Induction processes in blood-brain transfer of ketone bodies during starvation

ALBERT GJEDDE AND CHRISTIAN CRONE
Institute of Medical Physiology, Department A, University of Copenhagen, DK-2100 Copenhagen, Denmark

GJEDDE, ALBERT, AND CHRISTIAN CRONE. Induction processes in blood-brain transfer of ketone bodies during starvation. Am. J. Physiol. 229(5): 1165-1169. 1975.—Fed and starved rats were studied on successive days during a 5-day starvation period. The ability of ketone bodies to pass the blood-brain barrier was estimated by single common carotid injections of labeled ketone bodies and water, and results were expressed as the ratio between the normalized activities of tracers in tissue and blood, the brain uptake index (BUI). BUI of d-3-hydroxybutyrate and acetoacetate decreased as their total concentrations increased in the injectate bolus: BUI of d-3-hydroxybutyrate decreased significantly from 8% at 0.2 mM to 3-4% at 20.2 mM in fed rats and from 11.5% at 0.2 mM to 6% at 20.2 mM in starved rats, indicating saturation of the uptake mechanism. The BUI of both ketone bodies increased significantly with increasing duration of starvation, indicating adaptation to ketonemia. Enzymatic kinetics explained the uptake behavior of d-3-hydroxybutyrate in both fed and starved rats and involved a rise of $K_m$ and $V_{max}$ during starvation consistent with a doubling of the transport rate at the degree of ketonemia found in starved rats. The uptake of glucose was not influenced by starvation or ketonemia.

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**The Cerebral Respiratory Exchange Ratio (RQ)**

The cerebral respiratory exchange ratio (RQ) of 1 (12) suggests that glucose is the main energy substrate of the brain. It was, therefore, of great interest that the RQ for brain declined and arteriovenous concentration differences for d-3-hydroxybutyrate and acetoacetate increased in obese humans undergoing prolonged starvation, indicating that ketone bodies are metabolized by the brain under these circumstances (19).

In general, polar substances pass only slowly from blood to brain (3), but there are notable exceptions. D-Glucose (4), certain amino acids (18), and L-lactate (6) pass with apparent ease despite their hydrophilic properties. There is convincing evidence that special facilitating transport mechanisms are responsible for the rapid transfer of these solutes that would not penetrate the barrier if subject to simple diffusion only.

The ketone bodies are hydrophilic substances that present a similar problem. That ketone bodies pass into the brain at significant rates and are consumed rapidly during starvation may reflect adaptation of a transport mechanism across the blood-brain barrier in the prolonged presence of high concentrations.

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**Methods**

Injections were performed on male and female Wistar rats weighing 250-450 g. Control rats were fed standard food pellets and water ad libitum. Starving rats had access only to water and were kept in wire-bottom cages. Starvation extended from 1 to 5 days. The Oldendorf method (17) was applied in the following manner:

A 200-μl mixture of 2 μCi tritiated water and 0.5 μCi n-[3-14C]hydroxybutyrate or [14C]acetoacetate in a Ringer-HEPES solution was injected into the exposed, patent, right common carotid artery of rats anesthetized with pentobarbital sodium, 40 mg/kg, after a brief induction with diethyl ether. Fifteen seconds after the injection, the animals were decapitated with a rodent guillotine and the right cerebral hemisphere was removed for determination of labeled substances. According to Oldendorf (17), approximately 8% of the injected volume distributes to brain.

Following removal, the hemisphere was passed through the nozzle of a narrow-gauge syringe. The "homogenate" was divided in two halves, each approximately 200 μl, and left overnight at 40°C in a mixture of 1 ml Solucene (Packard) and 1 ml dioxane in capped vials. The next morning, the solution was bleached for 1 h at 0°C with 0.4 ml 35% hydrogen peroxide. Preparation was completed with 0.45 ml 1 M HCl. Finally, 15 ml Insta Gel (Packard) were added to the glass counting vials which were allowed to stabilize 0.5 h at counting temperature prior to counting.

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1. Ringer-HEPES solution: 8.59 g NaCl, 0.30 g KCl, 0.59 g CaCl₂ (2H₂O), 0.95 g 4 mM N,N-dihydroxylethylpiperazine-N'-2-ethansulphonic acid, distilled water ad 1,000 ml, titration ad pH 7.4.
Ten-microliter aliquots of the injection mixture and 10-μl blank samples of distilled water, respectively, were added to identical preparations of uninjected brain tissue, as were 14C-labeled standards of the original test substances. The constant composition of all samples made corrections for quenching unnecessary. Beta counting of 3H and 14C activities in the samples was performed with a Packard Tri-Carb 3005 liquid scintillation spectrometer.

Plasma samples were taken from fed and starved rats for spectrophotometric determination of endogenous concentrations of 3-hydroxybutyrate and acetoacetate according to the methods of Williamson and Mellanby (22) and Mellanby and Williamson (15), respectively.

Carbon-14-labeled compounds were supplied by the New England Nuclear Corp., including—by special order—the radiochemically pure b isomer of potassium 3-hydroxybutyrate, labeled in the C-3-position, and ethyl acetoacetate, also labeled in the C-3 position. Acetoacetate was prepared from 20 μl ethyl acetoacetate by hydrolysis with 1 M KOH to 10 ml, refrigeration of the mixture overnight at 4°C, titration with 1 M HClO4 to pH 7.0, and centrifugation. The supernatant contained acetoacetate, ethanol, and trace amounts of ethyl acetoacetate. At 4°C, very little acetoacetate evaporated within 10 h.

The specific activities of β-3-hydroxybutyrate and ethyl acetoacetate were 12.8 and 2.2 mCi/mmol, respectively.

ν-Glucose was labeled in the C-1 position with a specific activity of 5.6 mCi/mmol. Sucrose was labeled uniformly with a specific activity of 2.2 mCi/mmol.

Tritiated water with a specific activity of 1 mCi/g was supplied by the Radiochemical Centre, Amersham, England.

Each experiment was carried out on 13–17 rats. The extraction or brain uptake index (BUI) of test substances was calculated as tissue 14C activity relative to tissue 3H activity, divided by injectate 14C activity relative to injectate 3H activity, and expressed as a ratio or in percentage.

RESULTS

Blood-brain barrier impermeability was studied with sucrose. The BUI of sucrose was 2.9 % (SD 1.3 %, n = 15) in fed rats and 2.4 % (SD 0.4 %, n = 16) in rats starved for 5 days. These values were chosen to represent no uptake, because sucrose does not pass the blood-brain barrier at a significant rate (4).

Saturation kinetics in fed rats. Facilitated diffusion is generally thought to have Michaelis-Menten kinetic properties, including nonlinear dependence of transport rate on substrate concentration. Therefore, single carotid injections were performed at different concentrations of β-3-hydroxybutyrate in the injectate, obtained by addition of unlabeled racemic 3-hydroxybutyrate. Brain uptake was determined at total concentrations of β-3-hydroxybutyrate of 0.2 (concentration of β-[3-14C]hydroxybutyrate in the injectate), 1.2, 2.2, 5.2, 10.2, and 20.2 mM. The BUI of β-3-hydroxybutyrate declined as the total concentration increased, indicating saturation of a blood-brain barrier transport mechanism (Table 1).

Adaptation. The progressive development of adaptation of brain uptake of n-3-hydroxybutyrate and acetoacetate was studied during starvation. First, the degree of ketonemia obtained in the rats was measured (Fig. 1). Second, the BUI of both ketone bodies were determined on successive days of a 5-day starvation period, using 0.2 mM β-[3-14C]hydroxybutyrate or 1.1 mM [14C]acetoacetate in the injectate. Although the plasma concentration of these substances increased during fasting, as expected, the different degrees of ketonemia did not affect the uptake determination directly because the bolus cleared the vasculature at the moment of passage, thus exposing the blood-brain barrier only to the concentration present in the injectate. This particular feature of the Oldendorf method permitted the study of brain uptake of test substances at chosen concentrations rather than at the actual plasma concentrations.

With increasing duration of starvation, the BUI of β-3-hydroxybutyrate (Fig. 2) and acetoacetate (Table 2) both increased significantly.

Saturation kinetics in rats starved 5 days. Saturation of brain uptake of β-3-hydroxybutyrate was also studied in rats starved for 5 days. The BUI of n-3-hydroxybutyrate was always higher in the starved animals than in the fed, at equal concentrations of the ketone body in the injectate. It declined with higher concentration in the injectate, indicating saturation of the uptake capacity (Table 1).

Kinetics of brain uptake in fed and starved rats. The BUI values for β-3-hydroxybutyrate formed the basis for estimations of Vmax, maximal transport rate from blood to brain, and Km, ketone body affinity of an enzymatic prop-

TABLE 1. Self-suppression of brain uptake of n-[3-[14C]hydroxybutyrate in fed rats and rats starved 5 days

<table>
<thead>
<tr>
<th>Conc of n-[14C]hydroxybutyrate, mM:</th>
<th>0.2</th>
<th>0.2</th>
<th>0.2</th>
<th>0.2</th>
<th>0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc of unlabeled n-hydroxybutyrate, mM:</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Fed rats BUI, %</td>
<td>7.9</td>
<td>7.1</td>
<td>6.6</td>
<td>5.2</td>
<td>3.3</td>
</tr>
<tr>
<td>SD, %</td>
<td>1.4</td>
<td>1.3</td>
<td>2.0</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>17</td>
<td>16</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Starved rats BUI, %</td>
<td>11.5</td>
<td>9.6</td>
<td>11.3</td>
<td>8.8</td>
<td>6.8</td>
</tr>
<tr>
<td>SD, %</td>
<td>2.2</td>
<td>2.3</td>
<td>3.2</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>15</td>
<td>14</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

BUI, brain uptake index; SD, standard deviation; n, number of observations. Concentrations refer to concentrations in injectate.

![Graph](http://ajplegacy.physiology.org/1166/3A/GJEDDE/1975/04/20/Plascones.png)  
**FIG. 1.** Plasma concentrations of β-3-hydroxybutyrate and acetoacetate in rats during starvation. Bars represent 2 SD's.
CEREBRAL KETONE BODY TRANSPORT

The convergence of the experimental data presented in Table 1 toward this equation was examined in two ways.

First, a method of least-squares-normalized computerized optimization (1, 2) was applied. This method accomplished two things: it gave estimates of the constants $BUI_p$, $V'_{\text{max}}$, and $K_m$ (Table 3), and it fitted two curves to the data of Table 1 (Fig. 3).

Second, Eadie-Hofstee plots were constructed from the data presented in Table 1 after subtraction of the $BUI$ of sucrose (Fig. 4). The results of the Eadie-Hofstee plots were included in Table 3 with an estimation of the actual $V_{\text{max}}$ in fed rats, based on a whole-brain blood flow of the Wistar rat of 100 ml/100 g per min (7).

At the degree of ketonemia observed in the present study after 5 days of starvation, the estimates indicated that the blood-brain transport rate of $n$-3-hydroxybutyrate was 0.2 mmol/kg per min, rather than the 0.1 mmol/kg per min expected if no induction had taken place.

**Glucose uptake during ketonemia.** The mechanism responsible for a diminished net uptake of glucose during ketonemia (19) was studied in fed and starved rats. The $BUI$ of $d$-glucose was determined in the presence of a normoglycemic concentration of glucose and/or a high concentration of racemic 3-hydroxybutyrate obtained by addition of unlabeled $n$-glucose (5 mM) or unlabeled racemic 3-hydroxybutyrate (concentration of $d$-3-hydroxybutyrate: 10 mM) to the $d$-$[^{14}C]$glucose in the injectate.

### Table 2. Adaptation of brain uptake of $[^{14}C]$acetacetae in rats during starvation

<table>
<thead>
<tr>
<th>Duration of Starvation, days</th>
<th>0</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$BUI$, %</td>
<td>11.3</td>
<td>13.9</td>
<td>17.1</td>
</tr>
<tr>
<td>$\pm$ SD, %</td>
<td>3.2</td>
<td>3.7</td>
<td>5.2</td>
</tr>
<tr>
<td>$n$</td>
<td>28</td>
<td>28</td>
<td>28</td>
</tr>
</tbody>
</table>

$BUI$, brain uptake index; SD, standard deviation; $n$, number of observations. Increase from 0 to 5 days is significant ($P < 0.0125$).

Assuming that the $BUI$ values represented the unidirectional extraction of ketone body by brain tissue, i.e., the fractional amount of material passing from blood to tissue during one passage, and further assuming that the cerebral blood flow was constant in all sample populations, thus disregarding the effect of flow variations between individual rats, the transport rate was expressed as the tracer concentration in the injectate multiplied by the fraction extracted and the blood flow, or:

$$ V = \epsilon_a \times BUI \times CBF $$

or

$$ V' = \epsilon_a \times BUI $$

in which $V$ is the unidirectional transport rate and $V'$ equals $V/CBF$. It was considered likely that the experimentally determined $BUI$ values consisted of two components: a nonenzymatic "passive" component and an "active" component of facilitated transport, or:

$$ BUI = BUI_p + BUI_f $$

where $BUI_p$ = passive component, and $BUI_f$ = active component. Assuming that the active component had Michaelis-Menten properties,

$$ BUI = \frac{V'}{\epsilon_a} = BUI_p + \frac{V'_{\text{max}}}{K_m + \epsilon_a} $$

in which the equation $\epsilon_a$ and $BUI$ are the independent and the dependent variables, respectively, and $V'_{\text{max}} = V_{\text{max}}/CBF$. The convergence of the experimental data presented in Table 1 toward this equation was examined in two ways.

First, a method of least-squares-normalized computerized optimization (1, 2) was applied. This method accomplished two things: it gave estimates of the constants $BUI_p$, $V'_{\text{max}}$, and $K_m$ (Table 3), and it fitted two curves to the data of Table 1 (Fig. 3).

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### Table 3. Eadie-Hofstee plots and least-square normalized computer estimation of Michaelis-Menten kinetic constants in unidirectional brain uptake of $n$-3-hydroxybutyrate in fed rats and rats starved 5 days

<table>
<thead>
<tr>
<th>Plots</th>
<th>Estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed rats</td>
<td>Starved rats</td>
</tr>
<tr>
<td>$K_m$, mM</td>
<td>2.5</td>
</tr>
<tr>
<td>$V'_{\text{max}}$, mM</td>
<td>0.16</td>
</tr>
<tr>
<td>CBF, 1/kg per min</td>
<td>1</td>
</tr>
<tr>
<td>$V_{\text{max}}$, mmol/kg per min</td>
<td>0.16</td>
</tr>
<tr>
<td>$BUI_p$, %</td>
<td>2.9*</td>
</tr>
</tbody>
</table>

CBF, whole-brain blood flow of rat; $V'_{\text{max}}$, $V_{\text{max}}/CBF$; $BUI_p$, passive component of brain uptake index (see text). * $BUI$ of sucrose.
No remarkable change other than the self-depression of brain uptake of glucose was observed under different combinations of these conditions (Table 4), although a consistent 5% increase of BUI of glucose was seen during starvation.

DISCUSSION

It is necessary to emphasize the difference between a steady-state or "net" extraction of substances by the brain and the so-called unidirectional extraction measured in experiments with labeled substances (tracers).

Net extraction is defined as the fractional uptake or release of an unlabeled substance resulting from movement of diffusible substances from blood to brain and vice versa under steady-state conditions. Oxygen molecules, for example, move back and forth across the capillary membrane during a single transit, but the net result is a loss of oxygen from blood to tissue. The net extraction is calculated from the concentrations of oxygen in arterial and cerebral venous blood, normalized against the arterial concentration.

In contrast, the unidirectional transfer of oxygen from blood to brain can be estimated by the use of labeled molecules. If the dilution of labeled oxygen into the tissue oxygen sink is rapid, only negligible amounts of labeled oxygen diffuse back into blood, and the unidirectional transfer can be calculated from the activity of blood and tissue samples. If the fractions are expressed relative to the amount of labeled oxygen brought to the brain, the unidirectional transfer can be shown to be larger than the net loss.

Gottstein et al. (8) found net extractions of D-3-hydroxybutyrate and acetoacetate of 5.5 and 7.4%, respectively, in normal humans after 12-18 h fasting. The net uptake was approximately proportional to the arterial plasma concentration. Hawkins et al. (9) determined ketone body uptake in the brains of ketonemic rats and found that the net extraction of D-3-hydroxybutyrate remained quite constant at 4% at variations of serum ketone body concentration from 0.09 to 2.62 mM during a 96-h starvation period. Similar findings were reported by Zivin and Snarr (23) in studies on fed rats in which ketone body concentration was raised by infusion and in a variety of studies on suckling, infant, and adult animals (5). It is the consensus of these works that net utilization of ketone bodies is proportional to and a direct function of the serum concentration.

The consensus is not contrary to findings reported in the present study, in which fractional uptake of labeled ketone body decreased with increasing plasma concentrations, indicating saturation of a transport mechanism. The net utilization reflects the continuous removal of ketone body by brain tissue and may or may not remain constant with increasing plasma concentration. As long as the supply rate by blood is lower than an upper limit of tissue consumption, net extraction may very well be linearly related to the plasma concentration. However, when the limit of consumption is approached, net extraction begins to decrease. Therefore, proportionality of plasma concentration and ketone body net uptake exists only within a limited range of plasma concentrations.

In the present experiments with labeled ketone body, the situation is different. The isotope is supplied in a single bolus, and the tissue reservoir for labeled substance is probably large. In the brain, the rate-limiting step is the passage across the blood-brain barrier. However, although the transport process is nonlinear, it is nearly linear at plasma concentrations below 5 mM of D-3-hydroxybutyrate. The unidirectional transfer reflects the kinetics and capacity of the blood-brain transport mechanism and must always be equal to or higher than the rate of tissue consumption.

There are three reasons for believing that the sink function of brain tissue available for isotope dilution is sufficient to confirm the observed brain uptake indices in fed as well as in starved rats.

First, according to Miller, Hawkins, and Veech (16) and Hose and Duffy (10), the normal brain tissue content of D-3-hydroxybutyrate is 0.018-0.022 mmol/kg or at least 25 times the amount of that substance supplied in the labeled form by the injected bolus.

Second, according to Krebs et al. (13), the rate of the rate-limiting step in the breakdown of D-3-hydroxybutyrate in normal, adult rat brain exceeds the rate of supply of labeled substances by the injected bolus by at least two orders of magnitude.

Third, the rate of diffusion of ketone bodies along a con-
substrate affinity is induced with starvation. At the usual butyrate from blood to brain was twice the rate of transfer after 5 days' starvation, the transport rate of 3-hydroxy- butyrate and acetoacetate increased from 4.7 and 12% at 1 day's starvation to 12.3 and 14% at 8 days' starvation, respectively.

The enzymatic nature of the blood-brain barrier was postulated by Meister (14). In consequence of this nature, the increase in transport rate during starvation reported in the present study may be due to either an augmentation of the amount of protein believed to be involved in enzymatic transfer or to a change of the constant, $K_m$, indicating the appearance of a new enzymatic property with different Michaelis-Menten characteristics. In the latter case, we would expect the $K_m$ of the entire system to decrease in keeping with the development of greater substrate affinity. At higher substrate concentrations, however, it may be equally or even more efficient if $V_{max}$ increased rather than $K_m$. At intermediate substrate levels, changes in both constants could be expected.

In the present case, $V_{max}$ and $K_m$ both increased, $V_{max}$ more than $K_m$. The dual increase suggests the presence of two enzymatic properties, of which only one with a lower substrate affinity is induced with starvation. At the usual level of 3-hydroxybutyrate ketonaemia observed in rats after 5 days' starvation, the transport rate of 3-hydroxybutyrate from blood to brain was twice the rate of transfer to be expected if no induction had taken place. This difference is more striking at higher degrees of ketonemia at which the transport rate may be fourfold greater than the capacity of the uninduced mechanism at the same degree of ketonemia.

According to data presented by Owen et al. (19), the human net brain uptake of glucose decreased from 10 to 5.7% after 5–6 wk fasting. In the present study, the unidirectional transfer of D-glucose did not decrease significantly in the presence of ketone body. Hence it is unlikely that the mechanism responsible for a diminished net uptake is inhibition of glucose access to a transport process common to both glucose and ketone body. In agreement with Ide et al. (11), therefore, inhibition of tissue glycolysis may explain the decreased glucose utilization despite normoglycemia.

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Address reprint requests to A. Gjedde, Cerebrovascular Research Center, Department of Neurology, The New York Hospital–Cornell Medical Center, 525 E. 68 Street, New York, N.Y. 10021.

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