Limitations of Binding Potential as a Measure of Receptor Function: A Two-Point Correction for the Effects of Mass

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Using simulated time-activity curves, significant effects of mass of ligand on estimates of binding potential (BP) at reasonable mass doses for nonhuman primates have been demonstrated. Compartmental model theory predicts the dependence of BP on mass but this possibility is usually ignored—with justification. However, BP values for nonhuman primates may be more susceptible to artifacts because a typical mass dose per body weight of animal is higher than in a human. Time–activity curves were simulated for $^{[11]}$Craclopride in rhesus monkeys in order to gauge the likelihood of mass effects in positron emission tomography (PET) studies in which small (e.g., 10%) differences in BP between primate groups who may not have the same average body weight are detected. PET data and simulations in this study are in agreement that small procedural biases in ligand administration between groups could masquerade as changes in BP as large as those being sought. A two-point extrapolation technique based on the observations of Hume et al. (1995) to correct for mass artifacts is introduced and evaluated. Tests of the extrapolation with simulated data show that mass correlation can be reduced or eliminated without adding variability to data. The sensitivity of this correction method to noisy BP estimates favors the use of graphical rather than kinetic calculation of BP. Finally, a discrepancy between the Hume model and predictions regarding the impact of nonspecific binding on estimated BP is explored.

1. INTRODUCTION

Binding potential (BP), first introduced by Mintun et al. (1984), is a commonly used measure of receptor activity that can be derived graphically or kinetically from dynamic positron emission tomography (PET) studies whether or not a plasma curve has been measured (Logan et al., 1990, 1996; Lammertsma et al., 1996). That BP (or the closely related distribution volume ratio) is a fruitful and robust measure is reflected in the large number of literature citations (71) to one paper presenting a popular method for its graphical estimation (Logan et al., 1990). Estimates of BP in PET and single photon emission computed tomography (SPECT) research have been used in lieu of estimating available number of receptors ($B_{\text{max}}$) when the lack of blood data or convenience dictates. More recently, differences in BP before and after pharmacological perturbation have been used to infer the up- or downregulation of neurotransmitter concentrations in the synapse (Laruelle et al., 1997) or the action of one neurotransmitter system on another (Dewey et al., 1993, 1995). Nevertheless, there are some caveats to keep in mind when using binding potential as an index of receptor number or receptor occupancy.

This chapter sets out the simple theory behind a potentially important limitation on the interpretation of BP arising from its sensitivity to the mass of injected ligand. Through the use of realistic simulations of dynamic PET data with the $D_2$ antagonist $^{[11]}$Craclopride, those conditions (e.g., injected mass, subject body weight, receptor density) under which mass might present a significant confounding influence on BP estimates and their interpretation were explored. In addition, a “graphical” method for eliminating any dependence on mass and an evaluation of the proposed method are presented, based on how well it works with simulated time–activity data. Although the methods of BP estimation used here do not require blood curves,
these findings are consistent across methods and apply, in theory, to any methods that do not estimate $B_{max}$ and $K_D$ separately. This simulation study showed that masses of ligand (or conversely, specific activities) that are commonly considered in the PET literature to be immune from mass effects may not be without artifact in all circumstances and that the artifact is exacerbated by low receptor density. In any PET-receptor study, as a minimum, it is important to avoid erroneously attributing an effect of the experimental protocol (e.g., injected mass) to a change in receptor–ligand binding. This is most likely to occur when comparing results across groups of subjects where one population is more prone than the other to small body weight but where only the absolute radioactivity (and not the mass per body weight) has been held constant. Similarly, comparing the BP in regions of high and low BP might be subject to mass artifacts. The study presented here was intended to determine whether the mass of injectate was of concern in attempting to measure age-related changes in $D_2$ receptor activity in rhesus monkeys with PET.

II. THEORY

A. Definitions and Assumptions

BP is defined as the ratio of available receptor sites to the equilibrium distribution coefficient, $B_{max}/K_D$, but is calculated as the ratio of apparent forward binding to dissociation rate constants, $k_3/k_4$, whenever $B_{max}$ and $K_D$ cannot be separated. Such is the case in any single-injection PET investigation with a receptor ligand and is certainly true if an input function has not been measured. However, it is important to remember that $k_3/k_4$ is not identically equal to $B_{max}/K_D$. In the conventional three-compartment model (i.e., tissue = free + bound + nonspecific), the mass balance on the bound compartment is

$$\frac{dB}{dt} = k_{on}(B'_{max} - B - B')F - k_{off}B, \quad (1)$$

which is exactly equivalent to

$$\frac{dB}{dt} = k_{on}(B'_{max} - B/SA)F - k_{off}B \quad (2)$$

for single-injection experiments (Morris et al., 1996). $F$ and $B$ are the concentration of labeled tracer in free and bound compartments, respectively. $B'$ is the concentration of unlabeled ligand in the bound state. $k_{on}$ and $k_{off}$ are the true forward and reverse rate constants for the ligand and receptor and SA is the specific activity of the injectate. The steady-state volume of distribution between free and bound compartments is

$$\frac{B}{F}_{SS} = \frac{k_{on}(B'_{max} - B/SA)}{k_{off}} \quad (3)$$

$$= \frac{k_3}{k_4} \quad (4)$$

and is equal to the BP provided that $k_3$ is a constant. In other words, it is necessary that

$$B'_{max} > B/SA. \quad (5)$$

B. Model of BP Dependence on Mass

When the specific activity is low enough to violate Eq. (5), then the measured binding potential will decrease with the increasing total (labeled and unlabeled) ligand in the bound state, $B/SA$. It is reasonable to expect that the average amount of ligand in the bound state over the scanning period is proportional to the amount delivered to the region of interest and thus proportional to the mass of injectate per body weight of subject. An inverse relationship has been proposed by Hume et al. (1995) to relate BP (determined with PET) to the dose (in $\mu g/kg$):

$$BP = \frac{\alpha B_{max}/(\gamma K_D + M) + NS}{\alpha B_{max}/(\gamma K_D + M) + NS} \quad (6)$$

to explain the mass-response curves in rats administered raclopride in doses ranging from 0.36 to 360 $\mu g/kg$. $\alpha B_{max}$ and $\gamma K_D$ are constant terms related by SA to $B_{max}$ and $K_D$, respectively. NS is a constant offset term in BP due to nonspecific binding. $M$ is the mass of ligand injected per body weight.

C. Correction Method for Mass Dependence

Inverting the model in Eq. (6) suggests a simple linear correction to BP for mass artifacts. Consider the following two cases.

Case 1

If nonspecific binding is absent, Eq. (6) yields an expression for $1/BP$ that is linear in mass, $M$:

$$\frac{1}{BP} = M/\alpha B_{max} + \gamma K_D/\alpha B_{max} \quad (7)$$

In this case, any two BP estimates at different masses should be sufficient to extrapolate $1/BP$ at zero mass.

Case 2

If nonspecific binding of the ligand is present, linearity is preserved as long as $M < \alpha^2 K_D$. Two BP points should still be sufficient to extrapolate to zero mass provided that mass is small. With only two BP measurements, it will not be possible to fit the nonlinear model [Eq. (6)] for all three unknown parameters: $\gamma K_D$, $\alpha B_{max}$, and NS. Nevertheless, it is assumed that it is possible to choose two small but different mass values.
for which the following linearization holds:

\[
\frac{1}{BP} = M(\frac{B_{\text{max}}}{K_D} + \frac{1}{K_D}) + \frac{1}{K_D}.
\]

This expression predicts that two points are again sufficient to find the intercept on the \(1/\text{BP}\) axis. However, this intercept, \(\frac{1}{K_D} + \frac{1}{K_D}\), retains a contribution from nonspecific binding, \(\text{NS}\).

III. METHODS

A. Analysis

Test data sets [composed of a "striatum" and a "cerebellum" time–activity curve (TAC)] were generated by simulating the standard three-compartment model as implemented by Delforge et al. (1990). BP was estimated from simulated data using two reference region techniques that do not require a plasma input; rather, the receptor-free "cerebellum" curve was used as the reference. The kinetic method was originally introduced by Cunningham et al. (1991) and has been shown to yield BP estimates that correlate closely with the standard three-compartment kinetic analysis using a blood curve (Lammertsma et al., 1996). The graphical method used was introduced by Logan et al. (1996) as a bloodless modification of their earlier graphical method for estimating distribution volume ratios. All slopes from the graphical method were based on integrals of data from 30 to 62 min. Because the \(k_2\) value was the same for all simulations, and the \(B/F\) ratio was fairly constant after 30 min, the term including a population-average \(k_2\) was neglected as described by the authors.

B. Simulations

The simulation parameters were chosen to produce TACs that resembled experimental \(^{11}\text{C}\)raclopride data from rhesus monkeys, including a partial volume correction (Morris et al., 1998). Model parameters used were: \(K_1 = 0.06 \text{ ml/min/g, } k_2 = 0.06 \text{ min}^{-1}, k_{\text{on}} = 0.0032 \text{ ml/pmole/min, and } k_{\text{off}} = 0.075 \text{ min}^{-1}. B_{\text{max}}^*\) values for striatum curves were 5, 35, and 100 nM. These receptor densities corresponded to BP levels of 0.21, 1.5, and 4.27, respectively. "Cerebellum" was simulated with its \(B_{\text{max}}^*\) value set to 0. PET output was modeled as the continuous weighted sum of blood, free, bound, and nonspecific compartments integrated over each scan time. The blood volume fraction was fixed at 0.04. The plasma input curve was modeled as a biexponential with an initial 1-min time delay to the peak. Metabolite correction was not applied to the plasma curve. One standard blood curve was used to generate all simulated PET curves. The plasma curve was scaled so that the peak point equaled the total injected activity (10 mCi) normalized by the approximate blood volume of a 10-kg animal (1000 ml). Where nonspecific binding was included, both "striatum" and "cerebellum" were simulated with the same large forward and reverse rate constants for NS binding (\(k_6 - k_4 = 1.0 \text{ min}^{-1}\)). Poisson noise was added and the level of noise was chosen to resemble dynamic data acquired on the GE PC4096 plus whole body scanner. Pairs of "cerebellum" and "striatum" were simulated for a large range of injection masses (i.e., 10 mCi, where \(SA = [20,000 - 0.1] \text{ mCi/\mu mol}\) for each of three \(B_{\text{max}}^*\) values.

C. Test of Mass–Effect Correction

The simulated data was used to evaluate the correction method proposed earlier. Two-point extrapolation was tested for both cases (with and without NS binding). Every possible pair of BP estimates, for a given parameter set, were inverted and extrapolated to \(1/\text{BP}\) at zero mass. The reinverted intercepts were compared to the true BP values to determine bias and against each other to determine variability and the ability of the extrapolation to eliminate correlation of BP with mass.

IV. RESULTS AND DISCUSSION

A. Demonstration of (Experimental) Mass Artifact

Figure 1 displays the correlation between BP and mass that was found after two scans each of nine rhesus monkeys with \(^{11}\text{C}\)raclopride. The animals received between 0.5 and 2 mCi per kg of body weight with \(SA\) in the range 1200–1600 mCi/\mu mol, i.e., 0.1–0.5 \(\mu g/kg\). The BP values decline with increasing mass as predicted by the theory. Greater mass leads to more bound ligand molecules and greater impact for the term, \(B/SA\) relative to \(B_{\text{max}}^*\). The slope, \(-2.8 \text{ kg/\mu g, and the intercept, 7.3, translate to a decline of 3.8}\%\) in BP for every 0.1 \(\mu g/kg\) added to the mass injected. The BP estimates in Fig. 1 are all from the graphical method.

B. Simulated Mass Artifact

Figure 2 is a plot based on simulated data that is reminiscent of the artifact observed in Fig. 1. These simulations have no nonspecific binding and a \(B_{\text{max}}^*\) of 35 nM (true BP = 1.5). The slope is \(-0.16 \text{ kg/\mu g and...}
the intercept is 1.1, which is a decline of 1.5% in BP for each additional 0.1-μg/kg increase in the mass dose. These BP values were estimated with the graphical method. Within this limited mass range, BP appears to be linearly related to mass. However, if these simulations are extended to lower and higher masses (and to other values of \( B'_{\text{max}} \)) as shown in Fig. 3, the relationship is hyperbolic as predicted by Hume et al. (1995) [Eq. (6)]. Figure 3 displays graphically estimated BP over wide mass ranges of 0.018–360 μg/kg for \( B'_{\text{max}} \) values of 100, 35, and 5 nM without nonspecific binding. Each curve has a relatively flat (“safe”) portion over which the mass effect is negligible and a declining section where the dependence on mass is pronounced. The largest “safe” mass dose varies according to \( B'_{\text{max}} \). For a simulated 10-kg monkey with \( B'_{\text{max}} = 100 \) nM, this “safe” dose is between 1.44 and 3.6 μg/kg (probably outside the range required for humans or monkeys). For \( B'_{\text{max}} = 35 \) nM, the largest “safe” mass dose may be as low as 0.12 μg/kg (on the order of what is used in monkeys). For \( B'_{\text{max}} = 5 \) nM, the mass–response curve is noisy, but the largest safe dose appears to be no more than 0.024 μg/kg, within the range of doses administered to monkeys for SPECT (see Laruelle et al., 1997).

C. Comparison of Graphical and Kinetic Methods

Figure 4 shows that the mass dependence of BP is consistent across estimation methods. The kinetic method consistently shows a positive bias whereas the graphical method shows a negative one relative to the true BP value. Although the large variability in the kinetic estimates makes it difficult to identify the largest “safe” dose on the kinetic response curve, it is clear that they consistently overestimate the BP relative to the graphical method. Similar to graphical estimates, the kinetic estimates decline with mass beginning somewhere between 0.1 and 1.0 μg/kg. Graphical data (open squares) shown in Fig. 4 are the same points as the middle curve in Fig. 3 (simulations with \( B'_{\text{max}} = 35 \) nM).
FIGURE 4 Mass–response curves for BP based on kinetic (top curve) and graphical (bottom) estimates. $K_{\text{max}} = 35 \text{ nM}$ in all simulations. NS binding = 0. The solid line represents the value of the true BP = 1.5. Error bars on the kinetic points are based on the covariance matrix of the parameter estimates. No error bars are given for the graphical method because the points on the Logan plot are not independent.

D. Discrepancies between Models

Biases result from incompatibilities between the reference region techniques and the full three-compartment model. One obvious incompatibility is the presence of a blood component in the PET model. However, even with noiseless simulations and no blood volume fraction, the graphical method gives a low and the kinetic method a high estimate of BP. However, the reference region model is internally consistent. When it is used to both generate data and fit them, there is no bias, at least at moderate error levels, in BP estimates. The graphical technique assumes that the free and bound compartments are in equilibrium. This condition is not strictly satisfied with data presented here. Also, by using least-squares fitting of points that are dependent on the integral of data (i.e., contain cumulative error), the graphical method implicitly imposes a weighting of the points other than the inverse variance weighting prescribed by the unbiased nonlinear least-squares estimator. Thus, a priori, it appears that the Logan plot method is a biased estimator of BP, which has been confirmed by Hsu et al. (1997). Although the kinetic method properly weights data, it is also quite sensitive to the shape of the cerebellum curve. Thus, when the noisy cerebellum curve is smoothed, the resulting generated input function, $K_C$, may have a shape that is incompatible with the striatum and give a poor fit.

E. How Good is the Mass Correction?

Regardless of biases in the reference region methods, their performance in eliminating the mass effect, demonstrated earlier, on BP can be assessed. Figure 5 displays graphical BP estimates from simulated data for which $K_{\text{max}}$ is 35 nM; the mass range is 0.072–3.6 μg/kg and there is no nonspecific binding. These data correspond to activity doses of 10 mCi and a reasonable range of specific activities (5000–100 mCi/μmol) for [15C]raclopride at time of injection. [Note: Carson et al. (1997) injected 2–5 mCi of [15C]raclopride into 10-kg monkeys and observed mass effects in their BP estimates with SA of 363 ± 181 mCi/μmol.]

Figure 5a shows the expected decline in BP with increasing mass. These points are a subset of the points on the middle curve in Fig. 3. Figure 5b is a plot of 1/BP vs mass. The points lie roughly on a line with positive slope as predicted by Eq. (7). To correct 1/BP "graphically" (i.e., to locate the 1/BP intercept), any two points on Fig. 5b should suffice. The variability in the intercept values for all these points taken two at a

FIGURE 5 (a) BP vs mass for $K_{\text{max}} = 35 \text{ nM}$, NS = 0, (b) 1/BP vs mass, (c) histogram of corrected BP values, and (d) corrected BP values vs maximum of two masses used in correction.
time is displayed as a histogram of BP values in Fig. 5c. The histogram of corrected BP values, which is binned in 0.1 increments, is narrow and symmetric, reflecting good error properties of the correction. Figure 5d gives each of the corrected BP values plotted against the maximum of the two masses used to generate the corrected value. Regardless of mass correction, the Logan method underestimates the true BP. Prior to correction, the correlation coefficient (r) of BP with mass was -0.88 and the coefficient of variation (COV) was 9.7% in the BP values. Following correction, r was reduced to -0.41 and the COV was 6.6%. Much of the correlation with mass is eliminated without sacrificing precision. Unfortunately, if a similar test is performed with the kinetic estimates of BP, r of -0.59 is improved to -0.082, but the COV is aggravated from 35 to 1211%. The poor variability in the correction method reflects the large variability in kinetic estimates themselves.

F. Inclusion of Nonspecific Binding
To this point, the two-point correction has only been tested on parameters that were derived from data that had no nonspecific binding. If fast nonspecific binding is included in simulated data, the mass effect is retained but the amplitude of the mass–response curve is changed. Figure 6 displays three mass–response curves. The top curve is for data in the middle curve of Fig. 3 without nonspecific binding. The bottom curve is for data from the same parameters except that it includes fast nonspecific binding in both the “striatum” and the “cerebellum.” The largest “safe” mass dose remains unchanged (compare bottom curve to top), but the presence of nonspecific binding causes the mass-independent portion of the curve to be much lower than in the top curve. This fact, which is even clearer on an inverted plot, is consistent with case 2 of the Hume model in Eq. (8). The intercept is a function of NS. For seven simulations with NS binding and a mass range of 0.072–3.6 µg/kg, r improved dramatically from −0.9 to −0.12 after correction and COV improved slightly from 11.5 to 10.3%.

G. Disagreement with Hume Model
The middle curve in Fig. 6 (solid line) is the mass–response curve that Hume et al. (1995) fit to their [11C]raclopride PET data in rats. Although it shows a “safe” range of masses that is comparable to monkey simulations, it reveals a discrepancy with the results described in this chapter. As dictated by the offset term, NS, in Eq. (6), the Hume model predicts that in the presence of NS binding, one will measure a positive BP at least equal to the NS term for all mass doses no matter how large. Simulation data described in this study indicate otherwise. The bottom curve in Fig. 6 clearly goes to zero at a mass dose of 100 µg/kg. What is the reason for this discrepancy? In reviewing the paper by Hume et al. (1995), it appears that the model may not have been tested by an adequately large mass range to determine the need for an offset term. Most likely, Hume et al. (1995) were constrained by the lethal limits of raclopride. In any case, this finding has implications for the authors’ correction method. If the results shown here hold up (i.e., if all mass–response curves for BP go to zero at large masses, even with NS binding), then the prohibition on using a large mass dose to extrapolate to zero is removed (see case 2 in Section 11). Of course there may continue to be pharmacological reasons for avoiding large masses of tracer when the ligand, such as raclopride, is a psychoactive substance.

H. Conclusion
Using simulated time–activity curves, this chapter demonstrated significant effects of mass on estimates of binding potential at reasonable mass doses to nonhuman primates. As PET protocols are often prototyped in monkeys and basic science studies are often performed exclusively in monkeys, it is important not to mistake artifacts of mass for important findings. The same artifacts may occur in humans, most probably in tissues with low receptor density. At a minimum, these simulation results underscore the importance of uniform administration of ligand by mass. A two-point extrapolation method has been proposed and validated that corrects for the effects of mass based on a model.
of BP given by Hume et al. (1995). The correction performs better with graphical than with kinetic estimates of BP. Nonspecific binding will bias the corrected value for BP but, contrary to Hume, the authors' simulations suggest that any two estimates of BP, regardless of their injected masses, can be used for the extrapolation.

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References


