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INTERACTION OF CHLOROPROMAZINE WITH ADRENOCHROME AND INTERFERENCE OF A POSSIBLE ENDOGENOUS PSYCHOTOGENIC AGENT WITH SOME SYNAPTIC ENZYME ACTIVITIES

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Abstract—Chloropromazine is able to interact with adrenochrome yielding a complex more stable than the acetylcholine–adrenochrome complex postulated as the endogenous psychotogenic agent in mental illness. The toxicity of the acetylcholine–adrenochrome complex is related to its ability of inhibiting acetylcholinesterase and activating monoaminoxidase obtained from extracts of baboon brain.

IN PREVIOUS work it was shown that the chemical interaction of two neurotransmitters, acetylcholine (AC) and norepinephrine (NE), in a partially hydrophobic medium can originate a complex in which one component is rearranged to form an aminochrome.[1] The product is a strongly psychotogenic agent as shown by pharmacological and behavioural studies and both its components are necessary for a true psychotomimetic activity.[2]

A hypothesis has been put forward to correlate the formation of an endogenous psychotogenic agent, similar to the one found *in vitro*, with a possible chemical cause of some types of mental disturbance.[1]

In fact, since both cholinergic and adrenergic pathways coexist at the cortical level, any "short circuit", occurring as a result of genetic, traumatic or chemical causes might originate the psychotogenic complex. A rationale for the therapeutic use of nicotinamide (N)[3] was proposed in the framework of the short circuit hypothesis, after demonstrating that the affinity of N for adrenochrome (AD) *in vitro* was higher than the affinity of AC.

In this paper the mode of action of the drug mostly used for the treatment of different forms of mental illness, chloropromazine (CL), is investigated. In addition the action of the AD–AC complex is compared with the effects of different compounds on some synaptic enzyme activities. Among the compounds tested, lithium and cyclic AMP, which have been correlated recently[4] with the symptoms of depression and mania, are considered.

EXPERIMENTAL

The chemical interaction between AD and CL is documented both at the excited and at the ground state of the molecules with the methods described in the preceeding

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papers.[1,2] The results are summarized in Table 1. The equilibrium constant for the formation of an AD–CL complex was measured in tris–dioxane by a spectrophotometric method[2] and Table 2 reports the equilibrium constants of the different adrenochrome complexes obtained in the same medium.

TABLE 1. CHROMATOGRAPHIC MOBILITY AND SPECTRAL CHANGES IN THE ADRENOCHROME–CHLOROPROMAZINE (AD–CL) INTERACTION

Compound	Difference extinction		RF
	300 nm	485 nm	
Adrenochrome (AD)	—	—	0·29
Chloropromazine (CL)	—	—	0
AD–CL	0·140	0·050	0·16

0·1 ml of 1×10^{-3}M aqueous solution of AD (Sigma, St. Louis) and CL (Seca, Paris) were chromatographed on silica gel plates 48 hr after the preparation of the mixtures. The solvent was acetic acid–water 2%. Differential spectra were taken with an UNICAM SP 800 recording spectrophotometer 1 hr after mixing 1×10^{-4}M AD and 2×10^{-4}M CL in tris–dioxane (1 : 10) solvent.

TABLE 2. EQUILIBRIUM CONSTANTS OF ADRENOCHROME COMPLEXES IN TRIS–DIOXANE

Complex	Equilibrium constant	Relative magnitude
Adrenochrome–acetylcholine	$1{\cdot}75 \times 10^{-4}$M	1
Adrenochrome–nicotinamide	$4{\cdot}16 \times 10^{-3}$M	23·8
Adrenochrome–chloropromazine	$8{\cdot}00 \times 10^{-2}$M	457

Increasing concentrations of CL progressively decreased the two bands of AD at 300 nm and 485 nm. The existence of three discrete isosbestic points at 305, 440 and 560 nm indicates the presence of only one spectrophotometric species bound in addition to the free CL.[5]

The formation of an AD–CL complex has been ascertained also by the method of freezing solutions as described by Szent-Gyorgyi.[6] In fact by freezing a mixture of 1×10^{-4}M AD–CL (1 : 1) at −30° a mauve coloured frozen solution is obtained whereas the colour of AD alone in aqueous solution is red brown after freezing.

The AD–CL interaction is observable also polarographically (Table 3) since CL is able to inhibit the oxygen consumption induced by adding ascorbic acid to AD. On the other hand a spectrophotometric reduction of AD similar to the one induced

by ascorbic acid is not observable by effect of CL and direct formation of a melanoid pigment can be noticed after mixing AD and CL. In other words it seems that, in the presence of CL, the transformation of aminochrome to melanin may occur by-passing the trihydroxyindole step. The zinc induced formation of adrenolutin[7] from AD is inhibited by CL since the absorption band at 485 nm remains unchanged for a Zn–AD mixture in the presence of CL whereas in the absence of CL it disappears and the spectrum turns to the typical adrenolutin spectrum. CL has no catalytic effect on the NE autoxidation to aminochrome which has been observed with AC.[2]

TABLE 3. EFFECT OF CHLOROPROMAZINE (CL) ON THE POLAROGRAPHIC REDUCTION OF ADRENOCHROME (AD) INDUCED BY ASCORBIC ACID (AA)

Compound	Oxygen uptake (nat. O_2/min)
Adrenochrome	0
AD–AA (1 : 2)	102
AD–AA–CL (1 : 2)	72·5
AD–AA–CL (1 : 4)	55
AD–AA–CL (1 : 6)	17·5

To 2×10^{-4}M AD in 0·1M tris–HCl pH 7·4 ascorbic acid (Merck, Darmstadt) 4×10^{-4}M was added and the oxygen uptake was measured by a Clark oxygen electrode connected to a Sargent SR recorder. The different amounts of CL added ranged from 4×10^{-4}M to 12×10^{-4}M. Further addition of AA after the last addition of CL reported the rate of oxygen uptake to the initial value.

The effect of the AD–AC complex was investigated on acetylcholinesterase (AcChE) activity of synaptosomes and mitochondrial monoaminoxidase (MAO) activity of baboon brain. AD acts as a non competitive inhibitor of AcChE with an apparent K_i of 1×10^{-4}M whereas CL has a slight activating effect on AcChE and an inhibitory action on MAO.[8] A summary of the effects on AcChE and MAO activities of different compounds acting as stimulants or depressants of the central nervous system (CNS) is presented in Table 4.

The experimental details are given in caption to figures.

DISCUSSION

The different compounds tested together with AD were chosen on the basis of their relevance to a state of excitation or depression of higher nervous activities. Caffeine is a well known CNS stimulant whereas cyclic AMP is considered[4] a useful index of a clinical status of depression or mania, having been found low in depressed patients urine and high in maniac patients urine. Lithium has been reported to exert

TABLE 4. EFFECT OF DIFFERENT COMPOUNDS ON ACETYLCHOLINESTERASE (AcChE) AND MONOAMINOXIDASE (MAO) ACTIVITIES

Compound	Activation AcChE	(%) MAO	Inhibition AcChE	(%) MAO
Lithium	13 ± 3	—	—	68 ± 5
Caffeine	16 ± 3	—	—	60 ± 7
Chloropromazine	14 ± 5	—	—	20 ± 2
Cyclic AMP	—	53 ± 12	5 ± 2	—
Adrenochrome	—	10 ± 4	*	—
AD–AC	—	58 ± 18	10 ± 3	—

* The result is the same as obtained for AD–AC insofar as acetylcholine is always present as substrate.

AcChE activity of synaptosomes and MAO activity of mitochondria isolated from adult baboon brain with the sucrose gradient technique described by Marchbanks[9] were measured. AcChE was determined according to Ellman *et al.*[10] and MAO was measured with the oxygen electrode technique described by Tipton and Dawson.[11]

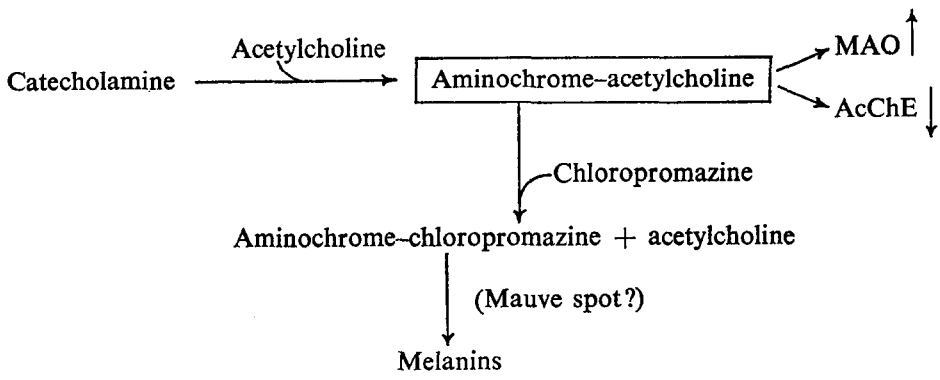
AcChE specific activity was 0·124 μmoles SH/min/mg protein and MAO specific activity with dopamine (Sigma, St. Louis) as a substrate was 2·3 nAt. O_2/min/mg protein.

The concentration of substrate acetylthiocholine (Sigma, St. Louis) was $0{\cdot}87 \times 10^{-3}$M and the concentration of substrate dopamine was 5×10^{-4}M. $LiSO_4$ (Merck, Darmstadt), caffeine (Merck, Darmstadt), cyclic AMP (Sigma, St. Louis), chloropromazine (Seca, Paris) and acetylcholine (BDH) were 5×10^{-4}M and adrenochrome was 4×10^{-5}M final concentration.

therapeutic effect in manic psychosis and this was related to the inhibition of cyclic AMP formation in brain.[12]

The way in which the different compounds tested influence two important synaptic enzyme processes appears to be almost complementary. The CNS stimulants used show an inhibitory effect on MAO and an activatory effect on AcChE, the CNS depressants seem to activate MAO and inhibit AcChE. In this respect AD seems to belong to the class of CNS depressants and its activatory action on MAO is potentiated by the presence of AC. This is in keeping with the decrease of higher nervous activities observed after administration of an AD–AC mixture to mice.[2]

CL is able to inhibit MAO[8] and can overcome the MAO activation and the AcChE inhibition induced by AD–AC. In general the antipsychotogenic activity of CL can be explained with the following scheme:



CL can disrupt the psychotogenic complex and bypass the formation of the still psychotogenic[13] trihydroxyindole while stimulating the catabolism of the aminochromes to melanins. This is in keeping with the observations showing that schizophrenics treated with phenotiazines present an increased melanogenesis.[14] The mauve colour of the AD–CL complex observed in the frozen state suggests that the "mauve spot" found in chromatograms of urines of psychiatric patients[15] might be the result of a CL treatment.

In the previous papers[1,2] the toxic effect of the endogenous psychotogenic complex has been ascribed generically to its permanence as an irritative "focus" at cortical level and to the possibility of inducing aberrant communications of synapses.

From the present data the toxic effect of the psychotogenic complex, thought to be one of the causes of the symptoms of mental disturbance, is documented at enzymatic level. It is possible that the simultaneous effect of inhibition of central adrenergic pathways and activation of central cholinergic pathways is related to the CNS depression.

In the psychotogenic complex an aminochrome (originated from either norepinephrine or dopamine) should be the main active agent, since the psychotomimetic activity of aminochromes has been well documented,[13] but a participation of the AC molecule itself, blocked within the complex in a hallucinogenic configuration,[16] cannot be excluded.

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