Asian Journal of Chemistry Vol. 5, No. 3 (1993), 612–616

Effect of L-Glutamate, L-Glutamine and γ-Aminobutyric Acid on Glucose Metabolism in Cerebral Cortical Slices of Albino Rats

P. VISWESWARAN†, V.S.S. AZAD‡,
S.P. SINGH, B.K. AMBASTA and B.P. VERMA*
Department of Chemistry
Magadh University
Bodh Gaya-824 234, India

Addition of L-glutamate decreases the C^{14} incorporation of U-C^{14} glucose into glycogen and carbon dioxide by cerebral cortical slices of normal rats and much more markedly by slices of diabetic rats. A similar effect could be demonstrated by the administration of glutamate intraperitonially. However, glutamine and γ-amino butyric acid which are closely related to glutamate do not have any effect under identical conditions both *in vivo* and *in vitro*.

INTRODUCTION

A marked resynthesis of glycogen was demonstrated with glucose as substrate by Lebaron[1] in cerebral cortical slices of guinea pigs and in rats by Brasannan *et al.*[2] Addition of 0.2U insulin to the incubation medium increases the C^{14} incorporation from U-C^{14} glucose[3] and the treatment of growth hormone increases the incorporation of U-C^{14} glucose[4] into glycogen and CO_2. However, addition of growth hormone does not produce any such effects. During deprivation of glucose, brain can take up non-carbohydrates as fuel. In conditions like fluroacetate poisoning and insulin induced hypoglycemia (due to lack of glucose) glutamic acid and γ-amino butyric acid are found to act[5]. Weil-Malherbe[5] further showed that L-glutamic acid is the only suitable amino acid that can replace glucose in oxidative metabolism of incubated slices. Hence attempts are made to study the effect of L-glutamate and related compounds on glucose metabolism in cerebral cortical slices of rats.

EXPERIMENTAL

The care and maintenance of albino rats and the preparation of slices and their incubation were as previously described by Prasannan *et al.*[6] The C^{14} from U-C^{14} glucose was assayed by the procedure given earlier by Visweswaran *et al.*[7]

†Department of Biochemistry,A. N. Magadh Medical College, Gaya, Bihar-823 001,India.
‡Department of Zoology, M.P. College, Mohania (Rohtas), India.

Normal albino rats weighing 150 gm were selected for this experiment and used in the fed state. L-Glutamic acid, L-glutamine and γ-amino butyric acid (each 50 mg) were given intraperitonially $3\frac{1}{2}$ hrs before killing. Rats were rendered diabetic by administering alloxan monohydrate 15 mg/100 gm body weight intraperitonially and those showing a blood sugar of 300-700 mg/100 ml of blood were used for this experiment. Bovine growth hormone 500 μg in saline (pH 7.4) was given intravenously to one group of normal rats and diabetic rats 6 hrs before killing.

RESULTS AND DISCUSSION

On treatment with L-glutamate $3\frac{1}{2}$ hrs before killing there is a marked depression of C^{14} from U–C^{14}glucose into glycogen and 50% into carbon dioxide. The results obtained in above experiments are given in Table 1 ($P < 0.001$). However, L-glutamine and γ-amino butyric acid do not have any such effect on glycogenesis. Hence the depressive effect of L-glutamate on glycogenesis is highly significant. In another series of experiments cerebral cortical slices of normal rats were incubated in a medium containing U–C^{14} glucose. To one of them L-glutamate or L-glutamine or γ-amino butyric acid (10 mμ each) was added. L-glutamate depresses significantly C^{14} incorporation by 50% both in glycogen and carbon dioxide. The other compounds do not have any effect on the glucose oxidation and glycogenesis. Thus L-glutamate effect is uniform both *in vivo* and *in vitro* [Tables 1 and 2 ($P < 0.001$)].

The above *in vitro* experiments were repeated after growth hormone treatment in normal and diabetic rats [Table 3 ($P < 0.001$)]. The depressive effect is dramatically shown in diabetic animals and uniformly shown in normal and hormone-treated animals. Addition of 0.2 U insulin could not annul the L-glutamate effect, thereby demonstrating the insulin effect obtained earlier [Table 4 ($P < 0.001$)]. In another series of experiments C^{14} from L-glutamate (U–C^{14}) was assayed and C^{14} from glutamate into glycogen was enhanced by addition of inactive glucose to the medium ($P < 0.001$) and not into CO_2 [Table 5].

A fall in ATP concentration in the presence of glutamate and diminished respiration in guinea pig cerebral cortex was reported by *Takagaki et al.*[8] Rolleston *et al.*[9] showed that on adding L-glutamate there is a decrease in concentration of glucose-6-phosphate, fructose-1,6-diphosphate and phosphoenol pyruvate in brain slices. It is possible that the diminished oxidation of glucose in the presence of L-glutamate observed in this study is related to the above findings.

The entry of K^+ into cerebral cortical slices is increased in presence of L-glutamate[10, 11] suggesting a glycolytic block when L-glutamate is added to glucose in cerebral cortical slices. Stern *et al.*[12] observed increased entry of K^+ with L-glutamate in brain slices. Kleinzeller *et al.*[13] reported that increased K^+ depletes glycogenesis in incubated cortical slices. Roister[14] reported that while L-glutamate causes increased entry of K^+ into brain cell, glutamine and γ-amino

butyric acid failed to show such effects. In these experiments it is possible that addition of L-glutamate to the slices was accelerating the entry of K^+ into cerebral cortical slices which might be depressing glycogenesis and oxidation. It is possible that L-glutamate may be playing a role in diminishing the permeability of glucose into brain cell or increasing the entry of K^+ and water into the slice which may explain the above *L-glutamate effect.*

TABLE 1

PRIOR ADMINISTRATION OF L-GLUTAMATE/L-GLUTAMINE/γ-AMINO BUTYRIC ACID (50 mg) EACH $3\frac{1}{2}$ HRS BEFORE SACRIFICE ON C^{14} INCORPORATION FROM (U-C^{14}) GLUCOSE INTO GLYCOGEN AND CARBON DIOXIDE BY CEREBRAL CORTICAL SLICES OF NORMAL RATS

Compound administered	Incorporation of C^{14} counts/min/g wet weight. of slice	
	Glycogen	Carbon dioxide
1. Normal (5)	9500 ± 608*	359,520 ± 18460
2. L-glutamate sodium salt 50 mg (6)	5461 ± 350	139,116 ± 4670
3. L-glutamine 50 mg (5)	8380 ± 408	134,144 ± 6160
4. γ-amino butyric acid 50 mg (5)	10,999 ± 508	130,932 ± 10470

*SEM P < 0.001 42% diminution in glycogen Mean ± SE

Cerebral cortical slices about (50 mg) were incubated in 3.5 ml of medium containing [U-C^{14}] glucose (10 mM). The total activity added to the medium was 1,688,000 counts/min Gas phase: oxygen. Temp. 38°C. Time 2 hrs. For other details see text.

TABLE 2

EFFECT OF ADDING L-GLUTAMATE (10 mM) OR L-GLUTAMINE OR γ-AMINO BUTYRIC ACID TO THE INCUBATION MEDIUM ON THE INCORPORATION OF C^{14} FROM [U-C^{14}] GLUCOSE INTO GLYCOGEN AND CARBON DIOXIDE BY CEREBRAL CORTICAL SLICES OF NORMAL RATS

Condition	Incorporation of C^{14} counts/min/g wet weight of slice	
	Glycogen	Carbon dioxide
1. [U-C^{14}] glucose (10 mM) (5)	9500 ± 608*	359,520 ± 1846
2. + (10 mM) L-glutamate (5)	4422 ± 503	169,224 ± 3532
3. + (10 mM) L-glutamine (5)	9265 ± 291	326,260 ± 26,000
4. + (10 mM) γ-amino butyric acid	7941 ± 385	331,870 ± 15,740

*Mean ± S.E. Values Col. 1 & 2 P < 0.001. 50% diminution.

Cerebral cortical slices (50 mg) were incubated in 3.5 ml of medium containing (U-C^{14}) glucose. The total activity added was 1,688,000 counts/min. To one of the slices L-glutamate or L-glutamine or γ-amino butyric acid was added. Gas phase : Oxygen. Time 2 hrs. Temp. 38°C.

TABLE 3

EFFECT OF ADDING L-GLUTAMATE (10 mM) TO THE INCUBATION MEDIUM C^{14} INCORPORATION FROM U-C^{14} GLUCOSE INTO GLYCOGEN AND CARBON DIOXIDE BY CEREBRAL CORTICAL SLICES OF DIABETIC RATS, DIABETIC RATS TREATED WITH GROWTH HORMONE (GH) AND GROWTH HORMONE TREATED RATS

	GH treated rats 6 hrs.		Diabetic rats treated with GH, 6 hrs.		Diabetic rats	
	U-C^{14} glucose	+L-glutamate	U-C^{14} glucose	L-glutamate	U-C^{14} glucose	+L-glutamate
1. Glycogen†	25,260 ±1256 (5)	6655 ±827 (5)	12,208 ±485 (5)	2783 ±184 (5)	6237 ±1023 (5)	1208 ±112 (5)
2. Carbon dioxide†	303,005 ±14,830	186,448 ±31,050	113,850 ±6508	63,134 ±4256	110,618 ±7464	69,640 ±5741

Mean ± S.E. $P < 0.001$ in all cases.

†Incorporation of C^{14} expressed in counts/min/g slice wt. Cerebral cortical slices (about 50 mg) were incubated in 3.5 ml of medium containing (U-C^{14}) glucose with a total activity of 1,688,000 counts/min for 2 hrs. Temp. 38°C. Gas phase: Oxygen. To one of the slices L-glutamate (10 mM) was added.

TABLE 4

EFFECT OF ADDING 0.2 U OF INSULIN TO THE INCUBATION MEDIUM ON THE INCORPORATION OF C^{14} FROM U-C^{14} GLUCOSE INTO GLYCOGEN AND CO_2 BY CEREBRAL CORTICAL SLICES

(In the experimental slice in addition to glucose + insulin l-glutamate 10 mM was added)

Condition	Incorporation of C^{14} counts/min/g wet wts. of slice.	
	Glycogen	Carbon dioxide
1. Control: U-C^{14} glucose (10 mM) + 0.2 U insulin	18,840 ± 1230 (5)	490,366 ± 15,960 (5)
2. Experimental: U-C^{14} glucose (10 mM) + 0.2 U of insulin + L-glutamate (10 mM)	6270 ± 483	311,166 ± 20,940
P value	$P < 0.001$	$P < 0.01$

Mean ± S.E.

Cerebral cortical slices weighing 50 mg were incubated in 3.5 ml medium containing U-C^{14} glucose (10 mM) with a total activity of 1,688,000 counts/min + and 0.2 U of insulin. To one of the slices L-glutamate (10 mM) was added. Temp. 38°C. Time 2 hrs. Gas phase: Oxygen.

TABLE 5
EFFECT OF ADDING INACTIVE GLUCOSE ON C^{14} INCORPORATION FROM U-C^{14} GLUTAMATE INTO GLYCOGEN AND CARBON DIOXIDE BY CEREBRAL CORTICAL SLICES OF NORMAL RAT

Condition	Incorporation of C^{14} counts/min/g wet wt. of slice	
	Glycogen	Carbon dioxide
1. [U-C^{14}] L-glutamate (10 mM)	1793 ± 68* (5)	129,720 ± 688 (5)
2. [U-C^{14}] L-glutamate (10 mM) + inactive glucose (10 mM)	2285 ± 98 (5)	122,480 ± 3890 (5)

Mean ± S.E. P value < 0.001.
Cerebral cortical slices (about 50 mg) were incubated in 3.5 ml of medium containing [U-C^{14}] glutamate with a total activity of 1,325,600 counts/min. Time 2 hrs. Temp. 38°. Gas phase: Oxygen.

REFERENCES

1. F.N. Le Baron, *Biochem. J.*, **61**, 80 (1955).
2. K.G. Prasannan and K. Subrahmanyam, *Indian J. Biochem.*, **2**, 94 (1965).
3. P. Visweswaran, K.G. Prasannan and K. Subrahmanyam, *J. Neurochem.*, **16**, 1389 (1969).
4. P. Visweswaran and K. Subrahmanyam, *J. Neurochem.*, **20**, 1493 (1973).
5. H. Weil-Malherbe, *Biochem. J.*, **30**, 665 (1936).
6. K.G. Prasannan and K. Subrahmanyam, *Endocrinology*, **82** (1968).
7. P. Visweswaran and K. Subrahmanayam, *J. Neurochem.*, **20**, 1493 (1973).
8. G. Takagaki, S. Hirano and Nagata, *J. Neurochem.*, **4**, 124 (1959).
9. Q.S. Rolleston and A. Newsholme, *Biochem. J.*, **104**, 524 (1967).
10. C. Terner, L.V. Eggleston and H.A. Krebs, *Biochem. J.*, **47**, 139 (1950).
11. M.M. Cohen and H.P.P.F. Cohen, *J. Neurochem.*, **13**, 811 (1966).
12. J.R. Stern, L.V. Eggleston, R. Hems and H.A. Krebs, *Biochem. J.*, **44**, 410 (1949).
13. A. Kleinzeller and R. Rybova, *J. Neurochem.*, **2**, 45 (1957).
14. R.J. Roister, *Can. J. Biochem. Physiol.*, **33**, 477 (1955).

(*Received*: 30 December 1991; *Accepted*: 15 October 1992) AJC-495