Biochimica et Biophysica Acta 818 (1985) 45–54
Elsevier

BBA 72667

A γ-aminobutyric acid-specific transport mechanism in mammalian kidney *

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(Received March 13th, 1985)

Key words: γ-Aminobutyric acid transport; Na^+ dependence; Membrane potential; Brush-border membrane; (Rat kidney)

We describe high-affinity, sodium-dependent transport of gamma-aminobutyric acid in slices exposing basal lateral membranes and brush-border membrane vesicles prepared from rat renal cortex. In the presence of aminooxyacetic acid, to block γ-aminobutyric acid oxidation, uptake into the intracellular space of slices was saturable (apparent K_t, 26 ± 4 μM, mean and S.E.) and concentrative (steady-state distribution ratio at 50 μM γ-aminobutyric acid, 47.7 ± 2.4, mean and S.E.). Brush-border membrane vesicles accumulated γ-aminobutyric acid in the presence of an inward-directed sodium chloride gradient, (apparent K_t, 30–36 μM) with the peak of 'overshoot' at 10 min. Uptake by vesicles responded to manipulation of the transmembrane potential gradient with valinomycin or impermeant anion. β-Alanine inhibited γ-aminobutyric acid transport by slices and brush-border membrane vesicles; inhibitors of neuronal-type γ-aminobutyric acid transport (e.g., nipecotic and diaminobutyric acids) did not. An 'ABC test' indicated that γ-aminobutyric acid and β-alanine do not share a single carrier in either the brush-border or basal-lateral membrane of renal cortex. Influx of γ-aminobutyric acid into brush-border membrane vesicles, at transequilibrium NaCl, was stimulated by *trans*-γ-aminobutyric acid but not by *trans*-taurine. Ion gradient-driven γ-aminobutyric acid co-transport was unaffected in freeze-thawed brush-border membrane vesicles; this treatment abolished β-alanine and taurine co-transport. We conclude that rat kidney membranes (brush-border and basal-lateral) possess a γ-aminobutyric acid-preferring, high-affinity transport mechanism.

Introduction

γ-Aminobutyric acid is an inhibitory neurotransmitter present throughout the central nervous system of vertebrates. It is synthesized from glutamate at GABAergic nerve terminals and released upon depolarization into the synaptic cleft, where it binds to specific post-synaptic receptors which mediate its neuroinhibitory function. Two high-affinity, sodium-dependent transport mechanisms have been identified in brain which may participate in terminating the action of γ-aminobutyric acid by removing it from the cleft through an uptake process in neuronal cells specifically inhibited by nipecotic and diaminobutyric acids, and an uptake process in glial cells inhibited by β-alanine [1].

Evidence showing that γ-aminobutyric acid is not confined to the central nervous system has accumulated. Certain extra-neural tissues [2], such as mammalian kidney, pancreatic islets and ovary,

* This is publication No. 85022 of the McGill University-Montreal children's Hospital Research Institute.

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GABA, γ-aminobutyric acid.

contain substantial quantities of γ-aminobutyric acid, yet, its physiologic roles there are unknown. Some evidence suggests that γ-aminobutyric acid may act as an inhibitory neurotransmitter in peripheral nerves [3,4]. However, in rat kidney, it is synthesized by a non-neural form of glutamate decarboxylase abundant in proximal tubules [5].

To determine whether mammalian kidney possesses a specific γ-aminobutyric acid transport mechanism similar to or different from those in neural systems, we examined uptake of γ-aminobutyric acid by cortical slices, which preferentially expose basal-lateral membranes [6,7], and in brush-border membrane vesicles prepared from rat kidney. In both preparations, high-affinity, sodium-dependent γ-aminobutyric acid transport was identified which was inhibited by β-alanine but not by nipecotic or diaminobutyric acids. In brush-border membrane vesicles, uptake occurred on a γ-aminobutyric acid-preferring system distinct from other mechanisms that mediate neutral amino acid transport.

Material and Methods

Animals. Long-Evans rats (male) weighing 200–225 g were obtained from Canadian Breeding Farms (St. Eustache, Quebec) and fed standard Purina rat chow.

Materials. We obtained reagent grade chemicals from Sigma Chemical Co. (St. Louis, MO) or Fisher-Scientific (Montreal, Quebec) and radiochemicals from New England Nuclear Canada (Lachine, Quebec): 4-amino[U-^{14}C]butyric acid, 200 mCi/mmol; β-[1-^{14}C]alanine, 40–60 mCi/mmol; D-[1(n)-^{3}H]glucose, 15–30 Ci/mmol; [*methylamine*-^{3}H(n)]muscimol, 5–20 Ci/mmol; and L-[5-^{3}H]proline, 5–15 mCi/mmol.

Preparation of tissues. Thin renal cortex slices (5–10 mg/slice, 0.08–0.16 mm thickness) were cut from hemi-kidneys with a Stadie-Riggs microtome and weighed on a Perkins-Elmer autobalance. Brush-border membrane vesicles were prepared from rat renal cortex by the method of Booth and Kenny [8]. Renal cortex from 6–8 rats was homogenized in 10 vol. of chilled 10 mM mannitol (10 mM)/2 mM Tris-HCl buffer (pH 7.1) to which was added magnesium chloride (10 mM final concentration). Membranes (brush-border membrane vesicles) sedimenting between 1500 × *g* and 15 000 × *g* were washed and resuspended in 300 mM mannitol/20 mM Hepes buffer (pH 7.4). Alkaline phosphatase activity in the brush-border membrane vesicles sample was enriched 12-fold over activity in the original cortical homogenate.

Transport conditions for slices. We measured uptake of γ-amino[U-^{14}C]butyric acid (10–100 μM) by slices at 37°C under 100% oxygen in a modified Krebs buffer containing D-glucose according to a published method [9] (5.6 mM). Slices ($n = 5–7$) were incubated in buffer (2 ml) then removed at specified times, blotted, weighed, and boiled in 0.25 ml H_2O before counting accumulated isotope by liquid scintillation spectrometry. Slices were also incubated in media containing [^{3}H]poly(ethylene glycol) for 45 min. We established that the extracellular water was 33.7% of wet slice weight (and intracellular water, 48.5% of wet slice weight) corroborating earlier observations [10]. Uptake rates (amount/ml intracellular water per unit time) on the mediated component and the distribution ratio (cpm/ml intracellular water/cpm/ml medium) at steady state (45 min) were calculated as previously described [9].

Transport conditions for brush-border membrane vesicles. We measured uptake of γ-amino[U-^{14}C]butyric acid (at 10–100 μM) by brush-border membrane vesicles in 10 μl aliquots of vesicles (10 μg protein/μl) suspended in buffered mannitol medium at 22°C, mixed with 90 μl of isosmotic 'incubation medium' containing 100 mM mannitol/20 mM Tris-Hepes (pH 7.4), with or without aminooxyacetic acid (0.1 mM), and either 100 mM NaCl or 100 mM KCl. To terminate transport, 3 ml chilled isosmotic buffered mannitol were added; the suspension was then rapidly filtered through 0.45 micron MF-millipore disc filters and washed three times with 3 ml chilled buffered mannitol. The filters were transferred to vials contining 5 ml Formula-963 (NEN) for liquid scintillation counting.

To measure trans-stimulation of γ-aminobutyric acid uptake, brush-border membrane vesicles were filled with control buffer 100 mM mannitol/100 mM NaCl/20 mM Tris-Hepes (pH 7.4)), or buffer containing 1 mM γ-aminobutyric acid or 1 mM taurine at 22°C for 30 min. Pre-loaded brush-border membrane (20 μl) were then mixed

with incubation buffer (180 μl) containing NaCl, to achieve the transequilibrium condition for NaCl, γ-amino[U-^{14}C]butyric acid ($1.6 \cdot 10^6$ cpm), and unlabelled γ-aminobutyric acid with or without taurine to final concentrations of 0.1 mM for each amino acid. Influx of γ-amino[U-^{14}C]butyric acid was measured at 1 and 10 min.

Statistics. Values are expressed as mean ± S.E. Statistical significance was determined by Student's *t*-test.

Results

Uptake of γ-aminobutyric acid by slices of rat renal cortex

We examined γ-aminobutyric acid by renal cortex slices to determine whether it is concentrative, influenced by substrate oxidation, and dependent on sodium ion.

Uptake of labelled γ-aminobutyric acid by slices was measured in the presence of 100 mM NaCl (100 mM) and [^{3}H]poly(ethylene glycol) (as a marker of extracellular space). After incubation for 45 min at 37°C, the isotopic distribution ratio of 50 μM γ-aminobutyric acid was 2.3. When γ-aminobutyric acid oxidation was blocked by addition of aminooxyacetic acid (0.1 mM), the ratio increased to 47.7 ± 2.4 (mean and S.E., n = 6). Partition chromatography of slice extracts confirmed that all the radiochemical was in a single band co-migrating with the γ-aminobutyric acid standard.

In the presence of aminooxyacetic acid (0.1 mM), uptake of γ-amino[U-^{14}C]butyric acid (50 μM) at 37°C was monotonic, approaching the steady-state at 45 min. Uptake was unaltered by the addition of a supplementary energy source (2 mM succinate) but was reduced to 11% of control when incubations were performed at 4°C or when NaCl (100 mM) in the external medium was replaced by choline chloride (100 mM) (Table I). γ-Aminobutyric acid uptake was unaffected by addition of nipecotic acid (0.5 mM), cysteine sulfinic acid (1 mM) or muscimol (1 mM) (Table I).

Net uptake of γ-aminobutyric acid in the presence of aminooxyacetic acid, showed monotonic saturation when the substrate concentration was raised from 2 to 100 mm. The mean apparent K_t for γ-aminobutyric acid uptake was 26 ± 4 μM (n = 5) (Fig. 1). β-Alanine (1 mM), an inhibitor of γ-aminobutyric acid transport in glial cells [11], reduced γ-aminobutyric acid uptake (at 50 μM) by renal slices (to 16 ± 3% of control); unlabelled γ-aminobutyric acid (at 1 μM) inhibited its own uptake equally well (to 10 ± 2% of control) (Table I). The mechanism of β-alanine inhibition was not clearly competitive (Fig. 1). Taurine (1 mM) was also inhibitory (66 ± 4% of control) but no more so than proline (56 ± 5% of control) or *p*-aminohippurate (60 ± 9% of control) at similar concentrations (Table I). The effect of *p*-aminohippurate was not competitive (Fig. 1).

These findings indicate that basal-lateral membranes of renal slices possess a high-affinity, sodium-dependent mechanism for concentrative uptake of γ-aminobutyric acid with properties resembling those in glial cells [1] but unlike those observed in GABAergic neurons [12]. γ-

TABLE I

UPTAKE OF γ-AMINOBUTYRIC ACID (50 μM) BY SLICES OF RAT RENAL CORTEX

Uptake of γ-amino[U-^{14}C]butyric acid (50 μM) into the intracellular space of rat renal cortex slices was measured after 45 min incubation in a modified Krebs buffer with aminooxyacetic acid (0.1 mM) at 37°C; control uptake was 7.9 ng γ-aminobutyric acid (GABA)/mg wet wt. per min. Where indicated, NaCl (100 mM) in the buffer was replaced with choline chloride (100 mM). Uptake in standard buffer was also measured at 4°C or in the presence of various potential inhibitors. Results are for six replicates. n.s., not significant.

Condition	Intracelllular GABA uptake (% of control)	
	mean ± S.E.	
Choline chloride (100 mM)	11 ± 1	$P < 0.01$
On ice (4°C)	11 ± 4	$P < 0.01$
+ Nipecotic acid (0.5 mM)	95 ± 11	n.s.
+ Muscimol (1 mM)	95 ± 12	n.s.
+ Cysteine sulfinic acid (1 mM)	102 ± 15	n.s.
+ Succinic acid (2 mM)	96 ± 10	n.s.
+ GABA (1 mM)	10 ± 2	$P < 0.01$
+ β-Alanine (1 mm)	18 ± 3	$P < 0.01$
+ Taurine (1 mM)	66 ± 4	$P < 0.01$
+ Proline (1 mM)	56 ± 5	$P < 0.01$
+ *p*-Aminohippuric acid (1 mM)	60 ± 9	$P < 0.01$

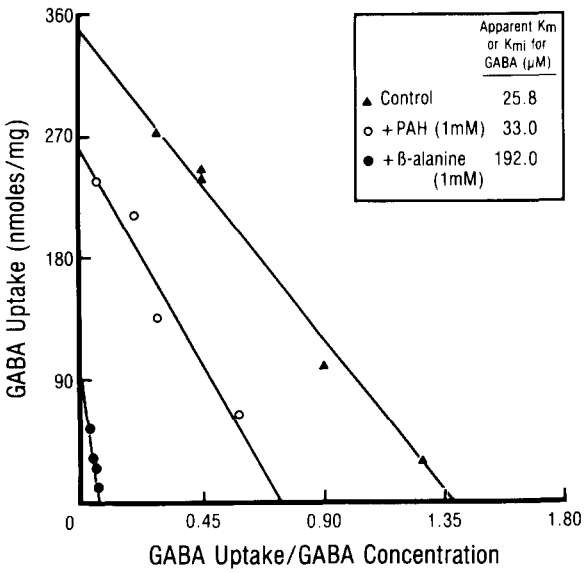

Fig. 1. Effect of substrate concentration and other substances (at 1 mM) on γ-aminobutyric acid (GABA) uptake in the concentration range (2–100 μM) by renal slices at steady-state in the presence of aminooxyacetic acid (0.1 mM). PAH, *p*-aminohippurate.

Aminobutyric acid oxidation influences net uptake.

Uptake of γ-aminobutyric acid by brush-border membrane vesicles

We then compared γ-aminobutyric acid uptake in brush-border membrane vesicles, with uptake in slices in which the basal-lateral membrane is preferentially exposed.

We measured time-dependent uptake of γ-amino[U-^{14}C]butyric acid (at 50 μM) by brush-border membrane vesicles at 22°C in an inward-directed gradient of NaCl (100 mM) or KCl (100 mM) (Fig. 2a). γ-Aminobutyric acid uptake occurred by an NaCl gradient-driven cotransport mechanism. In the presence of the NaCl gradient, there was an 'overshoot' with the peak at 10 min, representing 160% of the equilibrium value. γ-Aminobutyric acid uptake then declined until equilibrium was achieved at about 1 h. Overshoot was not observed when incubations were performed at 4°C or in the presence of external KCl.

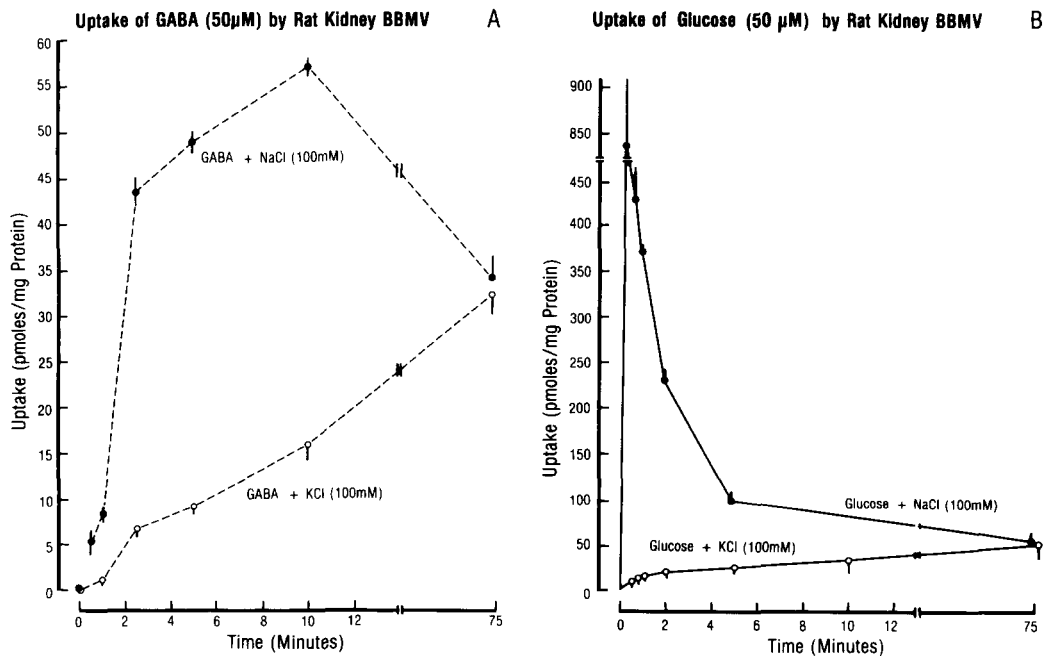

Fig 2. (A) Uptake of γ-amino[U-^{14}C]butyric acid (GABA) (50 μM) by renal brush-border membrane vesicles (BBMV) in the presence of an inward-directed NaCl or KCl gradient (100 mM outside, 0 mM inside), at 22°C in the presence of aminooxyacetic acid (0.1 mM). Each point is the mean ± S.E. of five replicates. (B) Simultaneous uptake of D-[1(n)-^{3}H]glucose (50 μM) in the same brush-border membrane vesicles preparations.

The findings resemble uptake of several other neutral organic solutes by renal brush-border membrane vesicles [13]. However, the overshoot phase for γ-aminobutyric acid uptake was delayed and was much smaller than that for the simultaneous uptake of D-glucose (at 50 μM) in the same brush-border membrane vesicles preparations (Fig. 2b). Intravesicular volume at equilibrium was 0.71 μl/mg protein (mean, $n = 3$) for γ-aminobutyric acid and 1.1 μl/mg protein ($n = 3$), for D-glucose.

We measured uptake of γ-aminobutyric acid by brush-border membrane vesicles in the presence of aminooxyacetic acid (0.1 mM) to block any γ-aminobutyric acid oxidation that might occur in contaminating mitochondria in the preparation; cytochrome *c* reductase activity in brush-border membrane vesicles was less than 10% of activity in crude homogenates of renal cortex. After incubation of labelled γ-amino[U-^{14}C]butyric acid (50 μM) in transport media with aminooxyacetic acid (0.1 mM) at 22°C for 75 min, there was no measurable release of $^{14}CO_2$ and all radioactivity was identified, by partition chromatography, in a single band co-migrating with the γ-aminobutyric acid standard.

To determine whether uptake of γ-aminobutyric acid represented transport into an osmotically sensitive space, we prepared brush-border membrane vesicles in the usual mannitol buffer, and incubated in media containing 50 μM γ-amino[U-^{14}C]butyric acid, 100 mM NaCl, 20 mM Tris-Hepes (pH 7.4) and varying amounts of sucrose (100–700 mM). γ-Aminobutyric acid uptake at equilibrium was inversely proportional to external osmolarity (Fig. 3). Extrapolation of the regression line to infinitely high osmolarity indicated less than 5% non-specific binding.

We measured the effect of transmembrane potential on the ion gradient-dependent γ-Aminobutyric acid transport. Brush-border membrane vesicles were preloaded with 100 mM KCl, 200 mM mannitol, 20 mM Tris-Hepes (pH 7.4) and the potassium ionophore valinomycin (8 μg/ml). Under these conditions, sodium gradient-dependent γ-aminobutyric acid uptake was stimulated at early time points (238% of control at 2.5 minutes, 173% of control at 7.5 min) (Table II). When incubations were performed wih a relatively impermeant anion in the medium, (Na_2SO_4, 50 mM), so that a favorable electronegative interior could not be generated by rapid anion influx [14–16] sodium gradient-dependent γ-aminobutyric acid uptake was abolished (Table II).

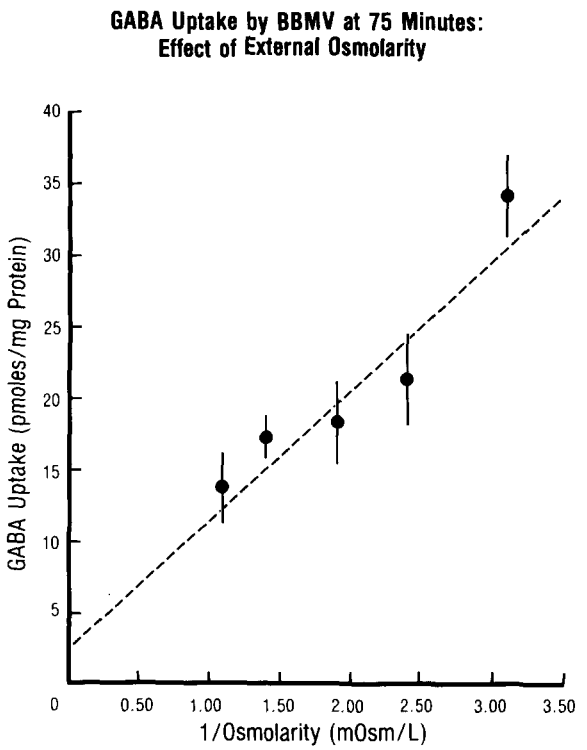

Fig. 3. Uptake of γ-aminobutyric acid (GABA) by renal brush-border membrane vesicles (BBMV) as a function of intravesicular volume. brush-border membrane vesicles were prepared in regular buffered mannitol (300 mM) and incubated in media containing 100 mM NaCl, 20 mM Hepes-Tris (pH 7.4), 50 μM γ-amino[U-^{14}C]butyric acid, 0.1 mM aminooxyacetic acid and varying amounts of sucrose (100–700 mM). Uptake of γ-aminobutyric acid at equilibrium (75 min) is expressed as the mean ± S.E. of five replicates.

We performed a kinetic analysis of sodium-dependent γ-aminobutyric acid uptake at 7.5 min; we were unable to make reliable measurements at earlier time points. Brush-border membrane vesicles demonstrated saturable, high-affinity uptake in the concentration range 10–100 mM γ-aminobutyric acid (Fig. 4). In three different experiments, the apparent K_t for γ-aminobutyric acid varied between 30 and 36 μM; the apparent V_{max} was 35–71 pmol/mg protein per 7.5 min. Uptake was inhibited by β-alanine but not by diaminobutyric acid or nipecotic acid; inhibition by β-alanine was apparently competitive (Fig. 4).

TABLE II

EFFECT OF THE ELECTRO-CHEMICAL GRADIENT ON γ-AMINOBUTYRIC ACID UPTAKE BY BRUSH-BORDER MEMBRANE VESICLES

Uptake of γ-aminobutyric acid (50 μM) by brush-border membrane vesicles was measured at 22°C in the presence of aminooxyacetic acid (0.1 mM) and a NaCl or KCl gradient (100 mM outside, 0 mM inside). [a] Brush-border membrane vesicles aliquots were pre-incubated in isosmotic buffer containing KCl (100 mM) and valinomycin (8 μg/ml) for 30 min at 22°C and compared to controls pre-incubated in regular buffered mannitol. [b] NaCl (100 mM) in the external medium was replaced with Na_2SO_4 (50 mM). Results are for five replicates.

External medium	Time (min)	Control uptake (%) (mean ± S.E.)	
		total	Na-dependent
NaCl (100 mM)	2.5	100 ± 2	100 ± 4
	7.5	100 ± 4	100 ± 7
KCl (100 mM)	2.5	39 ± 4	0 ± 9
	7.5	39 ± 6	0 ± 11
NaCl (100 mM) [a]	2.5	185 ± 8	238 ± 13
+ valinomycin (8 μg/ml)	7.5	167 ± 20	173 ± 18
Na_2SO_4 (50 mM) [b]	7.5	27 ± 6	−11 ± 9

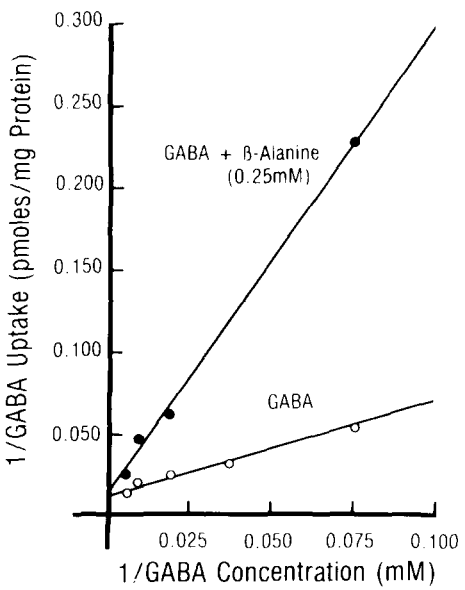

Fig. 4. Concentration-dependent uptake of γ-amino[U-^{14}C]butyric acid (GABA) was measured at 7.5 min under standard conditions. It was unaffected by addition (at 1 mM) of diaminobutyric acid (116 ± 10% mean ± S.E. of control, $P > 0.05$) or nipecotic acid (116 ± 6% of control, $P > 0.05$); data not shown. Inhibition by β-alanine (0.25 mM) was apparently competitive. Data shown are a typical experiment; three experiments gave similar findings.

TABLE III

EFFECT OF VARIOUS SUBSTRATES ON UPTAKE OF γ-AMINOBUTYRIC ACID OR β-ALANINE BY BRUSH-BORDER MEMBRANE VESICLES

The sodium-dependent component of uptake with γ-amino[U-^{14}C]butyric acid (GABA) 50 μM) or β-[U-^{14}C]alanine (50 μM) by brush-border membrane vesicles was determined at 7.5 min under standard conditions or in the presence of various compounds (at 1 mM). Results are for five replicates. n.s., not significant.

Unlabelled substrate added (1 mM)	Percent of control (mean ± S.E.)			
	β-alanine uptake (50 μM)		GABA uptake (50 μM)	
+ GABA	78 ± 12	$P < 0.05$	35 ± 3	$P < 0.01$
+ β-Alanine	20 ± 2	$P < 0.01$	51 ± 5	$P < 0.01$
+ L-Proline	75 ± 2	$P < 0.01$	89 ± 8	n.s.
+ D-Glucose	88 ± 9	n.s.	92 ± 9	n.s.
+ Taurine	27 ± 2	$P < 0.01$	83 ± 10	$P < 0.05$

γ-Aminobutyric acid uptake was unaffected by *p*-aminohippurate (1 mM) (data not shown).

Mammalian renal brush-border membrane vesicles possess a high-affinity transport process for β-amino acids [17,19]. We investigated whether γ-aminobutyric acid transport occurred primarily on a β-amino acid-preferring system or on its own system by examining the relative effects of various inhibitors on uptake of γ-aminobutyric acid (50 μM) and β-alanine (50 μM) (Table III), using the so-called 'ABC test' [18]. Sodium-dependent transport of γ-aminobutyric acid was inhibited more by the addition of unlabelled 1 mM γ-aminobutyric acid than by 1 mM β-alanine ($P < 0.01$). Conversely, sodium-dependent uptake of β-alanine was inhibited more by the addition of 1 mM β-alanine than by 1 mM γ-aminobutyric acid ($P < 0.01$). Furthermore, taurine (1 mM) strongly inhibited uptake of β-alanine, but had little effect on γ-aminobutyric acid uptake ($P < 0.01$). These findings imply that γ-aminobutyric acid is transported on a carrier different from that serving β-amino acids. A more rigorous test of this assumption is feasible with trans-stimulation experiments.

Influx of labelled γ-aminobutyric acid was measured at transequilibrium NaCl in brush-border membrane vesicles containing *trans*-γ-aminobutyric acid (1 mM), or *trans*-taurine (1 mM) and compared with entry into vesicles without *trans*-amino acid. γ-Aminobutyric acid influx was

TABLE IV

TRANS-STIMULATION OF γ-AMINOBUTYRIC ACID UPTAKE IN BRUSH-BORDER MEMBRANE VESICLES UNDER TRANSEQUILIBRIUM CONDITIONS FOR NaCl

Brush-border membrane vesicles (BBMV) were pre-incubated for 30 min at 22°C in buffered mannitol (100 mM) containing NaCl (100 mM) with or without the inside *trans*-amino acid (1 mM). Transport was initiated by mixing pre-loaded brush-border membrane vesicles with 9 vol. of an appropriate buffer containing at final concentration, 100 mM NaCl, 0.1 mM γ-amino[U-^{14}C]butyric acid (GABA) and 0.1 mM taurine. Uptake of γ-amino[U-^{14}C]butyric acid was measured at 1 and 10 min; results are mean ± S.E. of five replicates.

Inside BBMV	Uptake (pmol/mg protein)	
	1 min	10 min
NaCl (100 mM)	16.4 ± 1.5	48.7 ± 3.3
GABA (1 mM)	48.5 ± 4.6 [b]	118.3 ± 3.5 [b]
Taurine (1 mM)	19.8 ± 1.2	73.0 ± 9.0 [a]

[a] $P < 0.02$.
[b] $P < 0.01$.

markedly stimulated by *trans*-γ-aminobutyric acid (296 and 243% of control at 1 and 10 min, respectively) but only slightly by *trans*-taurine (121 and 156% of control at 1 and 10 min, respectively) (Table IV). Homo-trans-stimulation by γ-aminobutyric acid influx was abolished in the absence of sodium ion.

Hilden and Sacktor [20] found reduced sodium-dependent D-glucose transport after storage of brush-border membrane vesicles in buffered mannitol (300 mM) at −20°C. Brush-border membrane vesicles treated in this fashion lost the capacity for sodium-dependent γ-aminobutyric acid transport. However, when brush-border membrane vesicles were rapidly frozen at −70°C (iso-

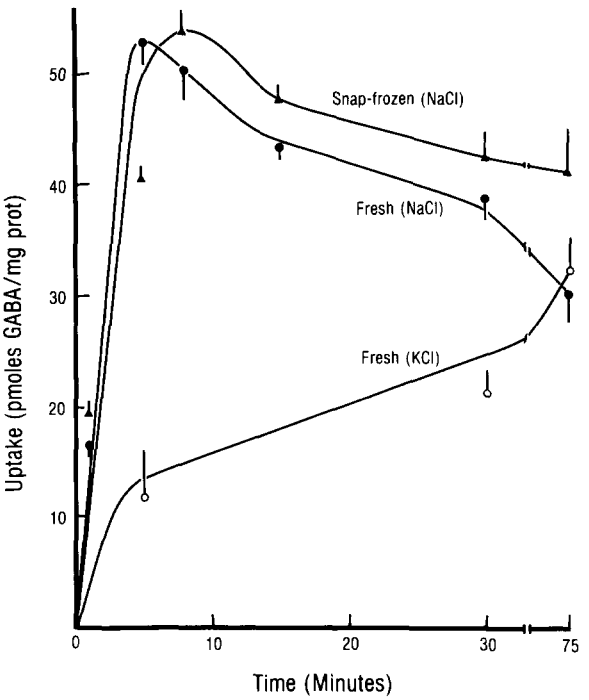

Fig. 5. Effect of freeze-thaw treatment of brush-border membrane vesicles on γ-aminobutyric acid (GABA) uptake. Transport of γ-amino[U-^{14}C]butyric acid (50 μM) was measured in the presence of an NaCl or KCl gradient (100 mM outside 0 mM inside). When brush-border membrane vesicles were subjected to three freeze-thaw treatments, uptake of γ-aminobutyric acid at 7.5 or 75 min was not significantly different from control values.

TABLE V

EFFECT OF RAPID FREEZE-THAW PROCEDURE ON SODIUM-DEPENDENT UPTAKE OF VARIOUS COMPOUNDS BY BRUSH-BORDER MEMBRANE VESICLES

Sodium-dependent uptake was measured (five replicates in each experiment) at the peak of overshoot. Treated aliquots of brush-border membrane vesicles (BBMV) were subjected to three freeze-thaw cycles. n.s., not significant. GABA, γ-aminobutyric acid.

Compound	Uptake (pmol/mg protein) (mean ± S.E.)		Ratio fresh/freeze-thaw
	fresh BBMV	freeze-thaw BBMV	
GABA (50 μM)	50 ± 1	54 ± 3	1.07 (n.s.)
L-Proline (26 μM)	130 ± 12	124 ± 8	0.95 (n.s.)
D-Glucose (50 μM)	193 ± 5	79 ± 8	0.41 ($P < 0.01$)
β-Alanine (98 μM)	297 ± 19	148 ± 8	0.50 ($P < 0.01$)
Taurine (44 μM)	586 ± 21	384 ± 40	0.65 ($P < 0.01$)

pentane on solid CO_2) and rethawed (22°C) three times, γ-aminobutyric acid transport was not altered (107% control at 10 min) (Fig. 5), whereas the sodium-dependent co-transports of D-glucose, β-alanine, and taurine were impaired (41, 50 and 65% of control, respectively) (Table V). γ-Aminobutyric acid uptake by snap-frozen brush-border membrane vesicles represented transport into the intravesicular space without non-specific binding, as shown by osmotic experiments. The freeze-thaw procedure had no effect on the diffusional component of uptake for any of these substrates.

Discussion

GABAergic neurons synthesize γ-aminobutyric acid from glutamate on a well-characterized glutamate decarboxylase (EC 4.1.1.15) [21], they exhibit high-affinity ($K_t = 30–50$ μM) sodium-dependent uptake of γ-aminobutyric acid which is selectively inhibited by nipecotic acid and diaminobutyric acid [12,22], and they innervate targets which possess γ-aminobutyric acid receptors intimately associated with chloride-selective channels at the cells surface [23,24]. Our earlier studies [5] demonstrated that renal glutamate decarboxylase is different from the neural enzyme. In the present study, we characterized renal γ-aminobutyric acid transport, using slices and brush-border membrane vesicles from rat renal cortex.

Slices demonstrate concentrative uptake when γ-aminobutyric acid oxidation is blocked. aminooxyacetic acid did not appear to impair energetics of γ-aminobutyric acid transport, since succinate did not further increase the rate of γ-aminobutyric acid transport or the observed slice/medium distribution ratio. The apparent K_t value at steady-state is analogous to the value at initial rates [9,25]. In the presence of aminooxyacetic acid, we observed a single high-affinity uptake mechanism (K_t approx. 26 μM) with properties typical of other systems mediating neutral amino acid transport in kidney slices, for example, dependence on sodium and temperature. Lack of inhibition by nipecotic acid and diaminobutyric acid indicates that the γ-aminobutyric acid transport system in kidney slices differs from that in GABAergic neurons.

γ-Aminobutyric acid transport by renal slices was impaired by *p*-aminohippurate. It is unlikely that this finding indicates a shared transport mechanism for the two compounds, since kinetic analysis sowed that inhibition was not competitive. The basal-lateral cell surface is predominantly exposed in slices [6,7]. *p*-Aminohippurate, an excellent substrate for the weak organic acid secretory mechanism at the basal-lateral surface [27], should therefore be readily transported. However, at millimolar concentrations, *p*-aminohippurate competes for energy and the sodium gradient and it may inhibit γ-aminobutyric acid uptake by these effects in the slice.

Transport of labelled γ-aminobutyric acid in renal slices was inhibited by β-alanine nearly to the same extent as by addition of equimolar unlabelled γ-aminobutyric acid. This finding suggests that γ-aminobutyric acid may share a β-amino acid-preferring transporter in basal-lateral membranes [15], or that γ-aminobutyric acid uptake, occurring on its own unique system, can be inhibited by β-amino acids. Taurine, a good substrate for the β-amino acid uptake system in slices [26], was no more effective as a competitive inhibitor than L-proline or *p*-aminohippurate; moreover the inhibition by β-alanine was not clearly competitive.

To determine whether γ-aminobutyric acid transport could be identified in preparations representative of the luminal surface of the proximal tubule, we studied γ-aminobutyric acid uptake by brush-border membrane vesicles. As is the case for other neutral amino acids and sugars, we observed that entry of γ-aminobutyric acid into the osmotically active space of brush-border membrane vesicles was stimulated by an inwardly directed sodium chloride gradient. In the presence of the imposed sodium gradient, γ-aminobutyric acid was transiently accumulated by brush-border membrane vesicles above its equilibrium value; overshoot was not observed in the presence of a KCl gradient. Furthermore, γ-aminobutyric acid uptake was affected by manipulations of the transmembrane potential gradient, suggesting electrogenic cotransport of γ-aminobutyric acid with one or more sodium ions. Brush-border membrane vesicles preloaded with KCl and valinomycin generate an electronegative interior [13]. Under these

conditions, Na gradient-dependent γ-aminobutyric acid co-transport was stimulated. When a relatively impermeant anion (sulfate) is substituted for a highly permeant anion (chloride) in the external medium, rapid anion influx favorable to the cotransport of sodium and neutral substrates is impaired [13]. Under these conditions, sodium-dependent γ-aminobutyric acid transport was abolished.

It is likely that γ-aminobutyric acid uptake by brush-border membrane vesicles represents uptake into vesicles derived from the proximal tubule luminal membranes rather than by contaminant basal-lateral membrane vesicles. Our brush-border membrane vesicles preparations were highly enriched in alkaline phosphatase activity (a brush-border enzyme). Furthermore, γ-aminobutyric acid uptake by brush-border membrane vesicles was reduced in the presence of other neutral organic substrates (D-glucose or L-proline). These compounds are transported across the luminal surface of the proximal tubule [14,15] and compete for the inward-directed sodium chloride gradient [13]. Brush-border membrane vesicles uptake of γ-aminobutyric acid was unaffected by *p*-aminohippurate.

Because sodium-dependent γ-aminobutyric acid co-transport by brush-border membrane vesicles is relatively slow compared to that of many α-amino acids and hexoses, we were unable to measure initial rates accurately enough for kinetic analysis. Instead, we calculated an apparent K_t by measuring uptake at 7.5 min. While this value may be different from the 'true' K_t calculated at the initial rates, it was useful for analyzing the effects of various transport inhibitors. As observed in slices, uptake of γ-aminobutyric acid by brush-border membrane vesicles was unaffected by established inhibitors of the neuronal γ-aminobutyric acid transport mechanisms (e.g., nipecotic and diaminobutyric acids), but it was substantially reduced by β-alanine, an inhibitor of γ-aminobutyric acid transport in glial cells. Kinetic analysis indicated that β-alanine was a competitive inhibitor of γ-aminobutyric acid transport by renal brush-border membrane vesicles. Three findings indicate a specific γ-aminobutyric acid-preferring system: (i) in the so-called 'ABC test' [18], labeled γ-aminobutyric acid uptake was inhibited by the addition of unlabelled γ-aminobutyric acid more than by β-alanine or taurine, whereas labelled β-alanine uptake was strongly inhibited by addition of unlabelled β-alanine or taurine, but minimally by γ-aminobutyric acid; (ii) influx of γ-aminobutyric acid into vesicles at NaCl equilibrium exhibited marked 'trans-stimulation' by γ-aminobutyric but not by taurine, an excellent substrate for the β-amino acid transporter [17,19]; (iii) γ-Aminobutyric acid uptake was preserved in brush-border membrane vesicles subjected to several rapid freeze-thaw procedures, whereas uptake of β-alanine, taurine, and D-glucose was substantially reduced. The freeze-thaw procedure did not change the time of peak uptake, the equilibrium uptake value or the sodium-independent diffusional component of uptake. Cryolability under these conditions is apparently a particular property of the transport protein involved rather than a non-specific effect on brush-border membrane vesicles integrity.

The role of dedicated γ-aminobutyric acid transport in mammalian kidney is unknown at this time. In rat blood, the concentration of γ-aminobutyric acid is about 0.8 μM [28], two or three orders of magnitude lower than the range for most other amino acids. In view of the extremely small filtered load it would seem irrelevant to have a specific γ-aminobutyric acid transport mechanism, if its sole function were to conserve endogenous γ-aminobutyric acid pools. A more appealing speculation is that high-affinity renal γ-aminobutyric acid transport, demonstrable in both renal slices and brush-border membrane vesicles, represents a mechanism by which the renal γ-aminobutyric acid content is maintained at a high level, or by which local extracellular γ-aminobutyric acid concentrations are minimized.

Acknowledgements

We acknowledge the excellent technical assistance of Miss Moira Mills. This work was supported by grants from the McGill University-Montreal Children's Hospital Research Institute and the Medical Research Council of Canada.

References

1 Schousboe, A. (1978) Adv. Exp. Med. Biol. 123, 263–280
2 Rozen, R., Goodyer, P.R. and Scriver, C.R. (1982) in Contemporary Metabolism Vol. II (Freinkel, N., ed.), pp. 189–237, Plenum Medical Book Company, New York
3 Bowery, N.G., Doble, A., Hill, D.R., Hudson, A.L., Shaw, J.S. and Turnbull, M.J. (1979) Proc. Br. Pharm. Soc. July, 444–445
4 Jessen, K.R., Mirsky, R., Dennison, M.E. and Burnstock, C. (1979) Nature 281, 71–74
5 Goodyer, P.R., Mills, M. and Scriver, C.R. (1982) Biochim. Biophys. Acta 716, 348–357
6 Arthus, M.F., Bergeron, M. and Scriver, C.R. (1982) Biochim. Biophys. Acta 692, 371–376
7 Cole, D.E.C., Koltay, M. and Scriver, C.R. (1984) Biochim. Biophys. Acta 776, 113–121
8 Booth, A.G. and Kenny, A.J. (1974) Biochem. J. 142, 575–581
9 Scriver, C.R. and Mohyuddin, F. (1968) J. Biol. Chem. 243, 3207–3213
10 Chesney, R.W., Jax, D.K., Mohyuddin, F. and Scriver, C.R. (1978) Renal Phys. 1, 166–170
11 Schon, F. and Kelly, J.S. (1975) Brain Res. 86, 243–257
12 Krogsgaard-Larsen, P. (1980) Mol. Cell Biochem. 31, 105–121
13 Sacktor, B. (1971) Curr. Top. Bioenerg. 6, 39–81
14 Beck, J.C. and Sacktor, B. (1975) J. Biol. Chem. 250, 8674–8680
15 Hammerman, M.R. and Sacktor, B. (1977) J. Biol. Chem. 252, 591–595
16 Schneider, E.G., Hammerman, M.R. and Sacktor, B. (1980) J. Biol. Chem. 255, 7650–7656
17 Hammerman, M.R. and Sacktor, B. (1978) Biochim. Biophys. Acta 509, 338–347
18 Scriver, C.R. and Wilson, O.H. (1964) Nature 202, 92–93
19 Rozen, R., Tenenhouse, H.S. and Scriver, C.R. (1979) Biochem. J. 180, 245–248
20 Hilden, S.A. and Sacktor, R. (1978) Kid. Int. 14, 279–282
21 Wu, J-Y. (1976) in Gaba in Nervous System Function (Roberts, E., Chase, T.N. and Tower, D.B., eds.), pp. 7–60, Raven Press, New York
22 Rososki, R. (1978) J. Neurochem. 31, 493–498
23 Andrews, P.R. and Johnston, G.A.R. (1979) Biochem. Pharmacol. 28, 2697–2702
24 Olsen, R.W., Meiners, B., Kehoe, P. and Ticku, M.K. (1977) Biochem. Soc. Trans. 5, 863–866
25 Tenenhouse, A. and Quastel, J.H. (1960) Can. J. Biochem. Physiol. 38, 1311–1326
26 Chesney, R.W., Scriver, C.R. and Mohyuddin, F. (1976) J. Clin. Invest. 57, 183–193
27 Podevin, R.A., Boumendil-Podevin, E.F. and Priol, C. (1978) Am. J. Physiol. 235(4), F278–F285
28 Ferkany, J.W., Smith, L.A., Seifert, W.E., Caprioli, R.M. and Enna, S.J. (1978) Life Sci. 22, 2121–2128