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EDITOR'S NOTE

A recent Letter to the Editor by Cumming P, Gjedde A and Reith J (*8*) and the replies by Wahl L and Nahmias C (*16*), and Dhawan V, Ishikawa T, Patlak C and Eidelberg D (*17*) raised issues which deserved further comment. This editorial was requested to provide an additional perspective on the issue of quantifying and modeling physiologic processes with PET.

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Letters to the Editor

Controversies Arising from Recent FDOPA Articles

TO THE EDITOR: In three recent articles, Dhawan et al. (*1*), Ishikawa et al. (*2*) and Wahl and Nahmias (*3*) examined the use of the tracer FDOPA to quantify the decarboxylation of DOPA to dopamine in human brain. Although we commend the authors for their desire to evaluate the issues pertaining to interpretation of positron emission tomograms of FDOPA metabolism in human brain, their citations are selective and their arguments noticeably biased. In particular, we feel that the current debate about the utility and merits of the several approaches to the assay of FDOPA metabolism with PET would benefit from consideration of the following points:

1. The authors' conclusions that it may be unnecessary to assay a specific biological variable (as opposed to a less specific variable) to obtain clinically relevant information.
2. The authors' demands that an uncertain disease process be the criterion of model of quantitation of a biological variable, and that the estimates of model parameters be independently clinically (as opposed to biologically) validated.
3. The authors' selections of cited articles.

Choice of Assay

With present PET methods, Dhawan et al. (*1*) conclude that FDOPA yields clinically more relevant information with the simple multiple-time graphical analysis of Patlak (*4–6*) than with the computationally more demanding compartmental techniques of Gjedde et al. (*7*) and Kuwabara et al. (*8,9*). According to Dhawan et al. (*1*), the information obtained with the simpler approach is clinically relevant because it agrees with clinical information already in evidence. It matters little whether the simpler approach yields any information of biological interest.

Diagnosticians and neuroscientists of course may have divergent interests in the outcome of specific tests, but we believe it is wrong to suggest that the only criterion of interest of such a measurement is whether or not it clearly distinguishes between specific groups of preselected subjects. For example, the failure of a particular assay to establish a conclusive difference between the activities of an enzyme in patients and healthy control subjects does not mean that the measurement is of no value to the understanding of the disease; on the contrary, the observation may be the key to that understanding. We only emphasize this truism because Dhawan and Ishikawa et al. incorrectly claim that attempts to measure the activity of the enzyme DOPA decarboxylase (DDC) in Parkinson's disease are misguided because another less specific measurement of the net transfer of FDOPA across the blood-brain barrier in their hands discriminates more clearly between patients and healthy volunteers. Ishikawa et al. (*2*) give an unintended but excellent example of this dilemma: The authors conclude that "Estimates of striatal DDC activity cannot discriminate between normals and Parkinson's disease patients as accurately as K_i^{FD} (i.e., the slope of the Patlak plot, not a "unidirectional" transfer constant as claimed by the authors) or SOR (i.e., striatum-occiput ratio)." The authors base this conclusion on the F-statistics of the measures which in reality is a reference to the precision rather than the accuracy of the measures.

Accuracy is the more illuminating property, which in the case of FDOPA may not apply to measures such as the Patlak slope and the SO ratio if they have no specific biological meaning. The accuracy of the two measures is placed in further serious doubt by the lack of correlation between the values of the SOR and measures of the disease's severity (UPDRS), as revealed by the authors' own Figure 3 (*2*) and by the restrictive biological bounds on the Patlak slope dictated by blood flow and the blood-brain permeability-surface area (PS) product of FDOPA. It is entirely possible that both the Patlak slope and the SO ratio fail to reveal this variation for methodological reasons.

In many scientific studies, the purpose of the assay is not to distinguish between patient groups but to measure a biological variable of interest to pathophysiology. In this respect, lack of discrimination need not be less revealing than discrimination. Also, were the activity of the enzyme that synthesizes dopamine from

DOPA not of clinical significance in Parkinson's disease because the measurement is too imprecise, as claimed by Ishikawa et al., the variable could be of interest in other states or to hypotheses of the regulation of dopaminergic neurotransmission in other conditions (*10*). To argue that a variable is not interesting because it is difficult to measure is absurd and reminiscent of the very ancient Sufi legend of the Dervish who dropped his spectacles one evening while whirling in the garden, but being of a fanatical bend, insisted on looking for them in the kitchen where the light was better.

Choice of Model

Dhawan et al. (*1*) require that model selection be biologically validated but establish a circular condition for the validation (i.e., that the model must accurately represent the underlying disease process). What they mean is that the results should not misrepresent the disease process, but we invite readers to think about this requirement for a moment: If the disease process is uncertain, as it is in Parkinson's disease, or when the process is the object of the assay, potential misrepresentation cannot be used as a criterion of choice of the model without begging the question. The model must be based on known biological facts but cannot be rejected solely on the basis of details of a disease that the model is being used to reveal. The multiple-time graphic analysis or "Patlak" plot (*4–6*) yields the net, not unidirectional, clearance of FDOPA from the circulation by exchange across the blood-brain barrier. Its use for the purpose of imaging DOPA metabolism in the human brain was explored during a meeting in Belgrade in 1987 (*11*). While the analysis is simple, it was argued, the information gained from the plot is useless because DOPA normally derives not from the circulation but from the tissue where it is derived in situ. The net transfer of FDOPA across the blood-brain barrier reflects a multiplicity of factors, of which plasma and brain amino acid concentrations, peripheral and central DOPA decarboxylase and COMT activities and cerebral blood flow are the most important.

Concurrent efforts were directed at the establishment of PET assays for a specific biochemical step of significance to DOPA metabolism. The efforts were undertaken by groups in Los Angeles (*12*) and Montreal (*7–9,13*) and by others, including the groups represented by Dhawan et al. (*1*), Ishikawa et al. (*2*) and Wahl and Nahmias (*3*). The goal has been to do so on the basis of a model with a minimum number of parameters, yet uniquely sensitive to the activity of the enzyme DOPA decarboxylase. Dhawan et al. (*1*) present the model proposed by us as their Figure 1.* To reduce the number of parameters to two transfer coefficients, and an initial volume of distribution, the Montreal group included two variables as constants, i.e., one symbolized as "q" which represents the ratio between the blood-brain transfer rates of FDOPA and 3-O-methyl-FDOPA (3OMFD), and the other by "V_e", which symbolizes the joint partition volume of large neutral amino acids (LNAA) in brain. The assumptions underlying our choice of values of q and V_e are physiologically reasonable. When the authors of the three papers variously criticize the assumptions on the grounds that they are at variance with the results of their actual PET observations, we insist that the authors look more carefully for pitfalls in the PET measurements.

Transport Radio

Dhawan et al., Ishikawa et al. and Wahl and Nahmias argue that:

1. In humans the value of q does not equal the value reported by us (q = 2.3).
2. Uncertainty about the value of q may bias the estimates of k_3, as reported by them in abstract.

They further claim that unity is closer to the correct value of the ratio in humans, and they base this claim on separate estimates of the blood-brain transfer constants of 3OMFD and FDOPA. However, a blood-brain transfer ratio can be determined accurately only in the absence of significant metabolism of the tracers, either when the transfer is measured for a period short enough to exclude significant metabolism, or when the tracers are not rapidly metabolized (as is the case for 3OMFD). This is not an academic point, because regression to uptakes maintained for long periods of time has fundamental difficulties distinguishing between noisy approaches to steady-state with and without metabolism, as documented by Kuwabara et al. (*8*). As shown by decades of research into glucose transport into red blood cells and across the blood brain barrier, it is simply not acceptable to measure blood-brain barrier transport rates in the presence of significant metabolism.

Ideally, q should be estimated as a separate parameter, not by subsequent numerical manipulation of average estimates of other regression analyses. No estimate of q, other than the original one of 2.3, determined as an abscissa intercept, actually meets these requirements. It is interesting that the only other estimate of q that come close to meeting these requirements, that of Dhawan et al., averages 1.8 in the cerebral cortex of healthy volunteers in whom the metabolism of FDOPA is minimal. It is true that the value of q determined in rats need not apply to humans and hence the use of this constant in human studies need not be warranted. Dhawan et al. (*1*) and Ishikawa et al. (*2*) cite their own preliminary evidence in support of this argument, but they neglect to discuss results of simulations which showed that the uncertainty about the exact value of q in the range 1.8–2.8 had little effect on the estimates of k_3 [see Figure 3B in Kuwabara et al. (*8*)]. We have recently obtained evidence in the rat that varying q from 0.5 to 3 had minimal effect on k_3 (Deep et al., unpublished data, 1997). In other words, our results indicate that it is safe to use the only correctly determined value of q also in human beings in which it has not yet been determined.

Partition Volume V_e

Our solution of the model equations assumes that V_e has the same value in every region of the brain. Dhawan et al. show that separately estimated FDOPA V_e values of frontal cortex and striatum are different. They neglect to say that this result was predicted by simulations reported by Kuwabara et al. (*8,* Figure 2A). There are several possible explanations of the result but the simplest is that V_e, as q, reflects processes involved in the blood-brain transfer of LNAAs which can only be measured correctly in the absence of significant metabolism of the tracer. In normal volunteers, Dhawan et al. reported a uniform distribution of 3OMFD in the brain. An entire body of literature of the transport of amino acids across the blood-brain barrier likewise shows that the partition volume of LNAAs is close to the water volume of brain because the total net consumption of LNAAs is minuscule in most regions of the brain (*14*), as is specific binding. In turn, this means that the total concentration of LNAAs in brain tissue is lower than, but close to, the total concentration in blood plasma.

The water volume of grey matter is close to 0.78 ml/cm^3 and this is the value that amino acid studies predict that V_e is likely to approach. In keeping with the prediction, Dhawan et al. (*1*) found no significant differences of V_e among any regions or any subject groups, when V_e was determined with a non-metabolized LNAA, 3OMFD; no value of V_e differed significantly from 0.78 ml/cm^3 in their study. A partition volume greater than the water volume of brain tissue, relative to plasma water, signifies a gradient of transport directed out of brain. This unphysiological situation means either that the brain tissue has a net export of one or more LNAAs, or that the LNAAs are transported into brain against their

*In the legend to Figure 1, Dhawan et al. (*1*) state that our "nomenclature was retained for comparison purposes." What does this statement mean? Is the original nomenclature actually wrong? Do Dhawan et al. have another that they reluctantly omit to mention? Is the use of the model adversely affected by the original nomenclature?

concentration gradient. None of these explanations should be entertained in the face of simpler methodological explanations.

As an argument in favor of higher partition volumes of LNAAs in the striatum than in cerbral cortex, Wahl and Nahmias refer (*3*) to their article in which the striatum is visible in 3OMFD images (*15*). A higher partition means a greater total content of LNAAs in striatum than in other regions of the brain, despite the relatively pronounced metabolism of tyrosine in striatum (*16*). As an alternative and more plausible explanation, we offer the speculation that 3OMFD may bind to DOPA decarboxylase without being metabolized. It would of course be a mistake to regard the value of V_e determined this way as representative of the partition volume of DOPA which is metabolized by DOPA decarboxylase. Wahl and Nahmias also refer to the findings of Wahl et al. (*15*) and other findings in the literature in which the steady-state distribution of non-metabolizable LNAAs such as 3OMFD appears to reflect a partition volume greater than the water volume, and sometimes higher than 1 ml/cm^3 or 1 ml/g. Rather than invoking unphysiological explanations involving export or active transport, we suggest that diffusion of LNAAs from plasma into erythrocytes after sampling of arterial blood artifically lowered the plasma concentration of these tracers, thus causing the calculated V_e to be too high. None of the papers give any indication of how blood samples were handled or of how rapidly plasma was separated from erythrocytes in these analyses.

Choice of Citations

Dhawan et al. (*1*), Ishikawa et al. (*2*) and Wahl and Nahmias (*3*) nelgect to cite the two early articles of the Patlak plot (*4,5,16,17*). Dhawan et al.'s citation of Firnau et al. (*18*) ignores the missing 3OMFD in that report, although the rapid peripheral metabolism of FDOPA to 3OMFD in humans had been described accurately by Boyes et al. (*19*). This oversight occurred again in Dhawan et al.'s references to Firnau et al. (*18,20*) in which the presence of large amounts of 3OMFD in tissue extracts was overlooked, apparently due to systematic misidentification of the radiochemical peaks in chromatographic fractionations. This misidentification has never been acknowledged or addressed, although it is in plain view. Wahl and Nahmias' references to Chan et al. (*21,22*) confirm a linear increase with time in the ratio of metabolites to FDOPA in circulation, but fail to acknowledge that this empirical result was first described by Boyes et al. (*19*).

The principle underlying the utility of FDOPA for PET sudies of DOPA metabolism is that FDOPA enters into the pathway for dopamine metabolism in living brain; i.e., that FDOPA is decarboxylated by DDC yielding F-dopamine, which is, in turn, eliminated from brain as deaminated metabolites. As such, Dhawan et al., Ishikawa et al. and Wahl and Nahmias quite properly begin their articles with a brief review of the pathway for dopamine synthesis and metabolism. However, none sees fit to acknowledge the body of work first describing the metabolic fate of FDOPA, and its similarity to that of endogenous DOPA. Dhawan et al.'s and Wahl and Nahmias' references to the largely confirmatory work of Melega et al. (*23,24*) ignore the detailed description of the peripheral and central metabolism of FDOPA by Boyes et al. (*19*) and Cumming et al. (*25–28*). Dhawan et al.'s references to blocking the formation of 3OMFD ignores earlier contributions by Cumming et al. (*26*), in which treatment of rats with the catechol-O-methyltransferase inhibitor U-0521 decreased the peripheral formation of 3OMFD, and so increased the synthesis of F-dopamine from FDOPA in striatum of living rats.

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