Glucose-6-Phosphatase Activity in Brain

Nelson *et al.* (*1*) have challenged our earlier finding (*2*) that the estimated rate of glucose metabolism in rat brain is greater when measured with [2-^{3}H]-glucose than when measured with [U-^{14}C]-glucose as a result of the apparent dephosphorylation of glucose-6-phosphate by brain in vivo. This challenge to our original report was based on (i) a comparison of the rates of glucose metabolism obtained between 2 and 7 minutes after intracarotid injection of variously labeled glucose (our original report measured the initial rates of glucose metabolism between 5 and 60 seconds after injection of the label) and (ii) a criticism of our original method of column fractionation of glucose.

As Table 1 and our previous report (*2*) show, the differences in the two measured rates of glucose metabolism are only significant within the first 30 seconds after intracarotid injection of label. Nelson *et al.* (*1*) report only the ^{3}H/^{14}C ratio of glucose from 2 to 7 minutes after injection. At 2 minutes, the specific activity of cerebral venous and systemic venous [2-^{3}H]glucose are already equilibrated, and the ^3H_2O formed in brain is already released (*2*). Without data from the first minute after injection, any conclusion about the magnitude of the rate of the dephosphorylation of glucose-6-phosphate, such as that drawn by Nelson *et al.* (*1*), is unwarranted.

Table 1. Observed average rates of the phosphorylation of glucose during the specified time interval after injection. The specific activity of brain glucose was determined at the end of the specified time period. Values are means ± SD; n = 4 or 5 (observations).

Time v from (sec)	v from [2-^{3}H]glucose (μmol min^{-1} g^{-1})	v from [U-^{14}C]glucose (μmol min^{-1} g^{-1})	Difference (μmol min^{-1} g^{-1})	P*
0–10	1.84 ± 0.1	1.48 ± 0.1	0.36	<0.01
0–20	1.38 ± 0.08	1.15 ± 0.06	0.24	<0.01
0–30	1.16 ± 0.24	1.02 ± 0.24	0.14	<0.08
0–60	1.12 ± 0.08	1.03 ± 0.09	0.09	<0.11

*Two-tailed t test.

The second methodological criticism raised by Nelson *et al.* (*1*) is entirely valid: in our original column fractionation of glucose we neglected to use a cation exchange column, with the consequence that we identified labeled glutamine and γ-aminobutyric acid in mass spectrometric studies of such extracts. Accordingly we have repeated our original study and included a cation exchange resin step similar to that used by Nelson *et al.* (*1*).

We herein report a repeat of our original experiments (*2*) in which we used this later method of fractionation of labeled glucose (*1*) from freeze-blown rat brains after intracarotid injection of radioactive label. All data were collected within 1 minute after injection of label. The rates of glucose phosphorylation (v) were calculated from $dP/dt = v \times SA(t)$, where dP/dt is the rate of formation of the labeled products of glucose phosphorylation, and $SA(t)$ is the specific activity of labeled glucose at time (t).

The initial rate of glucose utilization [$v = dP/dt/SA(t = 0)$] from data calculated at zero time for [2-^{3}H]glucose is 1.26 micromoles (μmol) per minute per gram of brain, while that from [U-^{14}C]glucose is 1.06 μmol per minute per gram, a difference of 0.20 μmol per minute per gram.

Our data show that the rate of the dephosphorylation of glucose-6-phosphate is about 19 to 24% of the rate of the phosphorylation of glucose estimated from [U-^{14}C]glucose.

MING-TA HUANG
RICHARD L. VEECH
Laboratory of Metabolism, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852

REFERENCES

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2. M-T. Huang and R. L. Veech, *J. Biol. Chem.* **257**, 11358 (1982).

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Response: Huang and Veech concede that the "glucose" fraction they extracted from brain, on which they based their conclusion of high glucose-6-phosphatase (G6Pase) activity (*1*), was contaminated with metabolic products of [2-^{3}H, U-^{14}C]glucose. They no longer mention a fall in the ^{3}H/^{14}C ratio in the glucose fraction, but base their claim of high G6Pase activity in brain on a fleeting difference between apparent rates of glucose utilization calculated from [^{3}H]glucose and [^{14}C]glucose disappearance. These rates are calculated by dividing the radioactivity in the labeled metabolic products by the specific activity of the respective labeled glucose fractions recovered from brain by their purification procedure. They offer no proof of quantitative recovery of labeled products and purity of the glucose fractions. In fact, neither of these requirements is met. They have now added to their purification procedure a cation exchange step, presumably applied to the Dowex-1–borate eluate in their previous procedure (*1*), which removes two ^{14}C-labeled contaminants identified by mass spectrometry. They provide no evidence, however, for the purity of the remaining glucose fraction and incorrectly cite our report (*2*) as providing evidence that the cation exchange step purifies the "glucose" fraction. We (*2*) used a different, more extensive purification procedure in our studies and found no significant change in the ^{3}H/^{14}C ratio of the brain glucose pool; the cation exchange step applied to the Dowex-1-borate eluate only partially purified the glucose.

The purification procedure of Huang and Veech, including the cation exchange step, does not exclude completely ^{14}C-metabolites from the [^{14}C]glucose fraction. The procedure also produces detritiated ^{14}C-labeled acidic derivatives of glucose and its metabolites at the Dowex-1-borate eluate step that are not removed by cation exchange. The procedure clearly leads to low recovery of ^{14}C-metabolites and overestimates of [^{14}C]glucose specific activity. These deficiencies are sufficient to explain why the rates of glucose utilization calculated with [^{14}C]glucose are lower than those calculated with [^{3}H]glucose, for which the contaminants are largely unlabeled. This difference in calculated rates is transient. As the loss of [^{3}H]water derived from brain [^{3}H]glucose metabolism becomes progressively more significant, the rate calculated with [^{3}H]glucose diminishes rapidly toward the already artifactually lower rate calculated with [^{14}C]glucose. Moreover, both rates are, in fact, erroneously inflated, particularly at early times, because they were calculated with the specific activities of precursor pools at kill time rather than with the correct integrated specific activities over the entire experimental period.

Contrary to the statement of Huang and Veech, a fall in the ^{3}H/^{14}C ratio of the glucose pool due to G6Pase would not disappear with time. It can be readily calculated (with worst-case estimates of the kinetics of glucose transport between blood and brain) that the magnitude of G6P dephosphorylation claimed by Huang and Veech would lead to an easily measurable reduction in the ^{3}H/^{14}C ratio in the glucose pool in brain, even under steady-state conditions,