INDUCTION OF CYTOCHROME P-450 ISOZYMES DURING LONG-TERM NIFEDIPINE ADMINISTRATION

L. F. Gulyaeva, A. Yu. Grishanova, V. M. Mishin, M. Koleva, Ts. Stoichev, and V. V. Lyakhovich

UDC615.224.015.4:612.015.1:577.152.121

KEY WORDS: nifedipine; induction; cytochrome P-450 isozymes.

The wide substrate specificity of the microsomal monooxygenase system, catalyzing oxidation of many xenobiotics and endogenous compounds, is determined by the existence of multiple forms of cytochrome P-450 [13]. A modern approach to the characterization and identification of the individual cytochrome P-450 isozymes is to study specific reactions catalyzed by these hemoproteins. There are substrates whose metabolism is more specific for a particular cytochrome P-450 isozyme. For example, the O-de-ethylation of 7-ethoxyresorufin is catalyzed by cytochrome P-450$_c$ [3]. The steroid hormones testosterone, androstenedione (AD), and progesterone are utilized as specific substrates by the microsomal monooxygenase of the liver, with well-marked regional and stereospecificity of metabolism [16]. The rate of formation of hydroxylated metabolites gives information on activity of different forms of cytochrome P-450. For example, the formation of 16α-OH-AD is catalyzed by cytochrome P-450$_h$, which is characterized only of male rats. The formation of 16β-OH-AD is catalyzed by P-450$_b$, that of 7α-OH-AD by cytochrome P-450$_a$, and that of 6β-OH-AD by cytochrome P-450$_p$ [15].

Thus the specificity of the reaction of metabolism of certain substrates can serve as an important criterion for the identification of many forms of cytochrome P-450. On the other hand, the immunochemical method using monospecific antibodies against individual cytochrome P-450 isozymes makes it quantitative and qualitative determination in microsomes possible. This combined approach can be used in pharmacologic investigations, especially those in which the drug influences the microsomal monooxygenase system.

The calcium antagonist nifedipine, widely used in cardiologic practice, is metabolized by cytochrome P-450$_p$ in rat liver microsomes [6] or by P-450$_{NF}$ in human liver microsomes [14], which are induced by the synthetic steroid pregnenolone-16-carbonitrile and steroids of the glucocorticoid class, such as dexamethasone and macrolide antibiotics [12].

Previously the writers described induction of a cytochrome P-450-dependent monooxygenase system after long-term administration of nifedipine to rats [7]. The aim of the present investigation was to discover what forms of cytochrome P-450 can be induced under these conditions.

EXPERIMENTAL METHOD

Nifedipine was injected intraperitoneally into 24 male Wistar rats in a dose of 10 mg/kg daily for 20 days. Nifedipine was dissolved in water with a few drops of Tween-80. Control rats received the same volume of solvent. The animals were decapitated on the 21st day (24 h after the last injection of nifedipine). The rats were given phenobarbital (80 mg/kg) intraperitoneally daily for 4 days and their microsomes were used to obtain cytochrome P-450 and b_5 [9]; NADPH-cytochrome P-450 reductase activity was estimated as the rate of reduction of cytochrome *c* at 22°C [10]. Protein was determined by Lowry's method.

Department of Cell Physiology and Pathology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Department of Drug Toxicology, Institute of Physiology, Bulgarian Academy of Sciences, Sofia. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 2, pp. 148-150, February, 1990. Original article submitted March 2, 1989.

 0007-4888/90/0002-0180$12.50

TABLE 1. Action of Nifedipine on Microsomal Monooxygenase System of Rat Liver ($M \pm m$)

Parameters	Control	Expt.
Cytochrome P-450, nmoles/mg	0,766±0,04	0,831±0,05
Cytochrome P-450$_{b/e}$, nmoles/mg	0,007±0,002	0,036±0,008
Cytochrome b_5, nmoles/mg	0,460±0,02	0,443±0,038
NADPH-cytochrome c reductase activity, nmoles/min	66,38±7,80	67,77±3,98
Mono-oxygenase activity, nmoles/min/mg protein		
benzpyrene-hydroxylas	0,500±0,15	0,368±0,07
7-ethoxyresorufin-O-de-ethylase	0,05±0,010	0,045±0,010
benzphetamine-N-demethylase	3,46±0,14	3,80±0,33
Androstenedione hydroxylase:		
6β-	2,04±0,44	4,02±0,58*
7α-	0,107±0,03	0,222±0,02*
16α-	3,32±0,24	4,27±0,30
16β-	0,154±0,02	0,589±0,13*

Legend. *$p < 0.05$ compared with control.

The rate of N-demethylation of benzphetamine was determined by measuring formaldehyde formation [8]. The rate of hydroxylation of benzpyrene was measured fluorometrically, using 3-OH-benzpyrene as the standard [12], and the rate of O-de-ethylation of 7-ethoxyresorufin was measured fluorometrically, using resorufin as the standard [3]. Androstenedione metabolism was studied as described in [16], using corticosterone as the internal standard; the metabolites were analyzed by high-pressure liquid chromatography [16].

Cytochrome P-450$_b$ was obtained from liver microsomes of phenobarbital-induced rats by the method in [6], using chromatographic sorbents 1,8-diamino-octyl-sepharose 4B and DEAE-sephacel. Preparations of cytochrome P-450$_b$ had a purity of 16 nmoles/mg protein and were electrophoretically homogeneous. Antibodies against cytochrome P-450 were obtained as described previously [1]. The quantity of cytochrome P-450$_b$ was determined by immunoelectrophoresis [11].

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The results given in Table 1 can be used to compare functional parameters of liver microsomes of the experimental and control rats. Clearly there were no significant differences in the total cytochrome P-450 and cytochrome b_5 levels. Activity of NADPH-cytochrome c reductase, as well as that of benzphetamine-N-demethylase and 7-ethoxyresorufin-O-de-ethylase, was unchanged, whereas activity of benzpyrene hydroxylase was reduced, though not significantly, after 20-day administration of nifedipine. The study of androstenedione metabolism revealed an increase in the rate of formation of its hydroxylated products 7α-, 16β-, and 6β-OH-AD in the liver microsomes of nifedipine-treated rats compared with the controls. The concentration of cytochrome P-450$_{b/e}$ in the microsomes of nifedipine-treated rats was determined by rocket immunoelectrophoresis, and was found to be 2-5% of the total cytochrome P-450 content, whereas in the control rats the cytochrome P-450$_{b/e}$ content did not exceed 1.2%.

The increase in the rate of formation of 7α-, 16β-, and 6β-hydrolylated products of AD suggests induction of cytochromes P-450$_a$, P-450$_b$, and P-450$_p$, catalyzing hydroxylation of AD in 7α-, 16β-, and 6β-positions, respectively. The nifedipine-induced increase in the cytochrome P-450$_{b/e}$ content, confirmed in our experiments by rocket immunoelectrophoresis, was smaller than that induced by the classical P-450$_{b/e}$ inducer phenobarbital (in the latter case the P-450$_{b/e}$ content was not more than 50% of the total cytochrome P-450 content [1]. Indirect proof of induction of cytochrome P-450 was obtained previously by the present writers, who found a decrease in the duration of hexobarbital sleep, suggesting an increase in metabolism of hexobarbital the substrate of cytochrome P-450$_b$ [7]. The 6β-hydroxylation of AD is catalyzed by cytochrome P-450$_p$ and by cytochrome P-450$_c$ less effectively. In our view, the increase in the rate of hydroxylation of AD following administration of nifedipine takes place through induction of cytochrome P-450$_p$, and not of P-450$_c$, for the rates of metabolism of benzpyrene and 7-ethoxyresorufin, cata-

lyzed by P-450$_c$, were not increased. Coordinated synthesis of cytochromes P-450$_b$ and P-450$_p$ during repeated injections of nifedipine is in agreement with data on the synchronous induction of these two isozymes during treatment by various inducers [4]. No marked increase in the total content of cytochrome P-450 was found after administration of nifedipine to the animals. Induction of cytochromes P-450$_a$, P-450$_b$, and P-450$_p$ by nifedipine may therefore be connected with repression of other forms of cytochrome P-450.

Nifedipine is thus not only a specific substrate of cytochrome P-450$_p$. Long-term administration of nifedipine to rats leads to induction both of this cytochrome and also of cytochromes P-450$_a$ and P-450$_b$.

The authors are grateful to Professor D. N. Kirk (University of London Queen Mary College, London, England) for providing the androstenedione metabolites.

LITERATURE CITED

1. N. I. Gutkina, V. M. Mishin, and V. V. Lyakhovich, Biokhimiya, **51**, 1223 (1986).
2. I. B. Tsyrlov and V. V. Lyakhovich, Biokhimiya, **44**, 1172 (1979).
3. M. D. Burke and R. T. Mayer, Chem.-Biol. Interact., **45**, 243 (1983).
4. G. A. Dannan, F. P. Guengerich, L. S. Kaminsky, and S. D. Aust, J. Biol. Chem., **258**, 1282 (1982).
5. F. P. Guengerich and M. V. Martin, Arch. Biochem., **205**, 365 (1980).
6. F. P. Guengerich, M. V. Martin, P. H. Beaune, et al., J. Biol. Chem., **261**, 5051 (1986).
7. M. Koleva and Ts. Stoichev (Stoytchev), C. R. Acad. Bulg. Sci., **41**, 147 (1988).
8. T. Nash, Biochem. J., **55**, 416 (1953).
9. T. Omura and R. Sato, J. Biol. Chem., **239**, 2379 (1964).
10. A. H. Phillips and R. G. Langdon, J. Biol. Chem., **237**, 2652 (1962).
11. C. B. Pickett, R. L. Jeter, J. Morin, and A. Y. H . Lu, J. Biol. Chem., **256**, 8815 (1981).
12. D. E. Ryan, P. E. Thomas, L. M. Reik, and W. Levin, Xenobiotica, **12**, 727 (1982).
13. E. G. Schuetz, S. A. Wrighton, J. L. Barwick, and P. S. Guzelian, J. Biol. Chem., **259**, 1999 (1984).
14. P. B. Watkins, P. Maurel, E. G. Schuetz, et al., Fed. Proc., **44**, 1206 (1985).
15. D. J. Waxman, Biochem. Pharmacol., **37**, 71 (1988).
16. A. W. Wood, D. E. Ryan, P.E. Thomas, and W. Levin, J. Biol. Chem., **258**, 8839 (1983).