Radiotracers for Cerebral Functional Imaging—A New Class

Conventional radiopharmaceuticals used for scanning the brain are excluded from normal brain tissue by the presence of an intact blood-brain barrier (BBB). Any radiopharmaceutical can be used as a crude brain scanning agent if it remains in the blood and is prevented from crossing the BBB by virtue of either its hydrophilicity, size, or protein binding ability. The fantastic selectivity of the BBB has greatly facilitated the development of brain scanning agents. In 1975 Konikowski (*1*) evaluated 16 different agents in mice, all of which were effectively excluded by the BBB and therefore capable of measuring its integrity.

Toward the end of the 1970s, however, nuclear medicine began placing less emphasis on measuring the integrity of the BBB and more emphasis on the regional determination of cerebral blood flow, glucose metabolism, and oxygen utilization. Measurements such as these require radiopharmaceuticals that are capable of crossing the BBB, a requirement currently met only by incorporating the positron-emitting isotopes C-11, N-13, O-15, and F-18 into suitable compounds. Whereas clinical results obtained using these short-lived radioisotopes are of intensive pharmacological interest, none of these agents has yet been used for the widespread delivery of diagnostic health care. For clinical nuclear medicine to develop a parallel capacity to quantitate cerebral function, it will require both high resolution single-photon tomographs and new radiopharmaceuticals capable of crossing the BBB and describing cerebral function. Single photon transaxial and longitudinal tomographs are now under development in academic centers and industry. Work in this area is progressing rapidly enough that the rate-limiting step in the routine quantitation of cerebral function appears to be the development of the required brain function agents that incorporate commonly available radioisotopes, such as Tc-99m and I-123.

The work of Kung and Blau (*2*) reported in this issue of the *Journal* can be viewed as the initial step in the development of a second generation of radiopharmaceuticals for the measurement of brain function. Their development of two new radiopharmaceuticals, PIPSE and MOSE, is significant not only because these radiopharmaceuticals gain access into the intracerebral space but also because their proposed uptake mechanism appears applicable to compounds that contain other radioisotopes, notably Tc-99m and I-123. PIPSE and MOSE are diamines that incorporate the bases piperidine and morpholine, respectively. Each molecule contains two N-substituted amines, separated from a centrally located Se-75 atom by an ethyl group. The selenium atom is presumed to have little influence on the in vivo distribution of the diamines other than to preserve their overall neutrality and lipophilicity.

An understanding of the mechanism by which PIPSE and MOSE cross the BBB is necessary for the translation of their sequential scintiphotos into functional information and for the design of second generation radiopharmaceuticals with enhanced cerebral specificity and better radionuclidic properties. Kung and Blau designed the two diamines to be taken up by the brain in proportion to the pH gradient that exists between the plasma and the intracerebral space. This mechanism of uptake, often termed the pH gradient hypothesis (*3*), is shown schematically in Fig. 1. It assumes that the un-ionized species is lipophilic and thus freely diffusible across the cell membrane ($[R - NH_2]_{Bl} = [R - NH_2]_{Br}$) whereas the cation, $R - NH_3^+$, is impermeable to the membrane. This situation is shown mathematically in equation 1 for a weak base with a dissociation constant pKa (*4*).

$$\frac{[R - NH_2]_{Br} + [R - NH_3^+]_{Br}}{[R - NH_2]_{Bl} + [R - NH_3^+]_{Bl}} = \frac{1 + 10^{pKa - pH_{Br}}}{1 + 10^{pKa - pH_{Bl}}} \quad (1)$$

Equation 1, the target to nontarget ratio, reaches its maximum value when pKa $\gg$ pH as shown in equation 2.

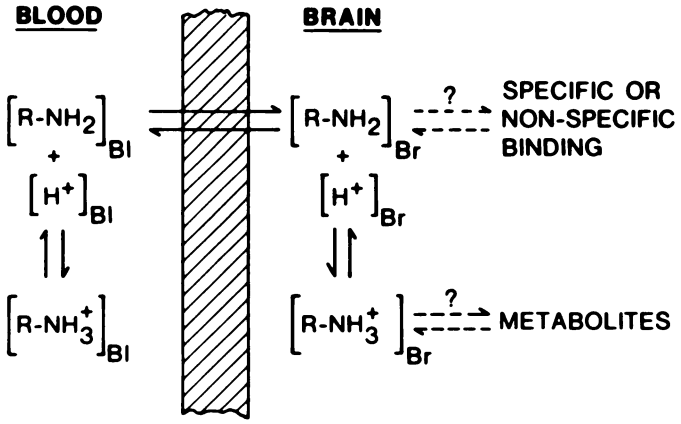

FIG. 1. Partitioning of weak bases across the blood-brain barrier.

$$\left[\frac{[R-NH_2]_{Br}+[R-NH_3^+]_{Br}}{[R-HN_2]_{BI}+[R-HN_3^+]_{BI}}\right]_{MAX}=\frac{[H^+]_{Br}}{[H^+]_{BI}} \qquad (2)$$

The pH gradient hypothesis predicts, therefore, that the distribution of amines across a semipermeable membrane should reflect the concentration of hydrogen ions across that membrane.

This model has been used to predict the absorption of weak acids from the gastric juice (pH 1.4) into the plasma (pH 7.4) and the excretion of weak bases from the plasma into the urine (pH 5.4) (*5*). The pH gradient hypothesis has also been used to predict the accumulation of amines in the lungs (pH ~6.7) and in the brain (pH 7.0). For example, nicotine has an in vivo lung-to-plasma concentration gradient of 5, a value in agreement with the pH gradient hypothesis (*6*). Similarly Anderson and coworkers (*7*) compared the magnitude of the lung accumulation of amphetamine (pKa 9.5) β-monofluoroamphetamine (pKa 8.6) and β,β'-difluoroamphetamine (pKa 6.7). They observed that lung uptake decreased with decreasing pKa and concluded that the degree of protonation was a significant factor in the lung extraction of amines. Fowler and coworkers (*8*) studied the lung uptake of a series of C-11 labeled aliphatic amines with chain lengths that varied from four to 13 carbons. Within this series of amines (pKa ~10.6) they observed that each compound's lung uptake at 1 min correlated well with its octanol to buffer partition coefficient. The magnitude of an amine's uptake by the lung is therefore a function of both the pKa of the amine and the lipophilicity of the un-ionized species.

The brain uptake of various amines has also been studied and the results compared with those predicted by the pH gradient hypothesis. In 1958 Warren and Nathan (*9*) found a correlation between blood pH and the entry of various ammonium salts into the brain. Stebanow and coworkers (*4*) systematically altered blood pH by both metabolic and respiratory alkalosis and observed a direct and predictable correlation between blood pH and tissue ammonia concentration. They explained their findings on the basis of the pH gradient hypothesis. More recently Carter et al. (*10*) studied the effect of blood pH on the brain uptake of N-13-labeled ammonia. They reported brain-to-blood ratios for ammonia of 0.8, 1.6, and 2.4 at plasma pHs of 7.1, 7.4, and 7.6, respectively. The pH gradient hypothesis (Eq. 1) would predict ammonia's initial brain- (pH 7.0) to-blood (pH 7.4) concentration ratio to be 2.5. It was concluded, therefore, that ammonia's initial brain-to-blood activity ratio exhibited an absolute magnitude and response to alteration in plasma pH consistent with the pH gradient hypothesis.

Although the pH gradient hypothesis has been successful in predicting the lung and brain uptake of various amines, there are several difficulties in applying it to the in vivo distribution of PIPSE and MOSE. The brain-to-blood concentration ratios for PIPSE are 7.72 and 14.1 at 30 min and 2 hr, respectively. These values far exceed those predicted based on the simple pH gradient hypothesis. Dr. Blau has observed, however, that should both pKa_1 and pKa_2 for PIPSE be very much greater than the pH, then the theoretical brain-to-blood ratio might be as high as 6 (Monte

Blau, personal communication). Were the pH gradient hypothesis to be the sole determinant of in vivo distribution, the lung-to-blood concentration gradient should be two to four times that of the brain-to-blood concentration gradient. The data do not support this. Finally, for the pH gradient hypothesis model to be applicable, the elimination phases of the blood and brain time-activity curves should parallel one another; i.e., as the radioactivity clears from the blood, it should also clear from the brain at the same rate. This was not seen to occur. It appears that whereas the pH gradient hypothesis may be used to account for the drug's initial rate of uptake into the brain, it cannot be used to predict its final concentration within the brain.

Other factors that might be responsible for the accumulation of the drug are indicated in Fig. 1. These include metabolism, as has been observed in the case of ammonia (*11*), and preferential partitioning of the un-ionized species, as has been observed for xenon (*12*). The time-activity curves for MOSE and PIPSE can be expected to be complicated functions of cerebral activity as has been observed for N-13 ammonia (*13*). It is possible that the initial uptake of these new Se-75 labeled compounds may be a function of flow as governed by the pH gradient hypothesis and that subsequent redistribution phases may occur secondary to either metabolism or binding within the brain.

The work of Kung and Blau is most significant in that it brings us closer to developing the cerebral perfusion agents suggested by Oldendorf (*14*). He proposed a new class of Tc-99m radiopharmaceutical that would be sufficiently lipophilic to penetrate the intact BBB. The relationship between lipophilicity and brain uptake has resulted in the clinical use of ^{123}I-4-iodoantipyrine as a cerebral perfusion agent (*15*). Winstead and coworkers (*16*) synthesized C-11 labeled α-p-iodoanilinophenylacetonitrile and observed that it rapidly concentrated in brain tissue. They suggested that this substituted aniline, when radiolabeled with radioiodine, would be useful for the evaluation of regional brain perfusion. Another amine, 2,5-dimethoxyphenylisopropylamine, has been radiolabeled in the 4 position with both Br-77 (*17*) and I-123 (*18*) and found to concentrate in the lungs (11.8%) and brain (2%). PIPSE and MOSE are significant not only in that their in vivo distribution is similar but also because the radiolabel appears to play a minor part in producing this in vivo distribution, but confirmation of the latter awaits further experimentation. If true, it may be possible to substitute lipophilic Tc-99m complexes for Se-75. Lipophilic Tc-99m complexes have been synthesized and shown to cross the BBB in quantities proportional to their lipophilicity (*19*). These compounds, however, were too highly protein bound for clinical use. A neutral, bis-structured, lipophilic Tc-99m complex might substitute well for the Se-75 in PIPSE and MOSE and yet remain unbound to proteins.

The work of Kung and Blau has brought us much closer to the goal of developing Tc-99m radiopharmaceuticals capable of crossing the intact BBB and thereby describing cerebral function. What is required now is an empirical optimization of those molecular parameters of PIPSE and MOSE that govern their cerebral extraction efficiency and a determination of the mechanism by which this extraction proceeds. Structure-activity data are needed to correlate the cerebral extraction efficiency of these compounds with the pKa of the amine, the lipophilicity of the free base, and the type of radiolabel. The mechanism by which these compounds cross the BBB should be examined using in vivo tissue distribution studies as a function of plasma pH and inhibitor concentration, sequential autoradiography, and radiochromatographic analysis for metabolites.

The BBB is the first and perhaps greatest barrier to the scintigraphic measurement of cerebral function. It is exciting to speculate that a new class of Tc-99m radiopharmaceuticals is in the offing, one capable of crossing the intact BBB.

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