Br. J. Pharmac. (1968), **34**, 70–75.

The effect of chlorpromazine on cerebral glucose, ATP, ADP, AMP and ATPase in the mouse

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1. Administration of chlorpromazine 10 mg/kg intraperitoneally to mice produced no alteration in cerebral adenosine triphosphate (ATP), adenosine diphosphate (ADP) or adenosine monophosphate (AMP). In these animals there was an increase in the glucose content of the supernatants of homogenates of liver and brain following acid hydrolysis.

2. Chlorpromazine 40 mg/kg intraperitoneally did not affect Mg^{++} activated adenosine triphosphatase (ATPase) activity in the brain.

Administration of chlorpromazine to mice results in an impairment of cerebral protein and lipid synthesis *in vivo* (Skinner & Spector, 1968). Dawkins, Judah & Rees (1959) have demonstrated that this drug uncouples oxidative phosphorylation in the brain *in vitro* and Quastel (1965) has presented evidence to suggest that drugs which depress the nervous system in general may act in this way by inhibiting oxidation of carbohydrates in the brain.

The process of oxidative phosphorylation is a mechanism by which oxidation of glucose is coupled to the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). In this way the energy released by carbohydrate oxidation is directed to form the terminal high energy bond in ATP. The synthesis of both protein and lipid is energy dependent and ultimately derives this energy from cleavage of the terminal phosphate group of ATP. If chlorpromazine produced a sufficient degree of respiratory uncoupling *in vivo* to lead to impairment of synthetic pathways, this would be as a result of a deficiency of ATP.

The following experiment includes the measurement of the ATP content of mouse brain following administration of chlorpromazine. In addition, cerebral ADP and adenosine monophosphate (AMP), adenosine triphosphatase (ATPase) and glucose were measured. If uncoupling occurred, a decrease in ATP would be accompanied by an increase in ADP. In the absence of uncoupling, a deficiency of ATP could be the result of a failure of nucleotide synthesis (and be accompanied by a deficiency of ADP and AMP), an increase in ATPase activity or a generalized failure of tissue utilization of glucose.

Methods

Colony bred *SAS/ICI* adult albino mice of either sex weighing 18–25 g were used. Chlorpromazine (10–40 mg/kg) was injected intraperitoneally and 1–2 hr later the mice were killed by stunning followed by immersion in liquid nitrogen (for nucleotide assay) or exsanguination (for ATPase and glucose assay) and the fore-brains were removed and weighed. For nucleotide assays the brains were homogenized in 0.3 M perchloric acid (0.5 g/4 ml.) and for ATPase assay the brains were homogenized in 0.1 M-phosphate buffer pH 7.4. These homogenates were then centrifuged at 2,000 *g* for 10 min. The following procedures were carried out.

ATP. The reagents and details of technique were obtained from C. F. Boehringer & Soehne, Mannheim, for this assay and for the ADP and AMP assays. The solutions used were: 0.1 M-triethanolamine buffer, pH = 7.6 containing 10 mM-$MgSO_4$ and 10 mM-D-3 sodium phosphoglycerate; 0.12 mM-NADH; a solution containing glyceraldehyde-3-phosphate dehydrogenase (4 mg/ml.) and phosphoglycerate kinase (1 mg/ml.). The following mixture was pipetted into a glass spectrophotometer cell: triethanolamine buffer, 2.4 ml.; NADH, 0.04 ml.; brain extract 0.2 ml. The optical density was measured at 340 and 366 mμ. A portion (0.04 ml.) of the glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase solution was then added and the optical density readings repeated after 5 min. The difference between the first and second set of optical density values is proportional to the initial ATP concentration in the cuvette. The actual ATP concentration was read off a standard plot of ATP/change in optical density. Phosphoglycerate kinase catalyses the reaction:

3-phosphoglycerate + ATP $\rightleftharpoons$ 1,3-diphosphoglycerate + ADP.

The progress of this reaction is followed by observing the utilization of NADH:

1,3-diphosphoglycerate + NADH + H^+ $\rightleftharpoons$ glyceraldehyde-3-phosphate + $HOPO_3^{2-}$ + NAD^+.

This reaction is catalysed by glyceraldehyde-3-phosphate dehydrogenase.

ADP and AMP. The methods of Lipman (1941) and Bucher (1953) were used. A dose effect curve was not constructed and the reagents were not analysed for AMP content, but the final calculations were based on the conversion of NAD^+ to NADH (see below). Triethanolamine buffer (1.0 M-triethanolamine hydrochloride, 1.3 M-K_2CO_3); phosphoenol pyruvate solution (1×10^{-2}M-phosphoenol pyruvate, 1.3 M-KCl, 0.4 M-$MgSO_4$); 5×10^{-3}M-NADH; lactate dehydrogenase (1 mg/ml.); pyruvate kinase (1 mg/ml.) and myokinase (2 mg/ml.) were used in the assay. The brain extract was mixed with triethanolamine buffer in the ratio 4:1 (v/v). The mixture was maintained at 0°–4° C for 15 min and then filtered. The pH of the filtrate was adjusted to 7.5 at 25° C with 0.1 N-NaOH using an EEL pH meter.

The following were pipetted into a glass spectrophotometer cuvette: 2 ml. filtrate (above) pH 7.5; 0.15 ml. phosphoenol pyruvate solution; 0.1 ml. 5×10^{-3}M-NADH and 0.02 ml. lactate dehydrogenase. After 5 min the optical density was read at 340 and 366 mμ. Pyruvate (0.02 ml.) kinase was then added and optical density measurements made at these wavelengths until a steady state was reached (about 5 min). Myokinase solution (0.02 ml.) was then added and optical density readings were taken at 1 min intervals at the same wavelengths until the new steady state was reached. The difference between the first and second set of optical density

readings is proportional to the initial ADP concentration in the cuvette, and the difference between the second and third readings is proportional to the initial AMP concentration: at 340 mμ, optical density difference between first and second readings × 159 = μg ADP in the cuvette; at 340 mμ optical density difference between second and third readings × 64.5 = μg AMP in the cuvette; at 366 mμ optical density difference between the first and second readings × 299.0 = μg ADP in the cuvette; at 366 mμ, optical density difference between second and third readings × 121.5 = μg AMP in the cuvette. The following reaction sequence is involved:

$$\text{ADP} + \text{phosphoenol pyruvate} \xrightleftharpoons{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate}.$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{lactate dehydrogenase}} \text{lactate} + \text{NAD}^+.$$

Thus the conversion of NADH to NAD^+ is dependent on the initial concentration of ADP.

$$\text{AMP} + \text{ATP} \xrightleftharpoons{\text{myokinase}} 2\ \text{ADP}$$

In the above equation the AMP in the initial filtrate reacts with the ATP formed in the first (pyruvate kinase) reaction. There is sufficient phosphoenol pyruvate and

TABLE 1. *Adenine nucleotides (mg/g fresh tissue) in mouse brain following administration of chlorpromazine (10 mg/kg, intraperitoneally)*

		Mean ± S.E.M.	*n*	*t*	*P*
ATP	Chlorpromazine	0·76 ± 0·015	9	0·68	0·5–0·6
	Control	0·69 ± 0·029	10		
ADP	Chlorpromazine	0·31 ± 0·004	9	2·0	0·05–0·1
	Control	0·23 ± 0·001	9		
AMP	Chlorpromazine	0·19 ± 0·012	9	1·3	0·2–0·3
	Control	0·22 ± 0·019	10		

n, Number of observations. *t*, Student's *t* test. *P*, Probability corresponding to the *t* deviate.

TABLE 2. *Effect of acid hydrolysis on glucose content (mg/g fresh tissue) of mouse liver and brain following administration of chlorpromazine (10 mg/kg intraperitoneally)*

	Brain				Liver			
	Non-hydrolysed		Hydrolysed		Non-hydrolysed		Hydrolysed	
	Control	Test	Control	Test	Control	Test	Control	Test
Mean	<0·01	<0·01	<0·01	0·02	2·66	3·10	4·98	6·39
S.E.M.	—	—	—	0·007	0·33	0·51	1·10	1·21
n	9	9	8	8	10	10	8	8
t	—		2·56		0·84		2·96	
P	—		0·02–0·05		0·4–0·5		0·01–0·02	

n, Number of observations. *t*, Student's *t* test. *P*, Probability corresponding to the *t* deviate.

TABLE 3. *Effect of chlorpromazine (40 mg/kg intraperitoneally) on mouse brain ATPase (Mg^{++} activated) (expressed as μ-moles phosphate released/5 min/mg wet weight)*

	ATPase activity	
	Control	Test
Mean	0·16	0·18
S.E.M.	0·041	0·044
n	16	16
t	0·94	
P	0·3–0·4	

n, Number of observations. *t*, Student's *t* test. *P*, Probability corresponding to the *t* deviate.

NADH for the newly formed ADP to form pyruvate and for this to be converted to lactate with the concomitant utilization of NADH.

Glucose assays. The brains were homogenized in ice-cold 10% trichloracetic acid in an M.S.E. rotary blade homogenizer. Following centrifugation glucose assays were carried out on the supernatants by a glucose oxidase method (Marks, 1959). In addition an aliquot of the supernatant was mixed with an equal volume of 5.6 M-HCl and heated in a boiling water bath for 30 min. Glucose assays were then performed as before. Liver was also examined by a similar technique.

Mg^{++} activated ATPase. The activity of this enzyme was measured by the rate of inorganic phosphate release on incubation of the supernatant of the brain homogenate with ATP and $MgCl_2$ as described by Kielley (1955).

Results

Table 1 shows the brain concentrations of adenine nucleotides following chlorpromazine administration. There was a small but non-significant rise in the mean brain ATP levels in the drug-treated animals. There was also no significant difference in the cerebral ADP and AMP in the test cerebra compared with controls.

The sensitivity of the glucose assays was such that concentrations of glucose less than 0.01 mg/g fresh tissue could not be accurately measured. Table 2 shows glucose could be measured in the fore-brain extracts of drug-treated animals only following acid hydrolysis. The fore-brain extracts from untreated mice contained no measurable amounts of glucose with or without hydrolysis. The difference between the glucose contents of hydrolysed fore-brain extracts from control and chlorpromazine treated animals was significant ($t=2.56$; $P=0.02$–0.05). The non-hydrolysed liver extracts showed no significant difference between tests and controls ($t=0.84$; $P=0.4$–0.5) but following hydrolysis the glucose contents were significantly higher in the drug treated animals compared with controls ($t=2.96$; $P=0.01$–0.02). Mg^{++} activated ATPase was measured in fore-brain extracts of mice which had been injected with chlorpromazine 10 mg/kg intraperitoneally. No significant difference in enzyme activity was detected in the two groups (Table 3).

Discussion

These experiments have shown that administration of chlorpromazine to mice *in vivo* does not alter the total cerebral ATP and ADP. This does not necessarily mean that the decrease in protein and lipid synthesis induced in the brain by this drug (Skinner & Spector, 1968) is not due to lack of ATP. Deficiencies of such high energy compounds could be localized to small groups of cells or be limited to particular intra-cellular organelles and therefore produce no measurable change in total brain ATP. The results do suggest, however, that the uncoupling of oxidative phosphorylation which occurs *in vitro* (Dawkins *et al.*, 1959) either does not occur *in vivo* following therapeutically effective doses of chlorpromazine or is of such a magnitude that it cannot be detected in homogenates made from the entire fore-brain.

In biochemical lesions of the brain in which one of the central abnormalities is a failure of energy production (for example, in anoxia) there is a decrease in the rate of nucleotide synthesis (as well as that of protein and lipid) (Yap & Spector,

1965) and this is accompanied by a fall in cerebral AMP, ADP and ATP (Spector, 1965) which is presumably at least partly the result of the inhibition of nucleotide synthesis. In the present experiment, no changes in cerebral AMP, ADP and ATP occurred, which suggests that the mode of action of chlorpromazine is not primarily an impairment of energy production. Simple estimations of tissue ATP do not give information about the turnover of high energy phosphate groups. For this it would be necessary to follow the metabolic fate of labelled phosphorus in the ATP molecule. One way by which constant tissue levels of ATP can be maintained in the face of a decreased production of high energy phosphate groups, however, is by inhibition of ATPase. Athough Dawkins *et al.* (1959) found that chlorpromazine inhibited this enzyme *in vitro*, no effect on Mg^{++} activated ATPase was detected *in vivo* in these experiments. Again, these negative findings in extracts of entire fore-brains do not exclude small localized biochemical changes which are of insufficient magnitude significantly to alter the net brain enzymic activity. In this work, however, following acid hydrolysis there were raised glucose concentrations in the brain extracts prepared from chlorpromazine-treated mice. It is thus possible that the underlying cause of this change occurs throughout the cerebrum. The precise nature of the acid hydrolysable material which yielded glucose has not yet been fully analysed but it includes glucose phosphates and glycogen (unpublished observations). The entire glycogen of the tissues was not measured because only the supernatant of the tissue homogenate was examined (Vrba, Gaitonde & Richter, 1962) and the post-hydrolysis glucose figures were lower than those found by conventional glycogen assays of liver and brain—2–38 mg/g and 0.8 mg/g, respectively (Albaum, Tepperman & Bodanski, 1946). Other experiments, however, have also shown that chlorpromazine produces an impaired conversion of glucose to non-carbohydrates in the brain with an increase in acid soluble metabolites of glucose (Skinner & Spector, 1968). This is consistent with a selective block in the glycolytic pathway as opposed to a generalized failure of all energy dependent mechanisms. There is evidence that some enzymes are selectively inhibited by phenothiazines. Iriye & Simmonds (1967) have shown inhibition of rat brain phosphorylase and De Waart, Brogt & Sietsma (1967) have inhibited rat brain *p*-nitro-phenylphosphatase with phenothiazines, whereas ATP dependent acetylation is not affected (Grenell, Mendelson & McElroy, 1955).

Another explanation of the metabolic changes induced by chlorpromazine is that the drug can induce hypothermia, and this in itself can produce a secondary change in the rate of biochemical reactions (Bartlet, 1965).

Although the relevance of these effects of phenothiazines on enzyme protein to their pharmacological properties is not known, these experiments have indicated that chlorpromazine can influence the metabolic fate of glucose in the absence of evidence of uncoupling of oxidative phosphorylation *in vivo*.

This work was supported by United Cerebral Palsy Research and Education Foundation, Inc., Grant No. R-212-67.

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(*Received April* 17, 1968)