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POTASSIUM MODULATION OF METHIONINE UPTAKE IN ASTROCYTES IN VITRO

C. J. CUMMINS[1,4], R. A. GLOVER[2], AND O. Z. SELLINGER[3]

[1] *Surgical Neurology Branch*
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health
Bethesda, Maryland 20205

[2] *Department of Anatomy*
The University of Michigan School of Medicine
Ann Arbor, Michigan 48109

[3] *Mental Health Research Institute*
The University of Michigan School of Medicine
Ann Arbor, Michigan 48109

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Methionine participates in a large variety of metabolic pathways in brain, and its transport may play an important regulatory role. The properties of methionine uptake were examined in a preparation of neonatal rat brain astrocytes. Uptake is linear for 15 minutes, up to 2.5 μM. At steady state conditions, methionine is concentrated 30–50-fold. Measured methionine homoexchange accounts for a significant fraction of uptake at concentrations greater than 10 μM. We recently reported that methionine uptake is decreased by elevations in extracellular K^+. Potassium induced efflux cannot account for this apparent effect; and thus for concentrations less than 2.5 μM, and for short times of incubation, measured rates of methionine uptake represent unidirectional flux. At extracellular concentrations of K^+ equal to 6.9 mM, the apparent V_{max} of methionine transport is 182 pmol/min/mg protein, and the K_m is 1.3 μM. Where K^+ is shifted to 11.9 mM, the K_m remains unchanged, and the V_{max} is reduced by half.

INTRODUCTION

Methionine (MET) is a precursor of proteins, other amino acids, polyamines, and the multifunctional methyl donor S-adenosyl methionine. MET

[4] To whom all correspondence may be sent: 10A/3E68, SNB, NINCDS, NIH, Bethesda, MD, 20205, (301) 496–5728; 496–6628.

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may be transported from blood to brain where the MET concentration approaches 20–40 μM (10). Thus transport may play a key role as the rate-limiting or regulatory step in many of the important pathways in which MET participates.

Brain appears similar to other tissues in many aspects of its amino acid transport (13). Amino acids are accumulated against a concentration gradient, and transport systems discriminate between classes of amino acids based on structural features. Sershen and Lajtha (13) have recently shown that MET appears to be transported primarily by the L (or leucine) preferring system in brain, but MET also antagonizes transport of the A (or alanine) preferring system, suggesting that MET may be transported by the A system as well.

Despite an intense interest, most aspects of neuronal-glial interaction remain obscure. One potential mechanism by which neuronal metabolic status may be communicated to glia is by elevations in extracellular K^+. Elevated extracellular K^+ has been shown to affect glial oxygen uptake (7), glucose uptake (4, 6) and oxidation state (14). Schousboe et al. (12) demonstrated that GABA uptake was modulated by elevations in extracellular K^+, and we demonstrated that elevations in extracellular K^+ modulated the uptake of methionine into primary cultures of rat brain astrocytes (6). We did not rule out possible confounding effects such as a potassium-induced efflux or homoexchange. We present here evidence to support a direct effect of extracellular K^+ on the kinetic properties of the transport system for methionine in primary cultures of rat brain astrocytes.

EXPERIMENTAL PROCEDURE

Radiolabeled methionine, L-[*methyl*-^{3}H]methionine, specific activity 53.7 mCi/mmol, or L-[^{14}C(U)]methionine, specific activity 213 mCi/mmol was obtained from New England Nuclear, Boston, Massachusetts.

Astrocytes were grown in culture as previously described by us (6). Confluent cultures show a typical astroglial morphology and ubiquitously stain with glial-specific histological stains. Comparably prepared cultures from neonatal rat brains are enriched in the astrocyte-specific GFA protein (3). Cultures were used at confluence 12–14 days after seeding, forty-eight hours following the last feeding.

The buffer used for the MET uptake experiments contained 5.3 mM KCl, 150 mM NaCl, 1 mM $CaCl_2$, 600 μM $MgCl_2$, 1.6 mM KH_2PO_4, and 4.3 mM Na_2HPO_4, pH 7.2. When the ionic composition of the buffer was varied, Na^+ was substituted iso-osmotically for K^+.

To determine MET uptake, 60 × 15 mm dishes of confluent astrocytes (10^4 cells) were washed free of medium with 5–7 ml of 37°C 0.9% NaCl solution. The rims of the dishes were blotted, and two ml of buffer containing the appropriate concentration of K^+, Na^+ and radiolabeled MET were added. The plates were returned to the incubator at 37°C, or for incubations shorter than 5 min, dishes were floated in a 37°C waterbath. At the appropriate time, the incubation medium was aspirated, and the dishes were rapidly rinsed in ice

cold isotonic saline (15–18 ml), and the edges were again blotted. Two ml of 0.2 M perchloric acid were added, and the dishes were stored at 4°C overnight. The perchloric acid soluble fraction was removed, neutralized, and duplicate or triplicate aliquots were counted.

To determine non-specific binding, astrocytes were rapidly washed free of tissue culture medium with ice cold 0.9% NaCl and two ml of ice cold buffer containing the same ionic and MET concentration as the experimental samples were rapidly added, swirled, and poured off (5–10 seconds). The cells were again washed in ice cold isotonic saline, disrupted in perchloric acid, and treated as above. Blank values obtained in this manner were subtracted from experimental samples, and the values presented here are corrected for the non-specific binding of tracer amino acid.

To load astrocytes for MET efflux or homoexchange experiments, cells were incubated 10 or 15 min in a complete buffer containing 6.9 mM K^+ and 500 nM radiolabeled MET. To estimate the residual MET at the end of the loading time, cells were washed and disrupted in perchloric acid as in the uptake experiments, and the quantity of acid soluble MET determined.

To determine efflux, loaded cells were briefly washed, and 37°C MET-free buffer was added, collected and replaced at intervals for the following 45 min. Duplicate 0.25 ml aliquots were counted. The data are expressed as log medium CPM as a percent of the total CPM taken up by the end of the loading period.

Protein was determined by the procedure of Lowry et al. (9) on dishes taken from the same batch, and on the same day as the experimental samples.

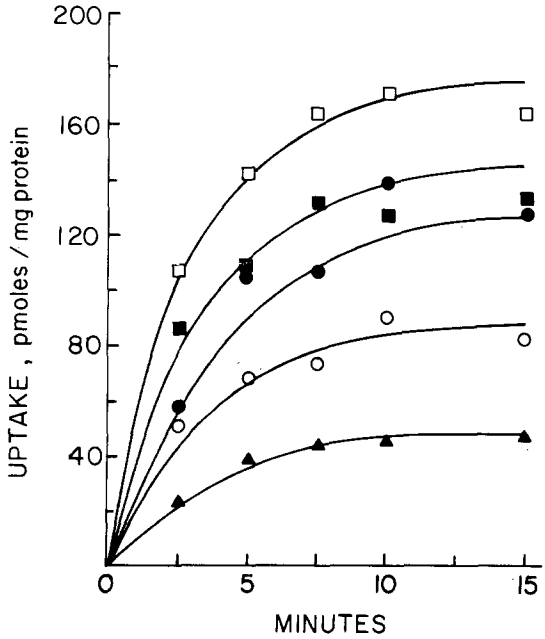

FIG. 1. The time course of methionine uptake is shown for a variety of extracellular methionine concentrations: uptake of 100–500 nM: 100 nM, (▲); 200 nM (○); 300 nM (●); 400 nM, (■); and 500 nM, (□). Each point represents duplicate determinations on 3–5 dishes. Standard error was less than 15% of the mean for all points.

RESULTS

To estimate the MET concentrative accumulation, ([intracellular]/[extracellular] or distribution ratio), cell protein was assumed to constitute 10%, and water to constitute 80% of the wet weight. Uptake indicated per mg protein is considered to be equivalent to uptake per 8 μl of cell water. MET appears in the perchloric acid insoluble fraction in incubations longer than 15 minutes. It is assumed that for short times of incubation (<15 min) MET is dispersed in the pool of intracellular water.

The time course of MET uptake is shown in Figure 1. MET uptake is linear for 2.5 min up to 2.4 μM extracellular MET (data not shown). The steady state (i.e. 15 min) distribution ratios varied from 37.5 with 100 nM extracellular MET to 47.5 with 500 nM MET.

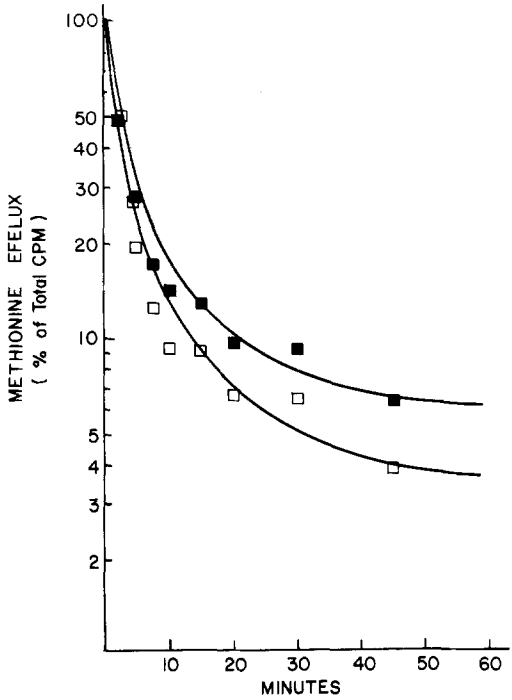

FIG. 2. Dishes of confluent astrocytes were loaded for 15 minutes in 500 nM methionine and 6.9 mM K^+ buffer. The subsequent washout was followed for 50 minutes in medium containing either 6.9 (□) or 11.9 (■) mM K^+. The results are presented as the efflux of label, as a percent of the total at the end of the loading period. The medium was replaced at the times indicated. Neither the maximum washout nor the initial velocities of efflux are significantly different. Each point represents duplicate determinations on two plates.

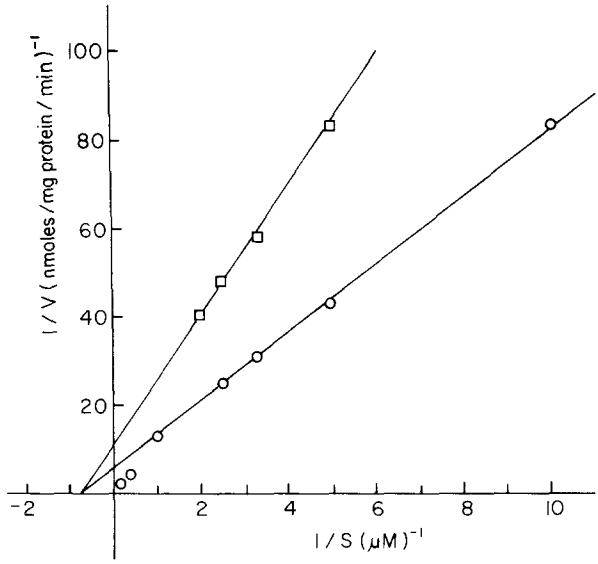

FIG. 3. The Lineweaver-Burk plot of methionine uptake in the presence of 6.9 mM K^+ (○) or 11.9 mM K^+ (□) is shown. The K_m for methionine uptake is 1.3 μM at either [K^+], and the V_{max} is 182 pmol/mg protein/min in the presence of 6.9 mM K^+ and 90 nmol/mg protein/min in the presence of 11.9 mM K^+. Each point represents the mean of duplicate determinations on 3–5 dishes. The standard error was less than 15% of the mean.

If elevations in extracellular K^+ increased the rate of MET efflux, and the rate of MET influx were unaffected, a specious decrease in MET transport would be observed. We therefore determined the effect of extracellular K^+ on methionine efflux. Cells were loaded with radiolabeled MET, and the effects of 6.9 or 11.9 mM extracellular K^+ on efflux into MET-free medium were examined (Figure 2). For times shorter than 2.5 minutes, initial rates of MET efflux did not differ significantly. The total MET efflux was also not significantly different for either K^+ concentration. We interpret these data to indicate that extracellular K^+ does not affect the rate or endpoint of MET efflux.

The transport of most L-system amino acids shows a homoexchange component (11). To test whether exchange diffusion was a significant aspect of uptake under the experimental conditions, cells were loaded with radiolabeled MET at 500 nM, for 10 minutes. The medium was then replaced with buffer containing 6.9 mM K^+ and various concentrations of unlabeled MET. Homoexchange was measured as efflux of radiolabeled MET "driven" by the concentration of unlabeled extracellular MET. Table I shows that at the concentrations used in this study (100–500 nM)

TABLE I
METHIONINE HOMOEXCHANGE

[MET] ADDED (μM)	[MET] Efflux (pmol/2.5 min/mg protein)	Homoexchange	% Total Pool Exchanged
None	268.1 ± 13.62		
0.1	239.7 ± 6.53	–0–	–0–
1.0	277.0 ± 9.39	8.9	5.0
10.0	336.3 ± 7.62	68.2	37.9
100.0	468.8 ± 15.83	200.7	111.5
500.0	476.3 ± 10.28	208.6	115.6

Dishes of confluent astrocytes were treated with 500 nM radiolabeled MET for 10 min in a buffer containing 6.9 mM K^+. At the end of the loading period the total intracellular MET pool was 180 pmol/mg protein. The medium was changed to buffer containing no MET (control) or various concentrations of unlabeled MET as indicated in column 1. Two and one half min later, the medium was removed, and the quantity of radiolabeled MET in the medium determined (column 2, mean ± SEM). The radiolabeled MET appearing after medium replacement in the absence of added MET (line 1) represents MET efflux (c. f. Figure 2), as well as residual MET adhering non-specifically. MET homoexchange (column 3) was calculated by subtracting the efflux value obtained in the absence of added MET (268.1 pmol/2.5 min/mg protein) from the values obtained in the presence of the various MET concentrations. The per cent of the total pool exchanged is calculated by dividing the MET homoexchanged by the total MET pool at the end of the loading period (180 pmol/mg protein).

homoexchange comprised less than 5% of the total apparent uptake. Thus, for concentrations of MET less than 1 μM, and at times less than 2.5 min apparent MET uptake cannot be accounted for by either homoexchange, efflux, or incorporation into protein, and represents true unidirectional flux.

Initial velocities were extrapolated from the timecourse data of Figure 1, and the calculated fraction of homoexchange was subtracted. A Lineweaver-Burk plot is shown in Figure 3. For MET uptake in the presence of 6.9 mM K^+ the apparent V_{max} is 182 pmoles/mg protein/min, and the K_m is 1.3 μM. In the presence of K^+ at 11.9 mM, the K_m is unchanged, but the V_{max} is markedly decreased, to 90.9 pmol/mg protein/min.

DISCUSSION

The general features of amino acid transport in other mammalian systems are also seen in brain slice preparations (13). MET uptake has not been widely examined in nervous tissue, and distribution ratios are not known. Distribution ratios vary from 37 to 47 in buffers containing 6.9

mM K^+. These values are higher than those reported by Belkhode and Scholefield (1) for non-nervous tissue.

The apparent uptake of MET was significantly reduced when the extracellular K^+ was increased to 11.9 mM, and elevations beyond 11.9 mM did not cause a further reduction in MET uptake (6). MET uptake in the range of 1 μM and less does not result from the interaction of potassium levels and mechanisms regulating homoexchange and efflux, but rather appears to affect the velocity of transport directly. The generality of this effect is still an open question, since extracellular K^+ levels do not effect the uptake of either β-alanine or α-aminoisobutyrate (4). Schousboe et al. (12) have recently demonstrated a K^+-induced alteration in the uptake of GABA, perhaps by a mechanism similar to the one observed here.

The upper range of extracellular K^+ observed in normal, stimulated intact cortex (measured by ion-specific electrodes) appears to be no greater than 12 mM (2, 14), and the concentrations of K^+ which affected MET uptake are within this physiological concentration. We reported (4, 6) that elevated K^+ and norepinephrine reciprocally modulated the uptake of the glucose analog, 2-deoxy-D-glucose, in rat brain astrocytes. This suggests that the production of potassium by rapidly firing neurons may alter important physiological processes of the surrounding glia, such as membrane potential, metabolism and amino acid uptake. Brain L-system amino acids include, among others, tyrosine and tryptophan (13). A potassium-induced decrease in the glial uptake of other L-system amino acids may therefore increase the availability of neurotransmitter precursors. Thus, the role of extracellular K^+ may represent an important physiological control in the neuronal modulation of astrocytic metabolism in vitro as well as in vivo.

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