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The acute effects of amphetamine, chlorpromazine, amitriptyline and lithium on adenosine 5-triphosphatase activity in the cortex of the rat brain

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A reuptake process appears to be the principle mechanism for the inactivation of noradrenaline, dopamine and serotonin following their physiological release from neurons. It has been demonstrated that the uptake of noradrenaline into both nervous and non nervous tissue is antagonized by ouabain [1, 2], a drug which has long been known to block the ATPase mediated flux of Na^+ and K^+ across cell membranes [3]. Bogdanski and Brodie [4] have proposed a model for the amine carrier mechanism. They propose that the affinity of the carrier for noradrenaline is dependent on the concentration of Na^+ and is a non energy requiring process. Once within the nerve ending, noradrenaline is mainly stored in specific vesicles and the Na^+ extruded from the cell by the energy dependent ATPase system. Thus any situation leading to a change in the cation flux across a nerve membrane would affect the affinity of the carrier for the neuro-transmitter. However, other invistigators [5] have shown that in cardiac tissue the onset of the ouabain inhibition of noradrenaline accumulation was uncorrelated with the changes in the intracellular Na^+ and K^+ concentrations. From such studies it would appear that although there is evidence which implicates Na^+, K^+ ATPase in the amine transport process, it is uncertain whether these processes are causally related. Furthermore most studies of the effect of drugs on ATPase activity and amine transport have been conducted *in vitro*. It was with a view to finding if there was a causal or coincidental involvement of Na^+, K^+ and Mg^+ dependent ATPases with amine transport into partially purified synaptosomes and vesicles that we decided to study the actions of some psychotropic durgs known to affect the uptake and metabolism of biogenic amines in the rat brain *in vivo*. Studies were made of the major tranquillizing drug chlorpromazine, of lithium chloride (a drug used most frequently in the treatment of manic depression), of the tricyclic antidepressant amitriptyline and of the stimulant drug D-amphetamine.

MATERIALS AND METHODS

Male Wistar rats (approximate weight 300 g) were used. The control and experimental groups consisted of 6 animals. The control group was injected with physiological saline and the experimental groups with D-amphetamine (5 mg/kg i.p.), chlorpromazine (10 mg/kg i.p.), amitriptyline (20 mg/kg i.p.) or lithium chloride (50 mg/kg i.p.). One hr after drug administration, the animals were decapitated; at this time all the drugs had clearly observable effects on the gross behaviour of the animals. The brains were rapidly removed, washed in cold saline, the excess saline removed and placed on ice. The cerebral cortex was removed from each brain. The cortices were homogenized for 5 min, in 10 volumes of 0.32 M sucrose containing 1 mM EDTA pH 7.4 at 0° using a Thomas teflon pestle tissue grinder (clearance 0.15 mm rotating at 1200 rev/min). The partially purified synaptosomes and vesicular fractions were separated by density gradient centrifugation using the method of Gray and Whittaker [6]. The vesicular fraction occurred at the junction of the 0.32 and 0.80 M sucrose band while the synaptosomal fraction occurred at the interface of the 1.0 and 1.2 M sucrose layer. The molarity of the sucrose containing the synaptosomal fraction was adjusted to 0.40 M and both the vesicular and the synaptosomal fractions centrifuged at 100,000 *g* for 30 mins. The pellets were resuspended in distilled water and retained at 4° for estimation of the ATPase activity. The incubation medium contained (final concentrations) 5 mM-$MgCl_2$, 15 mM-Kcl, 90 mM-NaCl and 5 uM ATP (sodium free); ouabain (1 mM) was added in some cases to inhibit Na^+, K^+ ATPase activity. Total ATPase activity was taken to be the activity occurring in the absence of ouabain while Mg^{2+} dependent ATPase activity was assumed to be that remaining after ouabain inhibition. ATPase activity was determined by measuring the release of inorganic phosphate from ATP after 5 min incubation at 37°. The enzyme activity is expressed as μmoles of inorganic phosphate formed per hr per mg protein. All estimations were carried out in duplicate. The statistical significance of the results was assessed using Student's *t*-test.

RESULTS AND DISCUSSION

The acute effects of amphetamine, chlorpromazine, amitriptyline and lithium chloride on vesicular and synaptosomal ATPase activity are summarized in Table 1.

Table 1. ATPase activities in synaptosomal fractions of rat cortex

Treatment	Mg^{2+} dependent ATPase	Na^+, K^+ dependent ATPase
Saline (control)	5.50 ± 0.56	10.00 ± 0.80
Amphetamine	6.00 ± 0.92	10.50 ± 0.50
Chlorpromazine	5.65 ± 0.72	6.52 ± 0.56*
Amitriptyline	6.00 ± 0.50	9.80 ± 0.75
Lithium	3.00 ± 0.52*	6.00 ± 0.54*

* Significantly different from control ($P < 0.05$). All activities expressed as μmoles inorganic phosphate formed per mg protein per hr. Results expressed as mean ± S.E.M. Each group consisted of six rats.

None of the drugs had any significant effect on the ATPase activity of this fraction. The activities of the Mg^{2+} and Na^+, K^+ dependent ATPases in the vesicular fraction of the control animals was 13.00 ± 0.97 and 5.52 ± 0.60 μmoles/mg/hr respectively. Na^+, K^+ dependent ATPase forms approximately 30 per cent of the total enzyme activity of this fraction in contrast to approximately 64 per cent of the total activity of the synaptosomal fraction.

In the synaptosomal fraction, chlorpromazine decreased the Na^+, K^+ ATPase activity while lithium chloride decreased both the Na^+, K^+ and the Mg^{2+} dependent ATPase activity. Neither amphetamine nor amitriptyline significantly changed the activity of the ATPases in the synaptosomal fractions.

Other investigators have shown that the uptake of catecholamines into intraneuronal storage vesticles is sensitive to the presence of ATP and Mg^{2+} [7, 8, 9]. Such studies implicate a Mg^{2+} dependent ATPase in the vesicular storage of these neurotransmitters. Carlsson *et al.* [10] have shown that chlorpromazine and several other tricyclic compounds actively inhibit the vesicular uptake of catecholamines. However, our findings show that following *in vivo* administration none of the psychotropic drugs studied had any appreciable effect on the activities of the ATPases in the vesicular fraction. The discrepancy between our findings and those of other investigators may be related to the fact that their work was undertaken on adrenal medullary vesicles. Furthermore, others have shown that Na^+, K^+ dependent ATPase activity is absent from the vesicular membrane [9, 11] which implies that our vesicular fraction may be slightly contaminated with other membrane fragments containing Na^+, K^+ ATPase.

Unlike the vesicular fraction, the ATPase activity of the synaptosomal fraction was significantly affected by both chlorpromazine and lithium chloride. The activities of both ATPases in the fraction obtained from the control animals were essentially similar to those reported by others [12]. The decrease in Na^+, K^+ activity following acute chlorpromazine and lithium chloride treatment and in the Mg^{2+} dependent activity in animals treated with lithium is consistent with our previous findings that the activities of these enzymes are decreased in the microsomal fraction from different brain regions [13]. Such results conflict with those of Hesketh [14] who reported that lithium chloride causes an increase in Mg^{2+} dependent ATPase activity in rat brain synaptosomes.

From this study it would appear that chlorpromazine and lithium preferentially inhibit synaptosomal ATPase activity without significantly affecting the enzyme activity of the vesicular fraction. It is, of course, possible that changes in the activities of these enzymes may have been more prenounced had studies been made on sub-cellular fractions from other brain regions. However, this seems unlikely as our previous study has shown that the effects of these drugs on the activities of the ATPases in other brain regions are, in general, qualitatively similar to those observed in the cortex [13]. It also seems unlikely that the changes observed in the ATPase activities in these subcellular fractions are causally related to the effects of the drugs on the uptake of biogenic amines *in vivo*, a situation which we have recently reviewed [15].

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