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INHIBITORY ACTION OF NEUROLEPTIC DRUGS AND SEROTONIN ON DOPAMINE AUTOXIDATION AND LIPID PEROXIDATION

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Abstract

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1. Dopamine, like other catecholamines, is normally metabolized by the enzymes monoamino oxidase and catechol-*ortho*-methyl transferase but it can also undergo oxidation to potentially toxic products, that, in turn, can generate free radicals.
2. In the present paper the effect of neuroleptic drugs (chlorpromazine, trifluoperazine and clozapine) and serotonin on the *in vitro* oxidation of dopamine and on lipid peroxidation was examined. Serotonin, clozapine, chlorpromazine and trifluoperazine inhibit autoxidation of dopamine both at pH 7.4 and pH 8.5. Trifluoperazine appears more efficient than chlorpromazine while serotonin shows an inhibitory effect intermediate between those of trifluoperazine and chlorpromazine; clozapine has only a moderate effect.
3. The catalytic effect of trace metal seems irrelevant since chelating agents do not show any significant inhibition.
4. All the substances used show a strong antiperoxidative activity.
5. It is concluded that the molecular and biochemical properties of serotonin and neuroleptic drugs on brain dopamine autoxidation and lipid peroxidation could be related to their physiological and clinical effects on mental illness in general and schizophrenia in particular.

Keywords: clozapine, dopamine, dopamine autoxidation, phenothiazines, serotonin.

Abbreviations: angiotensin converting enzyme (ACE), central nervous system (CNS), dopamine (DA), serotonin (5-HT).

Introduction

Dopamine, a constituent and transmitter in the central nervous system (Lindwall and Bijorklund, 1984), is involved in several diseases, such as Parkinson's disease, Huntington's chorea, schizophrenia and others as well. Its regional distribution in human CNS has been determined, the concentration varying from 0.3 μg/g fresh weight of tissue in cerebral cortex to 5.65 μg/g in putamen (Sourkes, 1981).

Studies "*in vivo*" by positron emission tomography (Wong et al. 1990) as well as post-mortem examination (Kleinman, 1990) of untreated schizophrenic patients demonstrated an increased DA receptor density. The spreading of receptor on cell surface may indicate a reduced sensitivity in the numerically increased receptors (Farde et al. 1990). The decreased synaptic affinity for DA may correspond to an increased availability of DA for its catabolism.

Catecholamines are usually metabolized by monoamino oxidase and catechol-*ortho*-methyl transferases but, due to the presence of the catechol moiety, they can also undergo oxidation, usually negligible at physiological pH, and higher at increasing pH or in the presence of metal ions. Several enzymatic systems are also involved in the stimulation of catecholamine oxidation (Bindoli et al., 1989).

The autoxidative pathway brings about the formation of potentially toxic products such as aminochromes and their derivatives such as adrenolutin and dihydroxyindoles. The oxidation products of catecholamines can give rise, possibly through a "redox cycling" process, to the formation of free radicals. Neurons are especially vulnerable to free radical attack that might be responsible for several pathological states including schizophrenia (Jesberger and Richardson, 1991). In particular adrenochrome, in the past, was considered to be responsible for some forms of mental illness (Hoffer et al. 1954). Adrenochrome can be metabolized either to adrenolutin, considered to be toxic or to 5,6-dihydroxy-N-methyl indole which is reported to be non-toxic (Heacock, 1971). Also dopamine easily undergoes autoxidation (Fig. 1) and, in addition, leads in the substantia nigra to the formation of neuromelanin (Sealy et al., 1980; Rogers and Curzon, 1975; Van der Vende and Spoerlein, 1963) the role of which is still not well established. In the present research the effect of neuroleptic drugs (clozapine, chlorpromazine and trifluoperazine) and that of serotonin on the oxidation of dopamine and on lipid peroxidation was examined. Bhatnagar (1972) reported that the *in vitro* melanin formation from dopamine is inhibited by serotonin and some centrally active drugs such as chlorpromazine and amitryptyline. It has also been reported that the autoxidation of adrenaline to adrenochrome, mediated by superoxide anion, is inhibited by the angiotensin converting-enzyme (ACE) inhibitors, such as captopryl. This inhibitory action appears to be referable to the presence of SH groups since N-2-mercaptopropionylglycine and N-acetylcysteine are effective as well (Westlin and Mullane, 1988).

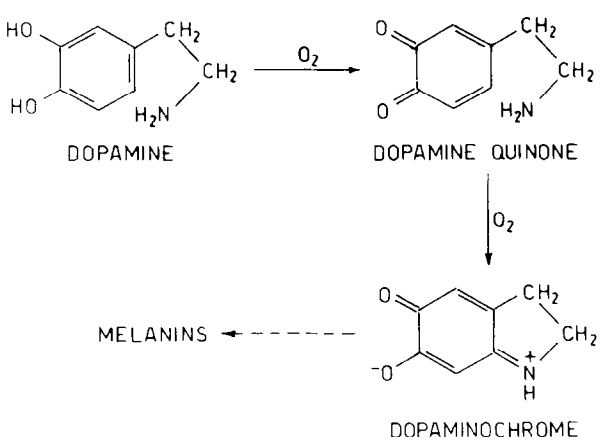

Fig. 1. Oxidative pathway of dopamine.

Methods

Animals

Wistar albino rats of both sexes, weighing 180-250 g were used for the preparation of rat liver and brain microsomes.

Drugs

Serotonin, chlorpromazine and trifluoperazine were supplied by Sigma Chem. Co.(St. Louis, Missouri, USA). Clozapine was a kind gift of Sandoz Pharma AG (Basel Switzerland).

Experimental Procedure

Rat liver microsomes were prepared according to Ernster and Nordenbrand (1967), and rat brain microsomes according to De Robertis et al. (1963).

Instrumental Assessment

Oxygen uptake, by aqueous solutions of autoxidizing dopamine was measured with an oxygen electrode of the Clark type (Estabrook, 1967). The oxidation of dopamine was also followed spectrophotometrically at 480 nm as dopaminochrome formation (Heacock, 1959). Lipid peroxidation was measured as malondialdehyde formation (Buege and Aust, 1978). Protein were measured by the biuret test (Gornall et al., 1949).

Data Analysis

The reported data are expressed as mean ± S.D. of at least 5 experiments. Statistical analysis was performed as indicated in the appropriate legend.

Results and Discussion

Oxidation of dopamine

Figure 2 illustrates the oxidation of dopamine followed as either oxygen uptake or dopaminochrome formation. There is a correspondence between the disappearance of oxygen and the formation of colored products, indicating that oxygen is utilized in the process of dopamine oxidation.

Inhibition of the oxidation of dopamine by serotonin and neuroleptic drugs

As shown in Fig. 3 (A and B) serotonin and the neuroleptic drugs chlorpromazine, trifluoperazine and clozapine inhibit the autoxidation of dopamine both at pH 7.4 and pH 8.5. Trifluoperazine appears more efficient than chlorpromazine while serotonin shows an inhibitory effect intermediate between those of trifluoperazine and chlorpromazine. Clozapine shows an effect slightly lower than that of chlorpromazine. The inhibition clearly appears to be biphasic showing a strong effect below 1 mM concentration, while at higher concentration the inhibition is lower. These results reinforce previous observations (Bhatnagar, 1972) showing that the "in vitro" melanin formation from dopamine, catalyzed by mitochondrial preparations, is strongly inhibited by serotonin and some centrally active drugs such as chlorpromazine and amitryptiline.

Effect of metal catalysis

Since metal catalysis appears to be involved in the oxidation of dopamine, chelating agents were employed in order to inhibit it, but neither EDTA (ethylenediaminetetraacetic acid) nor DTPA (diethylenetriamine pentaacetic acid) induced any significant inhibition (Table 1) indicating that, at least in the conditions used, the effect of metal ions is irrelevant. A similar behavior was found by Pilcblad et al. (1988) in studies on the autoxidation of dopamine and its interaction with ascorbate. Not only chelators, but also the classical antioxidant agent Trolox C is ineffective in counteracting the autoxidation of dopamine (Table 1).

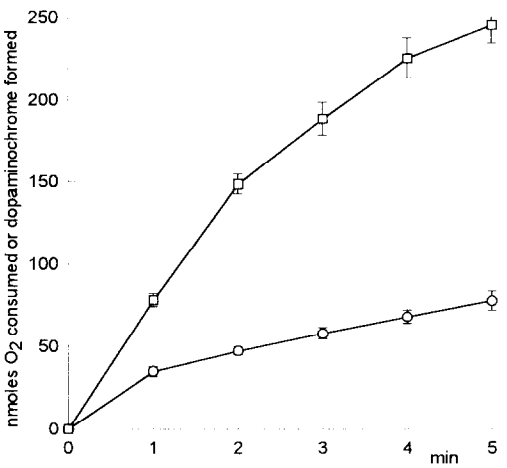

Fig. 2. Oxidation of dopamine. Oxidation of 16.6 mM dopamine was performed at 37 °C in 50 mM Tris·HCl buffer (pH 8.1) in a final volume of 1.5 ml and monitored as either oxygen uptake (□) or dopaminochrome (○) formation. Standard deviations for the mean values were <10%.

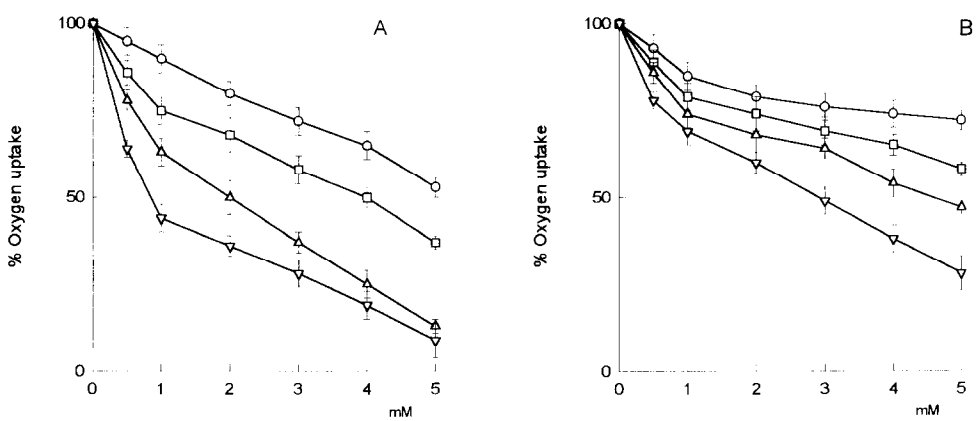

Fig. 3. Oxygen consumption by dopamine in the presence of increasing concentrations of clozapine,

chlorpromazine, trifluoperazine and serotonin. Dopamine (16 mM) was incubated at 37 °C in 50 mM Tris·HCl buffer at pH 7.4 (A) or 8.5 (B); the autoxidation was followed for about 10 minutes and the initial rate was considered. Clozapine (○), chlorpromazine (□), trifluoperazine (Δ) and serotonin (▽) were added at the indicated concentrations. The initial velocities for the non-inhibited reaction were about 10 nmol/min·ml and 80 nmol/min·ml at pH 7.4 and 8.5 respectively. Standard deviations for the mean values were <10%.

Table 1

Effect of Chelating Agents and Antioxidants on Oxygen Uptake Induced by Dopamine Oxidation.

	O_2 uptake	
	nmol/ml·min	%
None	56.73±5.20	100
2mM EDTA	57.07±4.35	101
7.5mM DTPA	60.78±5.80	108
0.5mM Trolox C	68.79±8.15	121

Dopamine (16 mM) was incubated in 50 mM Tris·HCl buffer at pH 8.5 and at 37 °C. The autoxidation was followed for about 10 minutes and the initial rates considered. No significant differences were found by statistical analysis.

Table 2.

Inhibitory effect of phenothiazines, clozapine and serotonin on lipid peroxidation induced by NADPH/Fe^{3+}/ADP on rat liver and brain microsomes.

	Liver microsomes		Brain microsomes	
	MDA formed	% inhibition	MDA formed	% inhibition
None	30.14±2.03	--	5.61±0.87	--
0.1mM Clozapine	11.31±1.89	63	2.86±0.54	49
0.2mM Clozapine	8.95±0.81	70	2.88±0.46	48
0.1mM Chlorpromazine	15.10±1.03	50	2.98±0.49	53
0.2mM Chlorpromazine	13.18±0.97	54	2.33±0.27	58
0.1mM Trifluoperazine	16.60±1.74	45	1.69±0.21	70
0.2mM Trifluoperazine	11.80±0.67	39	1.12±0.05	80
0.1mM Serotonin	5.67±0.24	81	1.13±0.09	79
0.2mM Serotonin	4.20±0.30	86	1.10±0.03	80

Liver or brain microsomes were incubated for 10 minutes at 30 °C in the presence of 0.5 mM NADPH, 20μM $FeCl_3$, 0.2mM ADP and the indicated concentration of the various drugs. Other conditions are indicated in Methods section.

Significant differences among group means were evaluated by one-way analysis of variance. Differences with the respect to the control were significant at the confidence level of 98% as evaluated with the Scheffé's test (Scheffé, 1959).

Effect of the neuroleptic drugs on lipid peroxidation

Even though the mechanism of the inhibitory action of clozapine, phenothiazines and serotonin on

catecholamines autoxidation needs more thorough studies, it might be linked to a free-radical process. The authors, therefore, tested the effect of these compounds on lipid peroxidation induced in brain and liver microsomes. All the compounds appear to inhibit lipid peroxidation (Table 2) directly preventing the deleterious action of free radicals. In addition their indirect preventive effect of oxidative stress is linked to the inhibition of catecholamine oxidation. The latter, upon autoxidation, give rise to potentially toxic products (aminochromes) able either to produce free radicals or act as nucleophiles towards biological molecules (Heacock, 1959; Bindoli et al. 1989). In addition some drugs such as amphetamines determine a displacement of dopamine into the cytosol (Fuller and Hemrick-Lueke, 1982) where it can undergo autoxidation. Consequently, phenothiazines and serotonin could have a therapeutic effect linked to the inhibition of the autoxidation of dopamine; they in fact decrease neuromelanin formation (Bhatnagar, 1972) but can also prevent or decrease the formation of toxic autoxidation products.

The reported results indicate that the spontaneous oxidation of dopamine appears to be inhibited by chlorpromazine, trifluoperazine and serotonin with a rather specific mechanism since both chelating agents and antioxidants are unable to inhibit this process.

The inhibitory effect on dopamine autoxidation and lipid peroxidation elicited by neuroleptic drugs and serotonin might explain in part their protective properties on neuropsychic impairment since the molecular and morphological basis of the latter seems to be referable to neuromelanin and lipofuscin formation (Hirano, 1985). Moreover, this inhibitory effect on dopamine autoxidation might be related to the "dopaminergic" pathogenetic hypothesis of schizophrenia because there are evidences that the antagonists of 5-HT_2 receptors (Nader and Hippius, 1990) can attenuate the effect of dopaminergic neurotoxins (Murphy et al., 1993). As already reported, aminochromes and their derivatives such as adrenolutin and dihydroxyindoles are potentially toxic products. Clozapine, by blocking 5-HT_2 receptors, allows serotonin, inactive on these receptors, to exert a direct antioxidant action "*in vivo*".

Central nervous system as a whole contains a relatively small amount (about 1%) of the total body 5-HT, while midbrain, brainstem and hypothalamus have high concentrations, cerebral cortex, hyppocampus and striatum a moderate concentration and cerebellum a low concentration; these concentrations ranges from 0.98 to 0.07 μgr/gr wet weight. Pineal gland concentration is very high: 73 μg/g wet weight (Brownstein, 1981).

The correlation between inhibitory action of 5HT and that of neuroleptic drugs on distinct anatomical areas of CNS with different biochemical properties still awaits further elucidation.

Conclusions

The inhibitory properties of serotonin and neuroleptic drugs on dopamine autoxidation and lipid peroxidation could throw some light on the pathogenesis of mental diseases and particularly of schizophrenia. In this illness a dopaminergic dearangement, mediated by a membrane impairment, is in fact apparent from neurochemical pathological data, as shown by positron emission thomography and anatomical pathological data disclosed by magnetic resononce imaging (Braff, 1991). Dopamine oxidation might be part of the above derangement and the mechanism of action of the present molecules could include their antioxidant effects.

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