Pyridine Carboxylic Acids as Inhibitors and Substrates of the *Escherichia coli* gab Permease Encoded by *gabP*

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Although considered selective for its natural substrate, 4-aminobutyrate, *gab* permease was inhibited by 1,2,3,6-tetrahydro-3-pyridinecarboxylate and 1,2,3,6-tetrahydro-4-pyridinecarboxylate. The former is a transported substrate, since its preloading into metabolically poisoned cells stimulated transient accumulation of 4-aminobutyrate via counterflow.

In order to study molecular recognition by *gab* permease (3, 4), we used a permease-negative [gabΔ *lacΔ (ZY)] *Escherichia coli* K-12 strain (SK35) to create additional strains harboring either an expression plasmid (strain SK45) or the same plasmid containing *gabP* under the control of a *lac* promoter (strain SK55). [3H]GABA (4-aminobutyrate) transport depended on the presence of *gabP* and its induction with IPTG (isopropyl-β-d-thiogalactopyranoside) (3a). Similarly, transport of the alternative substrate, [3H]nipecotic acid (3-piperidine carboxylic acid; compound 4), was *gabP* dependent and subject to inhibition (50% at 10 μM) by the natural substrate, 4-aminobutyrate (3a). We then asked whether several other compounds might likewise inhibit transport of these labeled *gab* permease substrates. These compounds were obtained either from Sigma (St. Louis, Mo.) or from Research Biochemicals (Natick, Mass.).

Although 4-aminobutyrate, the natural *gab* permease substrate (compound 1), appears to be structurally more similar to compounds 3 and 5 than to compounds 2 and 4 (Fig. 1), transport inhibition studies indicated that the carboxyl substituent in ring position 3 was better recognized than the substituent in position 4 (Fig. 2). The importance of substituent positioning was demonstrated by using twice-washed (100 mM potassium phosphate, pH 7.0; hereafter called buffer) cells that had been grown (Luria-Bertani medium plus 1 mM IPTG) to early logarithmic phase. In a total reaction volume of 0.1 ml, washed cells were exposed for 30 s to 10 μM [3H]GABA (5 μCi/ml) or for 15 s to 1 μM [3H]nipecotic acid (5 μCi/ml) as well as to the appropriate test compounds at the indicated concentrations (Fig. 2). These times were chosen to be in the linear range of the uptake time course. Transport was rapidly terminated with a stop solution (buffer containing 20 mM HgCl₂). Each datum point represents 0.1 to 0.2 mg of protein (bovine serum albumin standard and BCA Assay [Pierce, Rockford, Ill.]) or 0.5 to 1.0 μl of cytoplasmic water as determined by using 2H2O and 14C]taurine as a marker for the extracytoplasmic space (2, 6). The results were normalized to transport exhibited by SK55 cells that were not exposed to an inhibitor. The observed inhibitory effects (Fig. 2) prompted us to ask whether any of these heterocyclic compounds might be transported substrates of *gab* permease.

That *gab* permease translocates compound 2 across the membrane was confirmed by demonstrating that this heterocyclic molecule drives transient accumulation (counterflow) of [3H]GABA in metabolically poisoned (30 mM NaN₃) cells grown and washed as described above. Counterflow experiments were initiated by diluting cells (poisoned with 30 mM NaN₃ and preloaded with 10 mM compound 2) 200-fold into buffer containing 10 μM [3H]GABA (0.2 μCi/ml) and 30 mM NaN₃. Transient uptake of [3H]GABA by poisoned SK55 required preloading with compound 2, since only equilibration to the passive level occurred in the control experiment with nonpreloaded SK55 (Fig. 3). Transient uptake also required *gab* permease, since SK45 exhibited no activity (i.e., it exhibited activity equivalent to that exhibited by nonpreloaded SK55). Results were normalized to the presumptive equilibrium level of [3H]GABA uptake exhibited by poisoned, nonpreloaded SK55 cells. Compounds 3 and 5 did not support counterflow in analogous experiments (data not shown).

Counterflow is characteristic of carrier-mediated uptake mechanisms (1, 5, 7), and the rising phase (Fig. 3) of the counterflow time course reflects carrier-mediated exchange of the preloaded substrate for the labeled extracellular substrate. The falling phase (Fig. 3) of the counterflow time course reflects the exponential relaxation of the preloaded substrate.
toward equilibrium with the extracellular medium (i.e., the gradient of preloaded substrate collapses and with it collapses the energy to sustain concentrative uptake of the labeled substrate). Thus, counterflow provides two criteria by which to judge that compound 2 is a transported substrate of the \textit{gab} permease. We mention too that compound 4—which in labeled form is obviously transported by \textit{gab} permease (Fig. 2)—also supports transient [3H]GABA uptake when preloaded into poisoned cells (3a), indicating in concrete terms that counterflow reports on the translocation of heterocyclic compounds via the 4-aminobutyrate transporter.

In aggregate, these studies show that \textit{gab} permease recognizes both the piperidine carboxylic acids (compounds 3 and 5) and the pyridine carboxylic acids (compounds 2 and 4). These heterocyclic compounds are recognized with higher apparent affinity when the carboxyl group is in ring position 3. The observation that 1,2,3,6-tetrahydro-3-pyridinecarboxylic acid (compound 2) supports \textit{gabP}-dependent counterflow of [3H]GABA (Fig. 3) demonstrates for the first time that this heterocyclic molecule enters the \textit{gab} permease transport channel and is translocated across the membrane.

FIG. 2. Inhibition of \textit{gab} permease. Cells were exposed to either 10 \textmu M [3H]GABA (open symbols) or 1 \textmu M [3H]pipecolic acid (solid symbols) together with the indicated concentrations of compound 2 (●, ○), compound 3 (△, ◆), or compound 5 (■, □).

FIG. 3. Counterflow of [3H]GABA driven by compound 2. Metabolically poisoned \textit{E. coli} SK55 (●, ○) and SK45 (△) cells were (solid symbols) or were not (open symbols) preloaded with 10 mM compound 2 and then were diluted into 10 \textmu M [3H]GABA to initiate counterflow.

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