Substrate Specificity of the Escherichia coli 4-Aminobutyrate Carrier Encoded by gabP

UPTAKE AND COUNTERFLOW OF STRUCTURALLY DIVERSE MOLECULES*

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Casey E. Brechtel, Liaoyuan Hu, and Steven C. King‡

From the Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77555-0641

Transport of 4-aminobutyrate into Escherichia coli is catalyzed by gab permease (GabP). Although published studies show that GabP is relatively specific, recognizing the common ß-amino acids with low affinity, recent work from this laboratory indicates that a number of synthetic compounds are high affinity transport inhibitors (50% inhibition at 5-100 µM). Here we present evidence that many of these structurally heterogeneous compounds not only inhibit transport but also function as alternative GabP substrates (i.e. a set of observations inconsistent with the idea that the core of the GabP transport channel exhibits rigid structural specificity for the native substrate, 4-aminobutyrate).

The gab gene cluster is required for metabolism of 4-aminobutyrate in Escherichia coli. The cluster consists of a regulatory gene, gabC, two structural genes (gabD and gabT) encoding the metabolic enzymes, succinic semialdehyde dehydrogenase, and glutamate:succinic semialdehyde transaminase, and a third structural gene (gabP), encoding the 4-aminobutyrate transporter (gab permease or GabP). The GabP is a hydrophobic, 466-residue polypeptide (7) that is readily modeled as a transmembrane protein consisting of 12 transmembrane α-helical segments. The permease is active in whole cells as well as in right-side-out vesicles (3), and uptake of 4-aminobutyrate is stimulated by membrane potential and abolished by proton ionophores (7). Recently, we showed that a number of synthetic compounds are potent GabP inhibitors (8, 9). An unanswered question is whether any of these inhibitors might in fact be transported substrates of GabP. Here, we provide evidence consistent with the hypothesis that GabP transports at least nine different substrate analogs.

It is shown directly with radiolabeled compounds that two bulky and structurally distinct heterocyclic molecules are efficiently transported by the 4-aminobutyrate transporter expressed under control of a lac promoter. Transport of 3-piperidine carboxylic acid or 3-hydroxy-5-aminomethylisoxazole was dependent upon either the presence of plasmid-borne gabP or upon induction of gene expression with isopropylidinecarboxylic acid or 3-hydroxy-5-aminomethylisoxazole (muscimol); compound 3-aminopropylphosphonic acid; compound 4-aminobutyric acid; compound 4-amino-cis-2-butanolic acid, 3-piperidine carboxylic acid, cis-3-aminocyclohexyl carboxylic acid, 5-aminopentanoic acid, 3-aminobutanoic acid) could not be demonstrated directly since these were unavailable in radiolabeled form. However, the above named compounds exhibited behavior consistent with the hypothesis that they can serve as counterflow substrates of the 4-aminobutyrate transporter (i.e. under appropriate conditions GabP can translocate these compounds across the membrane). Among the inhibitors tested, GabP exhibited some preference for translocation of compounds that mimic a nonextended conformation of 4-aminobutyrate (modeled by 4-amino-cis-butanolic acid) over those that mimic the extended conformation (modeled by 4-aminotrans-butanolic acid). Regardless, the data indicate that the GabP transport channel can recognize and/or translocate a far more diverse range of chemical structures than previously imagined. It may be possible to exploit this diversity to develop both genetic and biochemical approaches aimed at identifying amino acid residues that affect ligand recognition and translocation.

EXPERIMENTAL PROCEDURES

Materials—[3H]GABA1 (31.6 Ci/mmol), [3H]muscimol (20 Ci/mmol), [3H]H2O (1 mCi/g), and [14C]taurine (180 mCi/mmol) were from DuPont NEN. The [3H]peptoid acid (28.5 Ci/mmol) was from Amersham Corp. Plasmid pSE380 was from Invitrogen (San Diego, CA), and pBLuescript II KS(−) was from Stratagene (La Jolla, CA). Restriction enzymes were from New England Biolabs (Beverly, MA). Kanamycin GenBlock was from Pharmacia Biotech Inc. Bacteriological media were Difco brand supplied by Fisher (Pittsburgh, PA). Transport inhibitor compounds were obtained from Sigma or Research Biochemicals (Natick, MA). DNA sequencing was performed with Sequenase from Amersham. Biocinchoninic acid protein assay reagents were from Pierce. Cellulose acetate filters (0.45 µm) were from Micron Separations, Inc. Westboro, MA). Silicic acid was Dow-Corning 510 fluid and 550 fluid. Liquiscint scintillation mixture was from National Diagnostics (Atlanta, GA). Other chemicals were from usual sources. Bacterial strains and plasmids are detailed in Table I.

Culture Conditions—LB medium (1% Bacto tryptone, 0.5% Bacto yeast extract, 1% NaCl) supplemented with ampicillin (150 µg/ml) was

1 The abbreviations and nomenclature used are: compound 2, 4-aminobutyric acid (GABA); IPTG, isopropyl-ß-d-thiogalactopyranoside; compound 1, 2-(aminomethyl)-5-hydroxy-4H-pyran-4-one; compound 3, 3-hydroxy-5-aminomethylisoxazole (muscimol); compound 4, 5-aminopentanoic acid; compound 5, 1,2,3,6-tetrahydro-3-pyridinocarboxylic acid; compound 6, 5-(aminomethyl)-3-2H-isothiazalzone; compound 7, 3-piperidinecarboxylic acid (nipeptid acid); compound 8, cis-3-aminocyclohexyl carboxylic acid; compound 9, 3-aminobutyric acid; compound 10, 3-aminopropionic acid; compound 11, 4-amino-cis-2-butanolic acid; compound 12, 6-aminohexanoic acid; compound 13, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; compound 14, 1,2,3,6-tetrahydro-4-pyrindinecarboxylic acid; compound 15, 3-piperidinecarboxylic acid; compound 16, 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol; compound 17, 3-aminopropylphosphonic acid; compound 18, 4-aminotrans-butanolic acid.

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Disintegrations per minute for each isotope were calculated as de-
mixed 7 parts 550 fluid with 3 parts 510 fluid, and after phase separa-
tion was empirically adjusted to an appropriate specific gravity (aqueous
medium by centrifugation through a silicone oil mixture, which
was washed with 4 ml of buffer containing 5 mM HgCl2. The filter was
dissolved in 20 mM HgCl2, and this was applied to the same filter. Finally, the filter
was washed with 4 ml of buffer containing 5 mM HgCl2. The filter was
dissolved in scintillation mixture, and the radioactivity (disintegrations
per minute) was counted for scintillation counting as described under “Experimental Pro-
cedures.” Control experiments indicated that compounds 1 (10 mM) and
6 (1 mM) had no inhibitory effect on the LacY-mediated transport of
[14C]methyl-β-o-thiogalactopyranoside, suggesting that the observed
inhibition of [3H]GABA transport by these compounds could not be
explained by a global effect on membrane energization (data not shown).

Strain or plasmid | Chromosome or Plasmid | Reference
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SK 45 | lac− Δ(ZY) gab pSKC-380 | 8
SK 55 | lac− Δ(ZY) gab pSKC-472A | 8
pSKC-472A | Amp′ lac′ tac O′ P− gabP− | 8
pSKC-380a | Amp′ lac′ tac O′ P− | 8

a This is the IPTG-inducible expression vector without the gabP in-
sert in its polylinker.

used to grow the Escherichia coli strains SK 45 and SK 55 for use in
experiments with cloned gabP. Cells grown overnight (16 h) were
diluted 100-fold into fresh medium (approximately Klett 10) containing 1
ml of IPTG to induce high level gabP expression. Cells were grown for
three to four doublings (Klett 130–150 with number 42 blue filter).

Transport—[3H]GABA or [3H]nipecotic acid transport was studied
under conditions in which gabP was expressed from the lac-inducible
plasmid, pSKC-472A, contained in E. coli strain SK 55. Log phase cells
were processed by washing twice with 100 mM potassium phosphate
(pH 7.0). The cell pellet was resuspended in this buffer using 25–50% of
the original culture volume such that the protein concentration was 1–2
mg/ml in such cells. “Washed” refers to bacteria that were “washed cells.”

Transport reactions were initiated by adding 80 μl of washed cells
with rapid vortex mixing to 20 μl of solution containing the radiolabeled
substrate and other additions (conditions indicated in the figure leg-
ends). A metronome was used to reproducibly time short uptake reactions
(2–20 s). Uptake was rapidly quenched by adding 1 ml of a “stop
solution” (buffer containing 20 mM HgCl2) to the rapidly mixing reaction
vessel. The “quenched” reaction was vacuum-filtered. The reaction ves-
sel was then washed with 1 ml of 100 mM potassium phosphate (pH 7.0),
5 mM HgCl2, and this was applied to the same filter. Finally, the filter
was washed with 4 ml of buffer containing 5 mM HgCl2. The filter was
dissolved in scintillation mixture, and the radioactivity (disintegrations
per minute) was counted for scintillation counting as described under “Experimental Pro-
cedures.” Control experiments indicated that compounds 1 (10 mM) and
6 (1 mM) had no inhibitory effect on the LacY-mediated transport of
[14C]methyl-β-o-thiogalactopyranoside, suggesting that the observed
inhibition of [3H]GABA transport by these compounds could not be
explained by a global effect on membrane energization (data not shown).

Nitrogen-limiting conditions induce in E. coli the ability to
utilize 4-aminobutyrate as a source of carbon and nitrogen
(3–6). This capability derives jointly from induction of the
requisite metabolic enzymes as well as from induction of a
transporter that catalyzes accumulation of 4-aminobutyrate from
the environment. Previous studies (3, 7) agree that the GabP
exhibits biological specificity, rejecting common constituents
from the cellular milieu (e.g. the 20 common α-amino acids,
ornithine, putrescine) that exhibit at least superficial struc-
tural similarity to 4-aminobutyrate.

On the other hand, studies (8, 9) show that a number of
synthetic compounds (e.g. Fig. 1) are potent GabP inhibitors.
Although these molecules bear some structural resemblance to
the native substrate, 4-aminobutyrate (compound 4), the
degree of dissimilarity was sufficient to warrant speculation that
some of these molecules (particularly the heterocycles) might
act at an allosteric inhibitory site rather than within the GabP
transport channel itself. The experimental evidence presented

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2 Transmembrane passage or “transport” of the preloaded compound
is suggested by both the rising and the falling phases of the counterflow
time course. The rising phase reflects trans-stimulation of uptake me-
diated by rapid exchange of the preloaded substrate for extracellular
[3H]GABA. That the unlabeled compound can participate in the ex-
change reaction implies that it is a substrate capable of transmembrane
passage via the same carrier (i.e. GabP) that transports [3H]GABA (10–14).
The transient nature of intracellular [3H]GABA accumulation suggests addi-
tionally that the carrier (i.e. GabP) has the capacity (i) to
catalyze net efflux of the preloaded substrate (reflected in the falling
phase) and (ii) to transduce energy, available in the transmembrane
gradient of preloaded substrate, to drive concentrative uptake of
[3H]GABA (reflected in the peak “GABA uptake ratio” defined under
“Experimental Procedures”).
coli cells were washed, resuspended in 100 mM potassium phosphate (pH 7.0), and then exposed to 100 μM [3H]nipecotic acid (0.5 μCi/ml) in the presence (open symbols) or absence (solid symbols) of 1 mM unlabeled nipecotic acid (compound 7). Inset, washed E. coli SK55 cells were exposed to 100 μM [3H]nipecotic acid (0.5 μCi/ml) along with the indicated concentrations of GABA for 60 s. The broken line indicating 50% inhibition was calculated from the uptake velocity measured in the absence of GABA. [3H]nipecotic acid uptake was rapidly quenched by the addition of a mercuric chloride stop solution. The cells were harvested by vacuum filtration, and radioactivity was quantitated as described ("Experimental Procedures").

The cells were grown in rich medium with (0.4 mM IPTG) along with the indicated concentrations of GABA prior to exposure (compound 4) or absence (compound 7) of 1 mM unlabeled GABA (Fig. 3). The observed transport of [3H]nipecotic acid and [3H]muscimol was consistent with the published Km (10–12 μM) of the GabP for GABA (3, 7). The observed transport of [3H]nipecotic acid and [3H]muscimol supports the proposal that these heterocyclic compounds occupy the GabP transport channel (rather than an allosteric site) to inhibit [3H]GABA uptake.

Additionally, it will be noted (Fig. 4) that nipecotic acid (compound 7) and [3H]muscimol (compound 3) are transported quite specifically by GabP (Figs. 2 and 3, respectively). Transport of these compounds depended strongly upon either the presence of plasmid-borne gabP (Fig. 2) or upon specific induction of gabP expression by IPTG (Fig. 3). Transport of [3H]GABA, the native substrate, was induced by IPTG in this expression system (8). Moreover, 10 μM GABA was found to inhibit transport of either [3H]nipecotic acid (Fig. 2, inset) or [3H]muscimol (Fig. 3, inset) by about 50%, an effect consistent with the published Km (10–12 μM) of the GabP for GABA (3, 7). The observed transport of [3H]nipecotic acid and [3H]muscimol supports the proposal that these heterocyclic compounds occupy the GabP transport channel (rather than an allosteric site) to inhibit [3H]GABA uptake.

Compounds 4 and 10 might in fact be transported GabP substrates. Compounds 4 and 10, like most of the inhibitory compounds described here and elsewhere (8), are unavailable in labeled form. Thus, an indirect method, entrance counterflow (10–14), was used to determine whether GabP might transport these nonradiolabeled inhibitors. First, the use of counterflow in this context was validated by showing that known GabP substrates support transient [3H]GABA accumulation (counterflow) in

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Fig. 2. The gabP dependence of nipecotic acid uptake by E. coli K-12. The cells were grown in rich medium supplemented with 0.6 mM IPTG to induce gabP expression. E. coli SK55 (●, ○) or SK45 (▲) cells were washed, resuspended in 100 mM potassium phosphate (pH 7.0), and then exposed to 100 μM [3H]nipecotic acid (0.5 μCi/ml) in the presence (open symbols) or absence (solid symbols) of 1 mM unlabeled nipecotic acid (compound 7). Inset, washed E. coli SK55 cells were exposed to 100 μM [3H]nipecotic acid (0.5 μCi/ml) along with the indicated concentrations of GABA for 60 s. The broken line indicating 50% inhibition was calculated from the uptake velocity measured in the absence of GABA. [3H]nipecotic acid uptake was rapidly quenched by the addition of a mercuric chloride stop solution. The cells were harvested by vacuum filtration, and radioactivity was quantitated as described ("Experimental Procedures").

Fig. 3. The gabP dependence of muscimol uptake by E. coli K-12. The cells were grown in rich medium with (solid symbols) or without (open symbols) 1 mM IPTG to induce gabP expression. E. coli SK55 (●, ○) or SK45 (▲) cells were washed, resuspended in 100 mM potassium phosphate (pH 7.0), and then exposed to 1 μM [3H]muscimol (0.4 μCi/ml) for the indicated times. [3H]Muscimol (compound 3) uptake was rapidly quenched by the addition of a mercuric chloride stop solution. The cells were harvested by vacuum filtration, and radioactivity was quantitated as described (see "Experimental Procedures"). Inset, washed E. coli SK55 cells were exposed for 15 s to 1 μM [3H]muscimol (0.4 μCi/ml) along with the indicated concentrations of GABA prior to quenching with stop solution as described above. The broken line indicating 50% inhibition was calculated from the uptake velocity measured in the absence of GABA.

Nipecotic Acid Uptake (mol/mg protein)

Time (sec)

Muscimol Uptake (mol/mg protein)

Time (sec)

[3H]muscimol (compound 3) is available as [3H]nipecotic acid so that transport has been directly demonstrated. Likewise, compound 7 is available as [3H]nipecotic acid so that transport has been directly demonstrated (Figs. 2 and 5). Compounds 8, 4, 10, and 9 are not available in labeled form, but transport has been inferred from the participation of these molecules in the counterflow reaction (Fig. 7). It will be noted that compound 2 is incorporated into the compound 8 ring structure. Likewise, compounds 4 and 10 are incorporated into the compound 7 ring structure. In addition to participating in counterflow, all of these molecules inhibit either [3H]GABA or [3H]nipecotic acid transport mediated by GabP.

Several lines of evidence suggest that both [3H]nipecotic acid (compound 7) and [3H]muscimol (compound 3) are transported quite specifically by GabP (Figs. 2 and 3, respectively). Transport of these compounds depended strongly upon either the presence of plasmid-borne gabP (Fig. 2) or upon specific induction of gabP expression by IPTG (Fig. 3). Transport of [3H]GABA, the native substrate, was induced by IPTG in this expression system (8). Moreover, 10 μM GABA was found to inhibit transport of either [3H]nipecotic acid (Fig. 2, inset) or [3H]muscimol (Fig. 3, inset) by about 50%, an effect consistent with the published Km (10–12 μM) of the GabP for GABA (3, 7). The observed transport of [3H]nipecotic acid and [3H]muscimol supports the proposal that these heterocyclic compounds occupy the GabP transport channel (rather than an allosteric site) to inhibit [3H]GABA uptake.

Additionally, it will be noted (Fig. 4) that nipecotic acid (compound 7) contains within its structure the basic elements of two open-chain amino acids, 5-aminopentanoic acid (compound 4) and 3-aminopropanoic acid (compound 10). Thus, nipecotic acid has the potential to be recognized by GabP in the context of either compound 4 or compound 10 (or both). Indeed, compounds 4 and 10 were found to inhibit [3H]nipecotic acid transport (Fig. 5) with IC50 values of 10 μM and 200 μM, respectively. These experiments are in accord with previous studies showing that 5-aminopentanoic acid and 3-aminopropanoic acid inhibit [3H]GABA transport with similar apparent affinities (8). A related and very important question is whether compounds 4 and 10 might in fact be transported GabP substrates.

Compounds 4 and 10, like most of the inhibitory compounds described here and elsewhere (8), are unavailable in labeled form. Thus, an indirect method, entrance counterflow (10–14), was used to determine whether GabP might transport these nonradiolabeled inhibitors. First, the use of counterflow in this context was validated by showing that known GabP substrates support transient [3H]GABA accumulation (counterflow) in
metabolically poisoned SK55 cells (Fig. 6). Then the capacity of many other nonradiolabeled transport inhibitors to support GabP-mediated counterflow was investigated (see Fig. 7 for summary).

Counterflow with Known Substrates—Preloading metabolically poisoned E. coli SK55 with either the native substrate, GABA (compound 2), or the alternative substrates, nipeptic acid (compound 7) and muscimol (compound 3), resulted in transient \(^{3}H\)GABA accumulation, which was not evident in SK55 cells that were not preloaded or in SK45 control cells lacking a functional GabP. 4-aminobutyrate is, of course, the best known GabP substrate (3, 7, 8), and transport of this molecule is clearly reflected in the counterflow data (Fig. 6). Likewise transport of compounds 3 and 7, shown here to be alternative GabP substrates (Figs. 2 and 3), is clearly reflected in the counterflow data (Fig. 6). This well known (10, 12–14) correlation between counterflow in metabolically poisoned cells and uptake in metabolically active cells can thus reasonably\(^{3}\) be used to implicate the GabP in the transport of other compounds that are not readily available in radioactive form (9). The results suggest that effective inhibitors are not always effective counterflow substrates (Fig. 7).

Counterflow with Test Compounds—Test compounds 18, 1, and 6 (all unsaturated) failed to support counterflow activity. Compound 18, 4-amino-trans-butenoic acid, was neither an inhibitor (Fig. 1), nor a counterflow substrate (Fig. 7), whereas its cis isomer (compound 11) was both a relatively potent inhibitor (Fig. 1) and a counterflow substrate (Fig. 7). In contrast, compounds 1 and 6 were relatively potent inhibitors (Fig. 1) but were not counterflow substrates (Fig. 7).

Within this series of unsaturated test compounds, those that failed to support counterflow share a common structural element (Fig. 8). The carboxyl carbon and the amine moiety are in the trans configuration relative to the double bond that these analogs share in common. It will be noted that the native substrate, 4-aminobutyrate (compound 2), rotates freely about the C2–C3 bond so that the molecule can assume conformations similar to either the cis or the trans isomers of 4-aminobutenoic acid. The profound functional differences between these conformationally restricted cis and trans isomers may imply something about the conformation(s) of GABA that are preferred by GabP.

Indeed, with appropriate bond rotations, the electronegative atoms in 4-amino-cis-butenoic acid (compound 11) can be made nearly isosteric with those of another conformationally restricted GABA analog, cis-3-amino-cyclohexyl carboxylic acid (Fig. 4, compound 8) so that either molecule might be imagined to dock (hydrogen bond) with the same complementary surface (eg. GabP).\(^{4}\) Like compound 11 (4-amino-cis-butenoic acid), compound 8 (cis-3-amino-cyclohexyl carboxylic acid) is not only

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\(^{3}\)Counterflow provides only an indirect indication that GabP can carry a particular preloaded substrate across the membrane. Under what circumstances might the presence or absence of a counterflow phenomenon be misleading? A genuine substrate having poor affinity for the inwardly oriented carrier might not significantly entrap \(^{3}H\)GABA entering from the outside, i.e. a small \(\gamma\) factor in the formalism of Wright (15). Alternatively, rapid degradation of a preloaded substrate could prevent observation of counterflow. However, it should be noted in this regard that GABA, clearly metabolized by GabT (16), nevertheless survives metabolic degradation long enough to allow experimental demonstration of counterflow (Fig. 6). Other investigators (3) using thin layer chromatography to analyze the state of intracellular GABA have also concluded that there is little degradation over the course of a transport experiment. A positive counterflow result (and the associated trans-stimulation) is more difficult to explain as an artifact. Possibly, a nonsubstrate inhibitor molecule might be metabolized to a substrate capable of trans-stimulating \(^{3}H\)GABA uptake. However, we doubt that such a fortuitous mechanism could occur frequently enough to make it an attractive artificial explanation for the present results (Fig. 7) in which nine different compounds were shown to support counterflow. Certainly this artificial mechanism is unattractive for compounds 2, 3, and 7 since direct methods have shown these to be GabP substrates.

\(^{4}\)The idea that GabP prefers a nonextended form of GABA may help to rationalize why compound 10 can be a substrate as well as why compounds 5 and 7 are preferred (8, 9) over compounds 14 and 15 (Fig. 7) even though the latter pair are more analogous to the extended conformation of GABA. Model building in fact substantiates that after appropriate rotations, the hydrogen bonding elements of compounds 4, 5, 7, and 10 can be made nearly isosteric with those of 4-amino-cis-butenoic acid (compound 11), a result consistent with the experimental observation that all five of these molecules are GabP substrates.
a GabP inhibitor but also a counterflow substrate (Fig. 7). When preloaded into metabolically poisoned E. coli SK55, compound 8 stimulated a transient accumulation of \([3H]GABA\), which was not observed in non-preloaded cells. It will be noted (Fig. 4) that part of the compound 8 ring structure indeed mimics the native substrate, GABA (compound 2), which is a high affinity ligand (Fig. 2). The remainder of the compound 8 ring mimics 6-aminohexanoic acid (compound 12), a relatively nonpotent inhibitor of \([3H]GABA\) transport (Fig. 7). We found no evidence that compound 12 could drive \([3H]GABA\) counterflow. Other nonradiolabeled compounds of interest are 5-aminopentanoic acid (compound 4) and 3-aminopropanoic acid (compound 10). As noted compound 4, a high-affinity GabP inhibitor (Fig. 5), can be recognized within the ring structure (Fig. 4) of
Cells resulted in transient accumulation of [3H]GABA and mM sodium azide to prevent active substrate accumulation (see "Experimental Procedures"). The poisoned cells were incubated with (solid symbols) or without (open symbols) an unlabeled compound (10 mM) in order to assess the effect of preloading the cells with noncyclic analogs of compound 7 (see Figs. 4 and 5). Countercurrent was initiated by diluting the poisoned cells 200-fold into medium containing 10 μM [3H]GABA (0.2 μCi/ml). The preloaded compounds were 5-aminopentanoate (compound 4), 3-aminopropanoate (compound 10), or 3-aminobutyrate (compound 9). Although modest, the uptake ratio for compound 10 was highly reproducible.

As mentioned, 5-aminopentanoate (compound 4) and 4-aminobutyrate (compound 2) are more potent GabP inhibitors (Fig. 7) than either the longer (6-aminohexanoate, compound 12) or the shorter (3-aminopropanoate, compound 10) open-chain amino acids (8). The separation distance between the amino and carboxyl groups could be a possible basis for the different inhibitory potencies. However, the behavior of these compounds in countercurrent suggests that the hydrocarbon skeleton is also significant.

Like 3-aminopropanoate (compound 10), 3-aminobutyrate (compound 9) is only a moderately potent GabP inhibitor (Fig. 7). On the other hand, the extra methyl group in compound 9 preserves the charge separation relative to compound 10 and improves countercurrent substantially (Fig. 9). The observed transient accumulation of [3H]GABA and trans-stimulation of [3H]GABA uptake are consistent with the notion that both 3-aminobutyrate (compound 9) and 3-aminopropanoate (compound 10) are molecules that GabP can recognize, transport, and couple to a GABA counterflux. That GabP effectively recognizes compound 9 (3-aminobutyrate) is consistent with other evidence (see Fig. 8 and Footnote 4) that the permease prefers analogs that mimic a nonextended conformation of the native substrate, 4-aminobutyrate.4

Irrespective of detailed mechanism, the data presented here support a structure/function model in which numerous molecules having different sizes and shapes are capable of interacting with GabP either as inhibitory ligands or as transported substrates. Perhaps most significantly, the countercurrent assay has provided the first evidence that several potent to moderately potent inhibitory compounds, unavailable in radiolabeled form, are likely to be transported substrates capable of traversing the core of the GabP transport channel. On the other hand, the countercurrent assay also suggested that some potent inhibitors (compounds 1 and 6) that mimic the extended conformation of 4-aminobutyrate do not appear to be countercurrent substrates.

In short, a broad range of structurally and perhaps functionally distinct GabP ligands has been identified. The availability of these ligands may allow successful implementation of strategies to select substrate specificity mutants and/or strategies to permit the synthesis of successful active site probes (affinity labels). Thus, dual approaches (genetic and biochemical) to identifying amino acid residues important in the recognition and/or translocation of substrates now appear more feasible than would have been suggested by previous models, which indicated that GabP might be highly selective and unable to recognize alternative ligands.

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