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EFFECTS OF NEUROTROPIC DRUGS ON GLUCOSE METABOLISM IN RAT BRAIN *IN VIVO*

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Abstract—The effects of centrally-active drugs on the incorporation of ^{14}C from glucose into rat brain α-keto acids and amino acids were tested *in vivo*. Chlorpromazine lowered the incorporation into all metabolites tested and nembutal into all except alanine and pyruvate. Morphine lowered the radioactivity of the isolated γ-aminobutyrate and aspartate. No significant effects were observed after treatment with the stimulants iproniazid or amphetamine.

INTRODUCTION

IT HAS long been established that of all the organs of the mammalian body, the brain demonstrates a unique requirement for glucose and utilizes a very high proportion of the total glucose of the body.[1–3] Whittam[4] has calculated that about 40 per cent of the energy produced from the metabolism of glucose by the brain is required for ion transport mechanisms important in maintaining the physiological function of the tissue. During short-time experiments *in vivo*, a high rate of incorporation of ^{14}C from glucose into the brain α-keto acids and amino acids has been demonstrated, as discussed in the preceding paper.[5] The rapid labelling of the amino acids is considered[5, 6] to reflect the rapid rate of glycolysis in the brain and to be due primarily to isotopic exchange between the α-keto acids and the amino acids.

The rapid incorporation of ^{14}C from glucose into such metabolites *in vivo* provides a useful system for studying the effects of centrally-active drugs on cerebral glucose metabolism. While the pharmacological actions of a number of neurotropic drugs have been extensively studied, very little is known of their effect on brain glucose utilization.

Earlier metabolic studies on the effects of drugs were made using central depressants *in vitro*, leading to the hypothesis that the depressants acted directly on respiratory mechanisms.[7, 8] However, the concentrations of the drugs which were required to cause respiratory inhibition *in vitro* were found to have a toxic rather than depressant effect when tested *in vivo*. McIlwain[9] suggested that "the action of the drugs as depressants *in vivo* was not likely to be inhibition of respiration". The evidence which has accumulated, particularly from studies on electrically-stimulated brain slices[9] also does not support an earlier view[10] that the centrally-active depressants act primarily by "uncoupling" oxidative phosphorylation, but indicates that the major effect of

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these drugs is on ion transport mechanisms[9] leading to secondary effects on general respiration.[11]

A similar state of confusion exists as to biochemical action of the tranquillizer, chlorpromazine. This drug has no obvious effect on brain respiration *in vivo*,[12] nor has it been observed to change the levels of organic phosphates[13] or oxidative phosphorylation[14] *in vitro* in brain. The primary site of action of chlorpromazine has also been suggested to be on membrane permeability.[15, 16]

This communication reports the effects of chlorpromazine, nembutal, iproniazid, DL-amphetamine and morphine on the incorporation of ^{14}C from the circulating glucose into rat brain α-keto acids and amino acids *in vivo*. In a previous study[17] of the effects of certain psychotropic drugs on the incorporation of ^{14}C from glucose into the free amino acid pools of the brain and other tissues of the rat, the individual amino acids were not separated. Nembutal was found to decrease ($P < 0{\cdot}001$) the radioactivity of the amino acid pool.

EXPERIMENTAL

Treatment of animals

Male rats (100–120 g) of the Wistar Albino Glaxo strain were injected intraperitoneally in groups of four with the drugs as shown in Table 1.

TABLE 1. DETAILS OF DRUGS INJECTED

Drug	Dose (mg/kg body weight)	Time*
Control	—	30 min
Iproniazid	100	18 hr
Chlorpromazine	25	60 min
DL-Amphetamine	10	30 min
Morphine	20	30 min
Pentobarbitone (sodium)	37	4 min

* Time between administration of drug and injection of ^{14}C-glucose.

The drugs were administered in isotonic NaCl; the control group was given 0·5 ml saline. When the times listed in Table 1 had elapsed, approximately 0·5 ml of carrier-free uniformly-labelled ^{14}C-glucose (10μc/100 g body weight) was injected intraperitoneally. After a further 20 min, the animals were killed by decapitation and the blood was immediately collected in heparinized beakers from which a measured volume (usually 1 ml) was pipetted into ice-cold 6% (w/v) perchloric acid. Also immediately after death the brain was rapidly excised and frozen by immersion in liquid N_2. The tissue was weighed and dispersed in 6% (v/v) perchloric acid (6 ml/g) in a Teflon pestle homogenizer (Type B, A.H. Thomas Co., Philadelphia).

The amino acids and α-keto acids were isolated from the brain perchloric acid extracts and glucose from the blood perchloric acid extracts as previously described.[5] The individual amino acids after separation by paper and thin-layer chromatography were estimated with ninhydrin and counted in a Packard Tricarb Model 314 EX

liquid scintillation counter; the α-keto acids were separated by thin layer chromatography of the dinitrophenylhydrazone derivatives and counted.[5] Glucose, after isolation by column and paper chromatography, was estimated using glucose oxidase and counted.[5]

Statistical analyses

The Student *t*-test was used throughout for estimating the significance of differences between means, after using the *F* (variance ratio) test to determine compatibility of variances. Where variances were incompatible, a modified *t*-test was used.[18] Results are expressed as Mean ± S.E. with the number of animals tested in parenthesis.

Drugs

Sodium pentobarbitone (Nembutal) was obtained from May and Baker Ltd., Dagenham, U.K.; chlorpromazine hydrochloride (Thorazine) and DL-amphetamine sulphate *Benzedrine* from Smith, Kline and French Pty. Ltd., Sydney, Australia and iproniazid from Roche Products Pty. Ltd., Sydney, Australia. Morphine hydrochloride was purchased from T. and H. Smith, Edinburgh, U.K.

RESULTS AND DISCUSSION

Since none of the drugs tested had any detectable effect on the radioactivity of the circulating glucose 20 min after ^{14}C-glucose injection (Table 2), the incorporation of ^{14}C into the brain α-keto acids and amino acids have been expressed as *relative specific activities* (counts/min/μmole metabolite vs. counts/min/μmole blood glucose) (Table 3); on that basis, the scatter due to variations in the specific activity of the circulating glucose between individual animals in the same test group is minimized.

TABLE 2. SPECIFIC ACTIVITIES OF THE CIRCULATING GLUCOSE IN DRUG-TREATED RATS

Drug	Specific activity
Control	39,500 ± 2600 (4)
Iproniazid	44,600 ± 4400 (4)
Chlorpromazine	39,900 ± 4500 (4)
DL-amphetamine	40,100 ± 3300 (3)
Morphine	31,700 ± 4200 (4)
Pentobarbitone (Sodium)	53,300 ± 8000 (4)

Specific activity: mean counts/min/μmole blood glucose ± S.E. (no of rats).

Chlorpromazine

The relative specific activities of all of the metabolites studied were significantly lowered in the brains from the chlorpromazine treated rats, indicating that the general rate of glucose utilization had been decreased, possibly due to a decreased rate of glucose uptake by the brain. The high sensitivity of the respiration of electrically-stimulated cerebral cortex slices to chlorpromazine has been ascribed to primary effects on membrane permeability[9, 15] rather than on aerobic glycolysis or the hexokinase reaction which have been shown to be affected.[19, 20] Chlorpromazine has also been suggested to act on adenosine triphosphatases (ATPases),[21] but the cerebral

TABLE 3. INCORPORATION OF ^{14}C FROM GLUCOSE INTO BRAIN α-KETO ACIDS AND AMINO ACIDS OF DRUG-TREATED RATS

Metabolite	Control	Iproniazid	Chlorpromazine	DL-Amphetamine	Morphine	Pentobarbitone (sodium)
Pyruvate	15·1 ± 1·0(4)	11·7 ± 2·7(3)	9·9 ± 0·2(4)†	11·7 ± 1·9(3)	14·1 ± 1·9(4)	12·2 ± 2·9(4)
α-Oxoglutarate	16·1 ± 2·6(3)	12·2 ± 2·9(3)	10·8 ± 0·2(4)†	10·0 ± 3·1(3)	12·3 ± 1·0(4)	9·1 ± 0·9(4)†
Alanine	18·2 ± 0·7(4)	12·3 ± 3·1(4)	8·5 ± 0·5(4)‡	13·8 ± 1·2(3)*	21·7 ± 1·5(4)	16·8 ± 0·8(4)
Aspartate	17·3 ± 0·8(4)	12·1 ± 2·2(4)	7·6 ± 0·3(4)‡	17·2 ± 3·6(3)	12·8 ± 0·6(4)†	9·2 ± 1·1(4)†
Glutamate	17·5 ± 0·9(4)	21·6 ± 4·2(4)	7·7 ± 0·3(4)‡	19·4 ± 3·2(3)	15·6 ± 0·7(4)	9·6 ± 0·6(4)‡
Glutamine	33·5 ± 4·8(4)	20·3 ± 3·2(3)	6·4 ± 0·2(4)†	24·4 ± 3·7(3)	23·4 ± 5·3(4)	9·2 ± 1·6(4)†
γ-Aminobutyrate	16·3 ± 0·6(4)	21·5 ± 4·7(3)	7·6 ± 0·1(4)‡	17·1 ± 2·4(3)	12·1 ± 0·9(4)†	8·8 ± 1·4(4)†

The brains were removed 20 min after ^{14}C-glucose injection.

Relative specific activity $= \frac{\text{counts/min/}\mu\text{mole metabolite}}{\text{counts/min/}\mu\text{mole blood glucose}} \times 10^2 \pm$ S.E. (No. of rats).

Significantly different from control: * $0.01 < P < 0.05$.
† $0.001 < P < 0.01$.
‡ $P < 0.001$.

microsomal Na^+, K^+-activated, Mg^{++}-dependent ATPase which is possibly involved with membrane ion transport[9, 22] is only slightly inhibited by relatively high doses of the drug.[23] Studies on the effects of the incorporation of ^{14}C-acetate into brain metabolites[24] have lent further support to the view[9, 15, 16] that the primary site of action of chlorpromazine is on membrane permeability. The results presented in the present study are consistent with this view. If the rate of glucose utilization is decreased due to an inhibition of glucose uptake by the brain, this would also be consistent with the hypothermia and hyperglycaemia known to be associated with chlorpromazine sedation.

Pentobarbitone (sodium)

Under Nembutal anaesthesia, the incorporation of glucose carbon was significantly lower into α-oxoglutarate, glutamate, glutamine, γ-aminobutyrate and asparate, but no change was observed in the radioactivity of pyruvate or alanine, in confirmation of the previous report[17] that the drug lowered the radioactivity of the general amino acid pool of brain. The carbon atoms of all the affected metabolites may be derived from glucose via the tricarboxylic acid cycle, whereas those of pyruvate and alanine are not derived so directly. Accordingly the results suggest that the anaesthetic is affecting glucose metabolism *in vivo* at a stage subsequent to pyruvate formation and possibly at the tricarboxylic acid cycle. This could be due to an inhibition of NADH dehydrogenase[25]—the resultant decrease of available NAD^+ would be expected to cause a slower turnover of the tricarboxylic acid cycle which would be reflected in a decrease in radioactivity of the intermediates. However, inhibition of NADH dehydrogenase *in vivo* might be expected to result in decreased levels of ATP and in turn cause a decrease in glycolysis. This does not appear to be the case since no change was observed in the radioactivity of pyruvate or alanine. Studies on electrically-stimulated cerebral tissues *in vitro* have indicated that the barbiturates do not act primarily by disturbing oxidative phosphorylation, but more likely by affecting membrane ion transport.[9] If the observed *in vitro* inhibition by barbiturates of the brain Na^+, K^+ ATPase[23] occurs *in vivo*, the ADP available for oxidative phosphorylation would become rate limiting—this would result in a lower rate of NADH oxidation and a slower turnover of the tricarboxylic acid cycle due to the decreased availability of NAD^+. This sequence of events could give rise to an apparent inhibition of NADH dehydrogenase, but as adequate concentrations of ATP would be present, no lowering of the glycolytic rate need follow. In this way the observations presented are consistent with an effect of pentobarbitone (sodium) on membrane ion transport.

Morphine

Only the radioactivity of the brain aspartate and γ-aminobutyrate were significantly lower than the control values; the α-keto acids and glutamate, glutamine and alanine were unchanged. This may be interpreted as an effect on the "γ-aminobutyrate shunt" (α-oxoglutarate-glutamate-γ-aminobutyrate-succinic semialdehyde-succinate) which can account for 40 per cent of brain glutamate metabolism;[26] a decrease in the incorporation into γ-aminobutyrate, due to an inhibition of glutamate decarboxylase, would ultimately lead to a lowered incorporation into aspartate. The incorporation into the the other intermediates studied would not be expected to be changed.

Amphetamine and iproniazid

The stimulant drugs had no effect on the incorporation into the metabolites studied, except for a slight lowering of the radioactivity of the brain alanine by amphetamine ($0{\cdot}01 < P < 0{\cdot}05$). However, this was not reflected by the labelling of the pyruvate, for which no difference from the control was observed.

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