Molecular Transport Mechanisms of Radiolabeled Amino Acids for PET and SPECT

Amino acids are important biologic substrates that play crucial roles in virtually all biologic processes. These ionic nutrients serve not only as basic modules of proteins and hormones but also as neurotransmitters, synaptic modulators, or neurotransmitter precursors. Transfer of amino acids across the hydrophobic domain of the plasma membrane is mediated by proteins that recognize, bind, and transport these amino acids from the extracellular medium into the cell, or vice versa. In the early 1960s, different substrate-specific transport systems for amino acids in mammalian cells were identified (*1*). General properties of mammalian amino acid transporters were revealed, such as stereospecificity and broad substrate specificity (i.e., several amino acids share the same transport system). Functional criteria such as the type of amino acid (e.g., basic, acidic) or thermodynamic properties (energy dependence of transport) were used to classify amino acid transporters. This classification has been retained to date in functional studies when the molecular mediators of transport are unknown. The molecular identification of amino acid transporters or related proteins started in the early 1990s, and studies on the structure–function relationship and the molecular genetics of the pathology associated with these transporters have generated considerable interest. The molecular identification of almost all physiologically characterized amino acid transporters in recent years has facilitated the functional analysis of this important class of transport proteins. These studies indicate that although several amino acid transporters are ubiquitous and serve to maintain nutritional demands, many cells additionally express specific transporters that allow the accumulation of particular amino acids. Tumor cells derived from specific cell types often continue to express cell-specific transporters and therefore accumulate certain amino acids more than others.

Radiolabeled amino acids have been considered in nuclear medicine since the early 1960s (*2*). With the advent of PET, many physiologic amino acids have been radiolabeled by the replacement of a carbon atom by ^{11}C, which does not chemically change the molecule (*3,4*). The most frequently used amino acid for PET is L-[methyl-^{11}C]-methionine (MET), and convincing clinical results, especially for the diagnostics of brain tumors, have been reported (*5*).

Because of the short—20-min—half-life of ^{11}C, however, the use of MET remains limited to the few PET centers with an on-site cyclotron. Stimulated by the promising results of PET studies on brain tumors with MET, research on the SPECT tracer 3-^{123}I-iodo-α-methyl-L-tyrosine (IMT) was intensified in the late 1980s (*6,7*), and many studies have investigated tumor imaging with this tracer (*5,8*). IMT exhibits increased uptake in many tumor types, although it is not incorporated into protein and remains metabolically unchanged within the cells. This is true for most radiolabeled amino acids presently used in nuclear medicine and indicates that an alteration of amino acid transport is the dominating process for increased uptake (*9*). Several studies have been undertaken to characterize the transport mechanisms of this unphysiologic amino acid by inhibition experiments in vitro, which have demonstrated that IMT transport is dominated by sodium-independent amino acid transport via system L (*8*).

The adoption of modern techniques of molecular biology such as expression of specific transporter subtypes in *Xenopus* oocytes allows a more specific evaluation of the transport characteristics of radiolabeled amino acids than can be provided by inhibition experiments in cell cultures alone (*10*). The first study using this approach to characterize IMT transport demonstrated that IMT is selectively transported by the LAT1 subtype of system L, whereas the natural parent L-tyrosine is transported by both LAT1 and LAT2 (*11*). This observation indicates that radiolabeled amino acids may be transported more selectively than natural amino acids and as a consequence might be more specific for the diagnostics of special pathologic processes.

On pages 1591–1596 of this issue of *The Journal of Nuclear Medicine,* Lahoutte et al. (*12*) investigate the transport characteristics of ^{123}I-2-iodotyrosine (2IT).

^{123}I-2IT shows some clinical advantages over IMT: lower renal accumulation and lower uptake in inflammatory tissue (*13*). The transport mechanisms of 2IT are analyzed and compared with those of IMT and 2 fluorinated amino acids currently under consideration for PET: *O*-(2-^{18}F-fluoroethyl)-L-tyrosine (FET) and 2-^{18}F-fluoro-L-tyrosine (2FT). FET is a promising new PET tracer because of efficient radiosynthesis and, like ^{18}F-FDG, offers widespread clinical applications (*14,15*). 2FT is incorporated into protein, in contrast to most other artificial amino acids, but radiosynthesis is not as efficient as for FET (*16,17*).

Expression of 4F2hc-LAT1 in *Xenopus laevis* oocytes shows that affinity of 2IT for LAT1 is similar to that of L-tyrosine. The experiments do not in-

Received Apr. 28, 2004; revision accepted May 20, 2004.

For correspondence or reprints contact: Karl Josef Langen, MD, Institut für Medizin, Forschungszentrum Jülich, Jülich, Germany D-52425.

E-mail: k.j.langen@fz-juelich.de

clude expression experiments with LAT2, and the question remains open of whether 2IT, like tyrosine, is also transported by LAT2 or other amino acid transporters (*11*). Furthermore, transstimulation experiments have shown that the influx rate of 2IT via LAT1 is comparable to that of IMT but lower than that of 2FT. In contrast, FET influx via LAT1 was found to be poor.

These experiments give interesting new insights into the variable transport characteristics of these different new radiolabeled amino acids. Especially, the poor transport of FET via LAT1 is surprising, since brain tumor imaging with this amino acid is comparable to that with MET and IMT (*18–20*). For FET, in contrast to MET and ^{18}F-FDG, a low uptake in inflammatory tissue has been observed (*21*,*22*). It is tempting to speculate that the selectivity of FET for certain transporter subtypes that are not expressed in those tissues may account for this phenomenon. Similarly, selective accumulation has been observed with other fluorinated amino acids, that is, the diastereoisomers *cis*-4-^{18}F-fluoro-L-proline and *trans*-4-^{18}F-fluoro-L-proline (*23*,*24*). Minor differences in transport characteristics have been identified and lead to considerable differences in the whole-body distribution in humans (*25*).

It appears that the diagnostic potential of radiolabeled amino acids will be amplified by enlarging knowledge of the molecular mechanisms involved in the transport processes. The application of radiolabeled amino acids with selective transport characteristics will offer a new tool for a more specific evaluation of pathologic processes. Furthermore, a new window may be opened for the pretherapeutic assessment of antitumor drug selectivity. For instance, the phenylalanine mustard melphalan is transported by LAT1 and accumulates in cancer cells (*26*).

The rapidly developing knowledge about the molecular genetics of amino acid transport systems and the capability of nuclear medicine techniques to measure such processes in humans offer great potential for improved diagnostics of numerous diseases.

Karl-Josef Langen, MD
Research Center Jülich
Jülich, Germany

Stefan Bröer, PhD
Australian National University
Canberra, Australia

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