Br. J. Pharmac. Chemother. (1968), 33, 129–135.

THE EFFECT OF CHLORPROMAZINE ON ^{14}C-GLUCOSE METABOLISM IN MOUSE LIVER AND BRAIN

BY

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(*Received November* 29, 1968)

Chlorpromazine in high concentrations decreases energy production in tissues *in vitro* by inhibiting cellular respiration and phosphorylation (Quastel, 1965). Berger (1957) found uncoupling of phosphorylation in liver, but not in brain mitochondria exposed to chlorpromazine. The precise site of uncoupling of the respiratory enzyme chain in the liver has been identified by Dawkins, Judah & Rees (1959), who also observed similar effects in the brain in the presence of 2×10^{-4}M chlorpromazine. Chance (1959) has demonstrated that the controlling metabolite for coupled respiration is adenosine diphosphate (ADP) and thus in normal conditions the rate of aerobic energy production is limited by the concentration of ADP. Chance & Baltscheffsky (1958) have employed the concept of respiratory control ratio which is defined as the ratio of respiratory rate in the presence of added ADP to that rate obtained following its expenditure. The use of this measurement enables more sensitive detection of loosely coupled respiration than that obtained by direct P/O ratios.

Administration of chlorpromazine to rats does not affect the oxygen uptake of homogenates of their tissues *in vitro* (Grenell, Mendelson & McElroy, 1955). In the presence of uncoupled respiration, however, impaired carbohydrate utilization can occur even though oxygen consumption is normal.

This paper describes an investigation of the fate of [U-^{14}C]-glucose in mice after the administration of chlorpromazine in order to detect alterations in energy dependent processes which would occur if significant respiratory uncoupling occurred in the intact animal. The ability of ADP to stimulate the respiration of brain and liver homogenates in the presence of chlorpromazine concentrations similar to those attained therapeutically has also been studied.

METHODS

SAS/ICI adult albino mice of either sex weighing 18–25 g were used.

Tissue respiration

Oxygen uptake of tissue homogenates was measured manometrically at 37° C in a Warburg respirometer. Untreated mice were killed by cervical fracture and exsanguination. The brain and liver were rapidly removed and immediately plunged into ice-cold Hanks balanced salt solution. The tissues were then blotted, weighed on a torsion balance and homogenized in ice-cold Hanks

solution (0.25 g/ml.) using an M.S.E. rotary blade homogenizer. The effects on respiration of adding 0.04M sodium succinate or lactate (A.R. grade, Hopkin & Williams Ltd.), 0.0012M ADP (Calbiochem) or 0.0117×10^{-4}M to 4.7×10^{-4}M chlorpromazine (May & Baker Ltd.) to the incubation mixture were assessed.

In vivo *(U-^{14}C)-glucose utilization*

Mice were given two intraperitoneal injections 3 hr apart of chlorpromazine 4 mg and 10 mg/kg, while controls received 0.1 ml. of isotonic saline. One hour later, 5 μc of (U-^{14}C)-D-glucose (0.3 mg) were intraperitoneally injected in 0.1 ml. of isotonic saline. After a further hour a 0.01 ml. sample of tail blood was taken and the animal was killed by light ether anaesthesia followed by exsanguination. The brain and liver were removed, washed in ice-cold water, blotted and weighed. The organs were homogenized (as in Vrba, Gaitonde & Richter, 1962) in 2 ml. of ice-cold 10% trichloracetic acid, and the suspension was then centrifuged and the pellet washed three times with ice-cold 10% trichloracetic acid. The washings were pooled and the trichloracetic acid extracted with ether. The remaining solution represented the acid soluble fraction which included sugars, amino-acids and other organic acids. The precipitate was successively washed with 2 ml. each of acetone, ethanol-ethyl ether (3:1 v/v), chloroform-methanol (2:1 v/v) and ether in that order. The pooled washings contained the tissue lipids and the precipitate consisted of protein and nucleotides. The latter was removed by stirring it with 3 ml. of 5% trichloracetic acid at 90° C for 15 min. Aliquots of the acid soluble, lipid, nucleotide and protein fractions were transferred to glass vials and dried under reduced pressure at room temperature. A scintillant was added consisting of 0.4% PPO (2,5-diphenyloxazole) and 0.01% POPOP (2,4-di(2-5-phenyloxazolyl)-benzene) dissolved in toluene. Carbon-14 was measured in a Beckman automatic liquid scintillation counter. The carbon-14 in 0.1 ml. of the original tissue homogenate and in 0.02 ml. of the tail blood was also measured.

RESULTS

The oxygen uptake of mouse brain *in vitro* was 12.8 μl./g wet weight/min (see Table 1). When chlorpromazine (0.59×10^{-4}M) was incorporated into the homogenate the mean rate of oxygen uptake was 11.3 μl./g wet weight/min. This reduction was not statistically significant. Higher concentrations of chlorpromazine (2.34×10^{-3}M) produced a significant reduction in oxygen uptake of brain to a mean value of 0.97 μl./g/min, which is 7.6% of controls ($n=12$; S.E.M. $=3.6\%$; $t=12.5$; $P<0.001$). Moreover, the difference in response of the brain to these two doses was significant ($t=2.1$; $P=0.02$–0.05). When liver homogenates were used 2.34×10^{-3}M chlorpromazine reduced oxygen uptake to 33.9% of normal and 1.17×10^{-3}M to 41.4% of normal. In each chlorpromazine concentration the diminished effect on liver compared with that of brain was significant (2.34×10^{-3}M drug, $t=3.1$; $P=0.001$–0.01 ; 1.17×10^{-3}M drug, $t=2.8$; $P=0.001$–0.01). The addition of 0.042M sodium lactate to brain homogenates produced a rapid increase in oxygen uptake, but when 2.34×10^{-4}M chlorpromazine was present no such stimulation occurred (Fig. 1). Sodium succinate 0.042M, however, increased the respiration of brain homogenates both with and without chlorpromazine (Fig. 2).

The effect of adding 0.0012M ADP *in vitro* to normal and chlorpromazine-treated brain is shown in Table 1. The concentration of chlorpromazine used was that which produced no significant change in respiration rate (0.59×10^{-4}M). In the absence of chlorpromazine, ADP produced a significant increase in oxygen uptake. When chlorpromazine (0.59×10^{-4}M) was incorporated in the incubation medium the addition of ADP resulted in a small but non-significant increase in respiration.

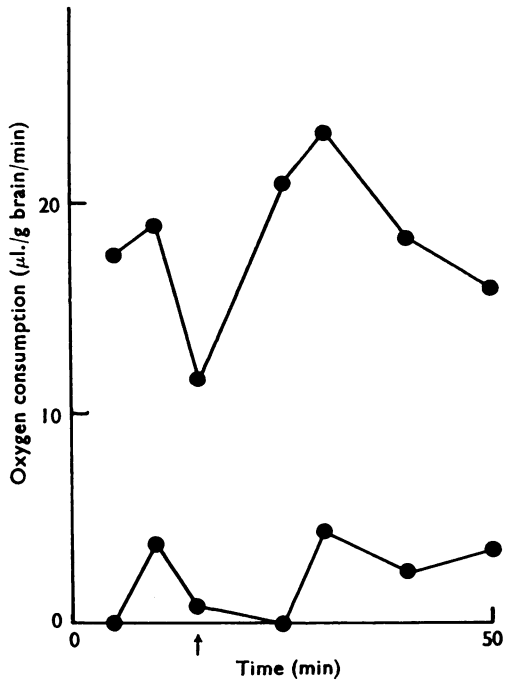

Fig. 1. Oxygen uptake of brain homogenate in phosphate buffer (upper curve) and in phosphate buffer with chlorpromazine 0.416 mg/ml. (lower curve). Succinate added at ↑.

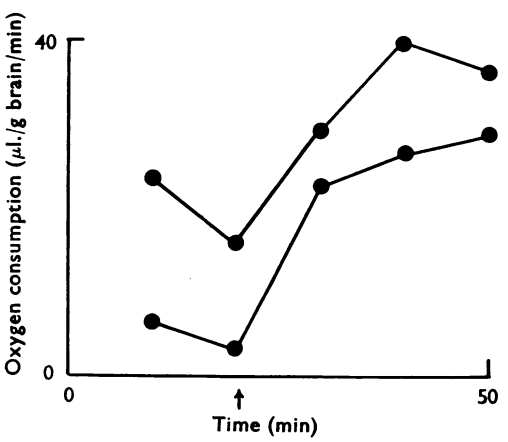

Fig. 2. Oxygen uptake of brain homogenate in phosphate buffer (upper curve) and in phosphate buffer with chlorpromazine 0.416 mg/ml. (lower curve). Sodium lactate added at ↑.

TABLE 1

EFFECT OF $0{\cdot}59 \times 10^{-4}$M CHLORPROMAZINE ON ADP-INDUCED STIMULATION OF *IN VITRO* RESPIRATION OF MOUSE BRAIN HOMOGENATES

* Student's *t* test.

		Oxygen consump. (μl./g wet wt./min)	*n*	S.D.	*t**	*P*
No chlorpromazine	No ADP	12·8	24	3·4	3·5	0·001
	0·0012M ADP	16·3	20	3·2		−0·01
$0{\cdot}59 \times 10^{-4}$M	No ADP	11·3	22	5·1	1·3	0·1
	0·0012M ADP	13·6	20	6·3		−0·2

TABLE 2

DISTRIBUTION OF CARBON-14 IN LIVER AND BRAIN AND IN CHEMICAL SUBFRACTIONS FOLLOWING THE ADMINISTRATION OF 5 μc OF (U-^{14}C)-D-GLUCOSE TO CHLORPROMAZINE-TREATED MICE

cpz, Chlorpromazine-treated animals.

		Brain				Liver			
		Mean	*n*	*t*	*P*	Mean	*n*	*t*	*P*
Radioactivity of homogenates (c.p.m./g of fresh tissue)	cpz.	59,319	6	1·24	0·2–0·3	174,487	6	0·26	0·8–0·9
	control	96,813	6			128,050	6		
Percentage of counts in acid and soluble fraction	cpz.	38·2	6	6·31	<0·001	11·1	5	2·66	0·02–0·05
	control	9·9	6			4·3	6		
Percentage of counts in lipid fraction	cpz.	40·7	6	4·04	0·001–0·01	78·8	6	0·58	0·5–0·6
	control	53·0	6			75·9	6		
Percentage of counts in protein fraction	cpz.	20·9	6	4·33	0·001–0·01	11·8	6	2·11	0·05–0·1
	control	37·1	6			19·6	6		
Protein/acid soluble ratio	cpz.	0·62	6	5·95	<0·001	1·49	5	1·53	0·1–0·2
	control	4·17	6			4·48	6		

TABLE 3

INFLUENCE OF CHLORPROMAZINE ADMINISTRATION ON THE RADIOACTIVITY IN MOUSE BLOOD, LIVER AND BRAIN FOLLOWING INTRAPERITONEAL INJECTION OF 5μc OF (U-^{14}C)-D-GLUCOSE

	Chlorpromazine		Control			
	Mean	*n*	Mean	*n*	*t*	*P*
Radioactive c.p.m./ml. tail blood	62,872	6	29,939	6	2·33	0·02–0·05
Ratio of radioactive c.p.m./g whole tissue (liver/brain)	2·89	6	1·43	6	1·91	0·05–0·1

In vivo *glucose metabolism*

The radioactive counts in blood, liver and brain and percentage distribution of tissue radioactivity in acid soluble, lipid, nucleotide and protein fractions of liver and brain are shown in Tables 2 and 3. There was a slight decrease in radioactive counts in brains of chlorpromazine-treated animals and a slight increase in the livers of these animals; however, these differences were not statistically significant. The tail blood carbon-14 of chlorpromazine-treated animals was significantly higher than that of controls. The ratio of radioactivity in liver to that in brain was higher in the test animals compared with controls ($P = 0.05$–0.01). In the fractionation experiments the percentage of radioactivity which appeared as nucleotides was always less than 5%. Table 2 shows that in the brains of drug-treated mice more counts appeared in the acid soluble fraction and less in the lipid and protein fractions compared with controls, whereas in the liver the only significant difference was found to be an increase in the radioactivity of the acid soluble fraction after chlorpromazine treatment.

DISCUSSION

The results show that a moderate degree of uncoupling of respiration occurs as measured by the ADP stimulation method *in vitro* in the presence of a concentration of chlorpromazine comparable with therapeutic levels (0.59×10^{-4}M; 0.2 mg/ml.). It is necessary to use higher concentrations of chlorpromazine (2×10^{-4}M) to detect uncoupling using direct measurements of the ratio of utilization of inorganic phosphate to oxygen uptake (Dawkins *et al.*, 1959). Brain oxygen consumption *in vitro* is lower than that estimated *in vivo*, although brain slices (but not homogenates) can be made to respire at *in vivo* rates by electrical or cationic stimulation (McIlwain, 1953). This enhanced respiration is particularly sensitive to inhibition by hypnotics and chlorpromazine (McIlwain & Greengard, 1957; Wallgren & Kulonen, 1960). The present work shows that ADP stimulated respiration is not only inhibited by chlorpromazine but the effect can also be demonstrated in brain homogenates. This suggests that a final common pathway in electrical and cationic stimulation is the production of ADP.

Respiration inhibited by chlorpromazine is increased when succinate (Fig. 2) but not when lactate is added to the incubation mixture (Fig. 1). This indicates that the electron transfer chain associated with succinate oxidation does not contain the rate limiting reaction (Krebs, 1959), but the metabolic block may possibly be present in electron

transfer chains containing NAD^+/NADH oxido-reductase systems. This ability of succinate to reverse drug-induced inhibition of brain respiration *in vitro* has been observed with anaesthetic agents (Quastel, 1965).

Grenell, Mendelson & McElroy (1955) have shown an increase in brain ATP following administration of chlorpromazine to rats, and this is consistent with the observations of Dawkins *et al.* (1959), who observed an inhibition of ATPase. This indicates that the influence of chlorpromazine on the tissue concentration of high energy phosphate bonds is effected more by inhibition of ATPase than by uncoupling. Furthermore, Grenell *et al.* (1955) showed that acetylation (which is ATP-dependent) is not impaired by the drug. Vrba, Gaitonde & Richter (1962) have shown that in rat brain there is a rapid conversion of ^{14}C-glucose to amino-acids (in the acid soluble extract), lipids, nucleic acids and protein. A similar distribution of radioactivity was observed in mouse brain in these experiments but in the chlorpromazine-treated animals the fraction of total tissue counts in the acid soluble fraction was significantly higher and that in the protein fraction was significantly lower than in controls. There was also a significant decrease in incorporation of carbon-14 into brain lipids in the test animals. Less obvious differences were seen in the liver—the only significant change being an increase in counts in the acid soluble fraction in the test animals. If the uncoupling which is found *in vitro* also occurs *in vivo* with chlorpromazine, then processes which are energy dependent—such as protein and lipid synthesis—would be impaired and there would probably be a relative increase in the metabolic precursors of these synthetic pathways. The results of the present experiments are consistent with this sequence of events, but in addition they show much smaller alterations in liver metabolism compared with brain. This effect is comparable with inhibition of incorporation of amino-acids into brain proteins in rats narcotized with ether or sodium pentobarbitone (Gaitonde & Richter, 1956).

Much of the total lipid turnover in the brain involves the phospholipid fraction—the most liable components being phosphoinositide, phosphatidic acid and phosphatidyl choline (Ansell & Dohmen, 1957). The possible impairment of the synthesis of these substances in the brain by drugs is of interest in relation to the evidence that phosphatidic acid may play a part in the ion-transporting system across cell membranes (Hokin & Hokin, 1958).

The uptake of glucose into the cell is itself an energy dependent mechanism. The experiments have shown chlorpromazine to impair uptake of the ^{14}C-glucose by the brain, but not by the liver (Table 2). Blood carbon-14 levels were higher in the treated mice compared with controls (Table 3), which is also consistent with a decrease in the net tissue uptake. Although the first step in metabolism of glucose is dependent on a hexokinase system in both liver and brain, these are distinct enzyme proteins with different kinetic constants in these two sites, and in addition the liver contains a glucokinase which is not present in the brain (Sols, Sillero & Salas, 1965). Thus the glucose phosphorylating systems in liver and brain are quite different and could well show different susceptibilities to chlorpromazine.

SUMMARY

1. Chlorpromazine (0.59×10^{-4}M) inhibits the stimulating effect of ADP on mouse brain respiration *in vitro*.

2. The administration of chlorpromazine to mice inhibits the incorporation of ^{14}C-glucose into the protein and lipid fractions of the brain, but does not significantly alter these in the liver. In both brain and liver the drug results in an increase in radioactive counts in the fraction which contained D-glucose and amino-acids.

We thank the United Cerebral Palsy Research (Grant No. R-212-67) for their financial support.

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