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INFLUENCE OF CHLORPROMAZINE ON THE LEVELS OF ADENINE NUCLEOTIDES IN THE RAT BRAIN AND HYPOTHALAMUS *IN VIVO*

C. L. KAUL, J. J. LEWIS and S. D. LIVINGSTONE*

Experimental Pharmacology Division, Institute of Physiology, University of Glasgow, Scotland, U.K.

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Abstract—An investigation has been made of the influence of chlorpromazine upon levels of adenine nucleotides, phosphocreatine and inorganic phosphate in the brain and hypothalamic region of the rat. The effects upon both whole brain and hypothalamus varied phasically with time between injection and killing. Changes in the hypothalamus were more marked than in the whole brain. In whole brain ATP was significantly lowered at 3 hr and significantly raised at 6 hr. In the hypothalamus the pattern was more complex and ATP rose at 1½ and 6 hr and fell at 3 hr. ADP levels varied in the opposite direction to ATP. Control ATP levels were found to vary with time. Chlorpromazine appears to suppress this variation in the whole brain.

INTRODUCTION

STUDIES on the influence of tranquillizers upon adenosine triphosphate (ATP) levels in brain have yielded conflicting results; thus ATP levels may rise,[1] be unchanged[2, 3] or fall.[4, 5] With chlorpromazine no change in brain ATP was found by Minard and Davis[2] or Weiner and Huls,[3] but Grenell *et al.*[1] found a rise. This study was made to see if chlorpromazine followed the pattern found by us for other tranquillizers.[4, 5] We have also attempted to localize its action in the brain and, because of reports[6, 7] that chlorpromazine acts partly on the hypothalamus, have investigated its effects on that region.

METHODS

Male Wistar rats weighing from 70 to 95 g were used. The rats were drawn from stocks housed twelve per cage and fed *ad lib.* upon cubes of diet No. 41 and water. The stock animals were kept in an air-conditioned room. Prior to experiment the required number of rats (two or three) were taken, weighed and injected intraperitoneally with the drug or control solution. Each rat was then placed individually into a cylindrical wire mesh cage, 9 cm long and 6 cm diameter (Fig. 1), and kept in a quiet, air-conditioned room under artificial light. At the end of the experimental period the cage and its contents were rapidly plunged into a vacuum flask of liquid nitrogen. After two minutes the cage was removed and the frozen rat taken out. It was decapitated and the head placed into liquid nitrogen. The brain was removed from the cranium, using bone chisels. To keep the temperature as low as possible, dissection was carried out in a glove box, through which air, chilled with methanol–carbon

* Present address: Department of Pharmacology, University of Toronto, Ontario, Canada.

dioxide was blown. During the dissection, the brain was frequently replaced in liquid nitrogen, and at no time thawed out.

The time of day at which the animals were killed was not fixed.

Drugs used were 10, 25 or 50 mg/kg chlorpromazine, or 20 mg/kg trifluprom azine. The drug solution was made in 0·9 per cent w/v sodium chloride and sterilized by filtration. Matching control solutions were given in a volume corresponding to the drug solution (0·2 ml/100 g body weight). Whole brain extracts were prepared, as described by Lewis and Van Petten.[8]

The hypothalamic region was removed by dissection from the brain, without thawing. The area taken was approximately that below the intermediary mass and between the optic chiasma and mammillary bodies. It was weighed and stored in liquid nitrogen, until required, for up to 24 hr. For assay the following modification of the method of Lewis and Van Petten[8] was used. The frozen hypothalamus was placed in a stainless steel centrifuge tube containing liquid nitrogen, standing in solid carbon dioxide. It was crushed by pounding with a stainless steel plunger. The centrifuge tube was then put in a beaker of crushed ice and, after two minutes, 1·0 ml of 0·3 M perchloric acid (PCA) added. The delay was necessary to prevent the PCA from freezing, when added to the frozen tissue. The mixture was homogenized for two min, using a previously cooled, loosely fitting, plastic plunger. 5 ml of ice-cold, distilled water was added and the mixture stirred for 20 sec and then centrifuged for 12 min (0°, 3500 rev/min). The supernatant was decanted into a glass centrifuge tube standing in crushed ice. 3 ml of the supernatant was added to a solution of 1·5 ml of 0·32 M succinate buffer (pH 6·1), 0·2 ml 1M $MgCl_2$ and 0·2 ml 0·1 M $CaCl_2$. The solution was adjusted to pH 6·1 and made up to 6 ml with 0·05 M PCA. 2·7 ml duplicates were assayed for adenosine monophosphate (AMP), adenosine diphosphate (ADP) and ATP. To the remaining supernatant were added 3 ml 0·05M PCA and 0·24 ml 0·02M $CuSO_4$. 5 ml of this solution was assayed for inorganic phosphate (IP) and phosphocreatine (PC). The methods of estimation have been described in an earlier publication.[8]

Rectal temperatures were determined by a thermistor probe inserted about 2·5 cm into the rectum; the temperatures were read directly from the previously calibrated scale.

For statistical analysis the order of treatments was as follows: the rats were placed in groups of two (one control and one drug-treated) and the order of treatment randomized, using a table of random numbers. This was done in all cases except for series 1–5, where the animals were placed in groups of three (control, 25 mg/kg dose and 50 mg/kg dose) in which the order of treatment was also randomized, using a 3×3 latin square design. The results were tested for significance, using an analysis of variance. To test the significance of the difference between similarly treated rats at different times, Duncan's[9] new multiple range test was used.

Each series of experiments was self-contained and was accompanied simultaneously by its own parallel series of controls, which received the appropriate volume of solvent. For this reason it was necessary to subject each series to separate statistical analysis.

RESULTS

Fifteen minutes after injection of 25 or 50 mg/kg chlorpromazine, the rats became lethargic and remained motionless. The temperature fell while the animals were motionless and rose as they again began to make voluntary movements (Table 1).

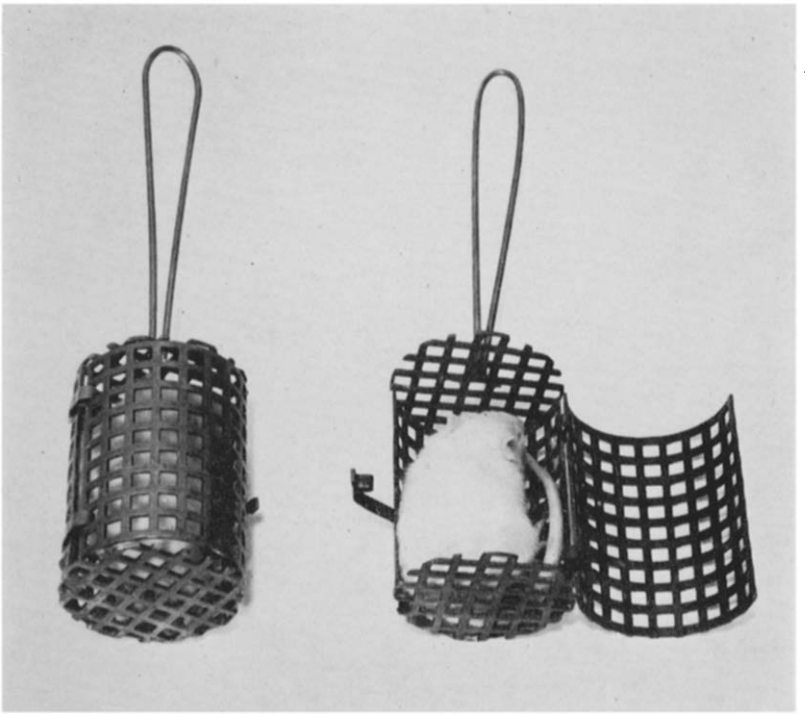

FIG. 1. Cages in which rats were placed after injection and prior to immersion in liquid nitrogen.

TABLE. 1. EFFECT OF CHLORPROMAZINE (25 MG/KG AND 50 MG/KG) ON THE RECTAL TEMPERATURE OF RATS AT DIFFERENT TIMES AFTER TREATMENT

Time after treatment in hours	0	½	1½	3	6	12	24	36	48
Control	35·5 ± 0·27(9)	35·3 ± 0·22(9)	35·4 ± 0·23(9)	35·8 ± 0·24(9)	35·9 ± 0·26(9)	35·2 ± 0·29(9)	35·4 ± 0·27(9)	35·5 ± 0·33(9)	35·9 ± 0·26(9)
25 mg	35·8 ± 0·35(9)	30·7 ± 0·58(9)†	28·7 ± 0·60(9)†	27·1 ± 0·61(9)†	26·8 ± 0·79(9)†	29·1 ± 1·27(9)†	32·6 ± 1·48(8)	33·9 ± 1·46(8)	34·8 ± 1·38(7)
50 mg	35·5 ± 0·38(9)	31·3 ± 0·35(9)†	28·7 ± 0·46(9)†	27·8 ± 0·64(9)†	27·7 ± 0·73(9)†	26·9 ± 1·00(9)†	29·3 ± 1·68(8)*	29·8 ± 1·49(8)*	29·8 ± 2·00(6)*

The number in parentheses indicates the number of observations taken. Significance of difference from control:
* $P<0.01$; † $P<0.001$.

TABLE 2. *IN VIVO* EFFECTS OF CHLORPROMAZINE AND TRIFLUPROMAZINE ON THE ADENINE NUCLEOTIDE, PHOSPHOCREATINE AND INORGANIC PHOSPHATE CONCENTRATIONS IN THE RAT BRAIN OR HYPOTHALAMIC REGION (SERIES 1–7)

Series	Treatment and Dose	Time after Treatment (Hr)	Concentrations in mμ mole/100 mg frozen tissue AMP	ADP	ATP	Total AMP + ADP + ATP	Ratio $\frac{ATP}{ADP}$
1	Control	½	36 ± 5	79 ± 12	49 ± 7	164 ± 21	0·67 ± 0·09
	Chlorpromazine (25 mg/kg)		41 ± 7	89 ± 12	34 ± 10	164 ± 15	0·44 ± 0·12
	Chlorpromazine (50 mg/kg)		35 ± 2	81 ± 9	29 ± 6	145 ± 11	0·42 ± 0·10
2	Control	1½	41 ± 4	81 ± 4	102 ± 5	224 ± 11	1·27 ± 0·06
	Chlorpromazine (25 mg/kg)		36 ± 5	85 ± 7	178 ± 17†	298 ± 22*	2·22 ± 0·34
	Chlorpromazine (50 mg/kg)		27 ± 2	77 ± 5	130 ± 13§	232 ± 15	1·73 ± 0·19
3	Control	3	36 ± 4	75 ± 7	119 ± 13	228 ± 16	1·70 ± 0·23
	Chlorpromazine§ (25 mg/kg)		44 ± 2	126 ± 16‡	44 ± 9‡	211 ± 21	0·36 ± 0·09‡
	Chlorpromazine (50 mg/kg)		29 ± 4	108 ± 5‡	21 ± 9‡	159 ± 11‡	0·20 ± 0·08‡
4	Control	6	32 ± 5	87 ± 7§	90 ± 7	204 ± 13	1·05 ± 0·01
	Chlorpromazine (25 mg/kg)		38 ± 5	28 ± 4‡	197 ± 26†	262 ± 25	8·93 ± 1·93†
	Chlorpromazine (50 mg/kg)		40 ± 7	44 ± 8‡	152 ± 19†§	238 ± 23	3·14 ± 0·22
5	Control	12	26 ± 4	59 ± 4	172 ± 6	257 ± 9	3·01 ± 0·18
	Chlorpromazine (25 mg/kg)		24 ± 3§	62 ± 7§	191 ± 6	277 ± 10	3·40 ± 0·38
	Chlorpromazine (50 mg/kg)		28 ± 5	67 ± 5	187 ± 7	282 ± 15	2·89 ± 0·17
6	Control	1½	37 ± 4	86 ± 4	118 ± 8	241 ± 12	1·37 ± 0·07
	Chlorpromazine (10 mg/kg)		36 ± 2	62 ± 6*	147 ± 12*	246 ± 13	2·57 ± 0·31†
7	Control	3	32 ± 3	76 ± 4	122 ± 8	222 ± 13	1·60 ± 0·08
	Chlorpromazine (10 mg/kg)		34 ± 2	94 ± 3	103 ± 4	232 ± 7	1·12 ± 0·07†
8	Control	½	104 ± 6	91 ± 4	255 ± 7	454 ± 12	2·83 ± 0·13
	Chlorpromazine (25 mg/kg)		104 ± 6	111 ± 8*	199 ± 9*	415 ± 16	1·87 ± 0·15‡

9	Control	1½	97 ± 6	87 ± 6	252 ± 13	436 ± 14	3·06 ± 0·30
	Chlorpromazine (25 mg/kg)		110 ± 8*	117 ± 4†	224 ± 9	452 ± 10	1·93 ± 0·10†
10	Control	3	77 ± 6	72 ± 5	286 ± 6	435 ± 6	4·19 ± 0·37
	Chlorpromazine (25 mg/kg)		73 ± 4	106 ± 4‡	244 ± 6‡	423 ± 9	2·33 ± 0·10‡
11	Control	6	89 ± 5	93 ± 4	253 ± 10	436 ± 7	2·80 ± 0·25
	Chlorpromazine (25 mg/kg)		87 ± 2	78 ± 5*	285 ± 9*	450 ± 9	3·79 ± 0·29†
12	Control	12	80 ± 5	76 ± 3	249 ± 8	406 ± 11	3·31 ± 0·15
	Chlorpromazine (25 mg/kg)		86 ± 7	85 ± 5	252 ± 6	422 ± 14	3·07 ± 0·17
13	Control	½	101 ± 8	78 ± 5	263 ± 11	441 ± 13	3·55 ± 0·30
	Chlorpromazine (50 mg/kg)		101 ± 5	116 ± 4†	221 ± 8*	438 ± 9	1·99 ± 0·17†
14	Control	1½	103 ± 5	92 ± 6	244 ± 7	439 ± 10	2·74 ± 0·18
	Chlorpromazine (50 mg/kg)		101 ± 7	108 ± 8	218 ± 8	428 ± 14	2·12 ± 0·17*
15	Control	3	92 ± 5	84 ± 4	291 ± 8	467 ± 8	3·57 ± 0·26
	Chlorpromazine (50 mg/kg)		95 ± 5	124 ± 9†	245 ± 9‡	464 ± 10	2·09 ± 0·18‡
16	Control	6	103 ± 5	99 ± 5	220 ± 7	418 ± 8	2·19 ± 0·12
	Chlorpromazine (50 mg/kg)		98 ± 4	86 ± 6	244 ± 7*	428 ± 7	2·92 ± 0·19*
17	Control	12	100 ± 7	87 ± 6	229 ± 4	416 ± 11	2·76 ± 0·22
	Chlorpromazine (50 mg/kg)		99 ± 8	108 ± 7	236 ± 8	443 ± 15	2·27 ± 0·16
18	Control	½	81 ± 8	84 ± 8	255 ± 9	419 ± 16	3·22 ± 0·29
	Chlorpromazine (10 mg/kg)		94 ± 6	101 ± 9	247 ± 12	442 ± 10	2·76 ± 0·39
19	Control	3	110 ± 3	93 ± 5	237 ± 10	439 ± 11	2·62 ± 0·17
	Chlorpromazine (10 mg/kg)		105 ± 7	112 ± 7‡	230 ± 14	448 ± 10	2·18 ± 0·25
20	Control	3	95 ± 5	86 ± 5	257 ± 8	439 ± 10	3·04 ± 0·20
	Trifluprazine (20 mg/kg)		94 ± 4	107 ± 9*	226 ± 9†	428 ± 9	2·33 ± 0·32

All values are expressed as mean ± (S.E.) of determination on 9 (Series 1 to 5) or 10 (Series 6 to 20) rats.
Significance of difference from control: * $P<0.05$; † $P<0.01$; ‡ $P<0.001$; § one missing value calculated.

Some animals given 50 mg/kg chlorpromazine died, while some were still lethargic after 48 hr. Animals given 10 mg/kg were active again at 3 hr.

The effects of chlorpromazine on the adenine nucleotide levels are summarized in Tables 2 and 3. There was no significant change in the levels of PC (except Series 4 and 10 where there was a rise; Series 8 and 18, a fall), IP (except Series 4 and 11 where there was an increase) and AMP (except Series 9 also an increase.)

TABLE 3. COMPARISON BETWEEN THE VALUES OF VARIOUS ADENINE NUCLEOTIDES IN THE WHOLE BRAIN OR HYPOTHALAMIC AREA OF THE RAT GIVEN SIMILAR TREATMENTS BUT KILLED AT DIFFERENT INTERVALS AFTER TREATMENT

	WHOLE BRAIN			
	Control (Time in hr)	25mg/kg Chlorpromazine (Time in hr)	Control (Time in hr)	50 mg/kg Chlorpromazine (Time in hr)
ATP	3 ½ 6 1½ 12 *	6 12 3 1½ ½	3 ½ 1½ 12 6	3 6 12 ½ 1½
ADP	6 ½ 1½ 12 3	1½ ½ 3 12 6	6 1½ 12 3 ½ *	3 ½ 12 1½ 6
ATP + ADP + AMP	½ 1½ 6 3 12 *	1½ 6 3 12 ½	3 ½ 1½ 6 12	3 12 ½ 6 1½
ATP / ADP	3 12 1½ ½ 6	6 12 3 1½ ½	3 ½ 12 1½ 6	6 12 1½ 3 ½

	HYPOTHALAMIC AREA		
	Control (Time in hr)	25 mg/kg Chlorpromazine (Time in hr)	50 mg/kg Chlorpromazine (Time in hr)
ATP	12 3 1½ 6 ½	6 12 1½ 3 ½	12 6 1½ ½ 3
ADP	6 1½ ½ 3 12	3 ½ 1½ 12 6	3 ½ 1½ 12 6
ATP + ADP + AMP	12 3 1½ 6 ½	1½ 12 6 3 ½	12 6 1½ 3 ½
ATP / ADP	12 3 1½ 6 ½	6 12 1½ ½ 3	6 12 1½ ½ 3

Only the time of killing has been indicated and not the actual value.
The order in which the times are given is obtained by placing them so that their values are in descending order.
Those times not underlined by the same line show significant ($P<0{\cdot}01$) differences in the values, except where* is shown the significance is $P < 0{\cdot}05$.

In this Table, it should be noted that, when any two or more time values are underlined by the same unbroken line, then there is no significant difference between the means at these times. For example, in the whole brain the control ATP value corresponding to 50 mg/kg chlorpromazine showed no significant difference between 3 and ½ hr; ½ and 1½ hr, 1½, 12 and 6 hrs, but the 3 hr value is significantly different from those at 1½, 12 and 6 hours.

In general, the levels of ATP in the drug-treated animals varied from the control values in the same direction in both the hypothalamus and whole brain, i.e. a significant increase at 6 hr and a significant decrease at 3 hr (Table 2). At ½ hr, however, the whole brain showed a significant decrease in ATP in the drug-treated rats, but

there was no significant difference in the hypothalamus. At 1½ hr only, the hypothalamic ATP levels in rats given 25 mg/kg were significantly raised.

The control ATP values in the whole brain also varied. If, for example, the 3 hr control ATP values at 25 and 50 mg/kg chlorpromazine are considered (Table 2) it can be seen that these are significantly higher than the other time values (Table 3). At 6 hr the ATP levels in the 25 mg/kg-treated rats were significantly higher than those at all other times. No differences were seen between the levels at different time intervals with the 50 mg/kg dose. Control ATP levels in the hypothalamus were significantly higher at 12 hr, and significantly lower at ½ hr than the control values at other times (Table 3). At 25 and 50 mg/kg the ½ and 3 hr ATP levels were significantly lower than those at the other time intervals.

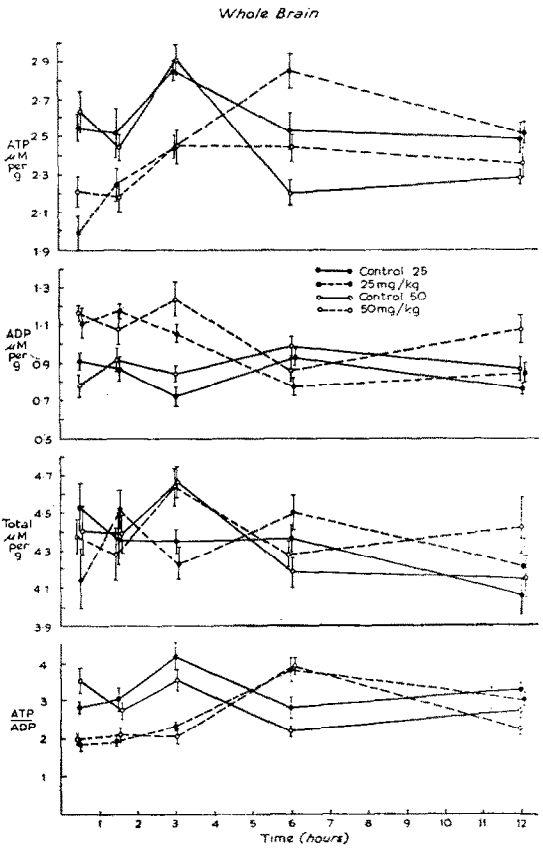

FIG. 2. Effect of chlorpromazine on ATP, ADP and total adenine nucleotide levels and on the ratio ATP/ADP in the whole brain. The vertical lines represent the standard error of the means.

The action of chlorpromazine on the ADP levels in the whole brain was more pronounced at 25 mg/kg than at 50 mg/kg. Thus, at 1½ hr the ADP levels were significantly increased, and at 6 hr significantly decreased with the 25 mg/kg dose, but not with 50 mg/kg (Figs. 2 and 3, Table 2). Both doses increased the ADP levels significantly at ½ and 3 hr. Significant changes in hypothalmic ADP levels occurred at 3 and 6 hr, when they were respectively significantly increased and decreased at both

doses (Figs. 2 and 3, Table 2). In whole brain the controls corresponding to the 25 mg/kg dose were significantly higher at 6 hr than at 3 hr; however, the other controls showed no significant differences from one another (Table 3). At 25 mg/kg the 6 hr level was significantly lower than the ½, 1½ and 3 hr levels, but at 50 mg/kg only the 3 hr level was significantly higher than the 6 hr level (Table 3). In the hypothalamus the control levels and 25 mg/kg dose levels showed no differences between the time intervals, but the 3 hr level of the 50 mg/kg dose was significantly higher than the others (Table 3).

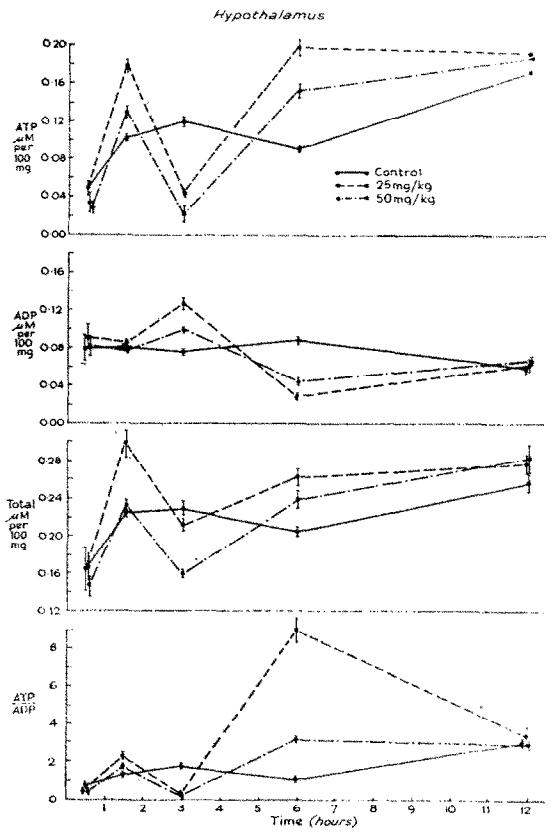

FIG. 3. Effect of chlorpromazine on ATP, ADP and total adenine nucleotide levels and on the ratio ATP/ADP in the hypothalamus. The vertical lines represent the standard error of the means.

The total amount of adenine nucleotides (AMP + ADP + ATP) in the whole brain did not change significantly from the controls (Table 2); but in the hypothalamus the total was raised significantly at 1½ hr with the 25 mg/kg dose and reduced significantly at 3 hr with the 50 mg/kg dose (Table 2).

In the drug-treated animals the total adenine nucleotide levels in the whole brain did not vary significantly from one another. Variations in the control values did occur and are shown in Table 3. When a comparison was made between time intervals in the hypothalamus, the ½ and 3 hr levels at both doses were found to be significantly lower than the others (Table 3). The ratio ATP/ADP rose significantly at 6 hr but fell

significantly at ½, 1½ and 3 hr in the whole brain with 25 and 50 mg/kg, but no significant changes were seen with the 10 mg/kg dose (Table 2). The hypothalamic ratio rose significantly at 1½ hr with the 10 mg/kg dose and at 6 hr with the 25 mg/kg dose, but fell significantly at 3 hr with all three doses (Table 2).

When comparing the ATP/ADP ratio between the time intervals, both sets of controls for the whole brain (Table 3) showed the 3 hr ratio to be significantly higher than the 6 hr one. The 25 mg/kg dose gave a ratio at 6 hr, which was significantly higher than all the others, and at 12 hr the ratio was significantly higher than the ratios at 3, 1½ or ½ hr. At 50 mg/kg the 6 hr ratio was higher than all the others, apart from that at 12 hr (Table 3). The ratios of the hypothalamic controls showed a significant rise at 12 hr, whereas the 25 mg/kg dose showed a significant rise at 6 hr. No significant differences between the ratios were detected with the 50 mg/kg dose (Table 3).

Trifluprομazine produced similar changes to chlorpromazine at 3 hr in the whole brain (Table 2).

Table 1 shows the changes in rectal temperature after chlorpromazine administration; it can be seen that the phasic changes found in the adenine nucleotides are not paralleled. There is an immediate drop in the temperature and a gradual rise after 6 to 12 hr.

DISCUSSION

Chlorpromazine was shown to cause hypothermia by Courvoisier *et al.*[10] We have also seen this, but the phasic change noted in brain and hypothalamic ATP levels were not paralleled by changes in rectal temperature. This supports the observations of Savchenko[11] that hypothermia has no marked effect on labile phosphate levels in whole brain. It seems improbable, therefore, that body temperature changes *per se* influence ATP levels.

Compounds which cause behavioural depression decrease brain ATP levels[4, 5] while behavioural stimulants increase them.[8, 12] At the same time ADP levels rise or fall respectively.[4, 5, 8, 12]

Examination of Table 2 shows that the control levels for the adenine nucleotides may undergo significant variation. This variation may be due to a number of factors; for example, variations in housing and handling, other environmental changes, a normal variation in brain nucleotide levels from one animal to the other and variations in the technique of injecting, killing, dissection, extraction and assay. Because of these factors, a control series of experiments was always carried out in parallel with the test series, and an appropriate statistical design employed. The changes in control ATP levels in whole brain and hypothalamus may thus be due to environmental changes or variations in experimental technique. An example of the former type of effect is seen in the marked variations in plasma corticosterone levels in rats under different types of housing.[13] A somewhat similar phenomenon was observed by Werdinius[14] with regard to brain serotonin levels in rats given injections of control solutions. Changes in hypothalamic ATP levels appear to influence the total hypothalamic adenine nucleotide pool, which varies in the same direction. The more marked changes in control hypothalamic ATP levels probably reflect the sensitivity of the hypothalamus to the stresses of the injection and change of environment. This is shown by the initial fall in hypothalamic control ATP and its subsequent rise towards levels found in whole brain at 12 hr.

The effect of 50 mg/kg chlorpromazine on the whole brain ATP seems to indicate a dampening of the control variation; at 25 mg/kg this effect is similar but less marked.

The phasic changes in the hypothalamus are much more pronounced than those in the whole brain, and the changes in the latter may be an indication of much larger changes in the former. For example, at 1½ hr the ATP levels in the hypothalamus are raised significantly, but in the whole brain there is no significant change. Yet at ½ and 3 hr the whole brain ATP levels fall significantly. Failure to record a similar fall at 1½ hrs may be due to the marked rise at this time in the hypothalamus.

Using chlorpromazine, Grenell *et al.*[1] also reported a greater effect on the hypothalamus than on the whole brain. In contrast to our observations, however, they found that both hypothalamic and whole brain ATP levels in rat brains increased half-an-hour after injection of chlorpromazine. Subsequently Grenell *et al.*[15] found no significant difference between control and chlorpromazine treated rats. The more marked effects on the hypothalamus are not surprising, since Berger *et al.*[7] found that chlorpromazine accumulated preferentially in this region, while Himwich[6] considered the posterior hypothalamus to be an important site of action of chlorpromazine. The position was summarized by Bradley,[16] who pointed out that many of the effects of chlorpromazine could be explained by an action on the hypothalamus and brain stem reticular formation.

Chlorpromazine suppresses the secretion of FSH, LH and TSH,[17–21] but reports upon its effects on ACTH secretion are conflicting. Thus, Talwalker *et al.*[22] and Woodbury[23] state that ACTH secretion is raised but Sulman and Winnik[17] indicate that it falls. Stress, however, stimulates secretion of ACTH and inhibits secretion of other anterior pituitary trophic hormones[24] so that the effect of chlorpromazine on the hypothalamus may initially resemble that of stress.

The complex phasic effects of chlorpromazine upon levels of ATP and ADP are difficult to explain. Both Weiner and Huls[3] and Grenell *et al.*[1] suggest that a rise in ATP may result from reduced utilization in the sedated animal. It can be argued that, when chlorpromazine enters the hypothalamus, it initially lowers ATP utilization, but synthesis remains normal or is increased. This would explain the increased ATP at 1½ hr. As the concentration of chlorpromazine in the brain increases, the synthesis of ATP is depressed, but ATP utilization is normal or only marginally reduced. This is seen at 3 hr as a fall in the ATP level. At 6 hr the concentrations of ATP is raised. This may be due to under-utilization of ATP because of the extreme state of depression and to recovery of synthesis. The fact that 25 mg/kg raises ATP significantly at 1½ hr, but 50 mg/kg does not, may be due to the higher dose producing a high hypothalamic concentration more quickly, so that ATP synthesis begins to decline earlier.

This view is supported by the observations of Abood,[25] that at 5×10^{-5}M, chlorpromazine uncouples oxidative physphorylation in rat brain mitochondria. At a higher concentration (10^{-4}M) it also inhibits ATPase activity. The latter concentration corresponds to 35 mg/kg chlorpromazine,[26] which is the same order as those used in this investigation. Thus, if the initial concentration of chlorpromazine is low, and only uncoupling can take place, the concentration of ATP will fall, providing that the rate of ATP utilization is unchanged. As the chlorpromazine concentration increases, ATPase activity is inhibited[25]; the rate of breakdown fails to equal the rate of synthesis and ATP rises. At this time the animals are severaly depressed, and utilization of ATP is probably also reduced. At this stage (3 hr) chlorpromazine acts

upon ATP and ADP levels in a manner similar to that of reserpine and other tranquillizers[4, 5] Changes in ATP and ADP at other time intervals are different from those seen with reserpine, and increased ATP levels are not associated with behavioural arousal. The rise in ATP at 1½ hr in the hypothalamus, as opposed to the whole brain at 25 mg/kg of chlorpromazine is interesting, in view of Berger's[27] suggestion that chlorpromazine stimulates the hypothalamus by lowering the threshold for electroshock seizures and chemical convulsants.

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