EDITORIAL

Compartments and Reaction Volumes of Brain Fluid Spaces: Shaken, Not Stirred

In this issue of *JNM*, Delforge et al. (*1*) raise the important question of the definition and assignment of the kinetic compartments assumed to be occupied by a tracer or its metabolites during a session of in vivo imaging. This question is particularly relevant to the imaging of the binding of radioligands. The authors advance the plausible hypothesis that the radioligand concentration may be lower in the vicinity of receptors than at the capillary wall, i.e., that significant tissue gradients of tracer concentration persist to the end of the scanning period. This consideration of the heterogeneity of the concentration is not routinely treated in existing receptor compartmental models.

Simplistically, the rate of binding to a receptor is believed to depend on the concentration of the ligand in the aqueous environment next to the receptor, as well as on the number of unoccupied receptors. The nooks and crannies of the spaces of the brain, and their unstirred nature, impede diffusion and hence delay the establishment of a uniform level of tracer in all parts of a compartment. The effect of tortuosity factors and unstirred layers may be magnified when particularly intense binding continuously depletes the ligand pool at the far end of the diffusion path.

These impediments interfere with the evaluation of the kinetic properties of concentration-driven tracer compartments because compartments are pools of tracer in which the concentration everywhere is the same. The need for this requirement stems from the solution of differential equations in which the parameters are the concentration-driven decay or relaxation constants of the individual compartments. The requirement has been a source of confusion and has led to puzzling ambiguities, such as the variable compartmental decay "constant" $k_3(t)$ in Equation 5 in Delforge et al.'s article (*1*). Such usage is at variance with the fundamental definition of a tracer compartment originally proposed by Rescigno and Beck (*2*).

As discussed in detail by Gjedde and Wong (*3*), a typical definition of a compartment obeying linear properties is a quantity (N) of tracer or tracer-derived molecules which obey the expression,

$$dN/dt = J - kN, \qquad \text{Eq. 1}$$

which simply means that the loss of molecules from the compartment is proportional to the number of remaining members, regardless of the flux (J) of new molecules to the compartment. This is the so-called "first-order" process of decay or relaxation, described by the relaxation constant k. If the driving force of the relaxation is the concentration ($C = N/[AV]$; A is Avogadro's number) of molecules in the solvent volume (V) of the compartment, the concentration must of course be uniform to allow the molecules to obey Equation 1. Note that compartments defined in this manner do not need physical boundaries and may indeed be completely mixed. Not all compartments, however, are concentration-based. Technically, molecules bound to receptors have no concentration. For this reason, Delforge et al. quite rightly define the members of a compartment by the symbol M for their molecular mass ($M = N/A$) rather than their concentration (C), which cannot be measured, as first proposed by Gjedde (*4*).

Compartments are highly artificial constructs that are at variance with a fundamental characteristic of living systems. Living systems establish steady-states ($dC/dt = 0$) that are never in complete equilibrium and are often far from equilibrium (no net flux; $dC/dx = 0$). The consequence is that the compartmental analysis gives more or less erroneous results when tracer molecules do not in fact obey Equation 1 exactly, due, for example, to significant concentration gradients.

The time-honored remedy to this problem is to subdivide the tracer pools into two or more compartments that adequately describe the kinetic behavior as a function of time. Delforge et al. use this remedy when they assign the molecules of the pool of unbound radioligand molecules in brain tissue to at least two compartments, one in which the concentration is uniformly equal to the aqueous concentration near the capillary wall and another in which the concentration is equal to the aqueous concentration near the receptor. Hence, the entire gradient is placed at the interface between these two compartments. Additional compartments can be defined as required, but kinetic analysis may then become increasingly ill-conditioned and may fail to distinguish between more than a few compartments. The number of compartments must be sufficient to yield accurate estimates of identifiable parameters.

Delforge et al. provide no evidence that the compartment defined as the "reaction" volume is of a constant magnitude with time, i.e., that the concentration gradient of unbound ligand between capillary and receptor is constant. It is plain, however, that no such evidence could be provided because the early establishment of transient equilibrium obscured the distinction between the individual subdivisions of the free radioligand pool.

Transient equilibrium is a kind of near-equilibrium situation, paradoxically reached during nonsteady-state conditions. Complete equilibrium is, of course, never present in living systems, and true steady-state is by definition impossible to attain in experiments in which the tracer is administered as a bolus (although with some programmed infusions, steady-state can be approached). In the absence of steady-state, some compartments nonetheless establish approximately constant ratios between their tracer masses, even after bolus administration of the tracer. This condition has no simple name. Delforge et al. speak inexactly of steady-state and equilibrium but the proper term for the condition is borrowed from nuclear physics in which a "transient" equilibrium exists when two compartments maintain an approximately constant mass ratio although the net flux between them is not zero.

When transient equilibrium is established between compartments, kinetic analysis fails to distinguish between the individual compartments. Because Delforge et al. cannot "see" the reaction volume kinetically, they are forced to deduce its existence from external evidence, including in vitro estimates of the Michaelis constant of binding. The danger of the concept is that it may serve as a "fudge" factor which adjusts in vivo results to already existing in vitro data. Only the careful analysis of all known physical and chemical properties of the

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radioligand, practiced by Delforge et al., avoids this pitfall.

The key to the description offered by Delforge et al. is the aqueous concentration of the tracer at a point near the tissue side of the capillary wall. Early introductions of a volume akin to the reaction volume used a similar point of departure. The use of diverse symbols, however, illustrates the confusion reigning in this field and complicate life for the uninitiated. Gjedde and Wong (*3*) based their description of the volumes on the definition of an in vivo "partition" volume, V_e, equal to the volume V_{DF} also defined as the K_1/k_2 ratio by Delforge et al. (*1*) (note the uppercase K_1; the tracer clearance from blood is not a relaxation constant and should not be symbolized by a lower case k). The partition volume should be distinguished from the physical property of lipophilicity (P) which is typically measured as the octanol-to-water ratio. The latter ratio does not reflect proteins or other components of biological fluids, making it less realistic for in vivo purposes.

The volumes V_e and V_{DF} incorporate two concepts: those of (a) the tracer's solubility coefficient in plasma or plasma water (α in Gjedde and Wong (*3*), λ_F by Delforge et al. (*1*), in which λ_F is not to be confused by the commonly used symbol λ for the partition coefficient of blood flow tracers which is conceptually equal to V_e and V_{DF}), depending on the inclusion or exclusion of plasma protein binding; and (b) the physical distribution volume in brain tissue [V_d in Wong et al. (*4*) and Gjedde and Wong (*3*) but not symbolized by Delforge et al. (*1*)]. The volume V_d is in fact $\lambda_F V_{DF}$ (see Eq. 2 below). Kinetically, the reaction volume is essentially an expansion (by a factor of, say, b) of the distribution volume which lowers the concentration below that at the capillary wall, such that $V_R = bV_d$, and $V'_R = bV_d(1 + \rho)$ when the radioligand is also subject to nonspecific binding with a binding potential of ρ [equal to k_5/k_6 in the terminology of Delforge et al. (*1*)]. Delforge et al. introduce the coefficient γ as a combined factor that relates the volumes to the partition volume and hence to the commonly used so-called "free" fractions f_1 and f_2 such that,

$$V_e = V_{DF} = K_1/k_2 = V_d/\alpha = V_R/[b\lambda_F]$$
$$= V'_R/[b\lambda_F(1 + \rho)] = \gamma V_R$$
$$= \gamma' V'_R = f_1/f_2, \qquad \text{Eq. 2}$$

which goes to show that the concept of the reaction volume is in reality none other than the mathematical description of the different free fractions in plasma (or plasma water) and brain tissue. Note that Delforge et al. (*1*) use f_2 in a different sense than the more common one, namely as a symbol for the reciprocal of the term $1 + \rho$ of Wong et al. (*4*) and Gjedde and Wong (*3*).

The concept of "reaction" volumes (V_d or V_R) which are different from the "partition" volumes (V_e or V_{DF}), has consequences primarily for the interpretation of the Michaelis constant K_d and hence for the definition of the binding potential. Like Gjedde and Wong (*3*), Delforge et al. (*1*) properly interpret the experimentally determined binding potential k_3/k_4 as representing the term $B'_{max}/[K_dV_d]$, termed V''_3 by Laruelle et al. (*5*), although it is not a volume. Mintun et al. (*6*) originally defined the binding potential as the B_{max}-to-K_d ratio, but the calculation of this entity requires knowledge of the true K_d of the radioligand in aqueous solution or the absolute magnitude of V_d as $1/f_2$ and V_e/f_1. Since the binding potential has practical significance for the quantitative interpretation of receptor maps, it is perhaps useful to redefine the binding potential as the k_3-to-k_4 ratio of an in vivo receptor study.

It is particularly interesting that the concept of the reaction volume provides an explanation for a correlation between estimates of B'_{max} and K_dV_d. This correlation has been observed by a number of PET researchers. If the estimates of V_e do not vary, the finding suggests that γ may vary in inverse proportion to B'_{max}. This variation could be caused by changes of radioligand binding in plasma (α), nonspecific binding in brain ($1 + \rho$) or by changes of the free tracer gradient in the tissue (b). We can only speculate which of these is the more significant.

In conclusion, the study by Delforge et al. illustrates the need for a common glossary of terms.

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