IV. 6. The Efflux Transport of Dehydroepiandrosterone Sulfate at the Blood-Brain Barrier


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We have investigated transport characteristics of dehydroepiandrosterone sulfate (DHEAS), a neuroactive steroid, at the blood-brain barrier (BBB) in functional in vivo study. The apparent BBB efflux rate constant of $[^{3}H]$DHEAS evaluated by Brain Efflux Index method was $2.68 \times 10^{-3}$ min$^{-1}$. The DHEAS efflux transport was a saturable process with a Michaelis constant ($K_m$) of 32.6 $\mu$M providing the direct evidence that most of DHEAS is transported from brain to the circulating blood across the BBB. This efflux transport of $[^{3}H]$DHEAS was inhibited by the common organic anion transporting polypeptide ( oatp) substrates such as taurocholate, cholate, sulfobromophthalein, estrone-3-sulfate demonstrating that DHEAS is predominantly transported from the brain to blood across the BBB.

Introduction

Dehydroepiandrosterone sulfate (DHEAS), which is termed ‘neurosteroid’, possesses multiple effects in the central nervous system, including interacting with GABA type A receptor and sigma receptor, an increase of memory and learning, and protection of neurons against excitatory amino acid-induced neurotoxicity$^{1}$. In multi-infarct dementia (MID) patient, DHEAS concentration in CSF was 400 ± 180 pg/ml, which was significantly lower than that in non-demented patients (800 ± 400 pg/ml)$^{2}$. This evidence raises that DHEAS concentration may afford the vulnerability of the brain.

The BBB is well recognized to regulate not only the entry of nutrients, and drugs into the brain from the circulating blood$^{3,4}$, but also the efflux of compounds such as $p$- aminohippuric acid, 3'-azido-3'-deoxythymidine, 2',3'-dideoxyinosine, L-glutamic acid, and L-aspartic acid$^{5-7}$.

The purpose of the present study was to investigate the DHEAS transport across the BBB in both the blood-to-brain and brain-to-blood directions in vivo.
Materials and methods

*Brain Efflux Index (BEI) Study*

The BEI study was performed according to the method of Kakee et al\(^8\)). Rats were anesthetized and mounted on a stereotaxic frame. Then, 0.50 \(\mu\)l of \(^{[3]}\text{H}\)DHEAS (0.08 \(\mu\)Ci) and \(^{[14]}\text{C}\)inulin (4 nCi) dissolved in the ECF buffer (122 mM NaCl, 25 mM NaHCO\(_3\), 10 mM glucose, 3 mM KCl, 1.4 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), 0.4 mM K\(_2\)HPO\(_4\), 10 mM HEPES, pH 7.4) was administered over 1 min via 5.0 \(\mu\)l-microsyringe fitted with a needle at a depth of 4.5 mm from the surface of the scalp, i.e., Parietal Cortex Area 2 (Par2) region. At predetermined time period, an aliquot of cerebrospinal fluid (CSF) was collected from the cisterna magna as reported previously\(^9\)). The whole brain was subsequently isolated and the brain specimen was divided into the left cerebrum, the right cerebrum and the cerebellum. After weighing, dissolving tissue in 2N NaOH, and mixing with scintillation cocktail, the associated radioactivity was measured with a liquid scintillation counter (LS-6500, Beckman, Fullerton, CA).

Determination of BEI from the Brain

The BEI was defined as equation (1) and the percentage of substrate remaining in the ipsilateral cerebrum was determined using equation (2).

\[
\text{BEI (\%)} = \frac{\text{test substrate undergoing efflux at the BBB}}{\text{test substrate injected into the brain}} \times 100 \tag{1}
\]

\[
100-\text{BEI (\%)} = \frac{\text{amount of test substrate in the brain/amount of reference in the brain}}{\text{concentration of test substrate injected/concentration of reference injected}} \times 100 \tag{2}
\]

As the percentage of DHEAS remaining in the brain is given by (100-BEI), apparent BBB efflux rate constant (K\(_{\text{eff}}\)) was estimated by fitting the semilogarithmic plot of (100-BEI) *versus* time data to the nonlinear least-squares regression analysis program, MULTIT\(^9\)).

Results

*In Vivo Brain-to-Blood Transport of DHEAS*

The in vivo brain-to-blood efflux of DHEAS was evaluated by means of the BEI method\(^8\)) over 20 min after intracerebral administration by comparing the BEI value with that of \(^{[14]}\text{C}\)inulin as a non-efflux compound. The \(^{[3]}\text{H}\)DHEAS in rat brain decreased in a time-dependent manner, with a K\(_{\text{eff}}\) of 2.68 x 10\(^{-2}\) ± 0.02 x 10\(^{-2}\) min\(^{-1}\) (mean ± S.D.) (Fig. 1), whereas \(^{[14]}\text{C}\)inulin did not significantly decrease over 20 min (data not shown).
Concentration-dependence of DHEAS Efflux from the Brain

The excess unlabeled DHEAS in the injectate solution reduced the DHEAS efflux transport from rat brain in a concentration-dependent manner (Fig. 2). Eadie-Scatchard plot exhibited the single saturable process of $[^3]$H DHEAS efflux (Fig. 2 inset). Nonlinear least-squares regression analysis provided a $K_m$ of $32.6 \pm 4.8 \mu M$ at cerebral concentration, which was calculated from the concentration of DHEAS in the injected solution and dilution factor as reported by Kakee et al.$^8$, and a $V_{max}$ of $4.14 \pm 0.39$ nmol/(min · g brain) (mean ± S.D.).

Effect of Several Organic Anions on DHEAS Efflux from the Brain

To characterize the $[^3]$H DHEAS efflux transport process at the BBB in vivo, the effects of several organic anions on $[^3]$H DHEAS efflux transport from rat brain was investigated (Table 1). Bile acids, at 20 mM (0.66 mM in the brain), such as taurocholate (TCA), and cholate (CA) inhibited $[^3]$H DHEAS efflux transport by $70.5 \pm 2.1\%$, and $47.9 \pm 14.6\%$ (p<0.001), respectively. A thyroid hormone, 3,5,3’-triiodo-L-thyronine at 5 mM (0.17 mM in the brain) inhibited $[^3]$H DHEAS efflux transport by $37.9 \pm 4.3\%$ (p<0.01). Sulfate conjugated hormones such as E$_1$S at 10 mM (0.33 mM in the brain) and E$_2$S at 20 mM inhibited $[^3]$H DHEAS efflux transport by $81.5 \pm 3.1$, and $81.5 \pm 6.8\%$ (p<0.001), respectively. Organic anions such as sulfobromophtalein (BSP) at 20 mM and probenecid at 100 mM (3.3 mM in the brain) also reduced $[^3]$H DHEAS efflux by $62.8 \pm 2.4\%$, and $87.5 \pm 3.8\%$ (p<0.001), respectively. By contrast, other compounds such as PAH and GABA at 100 mM did not affect the DHEAS efflux transport.

Discussion

In the present study, in vivo evidence provided that DHEAS, a neuroactive hormone, is transported via a carrier-mediated efflux transport process from brain to the circulating blood across the BBB. DHEAS is produced in the brain and plays physiological roles of neurosteroid in the brain$^1$. Our results exhibit that the BBB undergoes efflux of DHEAS, suggesting that the BBB acts as an efflux pump for DHEAS as is neurotransmitters such as L-Glu and L-Asp$^7$ and may regulate DHEAS concentration in the brain interstitial fluid. The efflux transport process of DHEAS from rat brain to the circulating blood across the BBB was saturable and concentration-dependent (Fig. 2). This result supports the hypothesis that DHEAS is transported via a carrier-mediated efflux transport process across the BBB.

Both oatp1 and oatp2, which transport DHEAS, are well known to play important roles of drug disposition in rat liver$^{10,11}$. The $K_m$ values of these transporters are also relatively similar to that determined by BEI method (Fig. 2)$^{10,11}$. The $[^3]$H DHEAS efflux transport from rat brain was significantly inhibited by TCA, CA, BSP, E$_1$S, E$_2$S, and probenecid (Table 1). This inhibition pattern was in good agreement with that of oatp1- and oatp2- mediated steroid hormone transport in the liver and kidney$^{11,12}$. Both oatp2 and oatp3 have been shown to mediate transport of the thyroid hormone such as T$_3$, and T$_4$, and

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the oatp3 is mainly expressed in the kidney\textsuperscript{13,14}. Taken together, DHEAS would be undergone efflux across the BBB via oatp2 which is located in the brain capillary\textsuperscript{15}.

In conclusion, DHEAS, a neurosteroid hormone, was eliminated from the rat brain. This is the first in vivo direct evidence of restricted brain distribution of DHEAS from circulating blood at the BBB, and is also important in helping us better understand how the BBB functions with regard to steroid hormone. Functional in vivo studies suggest that the BBB is involved in efflux transport of DHEAS at least via oatp2.

References
TABLE 1. Co-administration effect of several organic anions on [H]DHEAS efflux from rat brain

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Concentration in injectate (mM)</th>
<th>Concentration in ipsilateral cerebrum (mM)</th>
<th>No. studied</th>
<th>BE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>12</td>
<td>59.3 ± 1.8 (100)</td>
</tr>
<tr>
<td>p-aminohippuric acid (PAH)</td>
<td>100</td>
<td>3.3</td>
<td>4</td>
<td>50.6 ± 2.7 (85.4)</td>
</tr>
<tr>
<td>γ-aminobutyric acid (GABA)</td>
<td>100</td>
<td>3.3</td>
<td>4</td>
<td>58.1 ± 1.9 (97.9)</td>
</tr>
<tr>
<td>Sulfochromphthalein (BSP)</td>
<td>20</td>
<td>0.66</td>
<td>4</td>
<td>22.1 ± 1.4 (37.2)**</td>
</tr>
<tr>
<td>Taurocholate (TCA)</td>
<td>20</td>
<td>0.66</td>
<td>4</td>
<td>17.5 ± 1.2 (29.5)**</td>
</tr>
<tr>
<td>Cholate (CA)</td>
<td>20</td>
<td>0.66</td>
<td>4</td>
<td>30.9 ± 8.7 (52.2)**</td>
</tr>
<tr>
<td>Estrone-3-sulfate (E₁S)</td>
<td>10</td>
<td>0.33</td>
<td>4</td>
<td>11.0 ± 4.0 (18.5)**</td>
</tr>
<tr>
<td>Estradiol-3-sulfate (E₂S)</td>
<td>20</td>
<td>0.66</td>
<td>4</td>
<td>10.4 ± 3.0 (18.4)**</td>
</tr>
<tr>
<td>3,5,3'-Triiodothyronine (T₃)</td>
<td>5</td>
<td>0.17</td>
<td>4</td>
<td>36.9 ± 2.5 (62.2)*</td>
</tr>
<tr>
<td>Probenecid</td>
<td>100</td>
<td>3.3</td>
<td>4</td>
<td>7.44 ± 2.2 (12.5)**</td>
</tr>
</tbody>
</table>

[H]DHEAS was used at a concentration of 10 μM, i.e., 330 nM as a cerebral concentration.

a The cerebral concentration was estimated from the injectate concentration divided by the dilution factor, i.e., 30.3, which was reported previously (Kakee et al., 1996).

b Data, determined 20 min after intracerebral microinjection, are mean ± SEM values (percent of control).

* p<0.01, ** p<0.001, significantly different from control.

Fig. 1. Time-course of [H]DHEAS in the ipsilateral cerebrum following intracerebral microinjection to the Par2 region in the presence of [¹⁴C]inulin as an internal reference. The solid line was obtained by the nonlinear least-squares regression analysis program. Each point represents the mean ± S.E.M. (n=3).
Fig. 2. Concentration-dependence of DHEAS efflux from the brain across the BBB. The solid line was estimated using the nonlinear least-squares regression analysis program. The cerebral concentration was estimated from the injectate concentration divided by the dilution factor. Inset: Eadie-Scatchard plot for $[^{3}H]$DHEAS efflux transport at the BBB. Each point represents the mean ± S.E.M. (n=3-7).