Uptake and Release of Glutamate in Cerebral-Cortex Slices from the Rat

By K. OKAMOTO and J. H. QUASTEL

Kinsmen Laboratory of Neurological Research, Health Sciences Centre, University of British Columbia,
Vancouver 8, B.C., Canada

(Received 31 January 1972)

1. Cerebral-cortex slices from rat brain, loaded with labelled L-glutamate as a result of aerobic incubation with labelled glucose, lost less than 15% of this glutamate on subsequent incubation in the presence of unlabelled glucose and L-glutamate. This indicates that very little exchange occurs between extracellular L-glutamate and glutamate accumulated in the neurons as a result of glucose metabolism. 2. Slices, loaded with labelled L-glutamate as a result of aerobic incubation in a medium containing unlabelled glucose and labelled L-glutamate, lost more than half of this glutamate on subsequent incubation in the presence of unlabelled L-glutamate. This indicates that exchange occurs between extracellular glutamate and glutamate accumulated in brain slices as a result of its uptake from the incubation medium. 3. Evidence was obtained suggesting that only a part of the glutamate, accumulated in brain slices as a result of its uptake from an incubation medium containing both glucose and L-glutamate, entered the neurons; apparently almost all the rest entered the glia. 4. It is concluded that the slices contain a pool of glutamate, derived from glucose and located in the neurons, which is poorly exchangeable with extracellular glutamate, and another pool of glutamate, derived from extracellular glutamate and located in the glia, which is freely exchangeable with extracellular glutamate.

It has been shown, from studies of the effects of tetrodotoxin on amino acid release from rat brain-cortex slices, that the major location of glutamate is probably in the neurons (Benjamin & Quastel, 1972). We have carried out experiments to decide whether this conclusion holds independently of the pathway by which glutamate accumulates in the brain cells. The presence of glutamate in the brain cells is due to at least two processes: (a) intracellular formation from glucose via α -oxoglutarate; (b) uptake of glutamate from extracellular spaces. Both the neurons and the glia are involved.

The following questions were therefore considered: (1) is exogenous glutamate, well known to be accumulated by brain tissue *in vitro* against a concentration gradient, taken up for the most part in the neurons or is it partly taken up by the glia?; (2) does labelled glutamate, taken up by brain tissue by prior incubation in its presence, exchange freely with extracellular unlabelled glutamate?; (3) does labelled glutamate, derived from labelled glucose in brain tissue, exchange freely with exogenous unlabelled glutamate?

The results of experiments devised to answer these questions are described below.

Materials

Animals

Male adult rats of the Wistar strain weighing 150–200 g were used. They were obtained from Vol. 128

the Vivarium, University of British Columbia, Vancouver, B.C., Canada.

Chemicals

All common laboratory chemicals were of reagent grade and were used without further purification. [U - ^{14}C]Glucose, specific radioactivity 3 mCi/mmol, and L-[U - ^{14}C]glutamic acid, specific radioactivity 165 mCi/mmol, used after neutralization, were obtained from the Amersham/Searle Corp., Ont., Canada and the Volk Radiochemical Co., Chicago, Ill., U.S.A., respectively. Sodium isethionate was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Methods

Preparation and incubation of slices

Cerebral-cortex slices (not more than 0.4 mm thick) were prepared as described by Benjamin & Quastel (1972). The slices were weighed at once on a torsion balance and suspended in Krebs–Ringer phosphate medium of the following composition: 128 mM-NaCl, 5 mM-KCl, 2.8 mM- $CaCl_2$, 1.3 mM- $MgSO_4$, 10 mM- Na_2HPO_4 (adjusted to pH 7.4 with HCl) and 10 mM-glucose. Various substances were added to this incubation medium, but the final volume was always 3 ml. Incubation was carried out with the conventional Warburg manometric apparatus in an atmosphere

of O_2 at $37^\circ C$ for, usually, 60 min. Solutions (0.1 ml or 0.2 ml) of radioactive substances (0.5 or $1.0 \mu Ci$) were added to the medium in the main compartment of the manometric vessel 5 min after brief preliminary incubation at $37^\circ C$ for temperature equilibration.

Procedure for loading slices with labelled amino acids

In many of the experiments described below slices were loaded with radioactivity by pre-incubation with [^{14}C]glucose or L-[^{14}C]glutamate. Such slices were then incubated in medium containing either unlabelled glucose or a mixture of unlabelled glucose and unlabelled L-glutamate and the rates of release of radioactivity from the slices into the media were measured. The procedure for determining the contribution made by [^{14}C]glutamate and other labelled amino acids to the released radioactivity was as follows. Slices were incubated in O_2 at $37^\circ C$ for 1 h (or 30 min as described below) in medium containing 10 mM-[^{14}C]glucose or 10 mM unlabelled glucose and 5 mM-L-[^{14}C]glutamate. At the end of the incubation period they were divided into three equal portions. The first was assayed for its content of radioactivity and of labelled amino acids. The second was placed in 3 ml of medium containing 10 mM unlabelled glucose and the third was placed in 3 ml of medium containing 10 mM unlabelled glucose together with 5 mM unlabelled sodium L-glutamate. These two lots were incubated in O_2 at $37^\circ C$ for 1 h, after which time they were assayed for radioactivity and contents of labelled amino acids. The incubation media were similarly assayed for labelled amino acids.

Determination of amino acids in tissue and incubation medium

The determination of amino acids in the tissue and the incubation medium at the termination of the incubation was carried out in the manner described in detail by Benjamin & Quastel (1972), with a Beckman model 120B amino acid analyser.

Determination of ^{14}C -labelled amino acids

After incubation of the slices with radioactive substrates, they were extracted with trichloroacetic acid (Benjamin & Quastel, 1972) and the final ether- and acid-free extracts were applied to the chromatographic column of a Beckman model 120B analyser under the same conditions as those used for the analysis of unlabelled amino acids. The column, however, was disconnected from the ninhydrin reaction chamber and the eluate was collected directly from the outlet of the column with the use of a fraction collector, by which successive quantities of 1 ml of the eluate could be fractionated. Measurements of the radioactivities of each fraction were

made with the liquid-scintillation counter (Nuclear-Chicago; model Mk. I), each fraction being mixed with 10 ml of the scintillant medium. This consisted of equal volumes of toluene, dioxan and aq. 95% (v/v) ethanol containing 2,5-diphenyloxazole (5 g/l), 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.05 g/l) and naphthalene (80 g/l). The counting efficiency was 70% and then corrected by the channels-ratio technique. Amino acids in radioactive fractions were identified by comparison with standard amino acids run through the chromatographic column. Similar assays were carried out on portions of the incubation medium.

Measurement of acid-soluble radioactivity

The acid-soluble radioactivity in the slices, at the termination of incubation with either labelled glucose or with labelled glutamate, was measured as follows. The brain tissue was removed by forceps and rinsed quickly with the Krebs-Ringer medium, homogenized with 5 ml of 5% (w/v) trichloroacetic acid and centrifuged. A 0.5 ml sample of the supernatant was assayed for radioactivity in the liquid-scintillation counter and the results expressed as d.p.m./100 mg, or d.p.m./g initial wet wt. A portion of the medium was assayed in a similar manner and the radioactivity was expressed as d.p.m./ml, or d.p.m./3 ml of medium.

Determination of water uptake

Immediately after the incubation, the slices were removed from the media and drained. They were spread without folding on an ice-cold clean glass surface and excess of fluid around the slices and on the glass was absorbed with strips of filter paper. The slices were further drained by touching the glass surface several times until no cloud formed on the surface. The slices were then weighed on a torsion balance. The difference between this final wet weight and the initial wet weight of the slices gave a measure of the increase of water uptake. The difference (in mg) multiplied by $100/A$, where A was the initial wet wt. (in mg) of the tissue, gave the amount of water taken up by the tissue ($\mu l/100 mg$).

Estimations of tissue Na^+ and K^+

The brain slices, after incubation, were homogenized in 3 ml of 5% (w/v) trichloroacetic acid and the supernatants obtained after centrifuging were diluted with water to obtain final cation concentrations of about 0.05 – $0.10 \mu equiv./ml$ for both Na^+ and K^+ . The atomic absorptions were measured at 294.3 and 383.3 nm for Na^+ and K^+ respectively with a Perkin-Elmer model 303 atomic absorption spectrophotometer.

Results

Effect of exogenous glutamate on release of glutamate, derived from radioactive glucose, from rat brain-cortex slices

When brain slices, loaded with labelled glutamate derived from labelled glucose, were incubated with 5mM-sodium L-glutamate, there was only a small loss of radioactivity (about 14%) from the tissue during the 30min incubation period (Table 1). The percentage loss was still smaller when the second incubation period was prolonged to 60min. The total radioactivity in tissue and medium was smaller in the longer incubation period than in the shorter period because of the increased evolution of $^{14}\text{CO}_2$.

The proportions of the radioactive amino acids in the slices at the end of the initial (1h) incubation period are shown in Table 2(A). The labelled non-amino acid constituents (shown by fractionation to consist largely of glucose) in the tissue extracts accounted for 70% of the total radioactivity present. Glutamate accounted for 19.1%. Incubation of the tissue, subsequently, in the presence of unlabelled glucose, brought about a fall in the proportion of non-amino acid radioactive constituents in the tissue to 19% (Table 2B). This was expected as unlabelled glucose in the second incubation medium exchanged with the labelled glucose in the tissue. The proportion of labelled glutamate in the slices was only slightly decreased by incubation with sodium L-glutamate (Table 2B). Thus there was little exchange between external unlabelled glutamate and intracellular labelled glutamate derived from labelled glucose.

The calculated percentages of labelled glutamate and aspartate in the radioactive amino acid constituents of the tissue slices after the second incuba-

tion period, in the presence of glucose, assuming no loss of amino acids during this period, were $19.1 \times (100-19)/(100-70) = 51$ for glutamate and $5 \times (100-19)/(100-70) = 13$ for aspartate. The observed values of 45% for glutamate and 10% for aspartate indicated that the tissue retained much of its content of glutamate and aspartate during the second incubation period. The low value for glutamine (2.6%, Table 2B) after the second incubation period showed that leakage or loss of this amino acid took place during this period. The increased value for glutamine (6.6%, Table 2B), found when the tissue was incubated in the presence of both glucose and L-glutamate during the second incubation period, indicated that either the presence of glutamate diminished the loss of glutamine from the tissue or that glutamate promoted glutamine formation during this period.

Specific radioactivity of glutamate in brain-cortex slices incubated in glucose-L-[U- ^{14}C]glutamate media

Further evidence bearing on the possible exchange of extracellular glutamate with intracellular glutamate was sought in a different way. Slices were incubated in 3ml of medium containing 10mM-glucose and 5mM-sodium L-[U- ^{14}C]glutamate for 1h in O_2 at 37°C . At the end of this period the tissues were analysed for their content of glutamate, and the uptake of radioactivity into the tissues was determined. The glutamate content of slices incubated for 1h in medium containing 10mM-glucose was also determined. From these results it was possible to estimate the specific radioactivity of glutamate (d.p.m./ μmol) present in the incubated slices and to compare this value with the specific radioactivity of glutamate in the medium. If extracellular glutamate

Table 1. *Effects of sodium L-glutamate on release of radioactivity from rat brain-cortex slices previously incubated with [U- ^{14}C]glucose*

Slices were incubated in 3ml of Krebs-Ringer phosphate medium containing 10mM-[U- ^{14}C]glucose in O_2 for 1h at 37°C . They were then removed, rinsed rapidly with the medium containing 10mM unlabelled glucose, and divided into two equal portions: the first was incubated in O_2 for 30min (Expt. A) or for 1h (Expt. B) at 37°C in 3ml of Krebs-Ringer phosphate-10mM-glucose medium; the second was incubated under exactly similar conditions but with 5mM-sodium L-glutamate added to the medium. All experiments were carried out at least four times. Mean values $\pm$ S.D. are given.

Additions to final incubation medium	Expt. A (sp. radioactivity of glucose in initial incubation medium = 0.424 $\mu\text{Ci/ml}$)		Expt. B (sp. radioactivity of glucose in initial incubation medium = 0.33 $\mu\text{Ci/ml}$)	
	$10^{-4} \times$ Radioactivity retained in tissue	$10^{-4} \times$ Radioactivity released into medium	$10^{-4} \times$ Radioactivity retained in tissue	$10^{-4} \times$ Radioactivity released into medium
	(d.p.m./g)	(d.p.m./ml)	(d.p.m./g)	(d.p.m./ml)
Glucose	31.75 $\pm$ 1.24	2.84 $\pm$ 0.06	18.72 $\pm$ 0.46	1.66 $\pm$ 0.18
Glucose+sodium L-glutamate	27.19 $\pm$ 1.21	3.01 $\pm$ 0.06	16.16 $\pm$ 0.82	1.73 $\pm$ 0.06

exchanged with the whole of the intracellular glutamate, the specific radioactivity of the glutamate present in the tissue at the end of the 1 h incubation period would approximate to that in the medium. On the other hand, if extracellular glutamate did not fully exchange with the tissue glutamate then the specific radioactivity of the latter would be less than that of glutamate in the medium. The proportion of radioactive glutamate in the total radioactivity present in slices incubated for 1 h in medium containing glucose and 5 mM-sodium L-[U-¹⁴C]glutamate

amounted to 60% (Gonda & Quastel, 1962; and our own observations). The calculated specific radioactivity of the tissue glutamate amounted to 36% of the specific radioactivity of the glutamate present in the medium (Table 3). It was evident therefore that newly entered exogenous glutamate did not exchange freely with all the glutamate present in the brain slices. If the specific radioactivity of the tissue glutamate was calculated in terms of the amount of newly entered glutamate, its value became 54% of that of the glutamate in the medium. To interpret

Table 2. Analysis of radioactive constituents of rat brain-cortex slices, loaded with labelled glutamate derived from labelled glucose, after incubation in the presence of unlabelled glucose, with or without unlabelled L-glutamate

The slices were removed after incubation under the conditions described in Table 1 and the radioactivities of constituents were measured as described in the Methods section. The results recorded below give the amounts of the constituents expressed as percentages of the total radioactivity found in the tissue. All experiments were carried out at least four times. Mean values $\pm$ S.D. are given.

		Radioactive tissue constituents present at the end of first or second incubation periods (% of tissue radioactivity)			
(A) Addition to the first incubation medium	First incubation period	Non-amino acid constituents	Glutamate	Glutamine	Aspartate
[U- ¹⁴ C]Glucose		70 $\pm$ 3.5	19.1 $\pm$ 1.9	3.8 $\pm$ 0.1	5.0 $\pm$ 0.2
(B) Additions to the second incubation medium	Second incubation period				
Glucose		19 $\pm$ 1.8	45 $\pm$ 1.4	2.6 $\pm$ 0.8	10.0 $\pm$ 0.8
Glucose + sodium L-glutamate		14 $\pm$ 1.4	41 $\pm$ 3.4	6.6 $\pm$ 1.3	10.5 $\pm$ 1.0

Table 3. Specific radioactivity of labelled tissue glutamate in rat brain-cortex slices incubated in glucose-L-[U-¹⁴C]glutamate media

Slices were incubated in 3 ml of Krebs-Ringer phosphate media containing either 10 mM-glucose alone or 10 mM-glucose + 5 mM-sodium L-[U-¹⁴C]glutamate (specific radioactivity 0.092 μ Ci/ μ mol) for 1 h in O₂ at 37°C. At the end of this period, the slices were rinsed rapidly with Krebs-Ringer phosphate medium and their contents of radioactivity were measured. The total glutamate present in the tissue was measured as described in the text. Each experiment was carried out four times. Mean values $\pm$ S.D. are given.

Incubation conditions	Glutamate in tissue (μ mol/g)	10 ⁻⁶ $\times$ Radioactivity in tissue (d.p.m./g)	10 ⁻⁶ $\times$ Radioactivity in tissue due to glutamate (d.p.m./g)	10 ⁻⁶ $\times$ Specific radioactivity of tissue glutamate (d.p.m./ μ mol)	10 ⁻⁶ $\times$ Specific radioactivity of glutamate in medium (d.p.m./ μ mol)
10 mM-Glucose	8.4 $\pm$ 0.21	—	—	—	—
10 mM-Glucose + 5 mM-sodium L-[U- ¹⁴ C]glutamate	24.5 $\pm$ 1.0	3.76 $\pm$ 0.20	2.26	0.092	0.256 $\pm$ 0.013
Newly entered tissue glutamate	16.1	3.76	2.26	0.14	0.256 $\pm$ 0.013

these results, further information would be required about how the tissue glutamate is partitioned between glia and neurons.

Effects of exogenous glutamate on release of glutamate, derived from radioactive glutamate, from rat brain slices

In three experiments, in which the period of incubation of the slices, previously loaded with exogenous labelled L-glutamate, was varied from 30 min to 90 min, the presence of 5 mM or 10 mM unlabelled sodium L-glutamate caused significant falls in the amount of ^{14}C in the tissues and concomitant rises in the amount of ^{14}C in the medium (Table 4). The total (tissue and medium) radioactivity was but little affected by the presence of L-glutamate. In all cases the retention of radioactivity in the tissue slices was diminished by exogenous unlabelled glutamate to less than half that present in the glucose medium.

Measurements were made of the glutamate present in the tissue at the end of the second incubation period (60 min) in which the tissue was exposed to the presence of both glucose and unlabelled 5 mM-sodium L-glutamate. The amount was $14.48 \pm 1.5 \mu\text{mol/g}$ initial wet wt. of tissue. The radioactivity present in the tissue was $0.48 \pm 0.06 \times 10^6 \text{ d.p.m./g}$ (Table 4C); of this 60% (i.e. $0.29 \times 10^6 \text{ d.p.m./g}$) was found to be in the form of labelled glutamate. There-

fore the specific radioactivity of the glutamate in the tissue was $0.29/14.48 = 0.02 \times 10^6 \text{ d.p.m./}\mu\text{mol}$. This represented a large fall (78%) in the specific radioactivity of the tissue glutamate from that found in the tissue at the end of the first incubation period (i.e. $0.092 \times 10^6 \text{ d.p.m./}\mu\text{mol}$; Table 3).

Measurement of the radioactivity present in the incubation medium containing glucose and glutamate (5 mM), at the end of the second incubation period (1 h), showed this to contain $0.059 \pm 0.006 \times 10^6 \text{ d.p.m./ml}$, and the concentration of glutamate in the medium was found to be $4.34 \pm 0.04 \mu\text{mol/ml}$. The specific radioactivity of the glutamate in the medium, assuming the radioactivity to be largely due to released (or exchanged) glutamate, was therefore $0.059/4.34 = 0.0136 \times 10^6 \text{ d.p.m./}\mu\text{mol}$. It therefore appeared that the specific radioactivity of the glutamate present in the tissue at the end of the second incubation period approximated to that in the incubation medium.

These observations show that extracellular glutamate exchanges with the labelled glutamate which accumulates in the brain slices as a result of incubation of the tissue in its presence.

Glutamate uptake by the tissue and its effects on water and ion movements in chloride-deficient media

The results quoted above indicate that there are two separate pools of glutamate in brain cortex

Table 4. Release of labelled glutamate from rat brain cortex-slices, previously incubated in the presence of labelled glutamate, by exogenous unlabelled glutamate

Slices were incubated in 3 ml of Krebs-Ringer phosphate media containing 10 mM-glucose and 5 mM-sodium L-[U- ^{14}C]glutamate for 1 h (for Expts. A and C) or for 30 min (for Expt. B) in O_2 at 37°C . They were then rinsed rapidly with the Ringer glucose medium and transferred to 3 ml of Krebs-Ringer phosphate medium containing either 10 mM-glucose or 10 mM-glucose + sodium L-glutamate (5 mM or 10 mM, as stated below). The second incubation periods varied as shown below. Three sets of experiments were carried out, in which the initial specific radioactivity of the glutamate was: Expt. A, $0.033 \mu\text{Ci}/\mu\text{mol}$, Expt. B, $0.074 \mu\text{Ci}/\mu\text{mol}$ or Expt. C, $0.092 \mu\text{Ci}/\mu\text{mol}$. Each experiment was carried out four times. Mean values $\pm$ s.d. are given.

Expt.	Additions to the medium in the second incubation period	Length of second incubation period (min)	$10^{-5} \times$ Radioactivity present in tissue slices (d.p.m./100 mg initial wet wt.)	$10^{-5} \times$ Radioactivity in incubation medium (d.p.m./3 ml)	$10^{-5} \times$ Total radioactivity present (d.p.m.)	Retention of radioactivity in the tissue (% of total present)
A	Glucose	30	3.95 ± 0.13	1.29 ± 0.05	5.24	74
	Glucose + 10 mM-sodium L-glutamate	30	2.05 ± 0.14	3.81 ± 0.22	5.86	34
B	Glucose	90	0.67 ± 0.01	0.58 ± 0.02	1.25	54
	Glucose + 10 mM-sodium L-glutamate	90	0.22 ± 0.01	1.10 ± 0.02	1.32	17
C	Glucose	60	1.28 ± 0.08	1.09 ± 0.01	2.37	54
	Glucose + 5 mM-sodium L-glutamate	60	0.48 ± 0.06	1.77 ± 0.20	2.25	21

slices, one of which is exchangeable with extracellular glutamate, the other not (or much less so). As Benjamin & Quastel (1972) have presented evidence that the neuron contains the major pool of glutamate when brain slices are incubated aerobically in a glucose-containing medium, and as the results given above indicate that extracellular glutamate does not exchange freely with glutamate derived from glucose, it is likely that the pool of poorly exchangeable glutamate lies in the neurons. It follows that the pool of exchangeable glutamate is located in the glia. Experiments were therefore carried out to obtain additional evidence that might help to confirm or refute this conclusion.

As available evidence (Zadunaisky *et al.*, 1965; Gerschenfeld *et al.*, 1959; Tower & Bourke, 1966; Tower, 1967) indicates that the water uptake of incubated brain slices is largely confined to the glia, it follows that the increased water uptake found on incubation of slices in the presence of sodium L-glutamate is either due to uptake of glutamate into the glia or to uptake by the glia of K^+ (as KCl) released from the neurons by the excitatory activity of extracellular glutamate (Okamoto & Quastel, 1970). If the latter alternative is correct, it follows that if slices are incubated in a chloride-free medium, increased uptake of water by the glia in the presence of glutamate should not occur owing to the low extracellular chloride concentration. On the other hand, if the former alternative is correct, sodium glutamate should penetrate into the glia with accompanying water uptake to maintain iso-osmoticity.

Accordingly, experiments were carried out to observe the effects of sodium L-glutamate on water uptake and movements of Na^+ and K^+ in slices incubated in a glucose-containing medium in which sodium chloride was replaced by sodium isethionate.

Results showed that there was only little increased

water uptake when brain cortex slices were incubated in a glucose-isethionate medium but that the presence of 5 mM-sodium L-glutamate brought about a definite increase of water uptake (Table 5). There was no significant change in the tissue concentration of K^+ but that of Na^+ was increased. The accumulation of glutamate in the slices amounted to $13.5 \mu\text{mol/g}$. Presumably glutamate entered the tissue accompanied by Na^+ , as there was no significant change in the tissue content of K^+ . Assuming that glutamate entered the glia it would be accompanied by $13.5 \mu\text{equiv.}$ of Na^+/g . The actual rise in tissue Na^+ was $12.6 \mu\text{equiv./g}$, indicating an approximation of the Na^+ uptake to that of glutamate (Table 5). The increased water uptake was $138 \mu\text{l/g}$, which corresponded to an uptake of $138 \times 0.148 = 20.4 \mu\text{equiv.}$ of Na^+/g (Okamoto & Quastel, 1970). This indicated that much of the water uptake due to the presence of 5 mM-sodium L-glutamate in the glucose-isethionate media could be accounted for by the uptake of sodium L-glutamate directly into the glia.

Discussion

Results given in this paper lead to the conclusion that there exists in rat brain slices a pool of glutamate, derived from glucose and located in the neurons, which is poorly exchangeable with extracellular glutamate, and another pool of glutamate, derived from extracellular glutamate and located in the glia, which is freely exchangeable with extracellular glutamate.

The fact that extracellular labelled glutamate may enter neurons has been shown by Kuhar & Snyder (1970), who found localization of labelled glutamate in synaptosomes. It is possible, however, to demonstrate entry of extracellular glutamate into neurons by another method. Incubation of rat brain

Table 5. *Uptake of glutamate by brain-cortex slices, and its effect on cation contents in chloride-deficient media*

Slices were incubated in O_2 for 1 h at 37°C in 3 ml of Krebs-Ringer phosphate medium containing 10 mM-glucose or 10 mM-glucose + 5 mM-sodium L-glutamate, sodium isethionate being substituted for sodium chloride and potassium sulphate for potassium chloride. Sulphuric acid, instead of HCl, was used for pH adjustment. The concentration of Na^+ in the medium was maintained at 148 mequiv./litre, i.e. when sodium L-glutamate was added to the medium there was a corresponding adjustment of sodium isethionate. Na^+ and K^+ were assayed at the end of the experiment with a Perkin-Elmer atomic absorption spectrophotometer. Glutamate was estimated as described in the text. All experiments were carried out four times. Mean values $\pm$ S.D. are given.

Substances added to the incubation medium	Uptake of O_2 ($\mu\text{mol}/100\text{mg}$ initial wet wt.)	Uptake of water ($\mu\text{l}/100\text{mg}$ initial wet wt.)	Na^+ ($\mu\text{equiv./g}$ initial wet wt.)	K^+ ($\mu\text{equiv./g}$ initial wet wt.)	Glutamate ($\mu\text{mol/g}$ initial wet wt.)
Glucose	10.6 ± 0.3	8.0 ± 0.8	114.2 ± 4.1	50.2 ± 2.5	8.5 ± 0.2
Glucose + 5 mM-sodium L-glutamate	10.7 ± 0.2	21.8 ± 0.9	126.8 ± 1.4	47.8 ± 1.0	22.0 ± 1.0

cortex slices in a physiological saline-glucose medium in the presence of labelled L-glutamate gives rise to labelled γ -aminobutyrate, the amount of which increases as the concentration of extracellular labelled glutamate increases (Gonda & Quastel, 1962). Evidence indicates that glutamic acid decarboxylase and γ -aminobutyrate are mostly contained in nerve endings (Neal & Iversen, 1969) and that they have approximately the same distribution in central nervous tissue (Roberts & Eidelberg, 1960; Albers & Brady, 1959). It appears therefore that the major site of synthesis of γ -aminobutyrate is in the neurons (see also Balázs *et al.*, 1970) and therefore that extracellular labelled glutamate must be able to enter the neurons to give rise to an increased amount of labelled γ -aminobutyrate. Glutamine, proceeding from the glia, also acts as a precursor of the compound (Benjamin & Quastel, 1972). This possibility was presented as part of a comprehensive model by Van den Berg & Garfinkel (1971).

The question arises as to how much of exogenous glutamate accumulated by the tissue finds its way into the neurons. A partial answer may be given by the following consideration. Analysis of slices incubated aerobically in a physiological saline-5 mM-[U- 14 C]-glucose medium for 1 h at 37°C showed that the tissue content of glutamate increased from $8.93 \pm 0.25 \mu\text{mol/g}$ initial wet wt. in the absence of added glutamate to $29.65 \pm 1.15 \mu\text{mol/g}$ in the presence of 10 mM-sodium L-glutamate, and that of γ -aminobutyrate increased from $2.41 \pm 0.24 \mu\text{mol/g}$ in the absence of added glutamate to $4.00 \pm 0.09 \mu\text{mol/g}$ in the presence of the glutamate. If the accumulation of exogenous glutamate takes place only in the neurons, then the specific radioactivity of neuronal glutamate derived from radioactive glucose should fall owing to the entry of unlabelled glutamate. As labelled glutamate is the precursor of labelled γ -aminobutyrate in the brain slices it follows that if the specific radioactivity of the labelled glutamate falls, through dilution with unlabelled glutamate, then the specific radioactivity of the labelled γ -aminobutyrate should also fall, the amount being equal to the amount of dilution of labelled glutamate that has occurred. The recorded facts (Gonda & Quastel, 1962), together with additional data obtained by ourselves, showed that the specific radioactivity of glutamate in the incubated brain tissue slices fell from $0.95 \pm 0.04 \mu\text{g-atom of } ^{14}\text{C}/\mu\text{mol}$ of glutamate, found in the absence of added glutamate, to $0.19 \pm 0.01 \mu\text{g-atom of } ^{14}\text{C}/\mu\text{mol}$ of glutamate found in the presence of 10 mM-sodium L-glutamate in the incubation medium. The fall in specific radioactivity of the glutamate amounted to 80%. Under the same experimental conditions, the specific radioactivity of γ -aminobutyrate, found in the absence of added glutamate, was $0.92 \pm 0.06 \mu\text{g-atom of } ^{14}\text{C}/\mu\text{mol}$ of γ -aminobutyrate, i.e. a value almost identical with that found for glutamate, and

this value fell to $0.48 \pm 0.02 \mu\text{g-atom of } ^{14}\text{C}/\mu\text{mol}$ of γ -aminobutyrate in the presence of 10 mM-sodium L-glutamate in the incubation medium, i.e. a fall of 48%. These values indicate that a part of the accumulated glutamate in the brain slice does not enter the neurons and presumably therefore enters the glia.

A considerable fall, amounting to 55%, in the radioactivity of the tissue glutamine takes place, under the experimental conditions quoted above, when 10 mM-sodium L-glutamate is added to the incubation medium containing 5 mM labelled glucose. This would be expected if most of the unlabelled glutamate enters the cells in which glutamine biosynthesis takes place to a major extent and which form the major pool of glutamine, i.e. the glia.

The fact that extracellular glutamate brings about but little release from the tissue of labelled glutamate, derived from labelled glucose, i.e. of neuron-located glutamate, makes the action of glutamate quite distinct from that of protoveratrine (or of agents that influence excitability), which causes marked release of glucose-derived glutamate from the brain slices (Benjamin & Quastel, 1972). It is possible, however, that extracellular glutamate may cause localized release of glutamate, e.g. at the nerve endings, which may amount only to a small fraction of the total pool of glutamate in the neuron. This may not be measurable with accuracy by methods described in this paper.

Release of neuronal glutamate, as a result of synaptic activity, would lead to an increased concentration of extracellular glutamate, part of which might be taken up by the neurons and part by the glia. In the glia (Benjamin & Quastel, 1972) glutamate is converted into glutamine, which is released into the extracellular space and taken up by the neurons to be reconverted, after hydrolysis, into glutamate.

It may be pointed out, in this connexion, that an uptake mechanism for glutamate in the glia may be just as effective as that in the neurons for the decrease of an extracellular concentration of glutamate increased as a result of release from presynaptic sites, so long as glutamate continues to be made either from glucose in the neuron or from glutamine absorbed from the glia. In view of this consideration, it does not seem necessary to postulate an active uptake mechanism only in the neuron as essential for the transmitter activity of glutamate. As an example of this conclusion, it has been found (and confirmed by ourselves) that synaptosomes are unable to accumulate acetylcholine by an active uptake process (Ritchie & Goldberg, 1970), though brain slices are effective in this respect.

Results of experiments carried out in chloride-deficient isethionate-containing media led to the conclusion that much of the increased water uptake of brain slices that occurs when they are incubated in an

isethionate medium containing sodium L-glutamate may be accounted for by the accumulation of sodium glutamate in the glia. Lund-Andersen & Hertz (1970) have also observed that additional water uptake by incubated rat brain cortex slices occurs when 10mM-sodium L-glutamate is added to chloride-free media, the amount being less than that occurring in a chloride-containing medium, and moreover there is a smaller influx of Na^+ . They indicate that the increased water uptake does not take place in the absence of Na^+ from the incubation medium. This is to be expected, as it is well known (see Quastel, 1965) that cerebral active uptake of amino acids is a sodium-dependent process.

We acknowledge with gratitude financial assistance from the Medical Research Council of Canada. K. O. has been in receipt of a Canadian Muscular Dystrophy Fellowship. We are grateful to the Department of Soil Science, University of British Columbia, for use of their atomic-absorption spectrophotometer and to Mr. A. M. Benjamin for his assistance in amino acid analyses.

References

- Albers, R. W. & Brady, R. O. (1959) *J. Biol. Chem.* **234**, 926–928
- Balázs, R., Machiyama, Y., Hammond, B. J., Julian, T. & Richter, D. (1970) *Biochem. J.* **116**, 445–467
- Benjamin, A. M. & Quastel, J. H. (1972) *Biochem. J.* **128**, 631–646
- Gerschenfeld, H. M., Wald, F., Zadunaisky, J. H. & De Robertis, E. (1959) *Neurology* **9**, 412–425
- Gonda, O. & Quastel, J. H. (1962) *Biochem. J.* **84**, 394–406
- Kuhar, M. J. & Snyder, S. H. (1970) *J. Pharmacol. Exp. Ther.* **171**, 141–152
- Lund-Andersen, H. & Hertz, L. (1970) *Exp. Brain Res. (Berlin)* **11**, 199–212
- Neal, M. J. & Iversen, L. L. (1969) *J. Neurochem.* **16**, 1245–1252
- Okamoto, K. & Quastel, J. H. (1970) *Biochem. J.* **120**, 37–47
- Quastel, J. H. (1965) *Proc. Roy. Soc. Ser. B* **163**, 169–196
- Ritchie, A. K. & Goldberg, A. M. (1970) *Science* **169**, 489–490
- Roberts, E. & Eidelberg, E. (1960) *Int. Rev. Neurobiol.* **2**, 279–332
- Tower, D. B. & Bourke, R. S. (1966) *J. Neurochem.* **13**, 1099–1117
- Tower, D. B. (1967) in *Brain Edema* (Klatzo, I. & Seitelberger, F., eds.), pp. 313, 318, Springer, New York
- Van den Berg, C. J. & Garfinkel, D. (1971) *Biochem. J.* **123**, 211–218
- Zadunaisky, J. A., Wald, F. & De Robertis, E. (1965) *Progr. Brain Res.* **15**, 196–218