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EFFECTS OF PSYCHOTROPIC DRUGS ON THE CEREBRAL ENERGY STATE AND GLYCOLYTIC METABOLISM IN THE RAT: DIAZEPAM, CLOMIPRAMINE AND CHLORPROMAZINE

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Abstract—Phosphocreatine (PCr), *ATP, ADP, AMP, glucose, glucose-6-phosphate (G-6-P), lactate, and pyruvate were measured with the cerebral cortex tissues frozen in situ by liquid nitrogen after the intravenous administration of psychotropic drugs in rats, i.e. diazepam (0.25 mg/kg), clomipramine (2.0 mg/kg), and chlorpromazine (0.5 mg/kg). There were no significant changes in the levels of cerebral high energy phosphates or energy charge potential (ECP). There were also no significant changes in the levels of glycolytic intermediates or the lactate/pyruvate ratio (L/P ratio), except for an increase in glucose after the administration of chlorpromazine. Thus, none of these drugs appeared to impede the cerebral energy state in a therapeutic dose

A decrease in the cerebral metabolic rate for oxygen ($CMRO_2$) and a concomitant decrease in cerebral blood flow (CBF) with psychotropic drugs have been reported in our previous study [1]. Diazepam, clomipramine, and chlorpromazine of therapeutic doses decreased $CMRO_2$ by 16, 13 and 10 per cent, respectively, in the dog. Carlsson *et al.* [2] also reported that diazepam decreased $CMRO_2$ and CBF to about 60 per cent that of control when it was administered during 70 per cent nitrous oxide anaesthesia in the rat. From our study on the oxygen–glucose index and the cerebral venous oxygen tension, no evidence could be obtained for proceeding of anaerobic metabolism in cerebral tissue. In addition to these metabolic studies for oxygen consumption, it seems necessary to determine concentrations of metabolites in the cerebral tissue to evaluate the intracellular state of metabolism. As far as chlorpromazine is concerned, studies along this line have been done using rats or mice [3–6]. We are, however, unaware of any information on the cerebral levels of high energy phosphate compounds and glycolytic intermediates and end-product with diazepam or clomipramine. This paper described the results of determination of the above metabolites in the cerebral tissue frozen in situ by liquid nitrogen in rats after the administration of the psychotropic drugs under well controlled physiological conditions.

MATERIALS AND METHODS

Twenty unstarved male rats, weighing 300–410 g were anesthetized with 0.2% methoxyflurane, and 70% nitrous oxide in oxygen during the operation. The rats were ventilated with an animal ventilator (Rodent respiration pump 681, Harvard Apparatus Co., U.S.A.) through the tracheostomized catheter under the muscle relaxant *d*-tubocurarine, 0.5 mg/kg initially and 0.25 mg/kg following every 30 min. Catheterization of right femoral artery and vein were performed for monitoring direct arterial blood pressure, blood sampling, and the injection of fluid and drugs. The skin was reflected from the skull and the EEG was recorded from the fronto-pariental lead, using bipolar silver silver-chloride electrodes. After the completion of the operation, methoxyflurane was discontinued and the rats were ventilated with 70% nitrous oxide in oxygen, and thereafter, at least 30 min was allowed to elapse to obtain stable blood pressure, blood gas values, and body temperature. Arterial blood was sampled anaerobically in glass capillaries for micro analysis of blood gases and pH (BMS 2 MK 2 blood micro system and PHM 72 MK 2 digital acid–base analyzer, Radiometer Ltd., Denmark). The blood sample for gas analysis was taken at frequent intervals, including a sample immediately before the freezing. Blood loss due to sampling was replaced by fresh heparinized blood. Body temperature was kept at 37 ± 0.1° by warming blanket and hematocrit was maintained at 45 ± 1 per cent. The rats were randomized into four groups of five rats each, i.e. control (saline), diazepam (0.25 mg/kg), clomipramine (2.0 mg/kg), and chlorpromazine (0.5 mg/kg) groups. Five minutes after the administration of diazepam and 15 min after saline, clomipramine or chlorpromazine, the brain was frozen *in situ* following Pontén's method [7] by pouring liquid nitrogen into a funnel over the intact skull bone. Brain tissue samples were obtained by dissection in liquid nitrogen and were kept −196° until analysis. The cerebral tissue was extracted with HCl–methanol perchloric acid below 0° after weighing. The techniques of Lowry and Passonneau [8] were used for determination of PCr, ATP, ADP, AMP, glucose, G-6-P, lactate, and pyruvate concentrations in brain tissue. The ECP was calculated as suggested by Atkinson [9]. All enzymes and co-enzymes for

*Abbreviations: PCr, phosphocreatine; G-6-P, glucose-6-phosphate; ECP, energy charge potential; L/P, lactate–pyruvate ratio; $CMRO_2$, cerebral metabolic rate for oxygen; CBF, cerebral blood flow.

Table 1. The physiological parameters of control and psychotropic drug groups

	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	MAP (mmHg)
Control	110 ± 4	36.2 ± 0.8	7.401 ± 0.022	143 ± 4
Diazepam	112 ± 2	36.4 ± 0.8	7.421 ± 0.013	126* ± 3
Clomipramine	111 ± 7	33.1 ± 3.8	7.385 ± 0.018	136 ± 5
Chlorpromazine	107 ± 3	37.4 ± 0.9	7.364 ± 0.014	116* ± 4

* Significantly different from control ($P < 0.05$). PaO_2 = Arterial oxygen tension; $PaCO_2$ = Arterial carbon dioxide tension; MAP = Mean arterial blood pressure.

the assays were commercially available (Boehringer Mannheim GmbH, West Germany). All enzymatic analysis were duplicated by a Hitachi spectrophotometer (124, Hitachi Ltd., Tokyo, Japan) with an attached linear-log recorder except glucose and lactate. Statistical differences were compared between control and drug groups using the unpaired *t*-test. $P < 0.05$ was considered to be significant.

RESULTS

Table 1 shows mean arterial pressure, oxygen tension, carbon dioxide tension and pH of arterial blood before the brain sampling. There were no statistically significant differences in these physiological parameters between control and drug groups, except for MAP of the diazepam and chlorpromazine groups. Tables 2 and 3 shows the effect of three drugs on the high energy phosphate compounds, glycolytic intermediates and end-product. There were no statistical differences in the levels of these compounds between control and drug groups except for a significant increase in the glucose with chlorpromazine. Figure 1 shows representative EEG changes with three drugs. Each drug produced slow wave activity.

DISCUSSION

The present study clearly indicates that the levels of high energy phosphate compounds are stably maintained after the administration of three psychotropic drugs. Sari *et al.* [1] reported the decrease in $CMRO_2$ with diazepam, clomipramine, and chlorpromazine in dogs. Carlsson *et al.* [2] observed that $CMRO_2$ decreased with diazepam during 70% nitrous oxide anesthesia in rats. It is apparent from our results that the reported decreases in $CMRO_2$ are not accompanied by any significant changes in cerebral concentrations of adenine nucleotides and glycolytic intermediates, except glucose with chlorpromazine. The reduction in $CMRO_2$ may be a secondary effect of the drugs. Slow wave activity in EEG as shown in Fig. 1 is probably an indication of

Table 2. Effects of psychotropic drugs on cerebral energy states

	PCr (μmoles/g wet wt.)		ATP (μmoles/g wet wt.)		ADP (μmoles/g wet wt.)		AMP (μmoles/g wet wt.)		ECP	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	5.05	0.09	2.87	0.03	0.281	0.005	0.057	0.008	0.939	0.002
Diazepam	5.13	0.08	2.93	0.04	0.290	0.007	0.054	0.003	0.939	0.001
Clomipramine	5.23	0.10	2.94	0.04	0.288	0.012	0.049	0.009	0.941	0.002
Chlorpromazine	5.16	0.05	2.84	0.06	0.306	0.012	0.053	0.008	0.936	0.003

* Significantly different from control ($P < 0.05$). PCr = Phosphocreatine; ECP = Energy charge potential.

Table 3. Effects of psychotropic drugs on cerebral glycolytic metabolism

	Glucose (μmoles/g wet wt.)		G-6-P (μmoles/g wet wt.)		Lactate (μmoles/g wet wt.)		Pyruvate (μmoles/g wet wt.)		L/P	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	2.93	0.26	0.137	0.010	1.42	0.10	0.106	0.008	14.0	1.6
Diazepam	2.66	0.14	0.138	0.009	1.40	0.08	0.107	0.006	13.2	0.5
Clomipramine	2.90	0.15	0.151	0.005	1.41	0.08	0.102	0.004	13.8	0.9
Chlorpromazine	3.82	0.21*	0.129	0.023	1.64	0.07	0.112	0.005	14.8	0.9

* Significantly different from control ($P < 0.05$). G-6-P = Glucose-6-phosphate; L/P = Lactate-pyruvate ratio.

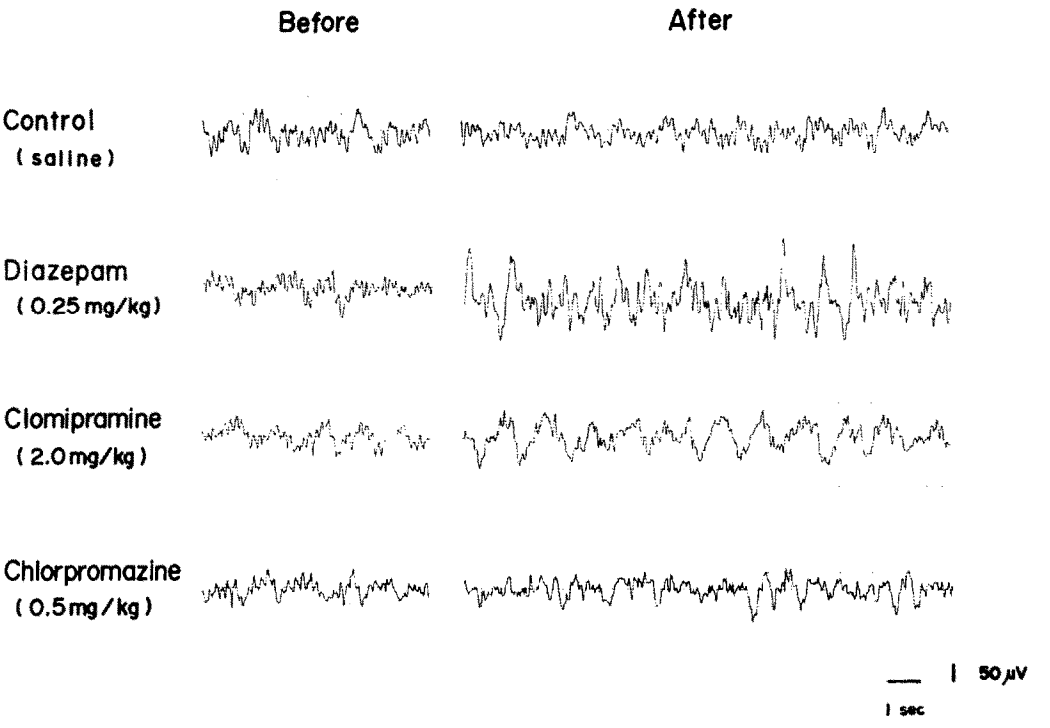

Fig. 1. Representative EEG pattern before and after the administration of diazepam, clomipramine or chlorpromazine.

depression of neuronal firing through neurotransmitter mechanisms [10, 11]. Under these circumstances, Na^+–K^+-ATPase which supports the transmembrane potential across neuronal membranes will be reduced. The decrease in the energy consumption will lead to the increase in ATP levels, which will, in turn, inhibit the rate of energy producing systems such as glycolysis and oxidative phosphorylation. Therefore, oxygen consumption will be reduced.

It has been reported that chlorpromazine inhibited oxidative phosphorylation [12]. The ratio of the moles of inorganic phosphate per atom of oxygen consumption in the brain slice *in vitro*, was decreased with chlorpromazine [12]. Diazepam inhibits oxidative phosphorylation and appeared to be a true uncoupler [13]. In spite of these observations *in vitro*, there was no change *in vivo* in the steady-state level of ATP, ADP, AMP and PCr with chlorpromazine and diazepam as shown in Table 2. Thus, the possibility arises as pointed out by Siesjö [14] that alterations in adenine nucleotide concentrations are confined to the microenvironment of the energy-producing system and that tight coupling with a high gain operates between energy producing and consuming systems.

The increase in glucose with chlorpromazine observed in this study is in agreement with the other reports [4–6], although they gave a large dose of this drug either intraperitoneally or subcutaneously. Gey *et al.* reported a decrease in concentrations of G-6-P, fructose-6-phosphate, and pyruvate, an increase in glucose, and no change in ATP [6]. Their results were interpreted either by a reduction in glycolytic flux rate [15] or an increased transport of glucose from the blood to the brain [4, 5]. The increase in cerebral glycogen and glucose concentrations has also been reported during anesthesia, although the mechanism is not fully understood. Recently, from the analysis of glycolytic intermediates at short time intervals following intravenous infusion of thiopental, Siesjö [14] demonstrated inhibition at the phosphofructokinase step. In order to obtain further insight into the intracellular metabolism, determination of flux rate of glucose metabolism is necessary in addition to the analysis of the steady-state levels of the metabolites.

In summary, diazepam (0.25 mg/kg), clomipramine (2.0 mg/kg), and chlorpromazine (0.5 mg/kg) did not cause any significant changes in the cerebral cortical energy state in rats.

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