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Chlorpromazine: differential effects on membrane-bound enzymes from rat brain*

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THE MECHANISM of action of chlorpromazine (CPZ) remains obscure despite a multitude of experimental studies documenting a variety of pharmacological and biochemical effects. As a unifying hypothesis to relate the many diverse effects of the drug, Guth and Spirtes[1] proposed that a major mode of action was through the modification of membrane properties. In this context we have examined the effects of a pharmacological concentration of CPZ on three membrane-bound enzymes involved with adenine nucleotide metabolism in brain. With a fresh microsomal preparation, CPZ inhibited slightly the Mg^{2+}-dependent adenosine triphosphatase (Mg^{2+}-ATPase), inhibited markedly the $Na^+ + K^+ + Mg^{2+}$-dependent ATPase (Na^+ K^+ Mg^{2+}-ATPase), but stimulated the adenylate kinase 2-fold. Treatment of the membrane preparation with a detergent abolished the ability of CPZ to stimulate adenylate kinase, whereas the ability to inhibit the Mg^{2+}-ATPase was increased.

METHODS

A microsomal fraction was prepared from brains of adult albino rats as previously described.[2] From this fraction the deoxycholate-treated microsomes (DOC-microsomes) were prepared as described by Järnefelt[3] using 1·5 mg deoxycholate/ml in 1·0 M KCl.[4] All incubations were performed within 4 hr after preparation.

For nucleotide phosphatase determinations, the standard incubation medium contained 50 mM Tris-HCl (pH 7·3), 3 mM $MgCl_2$, 3 mM adenosine triphosphate (ATP) or adenosine diphosphate (ADP) as the Tris salt, and about 0·3 mg microsomal protein (in 0·1 ml of 0·25 M sucrose) per ml.

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For studies on the total ATPase activity (i.e. Mg^{2+}-ATPase plus Na^+ K^+ Mg^{2+}-ATPase), 90 mM NaCl plus 10 mM KCl was added to the medium. Incubation was at 30° for 4 min with ATP as substrate or for 10 min with ADP; during these periods product formation proceeded linearly with time. The reaction was stopped by adding cold trichloroacetic acid to a final concentration of 5%. Activity was estimated[4] by measuring the production of inorganic phsophate (P_i) by the method of Lowry and Lopez.[5] All values were corrected for the P_i content in concurrent zero-time incubations; no P_i production beyond zero-time values occurred during incubations without tissue.

Adenylate kinase activity was estimated by measuring the formation of ATP and adenosine monophosphate (AMP) from ADP. In these experiments tracer quantities of 8-^{14}C-ADP were added to the standard incubation media containing 3 mM ADP. After incubation, the nucleotide products were extracted and separated by TLC on DEAE-cellulose,[6] and the radioactivity in AMP, ADP and ATP was measured as previously described.[7] Values were corrected for degradation as measured in concurrent zero-time incubations.

CPZ was a gift from Smith, Kline & French Laboratories, and 8-^{14}C-ADP was purchased from Schwarz BioResearch Inc.

Data are presented with S.D.'s and represent the averages of 5 or more experiments in duplicate. Statistical significance was calculated with the Student t test.

RESULTS AND DISCUSSION

The fresh microsomal preparation hydrolyzed ATP in the standard medium at the rate of 440 μmole P_i/g protein/min. There was essentially no activity in the absence of $MgCl_2$. Addition of 0·1 mM CPZ, a concentration in the range reported present in brain after therapeutic doses,[8] inhibited the Mg^{2+}-ATPase only 13 per cent (Table 1). This inhibition followed the kinetics of non-competitive

TABLE 1. EFFECTS OF CHLORPROMAZINE ON NUCLEOTIDASE ACTIVITY*

Preparation	Pathway	Activity (μmole P_i produced/g protein/min)	
		Control	with 0·1 mM CPZ
Fresh microsomes	Mg^{2+}-ATPase	440 ± 18 (12)	384 ± 21 (12)
	Total ATPase	691 ± 19 (6)	517 ± 20 (6)
	Adenylate kinase plus ATPase	159 ± 8 (10)	165 ± 12 (10)
DOC-microsomes	Mg^{2+}-ATPase	360 ± 15 (10)	259 ± 11 (10)
	Adenylate kinase plus ATPase	33 ± 2 (6)	26 ± 3 (6)

* Fresh microsomes or microsomes treated with deoxycholate (DOC-microsomes) were incubated at 30° in media containing 50 mM Tris-HCl (pH 7·3), 3 mM $MgCl_2$ and either 3 mM ATP (Mg^{2+}-ATPase) or 3 mM ADP (adenylate kinase plus ATPase), with or without 0·1 mM CPZ. Activity in media contaning, in addition, 90 mM NaCl plus 10 mM KCl is presented as Total ATPase. Activity was measured in terms of the liberation of P_i as described under Methods. Data are presented with S.D.'s and with the number of experiments in parentheses.

inhibition with a K_i of 0·7 mM. A previous report noted negligible inhibition by CPZ of the microsomal Mg^{2+}-ATPase.[9] With microsomes treated with deoxycholate, CPZ inhibited the Mg^{2+}-ATPase twice as much (Table 1).

In media containing added NaCl plus KCl, in which the total ATPase was measured, P_i production occurred at a rate of 691 μmole/g protein/min. Most of this 57 per cent increase in activity could be

blocked by 0·1 mM ouabain, which is known to inhibit specifically the Na^+ K^+ Mg^{2+}-dependent ATPase.[10] When 0·1 mM CPZ was added to media containing NaCl plus KCl, it inhibited total P_i production 25 per cent (Table 1). Assuming that the difference in activity between the total ATPase and the Mg^{2+}-ATPase represents the Na^+ K^+ Mg^{2+}-ATPase, then CPZ inhibited this latter enzyme about 46 per cent. Difficulties in the kinetic treatment of two enzymes that act on the same substrate and are differentially inhibited precluded specific kinetic analysis on the Na^+ K^+ Mg^{2+}-ATPase. Previous reports have noted inhibition of this ATPase by CPZ,[9,11] and an attempt has been made to correlate the therapeutic efficacy of a series of phenothiazines with the degree of inhibition of the Na^+ K^+ Mg^{2+}-ATPase.[12]

When ADP was incubated with the microsomal preparation in the standard medium, P_i production proceeded at a rate about 36 per cent of that with ATP. Previous studies[7] indicated that this production of P_i with ADP did not represent a nucleotide diphosphatase or an apyrase but rather conversion of ADP to ATP (and AMP) by adenylate kinase followed by liberation of P_i from the newly formed ATP by the Mg^{2+}-ATPase. With this pathway CPZ would be expected to inhibit P_i production with ADP to an extent equal to (or possibly greater than) that with ATP. However, 0·1 mM CPZ had little effect on P_i production with ADP (Table 1). A possible explanation for this discrepancy would invoke a stimulation of adenylate kinase by CPZ.

To investigate this possibility, adenylate kinase activity was measured directly by adding to the standard incubation mixture containing 3 mM ADP tracer quantities of 8-^{14}C-ADP. After incubation the production of ATP and AMP was estimated by measuring the radioactivity present in the nucleotides. The apparent rate of production of ATP was less than that of AMP due to the hydrolysis of ATP by ATPase (Table 2); under these experimental conditions AMP was not metabolized further.

TABLE 2. EFFECTS OF CHLORPROMAZINE ON ADENYLATE KINASE ACTIVITY*

Preparation	Nucleotide formation	Activity (μmole nucleotide formed/g protein/min)	
		Control	with 0·1 mM CPZ
Fresh microsomes	AMP formation	228 ± 16 (5)	406 ± 23 (5)
	ATP formation	69 ± 14 (5)	217 ± 20 (5)
DOC-microsomes	AMP formation	41·1 ± 2·4 (5)	34·3 ± 2·3 (5)
	ATP formation	8·0 ± 1·9 (5)	8·5 ± 2·0 (5)

* Fresh microsomes or microsomes treated with deoxycholate were incubated at 30° in media containing 50 mM Tris-HCl (pH 7·3), 3 mM $MgCl_2$, and 3 mM ADP with tracer quantities of 8-^{14}C-ADP. After incubation, the nucleotides were separated and the radioactivity was measured, as described under Methods. Data are presented with S.D.'s and with the number of experiments in parentheses.

The addition of 0·1 mM CPZ markedly stimulated the adenylate kinase reaction (Table 2). The percentage increase in ATP formation was greater than that of AMP formation, presumably due to the concomitant inhibition by CPZ of ATPase.

With microsomes treated with deoxycholate, however, CPZ inhibited P_i production over the adenylate kinase-ATPase pathway (Table 1) and did not stimulate adenylate kinase (Table 2).

These experiments thus demonstrate a differential effect of CPZ on three membrane-bound enzymes in rat brain: marked inhibition of the Na^+ K^+ Mg^{2+}-ATPase; slight inhibition of the Mg^{2+}-ATPase; and a 2-fold stimulation of adenylate kinase. Furthermore, the ability of CPZ to stimulate adenylate kinase could be abolished by physical modification of the membrane preparation, whereas the ability to inhibit the Mg^{2+}-ATPase was increased.

Although the stimulation of adenylate kinase by CPZ may be irrelevant to the pharmacological effects of the drug, the process itself appears of interest in light of the hypothesis of Guth and Spirtes[1] relating the effects of CPZ to alterations in membrane structure. Brain microsomal adenylate kinase

has been shown to be particularly sensitive to membrane organization; for example, increases in osmolarity and in ionic strength that either stimulated or had no effect on ATPase activity inhibited adenylate kinase, and this inhibition could be abolished reversibly by physical modifications of membrane structure.[7] Thus the micro-environment within the membrane, which may respond variously to drugs, could influence selectively the relative activity of the component enzymes and, in addition, the effect of the drugs on the enzymes would be related to the specific local membrane structure. Possibly, such considerations are involved in the reported lack of effect of CPZ on liver mitochondrial adenylate kinase.[13]

Certain reservations should be emphasized. The effects described were all with a uniform concentration of CPZ and of adenine nucleotides. The finding that either stimulation or inhibition of hexokinase occurred depending on CPZ : ATP ratios[14] must enforce caution in generalizing from such experiments *in vitro*. Changes in relative specific activities that occur after physical treatment may also be misleading. And although the enzymatic activities were present in the microsomal preparation, their original cellular location is unknown; microsomal fractions contain remnants of the endoplasmic reticulum and possibly of the plasma membrane. Mitochondrial contamination in this preparation, as indicated by electron microscopy and specific activities of succinic dehydrogenase and cytochrome oxidase, was low.[15]

The role of adenylate kinase in brain microsomes is uncertain; it may act to regenerate ATP from ADP for use in energy-requiring processes and to lessen product inhibition of ATPases. In any case, stimulation of adenylate kinase together with inhibition of the ATPases would tend to raise the ATP: ADP ratio. Since it has been shown with this membrane preparation[16] that ATP and ADP each induced particular structural changes in membrane organization, such a change in nucleotide concentrations could promote further membrane modification. Although an early study[17] on ATP levels after CPZ administration found increased levels of ATP, later reports have qualified this finding, and a recent study[18] described a complex relationship between ATP levels, location in the brain and time of CPZ administration.

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