CORRESPONDENCE

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The Importance of *In Vitro* Assay Temperature When Using Volatile Anesthetics

To the Editor:—The recent report by El-Maghrabi and Eckenhoff demonstrated that halothane and isoflurane disrupted [^{3}H]dopamine transport into synaptosomes from rat brain.[1] The authors suggest that the clinical relevance of this observation is validated, at least in part, by the fact that the IC_{50} of the inhibition by halothane was 0.7 mM at 22° C. Equilibration of 1% v/v halothane (1 MAC in rats)[2] with Tris buffer at 37° C results in an aqueous halothane concentration of 0.4 mM.[3]

We believe that the extrapolation of results obtained using volatile anesthetics at below physiologic temperatures must be qualified before any claim of clinical relevance can be maintained. Inhibition of amine transport by halothane at physiologic temperature would hold more credence with regard to clinical relevance than would similar inhibition at room temperature. That amine transport processes are temperature-dependent leads us to only speculate as to why the authors failed to measure anesthetic influences on dopamine transport at 37° C.

We maintain that this shortcoming fails to allow any valid extension of these results to the *in vivo* situation. In reporting the inhibition of [^{3}H]5-hydroxytryptamine (5-HT) transport by halothane at 37° C, we noted a somewhat less potent effect than the present results, where the IC_{50} value for halothane was approximately 1.0 mM.[4] Moreover, contrary to what El-Maghrabi and Eckenhoff stated, we noted a competitive kinetic interaction between halothane and 5-HT transport, implying an action by the anesthetic at the 5-HT transport recognition site.

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In Reply:—Martin and Merin raise at least two temperature-related issues, one being the influence of temperature on transport processes and the other being the relationship between temperature and volatile anesthetic concentration. There is no question that dopamine transport is temperature-dependent. This is the reason why the kinetic assays reported in our paper[1] were performed at 37° C; a fact clearly stated in the methods section. Only the inhibition experiments were performed at 22° C—to slow the initial rate of uptake and reduce the time-dependency of the assay. We have made the reasonable assumption that the IC_{50} value is *temperature-independent,* as verified by our finding that V_{max} in the presence of anesthetics (at 37° C) was reduced by an amount predicted by the 22° C IC_{50}.[1]

To examine the issue of temperature dependence of anesthetic concentrations, it is important to remember that anesthetic effects are ultimately related to the *molar concentration,* and not the gas concentration, despite the anesthesiologists' fixation on minimum alveolar concentration (really a compromise for measurement convenience and generally valid because of the relatively constant temperature of our patients). If the molar concentration is produced by equilibration with a gas phase, then temperature is important, because of the temperature-dependent nature of blood or buffer/gas partition coefficients. For example, 0.35 mM halothane in Kreb's buffer is produced by equilibration with about 1.0 vol% at 37° C or 0.5 vol% at 22° C. However, if the anesthetic is dissolved directly in a gas-tight system with no gas space (as we did), then the molar concentration is *temperature-independent.* Because most potency data for these agents are expressed in vol%, it is necessary for laboratory investigators to relate the (measured) molar concentration back to the clinically familiar gas concentration. In doing so, it is crucial that 37° C partition coefficients be used, regardless of the actual experiment's tempera-

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ture. The related and important question of how temperature influences partitioning between blood or buffer and the "effect" site is less well answered because the character of this site is not known. The small and inconsistent effect of temperature on buffer/tissue partition coefficients suggests, however, that this influence is small.

Lastly, though the [^{3}H]5-hydroxytryptamine (5-HT) and dopamine transporters are members of the same family, they are different proteins, so the fact that halothane may produce slightly different effects in one as compared to the other should not be surprising. What is perhaps surprising is the similarity between our results and those of Martin *et al.*[2] They reported that isoflurane inhibited 5-HT transport noncompetitively, and at a IC_{50} of 2.36 mM, compared to our noncompetitive IC_{50} for dopamine transport of 2.24 mM. Their data for halothane are less clear, however, because in one paper[3] they conclude that the inhibition of 5-HT transport showed competitive kinetics but then reported in a subsequent paper[4] that the inhibition was allosteric with respect to the 5-HT site (*i.e.*, noncompetitive). Martin and Merin do not discuss this apparent discrepancy in their letter or the Martin *et al.* paper,[4] but it has become increasingly clear that it is not a discrepancy at all; allosteric interactions can produce competitive kinetics, as is well documented by the situation with cocaine and the dopamine transporter,[5,6] for example. Overall then, it seems that our results are in rather good agreement with those of Martin and Merin, despite the minor methodologic differences.

We stand by our conclusion that dopamine transporter inhibition occurs with concentrations of halothane, but not isoflurane, that are used clinically. How or whether this can be reconciled with an *in vivo* effect, is not yet clear.

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