Integrity of Blood-Brain Barrier During Ethanol Intoxication and Withdrawal in the Rat: Normal Glucose Transfer and Permeability to Na⁺ and Cl⁻

RALF HEMMINGSEN, M.D., MARIANNE M. HERTZ, M.D., AND TOM G. BOLWIG, M.D.

SUMMARY The unidirectional brain uptake of 14-C-D-glucose, ¹⁴Na⁺ and ¹⁴Cl⁻ was measured by the Oldendorf tissue uptake method in order to test whether severe ethanol intoxication or withdrawal affected the glucose transfer across the blood-brain barrier (BBB) or the integrity of the BBB to small ions. The Oldendorf technique rests upon the assumption that water is freely diffusible across the BBB, but in the present study a correction was applied, which takes into account the fact that the water extraction actually depends upon the different cerebral blood flow (CBF) in the groups.

The ¹⁴Na⁺ and ¹⁴Cl⁻ extraction values were about 1% as reported in other studies and this suggests that there was no major effect on the BBB reaction to small ions during severe ethanol intoxication or during withdrawal. The permeability surface area product did not deviate significantly from control values in any group and thus there was no indication that glucose transfer across the BBB differed during ethanol intoxication or withdrawal.

IT IS EVIDENT from the literature that chronic ethanol intoxication and ethanol withdrawal is accompanied by a major reduction in the rate of cerebral protein synthesis, possibly due to abnormal ribosome function. Furthermore, it is known that acute ethanol intoxication significantly reduces cerebral consumption of glucose and oxygen and inhibits energy consuming transport systems in the brain probably through allosteric changes of enzyme molecules, whereas ethanol withdrawal is followed by increased neuronal activity in the brain as reflected through increased oxygen consumption and through changed EEG pattern. The exact mechanism by which ethanol causes central nervous system (CNS) suppression is unknown, but nonspecific membrane expanding effects may be of importance.

The purpose of the present study was to investigate 1) whether severe ethanol intoxication or withdrawal caused detectable perturbation of the facilitated (protein carrier mediated) diffusion of glucose across the blood-brain barrier (BBB) in rats and 2) whether under the same conditions the functional integrity of the BBB to small ions (Na⁺, Cl⁻) was disturbed.

For this study of blood to brain transfer of glucose and small ions we have used the methodology described by Oldendorf. Intracarotid injection of tritiated water as reference substance (ref.), together with the test substance, enables determination of brain content of test relative to reference substance and this leads to the so-called brain uptake index

$$\text{index (BUI)} = \left( \frac{c_{\text{test (brain)}}}{c_{\text{ref (brain)}}} \right) / \left( \frac{c_{\text{test (injectate)}}}{c_{\text{ref (injectate)}}} \right) = \frac{E_{\text{test}}}{E_{\text{ref}}}$$

where c is concentration and E is the unidirectional uptake, the extraction. The fact is stressed that $E_{\text{test}}$ is the relevant parameter in permeability studies whereas BUI in itself is without meaning.

Materials and Methods

Ethanol Intoxication and Withdrawal

Four groups of 250-300 g male Wistar rats were studied under conditions of 1) acute severe ethanol intoxication; 2) chronic severe ethanol intoxication, the intoxication period lasting 80 hours; 3) animals expressing a severe ethanol withdrawal reaction; and 4) normal controls.

Ethanol intoxication and the physical dependence on ethanol was established by intragastric intubation technique. Briefly, the intoxicated state was rated as follows: neutrality, sedation, ataxia, loss of righting reflex and coma. The withdrawal reaction was rated using the following signs: tremor, rigid posture, stereotyped movements, general seizure, enhanced startle reflex, tonic neck reflex, squealing. During intoxication the animals had severe ataxia or loss of righting reflex and during withdrawal all animals showed tremor, rigid posture, stereotyped movements, enhanced startle reflex and tonic neck reflex. The acutely intoxicated animals received a single intragastric ethanol dose of 8-10 g ethanol/kg, whereas the chronically intoxicated animals received 9-12 g ethanol/kg body weight/day.

General Procedure

The animals were anesthetized with 0.8% halothane in 70% $N_2O$:30% $O_2$ and ventilated with an animal respirator. After surgical exposure of the common carotid artery and catherization of a femoral artery and vein for blood sampling and infusions, the animals
were immobilized with succinyl choline 20 mg/kg i.v. Halothane was then switched off and the animals left undisturbed for 20 min. In all animals the following physiological variables were monitored and adjusted: arterial Pco₂ was kept between 32–42 mm Hg, arterial Po₂ above 90 mm Hg, pH between 7.31–7.50, mean arterial blood pressure between 130–160 mm Hg and rectal temperature at 37.0 ± 0.5°C. These variables did not differ significantly between the groups. Plasma glucose concentrations and blood ethanol concentrations were determined using enzymatic methods. A hypodermic needle (external diameter 0.4 mm) was placed in the common carotid artery and 50 μl of an isotopic mixture (1 μCi ¹⁴C-D-glucose (10 mmol/l), 3 μCi ¹¹²⁵I, 1 μCi ⁴Na, 0.5 μCi ¹³¹⁵Te, 5 μCi ¹²⁴I) was injected as a bolus; decapitation took place 15 sec later. The ipsilateral cerebral hemisphere was removed for isotope counting after preparation; these procedures have been described elsewhere.¹²

**Comments on Methodology**

Oldendorf's method originally rested on the assumption that the reference substance, water, is freely diffusible across the blood-brain barrier, i.e. that the extraction of water (E HOH) is 1.0. This assumption has been reported not to be valid¹³–¹⁸ and thus a correction has to be applied. E HOH is dependent upon cerebral blood flow (CBF) varying between 0.35 and 0.74 at a CBF of 465 ml/100 g/min and 0.74 at a CBF of 64 ml/100 g/min.¹⁴ BUI is in reality E test / E HOH, so BUI does not represent the unidirectional uptake of test substance unless E HOH in that specific situation is known and corrected for. When comparing normal with pathological states such a correction is mandatory, even though this fact has often been disregarded.

Pardridge and Oldendorf¹⁸ have introduced a correction factor based on E HOH = 0.75 obtained from studies of rhesus monkey,¹³ and Oldendorf and Braun¹⁷ have introduced an even more accurate modification of the method by shortening the time interval for decapitation and using a reference substance with an extraction of about 10% in order to diminish the influence of changing brain perfusion rates during pathological conditions.

In the present study we have corrected the BUI values of each individual group of animals as precisely as possible: we know the CBF values from measurements in exactly comparable groups;¹² (same strain of rats, same intoxication level, same blood gas values, same anesthesia, same laboratory), and are using Bolwig and Lassen's¹⁴ data to obtain E HOH. The figure represents the CBF values and the interpolated E HOH values in the groups. This procedure to arrive at the correction for varying uptake of reference substance may seem roundabout and introduces some uncertainty. However, no other avenue to solution is available at present. Finally, the E values were corrected for the amount of substance present within the cerebral vasculature and not really taken up by the brain: t¹²⁵Te-DTPA (molecular weight = 506 g/mole) remains completely outside the brain and was used for intravascular correction, so

\[
E_{\text{test}} = \frac{c_{\text{test}}(\text{brain})}{c_{\text{test}}(\text{bolus})} - \frac{c_{\ln\text{DTPA}}(\text{brain})}{c_{\ln\text{DTPA}}(\text{bolus})} \times E_{\text{HOH}}
\]

bolus denoting injectate. This equation is similar to the one arrived at by Oldendorf and Braun.¹⁷ The permeability surface area product (PS) was calculated according to Crone¹⁹ from the equation

\[
\text{PS} = \frac{-\text{CBF} \times \ln(1 - E)}{E_{\text{HOH}}}
\]

This is in accordance with our preliminary finding (Barry and Hemmingsen, unpublished data) that no

**Results**

Table 1 gives values of ¹⁴Na⁺ and ³⁵Cl⁻ extractions and the PS-products for these ions in the groups. The extractions were small, as expected, with a tendency to somewhat higher values during ethanol intoxication where CBF was lower. Within each group the extraction values for the 2 ions did not deviate. The PS-product in the experimental groups did not deviate significantly from control values. The blood ethanol concentration in the intoxicated groups were almost identical, i.e. about 4.7–4.8 g/l.

Table 2 gives the plasma glucose concentrations, the ¹⁴C-D-glucose extraction values and the PS-products for glucose. The plasma glucose concentration in the control group, the acutely intoxicated group and the withdrawal group did not differ whereas the plasma glucose concentration was significantly elevated in the chronically intoxicated group. Also the glucose extraction value was higher in the chronically intoxicated group. The PS-product in the experimental groups did not deviate significantly from the control value.

**Discussion**

The extraction values for ¹⁴Na⁺ of about 1% were in accordance with values reported earlier by the same and other methods in the rat,¹⁶ and the ³⁵Cl⁻ extraction values did not differ from those found in man.¹⁹ The tendency toward an increase in the extraction values for the 2 ions in the intoxicated groups was probably due to the lower CBF which has previously been shown to prevail during ethanol intoxication (see also figure). Topical application of an ethanol solution of a very high osmolality (corresponding to approximately 50% W/V ethanol solution) on the arachnoid surface may cause irreversible opening of the BBB to Evans blue albumin,²⁰ but in the present experiments in animals with a blood ethanol concentration compatible with life there was no major effect on the BBB reaction to the small ions we investigated. This is in accordance with our preliminary finding (Barry and Hemmingsen, unpublished data) that no
TABLE 1  Blood Ethanol Concentration, \(^{14}Na^+\) Extraction (\(E_{Na^+}\)) and \(^{14}Cl^-\) Extraction (\(E_{Cl^-}\)) and Permeability Surface Area Products (PS-products) for \(Na^+\) and \(Cl^-\) in the groups. Means ± SEM are given

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood ethanol concentration (g/l)</th>
<th>(E_{Na^+}) (%)</th>
<th>(E_{Cl^-}) (%)</th>
<th>PS-product (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 11)</td>
<td>—</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.49 ± 0.10 0.39 ± 0.10</td>
</tr>
<tr>
<td>Acute intoxication (n = 7)</td>
<td>4.8 ± 0.5</td>
<td>1.1 ± 0.3*</td>
<td>1.0 ± 0.3</td>
<td>0.66 ± 0.18 0.60 ± 0.18</td>
</tr>
<tr>
<td>Chronic intoxication</td>
<td>(n = 5)</td>
<td>4.7 ± 0.5</td>
<td>0.8 ± 0.1*</td>
<td>0.63 ± 0.08 0.47 ± 0.08</td>
</tr>
<tr>
<td>Withdrawal (n = 6)</td>
<td>—</td>
<td>0.3 ± 0.1</td>
<td>-0.2 ± 0.1</td>
<td>0.35 ± 0.12</td>
</tr>
</tbody>
</table>

*p < 0.05; Student's t-test for comparison with control values; n = number of animals.

TABLE 2  Plasma Glucose Concentration, \(^{14}C\)-D-glucose Extractions (\(E_{glucose}\)) and Permeability Surface for Glucose (PS-product) in Control Animals, Acutely and Chronically Intoxicated Animals and in Withdrawing Animals. Means ± SEM are given

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma glucose concentration (mmol/l)</th>
<th>(E_{glucose}) %</th>
<th>PS-product (glucose) (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 11)</td>
<td>10.6 ± 1.0</td>
<td>15.9 ± 1.7</td>
<td>17.0 ± 2.0</td>
</tr>
<tr>
<td>Acute intoxication (n = 10)</td>
<td>12.1 ± 1.2</td>
<td>18.2 ± 4.0</td>
<td>12.7 ± 3.1</td>
</tr>
<tr>
<td>Chronic intoxication (n = 5)</td>
<td>14.9 ± 0.8*</td>
<td>23.9 ± 1.9*</td>
<td>21.4 ± 2.2</td>
</tr>
<tr>
<td>Withdrawal (n = 6)</td>
<td>10.7 ± 0.9</td>
<td>17.5 ± 2.2</td>
<td>22.6 ± 3.2</td>
</tr>
</tbody>
</table>

*p < 0.05; Student's t-test for comparison with control values; n = number of animals.

EXTRAVASATION OF EVANS BLUE ALBUMIN OCCURRED IN THE RAT BRAIN DURING ETHANOL INTOXICATION AND WITHDRAWAL.

The mean glucose extraction value in the control group was in good accordance with the value (\(E_g = 12.5\)) reported by others using the indicator dilution technique of Crone\(^{18}\) where all methodological precautions were taken to obtain quantitatively correct values.\(^{11}\) During acute intoxication and withdrawal the glucose extraction did not deviate significantly from the control value.

The statistically insignificant tendency to a higher (15%) glucose extraction during acute intoxication was not unexpected because this group had a lower CBF than controls and hence the resulting PS-products were quite similar. During chronic ethanol intoxication the glucose extraction was significantly increased, and although the PS-product was not statistically different from control values it tended to be somewhat higher. This finding might be related to the high plasma glucose concentration in the group. Recently Lund-Andersen\(^{31}\) pointed at the possible role of accelerative exchange diffusion causing an increased unidirectional tracer flux when the glucose concentration is increased on the side of the membrane (the BBB) toward which the flux is directed. If we assume that the absolute CBF in one hemisphere in a rat brain is about 600 \(\mu l/min = 10 \mu l/sec\) then an intracarotid injection of a 50 \(\mu l\) bolus over less than 1 sec will definitely clear the cerebral vessels of that hemisphere for blood in a considerable part of the period from bolus injection to decapitation. If the bolus concentration of radioactively labelled glucose is lower than the plasma injection concentration of the animal then the essentially unchanged brain glucose concentration...
concentration during bolus transmission is higher than the intravascular concentration. Consequently an increased extraction of radioactively labelled glucose may be measured due to the accelerative exchange diffusion phenomenon. It may be concluded that during acute and chronic ethanol intoxication and during ethanol withdrawal there was no major functional perturbation of the specific D-glucose carrier system of the BBB in the rat.

References
10. Majchrowicz E: Induction of physical dependence upon ethanol and the associated behavioral changes in the rat. Psychopharmacologia (Berl.) 43: 245-254, 1975
15. Oldendorf WH, Braun LD: \( ^\text{3H} \) Tryptamine and \( ^\text{3H} \)-Water as diffusible internal standards for measuring brain extraction of radio-labelled substances following carotid injection. Brain Res 113: 219-224, 1976
Integrity of blood-brain barrier during ethanol intoxication and withdrawal in the rat: normal glucose transfer and permeability to Na+ and Cl-.
R Hemmingsen, M M Hertz and T G Bolwig

Stroke. 1980;11:141-144
doi: 10.1161/01.STR.11.2.141

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/11/2/141

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/