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COMMENT

COMPARTMENTATION IN THE BLOOD-BRAIN BARRIER: A Correction

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In 1981 we (1) presented evidence that the blood-brain barrier consisted of (at least) two compartments. Subsequently, Pardridge (2) took issue with our data and with our interpretation. In response, we have thoroughly reexamined our findings as well as related work in the literature (2–5). From this we have confirmed that the blood-brain barrier does consist of (at least) two compartments. We have also discovered that one of the equations in our article was in error and, consequently, that some of our comments were erroneous and require correction.

In our method (6, 7), the transport rate is mathematically defined by the slope of the straight line relating the molar accumulation of the tracer amino acid, in the brain, to the time elapsed since its injection. We found the blood-brain transport rate of tyrosine (Tyr) to be constant from about 2–15 min. After injection of the enzyme inhibitor methionine sulfoximine (MSO), however, the slope of the "transport line" was seen to decrease by 30–40% along with a marked increase in the value of the intercept at zero time.

In our paper (1) we sought to explain how the intercept of the "transport line" could be anything other than zero at zero time since our method measures a randomly chosen segment of a constant, ongoing process. Focusing on the data from the MSO treated animals, we concluded that there were (at least) two linear processes: one, feeding a relatively small tissue comparment whose specific activity came to equilibrium with that of the plasma earlier than 2 min and, because of the equilibrium, appeared to drop out. The second process was assumed to feed a larger tissue compartment that did not come to equilibrium with the plasma over the

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12–15 min course of our experiments. We concluded that the first process mediated entry into the astrocytes while the second mediated entry into the neurons. We concluded that while the two processes are sequential, they are also operating in parallel, to the extent that the astrocytes do not completely cover the capillary walls (about 15% exposed (8)) thus permitting some direct passage from the endothelial cells to the neurons. Unfortunately, we did not incorporate the 15% factor in Equation #3 of our article. This lead to a series of errors including the rather blatant one that Pardridge (2) pointed out. The equation should have read:

$$Q_T = t(K_1 + 0.15\ K_2)$$

As a result of the omission of the 0.15 factor it was not recognized that in the control mice K_1 and K_2 were almost certainly equal in value and that at times less than 5 min the small contribution from K_2 would have been evident not as a change in slope but rather as a small positive shift in the intercept at zero time. In the same way, we erroneously calculated that the initial in vivo rate was roughly equal to the uncorrected value found by Pardridge and Oldendorf (3) and therefore greatly in excess of the true value (2, 4, 5). This was the error commented on by Pardridge (2).

The omission of the 0.15 factor had a further consequence: it caused us to miss the salient observation that in the MSO treated animals although K_2 was decreased, K_1 was substantially increased. This latter finding is reminiscent of the elevated blood-brain transport of the large-neutral amino acids (LNAA) in rodents following experimental portacaval anastomosis (PCA) (9, 10). The astrocytic hypertrophy and hyperplasia following PCA (11) and the exceptionally high concentration of cerebral glutamine (Gln) (12), secondary to the increased ammonia level, probably account for the greatly increased transport of the LNAA found in that condition. Gln is produced in the astrocytes (13) and appears to exchange for the entering LNAA (14, 15). MSO produces a different picture: there is evidence of astrocytic swelling and pallor of the cytoplasm (16, 17), suggestive of an increase in membrane permeability. If so, this could account for the increased LNAA transport into the astrocytic space that our data indicate. Unlike PCA, however, MSO causes a marked depletion of Gln in the brain (18) which would cause a decreased rate of LNAA transport into the neurons. Our data are consistent with this effect.

We have recalculated the data in our original Table I (1), and obtained the values presented below:

The value 6.7 was obtained by assuming that the observations between 0.5 and 2.5 min were inflated by 15% and we corrected for this before

TABLE I

Condition	K_1*	K_2	Q'_1
Control	6.7	6.7	3.3
MSO	9.4	3.7	18.4

* Units of K_1 and K_2 are nmol/g brain/min; units of Q'_1 are nmol/g brain.

applying linear regression analysis. MSO is seen to have caused a 40.3% increase in K_1 and a 44.8% decrease in K_2.

Pardridge (2) may be correct in his assertion that there is no other direct evidence for the existence of compartmentation in the blood-brain barrier. From our studies it appears unreasonable to expect that compartmentation would be detected by observations limited to a single time, much shorter than 1 min. Similarly, it would be rather difficult to measure the subtle shift in rate in normal control animals. This doesn't mean that compartmentation does not exist but rather that not all methods are appropriate for observing it.

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