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THE ACUTE AND CHRONIC EFFECTS OF D-AMPHETAMINE, CHLORPROMAZINE, AMITRIPTYLINE AND LITHIUM CHLORIDE ON ADENOSINE 5-TRIPHOSPHATASES IN DIFFERENT REGIONS OF THE RAT BRAIN

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Abstract—The hypothesis that adenosine triphosphatase plays a primary role in the re-uptake process of biogenic amines in the rat brain was tested. Four centrally acting drugs, known to alter the turnover of biogenic amines in different ways, were studied for their *in vivo* effects on Na^+ K^+ ATPase and Mg^{2+} (EC 3.6.1.5) ATPase activities in high-speed supernatant fractions from ten brain regions. The acute and chronic administration of amphetamine, amitriptyline, chlorpromazine and lithium chloride altered the activities of the ATPases in several of the brain regions studied. In most cases, the regions in which the activity of these enzymes was most affected coincided with the areas in which other investigators have shown these drugs to have a pharmacological action. Amphetamine and amitriptyline increased the activity of the ATP'ases in several regions whereas chlorpromazine and lithium chloride had the opposite effects. While it seems possible that different classes of drugs can affect ATP'ases in different ways depending on their neuropharmacological profile, there is little evidence from this study to substantiate the view that changes in ATP'ase activity is directly involved in the activity of the re-uptake transport system for brain biogenic amines.

In the mammalian brain there is evidence that the re-uptake of physiologically released biogenic amine neurotransmitters is closely associated with an ouabain sensitive Na^+, K^+ dependent process [1, 2]. This suggests that Na^+-K^+-ATPase (EC 3.6.1.3) may have a regulatory function in synaptic transmission. However, other investigators have shown that in cardiac tissue the onset of the ouabain induced inhibition of noradrenaline accumulation was not related to the changes in intracellular Na^+ and K^+ concentrations [3]. The possibility therefore arises that while Na^+, K^+ ATPase activity may be involved in the re-uptake of biogenic amines, it is uncertain whether these processes are causally related.

In an attempt to investigate the possible relationship between ATPase activity and amine uptake *in vivo*, we studied the effects on ATPase activity of four drugs known to affect amine metabolism in the rat brain in different ways. It has already been shown by others that such centrally acting drugs as morphine, ethanol and *trans*- Δ9-tetrahydrocannabinol stimulate Na^+, K^+ ATPase activity in rat brain and that this effect may contribute to their neuropharmacological action [4]. Some of the results reported here have been previously communicated elsewhere [5, 6].

MATERIALS AND METHODS

In the *acute experiment*, groups of six male rats (Wistar, approximately 300 g) were injected intraperitoneally (i.p.) (1 ml/kg body wt) with 0.9% (w/v) sodium chloride (control group), D-amphetamine sulphate (5 mg/kg), amitriptyline (20 mg/kg), chlorpromazine (10 mg/kg) or lithium chloride (50 mg/kg) and decapitated 1 hr later. The brains were rapidly removed, washed in cold saline, the excess fluid removed and the brains then placed on ice. The brains were dissected into the following regions by the method of Popov *et al.* [7]: brain stem (pons + medulla), cerebellum, tegmentum + colliculus, thalamus + hypothalamus, hippocampus, striatum, olfactory lobes, neocortex, septum and amygdaloid cortex. The brain regions were homogenized by sonication for 1.5 min in 10 volumes of an extraction medium of 0.32 M sucrose containing 1 MM ethylene diamine tetra acetic acid and 0.15% deoxycholic acid pH 7.4. During sonication the temperature of the medium was maintained at 4°. The homogenates were then centrifuged at 20,000 *g* for 30 min at 4° and the resulting clear supernatants retained and stored for 24 hr at 4° for subsequent measurement of ATPase activities and protein determination. The supernatant of the sonicated material contained microsomes, broken mitochondrio and cytoplasm.

In the *chronic experiment*, groups of six rats were given the drugs daily for a period of 2 weeks. D-amphetamine was administered in the drinking water, together with an equal quantity of D-ascorbic acid as an antioxidant. The drug solution was changed daily. Initially, the concentration of amphetamine was 50 mg/l for 3 days which was increased to 100 mg/l for the remainder of the first week and then to 200 mg/l for the duration of the second week. The control group received the appropriate concentration of ascorbic acid in its drinking water. Lithium chloride was given in the drinking water of

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the second experimental group, the concentration being 400 mg/l for 2 weeks. The control group received tap water.

Chlorpromazine (2 mg/kg) and amitriptyline (5 mg/kg) were given by subcutaneous injection twice daily (0900–1800 hr) for 2 weeks. The corresponding control groups were injected at the same times with physiological saline. Two weeks after the commencement of treatment, the animals were decapitated, the brain regions dissected, homogenized and centrifuged as outlined above.

Determination of ATPase activity. ATPase activity was determined by the method of Schwartz *et al.* [8]. This consisted of adding 0.25 ml aliquot of the supernatant fractions to an incubation medium containing 5 mM $MgCl_2$, 15 mM KCl, 90 mM NaCl and 5 mM sodium free ATP at 37°; ouabain (1 mM) was added in some cases to inhibit Na^+, K^+ ATPase activity. The final volume of the incubation medium was 1.0 ml. The concentrations represent the final concentration in the incubation medium. The reaction was terminated after 30 min by adding 4 ml of a mixture of 1% (w/v) Lubrol WX (Sigma Chemicals) and 1% (w/v) ammonium molybdate in 0.9 M sulphuric acid solution. The inorganic phosphate released by ATPase during the incubation period was estimated by the method of Bowler and Tirri [9]. The protein content of an aliquot of the supernatant was estimated by the method of Lowry *et al.* [10] and the results expressed as μmoles inorganic phosphate formed/mg protein/hr. All estimations were carried out in duplicate. Total ATPase activity was taken to be the enzyme activity occurring in the absence of ouabain while Mg^{2+} dependent ATPase activity was assumed to be that remaining after ouabain inhibition.

The statistical significance of the results assessed using Student's '*t*' test; the α-level was chosen at 0.05.

RESULTS

Acute experiment

Effect of D-amphetamine. Following the acute administration of D-amphetamine, dyskinetic and stereotypic movements (gnawing, licking and biting bars of cage) were pronounced 1 hr after drug administration.

The cerebellum and midbrain of the control rats were found to possess the highest activity of Na^+, K^+ ATPase while the lowest activity was found in the septum (Table 1). Acute amphetamine administration resulted in an increased Na^+, K^+ ATPase activity in several brain regions but this only reached statistical significance in the olfactory lobes, hippocampus and thalamus. The activity of Mg^{2+} dependent ATPase was found to be highest in the amygdala and hippocampus of the control animals and lowest in the septum (Table 1). After acute amphetamine treatment, a statistically significant increase in Mg^{2+} dependent ATPase activity was found in the septum, hippocampus and amygdaloid cortex.

Effect of chlorpromazine. The rats were behaviourally depressed within 10 min of acute administration of chlorpromazine and remained in this state until they were decapitated 50 min later. Apart from a significant decrease in Na^+–K^+ ATPase activity of the hippocampus, acute chlorpromazine did not alter the activity of this enzyme in any of the brain areas investigated (Table 1). This drug did however significantly decrease the activity of the Mg^{2+} dependent enzyme in the olfactory lobes, striatum and amygdaloid cortex (Table 1).

Table 1. Acute effects of four centrally acting drugs on the activity of Na^+, K^+ and Mg^{2+} dependent ATPase activity

	Brain region									
	Cortex	Olfactory lobes	Septum	Hippocampus	Amygdaloid	Midbrain	Thalamus hypothal	Striatum	Cerebellum	Brain stem
	Mg^{2+} dependent ATPase activity									
Control	100±28	100±17	100±25	100±30	100±23	100±32	100±30	100±23	100±33	100±28
Amphetamine (5)	78±18	110±25	*252±30	*205±25	*150±20	137±30	104±15	105±10	81±16	133±32
Chlorpromazine (10)	72±24	*25±9	130±40	50±18	*60±10	65±25	63±12	*50±12	70±24	53±21
Amitriptyline (20)	81±25	200±15	140±45	67±20	100±14	105±20	90±10	108±24	93±12	95±10
Lithium chloride (50)	*45±16	*68±10	96±8	78±10	*62±8	*52±28	*50±5	*52±16	68±22	50±28
Control values (μmoles P/mg/hr)	2.34	2.40	2.08	2.52	2.58	2.10	2.31	2.36	2.10	2.16
	Na^+, K^+ dependent ATPase activity									
Control	100±31	100±35	100±26	100±20	100±23	100±37	100±18	100±30	100±12	100±26
Amphetamine	153±29	155±37	*150±20	76±18	133±28	164±32	95±10	*245±27	64±22	69±18
Chlorpromazine	70±18	104±33	108±28	82±10	110±20	50±25	100±12	155±32	95±16	73±20
Amitriptyline	150±20	*165±17	90±30	75±23	122±31	170±36	63±22	84±22	70±17	67±19
Lithium chloride	72±16	104±30	100±18	68±22	88±18	100±20	*60±10	80±20	*25±8	70±18
Control values (μmoles P/mg/hr)	1.43	0.92	1.73	2.93	1.57	1.79	2.08	1.40	1.93	2.64

Dose administered given in parenthesis (mg/kg). All values expressed as a percentage of the control (= 100%) ± S.D. *Difference between control and drug treated group significant at $P<0.05$. Absolute control values expressed as μmoles inorganic P formed/mg protein/hr.

Effect of amitriptyline. Acute administration of amitriptyline resulted in slight hyperactivity, characterized by rapid, jerky whole body movements. When the animals were left undisturbed this behaviour alternated with periods of reduced ambulation.

Amitriptyline caused a significant increase in the activity of Na^+, K^+ ATPase activity in the septum, amygdala and midbrain without producing a significant change in any of the other brain regions (Table 1). Mg^{2+} dependent ATPase activity was increased in the olfactory lobe region only.

Effect of lithium. Following the acute administration of this drug, the animals became behaviourally depressed throughout the 1 hr period of observation. They did not show any signs of motor incoordination or loss of righting reflex.

Acute lithium chloride treatment caused a significant reduction in Na^+, K^+ ATPase activity in the septum; while the activity of this enzyme in the other regions was reduced, the changes did not reach statistical significance (Table 1). A statistically significant reduction in Mg^{2+} ATPase activity occurred in the olfactory lobes, neocortex, striatum, midbrain, thalamohypothalamic complex and the amygdaloid cortex (Table 1). Thus the primary action of lithium appears to be in causing a reduction in Mg^{2+} ATPase activity without markedly affecting that of the Na^+, K^+ dependent enzyme.

Chronic experiment

Effect of D-*amphetamine.* Immediately before killing, rats which had been chronically treated with the drug showed stereotypic behaviour characterized by fast repetitive head movements and repeated licking and gnawing at the bars of the cage.

The activity of Na^+, K^+ ATPase was significantly increased in the olfactory lobes, thalamohypothalamic complex and hippocampus (Table 2). An increase in the activity of Mg^{2+} ATPase also occurred in the hippocampus and thalamohypothalamic complex whereas a marked decrease in enzyme activity occurred in the midbrain region (tegmentum+colliculus). No change in the activities of either enzyme occurred in the cerebellum.

Effect of chlorpromazine. After chronic chlorpromazine treatment, the rats showed little spontaneous exploratory activity but would react to external stimuli; there was no obvious loss of muscle tone.

Na^+, K^+ dependent ATPase activity was significantly decreased in the hippocampus after chronic drug administration; a significant decrease in Mg^{2+} dependent ATPase activity also occurred in the midbrain and while the activity of this enzyme was decreased in the olfactory lobes and striatum it did not reach statistical significance (Table 2).

Effect of amitriptyline. Chronic administration of this antidepressant resulted in exaggerated movement when the animals were disturbed. Slight stereotypic mouth movements were also evident.

Amitriptyline caused a significant increase in Na^+, K^+ ATPase activity in the midbrain, septum and amygdaloid cortex. A statistically significant increase in Mg^{2+} dependent ATPase was found to occur in the midbrain (Table 2).

Effect of lithium chloride. Apart from a slight decrease in spontaneous movement, lithium had little apparent effect in the gross behaviour of the rats.

Marked, and significant, decreases in the activity of Na^+, K^+ ATPase occurred in the brain stem and septum; the activity of this enzyme in most brain areas was decreased but did not reach statistical significance (Table 2). A decrease in Mg^{2+} dependent ATPase activity occurred in the septum, thalamohypothalamic complex and amygdaloid cortex.

DISCUSSION

Many investigators have provided evidence for the view that the central effects of D-amphetamine, particularly increased locomotor activity and stereotyped behaviour, are mediated through brain

Table 2. Chronic effects of four centrally acting drugs on activity of Na^+, K^+ and Mg^{2+} dependent ATPase activity

	Brain region: Cortex	Olfactory lobes	Septum	Hippocampus	Amygdala	Midbrain	Thalamus-hypothal	Striatum	Cerebellum	Brain stem
	Mg^{2+} dependent ATPase activity									
Control	100±27	100±21	100±14	100±18	100±10	100±21	100±22	100±31	100±26	100±18
Amphetamine	120±12	96±10	124±8	*132±12	102±11	68±10	*145±8	97±10	98±13	103±16
Chlorpromazine	100±12	88±12	100±16	96±10	96±9	*51±11	95±10	82±17	100±10	96±22
Amitriptyline	116±18	98±17	104±14	97±8	104±10	132±12	106±10	105±12	114±14	100±14
Lithium chloride	87±10	100±10	*56±8	94±6	*70±5	91±12	*65±8	100±18	95±18	95±12
	Na^+, K^+ dependent ATPase activity									
Control	100±20	100±18	100±20	100±14	100±11	100±8	100±18	100±22	100±25	100±16
Amphetamine	114±12	*132±14	120±8	*133±16	104±14	101±10	*142±14	122±10	100±14	98±20
Chlorpromazine	78±16	91±10	100±6	*70±8	100±9	100±12	94±15	78±18	82±16	88±17
Amitriptyline	81±17	87±13	*141±10	100±10	118±5	*140±15	105±19	88±17	100±21	91±21
Lithium chloride	98±18	90±12	*67±10	83±13	97±10	96±9	96±10	87±19	88±10	*75±18

Experimental groups treated with drugs for 14 days; doses given in Methods. All values expressed as percentage of control (= 100%)±S.D.

*Difference between control and drug treated group significant at $P < 0.05$.

catecholamines [11, 12]. Thus one would expect the biochemical changes caused by amphetamine to be most pronounced in those areas where the catecholamines have a neurotransmitter function. Noradrenaline is present in a relatively high concentration in the midbrain, hippocampus and amygdala while dopamine concentrations are high in the striatum and, to a lesser extent, the olfactory lobes. This differential distribution of the catecholamines may be causally related to their neurotransmitter role. Both acutely and chronically administered amphetamine caused increases in the activities of the ATPases in various parts of the limbic system and in the striatum. Thus the activity of the Mg^{2+} dependent enzyme was increased in the septum, hippocampus and amygdaloid cortex after acute administration and in the septum, thalamus and hippocampus after chronic administration. Na^+, K^+ ATPase activity also increased in the septum and striatum after acute administration and in the olfactory lobes, septum, thalamus and hippocampus after chronic administration. There is evidence that amphetamine acts by promoting the presynaptic release and blocking the re-uptake of catecholamines [13]. However, apart from a slight decrease in Mg^{2+} dependent ATPase activity in the midbrain region after chronic drug treatment the primary action of the drug is to increase the activities of these enzymes. If the hypothesis linking ATPase activity with amine re-uptake mechanism is correct then it would be anticipated that a drug which reduces amine re-uptake would decrease ATPase activity preferentially in those brain regions where the catecholamines function as neurotransmitters. This does not appear to be the case.

Chlorpromazine is a potent neuroleptic drug which, while affecting most parts of the central nervous system (CNS), primarily acts on the hypothalamus, limbic and reticular system [14]. It has been suggested that chlorpromazine acts by blocking post synaptic catecholamine receptor sites and, by feed-back stimulation of tyrosine hydroxylase, enhances the turnover of the catecholamines [32, 15]. After both acute and chronic administration of the drug, both Mg^{2+} dependent and Na^+, K^+ dependent ATPase activities were decreased. Other investigators have also shown, using *in vitro* preparations, that chlorpromazine decreases ATPase activity [16, 17]. However, Sikora and Krulik [18] found that rats treated with the drug for 10 days showed no significant change in cortical ATPase activity. Similarly, in the present chronic study, no change was found in cortical activity but a decrease in Na^+, K^+ ATPase occurred in the brain stem and hippocampus and in Mg^{2+} ATPase in the midbrain region. Our findings therefore substantiate and extend those of Sikora and Krulik [18].

It would be anticipated that a drug which increases catecholamine turnover would also increase ATPase activity if a causal relationship existed between the uptake mechanism and ATPase activity. Chlorpromazine, however, decreased ATPase activities thereby throwing some doubt on this hypothesis.

Amitriptyline is thought to act by selectively blocking the re-uptake of noradrenaline and serotonin into presynaptic terminals and thereby increases the availability of the neurotransmitters to the receptor sites [19]. As a consequence of the uptake inhibition, the "turnover" of these neurotransmitters is reduced [13, 20]. If the re-uptake of these amines is dependent on ATPase activity one would anticipate that amitriptyline would decrease the activity of this enzyme. In fact it has been shown in the present study that after both acute and chronic administration of the drug the activities of the ATPases increase. Qualitative differences, however, were found between the acute and chronic effects of amitriptyline. After acute administration the increase in enzyme activities occurred primarily in the olfactory lobe and, to a lesser extent, in the midbrain, septum and striatum. After chronic administration, amitriptyline caused an increase of these enzymes in the midbrain; Na^+, K^+ dependent ATPase activity increased in the septum and amygdaloid cortex. Whether these differences have any bearing on the clinical observation that this drug is only effective in the treatment of depression when given for a minimum period of 10–14 days remains to be investigated. Considerable attention has been directed to the action of the tricyclic-antidepressants on the amygdala and definite neuronal connections have been shown to exist between this area and the olfactory lobes in rats [22]. While it therefore seems unlikely that the changes induced in the ATPases by amitriptyline are causally related to its effect on the amine re-uptake system the possibility remains that the effects on these enzymes are correlated with the neuropharmacological action of the drug, particularly since the changes occur in those brain regions where other neuro-pharmacological studies have shown the drug to act.

Lithium salts have come to occupy an important place in the treatment of the affective disorders, particularly mania [23]. They are particularly effective during the manic phase of manic depressive psychosis and, when given prophylactically will also reduce the incidence of depression in such mental disorders [23]. There is evidence that mania is associated with an increased availability of noradrenaline at central receptor sites, and lithium has been shown to increase the concentrations of deaminated metabolites following the intracisternal administration of tritiated noradrenaline [24–26]. Colburn *et al.* [27] have shown that chronic lithium administration causes an increase in the re-uptake of catecholamines into the presynaptic nerve terminal and have suggested that this may be the primary action of the drug. Thus the lithium ion may exert its therapeutic action by causing a decrease in the concentration of noradrenaline at central receptor sites.

Besides affecting biogenic amine metabolism in the brain, there is evidence that the pharmacological action of lithium may be partly attributable to its ability to replace K^+ in the "sodium pump" [28, 29]. Lithium ions are therefore trapped intracellularly as they are transported out of the cell at a slower rate than Na^+, an effect which may be due to the fact that Li^+ stimulates ATPase less effectively than Na^+. Evidence for this view has been provided by Ploeger [29] who found that chronic lithium treatment inhibits ATPase activity in non-myelinated fibres. Gutman *et al.* [30] however found no change in either Mg^{2+} or Na^+, K^+ dependent ATPase activity in rat brain stem or cortex. In the present study, both acutely and chronically administered lithium chloride causes a decrease in the activities of both ATPases in most brain regions studied; the effect of acute drug treatment was more pronounced and involved more brain regions than did chronic administration. These effects would be anticipated from the previous observation that Li^+ reduces ATPase activity. If, however, lithium salts enhanced noradrenaline uptake then one would have anticipated that the drug would increase, rather than decrease, the activities of the ATPases should the hypothesis linking amine re-uptake with these enzymes be valid. It is well established that when lithium salts are given to manic-depressives the initial anti-manic action occurs within a few days whereas the drug must be given for several weeks before it is effective in the control of the depressive symptoms [23]. Whether these effects are a reflection of the qualitative differences found here between the acute and chronic drug administration remains to be further investigated.

We have previously shown that the ATPase activity of purified synaptosomal and vesicular fractions prepared by density gradient centrifugation from the cerebral cortex of rats treated acutely with the same four drugs used in the present study gave qualitatively similar results to those reported here [31]. Thus none of the drugs affected the activity of the vesicular fraction but lithium chloride significantly decreased both Na^+, K^+ and Mg^{2+} dependent ATPase in the synaptosomal fraction and chlorpromazine reduced the activity of Na^+, K^+ ATPase on this fraction. Clearly there is a need to extend these studies to include groups of animals treated chronically with the drugs.

From this study it can be concluded that all four drugs affect the activities of the ATPases in different brain regions. In some cases there may be a correlation between the changes in ATPase activity and the brain regions in which the drugs have been shown to exert their pharmacological actions. However, in most cases the qualitative nature of the changes in enzyme activity is the converse of that predicted and therefore it seems unlikely that changes in ATPase activity directly reflect the activity of the re-uptake system for the biogenic amine neurotransmitters.

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