Concept of Reaction Volume in the In Vivo Ligand-Receptor Model

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In vivo quantification of receptor concentration and ligand affinity using data obtained with PET is based on the compartmental analysis of ligand-receptor interactions. There is, however, an inconsistency between the assumed homogeneity of the ligand concentration in each compartment, a basic hypothesis of the compartmental analysis, and the obvious heterogeneity of the tissue. Our goal was to study the effects of the free ligand concentration heterogeneity on the parameters describing in vivo binding reaction and to introduce the concept of reaction volume, V_R, to account for that heterogeneity. **Methods:** The reaction volume is defined as the volume in which the free ligand mass present in 1 ml of tissue would have uniformly distributed with the same concentration as that in the vicinity of the receptor sites. The consequence of the heterogeneity of the free ligand concentration is that the equilibrium dissociation rate constant estimated from PET data corresponds to K_dV_R and not to K_d alone (defined by the ratio of the dissociation over the association rate constants). As a consequence, it is proposed to estimate the reaction volume as the ratio between the equilibrium dissociation constants obtained from in vivo and in vitro data (K_dV_R and K_d, respectively). **Results:** We used data obtained from studies performed with eight different molecules and found a correlation between the reaction volume and the molecule lipophilicity. This correlation can be used as a method to estimate the order of magnitude of V_R from the lipophilicity which is easily accessible experimentally. **Conclusion:** Reaction volume is an important parameter in in vivo ligand-receptor interaction modeling.

Key Words: distribution volume; reaction volume; receptors; PET; compartmental model; lipophilicity

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With the advent of PET and the recent developments of specific ligands labeled with short-lived positron emitters, it is now possible to study the distribution of various receptors in vivo in humans. One of the challenges of PET studies of ligand-receptor interactions is the quantitation of receptor density and ligand affinity for the receptor sites. Quantitative analysis of the ligand kinetics in vivo is essential for the understanding of the pharmacological properties of endogenous ligands and the role of binding sites in normal or pathological conditions.

PET, however, only measures the ligand concentration in a region of interest (ROI) and does not allow receptor concentration to be deduced directly; it is necessary to use kinetic data and a mathematical model describing the ligand-receptor interactions (*1–5*). All in vivo approaches are based on a mathematical model which includes at least two steps: first, a transport of the ligand from the blood to a free ligand compartment (a necessary step since the labeled ligand is injected intravenously) and, second, a classical ligand-receptor interaction similar to that used in in vitro studies. By including possible nonspecific binding, the usual four-compartment model is obtained, the rate constants of the transfers between the compartments being denoted by parameters k_i according to the numerotation shown in Figure 1. If the injected ligand doses are not tracer doses, parameter k_3 is not a constant and depends on time.

In this model, $C_p(t)$ is the concentration of free ligand in the blood (usually, the plasma concentration of the ligand unmetabolized and unbound to the proteins). In fact, this blood compartment is not a true compartment, since its kinetics are obtained from experimental data and not simulated by the model. The other compartments are defined by quantities of ligand per unit volume of tissue: $M_F(t)$, $M_B(t)$ and $M_{ns}(t)$ are the quantities of ligand which are free in the exchangeable pool, specifically or nonspecifically bound to receptor sites, respectively. The PET data correspond to their sum plus a fraction of the blood ligand concentration (denoted by F_V and corresponding to the fraction of blood present in the tissue volume). One uses quantities (or masses) per unit volume of tissue with symbol M, rather than concentrations with symbol C. This is due to the possible heterogeneity of the ligand concentration in each compartment. Consequently, the true local concentration in any point of a unit volume is unknown, since it can be different from the mean mass-to-volume ratio, which is the only estimated value by the modeling approach (*6,7*). This heterogeneity is not only the consequence of the limited resolution of the PET camera but also the result of the internal heterogeneity of the tissue. There is, however, an inconsistency between this fact and the assumed homogeneity of the molecule concentration in all compartments, which is a basic hypothesis of the compartmental analysis.

In theory, if the concentration is not homogeneous in a compartment, it is necessary to share this compartment in new subcompartments verifying that homogeneity property. Thus, new kinetic parameters have to be introduced in order to describe the exchanges between these new subcompartments and therefore have to be identified from the PET data. PET data, however, even with complex protocols, do not allow identification of more than 5–7 parameters [additional kinetic parameters would be unidentifiable (*8*)]. Another alternative is to account for heterogeneity without introducing a large number of new parameters, but with only one or two combined parameters which summarize the heterogeneity effects on the global kinetic. Examples of such approaches are illustrated by the parameter f_2 [introduced to take into account the nonspecific binding when the PET data do not allow to dissociate it from the free ligand compartment (*1*)] and by the apparent distribution volume [introduced in the blood-tissue exchanges when the single tissue compartment includes the bound ligand (*9*)].

Therefore, we studied the effects of ligand concentration heterogeneity on the parameters describing the binding reaction and to introduce the concept of volume of reaction to account for that heterogeneity. The relations between the parameter f_2, the distribution volume of the free ligand V_{DF} and the reaction volume V_R are established and discussed.

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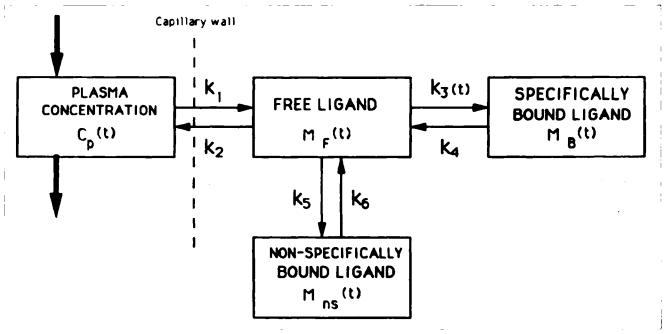

FIGURE 1. Usual compartmental model describing ligand-receptor interactions. All transfer probabilities of ligands between compartments are constant, except the binding probability, $k_3(t)$, which depends on the concentration of free receptor sites and, thus, is a function of the time if the ligand is not injected with the tracer amount.

METHODS

Distribution Volume

If the equilibrium state is reached, the distribution volume of the free ligand is defined by the following equilibrium ratio, which is independent of time:

$$V_{DF} = \frac{M_F(t)}{C_p(t)}. \qquad \text{Eq. 1}$$

In PET modeling studies, the distribution volume can also be defined as the ratio of the two model parameters describing the blood-tissue exchanges (*9–11*):

$$V_{DF} = \frac{k_1}{k_2}. \qquad \text{Eq. 2}$$

Its unit is ml_{blood}/ml_{tissue}.

To clarify the meaning of V_{DF} and allow comparison with the definition of the reaction volume (see next section), we used the parameter λ_F, which is defined as the equilibrium ratio between the concentrations of the free ligand on the two sides of the capillary wall. Thus, at the equilibrium state:

$$C_p(t) = \lambda_F C_{F,cap}(t), \qquad \text{Eq. 3}$$

where $C_{F,cap}(t)$ is the tissular concentration of the free ligand in the vicinity of the capillary wall (Fig. 2). Therefore, the volume of distribution can also be defined by the following equation:

$$V_{DF} = \frac{M_F(t)}{\lambda_F C_{F,cap}(t)}. \qquad \text{Eq. 4}$$

This usual meaning of V_{DF} is based on the assumption that the concentration of the free ligand is identical on the two sides of the capillary wall ($\lambda_F = 1$, which can be justified if the exchanges are passive and if an equilibrium state is rapidly reached). The distribution volume is then defined as the volume of the tissue in which the free ligand mass present in 1 ml of tissue ($M_F(t)$) would have distributed with the same concentration as in the blood ($C_p(t)$). In such a case, it is greater than 1 ml/ml if the free ligand concentration in the blood is smaller than the mean free ligand concentration in the tissue.

The k_1 to k_2 ratio also corresponds to the concept of "partition coefficient" used by some authors (*5,12*). If the mathematical definitions of these two concepts are similar, however, Huang et al. (*10*) recalled that the implicit meanings are different. The partition coefficient concept assumes that the tracer can distribute over the entire tissue space but that the capillary wall forms a partition such that the tracer concentrations on the two sides are not equal. On the other hand, the distribution volume concept assumes that the concentration of free ligand is identical on the two sides of the capillary wall, but that the volume occupied by the molecules does not correspond to the entire tissue volume. Both definitions imply that the concentration of the free ligand is homogeneous in the volume occupied in the tissue.

Due to tissue heterogeneity, however, the free ligand may not be uniformly distributed (it can have different concentrations in different locations in the tissue, even if a small volume of tissue is considered) and some parts of the tissue may not be reachable by the free ligand. Moreover, even if the exchanges are passive transfers, it is difficult to be sure that the ligand concentration measured in the blood samples correspond exactly to the concentration of the free ligand present in the vicinity of the capillary wall and available for crossing this barrier (for example, a bias can result from unknown protein binding or other sequestrations and inhomogeneities in blood) (*11*).

Distribution volume has to be considered as a virtual (not

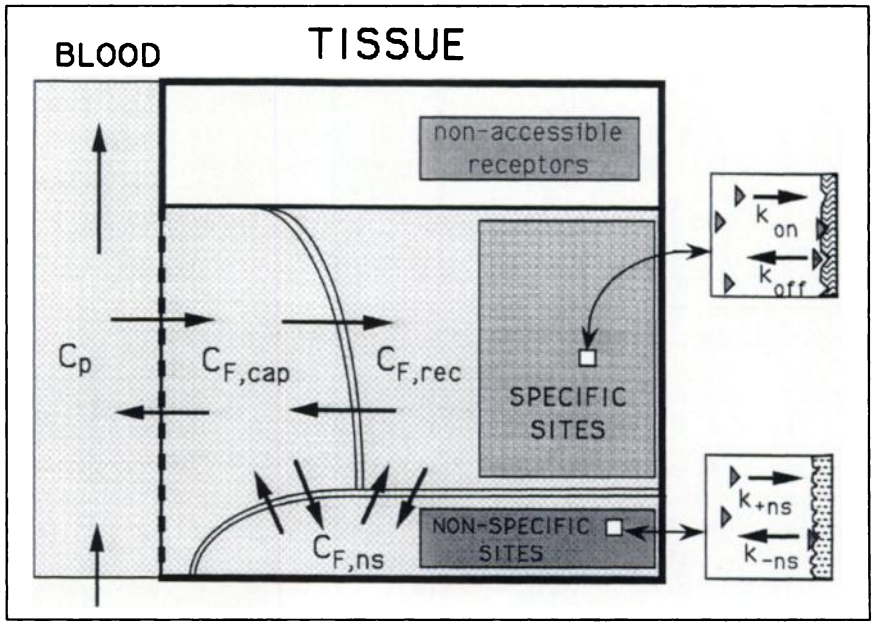

FIGURE 2. Tissue heterogeneity leads to heterogeneity of the free ligand concentration which can differ in the capillary wall ($C_{F,cap}$) and in the vicinity of specific or nonspecific receptor sites ($C_{F,rec}$ and $C_{F,ns}$, respectively). Parameters k_{on} and k_{off} (k_{+ns} and k_{-ns}) are local association and dissociation rate constants for the specific (nonspecific) binding. It is possible that part of the receptor sites are not accessible by the ligand and are not included in the receptor concentration estimated by the modeling method.

physical) volume which can be greater than 1 ml/ml and reflects the effect of free ligand heterogeneity in blood-tissue exchanges.

Ligand-Receptor Interactions

Whereas the distribution volume concerns the transfers between the blood and the free ligand compartment, the concept of reaction volume is defined from the binding of the free ligand with the receptor sites. The parameter B'_{max} represents the unknown concentration of receptor sites available for ligand binding and, thus, only takes into account the receptor sites which are reachable by the ligand (see Fig. 2) and not occupied by an endogeneous ligand. At any time t, the concentration of the free receptor sites is equal to $[B'_{max} - M_B(t)]$, where $M_B(t)$ is the quantity of labeled ligand bound to receptors in 1 ml of tissue. The in vivo ligand-receptor interactions are assumed to be similar to the in vitro ones. By definition of the association rate constant (denoted by k_{on}), the quantity of ligand bound to the receptor sites, per unit time and per unit volume, is equal to the product of k_{on} by the free receptor site concentration ($B'_{max} - M_B(t)$) and by the concentration of the free ligand in the vicinity of the receptor sites (denoted by $C_{F,rec}(t)$). This quantity is also denoted by $k_3(t)M_F(t)$ in the usual equation system (Fig. 1), and therefore one obtains:

$$k_3(t)M_F(t) = k_{on}[B'_{max} - M_B(t)]C_{F,rec}(t). \qquad \text{Eq. 5}$$

The concentration $C_{F,rec}(t)$ can be different from $M_F(t)$ due to the heterogeneity of the local free ligand concentration.

The rate constant for the dissociation of the specifically bound ligand is denoted by k_{off} and the equilibrium dissociation rate constant K_d is given by the ratio k_{off}/k_{on}. It is assumed that only one type of receptor site is occupied by the ligand. A study with several receptor types is possible but includes a larger number of parameters.

Reaction Volume

Reaction volume has been introduced to take into account the possible heterogeneity of the free ligand concentration in the tissue, resulting from the tissue heterogeneity and from the ligand properties, such as lipophilicity. Indeed, the concentration in the receptor site vicinity ($C_{F,rec}(t)$) may not be equal to the mean concentration in 1 ml used in the mathematical model ($M_F(t)$), and estimated by modeling the PET data (*2,7,13*). If, however, an equilibrium state is assumed to be rapidly reached inside of the free ligand compartment, the ratio $M_F(t)/C_{F,rec}(t)$ is a constant which defines reaction volume:

$$V_R = \frac{M_F(t)}{C_{F,rec}(t)}. \qquad \text{Eq. 6}$$

Reaction volume is then defined as the volume in which the free ligand mass present in 1 ml of tissue ($M_F(t)$) would have uniformly distributed with the same concentration as in the vicinity of the receptor sites ($C_{F,rec}(t)$).

If $M_F(t)$ includes a nonspecific binding, the reaction volume is denoted by V'_R and called the apparent reaction volume. Like the distribution volume, the volume of reaction is a fictive volume with dimension ml_{tissue}/ml_{tissue}.

The model parameter $k_3(t)$ is defined from Equations 5 and 6 by:

$$k_3(t) = \frac{k_{on}}{V_R}[B'_{max} - M_B(t)], \qquad \text{Eq. 7}$$

where the combined parameter k_{on}/V_R is called the macroscopic bimolecular association rate constant. Therefore, the association rate constant estimated in vivo with PET is k_{on}/V_R and not k_{on} alone. In conclusion, the reaction volume can also be defined as the ratio of the microscopic (or local) association rate constant (k_{on}) to the macroscopic (or global) one (k_{on}/V_R).

Relation between V_R and V_{DF}

From the definitions of the distribution volume (Eqs. 2 and 4) and of the reaction volume (Eq. 6), we can deduce their relation by way of the following relation:

$$\frac{V_{DF}}{V_R} = \frac{C_{F,rec}(t)}{C_p(t)} = \frac{C_{F,rec}(t)}{\lambda_F C_{F,cap}(t)} = \gamma, \qquad \text{Eq. 8}$$

where $C_{F,rec}(t)$ and $C_p(t)$ are ligand concentrations at equilibrium; therefore, γ is a constant. It results that V_{DF}/V_R only depends on the free ligand concentrations in the vicinities of the receptor sites and of the capillaries.

Reaction Volume Estimation

According to its definition, the reaction volume can be obtained from the ratio of the microscopic association rate constant (k_{on}, estimated using in vitro methods) to the macroscopic one (k_{on}/V_R, estimated in vivo by modeling the PET data). Both in vitro and in vivo methods, however, estimate the equilibrium dissociation rate constant (K_d and K_dV_R, respectively) more precisely than the association (k_{on} and k_{on}/V_R, respectively) rate constants. Thus, despite the necessary cautions when comparing between in vivo and in vitro results, we suggest estimating the order of magnitude of V_R from the ratio of K_dV_R estimated in vivo to K_d estimated in vitro.

If the free ligand concentrations in the receptor site and the capillary vicinity are similar (then $\gamma = 1/\lambda_F$, Eq. 8) and equal to this concentration in the blood (then $\lambda_F = 1$, Eq. 3), it results that the reaction volume is equal to the distribution volume (Eq. 8, since $\gamma = 1$) and, consequently, that V_R can also be estimated from the ratio k_1/k_2 (Eq. 2). An example is given in the following section.

RESULTS

Since it is impossible to measure the free ligand concentration in the vicinity of the receptor sites, it is difficult to prove the need to introduce the reaction volume and the validity of its estimate from the in vivo and in vitro affinity measurements. Some experimental results, however, provide supporting arguments and indirect justifications. Three examples are discussed below. In the first one, three estimates of the reaction volume corresponding to a hydrophilic molecule were obtained using three different methods. The estimate agreement is a strong argument in favor of the validity of these three methods. In the second example, the introduction of V_R seems to be the only simple explanation to an observed correlation between the receptor concentration and the ligand affinity in the flumazenil-benzodiazepine interaction model. In the third example, a correlation between V_R and the lipophilicity coefficient P of eight different molecules is in agreement with the known influence of the ligand lipophilicity.

Hydrophilic Molecule

The first example of the estimation of a reaction volume deals with the interactions between myocardial muscarinic receptors and ^{11}C-labeled methylquinuclidinyl benzilate (MQNB). This muscarinic receptor antagonist is a nonmetabolized, hydrophilic molecule (*14*). The latter property is used for the first estimation of the reaction volume, which should be close to the fraction of extracellular fluid in the tissue. This hypothesis, also used by Gjedde and Wong (*11*) and Carson et al. (*15*), leads to a reaction volume estimate of 0.15 ml/ml in the heart (*16*).

A second estimate of this parameter can be obtained by assuming that the ligand concentration on the two sides of the capillary wall are equal ($\lambda_F = 1$, an implicit hypothesis of the distribution volume concept) and that the concentration in the free ligand compartment is homogeneous. In this case, the reaction and distribution volumes have the same value (Eq. 8

TABLE 1
Estimation of Reaction Volume (V_R) in the (MQNB)-(Myocardial Muscarinic Receptors) Interaction Model

METHODS			RESULTS	
Basic	Principle	Hypothesis or Property	V_R ml/ml	Ref.
Molecule property	Water content of tissue	Hydrophilic molecule	0.15	16
Blood/Tissue exchanges	Ratio $\frac{k_1}{k_2}$	$\lambda_F = 1$ (Passive transfers)	0.16 ± 0.04 (dog)	17
		Homogeneous free ligand and	0.16 ± 0.06 (human)	7
Binding interactions	Ratio $\frac{(K_d V_R)_{invivo}}{(K_d)_{invitro}}$	$(K_d)_{in\ vivo} = (K_d)_{in\ vitro}$	0.15 ± 0.05 (dog)	17

with $\lambda_F = 1$ and $C_{F,cap}(t) = C_{F,rec}(t)$) and, thus, these two volumes can be estimated from the k_1/k_2 ratio. Using the k_1 and k_2 estimates obtained with a multi-injection approach in dog (*17*) and in human (*7*), two identical estimates of V_{DF}, and thus of V_R, are deduced: 0.16 ± 0.04 ml/ml in dog and 0.16 ± 0.06 ml/ml in human.

A third estimation of V_R is based on the binding interactions and is obtained by comparing in vivo and in vitro affinity estimates. In a dog study, the K_dV_R value found in vivo using PET (0.072 ± 0.021 pmole/ml_{tissue}) has been compared with the K_d measured using in vitro method (0.49 ± 0.06 pmole/ml_{tissue}). This led to a V_R value equal to 0.147 ml/ml (*17*).

Therefore, these three reaction volume estimates, obtained with three completely independent methods, are very close to 0.15 ml/ml (Table 1). The similarity of these estimates strongly favors the hypotheses used (i.e., the reaction volume is the extracellular fluid volume in the tissue and the free ligand is homogeneous in this volume after a very fast diffusion) (*18*).

This result has helped us interpret the data obtained from the following study. We studied the in vivo quantification of the muscarinic receptors with MQNB in normal and transplanted patients using the multi-injection approach. Whereas the receptor concentration was not modified by the transplantation, the study showed an increase of k_dV_R in the transplanted patients, which had first been interpreted as an increase of K_d (*19*). This result was not easy to explain physiologically. After the first studies on the reaction volume, however, a new examination of the k_1 and k_2 estimates indicated that the distribution volume was increased in the transplanted patients. Based on our previous analysis and on the identical value of the reaction and distribution volumes, calculations showed that K_d was in fact not modified by the transplantation and that the increase in K_dV_R only resulted from an increase in V_R (*20*). Our conclusion is strongly supported by the heart edema affecting all transplantation patients which increases the fraction of extracellular fluid in the tissue.

Correlation between B'_{max} and K_dV_R

Earlier studies using a kinetic approach based on a multi-injection protocol (*6,7*) found a linear correlation between benzodiazepine receptor density and apparent flumazenil affinity (*21*) (Fig. 3). This correlation was surprising at first, since we usually considered that the parameter K_dV_R (usually denoted by K_d) estimated in vivo using PET is a constant independent of the receptor concentration. No dependence between receptor concentration and affinity has been observed in in vitro studies and would be difficult to explain.

To test whether our result was consistent with other published results, a positive linear correlation between B'_{max} and K_dV_R was tested with all 12 published data sets (*21*). It appeared that no set of available experimental data was in contradiction with this correlation, whereas all correlation coefficients, r, were greater than 0.70, and on all but two datasets were greater than 0.85 for five. The probability that such correlation was obtained randomly was less than 6% for 8 datasets and less than 0.05% for all the studies, including more than 14 couples of values (B'_{max}, K_dV_R).

This correlation between B'_{max} and K_dV_R can be attributed to a bias due to the hypotheses introduced in the parameter estimation methods. All the methods used in the studies reported in (*21*) were different with various hypotheses, and the only common hypothesis to all approaches to explain a common bias was the structure of the model. Using simulations, other hypotheses have been tested, such as a possible irreversible nonspecific binding or a possible endogeneous ligand. In all cases, it was concluded that the simulated bias obtained could not explain the observed correlation.

Therefore, the simplest explanation of the positive linear correlation found in vivo between B'_{max} and K_dV_R is the existence of a positive linear correlation between B'_{max} and V_R, assuming that the affinity is a constant. This hypothesis is supported by the experimental results showing that the dissociation rate constant k_{off} appeared independent of B'_{max}, whereas the apparent association rate constant (k_{on}/V_R) correlated with B'_{max} (*21*). Based on Equation 8 and the fact that the equilibrium state is rapidly reached with flumazenil (*6*), the reaction volume can be written as:

$$V_R = \frac{V_{DF}C_p(t)}{C_{F,rec}(t)}. \qquad \text{Eq. 9}$$

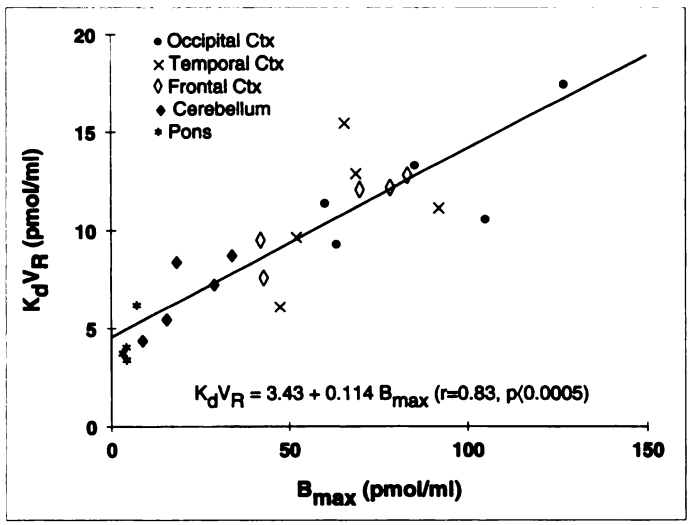

FIGURE 3. Correlation between B'_{max} and K_dV_R estimates in flumazenil-benzodiazepine interactions. These parameters have been estimated in humans using a multi-injection approach (*6,7*). The different symbols correspond to various ROIs and the straight line represents linear correlation.

TABLE 2
Estimates of Volume Reaction (V_R) and Volume Distribution (V_{DF}) with Various Molecules

Molecules	In Vitro K_d estimates		In Vivo K_dV_R estimates		V_R*	V_{DF}†	log P (oct/water, pH 7.4)	
	nM	Ref.	nM	Ref.	ml/ml	ml/ml		Ref.
NM-Spiperone	0.075	30	1.25	27	11.3		3.2	26
	0.16§	31					2.9	Loc'h (personal communication)
	0.097¶¶	5					3.0	43
Bromolisuride	0.30***	32	1.9***	13	6.3	1.5	3.1	Loc'h (personal communication)
Spiperone	0.1‡‡	11	1.5‡‡	11	12.6	3.1	2.7	26
	0.06	33	0.52	27			2.6	43
Raclopride	2.0‡	34	9.8§	38	4.0	0.40	1.33	44
	1.4	30	3.8‡‡	10			2.1	45
	3.9§	31		40			2.6	28
	1.2	35		Loc'h C (personal communication)				
Diprenorphine	0.22§§	36	0.68§§	41	3.7	1.5	1.7	29
	0.14¶¶¶	29						
Cyclofoxy	0.7‡‡‡	37	1.9§§§	42	2.1		0.8	46
Flumazenil	6.1¶	39	12.5**	6	1.4	0.59	1.14	Maziere M (personal communication)
	8.9††	39	9.0††	6		0.73		
MQNB	0.49†††	8	0.072†††	8	0.15	0.16	−0.5	

*Estimated by the ratio of K_dV_R estimated in vivo to K_d estimated in vitro. If several estimates are available, the mean values are used.
†Estimated by the ratio k_1/k_2, the parameter values are those given in the same references than in vivo K_dV_R estimates.
‡Human brain homogenates. §Human putamen. ¶Human neocortex. **Human occipital. ††Human cerebellum. ‡‡Human striatum. §§Human frontal cortex. ¶¶¶Human caudate. ***Baboon striatum. †††Dog heart. ‡‡‡Rat brain membranes. §§§Frontal cortex in rat brain. ¶¶¶¶Rat brain homogenates.

Since the distribution volume is nearly constant in all brain regions (*6*), $V_{DF}C_p(t)$ is independent of the receptor concentration. Therefore, the correlation between B'_{max} and V_R implies that the local free ligand concentration in the receptor site vicinity ($C_{F,rec}(t)$) is decreasing with the receptor site density. This conclusion is coherent since the larger the receptor density, the easier the binding of the free ligand and, thus, the smaller the resulting local concentration of this free ligand.

Correlation between V_R and the Lipophilicity Coefficient

The partition coefficient, measured using the octanol-water concentration ratio and denoted by P, is usually used as the measure of the lipophilicity of a molecule. It is known that the increasing partition coefficient P tends to enhance permeation through the blood-brain barrier, increasing the nonspecific binding in tissue and fat. This nonspecific binding increase should lead to a decrease of the free ligand quantity available for the specific binding, and thus to a decrease of the local concentration of the free ligand in the receptor site vicinity ($C_{F,rec}(t)$). This should lead to an increase of the reaction volume (Eq. 9). Therefore, according to the known relation between the lipophilicity and the nonspecific binding, one expects to observe a positive linear correlation between V_R and P.

The experimental data necessary to study this correlation are difficult to obtain since it is necessary to know simultaneously the K_d estimate measured in vitro, the K_dV_R estimate measured in vivo with PET and the estimates of the lipophilicity coefficient P. The number of available molecules is limited by the PET estimates of K_dV_R, since many molecules are used without parameter quantitation and some quantitation methods do not estimate K_dV_R (*5,22*). It is well known that the in vitro estimates of K_d are often different according to the experimental conditions used. Therefore, when several estimates are available, V_R has been calculated from the average value.

The results given in Table 2 and plotted in Figure 4 show a linear correlation ($r = 0.95$, $p < 0.0005$) between the logarithm of the reaction volume (estimated from the ratio of K_dV_R measured in vivo to K_d measured in vitro) and the logarithm of the lipophilicity (measured by the octanol-water partition coefficient P at pH 7.4):

$$\log V_R = -0.36 + 0.48 \log P, \qquad \text{Eq. 10}$$

which leads to the relationship between the reaction volume and the lipophilicity P:

$$V_R = 0.43P^{0.48}. \qquad \text{Eq. 11}$$

A similar linear correlation is also found between the distribution volume and the lipophilicity,

$$\log V_{DF} = -0.59 + 0.30 \log P, \qquad \text{Eq. 12}$$

but with a smaller correlation coefficient ($r = 0.82$, $p = 0.05$). This result agrees with the known increase of the permeation through the blood-brain barrier as a function of the lipophilicity.

DISCUSSION

Reaction Volume

Reaction volume results from the nonlinearity of the ligand-receptor interactions and from the tissue heterogeneity because binding depends on the local concentrations of ligand and of receptor sites and not on the global quantities in a tissue volume. This was pointed out and experimentally verified by Selikson et al., who used an equilibrium dialysis method (*23*). In PET studies, a volume effect in the binding reaction only appeared in some papers where it was obvious or well known that the available receptors are located only in part of the tissue volume observed by PET. Vera et al. (*3*) introduced a volume to describe the pharmacokinetics of ^{99m}Tc-galactosylneoglycoalbumin, this volume being defined as the hepatic plasma volume. It did not appear, however, any more in their recent publications (*24*). Wong et al. introduced in the (D2 dopamine receptor)-(N-methylspiperone) interaction model as a "physical distribution volume of the ligand" (*5*) or "the volume of tissue in which the ligand is dissolved" (*11,12*). In a recent paper

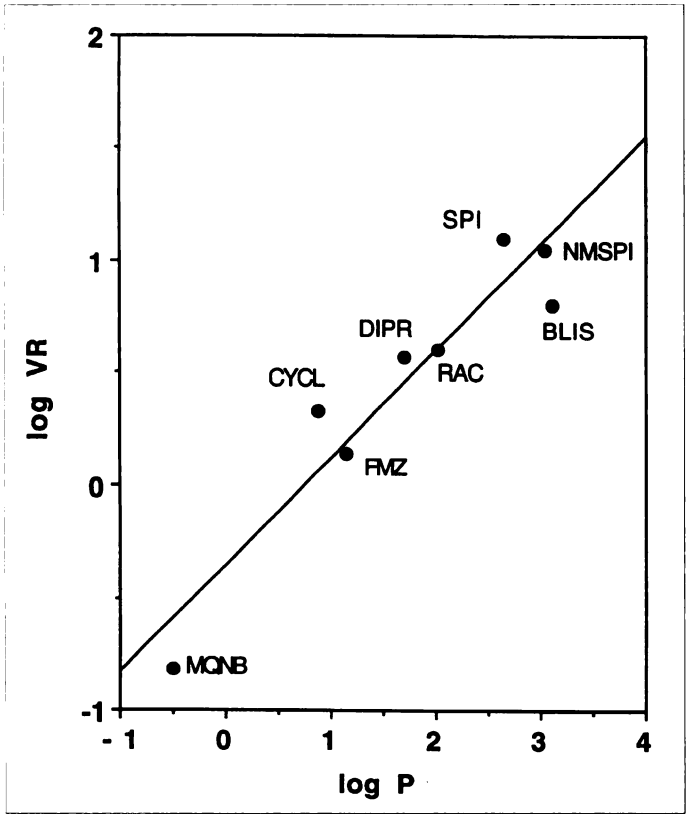

FIGURE 4. Linear correlation ($r = 0.95$, $p < 0.0005$), obtained from results given Table 2, between the logarithm of reaction volume and lipophilicity (NMSPI = NM-spiperone, SPI = spiperone, BLIS = bromolisuride, RAC = raclopride, DIP = diprenorpine, CYC = cyclofoxy, FMZ = flumazenil, MQNB = methylquinuclidinyl benzilate).

from Carson et al. (*15*) describing the kinetics of Cyclofoxy (an antagonist of opiate receptors), the water content of tissue (denoted by ω_T) had the same dimensionality and a similar significance to that of the reaction volume.

The choice of the notations used to describe the model parameters is very important since these notations may include some implicit hypotheses. Usually, authors do not include a reaction volume in the PET ligand-receptor model, which is thus implicitly set to 1 and, consequently, the K_d values obtained by these authors correspond to estimates of K_dV_R. Not only does it affect the comparison between the in vivo and in vitro results, but it can also lead to misinterpretation of the differences observed in the affinity constants with normal volunteers and with patients. An example has been provided by the in vivo quantification of the muscarinic receptors with MQNB in normal and transplanted patients using the multi-injection approach: without the reaction volume, this study would conclude to an increase of K_d, whereas it has been proved that the increase of the apparent equilibrium dissociation rate constant K_dV_R is easily explained by a V_R increase resulting from a known edema affecting the transplanted patients (*20*).

Relation between V_R and V_{DF}

By definition (Eq. 8), the V_{DF} to V_R ratio is equal to the equilibrium ratio γ of the free ligand concentration in the vicinity of the receptor sites to that in the blood. The parameter γ can thus easily be estimated if both V_R and V_{DF} values are known. For example, using the values given in Table 2, the γ value is found to be equal to 0.10 for raclopride, 0.24 for spiperone, 0.47 for flumazenil, 0.41 for diprenorphine and reaches about 1.0 for MQNB. It appears that the free ligand concentration in the vicinity of the receptor sites is less than the free ligand concentration in the blood for all brain molecules used in Table 2.

With a hydrophilic molecule, the usual assumption is that the ligand is dissolved in the extracellular fluid of the tissue and that the free ligand concentration is homogeneous in this volume. The MQNB example gives strong arguments in favor of the validity of these hypotheses. Indeed, our results based on three independent methods conclude that the reaction volume and the distribution volume are equal and both close to the water content in the tissue (0.15 ml/ml, see Table 1). According to the theoretical results of this study, the equality between V_R and V_{DF} is explained both by the homogeneity of the free ligand concentration in the tissue and by an equal concentration in the two sides of the capillary wall.

Nonspecific Binding Effects

A usual difficulty is the presence of a nonspecific binding. If part of this nonspecific binding is irreversible or has sufficiently slow kinetics, parameters k_5 and k_6 can be identified (*13,17*). If, however, the exchanges or part of the exchanges between the free ligand compartment and a nonspecifically bound ligand compartment are very fast, compared with the exchanges between the other compartments, an internal equilibrium state is reached very quickly and this nonspecifically bound ligand compartment is lumped with the free ligand in a single compartment whose concentration is denoted by M_{F+ns} (*4,10*):

$$M_{F+ns}(t) = M_F(t) + M_{ns}(t). \qquad \text{Eq. 13}$$

In this case, the usual method consists of including a new parameter f_2, not directly identifiable from PET data (like V_R), and defined by:

$$M_F(t) = f_2 M_{F+ns}(t). \qquad \text{Eq. 14}$$

At the equilibrium state, this parameter is related to the k_i parameters according to the following equation:

$$f_2 = \frac{k_6}{k_5 + k_6}. \qquad \text{Eq. 15}$$

The observed differences between in vivo and in vitro affinity estimates were often explained only by a nonspecific binding and quantified using parameter f_2. For example, Farde et al. (*25*) explained the differences between the in vivo and in vitro affinity estimates of the raclopride affinity for the D2 receptors, by a very high nonspecific binding in the F + ns compartment (90%, since f_2 is estimated to 10%). This difference can also be explained, however, without a very high nonspecific binding, through the influence of the free ligand concentration heterogeneity. The reaction volume thus estimated is coherent with the curve shown in Figure 4 (see Table 2).

The two parameters f_2 and V_R are related since, when a nonspecific binding is included in the free ligand compartment, it is easy to prove that the apparent reaction volume V'_R is given by:

$$V'_R = \frac{V_R}{f_2} = \left(1 + \frac{k_5}{k_6}\right)V_R. \qquad \text{Eq. 16}$$

It is clear, however, that f_2 and V_R have two different meanings, since it is still necessary to introduce a volume of reaction ($V_R \neq 1$) in the association rate constant when there is no nonspecific binding ($f_2 = 1$).

In fact, this difficulty is related to the definition of the nonspecific binding. According to the model structure, shown

in Figure 1 and including a nonsaturable nonspecific binding, a molecule is nonspecifically bound if it is not instantaneously available for binding to a receptor site or for escaping into the blood microcirculation. In Figure 2, however, the free molecules located in the vicinity of the nonspecific binding sites verified this definition, and therefore, are considered in the Figure 1 model as nonspecifically bound. In fact, the problem is not to know if the molecules of the free ligand compartment are really free or nonspecifically bound: the nonspecific binding has an influence on the kinetics only because it is one cause (but not the only one) for the heterogeneity of the local free ligand concentration. The parameter f_2 only takes into account the heterogeneity resulting from the nonspecific binding, whereas V'_R (Eq. 16) includes both this phenomenon and the intrinsic heterogeneity of the free ligand compartment. Finally, we conclude that the reaction volume concept integrates the nonspecific binding phenomenon, in the same way that it includes the inhomogeneity of the free ligand. It is clear that $V'_R = V_R/f_2$ verifies the reaction volume definition (Eq. 6, in which M_F include a unknown part of nonspecific binding) and thus can be considered as a true reaction volume.

Correlation between V_R and the Lipophilicity Coefficient

The partition coefficient, measured using the octanol-water concentration ratio is usually used as the measurement of the lipophilicity of a molecule. It is known that the increasing partition coefficient P tends to enhance permeation through the blood-brain barrier and to increase the nonspecific binding. Moerlein et al. (*26*), however, showed that increasing the lipophilicity of spiperone analogues does not have straightforward effects on the cerebral localization properties of the radiolabeled compounds. Regardless of the measurements (concentrations in striatum, cerebellum, blood and whole brain or striatum/cerebellum and brain/blood ratios), the curves as a function of lipophilicity were first increasing and second decreasing, the optimum being reached for a lipophilicity (log P) ranged from 3 to 4.1 depending of the measurement which is chosen. This author concluded that this result was not in contradiction with the increasing uptake as a function of lipophilicity, but that this effect was ultimately limited due to competitive binding to plasma proteins and/or precipitation in the blood. Similarly, Stocklin et al. (*27*) studied the butyrophenone neuroleptics with P ranging from 2.7 to 4.3 and Kessler et al. (*28*) studied D2 receptor radioligands with P ranging from 2.1 to 3.5. Both found no general trend when the lipophilicity of the ligand is plotted against the striatal uptake and the striatum-to-cerebellum ratio. These results are not necessary in contradiction with the assumed influence of the lipophilicity, but pointed out that the used criteria are perhaps not pertinent.

The influence of the lipophilicity appears clearer in the binding reactions if the affinity is taken into account. Using opiate receptor ligands, Frost and Wagner (*29*) pointed out a correlation between a clearance rate constant and the product K_dPM, where M is the molecular weight. These authors proposed a method allowing to predict the in vivo K_d from the in vitro results and they showed that the correlation between in vivo and in vitro measurements of K_d is better when the lipophilicity of the ligand is incorpored into their methods. In a study of the D2 receptor ligands with P ranging from 0.8 to 2.8, Kessler and al. (*28*) found a linear correlation ($r = 0.92$, $n = 10$) between the logarithm of the striatal/cerebellum concentration ratio and the logarithm of K_dP:

$$\log (\text{stri./cereb.}) = 2.87 - 0.82 \log (K_dP). \quad \text{Eq. 17}$$

By using the following approximations, striatum-to-cerebellum $\approx$ bound-to-free $\approx B'_{max}/(K_dV_R)$ (which are justified if the ligand injections are tracer injections and if an equilibrium state is rapidly reached), $K_d^{0.82} \approx K_d$ and $B'_{max} \approx 80$ pmole/ml (which is the estimated value of D2 receptor concentration in the human striatum (*13*), we deduced a linear correlation between V_R and P:

$$\log V_R \approx -1 + 0.82 \log P, \quad \text{Eq. 18}$$

and therefore to the relationship:

$$V_R \approx 0.1\ P^{0.82}, \quad \text{Eq. 19}$$

similar to our result in Equation 11.

If the free ligand compartment included the nonspecific binding, the reaction volume V'_R defined by Equation 16 is only a function of parameters k_5 and k_6 and of the volume of reaction V_R estimated without nonspecific binding. By assuming the validity of the following approximations (which are in agreement with the known influence of the lipophilicity on the nonspecific binding):

$$\left(1 + \frac{k_5}{k_6}\right) \approx \frac{k_5}{k_6} \approx \alpha P, \quad \text{Eq. 20}$$

one deduces from Equation 16 that:

$$V'_R \approx \alpha' P, \quad \text{Eq. 21}$$

where the order of magnitude of $\alpha' = \alpha V_R$ is assumed to be independent of the molecules. Obviously, this calculation is only indicative and it is not surprising that the powers of P deduced from experimental data are not equal to 1 (0.48 in our results and 0.82 in our estimates from the Kessler's results).

It is clear that the increase of the reaction volume as a function of the lipophilicity can be explained by the increase of the nonspecific binding in the F + ns compartment. This, however, only corresponds to an increase of the heterogeneity of the ligand concentration in this compartment (increase of the ratio $M_{ns}(t)/M_F(t)$), and not necessarily to an increase of the nonspecific binding ($M_{ns}(t) \approx M_{F+ns}(t)$) compared to the specific one ($M_B(t)$). Bromolisuride is an example of the molecule with a high lipophilicity and a weak concentration of the nonspecific binding compared to the specific one (*13*).

This correlation between V_R and P would be interesting if in the future it is confirmed by using a larger number of molecules. Indeed, it can be used as a method to provide a first estimate of V_R from the lipophilicity measured by octanol-water concentration ratio, which is an easily accessible experimental measurement.

For a given molecule, however, the reaction volume can differ as a function of the tissue properties, and thus, only the order of magnitude of V_R can be estimated from Figure 4 (see the MQNB example, where the measured volumes of reaction were 60% higher in patients compared to normal volunteers). Moreover, it will be necessary to estimate the reaction volume for ligands with higher lipophilicities in order to verify that the correlation between log V_R and log P shown in Figure 4 remains linear for high P values.

CONCLUSION

The in vivo quantification of the receptor concentration and of the ligand affinity, derived from PET data and the modeling approach, has been used by many groups with various molecules. The interest of this quantification, however, is conditioned by the validity of the estimated parameters (often related to the validity of the hypotheses included in the model), but also

by the pertinence of the biological interpretation of these parameters.

In the four-compartmental model usually used in PET, the assumed homogeneity of the concentration in the free ligand compartment seems inconsistent with the well known heterogeneity of the tissue. As a result, the inverse of the reaction volume has to be introduced in the specific binding rate constant. The reaction volume can explain most of the differences between the K_d estimates obtained from in vivo and in vitro methods, and its order of magnitude can be estimated from this method.

The correlation found between the reaction volume and the lipophilicity of the molecule is coherent with the known increase of the nonspecific binding as a function of lipophilicity. This correlation can also be used as a method to provide a first estimate of V_R from the lipophilicity measured by octanol-water concentration ratio, which is an easily accessible experimental measurement.

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