The concern Garcia-Buñuel expresses regarding the possibility that our results may be confounded by interactions between the paralytic we used and the specific substances under study is important and addresses an issue that is often ignored in pharmacological experiments. However, the gallamine-dopamine interaction hypothesized by Garcia-Buñuel is extremely unlikely.

Garcia-Buñuel’s hypothesis necessitates at least three assumptions: gallamine crosses the blood-brain barrier, gallamine affects dopaminergic systems, and gallamine affects dopamine receptor sensitivity. We address each assumption in order.

Because charged molecules cross lipid membranes with difficulty, substances containing one quaternary amino group usually do not cross the blood-brain barrier in pharmacologically significant amounts (1); gallamine contains three quaternary amine groups. Thus, radioactively labeled gallamine has been shown not to enter the central nervous system (CNS) (2). However, some of the papers referred to by Garcia-Buñuel do present evidence that small amounts of gallamine may enter the CNS, and this possibility must be considered. In our case, however, even the crossing of gallamine into the brain could not have confounded our results, as shown below.

The references given by Garcia-Buñuel for dopamine-acetylcholine interactions deal with muscarinic drugs, whereas gallamine is a nicotinic antagonist. The paper referenced by Garcia-Buñuel to argue for anatomical dopaminergic systems overlap shows the substantia nigra to have one of the lowest concentrations of acetylcholine in the brain. Although acetylcholinesterase is found in substantia nigra, it is well recognized that it is not a reliable marker for acetylcholine input (3).

Even if gallamine did affect the nigrostriatal system directly, a direct action of gallamine at the dopamine receptor must be hypothesized in order to confound our results, because microiontophoresis allows one to study the direct effect of dopamine on neurons. To our knowledge, no evidence has been advanced relating an interaction of acetylcholine with dopamine receptor sensitivity.

In addition, we have found that the dose response curves for dopamine neuron activity in rats with respect to apomorphine and dopamine are the same whether the animals are anesthetized with chloral hydrate or paralyzed with gallamine. Intravenous administration of gallamine to a respirated rat anesthetized with chloral hydrate does not alter dopamine cell firing rate or pattern. Furthermore, cholinergic drugs (for example, scopolamine and physostigmine), which pass the blood-brain barrier more easily than gallamine, have no detectable effect on dopamine cell activity.

From these data we conclude that in our studies gallamine did not confound the results.

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References

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Prediction of Hypolimnetic Oxygen Deficits: Problems of Interpretation

The recent report of Cornett and Rigler (1) presented a model to predict the areal hypolimnetic oxygen deficit (AHOD). This model was accurate over the range of the 12 lakes investigated (the total amount of variation $R^2 = 0.75$). The regression equation developed was

$$\text{AHOD} = -277 + 0.5R_p + 5.0\ T_h^{1.74} + 150\ln(Z_H)$$

where $R_p$ is the areal phosphorus retention (in milligrams per square meter per year) from Dillon and Rigler (2), $T_h$ is the mean volume-weighted temperature of the hypolimnion (in degrees Celsius), and $Z_H$ is the mean thickness of the hypolimnion (in meters). But, despite the accuracy of this model to predict AHOD, we feel there are some logical and computational flaws in the approach used to generate the model.

The logical flaw centers around the term $R_p$, which appears to have been applied outside the limits of its normal variability in the Cornett and Rigler model. Nevertheless, this is a common problem with retention models (2) and is not too serious here.

The computational flaws are somewhat more serious than the logical flaw and jeopardize the validity of the model. A basic assumption of all least-squares multiple regression analyses is that the independent variables are not correlated (3). Although often overlooked in many multiple regression analyses, this assumption was particularly violated in the AHOD model of Cornett and Rigler. The negative correlation between $T_h^{1.74}$ and $\ln(Z_H)$ is very high, $-0.69$. A consequence of this high correlation is that the order in which the variables are entered into the stepwise multiple regression analysis will dramatically affect the predictive equation. The temperature term $T_h^{1.74}$ was not significant in the original analysis, but this term may have been significant if added to the stepwise analysis before the hypolimnion thickness term, $\ln(Z_H)$. The high negative correlation between $T_h^{1.74}$ and $\ln(Z_H)$ suggests that, if temperature were added to the stepwise regression first, the hypolimnion term might be nonsignificant. Should this be the result, the major point of the Cornett and Rigler report would be negated, that is, that $Z_H$ played an important and unexpected role in predicting AHOD. We suggest a more detailed analysis of the significance of $T_h$ and $Z_H$ be made before any limnological conclusion about cause and effect be drawn. In particular, the stepwise regression analysis approach should be avoided.

We applied the Cornett and Rigler model with data from the Great Lakes. The predicted AHOD for Lake Michigan is 504 mg of $O_2$ per square meter per day for a hypolimnion 70 m thick and a total phosphorus concentration of $8 \text{ mg/m}^3$ (4); for Lake Superior the predicted AHOD is 486 mg of $O_2$ per square meter per day for a hypolimnion 130 m thick and a total phosphorus concentration of $4 \text{ mg/m}^3$ (5). We found that these predictions, although possible, are too high for both Lake Michigan and Lake Superior and have never been observed. Our conclusion is that the model appears very sensitive to $Z_H$ and yields high AHOD values for any lake with a hypolimnion over 50 m thick.

An additional but less important problem with the computational technique is the transformation of the $Z_H$ and $T_h$ variables. The transformed variables, although statistically correct (6), are difficult to understand in terms of meaningful units. The temperature variable, in particular, is hard to decipher when raised to a power of 1.74. In some cases, transformation could change the meaning of an independent variable in the predictive model: that is, is $\ln(Z_H)$ a change...
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