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SUPPRESSION OF GLYCOLYSIS IN RAT BRAIN *IN VIVO* BY CHLORPROMAZINE, RESERPINE, AND PHENOBARBITAL

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Abstract—In starved and fed rats having received single intraperitoneal injections of 25 mg/kg chlorpromazine, of 2 mg/kg reserpine, and of 100 mg/kg phenobarbital respectively, the level of glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate dihydroxyacetone phosphate, adenosine 5′-triphosphate (ATP), inorganic phosphate, and glycogen was estimated in blood and brain. In the brain, but not in the blood, the three drugs decreased glucose-6-phosphate and fructose-6-phosphate at the time of maximal depression of the motor activity. The other phosphorylated metabolites as well as the inorganic phosphate showed no regular alterations. Reserpine and phenobarbital, but not chlorpromazine, caused an accumulation of glycogen following the time of maximal sedation. The finding of decreased hexose phosphates in the brain supports the hypothesis that central depressant drugs suppress glycolysis in the central nervous system *in vivo* possibly by a diminution of glucose phosphorylation.

THE ENERGY metabolism of the brain depends almost completely on glucose.[1,2] Drugs depressing central nervous functions, such as chlorpromazine and phenobarbital, seem to diminish aerobic and anaerobic glycolysis *in vitro*.[3–7] Inhibition of cerebral glycolysis by central depressant drugs might also occur *in vivo* since in the brain reserpine, chlorpromazine, and phenobarbital increase the glucose,[8–12] but decrease the pyruvate and lactate.[10–18] Phenobarbital and reserpine also cause an accumulation of cerebral glycogen,[9–12, 15–23] whereas with chlorpromazine contradictory results were reported.[8, 20, 24] Finally, phenobarbital has been found to change the cerebral level of phosphorylated metabolites of the glycolytic pathway.[12]

In order to further investigate the alteration of the cerebral carbohydrate metabolism by central depressant drugs, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, dihydroxyacetone phosphate, adenosine 5′-triphosphate (ATP), inorganic phosphate, and glycogen were measured in the brain of rats treated with chlorpromazine, reserpine, or phenobarbital. Intracellular brain levels were calculated from the values of total brain and blood.

METHODS

Animals. Female Wistar rats weighing 100–150 g, kept at 25°, received intraperitoneal injections of 25 mg/kg chlorpromazine-HCl, 2 mg/kg reserpine, 100 mg/kg phenobarbital-sodium, and physiological saline (controls) respectively.

Tissues. Blood was obtained by decapitation. The brain of the rats killed by immersion in liquid nitrogen was chiselled out and powdered under liquid nitrogen. Deproteinization and extraction of brain and blood were performed with

the following solutions: 7% $HClO_4$ (w/v) in acetone at −78° for the phosphorylated intermediates of glycolysis and ATP;[25] ice-cold 10% CCl_3COOH (w/v) in a VirTis-homogenizer with glass beads for glycogen,[26] and in a Potter–Elvehjem homogenizer for inorganic phosphate.

Analysis of metabolites. The various metabolites were measured spectrophotometrically as follows: glucose-6-phosphate and fructose-6-phosphate by means of glucose-6-phosphate dehydrogenase without and with addition of phosphohexose isomerase respectively; dihydroxyacetone phosphate by glycerine-1-phosphate dehydrogenase; fructose-1,6-diphosphate by the same enzyme in the presence of aldolase and triosephosphate isomerase; pyruvate and lactate by lactate dehydrogenase; ATP by hexokinase and glucose-6-phosphate dehydrogenase;[27] inorganic phosphate by a modified Fiske-Subbarow method (reaction with ammonium molybdate at 2° for 3 min).[28] Glycogen was determined with the anthrone reagent after precipitation by 60% ethanol (v/v) and washing with diethyl ether;[29] comparative measurements by glucose oxidase gave corresponding results.

Calculation of intracellular brain levels. The intracellular values of the phosphorylated metabolites were estimated by deducting 5 per cent of the blood values from the values of total brain, assuming a 5 per cent blood content in rat brain. This seems to be justified because the phosphorylated intermediates (unlike pyruvate and lactate) are mainly associated with blood cells and hardly at all with extracellular fluid. The intracellular brain level of inorganic phosphate was approximated by deducting 10 per cent of the blood values from the values for the total brain. This approximation is based on the assumption that the extracellular space of rat brain is of the order of 10 per cent[30–33] and that the concentration in the extracellular space equals that in blood.

Psychomotor behaviour. The following scale was used:

1 Spontaneous motor activity as well as reaction to acoustic and tactile stimuli slightly diminished.

2 Little spontaneous motor activity; markedly reduced reactions to stimuli.

3 No spontaneous motor activity; no reactions to stimuli, but righting reflex still present.

4 Narcosis; righting reflex absent.

RESULTS

Chlorpromazine and reserpine. In the blood (Tables 1 and 2), both drugs increase the glucose-6-phosphate in starved rats more markedly than in fed rats. The other phosphorylated metabolites of the blood are either not significantly changed or even decreased, for example fructose-6-phosphate by chlorpromazine in fed rats, fructose-1,6-diphosphate by both drugs in starved rats, dihydroxyacetone phosphate by chlorpromazine in starved rats.

In the brain (Tables 1 and 2) of fed rats, both neuroleptics cause a distinct diminution of glucose-6-phosphate as well as of fructose-6-phosphate at the time of maximal sedation (Fig. 1); dihydroxyacetone phosphate is decreased by reserpine. In starved animals, no significant alteration of the brain levels of phosphorylated carbohydrates can be seen.

In both blood and brain, ATP and inorganic phosphate (Tables 1 and 2) remain unaffected by the neuroleptics with the exception of ATP in starved rats which shows a slight decrease after reserpine.

TABLE 1. EFFECT OF CHLORPROMAZINE, RESERPINE, AND PHENOBARBITAL ON THE LEVELS OF PHOSPHORYLATED METABOLITES AND INORGANIC PHOSPHATE IN BLOOD AND BRAIN OF FED RATS

Metabolite	Tissue	Controls (NaCl i.p.; 1/2–3 hr)		Chlorpromazine (25 mg/kg i.p.; 1/2 hr)	Reserpine (2 mg/kg i.p.; 3 hr)	Phenobarbital (100 mg/kg i.p. 1 hr)
		μmoles/g	%	%	%	%
Glucose-6-phosphate	blood	0·028 ± 0·001	100 ± 5	121 ± 4	115 ± 4	136 ± 6†
	brain total	0·095 ± 0·005	100 ± 5	83 ± 4†	75 ± 7†	61 ± 7†
	brain i.c.	0·094 ± 0·005	100 ± 5	83 ± 4†	75 ± 7†	61 ± 7†
Fructose-6-phosphate	blood	0·006 ± 0·001	100 ± 8	76 ± 5†	101 ± 3	138 ± 7†
	brain total	0·029 ± 0·002	100 ± 7	71 ± 3†	81 ± 3*	69 ± 2†
	brain i.c.	0·029 ± 0·002	100 ± 7	71 ± 3†	81 ± 3*	69 ± 2†
Fructose-1,6-diphosphate	blood	0·010 ± 0·001	100 ± 5	93 ± 10	106 ± 6	93 ± 10
	brain total	0·098 ± 0·008	100 ± 8	93 ± 2	85 ± 9	128 ± 8
	brain i.c.	0·097 ± 0·008	100 ± 8	93 ± 2	85 ± 9	128 ± 8
Dihydroxyacetone phosphate	blood	0·011 ± 0·001	100 ± 5	104 ± 9	103 ± 14	79 ± 7†
	brain total	0·039 ± 0·002	100 ± 6	100 ± 6	78 ± 10†	101 ± 10
	brain i.c.	0·039 ± 0·002	100 ± 6	100 ± 6	78 ± 10†	101 ± 10
ATP	blood	0·414 ± 0·018	100 ± 4	106 ± 3	104 ± 4	85 ± 8
	brain total	2·135 ± 0·50	100 ± 3	106 ± 5	112 ± 4	96 ± 3
	brain i.c.	2·105 ± 0·50	100 ± 3	106 ± 5	111 ± 4	96 ± 3
Inorganic phosphate	blood	1·40 ± 0·07	100 ± 5	91 ± 7	95 ± 9	96 ± 4
	brain total	5·40 ± 0·30	100 ± 6	102 ± 9	104 ± 15	98 ± 7
	brain i.c.	5·26 ± 0·30	100 ± 6	102 ± 9	104 ± 15	98 ± 7

* $P<0.05$

† $P<0.01$

The results are expressed in μmoles per g wet tissue and in per cent of controls repectively. The experimental values represent averages ± S.E of 2–6 determinations in blood (pools of 4 animals each) and of 4–8 determinations in brains (individual animals); the controls represent averages ± S.E. of 7–20 determinations; i.c. means intracellular levels.

TABLE 2. EFFECT OF CHLORPROMAZINE, RESERPINE, AND PHENOBARBITAL ON THE LEVELS OF PHOSPHORYLATED METABOLITES AND INORGANIC PHOSPHATE IN BLOOD AND BRAIN OF RATS STARVED FOR 16 HOURS

Metabolite	Tissue	Controls (saline i.p.: 1/2–3 hr)		Chlorpromazine (25 mg/kg i.p.: 1/2 hr)	Reserpine (2 mg/kg i.p.: 3 hr)	Phenobarbital (100 mg/kg i.p.: 1 hr)
		μmoles/g	%	%	%	%
Glucose-6-phosphate	blood	0·028 ± 0·001	100 ± 5	161 ± 11*	132 ± 11*	117 ± 5
	brain total	0·092 ± 0·005	100 ± 5	102 ± 5	88 ± 14	78 ± 4*
	brain i.c.	0·090 ± 0·005	100 ± 5	101 ± 5	88 ± 14	78 ± 4*
Fructose-6-phosphate	blood	0·007 ± 0·001	100 ± 10	122 ± 12	122 ± 20	117 ± 10
	brain total	0·031 ± 0·002	100 ± 6	89 ± 4	98 ± 14	78 ± 6*
	brain i.c.	0·030 ± 0·002	100 ± 6	89 ± 4	98 ± 10	78 ± 7*
Fructose-1,6-diphosphate	blood	0·013 ± 0·001	100 ± 8	79 ± 5*	77 ± 5*	82 ± 5*
	brain total	0·066 ± 0·004	100 ± 6	108 ± 6	92 ± 3	91 ± 7
	brain i.c.	0·065 ± 0·004	108 ± 6	108 ± 6	92 ± 3	91 ± 7
Dihydroxyacetone phosphate	blood	0·015 ± 0·001	100 ± 8	76 ± 5*	83 ± 8	77 ± 4*
	brain total	0·041 ± 0·001	100 ± 3	96 ± 10	103 ± 10	102 ± 3
	brain i.c.	0·041 ± 0·001	100 ± 3	95 ± 10	103 ± 10	102 ± 3
ATP	blood	0·478 ± 0·030	100 ± 6	99 ± 6	100 ± 6	87 ± 3
	brain total	2·360 ± 0·050	100 ± 3	101 ± 3	90 ± 3*	96 ± 2
	brain i.c.	2·340 ± 0·050	100 ± 3	101 ± 3	90 ± 3*	96 ± 2
Inorganic phosphate	blood	1·39 ± 0·01	100 ± 1	102 ± 2	137 ± 6	106 ± 5
	brain total	7·20 ± 1·30	100 ± 18	102 ± 6	121 ± 4	85 ± 8
	brain i.c.	7·06 ± 1·30	100 ± 18	102 ± 6	120 ± 5	84 ± 8

* $P<0.05$
† $P<0.01$
(Details as in Table 1).

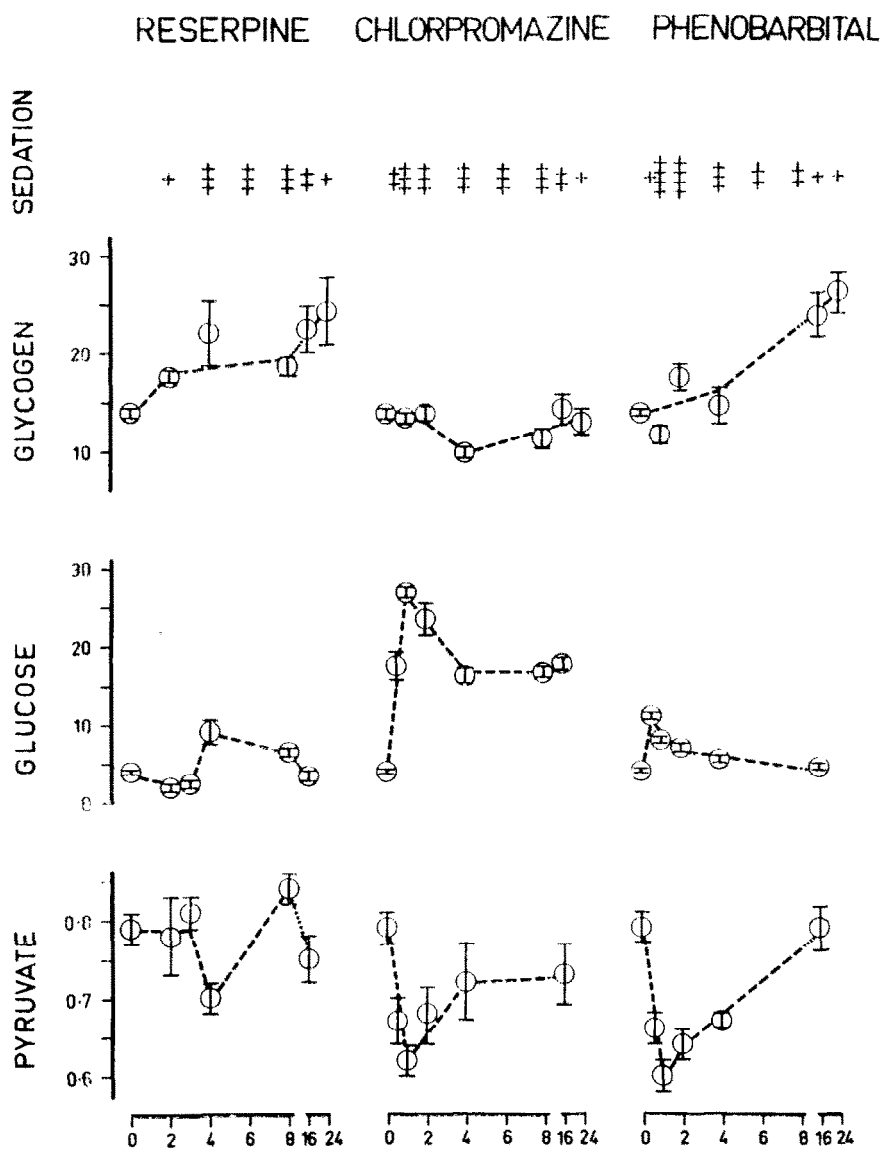

FIG. 1. Effect of reserpine, chlorpromazine, and phenobarbital on psychomotor behaviour, cerebral glycogen (calculated as mg % glucose) as well as on intracellular levels of glucose and pyruvate in brain (mg %) of rats. Abscissa: hr after intraperitoneal injection of the drugs. The values for glucose and pyruvate were taken from a previous paper[10] for the sake of comparison. The animals were starved for 16 hr. The results represent means ± S.E. of the following number of experimental values: 6–18 for glycogen, 3–8 for glucose, 3–20 for pyruvate. The values of controls did not vary significantly within 24 hr. The results differed from controls with $P < 0.001$ (< 0.05) at the following time intervals: glycogen 16 and 24 hr after reserpine and phenobarbital respectively; glucose 4 (2–16) hr after reserpine, 1/2–16 hr after chlorpromazine and 1/2–1 hr after phenobarbital; pyruvate 4 hr after reserpine, 1 (1/2–2) hr after chlorpromazine, and 1–2 (1/2–4) hr after phenobarbital.

Cerebral glycogen slightly accumulates in starved rats (Fig. 1) 16 hr after reserpine, i.e. subsequent to the maximal sedation. Chlorpromazine has no significant effect.

Phenobarbital. In the blood (Tables 1 and 2), the drug increases glucose-6-phosphate and fructose-6-phosphate in fed rats; it diminishes the fructose-1,6-diphosphate in starved rats and the dihydroxyacetone phosphate in both fed and starved animals.

In the brain (Tables 1 and 2), phenobarbital markedly decreases the glucose-6-phosphate and fructose-6-phosphate in both starved and fed rats. The other phosphorylated intermediates including ATP as well as inorganic phosphate show no significant alterations. Brain glycogen in starved rats (Fig. 1) continuously increases up to 24 hr, without relation to changes in behaviour.

DISCUSSION

Alterations of blood levels. The present results with blood (Tables 1 and 2) confirm that chlorpromazine, reserpine, as well as phenobarbital alter the extracerebral metabolism of carbohydrates (*cf.* Ref. 10). These changes, however, do not in general parallel the alterations in cerebral carbohydrate metabolism. For instance, glucose-6-phosphate and fructose-6-phosphate are rather increased in the blood cells, but decreased in the brain. The alterations in the extracerebral carbohydrate metabolism might be due to a reduced glucose uptake by extracerebral tissues,[34–36] to functional alterations in the autonomic nervous systems and/or to an endocrine imbalance, for example to increased gluconeogenesis mediated through the adrenals.[23, 31, 37]

Alterations of cerebral hexose-6-phosphates. The present results (Tables 1 and 2) show that the neuroleptics chlorpromazine and reserpine as well as the hypnotic phenobarbital decrease the hexose-6-phosphates in rat brain. The three drugs do also not differ in their effect on the intracellular levels of glucose and pyruvate in the rat brain.[10] (Fig. 1.) These findings indicate that *in vivo* the cerebral carbohydrate metabolism of the rat is altered by neuroleptics as well as by hypnotics in a qualitatively similar, unspecific way.

It has been suggested that chlorpromazine, reserpine, and phenobarbital cause a suppression of cerebral glycolysis, since these drugs diminish the pyruvate, but increase the glucose in the brain.[9] The present results showing a decrease in cerebral glucose-6-phosphate and fructose-6-phosphate support this hypothesis. The inhibition of glycolysis is almost certainly not due to a lack of total inorganic phosphate. Thus the latter is not influenced by the three drugs, confirming earlier experiments with reserpine.[38] The diminution of hexose-6-phosphates rather indicates an interference of the three neurotropic drugs with the ATP-dependent phosphorylation of glucose, which is a rate-limiting reaction of cerebral glycolysis.[1, 2] A reduced formation of glucose-6-phosphate in central nervous depression is also suggested by previous findings. *In vitro*, high concentrations of chlorpromazine inhibit hexokinase at relatively low levels of ATP.[3, 39–41] Furthermore, *in vivo*, amobarbital decreases the formation of glucose-6-[$^{32}PO_4$],[42] and central nervous stimulation induces an activation of both hexokinase activity[43] and glucose-6-[$^{32}PO_4$] formation.[42] The inhibition of glucose phosphorylation by central depressants might be related to a reduced availability of ATP as suggested by a reduction of the [^{32}P]P_i-ATP exchange, by an inhibition of "ATP-ases" and of the postmortem decrease of ATP.[3, 12, 18, 25, 41, 44–47] A reduced availability of ATP is possible in spite of the present and previous experiments which

show no diminution in the total level of brain ATP by phenobarbital and chlorpromazine and only a slight decrease by reserpine.[12, 17, 18, 25, 38, 48]

It has to be considered that inhibition of glycolysis by central depressant drugs might be associated with an activation of the pentose pathway, especially in the hypothalamus and the reticular formation of the central nervous system.[49, 50] In the present experiments with the whole brain, however, such a mechanism is doubtful, since the pentose pathway seems to be of quantitatively inferior importance in the central nervous system of the rat.[51] Furthermore, in experiments with slices of brain cortex, chlorpromazine did not change the relation between the pentose and the glycolytic pathway.[6]

Some authors investigating the mice brain found, in contrast to the present results, an increase of the hexose-6-monophosphates and a decrease of fructose-1,6-diphosphate 1 hr after 150 mg/kg phenobarbital.[12] This discrepancy might be due to differences of the animal species, of the feeding state, and at most of the experimental technique. Thus, freezing the decapitated head instead of the whole animal may give different results.[10]

Alteration in cerebral glycogen. The increase of glycogen in the brain may not be primarily related to the other alterations in the carbohydrate metabolism mentioned above. Thus after reserpine and phenobarbital, the changes in cerebral glycogen occur subsequently to those of glucose and pyruvate, and chlorpromazine has no effect on glycogen at all (Fig. 1). The changes in cerebral glycogen (in contrast to those of the intracellular levels of glucose and pyruvate[10]) also show no parallelism to the degree and time course of sedation and seem therefore not to be directly related to the latter. The glycogen accumulation due to reserpine and phenobarbital might result from an increased gluconeogenesis[23, 24] rather than from a decreased activity of the glycogen breakdown by phosphorylase.[18, 52–55]

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