Reexamination of Glucose-6-Phosphatase Activity in the Brain in Vivo: No Evidence for a Futile Cycle

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Abstract. Glucose-6-phosphatase activity in the rat brain in vivo was estimated by
measuring the differential loss of tritium and carbon-14 from the glucose pool labeled
by a mixture of [2-3H]glucose and [U-14C]glucose. The results provide no evidence of
significant dephosphorylation of glucose-6-phosphate and do not support the hy-
pothesis of a futile cycle involving glucose-6-phosphatase activity in the brain.

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It is widely believed that the mammali-
an brain is not a gluconeogenic organ and
contains negligible glucose-6-phospha-
tase (G6Pase) activity (1). The presence of
G6Pase in the brain has been demon-
strated by histochemistry (2, 3), but qua-
titative assays of the enzyme activi-
ty in cerebral tissues in vitro have usual-
ly shown it to be a small fraction of that
of known gluconeogenic organs, such as
der liver and kidney (4, 5), and supported the
view that G6Pase activity has little, if
any, role in the carbohydrate metabolism
of the brain. This belief was recently
challenged by Huang and Veech (6), who
reported that there was sufficient G6Pase
activity in the rat brain to maintain a
steady-state hydrolysis of glucose-6-
phosphate (G6P) equal to at least 35
percent of its rate of formation by hexo-
kine-catalyzed phosphorylation of glu-
cose. Since the brain has a very high rate
of glucose utilization (7), a rate of de-
phosphorylation of G6P equal to one-
third the rate of glucose phosphorylation
by hexokinase would represent a level of
G6Pase activity approaching that found in
the rat liver after feeding (8).

Huang and Veech injected a mixture of
[2-3H]glucose and [U-14C]glucose into
one carotid artery of rats, removed the
brains by freeze-blowing (9) at various
times up to 5 minutes later, separated
free glucose from the tissue, and deter-
mined its 3H/14C ratio by liquid scintilla-
tion counting. The principle behind these
procedures is as follows. Any [2-3H]glu-
cose metabolized as far as fructose-6-
phosphate in the glycolytic pathway
loses most of the 3H label, but [14C]fruc-
tose-6-phosphate retains the 14C (8).
Some fructose-6-phosphate is converted
back to G6P by reversal of the reac tion
catalyzed by hexosephosphate isomeras-
ease, and, if there is G6Pase activity, the
glucose moiety is returned to the free
glucose pool with its 14C but without 3H.
The 3H/14C ratio in the free glucose pool
should then decline progressively with
time if there is G6Pase activity. Huang
and Veech reported such a decline, sug-
gesting previously unsuspected levels of
G6Pase activity in the brain.

A key issue is the purity of the free
glucose pool in which they measured the
3H/14C ratio. Most products of glucose
metabolism beyond the G6P step lose the
3H but not the 14C label; if any of these
products were to contaminate the glu-
cose fraction, they would lead to low 3H/
14C ratios. In their experiments Huang
and Veech relied on Dowex 1-formate
and Dowex 1-borate column chromatog-
raphy of acidic metabolites of glucose-6-
phosphate to determine the 3H/14C ratio.
In a subsequent study (10) they added derivatization of the glucose fra c tion
by hexokinase-catalyzed phospho-
rylation.

We repeated the experiments of
Huang and Veech (6) but with special
efforts to ensure the purity of the glucose
fraction in which the 3H/14C ratio was
measured. Normal male Sprague-Daw-
ley rats (330 ± 9 g) were anesthetized
with pentobarbital (30 mg/kg, intraperi-
toneally), and one femoral artery and
one external carotid artery were catheter-
ized in each animal. The carotid catheter
was inserted 5 mm past the carotid
bifurcation into the common carotid
artery. Both catheters were secured by
ligatures. In one group of seven rats the
internal carotid artery contralateral to
the side of catheterization was ligated as
in the studies of Huang and Veech (6).
Because these animals occasionally ex-
hibited neurological and behavioral de-
fects at the time of the experiment, a
second group of eight animals was simi-
larly prepared except that the contralat-
eral internal carotid artery was not per-
manently ligated but was encircled with
a loose ligature that was temporarily
pulled taut during injection of the labeled
glucose through the carotid catheter to
ensure bilateral distribution of tracer.

Approximately 24 hours after surgery
a mixture of 50 μCi of [2-3H]glucose, 5
μCi of [U-14C]glucose (11), and 5.1 mM
glucose in 0.05 ml of 0.9 percent saline
was injected through the carotid cathete-
ry. At various times between 2 and 7
minutes after the injection the brain was
removed by freeze-blowing (9). Several
blood samples were drawn from the fem-
oral artery at various times during the
interval between the injection of labeled
glucose and freeze-blowing.

The frozen brains were powdered un-
der liquid nitrogen in a cryostat at −35°C
and perchloric acid (0.6M) extracts of
the brain tissue and plasma were pre-
pared. Acidic metabolites were removed
from the neutralized supernatant frac-
tions by passage through columns con-
taining 2 ml of Dowex AG 1-8X formate
(200 to 400 mesh). After adjustment of
the pH of the effluent to 4.0, basic
metabolites were removed by cation-ex-
change chromatography on columns
with 2 ml of Dowex AG 50-X8 H+ (200
to 400 mesh). The final effluent contained
approximately 96 percent of the glucose
originally present in the perchloric acid
extracts. Tritiated water released by the
metabolism of [2-3H]glucose was also pre-
sent.

The extracts, now depleted of all an-
ionic and cationic metabolic products of
hexokinase-catalyzed phosphorylation of
hexokinase, were evaporated to dryness to
eliminate the [3H]H2O and chromatographed as 4-cm bands on
Whatman 3MMChr paper in the ascend-
ing direction with a solvent system con-
sisting of isobutyric acid, water, and
concentrated ammonium hydroxide
dards were chromatographed in parallel
lanes on the same chromatographic pa-
pers. Bands migrating to the same posi-
tion relative to the solvent front (RF) as
authentic [14C]glucose were eluted with
water; this eluate contained 25 to 40
percent of the total \(^{14}\text{C}\) applied to the chromatogram. Half of each of these samples was assayed for \(^{3}\text{H}\) and \(^{14}\text{C}\) concentrations by liquid scintillation counting calibrated with internal \(^{3}\text{H}\)toluene and \(^{14}\text{C}\)toluene standards; the other halves were converted to G6P by incubation with adenosine triphosphate, yeast hexokinase, and MgCl\(_2\) and chromatographed on paper as before. Bands migrating with the same \(R_f\) as authentic \(^{14}\text{C}\)G6P were eluted with water and assayed to determine the \(^{3}\text{H}/^{14}\text{C}\) ratios.

The \(^{3}\text{H}/^{14}\text{C}\) ratios of the plasma and brain extracts were normalized to the \(^{3}\text{H}/^{14}\text{C}\) ratio of the injectant used in each experiment. The \(^{3}\text{H}/^{14}\text{C}\) ratios of the injectants were determined in samples purified by the same procedures used for the plasma and brain extracts to eliminate radiochemical impurities that might have contaminated the injected labeled glucose. Best-fitting straight lines representing the normalized \(^{3}\text{H}/^{14}\text{C}\) ratios of the labeled glucose and its G6P derivative with respect to time were calculated by least-squares regression.

The results for the two groups did not differ significantly and therefore were pooled for statistical analysis. The glucose fractions separated from plasma showed a slight, progressive decline in the \(^{3}\text{H}/^{14}\text{C}\) ratio below that of the injectant over the 2- to 7-minute interval after injection (Fig. 1A); this decline probably reflects the consequences of glucose metabolism throughout the body. The glucose fractions from the brain showed a slightly greater progressive decline in \(^{3}\text{H}/^{14}\text{C}\) ratios, but the difference was not statistically significant (Fig. 1B), indicating no differential loss of \(^{3}\text{H}\) and \(^{14}\text{C}\) in the glucose pool in the brain.

To ensure that the \(^{3}\text{H}/^{14}\text{C}\) ratios represented uncontaminated glucose, portions of the fractions derived from plasma, brain, and injectants were derivatized to G6P by hexokinase-catalyzed phosphorylation. The labeled G6P was separated by paper chromatography and assayed to determine the \(^{3}\text{H}/^{14}\text{C}\) ratios. The fractions from plasma so treated showed a statistically insignificant, time-dependent trend toward reduced \(^{3}\text{H}/^{14}\text{C}\) ratios (Fig. 2A), and the fractions from brain tissue exhibited no greater change in \(^{3}\text{H}/^{14}\text{C}\) ratio with time (Fig. 2B). In contrast to the results of Huang and Veech (6), there was no evidence of differential loss of \(^{3}\text{H}\) and \(^{14}\text{C}\) from glucose in the brain after intracarotid injection of [2-\(^{3}\text{H}\)]glucose and [U-\(^{14}\text{C}\)]glucose.

It appears that Huang and Veech inadequately purified the fraction extracted from brain tissue which they designated as glucose and in which they measured the \(^{3}\text{H}/^{14}\text{C}\) ratio. The labeled glucose in brain tissue gives rise to many metabolic products, most of which lose the \(^{3}\text{H}\) but not \(^{14}\text{C}\). Experiments in our laboratory have confirmed their results but have demonstrated that the sequential column chromatography of perchloric acid extracts of brain tissue on Dowex AG 1-X8 formate and Dowex AG 1-X8 borate does not isolate glucose uncontaminated by other labeled products. The eluate from the Dowex AG 1-X8 borate column does have a low \(^{3}\text{H}/^{14}\text{C}\) ratio but contains several labeled components other than glucose when chromatographed on paper. At 7 to 8 minutes after the injection, 40 to 50 percent of its \(^{14}\text{C}\) content are in contaminants that contain little tritium, but the \(^{3}\text{H}/^{14}\text{C}\) ratio in its glucose component is similar to that of the injectant. When the eluate from the Dowex AG 1-X8 borate column is chromatographed on a cation-exchange column, the \(^{3}\text{H}/^{14}\text{C}\) ratio in the effluent is increased. Treatment of the effluent with glucose oxidase, which converts glucose to gluconic acid, and separation of this derivative by anion-exchange chromatography raises the \(^{3}\text{H}/^{14}\text{C}\) ratio to that of the plasma and close to that of the injectant. Hexokinase-catalyzed phosphorylation of the eluate from the Dowex AG 1-X8 borate column is inadequate to ensure purity of the glucose; hexokinase is less specific than glucose oxidase and would phosphorylate any fructose or glucosamine that might be present in that fraction.

Our results do not support the finding of significant G6Pase activity in the brain in vivo. The activity of the small amount of enzyme that is present is limited by intracellular compartimentation. In the cell, G6Pase is present on the inner surfaces of the cisterns of the endoplasmic reticulum (ER) (1, 12); the G6P is formed in the cytosol. In tissues with high gluconeogenic activity there is a specific

![Fig. 1](image-url)  
Fig. 1 (left). Time course of \(^{3}\text{H}/^{14}\text{C}\) ratios in glucose purified from plasma (A) and brain (B) after intracarotid injection of a mixture of [2-\(^{3}\text{H}\)]glucose and [U-\(^{14}\text{C}\)]glucose. Symbols: (▲) results for animals with carotid ligation contralateral to side of carotid catheterization and (●) results for animals with contralateral carotid obstruction only during the injection. The \(r\) represents the product-moment correlation coefficient of the normalized \(^{3}\text{H}/^{14}\text{C}\) ratio on the ordinate with respect to time; \(P\) represents the probability value of the correlation coefficient. Fig. 2 (right). Time course of \(^{3}\text{H}/^{14}\text{C}\) ratios in G6P derivatized from the labeled glucose isolated from plasma (A) and brain (B) in Fig. 1. The symbols are explained in the legend to Fig. 1.
carrier in the ER that transports the substrate across the membrane to the phosphatase (12, 13). Karnovsky et al. (14) found that this carrier is absent in the brain and that G6P gains access to the phosphatase only by slow diffusion across the ER membrane. This would further slow the phosphohydrolytic activity of whatever G6Pase is present in the brain.

The report of Huang and Veech (6) has led to debate on the role of G6Pase in the brain and to speculations about futile cycles in cerebral tissue (15). It has also been used to argue against the validity of the deoxyglucose method for measuring local utilization of glucose in the brain in animals and humans (6, 14), a method that in its earliest form assumed negligible loss of deoxyglucose-6-phosphate in the brain during the experimental period (16). It now seems that such speculations and extrapolations are without foundation.

References and Notes


11. The radionuclides n-[2-3H(N)]glucose (specific activity, 24.0 Ci/mmol), n-[U-14C]glucose (14.4 mCi/mmol), and n-[1-14C]G6P (51.4 mCi/mmol) were purchased from New England Nuclear.


