Influence of Hypovolemia on the Pharmacokinetics and the Electroencephalographic Effect of Etomidate in the Rat

PETER DE PAEPE, FRANS M. BELPAIRE, GERT VAN HOEY, PAUL A. BOON, and WALTER A. BUYLAERT

Heymans Institute of Pharmacology (P. De P., F.M.B.), Electroencephalography Laboratory, Department of Neurology (P.A.B.), Department of Electronics and Information Systems (G. Van H.), and Department of Emergency Medicine (W.A.B.), University of Ghent, Medical School, Ghent, Belgium

Accepted for publication April 15, 1999   This paper is available online at http://www.jpet.org

ABSTRACT

The influence of hypovolemia (removal of 30% of the blood volume) on the pharmacokinetics and pharmacodynamics of etomidate was investigated in the rat. Chronically instrumented animals were randomly allocated to either a control (n = 9) or a hypovolemia (n = 9) group, and etomidate was infused (50 mg/kg/h) until isoelectric periods of 5 s or longer were observed in the electroencephalogram. The changes observed in the electroencephalogram were quantified using aperiodic analysis in the 2.5- to 7.5-Hz frequency band and used as a surrogate measure of hypnosis. The righting reflex was used as a clinical measure of hypnosis. The etomidate dose that had to be infused to reach the electroencephalographic endpoint was almost 40% lower (p < .01) in the hypovolemic animals than in the control animals. This difference could be attributed to a decrease in clearance (−20%; p = .06) and distribution volume (−30%; p < .01) of etomidate. Protein binding was similar in both groups. To investigate changes in end organ sensitivity during hypovolemia, the electroencephalographic effect-versus-effect-site concentration relationship was studied. The effect-plasma concentration relationship was biphasic, exhibiting profound hysteresis in both hypovolemic and control animals. Semiparametric minimization of this hysteresis revealed similar equilibrium half-lives in both groups, and the biphasic effect-concentration relationship was characterized nonparametrically by descriptors. With these descriptors, a slightly increased potency of etomidate during hemorrhage was observed. The concentration at the return of righting reflex was 16% (p < .05) lower in the hypovolemic animals. In conclusion, an increased hypnotic effect of etomidate was observed during hypovolemia that is mainly attributed to pharmacokinetic changes. Our data also suggest a small increase in central nervous system sensitivity for etomidate in hypovolemic animals.

Patients presenting to the emergency department with hemorrhagic shock frequently require analgesic and anesthetic agents for pain relief and hypnosis. Pathophysiologic changes during hypovolemia may alter the effect of these drugs by influencing the pharmacokinetics or by changing end organ sensitivity.

We observed an increased sensitivity to the analgesic effect of morphine in rats subjected to hypovolemia. This could be attributed at least partially to the higher morphine concentrations in these animals compared with normovolemic animals (De Paep et al., 1998). An increased central nervous system sensitivity during hypovolemia has been described for barbiturates and benzodiazepines in the rat (Klockowski and Levy, 1988a,b) and the dog (Adams et al., 1985). During moderate bleeding in the pig, the anesthetic requirement of thiopentone and ketamine decreases (Weiskopf and Bogetz, 1985). These changes have been ascribed to alterations in both pharmacokinetics (Klockowski and Levy, 1988b) and end organ sensitivity (Adams et al., 1985; Klockowski and Levy, 1988a,b).

No data are available for the anesthetic etomidate, a potent and short-acting i.v. hypnotic agent, which is frequently used in hemodynamically compromised patients for the induction of hypnosis because of its interesting hemodynamic profile (Colvin et al., 1979; Ebert et al., 1992; Simon and Young, 1994). Human data concerning the pharmacology of etomidate during hypovolemia are lacking. Because it is almost impossible to study this in a clinical setting, we decided to study the pharmacokinetics and pharmacodynamics of etomidate in a hypovolemia model in the rat. Etomidate was administered in a continuous infusion. The righting reflex was used as a clinical measure of depth of hypnosis, but...

ABBREVIATIONS: EEG, electroencephalographic; AMP, amplitude per second; MAP, mean arterial blood pressure; HR, heart rate; k_{10}, first-order rate equilibrium constant; t^{1/2}_{1/2}, equilibrium half-life; E_0, baseline effect; E_{max}, maximal activation of the electroencephalographic effect; EC_{m}, concentration required to produce maximal electroencephalographic activation; EC_{50}, concentration required to obtain 50% activation of the electroencephalographic effect; EC_{max}, concentration required to produce the baseline effect between maximal electroencephalographic activation and maximal electroencephalographic inhibition.
because this provides only a single endpoint, continuous electroencephalographic (EEG) registration was applied because this is considered to be a useful surrogate measure of hypnosis (De Paepe et al., 1999).

**Materials and Methods**

**Animal Instrumentation.** The study protocol was approved by the Ethics Committee for Animals of the Ghent University Medical School.

Male Wistar rats (280–320 g) were purchased from Iffa Credo and kept at 21°C with a 12-h light/dark cycle. Surgery for the instrumentation was carried out under pentobarbital anesthesia (60 mg/kg i.p.).

One week before the experiment, epidural EEG electrodes were implanted (Mandema and Danhof, 1990). During atrumatic fixation in a stereotaxic device, seven holes were drilled into the skull without penetration of the dura at the following locations: 11 mm anterior and 2.5 mm lateral (F1 and F2); 3 mm anterior and 3.5 mm lateral (C1 and C2); 3 mm posterior and 2.5 mm lateral (O1 and O2) to lambda. A reference electrode was placed on lambda. The lead wires from each electrode were connected to a connector, which was insulated and fixed to the skull with dental acrylic cement.

Two days before the experiment, polyethylene catheters (PE 10) filled with heparin solution (100 IU/ml) were inserted into the femoral artery and vein through a small incision in the groin. The catheters were threaded under the skin of the back and exteriorized at the nape of the neck.

To minimize restraining stress during the experiment, the animals were put in a restraining cage on several occasions before the actual experiment.

Arterial blood pressure was registered via the arterial line on a Beckman dynograph type R recorder. Heart rate (HR) was directly derived from the pulse signal. Data were saved on a hard disk using a hemodynamic data acquisition software system (HDAS; University of Maastricht, Maastricht, the Netherlands). The core temperature was measured with a flexible thermistor probe inserted rectally to a depth of 5 cm.

The EEG was measured and recorded using a D/EEG Lite EEG recorder (Telefactor, Zwolle, the Netherlands) at a sampling rate of 200 Hz. The high- and low-pass filter cutoff frequencies were set at 1 and 70 Hz, respectively.

The return of righting reflex was used as a clinical parameter of depth of anesthesia.

**Experimental Protocol.** After overnight fasting, the rat was loosely restrained in a cage. All experiments started between 9:00 and 10:00 AM. The arterial line was filled with 0.2 ml of heparinized saline (100 IU/ml) and connected to a blood pressure transducer.

After 20 min of baseline hemodynamic and EEG recording, the animals were randomly assigned to undergo either the control or the hypovolemia procedure. Hypovolemia was induced in nine animals by removing 30% of the initial blood volume (assumed to be 60 ml/kg) in six increments over 30 min through the arterial line. An additional 30 min, the hypovolemic animals received an i.v. infusion of etomidate dissolved in propylene glycol (Hypnomidate; Janssen Pharmaceutica, Beerse, Belgium). It was administered at a rate of 50 mg/kg/h. The infusion was terminated when the EEG indicated burst suppression with isoelectric periods of 5 s or longer. Arterial blood samples of 100 μl were taken for determination of etomidate plasma concentrations at the following time intervals: 0.5, 1, 2, 4, 6, and 8 min after the start of the infusion; at the time of termination of the infusion; and 0.5, 1, 2, 4, 8, 15, 25, 35, 50, 70, 90, 120, 150, and 180 min thereafter. Sampled blood was replaced with the same volume of saline. At the end of each experiment, an arterial blood sample (500 μl) was withdrawn for measurement of hematocrit, blood gases, protein concentration, and protein binding of etomidate.

Control animals (n = 9) underwent the same experimental protocol but without the removal of blood.

The time course of the etomidate protein binding was investigated in six animals that were randomly assigned to undergo either the hypovolemia (n = 3) or the control (n = 3) procedure; propylene glycol was infused, and three arterial blood samples (1 ml) were taken for the determination of protein binding at different time intervals (at baseline, before the start of the infusion, and at the end of the experiment).

**Drug Assay.** Blood was collected in tubes (4°C) containing sodium fluoride (1 mg/ml) to block plasma esterase activity. After centrifugation, plasma was stored at −20°C until analysis.

Concentrations of etomidate in plasma (50 μl) were assayed by HPLC according to the slightly modified method of Le Moing and Levron (1990). Detection was performed by UV at 240 nm. The coefficients of variation for the determination of etomidate at concentrations of 400, 800, and 4000 ng/ml were always less than 8.4%; overall accuracy (analytical recovery) ranged from 85.4 to 116.3% (n = 35, 18 assays). The lower limit of quantification of etomidate was 50 ng/ml using 50 μl of plasma.

**Protein Binding.** Protein binding of etomidate was measured by equilibrium dialysis for 2.5 h at 37°C as described previously (Bel-paire and Bogaert, 1990). Then 200 μl of plasma spiked with 1 μg/ml etomidate and 1 mg/ml sodium fluoride and adjusted to pH 7.4 were dialysed against 200 μl of phosphate buffer (0.15 M, pH 7.4). Etomidate concentrations after dialysis were determined in 100-μl aliquots of dialysate as described above.

**Analysis of Data.** The pharmacokinetics and pharmacodynamics of etomidate were quantified for each rat. The plasma concentration-time profiles during and after infusion were described by a polynomial equation using WinNonlin version 1.5 (Pharsight Corporation, Palo Alto, CA). Two- and three-compartmental models were evaluated, and the most suitable model was chosen according to the Akaike Information Criterion and according to the precision of the parameter estimates. The estimated intercepts and slopes were used for calculation of the pharmacokinetic parameters (Gibaldi and Perrier, 1982).

The effect of etomidate was assessed from the EEG signal processed by aperiodic analysis (Gregory and Pettus, 1986). The aperiodic EEG analysis algorithm determines the amplitude and period of each EEG signal on a wave-by-wave basis. From this analysis, the total number of waves per second and the amplitude per second (AMP), the two basic parameters derived from aperiodic EEG analysis, can be calculated in several freely selectable frequency bands (Mandema and Danhof, 1990). The AMP from 2.5 to 7.5 Hz in the left fronto-occipital lead was used as a measure of the effect of etomidate because this parameter was shown to provide an optimal measure for the effect of etomidate on the central nervous system (De Paepe et al., 1999). The EEG data were averaged over predetermined intervals. The interval duration (10 s to 2 min) depended on the rate of change of the signals.

Hysteresis in the EEG effect-versus-plasma concentration curve was minimized by a semiparametric approach using a FORTRAN written program (Verotta and Sheiner, 1987) to reveal the apparent effect-versus-effect-site concentration relationship and to estimate the first-order rate equilibrium constant (k eq). Etomidate plasma concentration-time curves were fitted based on the compartmental model obtained in each individual rat.

After hysteresis minimization, the EEG effect-versus-effect-site concentration curve was characterized nonparametrically with the use of descriptors without invoking a pharmacodynamic model (Ebling et al., 1991). The descriptors used are the baseline effect (E b), the maximal activation of the EEG effect (E max), the concentration required to produce the maximal EEG activation (ECmax), the concentration required to obtain 50% activation of the EEG effect (EC50), and the concentration required to produce the Ec between maximal activation and maximal inhibition (ECb). E b, E max, and ECb values were directly obtained from the data, and EC50 and ECb values were derived by linear interpolation between the two closest points. The
etomidate concentration at the return of righting reflex was also derived by linear interpolation.

The results are expressed as mean ± S.E.M. Comparison of physiological parameters and pharmacokinetic and pharmacodynamic estimates between hypovolemic and control animals were made using multivariate multiple regression. Hemodynamic data were compared by using a two-way ANOVA for repeated measures. A value of \( p < .05 \) was considered as statistically significant.

## Results

Eighteen animals were randomly allocated to either the control (\( n = 9 \)) or the hypovolemia (\( n = 9 \)) group. All animals fell asleep with a loss of righting reflex within the first minutes after the start of the infusion of etomidate.

In the hypovolemic animals, a significantly lower dose of etomidate was needed to reach the endpoint of 5-s isoelectric EEG (5.55 ± 0.30 versus 8.79 ± 0.53 mg/kg, \( p = .001 \)), corresponding to a mean infusion duration of 6.7 ± 0.4 and 10.5 ± 0.6 min, respectively. In the hypovolemia group, two animals died 90 and 120 min, respectively, after the end of the infusion; there were no deaths in the control group.

Table 1 shows the mean arterial blood pressure (MAP) and HR in control and hypovolemic rats before, during, and after the infusion of etomidate. Baseline MAP and HR before induction of hypovolemia were not different between the hypovolemia group and the control group (125 ± 2 versus 119 ± 3 mm Hg, \( p = .112 \); 446 ± 9 versus 418 ± 15 beats/min, \( p = .132 \)). In the hypovolemic animals, MAP decreased to a minimum of 78 ± 9 mm Hg at the end of the hypovolemia procedure and then gradually increased again to 107 ± 4 mm Hg in the period preceding the infusion of etomidate. HR slightly increased during the hypovolemia procedure and remained elevated until the start of the infusion. In the control group, MAP and HR remained stable during the predrug period. Immediately after the start of the etomidate infusion, a decrease in MAP was observed in both hypovolemic and control animals, becoming maximal at the end of the infusion. This was accompanied by a decrease in HR in both groups. In the control animals, MAP and HR were not different at the end of the experiment (i.e., after 3 h) from the preinfusion values. In the hypovolemic animals, however, MAP and HR remained significantly below preinfusion values and were significantly lower in the hypovolemia group than in the control group at the end of the experiment.

Hypovolemia caused a significant reduction in body temperature, arterial pCO\(_2\), plasma HCO\(_3^-\), hematocrit, plasma albumin, and plasma total protein compared with the control group; in contrast, the arterial pO\(_2\) was significantly higher (Table 2).

The time course of the plasma concentrations of etomidate in both hypovolemic and control animals during the first 30 min after the start of the infusion is shown in Fig. 1. Etomidate concentrations in both groups were most adequately fitted using a three-exponential model, except for one animal in each group in which a two-exponential model was better.

The pharmacokinetic parameters of etomidate for both groups of animals are shown in Table 3. Two animals in the hypovolemia group could not be included in the pharmacokinetic analysis because they died before the end of the experiment. The maximal etomidate concentration at the end of the infusion was similar in both groups. Systemic clearance was slightly lower in the hypovolemia group. The volume of distribution of the central compartment and at steady state was lower in the hypovolemic animals, but only that at steady state was statistically significant. No significant difference between the two groups was observed for mean residence time and half-lives.

The free fraction of etomidate at the end of the experiment was slightly but not significantly higher in the hypovolemic animals (19.6 ± 0.5% in the hypovolemia group versus 18.8 ± 0.7% in the control group, \( p = .2 \)). Preliminary experiments showed that protein binding remained stable in both control (\( n = 3 \)) and hypovolemic (\( n = 3 \)) animals during the experiment, and no differences could be observed between the two groups (data not shown).

The time course of the EEG parameter, AMP in the frequency range of 2.5 to 7.5 Hz, showed a biphasic response in each animal followed by a return to baseline values as described previously (De Paepe et al., 1999). The EEG amplitude-versus-plasma concentration relationship showed profound hysteresis. This hysteresis was collapsed for both control and hypovolemic animals by estimating \( k_{on} \), with use of the hysteresis minimization program, resulting in a biphasic EEG effect-versus-effect-site concentration relationship of etomidate as shown in Fig. 2. This relationship was characterized by descriptors that are shown in Table 4 for both control and hypovolemic animals. The \( k_{on} \) and the equilibrium half-life (\( T^{1/2}_{k_{on}} \)) were similar in the two groups, as were the \( E_{50} \) and \( E_{max} \) values. The \( EC_{50} \), \( EC_{mi} \), and \( EC_{p} \) values

![Fig. 1. Plasma concentration of etomidate as a function of time in control (straight lines; \( n = 9 \)) and hypovolemic (dotted lines; \( n = 7 \)) rats during and after an i.v. infusion of etomidate (50 mg/kg/h). Infusion is started at time 0 and terminated when the EEG indicated burst suppression with isoelectric periods of 5 s or longer.](image-url)
tended to be lower in the hypovolemia group compared with the controls, but only the difference in $E_{C_{m}}$ attained statistical significance. The concentration at the return of righting reflex was significantly lower in the hypovolemic animals ($0.36 \pm 0.01 \mu g/ml$ in the hypovolemia group versus $0.43 \pm 0.03 \mu g/ml$ in the control group, $p = 0.04$).

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypovolemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal concentration ($\mu g/ml$)</td>
<td>$3.91 \pm 0.13 (9)$</td>
<td>$4.18 \pm 0.17 (7)$</td>
</tr>
<tr>
<td>Systemic clearance ($ml/min/kg$)</td>
<td>$104 \pm 7 (9)$</td>
<td>$86 \pm 5 (7)$</td>
</tr>
<tr>
<td>Volume of distribution of the central compartment (liters/kg)</td>
<td>$0.26 \pm 0.02 (9)$</td>
<td>$0.19 \pm 0.02 (7)$</td>
</tr>
<tr>
<td>Volume of distribution at steady state (liters/kg)</td>
<td>$3.89 \pm 0.21 (9)$</td>
<td>$2.80 \pm 0.21^* (7)$</td>
</tr>
<tr>
<td>Mean residence time (min)</td>
<td>$39.2 \pm 3.8 (9)$</td>
<td>$33.2 \pm 3.0 (7)$</td>
</tr>
<tr>
<td>Initial half-life (min)</td>
<td>$0.4 \pm 0.0 (8)$</td>
<td>$0.4 \pm 0.0 (6)$</td>
</tr>
<tr>
<td>Intermediate half-life (min)</td>
<td>$7.3 \pm 1.6 (8)$</td>
<td>$6.7 \pm 1.5 (6)$</td>
</tr>
<tr>
<td>Terminal half-life (min)</td>
<td>$52.9 \pm 8.0 (9)$</td>
<td>$39.4 \pm 4.9 (7)$</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M. The number of animals is indicated in parentheses.

* $p < 0.05$, ** $p < .01$, compared with the control group; multivariate multiple regression.

### Discussion

The aim of this study was to investigate the influence of hypovolemia on the pharmacokinetics and the EEG effect of etomidate. Hypovolemia was induced in unanesthetized rats by removal of 30% of the blood volume. This model, described by Klockowski and Levy (1988a,b), corresponds to a moderate hypovolemia. The moderate drop in MAP to $\pm 100$ mm Hg and the presence of pH in the physiological range at the end of the experiment indicate that indeed only a moderate hypovolemia was induced. pH was well maintained in the hypovolemic animals as the fall in $HCO_3^-$ was entirely compensated by a decrease in $pCO_2$ due to hyperventilation, resulting also in an increase in $pO_2$. Moderate hypothermia was observed in the hypovolemic animals at the end of the experiment. A possible influence of hypothermia on the pharmacokinetics and/or pharmacodynamics of etomidate cannot be excluded. Hypothermia has been shown to decrease the clearance of, for example, remifentanil, propofol, and pentobarbital (Boucher and Hanes, 1998), and to alter the pharmacodynamics of pentylenetetrazol (Walker and Levy, 1991). Hypothermia was also shown to affect the EEG effect-versus-concentration relationship of alfentanil (Cox et al., 1997). However, the decrease in body temperature in most of these studies was much more pronounced than that in our study. Therefore, it is unlikely that the moderate hypothermia occurring in our experiments explains the alterations in the pharmacology of etomidate.

In the hypovolemic animals, the infusion of etomidate was accompanied by a decrease in MAP and HR. This contrasts with findings by Wauquier (1983), who described an increase in MAP to normal levels and a slight increase in HR after a long-term infusion of etomidate in hypovolemic dogs. Pascoe et al. (1992) found, with an i.v. bolus administration of etomidate in hemorrhagic dogs, no change in MAP and a decrease in HR. The discrepancies with our data may be explained by the fact that in these studies, a more pronounced fall in blood pressure was provoked, so a pressor response is more readily observed when blood pressure is low; obviously, the role of species differences and differences in dose regimens cannot be excluded.

The etomidate dose needed to reach the endpoint of 5-s isoelectric EEG was almost 40% lower in the hypovolemic animals than in the control animals. This difference can be due to changes in pharmacokinetics and/or end organ sensitivity.

An argument for a role of pharmacokinetic changes is the

---

**Table 2**

<table>
<thead>
<tr>
<th>Effect of hypovolemia on some physiological characteristics</th>
<th>Control</th>
<th>Hypovolemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>295 ± 9 (9)</td>
<td>292 ± 6 (9)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.9 ± 0.2 (9)</td>
<td>37.9 ± 0.2 (9)</td>
</tr>
<tr>
<td>Prestudy</td>
<td>36.7 ± 0.2 (9)</td>
<td>35.3 ± 0.3** (7)</td>
</tr>
<tr>
<td>pH</td>
<td>7.46 ± 0.01 (9)</td>
<td>7.48 ± 0.03 (7)</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>38.5 ± 1.3 (9)</td>
<td>24.2 ± 2.3** (7)</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>108.1 ± 3.8 (9)</td>
<td>129 ± 4.1** (7)</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/liter)</td>
<td>27.4 ± 0.8 (9)</td>
<td>18.3 ± 1.6** (7)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36.1 ± 0.5 (9)</td>
<td>25.0 ± 0.9** (7)</td>
</tr>
<tr>
<td>Plasma albumin (g/100 ml)</td>
<td>2.33 ± 0.04 (9)</td>
<td>1.91 ± 0.12** (7)</td>
</tr>
<tr>
<td>Plasma total protein (g/100 ml)</td>
<td>5.08 ± 0.17 (9)</td>
<td>4.32 ± 0.25** (7)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M. The number of animals is indicated in parentheses.

* $p < 0.05$, ** $p < 0.01$, compared with the control group; multivariate multiple regression.

---

**Table 4**

<table>
<thead>
<tr>
<th>Pharmacodynamic descriptors of the biphasic EEG effect-concentration relationship in control and hypovolemic animals on i.v. infusion of etomidate</th>
<th>Control</th>
<th>Hypovolemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{C_0}$ ($\mu V/s$)</td>
<td>443 ± 21</td>
<td>450 ± 26</td>
</tr>
<tr>
<td>$E_{C_{50}}$ ($\mu g/ml$)</td>
<td>0.32 ± 0.05</td>
<td>0.29 ± 0.05</td>
</tr>
<tr>
<td>$E_{C_{max}}$ ($\mu g/ml$)</td>
<td>1435 ± 94</td>
<td>1326 ± 73</td>
</tr>
<tr>
<td>$E_{C_{m}}$ ($\mu g/ml$)</td>
<td>0.74 ± 0.04</td>
<td>0.60 ± 0.03*</td>
</tr>
<tr>
<td>Concentration at return of righting reflex ($\mu g/ml$)</td>
<td>2.98 ± 0.20</td>
<td>2.82 ± 0.05</td>
</tr>
<tr>
<td>$k_{reflex}$ (min⁻¹)</td>
<td>0.43 ± 0.03</td>
<td>0.36 ± 0.01*</td>
</tr>
<tr>
<td>$\tau_{reflex}$ (min)</td>
<td>2.60 ± 0.12</td>
<td>2.32 ± 0.15</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M.

* $p < 0.05$, compared with the control group; multivariate multiple regression.

---

**Fig. 2.** Biphasic relationship between EEG effect and apparent effect-site concentration for control (straight lines; $n = 9$) and hypovolemic (dotted lines; $n = 7$) rats.
fact that although a lower dose of etomidate was infused in the hypovolemic animals, maximal plasma concentrations of etomidate did not differ between control and hypovolemic animals. Analysis of the pharmacokinetic parameters showed that the systemic clearance of etomidate was almost 20% lower in the hypovolemia group \( p = .08 \). Etomidate is cleared by ester-hydrolysis in the liver and in plasma (Lewi et al., 1976). The latter is not expected to be changed by moderate hemorrhage. However, because etomidate is assumed to be a high extraction drug (De Boer and Breimer, 1984), its liver clearance is expected to decrease when blood flow diminishes. A reduced liver blood flow during hypovolemia was also proposed in other studies to explain the reduced clearance for midazolam (Adams et al., 1985) methylprednisolone succinate (Toutain et al., 1987), prednisolone (Hankes et al., 1985), lidocaine (Benowitz et al., 1974), and morphine (De Paepe et al., 1998).

In contrast with the small difference in clearance is the marked reduction by almost 30% in the volume of distribution of etomidate at steady state observed in the hypovolemic rats compared with the control animals. This reduction in distribution volume is probably explained by a reduction of circulating blood volume and cardiac output, which may cause homeostatic redistribution of blood flow away from less vital organs with preservation of blood flow to heart and brain (Blahitka and Rakusan, 1977). The same mechanisms were suggested to explain the reduced distribution volume during hypovolemia in animal experiments with lidocaine (Benowitz et al., 1974), atropine (Smallridge et al., 1989), prednisolone (Hankes et al., 1985), and morphine (De Paepe et al., 1998).

Changes in protein binding during hypovolemia could theoretically also explain the increased hypnotic effect of etomidate. The decrease in albumin concentration in the hypovolemic animals could indeed lead to an increased free fraction of etomidate, which may result in an increased distribution volume and increased free concentrations of etomidate. However, although plasma albumin concentration decreased significantly in the hypovolemic animals, protein binding of etomidate was comparable in both groups and was similar to the value of about 80% reported by others (Meuldermans and Heykants, 1976).

In addition to these pharmacokinetic changes, pharmacodynamic alterations might contribute to the increased hypnotic effect of etomidate. To test this hypothesis, the relationship was studied between the effect-site concentration and effect of etomidate. The changes observed in the raw EEG signal were used as a surrogate measure of the hypnotic effect of etomidate. In this context, it should be considered that the EEG might be affected in the hypovolemia group by other factors than the anesthetic (e.g., by the hypothermia and hypocapnia combined with the etomidate-induced hypotension (Morawetz et al., 1979; Gregory et al., 1981; Artru and Colley, 1984). However, preliminary experiments in three rats not receiving etomidate but subjected to the hypovolemia procedure and the ensuing moderate hypothermia showed no changes in the EEG. In addition, the fact that the EEG activity in the hypovolemia group returned to baseline values after etomidate infusion corroborates this view. Moreover, preliminary experiments with repetitive pH and blood gas measurements showed that normocapnia was present in hypovolemic animals during and after etomidate infusion, and that hypocapnia appeared only when the animals became fully awake and EEG activity had almost completely returned to baseline values. An influence of etomidate-induced hypotension on the EEG seems unlikely in that preliminary experiments in which the time course and degree of the hypotension were mimicked by the infusion of sodium nitroprusside showed no effect on the EEG. These findings are in agreement with data of Wauquier (1983), in which hypovolemia-induced hypotension in conscious dogs caused only slight changes in the EEG.

We believe that the EEG effects are due to the anesthetic agent itself. The EEG changes were quantified using aperiodic analysis resulting in an EEG parameter, AMP in the 2.5- to 7.5-Hz frequency band, reflecting the central nervous system effect of etomidate as previously shown in control animals (De Paepe et al., 1999).

After plotting the EEG amplitude-versus-etomidate plasma concentration relationship, a figure-eight-shaped hysteresis was observed. Subsequently, this hysteresis was collapsed by estimating \( k_{eq} \). The equilibrium rate constant \( k_{eq} \) and the equilibrium half-life \( T^{1/2}_{k_{eq}} \) both measures of the equilibration delay between plasma and effect-site, were similar in hypovolemic and control animals. Equilibration in the hypovolemic animals tended to occur even faster, suggesting that brain blood flow is not reduced and that blood-brain barrier is not altered during hypovolemia. This observation confirms the data of Benowitz et al. (1974), which showed in rhesus monkeys an increased brain blood flow after 30% exsanguination at the expense of blood flow to peripheral organs.

After collapsing hysteresis, a biphasic EEG effect-versus-effect-site concentration relationship that can be considered equivalent to an effect-plasma concentration relationship under steady-state conditions was observed in both hypovolemic and control animals. Quantification of this relationship was subsequently done by using nonparametric descriptors as Ebling et al. (1991) pointed out that these are of potential interest for quantitative measurement of changes in pharmacodynamics resulting from disease states. A pharmacodynamic model was not used because parameters of biphasic pharmacodynamic models are not estimable (Dutta et al., 1997).

\( E_o \) did not differ between hypovolemic and control animals, confirming that the hypovolemia procedure did not influence the EEG parameter. \( EC_{50} \), \( EC_{m} \), and \( EC_p \) values tended to be lower in the hypovolemia group, but only the difference in \( EC_m \) was significant. These observations indicate that an increased potency of etomidate during hypovolemia cannot be excluded. However, because the differences are not very pronounced, this enhanced sensitivity probably contributes little to the increased hypnotic effect of etomidate in the hypovolemic animals. The etomidate effect-site concentration at the return of righting reflex was slightly but significantly lower in the hypovolemic rats, which is another argument in favor of a small increased end organ sensitivity. It should be kept in mind that our study evaluated end organ sensitivity indirectly by estimating effect-site concentrations. More direct information on end organ sensitivity could be provided by intracerebral microdialysis, which allows the measurement of effect-site concentrations. However, with this technique, one should take into account that drug distribution might differ in the different brain regions and that the exact
site of action for anesthetic agents is not known. An increased central nervous system sensitivity during hypovolemia was also proposed for midazolam in the dog (Adams et al., 1985) and for phenobarbital and desmethylazepam in the rat (Klockowski and Levy, 1988a,b). However, none of these studies provided direct evidence for increased end organ sensitivity during hypovolemia.

In conclusion, the present study demonstrates an increased hypnotic effect of etomidate during hypovolemia in the conscious rat, which can mainly be attributed to changes in the pharmacokinetics.

Acknowledgments

We thank Dr. Davide Verotta (University of California, San Francisco) for kindly providing the FORTRAN program for hysteresis minimization, Dr. M. Bogaert for critically reading the manuscript, Belpaire (Cisco) for kindly providing the FORTRAN program for hysteresis minimization, and Marleen De Meulemeester for technical assistance.

References


Send reprint requests to: Dr. Peter De Paepe, Heymans Institute of Pharmacology, De Pintelaan 185, B-9000 Ghent, Belgium. E-mail: Peter.DePaepe@rug.ac.be