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THE EFFECT OF PSYCHOTROPIC DRUGS ON THE UTILIZATION OF GLUCOSE CARBON ATOMS IN THE BRAIN, HEART AND LIVER OF THE RAT

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Abstract—Measurements were made of (a) the concentration of free amino acids, (b) the content of ^{14}C in the total acid-soluble fraction and (c) the incorporation of ^{14}C into amino acids of the rat brain, heart, liver and blood at 20 min after injection of [U-^{14}C]glucose. The effect of reserpine, iproniazid, chlorpromazine, pentobarbitone (sodium), ethylether, ethanol, electroshock and restraint by binding were examined. Reserpine inhibited markedly the uptake of ^{14}C from [^{14}C]glucose in the heart. Pentobarbitone (sodium) inhibited the incorporation of ^{14}C into the free amino acids in brain and liver. Reserpine and ethanol inhibited the incorporation of ^{14}C into amino acids in the liver, but not in the brain.

WHEN ^{14}C-labelled glucose is administered to the rat, a part is oxidized to CO_2 and a part of the ^{14}C is incorporated into a number of different tissue constituents. In most organs the ^{14}C remains for an appreciable time combined in carbohydrate, and the labelling of amino acids is relatively low; but in brain the ^{14}C rapidly passes into the free amino acids. Within 20 min of subcutaneous injection of [U-^{14}C]glucose more than 70 per cent of the ^{14}C in the brain is found in the free amino acid fraction, mainly in glutamic and aspartic acids.[5, 6] A rapid incorporation of glucose carbon into amino acids has been reported also in the mouse brain,[4] cat brain[2] and in brain slices *in vitro*.[1, 3]

Since the rapid and extensive incorporation of glucose carbon into amino acids is apparently a significant feature of brain metabolism, it appeared of interest to see whether this process is affected by drugs which are known to influence the physiological functions of the brain: the effects of reserpine, iproniazid, chlorpromazine, pentobarbitone (sodium), ethanol and ethylether were examined. The effects of electrically induced convulsions and of physical restraint on the labelling of amino acids were also studied.

EXPERIMENTAL

Female rats (90–110 g) of Wistar albino strain were used, and the animals were fed *ad libitum*. Each animal received by subcutaneous injection 10 μc of [U-^{14}C] glucose together with 1 mg carrier glucose dissolved in 0·20 ml water. D-[U-^{14}C]glucose (410 μc/mg) was supplied by the Radiochemical Centre, Amersham. In the experiments with drugs care was taken to maintain the body temperature. Groups of four or more rats were treated with the following drugs:

Reserpine (L. Light & Co., Colnbrook; 5 mg/kg) was injected intraperitoneally

18 hr before glucose: it was administered in the form of a 0·1% (w/v) solution made by dissolving 10 mg reserpine in 0·02 ml glacial acetic acid and diluting to 10 ml.

Iproniazid (10% w/v solution, 'Marsilid', Roche Products Ltd., 100 mg/kg) was injected intraperitoneally 18 hr before glucose administration.

Chlorpromazine (1% w/v solution, "Largactil", May & Baker Ltd.; 25 mg/kg) was injected subcutaneously 1 hr before glucose injection.

Pentobarbitone (sodium) (Veterinary nembutal, 60 mg/ml, Abbott Laboratories Ltd., 75 mg/kg) was injected intraperitoneally. The glucose was administered as soon as anaesthesia was observed.

Ethanol (diluted 1: 1 (v/v) with water; 5 ml/kg) was injected intraperitoneally 1 hr before glucose administration.

Ethylether. The animals were kept under deep ether narcosis for the period of 20 min from the injection of glucose until decapitation.

Electroshock: [^{14}C]Glucose was injected, and 10 min later convulsions were produced by applying 110 V for 1–2 sec by two electrodes on the parietal region of the skull.

Physical restraint. One group of animals was restrained by binding for the period of 20 min from glucose administration until decapitation.

All rats were killed by decapitation 20 min after injection of glucose. Samples (1 ml) of blood were collected in 5 ml of 5% (w/v) perchloric acid and the brain, heart and liver were rapidly excised and frozen in liquid nitrogen. Perchloric acid (5 ml of 5% w/v $HClO_4$) was added to the tissues (1 g) which were dispersed in a Teflon-pestle homogenizer (A. H. Thomas & Co.) at 0°. The residues after centrifugation at 0° and 2500 *g* for 15 min in a M.S.E. Major refrigerated centrifuge were treated three times with 5% perchloric acid at 0°. The total extract in each case was neutralized with 6N-KOH, the precipitated $KClO_4$ was removed by centrifugation at 0° and the residue was washed twice with ice-cold water. The combined supernatant and washings were made up to 25 ml with water.

Radioactivity was measured by plating the extract at "infinite thinness" on planchets and counting in an automatic Nuclear Chicago gas-flow counter, fitted with a Micromil window. When necessary, corrections were made for self-absorption and all measurements were corrected for background counting rate. The amino acid fraction was separated from the neutralized perchloric acid extract by passage through a column of Zeokarb 225 (H^+ form).[6] The amino acids were eluted from the column with 1N ammonia and the eluate was evaporated to dryness until free from ammonia. The residue containing amino acids was dissolved in water and portions were taken for ^{14}C and amino nitrogen determination. Amino nitrogen was determined by the method of Yemm and Cocking[8] after removal of traces of ammonia from the samples.[6]

RESULTS

Free amino acid content of the tissues

The concentration of free amino acids in the acid-soluble fraction of the tissues examined was not greatly affected by the drugs or other procedures used (Table 1). A significant increase of 38 per cent in the amino nitrogen content of the blood was noted after treatment with iproniazid.

Total acid-soluble ^{14}C after injection of [^{14}C]glucose

The ^{14}C contained in the acid-soluble fraction of the tissues at 20 min after injection of [^{14}C]glucose was increased very considerably by iproniazid (Table 2). This was observed in all organs examined after iproniazid administration. A similar effect, i.e. an increase of the ^{14}C content in the acid soluble fraction of the brain could also be produced in animals fasted for 18 hr. At 20 min after injection of [U-^{14}C]glucose to a group of four rats the ^{14}C content of the brain of the animals fed *ad libitum* corresponded to 31,600 counts/min/g fresh weight of brain, whereas after 18 hr of fasting an identical dose of [U-^{14}C]glucose resulted in 45,100 counts/min/g fresh weight of brain (means of four animals).

TABLE 1. FREE AMINO ACID CONTENT OF RAT TISSUES AFTER VARIOUS TREATMENTS.

Treatment	Conditions	Time from treatment to decapitation	Blood	Liver	Brain	Heart
Control	—	—	47 ± 2(6)	231 ± 11(8)	335 ± 14(8)	268 ± 28(6)
Reserpine	5 mg/kg, i.p.	18 hr	45 ± 5(3)	235 ± 31(4)	295 ± 13(4)	268 (2)
Iproniazid	100 mg/kg, i.p.	18 hr	65 ± 4(3)*	248 ± 22(4)	325 ± 21(4)	384 ± 76(3)
Chlorpromazine	25 mg/kg, i.p.	1 hr	48 ± 5(3)	246 ± 22(4)	316 ± 19(4)	274 ± 18(3)
Pentobarbitone (sodium)	75 mg/kg, i.p.	23 min	55 ± 6(3)	207 ± 13(4)	331 ± 24(4)	257 ± 33(3)
Ethylether	narcosis by	20 min	34 ± 8(3)	233 ± 23(4)	335 ± 21(4)	308 ± 16(3)
Ethanol	5 ml (1:1v/v)/kg, i.p.	1 hr	54 ± 6(3)	221 ± 9(4)	345 ± 13(4)	289 ± 22(3)
Electroshock	110V, 1–2 sec	10 min	48 ± 4(3)	248 ± 25(4)	344 ± 20(4)	287 ± 30(3)
Restraint	Binding	20 min	46 ± 1(3)	214 ± 16(4)	326 ± 20(4)	294 ± 21(3)

Results expressed as μg α-amino-N/g fresh tissue/ml blood ± M.E.M. (no. of animals).
(M.E.M., MEAN ERROR OF THE MEAN)

* $P < 0.01$ † Ethylether narcosis produced by inhalation.

TABLE 2. TOTAL ^{14}C FROM [U-^{14}C]GLUCOSE IN THE ACID-SOLUBLE FRACTION OF RAT ORGANS AFTER VARIOUS TREATMENTS.

Treatment	Blood	Liver	Brain	Heart
Control	198 ± 14(7)	238 ± 39(8)	222 ± 19(8)	218 ± 15(6)
Reserpine	232 ± 8(4)	286 ± 40(4)	195 ± 41(4)	88 ± 20(4)*
Iproniazid	310 ± 25(4)†	421 ± 87(4)	341 ± 58(4)	275 ± 17(3)
Chlorpromazine	196 ± 10(4)	229 ± 41(4)	185 ± 37(4)	218 ± 56(3)
Pentobarbitone (sodium)	214 ± 38(4)	283 ± 62(4)	176 ± 34(4)	360 ± 97(3)
Ethylether	193 ± 8(4)	265 ± 19(4)	189 ± 23(4)	251 ± 33(3)
Ethanol	205 ± 34(4)	269 ± 51(4)	183 ± 25(4)	154 ± 19(3)‡
Electroshock	183 ± 15(4)	207 ± 19(4)	180 ± 16(4)	270 ± 25(3)
Restraint	199 ± 14(4)	233 ± 7(4)	174 ± 11(4)	248 ± 48(3)

Conditions as in Table 1. Animals were injected with 10 μC [U-^{14}C] glucose 20 min before decapitation.

Results expressed as counts × 10^{-2}/min/g fresh tissue ± M.E.M. (no. of animals).

* $P < 0.001$.
† $P < 0.01$.
‡ $P < 0.05$.

Reserpine produced a considerable decrease (−60%: $P<0.001$) in the uptake of ^{14}C by the heart: this effect of reserpine was not observed in the brain or liver. Of the other compounds tested only ethanol appeared to reduce the uptake of ^{14}C by the heart, but the effect was smaller (−31%: $P<0.05$) than that of reserpine.

Incorporation of ^{14}C from [^{14}C]glucose into the free amino acid fraction

The rate of incorporation of ^{14}C from labelled glucose into amino acids is indicated by data giving (a) the percentage of the total acid-soluble ^{14}C contained in the free amino acids and (b) the relative specific activity of the amino acid fraction at 20 min after injection of [^{14}C]glucose (Table 3). The process was strongly inhibited in liver by nembutal, ethanol and reserpine, whereas only nembutal produced a highly significant (−66%: $P<0.001$) inhibition in the brain. Apart from nembutal, only ether appeared to inhibit this process in the brain: it is of interest to note that reserpine, ethanol and electroshock appeared to inhibit it in the heart.

DISCUSSION

It was previously shown that at 20 min after subcutaneous injection of [^{14}C]glucose, a considerable portion of the ^{14}C retained in the tissues is contained in the acid-soluble amino acids.[2, 5, 6] The data reported here give evidence that certain psychotropic drugs can influence the incorporation of glucose carbon into amino acids in the brain and other organs of the rat. The drugs tested had relatively little effect on the free amino acid concentration in blood, liver, brain or heart.

The action of iproniazid in increasing the free amino acid of blood (Table 1) and the total ^{14}C content (Table 2) of the acid soluble fraction of the tissues may be related to the observation that there was a decreased intake of food after administration of iproniazid under the conditions of these experiments also, and consequdntly an increased break-down of proteins. This results in a fall in the blood glucose level, as in the case of fasted animals.[7] The specific activity of the blood glucose in fasted animals was higher than in non-fasted animals since there was lessdilution of the labelled glucose.

The reduced level of ^{14}C in the heart (Table 2) is due apparently to a specific action of reserpine on the heart. In this connection it is relevant that other workers have reported histological evidence that reserpine exerts a damaging effect on the heart.[9]

The factors determining the rapid incorporation of glucose carbon into amino acids in the brain have been discussed elsewhere.[2] The present observations indicate that this process is specifically inhibited by pentobarbitone (sodium).

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TABLE 3. PERCENTAGE OF TOTAL ^{14}C IN FREE AMINO ACIDS AND RELATIVE SPECIFIC ACTIVITY OF AMINO ACIDS AFTER VARIOUS TREATMENTS

Treatment	Blood		Liver		Brain		Heart	
	(a) % total ^{14}C	(b) Relative specific activity	(a) % total ^{14}C	(b) Relative specific activity	(a) % total ^{14}C	(b) Relative specific activity	(a) % total ^{14}C	(b) Relative specific activity
Control	2·8 ± 0·1(6)	61 ± 9	15·3 ± 1 6(7)	65 ± 11	71·0 ± 9·7(8)	220 ± 11	30·1 ± 4·6(6)	104 ± 12
Reserpine	2·3 ± 0·4(3)	51 ± 7	6·2 ± 1·9(4)†	29 ± 10	53·5 ± 6·8(4)	181 ± 26	11·3 (2)	44
Iproniazid	2·5 ± 0·2(3)	38 ± 1	11·1 ± 2·1(4)	44 ± 6	74·1 ± 5·7(4)	213 ± 11	17·8 ± 3·9(3)	50 ± 16
Chlorpromazine	2·5 ± 0·3(3)	53 ± 4	12·8 ± 0·6(4)	53 ± 3	65·0 ± 0·7(4)	212 ± 15	21·3 ± 4·1(3)	83 ± 20
Pentobarbitone (sodium)	2·6 ± 0·1(3)	47 ± 7	8·6 ± 1·0(4)†	41 ± 3	23·9 ± 0·8(4)*	73 ± 6	18·9 ± 2·9(3)	65 ± 6
Ethylether	2·1 ± 0·4(3)	73 ± 23	10·1 ± 1·8(4)	47 ± 12	42·9 ± 6·8(4)‡	132 ± 30	35·8 ± 1·9(3)	117 ± 13
Ethanol	3·0 ± 0·4(3)	54 ± 2	8·5 ± 1·1(4)†	38 ± 4	61·6 ± 3·9(4)	180 ± 13	16·6 ± 1·3(3)‡	58 ± 2
Electroshock	3·0 ± 0·8(3)	64 ± 19	12·1 ± 2·4(4)	48 ± 6	53·1 ± 3·0(4)	161 ± 14	16·8 ± 0·7(3)‡	60 ± 8
Restraint	2·2 ± 0·3(3)	49 ± 7	11·3 ± 0·9(4)	53 ± 3	64·0 ± 5·0(4)	197 ± 13	27·2 ± 1·5(3)	94 ± 8

Results expressed as (a) percentage of total acid-soluble ^{14}C in the organ incorporated into free amino acids ± M.E.M. (no. of animals) and (b) counts × 100 min/mg α-amino-N per counts/min/g fresh tissue ± M.E.M. Animals were injected with 10 μC [^{14}C] glucose 20 min before decapitation. Conditions as in Table 1.

* $P < 0·001$.
† $P < 0·01$.
‡ $P < 0·05$.