Biochemical Pharmacology, Vol. 20, pp. 2371–2384. Pergamon Press, 1971. Printed in Great Britain

EFFECT OF DIHYDROXYPHENYLALANINE ADMINISTERED WITH A MONOAMINE OXIDASE INHIBITOR ON GLUCOSE METABOLISM IN RAT BRAIN

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(*Received* 5 *October* 1970; *accepted* 4 *January* 1971)

Abstract—Injection of L-3,4-dihydroxyphenylalanine (L-dopa) without and with an inhibitor (β-phenylisopropylhydrazine; JB516) of the enzyme, EC 1.4.3.4 monoamine: oxygen oxidoreductase (deaminating), induced hyperglycemia in fed, but not in fasted, rats. The rates of entry into and of removal of glucose from the glucose space were higher in fed rats treated with L-dopa and JB516 than in control rats. Thus, uptake of glucose by peripheral tissues is increased after treatment with L-dopa and JB516. Concentrations of glucose in brain were higher in rats treated with L-dopa and JB516 than in control rats, but uptakes of glucose-^{14}C by brain were not meaningfully different in controls and treated rats. At 15 min after a pulse injection of glucose-^{14}C, flux of ^{14}C into lactate and alanine in brain was the same in control rats as in rats treated with L-dopa and JB516; the flux of ^{14}C into glutamate, glutamine, aspartate, and γ-aminobutyric acid was markedly decreased in the treated rats.

It is concluded that dopa injected after a monoamine oxidase inhibitor inhibits the metabolism, in brain, of glucose to the tricarboxylic acid cycle intermediates. The accumulation of unmetabolized glucose in brain in the treated rats suggests a second point of inhibition, possibly at the formation of glucose 6-phosphate.

LARGE doses of L-dihydroxyphenylalanine (L-dopa) are used to treat patients with Parkinson's disease. This therapy is based on the premise that the L-dopa is taken up by brain and there converted to dihydroxyphenylethylamine (dopamine). Dopamine is an inhibitory transmitter substance in certain centers in the brain, and its concentration in brains of patients with Parkinson's disease is decreased.

Dopa is a precursor of the catecholamines, hormones which produce increases in blood glucose concentration. Kakimoto *et al.*[1] have shown that the concentration of glycogen decreases and the concentration of lactate increases in brains of mice after giving L-dopa with the inhibitor, β-phenylisopropylhydrazine (JB516) of the enzyme EC 1.4.3.4 monoamine:oxygen oxidoreductase (deaminating) (monoamine oxidase); the concentration of glucose in brain also was increased, but this was attributed to the hyperglycemia which occurred.

Brain is unique among tissues: it uses glucose as its principal source of energy and metabolizes it chiefly to glutamate, aspartate, γ-aminobutyric acid (GABA), and glutamine, compounds in equilibrium with the tricarboxylic acid intermediates.[2] This pattern of metabolism is not fully developed in immature brain[3] and is inhibited in adult brain under a variety of conditions: after hepatectomy,[4–6] during hypoxia,[7] alcohol intoxication,[8] triethyl tin and triethyl lead intoxication,[9] and picrotoxin and pentylenetetrazole seizures.[7]

This report describes the effect of L-dopa given with JB516 on the uptake and metabolism of radioactive glucose by brain in rats. A preliminary report has been presented.[10]

EXPERIMENTAL

Male Sprague–Dawley rats, maintained on a Rockland rat diet and weighing 265–360 g, were used. Rats were either fasted for 18 hr or fed *ad lib.* until the beginning of the experiment. Each rat was anesthetized with ether and its tail vein was cannulated (polyethylene tubing, PE 10) for injections; in some experiments the femoral artery also was cannulated (polyethylene tubing PE 50) so that serial samples of blood could be collected. All rats were placed in restraining cages without food[11] and were infused with isotonic saline at a rate of 1·25 ml/hr. Although the animals recovered rapidly from the brief ether anesthesia, an hour was allowed to elapse before other injections were made.

The dose of L-dopa (Mann Research Laboratories) was 100 mg/kg in all experiments and was injected intraperitoneally as a 2% suspension in a gum acacia gel (5% gum acacia in isotonic saline). The dose of JB516 was 20 mg/kg in all experiments; it was dissolved in saline and also injected intraperitoneally. Control rats received injections of saline or gum acacia.

In studies of the effect of L-dopa and JB516 (separately or in combination) on the concentration of glucose in blood of fed and fasted rats, small samples of blood were collected immediately before and at half-hour intervals after the injection. When both substances were given, JB516 was given 2 hr before L-dopa.

In experiments with glucose-U-^{14}C, the tracer (20 μc in 0·24 mg) was injected intravenously to fed rats 2¼ hr after the JB516 and 15 min after the L-dopa. The infusion of saline was then resumed. In some rats, small samples of blood from the femoral artery were collected at 5, 15 and 30 min after the glucose-^{14}C injection for determination of radioactivity and glucose concentration in plasma and calculation of rates of entry and disappearance of glucose. In other rats, small samples of blood were collected at 2, 5, 10 and 14 min after the glucose-^{14}C injection and then the brain was frozen *in situ* (rats were given 20 mg of sodium pentobarbital 30 sec before and then were dropped head-first into liquid nitrogen) 1 min later (15 min after injection) for determination of uptake and metabolism of glucose. The frozen brains were chiselled out for analysis.

The amount of residual blood in frozen brain was determined in some rats by giving erythrocytes labeled with ^{51}Cr according to the method of Cooper and Owen.[12]

Chemical determinations. The methods used for making extracts of blood and brain have been described in detail.[4,6] Somogyi filtrates were made of plasma. Frozen brains were rapidly weighed and homogenized in ice-cold 0·1 N HCl, and the proteins were immediately precipitated with perchloric acid. Glucose was determined in these filtrates by the hexokinase-glucose dehydrogenase method[13] and lactate, by the lactic acid dehydrogenase method.[14] Concentrations of free amino acids were measured on ion exchange columns in an amino acid analyzer using spherical resins Beckman PA 28 and PA 35 as described by Benson and Patterson.[15] Insulin concentrations in plasma were measured, in a number of experiments, by the double antibody system of Morgan and Lazarow[16] with pork insulin as standard.

Radioactivity measurements. Radioactivity in plasma and in protein-free extracts of blood was measured in a 2:1 mixture of toluene-PPO-DMPOPOP and Triton X 100[17] in a liquid scintillation counter; corrections for quenching were made by using an external standard. Radioactivity in the effluent of the column was measured in a 1-ml flow cell (packed with anthracene) in a liquid scintillation counter before the effluent entered the mixing manifold for reaction with ninhydrin. Radioactivity as glucose and as lactate was measured in the effluent from the amino acid analyzer.

The specific activity of glucose in blood was determined by dividing total radioactivity in blood by the concentration of glucose. The specific activity of blood glucose also was determined by conversion to and isolation of the derivative D-phenylglucosotriazole, by the method of Hann and Hudson[18] and Steele *et al.*,[19] and counting this in the liquid scintillation counter. The specific activities of glucose and lactate in brain were determined by dividing total ^{14}C in each radioactive peak from the column by the concentration measured by enzymatic procedures in separate aliquots of the same brain. The specific activities of amino acids were determined by dividing total ^{14}C in each peak by the concentration of the amino acid measured by the ninhydrin reaction.

Calculations. Rates of entry and disappearance of glucose into and from the glucose space for the interval between 5 and 30 min after the pulse injection of glucose-^{14}C were calculated by the method of Dunn *et al.*[20] from changes in glucose concentration and specific activity of glucose in plasma.

Total ^{14}C in brain at 15 min after the pulse injection of glucose-^{14}C was expressed as a function of the average specific activity of glucose in plasma to obtain the plasma glucose equivalent in brain. The flux of ^{14}C from glucose in plasma to amino acids in brain was measured by expressing ^{14}C in these metabolites as a function of the average specific activity of glucose in plasma, as described by Roberts *et al.*[21] The plasma glucose equivalent in these compounds was thus obtained.

Brain glucose and brain glutamate equivalents in metabolites in brain were also calculated; the radioactivity (dis./min/g) in each metabolite was expressed as a function of the specific activity (dis./min/μmole) of the precursor.

RESULTS

Physiologic changes. When L-dopa was administered to rats 2 hr after JB516, the animals became irritable and showed increased locomotor activity, a fast respiration, piloerection, frequent urination and seminal ejaculation.

Effect on blood volume. At 30 min after injection of L-dopa and 2½ hr after JB516, the mean blood volume was 4·63 per cent of the body weight (S.E. = 0·15; $N = 9$) compared to 5·72 per cent in control rats (S.E. = 0·21; $N = 8$). This difference was statistically significant ($P < 0{\cdot}001$) and reflected somewhat higher hematocrit values in the treated rats (48·3 per cent [S.E. = 0·6; $N = 4$] in treated rats and 39·3 per cent [S.E. = 0·4; $N = 5$] in control rats). In four rats, at 2 hr after JB516 (no L-dopa) the mean hematocrit value was 42·6 per cent (S.E. = 0·7).

Effect on concentrations of glucose and insulin in plasma. During the 3 hr after the injection of L-dopa (without JB516) in fed rats the concentration of glucose in blood increased by about 50 per cent (Fig. 1). The injection of JB516 (without L-dopa) did not change blood glucose concentrations in four of six fed rats; however, two rats

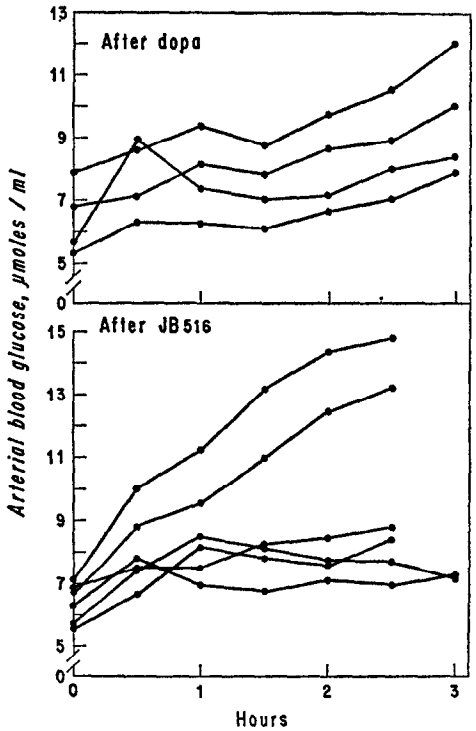

FIG. 1. Concentration of glucose in femoral arterial blood of individual fed rats at different times after intraperitoneal injection of L-dopa (100 mg/kg) or JB516 (20 mg/kg).

showed considerable increases (Fig. 1). When dopa and JB516 were given together to eight fed rats, blood glucose concentrations increased sharply in seven rats in the first 30 min (Fig. 2). This increase was followed by a decrease between 1½ and 3 hr; in three rats this decrease was to hypoglycemic levels. The amounts of insulin in the plasma were decreased slightly even when blood concentrations were high.

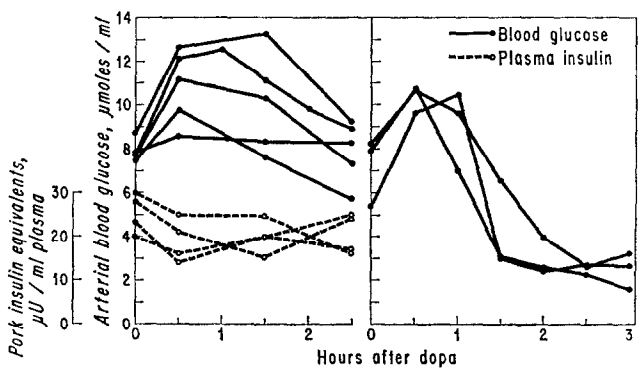

FIG. 2. Concentrations of glucose in femoral arterial blood and of insulin in plasma of fed rats at different times after intraperitoneal injection of L-dopa (100 mg/kg) (JB516, 20 mg/kg, was injected 2 hr before time 0). Data separated at right are from three rats which showed frank hypoglycemia at about 2 hr after the L-dopa injection.

Similar administration of L-dopa with JB516 to four fasted rats failed to increase blood glucose concentration; in one rat, blood glucose concentration decreased (Fig. 3).

All further experiments on disappearance of glucose-^{14}C from plasma and uptake and metabolism of glucose-^{14}C by brain were done on fed rats during the first 30 min after L-dopa was given (JB516 having been given 2 hr before the L-dopa). During these 30 min, plasma glucose concentrations were consistently increased (Fig. 2); after this time unpredictable changes in glucose concentration occurred which would have made interpretation of the results difficult.

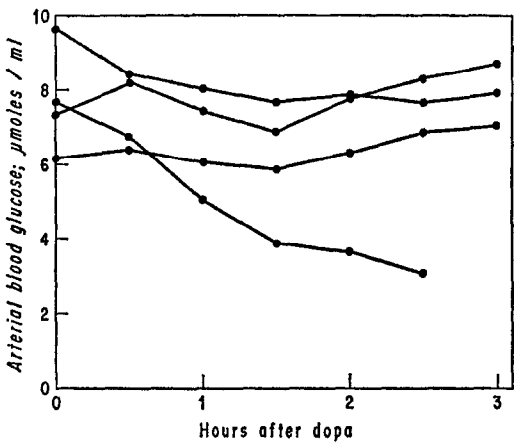

FIG. 3. Concentrations of glucose in femoral arterial blood of fasted rats at different times after injection of L-dopa (100 mg/kg) preceded, by 2 hr, by injection of JB516 (20 mg/kg).

Concentration of glucose in plasma (Table 1) at 14 min after glucose-^{14}C injection and after small samples of blood were taken at 2, 5 and 10 min was somewhat higher than the concentration of glucose in blood (Fig. 2) found when only 1 sample of blood was taken (assuming that concentration of glucose in plasma = 1·4 × concentration of glucose in blood).[22] Similar small steady increases in blood glucose had been observed previously[8] when several small samples of blood were collected from polyethylene tubing in the femoral artery with minimal disturbance to the rat.

Radioactive compounds in plasma. At 15 min after the injection of glucose-^{14}C to three rats treated with L-dopa and JB516, column chromatograms showed that 94·5, 95·6 and 94·1 per cent of the radioactivity in plasma was glucose-^{14}C and 3·9, 4·5 and 5·2 per cent was lactate-^{14}C. An aliquot of the glucose peak from a column chromatogram of an extract of plasma of one of these rats was converted to the D-phenylglucosotriazole; the specific activity of this derivative was 98 per cent of that obtained by dividing total ^{14}C in the glucose peak in the effluent from the column by the concentration of glucose in plasma (determined enzymatically). Therefore, the glucose peak from the column was indeed only glucose and most of the radioactivity in blood was in glucose. In control rats, percentages of total ^{14}C in plasma as glucose-^{14}C (95·1 and 94·7 per cent) and as lactate-^{14}C (3·6 and 4·2 per cent) were similar to those found in the treated rats.

TABLE 1. PLASMA GLUCOSE EQUIVALENTS IN BRAIN

Parameter	Control rats $N=8$ Mean	S.E.	Treated rats* $N=8$ Mean	S.E.	P
1. Plasma glucose (μmoles/ml)	9·79	1·07	17·90	1·31	<0·001
2. Brain glucose (μmoles/g)	0·97	0·07	2·89	0·30	<0·001
Sp.act. of plasma glucose (10^3 dis./min/μmole)					
3. At 14 min	41·11	2·40	23·80	2·24	<0·001
4. Average, 0–14 min†	59·48	3·91	42·67	3·50	<0·01
5. Total brain ^{14}C (10^3 dis./min/g) at 15 min	359·9	20·6	230·0	11·1	<0·001
6. Plasma glucose equivalents in brain at 15 min (5 ÷ 4)‡	6·10	0·27	5·51	0·31	NS
For lactate + alanine					
7. Per cent of total ^{14}C in brain at 15 min	21·2	0·8	19·6	0·8	NS
8. Plasma glucose equivalents (μmoles/g) (6 × 7)‡	1·29	0·06	1·19	0·06	NS
For amino acids except alanine					
9. Per cent of total ^{14}C in brain at 15 min	59·9	1·1	38·5	2·7	<0·001
10. Plasma glucose equivalents (μmoles/g) (6 × 9)‡	3·65	0·02	2·08	0·11	<0.001

* L-dopa and JB516; see text.

† The average specific activity of glucose in plasma from 0 to 14 min was calculated from the mean of values read at 1·25-min intervals from the curves of specific activity of glucose in plasma for each rat. The factor used to convert specific activity of plasma glucose at 14 min to the average specific activity from 0 to 14 min was 1·45 (S.E. = 0·04) in control rats and 1·81 (S.E. = 0·05) in treated rats.

‡ Means of values calculated for individual rats.

Rate of disappearance of glucose-U-^{14}C from plasma. In both control rats and rats given L-dopa and JB516, after the injection of glucose-^{14}C, radioactivity disappeared from plasma rapidly for 5 min and then more slowly for the next 9 min (Fig. 4). More radioactivity was present in plasma of treated rats than of control rats until 14 min after the glucose-^{14}C was given. The specific activity of glucose in plasma was lower in treated rats than in control rats after the pulse injection of glucose-^{14}C, due to the higher concentrations of plasma glucose in the former group (Table 1). The half-life of plasma glucose (calculated from the slope of the line of best fit for log [specific activity of blood glucose] vs. time for 5–14 min) was 16·9 min in control rats and 12·4 min in the treated rats.

Rates of entry and disappearance of glucose into and from glucose pool. Between 5 and 30 min after glucose-^{14}C injection, the rates were much greater in rats treated previously with L-dopa (15 min before the glucose-^{14}C) and with JB516 than in control rats (Table 2).

Concentrations of glucose, lactate, and ninhydrin-reacting substances in brain. Brain glucose concentrations were higher in rats treated with L-dopa and JB516 than in control rats (Table 1). The amount of residual blood in brain was not different in the two groups (treated rats, 2·53 per cent, S.E. = 0·10, $N = 9$; control rats, 2·65 per cent, S.E. = 0·14, $N = 8$). The amount of glucose in brain that could be attributed to the glucose in plasma was 0·18 μmole/g in control rats and 0·32 μmole/g in treated

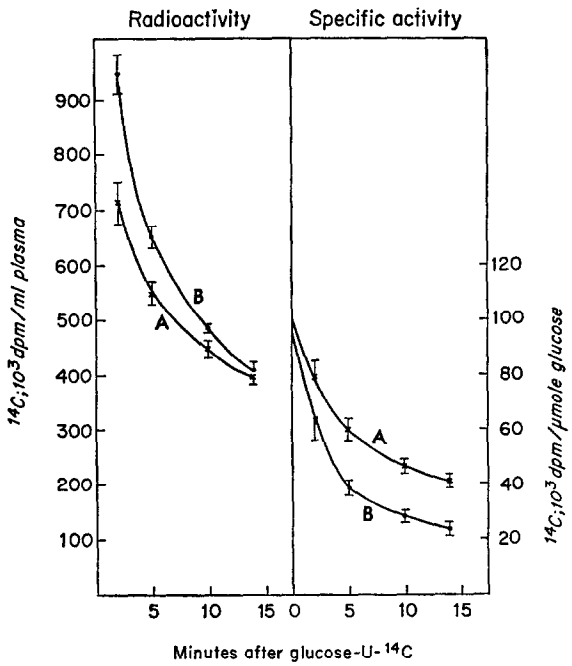

FIG. 4. Left: Total radioactivity in plasma from femoral arterial blood during 14 min after pulse injection of 20 μc of glucose-U-^{14}C into tail vein of control rats (A) and of treated rats (B) (given L-dopa [100 mg/kg] and JB516 [20 mg/kg] 15 min and 2½ hr previously, respectively). Means and standard errors of eight determinations are shown. Right: Corresponding specific activity of plasma glucose.

TABLE 2. RATES OF ENTRY AND DISAPPEARANCE OF GLUCOSE INTO AND FROM GLUCOSE POOL BETWEEN 5 AND 30 min AFTER GLUCOSE-^{14}C INJECTION

Parameter	Control rats $N = 4$		Treated rats* $N = 4$		P
	Mean	S.E.	Mean	S.E.	
Plasma glucose (μmoles/ml)					
At 5 min†	8·44	0·56	17·80	0·92	<0·001
At 30 min†	8·36	0·36	15·40	1·27	<0·001
Specific activity of plasma glucose (10^3 dis./min/μmole)					
At 5 min†	48·8	2·2	31·2	1·9	<0·001
At 30 min†	23·8	1·3	11·1	1·6	<0·001
Rate of entry (μmoles/100 g/min)‡	6·11	0·52	17·54	1·80	<0·001
Rate of removal (μmoles/100 g/min)‡	6·19	0·61	19·94	1·83	<0·001

* L-dopa and JB516; see text.

† Time after glucose-^{14}C was given.

‡ After equations of Dunn *et al.*:[5,20] rate of entry $= R_1 = [(B_0 - B_t) \log (i_0/i_t)] \div t \log (B_0/B_t)$ and rate of disappearance $= R_2 = [(B_0 - B_t)/t] + R_1$, in which B = glucose concentration in total body glucose space (= plasma glucose concentration × 25 on basis of glucose space of 25 ml/100 g body weight), i = specific activity of glucose in plasma, and subscripts "o" and "t" indicate values at initial and final times of the experimental period—5 and 30 min after glucose-^{14}C injection.

rats (using the factor 1/1·4 to convert plasma glucose to blood glucose).[22] In treated rats, the concentrations of lactate, alanine, and GABA in brain increased and the concentration of aspartate decreased (Table 3). The concentrations of the other ninhydrin-reacting substances measured did not change.

Radioactivity in brain. Although the separations were on cation exchange columns, distinct peaks of radioactivity were obtained for glucose and lactate[5,23,24] as well as for a number of amino acids (aspartate, glutamine, glutamate, alanine and GABA) in extracts of brain at 15 min after glucose-^{14}C injection. Very small amounts of ^{14}C

TABLE 3. CONCENTRATIONS (μmoles/g) OF AMINO ACIDS AND LACTATE IN BRAIN

Substance	Control rats $N = 8$ Mean	S.E.	Treated rats* $N = 8$ Mean	S.E.	P
Lactate	4·66	0·35	7·47	1·33	<0·05
Alanine	0·34	0·02	0·47	0·04	<0·01
Glutamate	10·56	0·24	10·80	0·24	NS
Glutamine	5·76	0·19	6·14	0·10	NS
Aspartate	2·70	0·06	2·35	0·09	<0·01
GABA	1·51	0·05	1·66	0·02	<0·05
Glycine	0·91	0·05	0·93	0·06	NS
Serine	0·85	0·02	0·79	0·04	NS
Threonine	0·54	0·02	0·58	0·03	NS
Taurine	4·53	0·37	4·87	0·31	NS

* L-dopa and JB516; see text.

were found in an unknown substance which emerged before glucose,[23] and in glycine, serine and urea. Recoveries in these metabolites, calculated by collecting the effluent in a fraction collector and measuring ^{14}C in aliquots of each effluent, have previously been shown in our laboratory to average 99·1 per cent (S.E. = 0·4) in 66 experiments.[4] At 15 min after the injection of glucose-^{14}C, it accounted for only 12·5 per cent of the total radioactivity in brain of control rats (Table 4). Glutamate-^{14}C was the most abundant radioactive metabolite in brain, and lactate was the next most abundant. Glutamine and aspartate accounted for almost equal amounts of radioactivity; smaller amounts of radioactivity were present as GABA-^{14}C and alanine-^{14}C. The total ^{14}C in brain was less in treated rats than in control rats. More ^{14}C was present as unmetabolized glucose but smaller percentages were found in all the metabolites of glucose except lactate and alanine.

At 15 min after injection of glucose-^{14}C, the specific activity ratios of brain glucose to plasma glucose were about 1 in control rats and in rats treated with L-dopa and JB516 (Table 5). In the treated rats, significant decreases were found in the ratios of specific activities of glutamate–glucose and GABA–glutamate, and there was an increase in the aspartate–glutamate ratio.

Glucose uptake and metabolism in brain. At 15 min after the injection of glucose-^{14}C, the total radioactivity in brain was less in rats treated with L-dopa and JB516 than in control rats (Table 1). However, the plasma glucose equivalents were not significantly different in the two groups of rats. Also the plasma glucose equivalent in the fraction

TABLE 4. RADIOACTIVITY IN BRAIN

Substance	^{14}C (10^3 dis./min/g)					Per cent of total ^{14}C in brain				
	Control rats $N = 8$		Treated rats* $N = 8$			Control rats $N = 8$		Treated rats* $N = 8$		
	Mean	S.E.	Mean	S.E.	P	Mean	S.E.	Mean	S.E.	P
Total	359·9	20·6	230·0	11·1	< 0·001					
Glucose	44·5	2·6	68·7	5·2	< 0·001	12·5	0·8	30·5	2·7	< 0·001
Lactate	70·0	4·0	50·4	6·9	< 0·05	19·6	0·8	21·9	2·6	NS
Alanine	6·1	0·3	4·2	0·6	< 0·01	1·7	0·02	1·8	0·2	NS
Glutamate	130·5	9·4	53·8	6·4	< 0·001	36·1	0·7	23·2	1·8	< 0·001
GABA	17·9	1·4	6·9	0·9	< 0·001	4·9	0·2	3·0	0·2	< 0·001
Glutamine	35·0	2·8	15·9	1·4	< 0·001	9·6	0·3	6·9	0·4	< 0·001
Aspartate	31·5	2·3	12·5	1·5	< 0·001	8·7	0·2	5·4	0·4	< 0·001

* L-dopa and JB516; see text.

TABLE 5. RATIO OF SPECIFIC ACTIVITIES (dis./min/μmole) IN BRAIN

Ratios	Control rats $N = 8$ Mean	S.E.	Treated rats* $N = 8$ Mean	S.E.	P
Brain glucose–plasma glucose	1·15	0·07	1·07	0·06	NS
Brain lactate–brain glucose	0·34	0·04	0·28	0·01	NS
Brain alanine–brain glucose	0·40	0·03	0·36	0·02	NS
Brain glutamate–brain glucose	0·27	0·02	0·20	0·01	<0·01
Brain glutamine–brain glutamate	0·49	0·02	0·53	0·02	NS
Brain aspartate–brain glutamate	0·94	0·02	1·07	0·03	<0·01
Brain GABA–brain glutamate	0·96	0·03	0·84	0·05	<0·05

* L-dopa and JB516; see text.

actate + alanine in treated rats was not different from that in control rats. However, the plasma glucose equivalent in the amino acids derived from the tricarboxylic acid cycle intermediates was significantly less in the treated rats than in control rats.

Because the radioactivity in brain increases during the first 15 min after injection of glucose-^{14}C,[4,6] we divided the total brain ^{14}C at 15 min by the average specific activity of plasma glucose during that time to obtain the plasma glucose equivalents in the above calculations. Flux of ^{14}C from glucose in plasma to metabolites in brain (as observed at 15 min after glucose-^{14}C was given) also was calculated in relation to the specific activity of glucose in plasma at 2 min after injection of glucose-^{14}C (Table 6). During the first 3 min after glucose-^{14}C injection, specific activity in glucose would be very high and would be decreasing rapidly. Total plasma glucose equivalents in brain were slightly less in the treated rats than in controls. Plasma glucose equivalents in lactate + alanine were not different, but plasma glucose equivalents in amino acids derived from the tricarboxylic acid cycle were significantly less in the treated rats than in control rats.

Brain glucose equivalents and brain glutamate equivalents in brain. Brain glucose equivalents in lactate and in alanine were the same in controls and treated rats; brain glucose equivalents in all other metabolites, however, were significantly less in treated rats (Table 7).

TABLE 6. PLASMA GLUCOSE EQUIVALENTS IN BRAIN IN RELATION TO SPECIFIC ACTIVITY OF GLUCOSE IN PLASMA AT 2 min AFTER GLUCOSE -U-^{14}C INJECTION*

Parameters	Control rats $N = 8$ Mean	S.E.	Treated rats $N = 8$ Mean	S.E.	P
1. Sp.act. of plasma glucose (10^3 dis./min/μmole) at 2 min	79·41	5·93	62·91	4·22	<0·05
2. Total brain ^{14}C (10^3 dis./min/g) at 15 min	359·9	20·6	230·0	11·1	<0·001
3. Plasma glucose equivalents in brain at 15 min (2 ÷ 1)	4·60	0·25	3·72	0·21	<0·05
Plasma glucose equivalents in brain					
4. For lactate + alanine	0·97	0·05	0·90	0·16	NS
5. For other amino acids except alanine	2·75	0·14	1·42	0·12	<0·001

* Plasma glucose equivalents in metabolites in brain were calculated as in Table 1.

TABLE 7. GLUCOSE AND GLUTAMATE EQUIVALENTS IN METABOLITES IN BRAIN

Metabolite	Brain glucose equivalent (μmoles/g)					Brain glutamate equivalent (μmoles/g)				
	Control rats $N = 8$		Treated rats* $N = 8$			Control rats $N = 8$		Treated rats* $N = 8$		
	Mean	S.E.	Mean	S.E.	P	Mean	S.E.	Mean	S.E.	P
Lactate	1·53	0·12	2·07	0·30	NS					
Alanine	0·13	0·01	0·17	0·02	NS					
Glutamate	2·81	0·15	2·13	0·09	< 0·001					
GABA	0·38	0·02	0·27	0·01	< 0·001	1·45	0·07	1·39	0·09	NS
Glutamine	0·74	0·04	0·64	0·02	< 0·05	2·83	0·12	3·24	0.12	< 0·05
Aspartate	0·67	0·03	0·49	0·02	< 0·001	2·55	0·09	2·49	0·06	NS

* L-dopa and JB516; see text.

Brain glutamate equivalent in glutamine was higher in the treated rats than in controls. Brain glutamate equivalents in GABA and in aspartate were not different in the two groups.

DISCUSSION

Some of the physiologic effects of giving L-dopa together with JB516 noted in these experiments are similar to those described previously by Ernst[25] after giving dopa and N^1-isonicotinoyl-N^2-isopropylhydrazine (Iproniazid).

The hyperglycemic action of L-dopa and JB516 observed in our rats is similar to that noted previously by Håkanson *et al.*[26] in mice and by Hayashi *et al.*[27] in rabbits. Both groups of investigators concluded that the hyperglycemia was caused by dopamine, because no changes in blood glucose occurred if L-dopa was given with an inhibitor of the enzyme EC 4.1.1.26 3,4-dihydroxy-L-phenylalanine carboxy-lyase. An inhibition of release of insulin by dopamine was postulated[26] to be responsible for the hyperglycemia. We found that insulin was not released in the rats treated with L-dopa and JB516 even when blood glucose concentrations were increased. Giving L-dopa with JB516 failed to increase blood glucose concentration in fasted rats, and this suggests that increased glycogenolysis contributed to the hyperglycemia.

During the 15 min after a pulse injection of glucose-^{14}C, virtually all the ^{14}C in plasma was in glucose in control rats and in rats treated with L-dopa and JB516. This has been found to be the case in many conditions in rats in our laboratory.[5,6,23,28] Berson *et al.*[29] also have shown that all ^{14}C in plasma was glucose-^{14}C for 90 min after glucose-^{14}C injection in rabbits.

The plasma glucose equivalent in brain provided an index of uptake of glucose by brain. Because some glucose-^{14}C was metabolized to $^{14}CO_2$, which could not be measured, this index was not a precise one. After L-dopa and JB516 were given, uptake of glucose by brain, as measured by the plasma glucose equivalent in brain, was similar to normal. This suggests that brain differs from peripheral tissues, because rate of exit of glucose from the glucose space was increased after L-dopa and JB516, indicating an increased uptake by peripheral tissues.

It is well known that the determination of glucose in brain is fraught with difficulties. Bachelard[30] contends that most of the glucose in brain can be accounted for by glucose in the residual blood in brain, in cerebrospinal fluid, or in extracellular fluid in brain; true intracellular glucose may approach zero. On the other hand, Lowry *et al.*[31] contend that glucose concentrations *in vivo* may be considerably higher than the reported values suggest because rapid losses of glucose occur within seconds during the removal and extraction of brain. The increase in brain glucose found in this study probably is correct because proper precautions were taken for rapid freezing of the brains, the amounts of residual blood in brain were not different in the two groups of rats, and most significantly the ratio of brain glucose concentration to plasma glucose concentration was increased in the treated rats.

In the present investigation, the flux of ^{14}C calculated in terms of the average specific activity of the precursor in plasma or the specific activity at 2 min after glucose-^{14}C injection clearly shows that metabolism of glucose in brain is altered by treatment with L-dopa and JB516. Metabolism of glucose to lactate was not altered,

but there was a decrease in the metabolism of glucose to intermediates (amino acids) derived from the tricarboxylic acid cycle. The accumulation of glucose-^{14}C in brain after the treatment suggests a second point of inhibition, possibly at the formation of glucose-6-phosphate. Also, when flux of ^{14}C was calculated in terms of the specific activity of brain glucose, similar disturbances in glucose metabolism were noted. This indicated that the smaller amounts of radioactivity in metabolites in brain could not be merely the result of dilution of glucose-^{14}C from plasma in the large pool of glucose in the brains of the treated rats.

The mechanism whereby L-dopa and JB516 exert their effect on glucose metabolism in brain is not clear. It is well documented that compartmentation of tricarboxylic acid intermediates and metabolites occurs in brain,[32] and it could be that these drugs alter the accessibility or the size of these pools. It is also possible that large amounts of dopa in brain would compete for cofactors (particularly pyridoxal phosphate), or that transamination of dopa in brain could deplete the Krebs cycle of normal intermediates. However, this seems unlikely because Kakimoto *et al.*[1] found the concentration of dopa in brain to be only about 1 μg/g and the concentrations of the metabolites dopamine and norepinephrine to increase by only 1–5 μg/g at 0–60 min after giving mice L-dopa and JB516 in the same doses used in the present experiments. In the present study, on analysis of brain, barely detectable amounts of dopa were found in the effluent from the amino acid columns, amounts insignificant in comparison to the concentrations of the more abundant amino acids in brain. Most of the dopa given to animals undergoes rapid metabolism by liver even when an amine oxidase inhibitor is given,[33] and only small amounts are available to the brain.

High concentrations of other aromatic amino acids (phenylalanine and tryptophan) added to minces of rat brain incubated with glucose-^{14}C have previously been shown to inhibit $^{14}CO_2$ production, O_2 utilization and incorporation of ^{14}C into amino acids.[34] Giving phenylalanine at 1 mg/g to 10-day-old rats has been shown to decrease the concentrations in brain of the nonessential amino acids, glutamic acid, aspartic acid, alanine and serine.[35]

Hochstein and Cohen[36] previously demonstrated that in cell-free preparations of brain (but not of liver or of melanoma) the formation of lactic acid from glucose was inhibited in the presence of certain catechols, including 2,5-dopa and 3,4-dopamine, in concentrations of 50 μg/ml of the incubation medium. Barondes[37] has shown that dopamine, norepinephrine, and other amines stimulated the production of $^{14}CO_2$ from glucose-1-^{14}C but not from glucose-6-^{14}C in rat brain slices. This stimulation of the hexose monophosphate shunt was abolished if a monoamine oxidase inhibitor was given together with the amine, and the aldehyde was considered to be the active compound. However, the hexose monophosphate shunt is probably not a major pathway for glucose metabolism in brain *in vivo*.[38]

Theoretically, if a monoamine oxidase inhibitor were given with L-dopa to patients with Parkinson's disease, it would be possible to conserve the dopamine formed in brain, and thus a smaller dose of L-dopa would then be feasible. However, giving the two drugs together has been shown to induce hypertensive attacks,[39] and the present experiments indicate that the combination of L-dopa with a monoamine oxidase inhibitor also seriously changes the metabolism of glucose in brain.

Further experiments are in progress to determine the effects of large doses of L-dopa and chronic L-dopa administration (without giving a monoamine oxidase inhibitor) on

glucose-^{14}C metabolism in brain. It would also be of interest to study the effect of JB516 and other monoamine oxidase inhibitors (without giving L-dopa) on glucose metabolism. The possibility must be considered that the effect of JB516 on glucose metabolism is not related to its properties as an inhibitor of monoamine oxidase.

Acknowledgements—The author thanks Drs. E. V. Flock and C. A. Owen, Jr., for advice and criticism and Dr. G. D. Molnar for carrying out the insulin assays. The investigation was supported in part by grant NS-9143 from the National Institutes of Health, Public Health Service, and by a donation from Mrs. Charles S. Lips.

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