it is very unlikely that selenium is a constituent of either cytochrome P-450 or cytochrome b_5 because each is present in rat liver in a concentration of around 40 nmoles per g (7) while microsomal selenium content is approximately 0.8 nmoles per g of liver by calculation from Table I and published liver selenium contents (8).

In the present study the hemoproteins P-450 and b_5 were assayed spectrophotometrically, and thus the heme portion of the molecule was the assayed moiety. Preliminary studies from this laboratory, in which microsomal proteins have been separated by sodium dodecyl sulfate-urea polyacrylamide gel electrophoresis, indicate that in both selenium-deficient and selenium-replete animals the apoprotein moiety of cytochrome P-450 increases in response to phenobarbital stimulation. The apparent non-responsiveness of cytochromes P-450 and b_5 to phenobarbital stimulation in the selenium-deficient rat may, therefore, be a manifestation of a primary role for selenium in hepatic heme synthesis, intracellular heme transport, or possibly the attachment of heme to the apoprotein moiety of these cytochromes.

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