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LIPID PEROXIDATION AS THE CAUSE OF THE ASCORBIC ACID INDUCED DECREASE OF ADENOSINE TRIPHOSPHATASE ACTIVITIES OF RAT BRAIN MICROSOMES AND ITS INHIBITION BY BIOGENIC AMINES AND PSYCHOTROPIC DRUGS

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Abstract—The inhibitory effect of ascorbic acid on microsomal Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of rat brain and the ability of several mediator substances and of many drugs acting on the nervous system to antagonize this inhibition was studied. The maximal effect of ascorbic acid on ATPase activities was completely antagonized by catecholamines, apomorphine, oxypertine, reserpine, tetrabenazine, phenothiazines (chlorpromazine and promethazine) and yohimbine at a concentration of 10^{-4} M or below. Apomorphine proved to be the most effective compound, fully antagonizing the effect of ascorbic acid at a concentration of 10^{-6} M. A partial inhibition of the effect of ascorbic acid was induced by 10^{-4} M serotonin, desipramine, imipramine and LSD. During the incubation of the microsomes for ATPase activity determinations in the presence of ascorbic acid, a significant amount of lipid peroxide was formed. Compounds which antagonized the effect of ascorbic acid on the ATPase activities inhibited at the same concentrations the lipid peroxide formation. The well-known inhibitors of lipid peroxidation eliminated the effect of ascorbic acid on the ATPase activities. It has been established that the inhibition of ATPase activities by ascorbic acid is a consequence of lipid peroxidation. The mechanism of action of the antagonizing compounds is discussed.

In earlier publications from this laboratory it was reported that the soluble fraction of rat brain contains a heat-stable, dialyzable substance inhibiting the ATPase activities of the particulate fractions, the effect of which can be antagonized by catecholamines and by tetrabenazine or chlorpromazine [1, 2]. A similar observation concerning the catecholamines was described by Yoshimura, who found that Na^+, K^+-ATPase activity of total homogenates prepared from various regions of rat brain was considerably increased by catecholamines [3]. In our previous work the inhibitory substance in the soluble fraction playing a role in the phenomenon was identified as ascorbic acid and it was established that an oxidizable–reducible heavy metal ion bound to the membrane structures, presumably iron is responsible for the effect of ascorbic acid [4].

It is known that ascorbic acid can induce the formation of lipid peroxides in subcellular fractions prepared from liver and brain [5–9]. Experiments on liver microsomes indicates that a non-haem iron component of the microsomes is essential for the lipid peroxidation brought about by ascorbic acid or NADPH [10]. By the oxidative deterioration of the unsaturated fatty acids of the membrane phospholipids, lipid peroxidation results in the damage of the membrane structures and the functions related to them [11, 12]. On synaptosomal membranes isolated from the brain, lipid peroxidation induced by soybean lipoxygenase–H_2O_2 treatment brings about a structural change resulting in a marked inactivation of Na^+, K^+-ATPase activity; at the same time, however, acetylcholinesterase activity is not influenced [13].

Our present work shows that the inhibition of ATPase activities of the membrane structures of the brain by ascorbic acid characterized in our previous work [4], is related to lipid peroxidation. On the basis of previous observations concerning the effects of catecholamines and certain neuroleptics [1, 2] we tried to characterize the action of biogenic amines and psychotropic drugs in the phenomenon in order to reveal the significance of these *in vitro* effects.

MATERIALS AND METHODS

CFE rats of both sexes weighing 140–200 g were used in the experiments. The microsomal fraction was isolated from whole brains according to the cell fractionation method of De Robertis *et al.* [14, 15] with the alterations described previously [4]. The microsomes were suspended in 0·32 M sucrose, the protein content of the final suspension being 0·6–0·9 mg/ml. Preparations were stored at −4°.

ATPase activities were measured as described previously [2]. In the present experiments Na_2 ATP was used, therefore Mg^{2+}-ATPase activity was measured in the presence of 0·5 mM ouabain.

Acetylcholinesterase was assayed according to Ellman *et al.* [16] with acetylthiocholine as the substrate.

Lipid peroxide was measured by the thiobarbituric acid reaction as described by Wilbur *et al.* [17]. The amount of malonaldehyde formed was calculated by the molar extinction coefficient, ϵ_{530} nm = $1{\cdot}56 \times 10^5$ cm/m-mole, reported by Sinnhuber, Yu and Yu [18] and was used to express the extent of peroxidation.

Protein was determined by the method of Lowry *et al.* using bovine serum albumin as a standard [19].

Chemicals: ATP disodium, Sigma, St. Louis; L-ascorbic acid, Merck, Darmstadt; acetylthiocholine iodide, *N*-*N*′-paraphenylene diamine dihydrochloride, Fluka, Buchs; 2-thiobarbituric acid, Reanal, Budapest.

The following drugs (found to be effective) were used: L-noradrenaline bitartrate, dopamine hydrochloride, Sigma, St. Louis; L-dopa, Fluka, Buchs; promethazine hydrochloride, chlorpromazine hydrochloride, imipramine hydrochloride, E.G.Y.T., Budapest; reserpine phosphate, Ciba-Geigy, Basel; oxypertine, Winthrop, Surbiton; tetrabenazine (Nitoman ampullas), Hoffman–La Roche, Basel; apormorphine hydrochloride (Ph. Hg. VI.); yohimbine hydrochloride, Chinoin, Budapest; desipramine hydrochloride, Geigy, Basel; *d*-lysergic acid tartrate (LSD), Sandoz, Basel.

RESULTS

Antagonization of the effect of ascorbic acid on ATPase activities by biogenic amines. In the presence of 5×10^{-5} M ascorbic acid, sufficient to obtain maximal inhibition in the microsomal ATPase activities, the effects of the following mediator substances and/or of their precursors were studied: noradrenaline, dopamine, dopa, serotonin, 5-hydroxytryptophan, acetylcholine, γ-aminobutyric acid and histamine.

In addition to catecholamines, which are known to antagonize the inhibition by ascorbic acid [4], serotonin was found to antagonize the effect of ascorbic acid, while the other compounds proved to be ineffective. In Table 1 the effect of a catecholamine, dopamine and of serotonin at different concentrations are compared. In addition to dopamine, the efficiency of the two other catecholamines was also studied, but no substantial difference was observed as compared to dopamine. Serotonin was less effective than catecholamines. Dopamine and serotonin antagonized completely the effect of ascorbic acid on Mg^{2+}-ATPase activity at a concentration of 5×10^{-5} M and 10^{-4} M, respectively. In the presence of 10^{-4} M dopamine, Na^+, K^+-ATPase activity reached the value of the control measured in the absence of ascorbic acid, but the antagonization by serotonin was still not complete even at the highest concentrations tested (2×10^{-4} M).

Na^+, K^+-ATPase activity was considerably increased by dopamine also in the absence of ascorbic acid. Similar activation could be measured also in the presence of dopa and noradrenaline. This effect of catecholamines is probably chelatory, since a similar activation could be obtained by chelators. The increase in Na^+, K^+-ATPase activity brought about by dopamine and chelating agents, already known [4] was highly variable and did not occur in every preparation.

Antagonization of the effect of ascorbic acid on the ATPase activities by psychotropic drugs. In similar way the following drugs, acting on the nervous system were tested for their ability to antagonize the inhibitory effect of ascorbic acid on the microsomal ATPase activities: phenobarbital, glutethimide, morphine, meperedine, harmine, mescaline, LSD, cocaine, procaine, lidocaine, meprobamate, trimetozine, chlordiazepoxide, diazepam, chlorpromazine, promethazine, reserpine, tetrabenazine, oxypertine, haloperidol, trifluperidol, desipramine, imipramine, trimipramine, amitriptyline, nortriptyline, nialamide, pentylenetetrazole, strychnine, trimethadione, diphenylhydantoin, nicotine, tetraethylammonium, quanethidine, bretylium, dibenamine, phentolamine, yohimbine, phenoxybenzamine, propranolol, pronethalol, prenylamine, atropine, methantheline, physostigmine, tubocurarine, decamethonium, theophilline, apomorphine and tremorine.

The compounds were tested at a concentration of 10^{-4} M and those that were effective were investigated also at lower concentrations. The results are shown in Table 2. Since at the concentrations shown in the table these compounds did not influence the

Table 1. Antagonization of the inhibitory effect of ascorbic acid on microsomal ATPase activities by dopamine and serotonin*

Substance	Concentration (M)	ATPase activity (% of control) Without ascorbic acid Na^+, K^+-ATPase	Without ascorbic acid Mg^{2+}-ATPase	With 5×10^{-5} M ascorbic acid Na^+, K^+-ATPase	With 5×10^{-5} M ascorbic acid Mg^{2+}-ATPase
Dopamine	0	100·0	100·0	34·8 ± 2·8	52·8 ± 4·3
	10^{-5}	104·7 ± 6·7	106·3 ± 6·2	46·9 ± 7·9	69·7 ± 2·2
	5×10^{-5}	128·4 ± 1·6	110·4 ± 4·6	99·3 ± 3·7	104·8 ± 10·8
	10^{-4}	137·4 ± 6·6	109·5 ± 7·5	129·4 ± 1·4	112·2 ± 8·2
	2×10^{-4}	164·7 ± 5·7	117·5 ± 8·5	166·7 ± 9·3	121·1 ± 7·2
Serotonin	0	100·0	100·0	39·9 ± 3·4	57·1 ± 5·4
	5×10^{-5}	115·2 ± 3·5	102·3 ± 0·7	45·7 ± 5·7	84·4 ± 3·1
	10^{-4}	115·4 ± 5·7	106·8 ± 1·8	60·2 ± 2·1	97·8 ± 1·4
	2×10^{-4}	119·4 ± 3·9	109·6 ± 1·3	92·2 ± 4·5	112·3 ± 1·5

* Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of the control samples varied from 4·8 to 6·2 and from 5·6 to 6·9 μmoles P_i/mg protein per 15 min, respectively.

Results are means of three experiments in duplicate ± S.E.

Table 2. Antagonization of the inhibitory effect of ascorbic acid on microsomal ATPase activities by drugs*

Substance	Concentration (M)	ATPase activity (% of control) Na^+, K^+-ATPase	Mg^{2+}-ATPase
Apomorphine	0	37·3 ± 3·2	61·0 ± 0·6
	10^{-7}	39·3 ± 0·9	70·1 ± 1·1
	5×10^{-7}	82·3 ± 9·8	111·5 ± 1·4
	10^{-6}	104·9 ± 1·9	110·1 ± 3·4
Oxypertine	0	33·6 ± 4·1	54·3 ± 1·2
	5×10^{-6}	46·0 ± 2·0	77·3 ± 1·6
	10^{-5}	91·2 ± 12·5	106·6 ± 5·1
Reserpine	0	31·2 ± 4·7	57·3 ± 2·0
	10^{-5}	61·7 ± 10·7	67·1 ± 2·3
	$2·5 \times 10^{-5}$	95·2 ± 5·2	99·9 ± 1·4
Promethazine	0	40·8 ± 2·0	54·8 ± 0·8
	10^{-5}	50·7 ± 0·7	84·5 ± 4·5
	$2·5 \times 10^{-5}$	98·7 ± 13·0	98·4 ± 4·4
Tetrabenazine	0	28·9 ± 4·0	55·7 ± 4·2
	$2·5 \times 10^{-5}$	65·4 ± 8·5	65·6 ± 2·7
	5×10^{-5}	97·1 ± 5·0	83·1 ± 1·7
	10^{-4}	104·8 ± 4·8	100·0 ± 2·6
Yohimbine	0	31·9 ± 4·8	55·7 ± 2·4
	5×10^{-5}	39·9 ± 3·0	69·1 ± 5·2
	10^{-4}	91·9 ± 8·1	92·1 ± 5·7
Desipramine	0	34·3 ± 2·3	60·6 ± 3·7
	5×10^{-5}	64·3 ± 9·7	82·9 ± 3·9
	10^{-4}	78·4 ± 6·6	91·9 ± 4·6
Imipramine	0	35·2 ± 4·5	59·6 ± 4·0
	10^{-4}	62·7 ± 6·5	79·5 ± 2·7
LSD	0	33·2 ± 4·7	50·9 ± 5·5
	10^{-4}	43·2 ± 0·9	82·0 ± 5·6

* Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of the control samples varied from 4·1 to 7·3 and from 4·6 to 7·7 μmoles P_i/mg protein per 15 min, respectively.

Results are means of three experiments in duplicate ± S.E.

ATPase activities in the absence of ascorbic acid, only the values measured in the presence of ascorbic acid are shown. Chlorpromazine was effective and it has been shown previously [2] to antagonize inhibition by the soluble fraction of brain, but it is not included in the table because of its inhibitory effect on the ATPase activity. Instead the efficiency of another phenothiazine, promethazine, is presented, which did not inhibit the ATPase activity at the concentrations shown in the table.

Apomorphine proved to be the most effective compound, completely antagonizing the effect of ascorbic acid on Mg^{2+}-ATPase activity and almost completely that on Na^+, K^+-ATPase already at a concentration of 5×10^{-7} M. 10^{-5} M oxypertine and $2·5 \times 10^{-5}$ M reserpine and promethazine eliminated the effect of ascorbic acid. Na^+, K^+-ATPase activity was preserved by 5×10^{-5} M tetrabenazine, though Mg^{2+}-ATPase activity was inhibited to a small extent. Yohimbine antagonized the effect of ascorbic acid at a concentration of 10^{-4} M. Desipramine also was effective, at a concentration of 10^{-4} M but it did not antagonize completely the effect of ascorbic acid on Na^+, K^+-ATPase activity. Imipramine and trimipramine were even less effective. The latter was omitted from the table. LSD at 10^{-4} M caused 60% inhibition of the effect of ascorbic acid on Mg^{2+}-ATPase but was ineffective in the case of Na^+, K^+-ATPase activity.

Inhibition of the ascorbic acid induced lipid peroxidation by biogenic amines and psychotropic drugs. During the incubation of the microsomes for ATPase activity determinations in the presence of ascorbic acid, a significant amount of lipid peroxide was formed, as measured by the thiobarbituric acid reaction. Table 3 shows the effect of the compounds found to antagonize the inhibition by ascorbic acid in the ATPase experiments on the ascorbic acid induced lipid peroxide formation in the microsomes. The system tested was the incubation mixture for the total ATPase assay. Substances were introduced to the system in concentrations sufficient to obtain full antagonization of the ascorbic acid effect on ATPase activity or, in case of serotonin and desipramine, the highest concentration tested was added.

Except for serotonin and desipramine, all the compounds tested completely inhibited lipid peroxidation induced by ascorbic acid. The table includes the chlorpromazine which at 5×10^{-5} M inhibited lipid peroxide formation completely.

The ascorbic acid induced lipid peroxidation, like the effect of ascorbic acid on ATPase activity was not completely inhibited by serotonin and desipramine in the concentrations tested.

Comparison of the effects of EDTA, EGTA, N-N'-paraphenylene diamine, Co^{2+} and Mn^{2+} on ascorbic acid induced lipid peroxidation and on inhibition of ATPase activity by ascorbic acid. It is well known that

Table 3. Inhibition of ascorbic acid induced lipid peroxidation in rat brain microsomes by biogenic amines and psychotropic drugs*

Substance	Concentration (M)	Lipid peroxide formation induced by ascorbic acid (nmoles of malonaldehyde/mg of protein per 10 min)
Control		6·65 ± 0·37
Noradrenaline	$7{\cdot}5 \times 10^{-5}$	0
Dopamine	$7{\cdot}5 \times 10^{-5}$	0
Dopa	$7{\cdot}5 \times 10^{-5}$	0
Serotonin	2×10^{-4}	0·98 ± 0·16
Apomorphine	10^{-6}	0
Oxypertine	10^{-5}	0
Reserpine	$2{\cdot}5 \times 10^{-5}$	0
Promethazine	$2{\cdot}5 \times 10^{-5}$	0
Chlorpromazine	5×10^{-5}	0
Tetrabenazine	$7{\cdot}5 \times 10^{-5}$	0
Yohimbine	10^{-4}	0
Desipramine	10^{-4}	1·32 ± 0·14

* In a total volume of 2 ml the incubation system contained 0·16–0·20 mg of microsomal protein, 50 mM Tris buffer pH 7·4, 3 mM $MgCl_2$, 100 mM NaCl, 30 mM KCl (incubation mixture for total ATPase assay), 5×10^{-5} M ascorbic acid and various amounts of investigated compounds. Incubation was carried out for 10 min at 37°.

The results are means of three experiments in duplicate ± S.E.

lipid peroxidation is inhibited by chelating agents, antioxidants and certain heavy metals, e.g. Co^{2+} and Mn^{2+} [5, 8, 9]. Previously we reported that the effect of ascorbic acid on ATPase activity can be prevented by the addition of chelators [4]. Excess Ca^{2+} and Ni^{2+} were found to eliminate the effect of EGTA and EDTA, respectively. As shown in Table 4, the inhibition of lipid peroxidation by EGTA can be prevented by an excess of Ca^{2+}. On the other hand, an excess of Ni^{2+} had no influence on the effect of EDTA. It is noteworthy that in the absence of EDTA, 5×10^{-5} M Ni^{2+} stimulates the ascorbic acid induced lipid peroxide formation.

Table 4 also shows the effects of Co^{2+} and Mn^{2+} and of an effective antioxidant, *N-N'*-paraphenylene diamine. These substances at a concentration of 5×10^{-5} M completely inhibited the ascorbic acid induced lipid peroxide formation. Table 5 shows that the same concentrations of these substances also antagonize the effect of ascorbic acid on ATPase activities.

The effect of ascorbic acid on acetylcholinesterase activity. The effect of ascorbic acid was tested on the activity of another membrane-bound enzyme, namely, acetylcholinesterase activity. As can be seen in Fig. 1, microsomal acetylcholinesterase activity was not changed by the presence of ascorbic acid in the incubation system. The same result was obtained, when the preparation was preincubated for 20 min, at 37° in the presence and absence of ascorbic acid.

DISCUSSION

Our results show, that the effect of ascorbic acid on ATPase activities and on lipid peroxidation can both be inhibited by certain biogenic amines and psychotropic drugs. Moreover, compounds, which even in the highest concentration tested did not inhibit totally the effect of ascorbic acid on ATPase activities, proved to be only partially effective in inhibiting the ascorbic acid induced lipid peroxide formation. On

Table 4. Effects of EGTA, EDTA, N-N'-paraphenylene diamine, Mn^{2+}, Co^{2+}, Ca^{2+} and Ni^{2+} on ascorbic acid induced lipid peroxidation in rat brain microsomes*

Substances	Lipid peroxide formation induced by ascorbic acid (nmoles of malonaldehyde/mg of protein/10 min)
Control	7·06 ± 0·22
EGTA	0
EDTA	0
Mn^{2+} (sulphate)	0
Co^{2+} (nitrate)	0
Ca^{2+} (chloride)	7·35 ± 0·15
Ni^{2+} (sulphate)	9·60 ± 0·59
EGTA + Ca^{2+}	7·57 ± 0·26
EDTA + Ni^{2+}	0
Paraphenylene diamine	0

* Conditions of experiment were the same as given under Table 3.

Results are means of three experiments in duplicate ± S.E.

The concentration of the metal ions and N-N'-paraphenylene diamine and the concentration of the chelators were 5×10^{-5} M and 10^{-4} M, respectively. In samples containing chelators together with metals, metal ions were in an excess of 5×10^{-5} M. In the absence of ascorbic acid no lipid peroxide formation was induced by the metal ions tested.

Table 5. Antagonization of the effect of ascorbic acid on microsomal total ATPase activity by Co^{2+}, Mn^{2+} and *N-N'*-paraphenylene diamine

Substances (5×10^{-5} M)	Total ATPase activity (% of control)
Co^{2+} (nitrate)	99·6 ± 3·8
Mn^{2+} (sulphate)	104·8 ± 4·4
Paraphenylene diamine	103·0 ± 3·6
Ascorbic acid	51·8 ± 2·2
Ascorbic acid + Co^{2+}	103·1 ± 5·7
Ascorbic acid + Mn^{2+}	107·0 ± 5·1
Ascorbic acid + paraphenylene diamine	105·0 ± 2·2

Activity of the control samples was 11·82 ± 0·60 μmoles P_i/mg protein per 15 min.

Results are means of three experiments in duplicate ± S.E.

the other hand well known inhibitors of lipid peroxidation like chelators, or a potent antioxidant such as *N-N'*-paraphenylene diamine, as well as Co^{2+} and Mn^{2+} ions could antagonize the action of ascorbic acid on ATPase activities. Both phenomena-ascorbic acid induced lipid peroxidation and decrease of ATPase activities-present a maximum curve-type dependency on ascorbic acid concentration [4, 8, 9]. In contrast to ATPase activities acetylcholinesterase activity was not affected by ascorbic acid (Fig. 1). It is already known, that whereas Na^+, K^+-ATPase activity undergoes marked inactivation, acetylcholinesterase activity remains unaffected following lipid peroxidation brought about by soybean lipoxygenase-H_2O_2 treatment of synaptosomal membranes [13].

From these results it can be concluded that the ascorbic acid-mediated inhibition of ATPase activities is caused by the lipid peroxidation induced by ascorbic acid. Only one difference was observed in the course of experiments on the two phenomena: while in ATPase experiments the antagonizing effect of EDTA seemed to be prevented by an excess of Ni^{2+} [4], lipid peroxidation induced by ascorbic acid was inhibited by EDTA in the presence of an excess of Ni^{2+}. The inhibition of ATPase activity in the presence of ascorbic acid, EDTA and Ni^{2+} may proceed according to some other mechanism, not related to lipid peroxidation.

The inactivation of Na^+, K^+-ATPase activity by lipid peroxidation can be explained by the deterioration of the phospholipid structure of the membranes. Na^+, K^+-ATPase activity of synaptosomal membranes has been shown to depend on the structural integrity of the membrane [20–23] and it has been demonstrated that membrane phospholipids are essential for this enzyme activity [21 25]. Moreover polyunsaturated fatty acids of membrane phospholipids, which are the sites of the peroxidative damage, have been implicated to be involved in Na^+, K^+-ATPase activity [13, 23].

As indicated by experiments with synaptosomal membranes, Na^+, K^+-ATPase activity is not affected by peroxidized oleic or linoleic acid, showing that the inhibition of enzyme activity cannot be attributed to some toxic product of lipid peroxidation [13]. Inhibition of Mg^{2+}-ATPase activity in our experiments by ascorbic acid is also likely to be caused by damage of membrane structures, although in this case the role of lipoxidative products cannot be excluded.

The marked effect of experimental alterations of membrane phospholipids on Na^+, K^+-ATPase activity also suggest that eventual changes taking place in the phospholipid structure of the membranes *in vivo*, may have an important role in the regulation of Na^+, K^+-ATPase activity.

Lipid peroxidation, however, probably has no significance from this point of view. Under *in vivo* conditions deterioration of membrane phospholipids by lipid peroxidation occurs in some pathological cases and in connection with aging [12]. Processes involving free radical intermediates, which can induce *in vitro* the formation of lipid peroxides proceed in the living organism under strictly controlled conditions and do not lead to lipid peroxidation causing the damage of cell structures [26].

The high ascorbic acid concentration of the brain may have an important role in the prevention of peroxidative damage of brain tissues. Lipid peroxide formation induced by ascorbic acid follows a maximum curve as a function of ascorbic acid concentration [8, 9]. In high concentrations, where ascorbic acid antagonizes its own effect it is also able to inhibit the enzymatic lipid peroxidation induced by NADPH [8] and lipid peroxidation induced by Fe^{2+} (unpublished result). The ascorbic acid concentration in the brain is about 2×10^{-3} M, [4, 27] a concentration not inducing any peroxidation in our experiments. Thus it can be stated, that the physiological level of ascorbic acid in the brain corresponds to a concentration at which ascorbic acid acts as a protective compound only.

Our experiments on the other hand show that lipid peroxidation induced *in vitro* by ascorbic acid in microsomes prepared from rat brain can be antagonized by biogenic amines and psychotropic drugs. Consi-

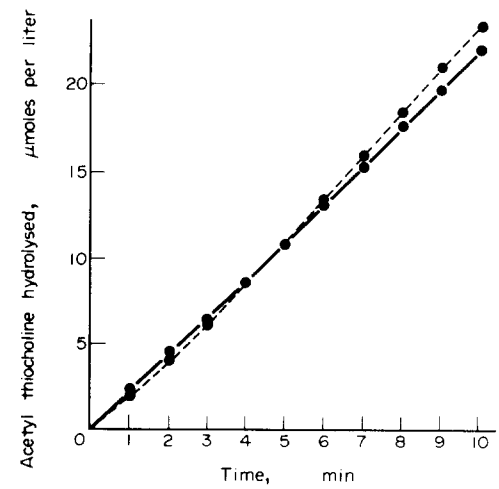

Fig. 1. Acetylcholinesterase activity of rat brain microsomes in the presence (dashed line) and in the absence (continous line) of 10^{-4} M ascorbic acid. Activity of acetylcholinesterase was measured spectrophotometrally according to Ellman *et al.* [16]. The assay solution contained 26·7 μg of microsomal protein in a total volume of 3·1 ml.

dering that the majority of these compounds belong to a relatively well circumscribed group of CNS active drugs, the effect of these compounds is rather specific. Only yohimbine, an α-adrenergic blocking agent seems to be an exception. Injected intraperitoneally in high doses however, yohimbine has a sedative action [28, 29] and intraventricular administration of high doses of yohimbine induces central depression [30]. Disregarding apomorphine, the most effective drugs were neuroleptics and except for butirophenones all the neuroleptics tested were found to be effective.

The expected inhibitors of lipid peroxidation are those compounds, which posses chelating or antioxidant properties. Among the compounds found to be effective there are several presenting well known chelating or antioxidant properties. Catecholamines, phenothiazines and serotonin had already been shown to be powerful inhibitors of lipid peroxide formation in mitochondria or in microsomes prepared from rat liver [6, 31, 32]. The inhibitory effect was attributed to their antioxidant properties. However, there was no correlation between the chelating or antioxidant properties on one side and effectiveness of the active compounds on the other side. Therefore it is also possible, that these compounds interact with membrane phospholipids, confering a protection to them.

The elucidation of the exact mechanism of the action of the compounds in question on lipid peroxidation requires further research. However, considering that the effect described above seems to be rather specific to a certain group of CNS active drugs, it can be supposed that biochemical characteristics of these compounds underlying this *in vitro* effect also have a role in their *in vivo* pharmacological action.

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