NADPH- AND ORGANIC HYDROPEROXIDE-DEPENDENT OXIDATION OF ADRENALIN INTO ADRENOCHROME IN LIVER AND BRAIN MICROSOMES

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The object of this investigation was to study possible pathways of oxidation of adrenalin into adrenochrome during NADPH- and NADH-dependent generation of superoxide anion-radicals in the microsomal fraction of liver and brain, and also during its interaction with organic hydroperoxides.

EXPERIMENTAL METHOD

Microsomal fractions of liver and brain were isolated by methods described previously [1, 12]. To obtain lipsomes, lipids isolated by the method of Folch et al. [4] were treated on an MSE (150 W) ultrasonic disintegrator for two periods of 30 sec, with an amplitude of 6 μ and maximal power. The content of lipid peroxidation products (LPP) was determined by the method of Kohn and Liversedge [7], and NADPH-cytochrome C-reductase activity was determined by the method of Richter et al. [12]. The rate of formation of adrenochrome was measured in constant-temperature cuvettes (37°C) on a Shimadzu MPS-50L spectrophotometer. The standard incubation mixture (3 ml) contained (in mM): 100 NaCl, 50 Tris-HCl buffer, $pH_{37}°$ 7.4, 0.1 adrenalin (noradrenalin), and 0.5 NADPH (NADH). The reaction was started by the addition of microsomes (to a protein concentration of 1 mg/ml). The hydroperoxide of diphenylethane (DPE) and hydroperoxide of phosphatidylethanolamine (PEA) were added as solutions in methanol (the final concentration of methanol did not exceed 0.1%). Oxidation of adrenalin (noradrenalin) was recorded as the formation of adrenochrome (λ_{max} = 480 nm), using a molar extinction coefficient $\varepsilon = 402\ \Omega^{-1} \cdot cm^{-1}$ [5, 9]. Hydroperoxide of PEA was obtained by subjecting oxidation products of phosphatidylethanolamine by lipoxygenase to preparative thin-layer chromatography (chloroform–methanol–28% NH_4OH 13:7:1) on Ferak (West Germany) silica gel. The preparation of DPE hydroperoxide was obtained from the Institute of Chemical Physics, Academy of Sciences of the USSR.

TABLE 1. Rate of Adrenochrome Formation during NADPH- and NADH-Dependent Oxidation of Adrenalin and NADPH- and NADH-Cytochrome c-Reductase Activity in Liver and Brain Microsomes

Test object	Maximal rates of adrenochrome formation nmoles · mg^{-1} · min^{-1}		Cytochrome c-reductase activity, nmoles · mg · min^{-1}	
	NADPH	NADH	NADPH	NADH
Liver microsomes	6,01±0,50	0,6±0,05	165±12	358±23
Brain microsomes	0,40±0,03	0,1±0,02	45±3	61±4

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TABLE 2. Action of Inhibitors, Acceptors, and Traps of Free Active Forms of Oxygen on Adrenochrome Formation in Liver and Brain Microsomes in Presence of NADPH or Organic Hydroperoxides

Inhibitors, acceptors, and traps of free active forms of oxygen	Concentration, M	Rate of adrenochrome formation, nmoles · mg^{-1} · min^{-1}				nmoles · min^{-1}
		liver microsomes		brain microsomes		Without microsomes
		NADPH	hydroperoxide of DPE, $1 \cdot 10^{-4}$ M	NADPH	endogenous lipid peroxides	hydroperoxide of DPE $1 \cdot 10^{-4}$ M
Control	—	6,01	0,61	0,40	0,12	0,23
1,3-diphenylisobenzofuran	$1 \cdot 10^{-4}$	5,53	0,41	0,40	0	0,18
β-carotene	$1 \cdot 10^{-4}$	5,02	0,10	—	—	0,07
1,4-diazobicyclo(2,2,2)-octane	$1 \cdot 10^{-4}$	5,66	0,17	0,40	—	0,08
Superoxide dismutase	5 μg/ml	0	0	0	0	0
$Cu(Tyr)_2$	$1 \cdot 10^{-6}$	17,4	1,57	4,5	0,61	3,2
Mannitol	$1 \cdot 10^{-4}$	5,75	0,58	—	0	0,20
Ethanol	$2 \cdot 10^{-4}$	5,85	0,62	0,40	—	0,23
Methanol	$2 \cdot 10^{-4}$	5,87	0,64	0,40	—	0,24
4-Methyl-2,6-di-tert-butylphenol	$1 \cdot 10^{-5}$	0	0	0	0	0
7,8-benzoflavone	$1 \cdot 10^{-4}$	5,68	0,20	0,40	—	0,12

EXPERIMENTAL RESULTS

The rate of adrenochrome formation did not correlate with the rates of electron transport in NADPH- and NADH-electron-transport chains (Table 1). As the data given in Table 2 show, NADH-dependent adrenochrome formation occurred practically entirely on account of interaction between adrenalin and the superoxide anion-radical, for neither quenchers of singlet oxygen ($^1O_2$) nor hydroxyl radical ($^{\cdot}OH$) traps had any effect, whereas superoxide dismutase (SOD) completely inhibited the oxidation of adrenalin in the presence of NADPH. During accumulation of peroxidation products in liver and brain microsomes, adrenochrome formation also took place in the absence of a source of reducing equivalents: NADPH or NADH. The rate of oxidation of adrenalin under these circumstances depended on the content of LPP in the membranes, in the same way as is observed during interaction between adrenalin and individual organic hydroperoxides (hydroperoxides of DPE and PEA) (Fig. 1).

It can be tentatively suggested that the catalyst for oxidation of adrenalin into adrenochrome in membranes of liver microsomes, but not brain microsomes, in the presence of organic hydroperoxides is cytochrome P-450, for its specific inhibitor 7,8-benzoflavone has a stronger inhibitory action in liver microsomes than during direct interaction between DPE hydroperoxide and adrenalin (without microsomes) (Table 2). Furthermore, 7,8-benzoflavone inhibits oxidation of adrenalin in liver microsomes in the presence of DPE hydroperoxide and has no effect on this reaction in the microsomal fraction of liver and brain in the presence of NADPH, i.e., in the case when oxidation of adrenalin takes place mainly on account of generation of superoxide anion-radicals. Further evidence of the catalytic role of cytochrome P-450 in this reaction is given by the fact that the rate of adrenochrome formation if the reaction is induced by DPE hydroperoxide in liposomes prepared from membranes of the microsomal fraction of the liver is lower (0.40 nmole·mg^{-1}·min^{-1}) than in the membranes themselves (0.61 nmole·mg^{-1}·min^{-1}).

Oxidation of adrenalin can thus take place through interaction with organic hydroperoxides (synthetic and of natural origin) and cytochrome P-450 may be the catalyst for this reaction.

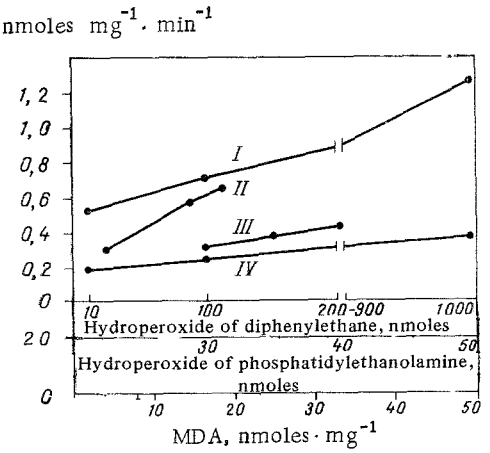

Fig. 1. Rate of oxidation of adrenalin into adrenochrome in the presence of organic hydroperoxides (HP). I) HP of DPE+ liver microsomes (1 mg protein/ml); II) endogenous LPP in liver microsomes (1 mg protein/ml); III) HP of PEA + liver microsomes (1 mg protein/ml); IV) HP of DEP. Ordinate, rate of accumulation of adrenochrome (in nmoles·mg^{-1}·min^{-1}).

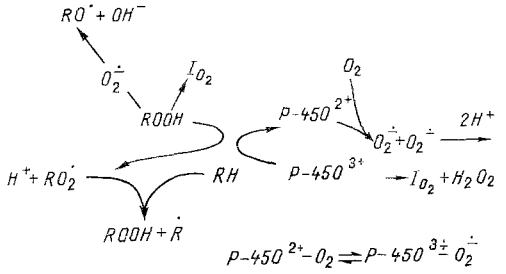

Fig. 2. Scheme of generation of active forms of oxygen during lipid peroxidation induced by organic peroxides in liver microsomal membranes.

Oxidation of adrenalin is known to take place through its interaction with active forms of oxygen such as superoxide anion-radical and hydroxyl radicals [2, 3, 9, 10]. It follows from the data in Table 2 that SOD completely inhibits adrenochrome formation from adrenalin both in the presence of NADPH and after addition of organic hydroperoxides.*

Traps of hydroxyl radicals (ethanol, methanol, mannitol), on the other hand, do not affect the reaction of adrenalin oxidation regardless of the method of induction. Meanwhile, quenchers of singlet oxygen have a weak inhibitory action in the presence of organic hydroperoxides and have practically no effect on adrenalin oxidation due to NADPH-dependent generation of superoxide anion-radicals in microsomal membranes.

It can be concluded from these results that mainly superoxide anion-radicals are utilized in the reaction of adrenochrome formation from adrenalin taking place in membranes in the presence of sources of reducing equivalents. Meanwhile, during oxidation of adrenalin dependent on organic peroxides, a definite role may be played by singlet oxygen, the source of which may be organic hydroperoxides [6, 8]. Hydroxyl radicals in the cases examined above evidently do not take part in the process of adrenalin oxidation. The possible role of cytochrome P-450 in the formation of active forms of oxygen during interaction with organic hydroperoxides is illustrated by the scheme in Fig. 2.

*The $Cu(Tyr)_2$ complex accelerates the reaction sharply and it cannot therefore serve as criterion for evaluation of the role of the superoxide anion-radical in NADPH-dependent and hydroperoxide-dependent adrenochrome formation. This conclusion is in agreement with observations showing acceleration of catecholamine oxidation by Cu^{++} complexes and by copper-containing proteins [13].

It must also be pointed out that oxidation of adrenalin into adrenochrome in membranes containing LPP may be the source of errors in the analytical procedure of determination of SOD. The velocity of the adrenalin–adrenochrome reaction in the presence of membrane fragments will in fact be determined not only by the ability of SOD to catalyze the reaction of dismutation of superoxide anion-radicals:

$$O_2^{\cdot} + O_2^{\cdot} \xrightarrow[SOD]{2H^+} H_2O_2 + {}^3O_2,$$

but also by conversion of adrenalin into adrenochrome on account of LPP contained in the membrane, independent of the superoxide anion-radical.

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ROLE OF ATP IN SPECIFIC BINDING OF ^{125}I-INSULIN WITH CYTOPLASMIC RECEPTORS OF LIVER AND MUSCLE MEMBRANES OF CONTROL ANIMALS AND OF RATS WITH DIABETES

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The mechanism of the decrease in sensitivity of the cells of various organs and tissues to insulin in diabetes is not yet clear. There is ample evidence to indicate a reduction in the binding of insulin with its receptors in some tissues in states characterized by resistance to endogenous or exogenous insulin [2, 5, 7, 8, 10]. However, insular insufficiency may be manifested in the presence of normal or even increased binding of the hormone with its specific receptors, and it is probably caused by disturbances of glucose metabolism at stages following activation of insulin receptors. The possibility cannot be ruled out that the processes responsible for tissue sensitivity to insulin are regulated by ATP, the effector component of the plasma membrane, in both normal and resistant states or against the background of deficient production of the hormone. It has been suggested that the ability of receptors to bind insulin is regulated by phosphorylation of membrane proteins, possibly even of the receptors themselves, and that ATP has a modulating influence on the binding of insulin, and in that way it affects its biological action [3, 13].

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