ENZYMIC PEROXIDATION OF LIPIDS AND OXIDATIVE METABOLISM OF CHLORPROMAZINE IN BRAIN MICROSOMAL FRACTION

I. A. Eluashvili, L. L. Prilipko, and V. E. Kagan

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Enzymic NADPH-dependent systems of lipid peroxidation and chlorpromazine metabolism were shown to be localized in rat and human brain microsomal fractions (BMF). Hydroxy-derivatives of chlorpromazine [7-hydroxychlorpromazine (7-OH)] formed as a result of enzymic NADPH-dependent metabolism possess antioxidant action and inhibit enzymic peroxidation of lipids (POL) in brain microsomes. The properties of enzymic NADPH-dependent oxygenase systems in the membranes of the endoplasmic reticulum of the liver and BMF are compared.

KEY WORDS: peroxidation of lipids; chlorpromazine metabolism; liver and brain microsomes.

Enzymic generators of active forms of oxygen, capable of inducing peroxidation of lipids (POL), are localized in the brain microsomal fraction (BMF) [7, 14]. The accumulation of POL products in biomembranes may be the cause of inhibition of membrane-bound enzymes [10, 15], including Na,K-ATPase in BMF [12]. In this connection it is extremely important to study endogenous mechanisms of regulation of free-radical oxidation of lipids, and also methods of deliberate intervention into POL processes in the membranous structures of the brain. Hydroxylation products of hydrophobic xenobiotics by systems of multipurpose oxygenases in the membranes of the endoplastic reticulum (EPR) of the liver [3], including hydroxylated derivatives of the psychotropic compound chlorpromazine [4], are known to possess antioxidant action.

In the investigation described below the mechanisms of interaction between the reactions of POL and oxidative metabolism of chlorpromazine by BMF were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male albino rats weighing 150-200 g, and also on human brain tissue taken from clinically healthy subjects aged 50-70 years 2-4 h after death resulting from cardiovascular failure. The microsomal fraction was isolated from rat liver by the method described in [1] and the microsomal fraction from rat and human brain as described in [14], with additional centrifugation at 105,000g for 60 min in a medium of 50 mM Tris-HCl + 100 mM NaCl, pH 7.0, to remove sucrose. Incubation of the microsomes and the method of determining the content of POL products and chlorpromazine metabolites were fully described in [4]. The concentration of cytochrome P-450 and NADPH-cytochrome c-reductase and activity of 3,4-benz(a)pyrenehydroxylase were determined by methods suggested in [2, 8, 11].

EXPERIMENTAL RESULTS AND DISCUSSION

The results illustrated in Fig. 1 show that during aerobic incubation of rat BMF in the presence of reduced pyridine-nucleotides (NADPH, NADH) POL products accumulate. The enzymic nature of the POL process was confirmed by inhibition of the reaction of accumulation of TBA-active* products by heat and by p-chloromercuribenzoate (Table 1).

*TBA — thiobarbituric acid.

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TABLE 1. Accumulation of TBA-Active Products during Enzymic NADPH-Dependent POL in Rat Brain Microsomes in Presence of Inhibitors (after incubation for 30 min, in % of control)

Experimental conditions	Concentration	MDA, %
Control		100±11
Preparation of rat brain plasma membranes		93±9
2-Methyl-1,4-naphthoquinone	$5 \cdot 10^{-4}$ M	21±4
p-Chloromercuribenzoate	$5 \cdot 10^{-4}$ M	0
7-OH	$5 \cdot 10^{-4}$ M	0
Heating	3 min, 80°C	0

TABLE 2. Effect of Inhibitors of NADPH-Specific ETC on Chlorpromazine Metabolism in Rat Liver and Brain Microsomes

R_f	Substance	Liver			Brain
		Control	CO	2-methyl-1,4-naphthoquinone (0.5 mM)	
0,23	X	3,2±0,8	0,7±0,3	0	1,8±0,3
0,57	Y	10,0±1,5	2,9±0,5	0	0
0,66	SO	8,1±0,7	8,7±1,1	5,7±1,4	5,8±0,8
0,78	7-OH	6,6±1,1	4,7±1,0	0	2,9±0,6
0,90	C	72,1±5,0	83,0±4,9	94,3±5,4	89,5±6,1

Legend: 1. C) chlorpromazine, SO) chlorpromazine sulfoxide, X and Y) unidentified metabolites of chlorpromazine. 2. Concentration of chlorpromazine and its derivatives given as a percentage of initial chlorpromazine concentration.

The NADPH-dependent system of POL, which belongs to the membranes of rat BMF, incidentally, is probably present also in plasma membranes, for in a preparation enriched with plasma membranes (by the method described in [5]), in which Na^+,K^+-ATPase activity was 188 nmoles P/(min•mg protein), compared with 56 nmoles P/(min•mg protein) in standard preparations, the reaction velocity of POL induced by NADPH remained at the same level as with BMF isolated by the standard method (Table 1). The essential point is that enzymic NADPH-induced POL was also a feature of human BMF (Fig. 1), but its velocity showed considerable individual variations [from 0.05 to 0.3 nmoles MDA/(min•mg protein)].*

Just as in liver microsomes, 2-methyl-1,4-naphthoquinone, an electron acceptor from flavoprotein components of electron-transport chains (ETC), is also an inhibitor of POL in BMF (Table 1) [1]. Whereas in liver microsomal fractions the velocity of NADPH- and NADH-dependent POL differs sharply (by 4-6 times), in rat and human brain microsomes both pyridine nucleotides are equally effective inducers of POL (Fig. 1), pointing to significant differences in the properties of the lipid peroxide generators in these two types of membranes. Cytochrome P-450 (P-448), the concentration of which in preparations in [1] reached 0.7-1.4 nmole/mg protein (in the present experiments it was 0.78 ± 0.20 nmole/mg protein), is a terminal component of oxygenases with mixed function in EPR membranes [1]. Meanwhile the concentration of cytochrome P-450 in brain microsomes is extremely low. For instance, frequent attempts to find cytochrome P-450 in brain microsomes gave negative results [7]. More recently, however, the characteristic absorption spectra of a CO-complex with reduced cytochrome P-450 in BMF have been recorded [9]. During calculation of the cytochrome P-450 concentration in these membranes from data in [9], taking the extinction coefficient at λ = 450 nm to be $91 \cdot 10^3$ $cm^{-1} \cdot M^{-1}$, a value of 0.01 nmole cytochrome P-450/mg BMF protein was found, i.e., one-hundredth of its concentration in liver microsomal fractions. Meanwhile, comparison of

*MDA — malonic dialdehyde.

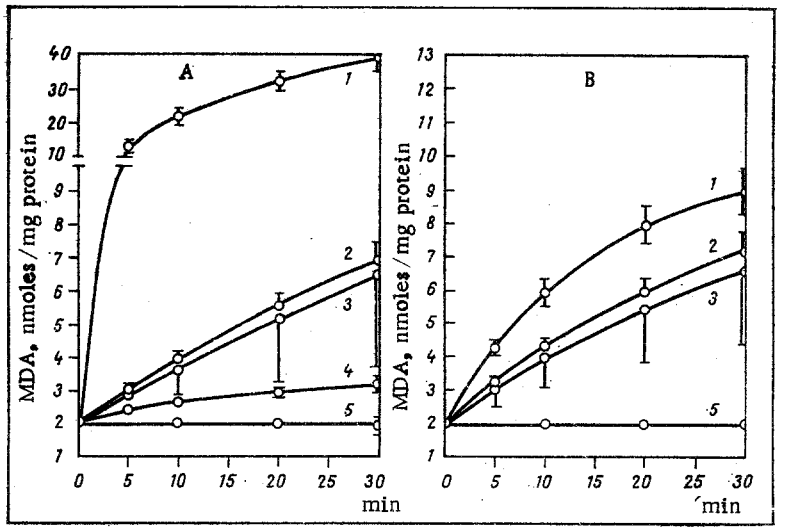

Fig. 1. Kinetics of accumulation of TBA-active products during NADPH-dependent (A) and NADH-dependent (B) enzymic POL in microsomal fraction of rat liver (1) and microsomal fractions of rat (2) and human (3) brain. Effect of chlorpromazine (4) and of 7-OH (5).

the activity of NADPH-cytochrome c-reductase activity in liver and brain microsomal fractions showed that it is four times higher in the liver (in the present experiments 165 and 45 nmoles cytochrome c/min/mg protein respectively). Assuming that in membrane ensembles of oxygenases with mixed function in EPR the molar ratio between cytochrome P-450 and NADPH-cytochrome c-reductase is 12:1 [13], allowing for the data given above on the concentrations of cytochrome P-450 and activity of NADPH-cytochrome c-reductase in brain microsomes, this ratio is 1:2. This means that besides oxygenase complexes with the participation of NADPH-cytochrome c-reductase and cytochrome P-450, in brain microsomal membranes there must also exist NADPH-cytochrome c-reductase sites which do not interact with cytochrome P-450. This explains the special features of NADPH-dependent oxidative conversions of hydrophobic substrates taking place in the BMF. For instance, whereas in liver microsomal fraction high activity of 3,4-benz(a)pyrene-hydroxylase activity is recorded (600 pmoles/mg protein/h), in BMF hydroxylation of 3,4-benz(a)pyrene takes place only one-twentieth as effectively [6]. Whereas during NADPH-dependent oxidation of chlorpromazine by microsomal oxygenases of the liver a wide spectrum of metabolites is formed, the range of derivatives of chlorpromazine formed as a result of its enzymic conversions in BMF is much narrower (Table 2).

However, it should be pointed out that, despite sharp differences in the cytochrome P-450 concentration, the velocity of NADPH-dependent POL in the microsomal fractions of the liver was only 10 times higher than that in the brain (2.2 and 0.22 nmole MDA/mg protein respectively). Hence it follows that NADPH-cytochrome c-reductase evidently participates in the enzymic catalysis of POL by BMF. The formation of hydroxylated derivatives in the course of NADPH-dependent metabolism of chlorpromazine suggests that chlorpromazine must have an inhibitory action on the enzymic NADPH-induced POL in BMF by two mechanisms: a) by competing for reduced components of NADPH-dependent ETC in the microsomal membranes and b) by catalyzing the formation of antioxidants [7-hydroxychlorpromazine, 8-hydroxychlorpromazine (7-OH and 8-OH)] in the course of enzymic metabolism of chlorpromazine in much the same way as has been shown for liver EPR membranes [4]. In fact, the character of the inhibitory action of chlorpromazine on the enzymic NADPH-dependent POL in the membranes of liver and brain microsomal fraction was found to be similar: an increase in the inhibitory effect of chlorpromazine in the initial stages and achievement of the full inhibitory effect only after incubation for several minutes (Fig. 1) [4]. Addition of 7-OH to a suspension of brain microsomes completely prevented the accumulation of TBA-active products immediately, i.e., inhibited POL (Table 1). Hence, the very low level of cytochrome P-450 (P-448) — the terminal component of microsomal NADPH-dependent oxygenases of EPR membranes — is no obstacle to induction of NADPH-dependent POL and oxidative metabolism of the cytotropic compound chlorpromazine in membranes of rat and human BMF. Hydroxylated derivatives of chlorpromazine formed under these circumstances have a marked antioxidant action and are capable of inhibiting enzymic POL induced in brain microsomal membranes by NADPH.

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