

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

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Background: **Hypovolemia decreases the dose requirement for anesthetics, but no data are available for propofol. As it is impossible to study this in patients, a rat model was used in which the influence of hypovolemia on the pharmacokinetics and pharmacodynamics of propofol was investigated.**

Methods: **Animals were randomly allocated to either a control (n = 9) or a hypovolemia (n = 9) group, and propofol was infused (150 mg · kg^{-1} · h^{-1}) until isoelectric periods of 5 s or longer were observed in the electroencephalogram. The changes observed in the electroencephalogram were quantified using aperiodic analysis and used as a surrogate measure of hypnosis. The righting reflex served as a clinical measure of hypnosis.**

Results: **The propofol dose needed to reach the electroencephalographic end point in the hypovolemic rats was reduced by 60% ($P < 0.01$). This could be attributed to a decrease in propofol clearance and in distribution volume. Protein binding was similar in both groups. To investigate changes in end organ sensitivity during hypovolemia, the electroencephalographic effect versus effect–site concentration relation was studied. The effect–blood concentration relation was biphasic, exhibiting profound hysteresis in both hypovolemic and control animals. Semiparametric minimization of this hysteresis revealed similar equilibration half-lives in both groups. The biphasic effect–concentration relation was characterized by descriptors showing an increased potency of propofol during hemorrhage. The effect–site concentration at the return of righting reflex was 23% ($P < 0.01$) lower in the hypovolemic animals, also suggesting an increased end organ sensitivity.**

Conclusions: **An increased hypnotic effect of propofol occurs during hypovolemia in the rat and can be attributed to changes in both pharmacokinetics and end organ sensitivity. (Key words: Anesthesia; effect–site; hemorrhage; shock.)**

PROVIDING analgesia and general anesthesia to hypovolemic patients remains a major challenge. In 1943, Halford[1] reported an increased anesthetic-associated mortality after the administration of thiopental to hypovolemic war casualties. An observational study by Harrison[2] found that the induction of anesthesia in hypovolemic patients was the most common cause of death attributed to anesthesia.

It is common clinical practice to reduce the anesthetic dose in hypovolemic patients. The rationale for this is mainly based on clinical experience and is supported by only a small number of studies. Because it is hardly possible to study this in critically ill patients, these investigations are almost exclusively performed in animals. An increased anesthetic effect during hypovolemia has been observed in animal experiments for barbiturates,[3,] benzodiazepines,[5,6] and ketamine,[4] and we have shown an increased analgesic effect of morphine in hypovolemic rats.[7] The increased response to anesthetics and analgesics during hypovolemia has been ascribed to pathophysiologic changes that alter the pharmacokinetics[5,7] or the pharmacodynamics (end organ sensitivity).[3,5,6]

For etomidate, we recently studied the influence of hypovolemia on the pharmacokinetics and the anesthetic effect in the rat in which the electroencephalogram was used as a surrogate measure of hypnosis.[8] An increased hypnotic effect of etomidate was observed during hypovolemia that was mainly attributed to pharmacokinetic changes with a small increase in central nervous system sensitivity for etomidate in hypovolemic animals.

We decided to study the anesthetic propofol in the same model for several reasons. Propofol is used for continuous anesthesia–sedation rather than etomidate because of the adrenocortical suppression observed with this drug. Moreover, there is much interest for target controlled infusion and closed-loop control systems using the electroencephalogram as a measure of depth of anesthesia, but there are no data available about the use of these recent techniques in hypovolemia. Finally, in contrast with etomidate, propofol is extensively bound to plasma proteins and erythrocytes,[9] and therefore the pharmacokinetic changes expected during hypovolemia cannot be extrapolated from the etomidate experiments.

Materials and Methods

Animal Instrumentation

The study protocol was approved by the Ethics Committee for Animals of the Ghent University Hospital. Male Wistar rats (310–370 g) were purchased from Iffa Credo and kept at 21°C with a 12 h–12 h light–dark cycle.

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Surgery for the instrumentation was conducted with pentobarbital anesthesia (60 mg/kg intraperitoneally).

Animals were instrumented for electroencephalographic, blood pressure, heart rate (HR), and body temperature recording as described previously.[8] Briefly, 1 week before the experiment, seven epidural electroencephalographic electrodes were implanted. Two days before the experiment, polyethylene catheters were inserted into the femoral artery and vein.

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

Arterial blood pressure was registered *via* the arterial line on a Beckman recorder, and 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, the Netherlands). The core temperature was measured with a flexible thermistor probe inserted rectally to a depth of 5 cm.

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

Experimental Protocol

After overnight fasting, the rat was loosely restrained in a cage. All experiments started between 9 and 10 AM. The arterial line was filled with 0.2 ml of heparinized saline (100 IU/ml) and connected to a blood pressure transducer. Before the experiment, all animals received an intravenous bolus dose of heparine (1 IU/g body weight).

After 20 min of baseline hemodynamic and electroencephalographic 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. After another 30 min, the hypovolemic animals received an intravenous infusion of propofol (Diprivan 1%; AstraZeneca, Wilmington, DE). It was given at a rate of 150 $mg \cdot kg^{-1} \cdot h^{-1}$. The infusion was terminated when the electroencephalogram indicated burst suppression with isoelectric periods of 5 s or longer. Arterial blood samples of 100 μl were taken for determination of propofol blood concentrations at the following time intervals: 1, 2, 3, and 4 min after the start of the infusion; at the time of termination of the infusion; and 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, and protein concentration.

Control animals (n = 9) underwent the same experimental protocol but without removal of blood. During propofol infusion, blood samples were taken at 1, 2, 4, and 6 min, and the next samples were taken at the same time intervals as in the hypovolemic animals. The return of righting reflex was used as a clinical parameter of depth of anesthesia.

Drug Assay

Immediately after collection, whole blood samples (75 μl) were hemolysed in 375 μl of deionized water, vortexed, and stored at 4°C until analysis. Concentrations of propofol in hemolysed blood (300 μl) were assayed by high-performance liquid chromatography according to the slightly modified method of Plummer.[10] Diluted blood samples were mixed with 50 μl methanolic solution containing internal standard (thymol, 1 μg/ml) and 1 ml 0.025 M KH_2PO_4. After extraction with 4.5 ml of n-hexane, the organic layer was evaporated to dryness at 55°C with nitrogen, and the residue was reconstituted in the mobile phase and analyzed on a Supelco LC-8-DB column (Sigma-Aldrich, Bornem, Belgium; 15 cm × 4 mm). The mobile phase was a mixture of 23 mM H_3PO_4 (pH 1.9)–acetonitrile (35:65). The flow rate was set at 1 ml/min. Detection was performed by fluorescence (excitation wavelength of 276 nm and emission wavelength of 295 nm).

The interday coefficients of variation for the determination of propofol at concentrations of 0.5, 4, and 20 μg/ml were always less than 9.7%, and the overall accuracy ranged from 94.3% to 99.7% (n = 16). The lower limit of quantitation of propofol was 0.1 μg/ml using 300 μl hemolysed blood.

Protein Binding

Propofol blood binding was investigated in 12 animals randomly assigned to undergo either the hypovolemia (n = 6) or the control (n = 6) procedure; the solvent of propofol (Intralipid 10%, Pharmacia & Upjohn, Peapack, NJ) was infused, and four arterial blood samples (600 μl) were taken for determination of whole blood binding of propofol at different time intervals (before the start of the infusion and at 1, 2, and 3 h after the end of the infusion).

Propofol blood binding was measured immediately after the collection of the blood samples by equilibrium dialysis for 3 h at 37°C.[11] Two hundred microliters of blood, spiked with 2 μg/ml propofol and adjusted to pH 7.4, was dialysed against 200 μl isotonic phosphate buffer (16 mM Na_2HPO_4, 4 mM KH_2PO_4, 100 mM NaCl, pH 7.4). After dialysis, propofol concentrations were determined in blood after extraction as described previously and in dialysate by direct injection on the high-performance liquid chromatography column at an excitation wavelength of 200 nm and an emission wavelength of 295 nm. A higher sensitivity for propofol at these wavelengths was obtained than at the wavelengths used for the assay in blood. The interday coefficients of vari-

ation for the determination of propofol in dialysate at concentrations of 30 and 60 ng/ml were less than 11.5%, and the overall accuracy was 103% and 110%, respectively (n = 6). The lower limit of quantitation of propofol was 12.5 ng/ml using 100 μl dialysate.

Blood binding was measured in duplicate in each sample. Propofol blood binding was constant over the concentration range studied (1–20 μg/ml). After spiking blood with 2 μg/ml propofol, propofol recovery after dialysis (sum of concentration in blood and dialysate) was 88% (n = 17), and within-day variability of propofol free blood fraction was 6% (n = 5). Interday variability of propofol free fraction in plasma spiked with 2 μg/ml propofol was 11% (n = 6). Plasma was chosen instead of blood for determination of interday variability because blood would disintegrate over the time course of the different assays.

Analysis of Data

The pharmacokinetics and pharmacodynamics of propofol were quantified for each individual rat. The blood concentration time profiles during and after infusion were described by a polyexponential equation using Winnonlin version 1.5 (Pharsight Corp., 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. Calculation of the pharmacokinetic parameters was performed according to Gibaldi and Perrier.[12]

Propofol drug effect was assessed from the electroencephalographic signal processed by aperiodic analysis using the amplitude per second from 11.5 Hz to 30 Hz in the left fronto-occipital lead.[13] The electroencephalographic data were averaged over predetermined intervals. The interval duration (10 s to 2 min) depended on the rate of change of the signals. Details about the electroencephalographic recording and processing have been reported elsewhere.[14] Hysteresis in the electroencephalographic effect *versus* blood concentration curve was minimized by a semiparametric approach using a FORTRAN-written program to reveal the apparent effect–site concentration–effect relation and to estimate the first-order rate equilibration constant, k_{eo}, using a first-order monoexponential equilibrium model.[15] Propofol blood concentration time curves were calculated based on the compartmental model obtained in each individual rat.

After hysteresis minimization, the electroencephalographic effect versus effect–site concentration curve was characterized nonparametrically with the use of descriptors without invoking a pharmacodynamic model because the parameters of the biphasic models are not estimable.[16] The descriptors used are the baseline effect (E_0), the maximal activation of the electroencephalographic effect (E_{max}), the concentration required to produce the maximal activation (EC_m), the concentration required to obtain 50% activation of the electroencephalographic effect (EC_{50}), the concentration required to produce the baseline effect between maximal activation and maximal inhibition (EC_b), and the concentrations required to obtain 50% and 90% reduction of the electroencephalographic effect below baseline ($EC_{i,50}$ and $EC_{i,90}$, respectively). E_0, E_{max}, and EC_m were directly obtained from the data, and EC_{50}, EC_b, $EC_{i,50}$, and $EC_{i,90}$ were derived by linear interpolation between the two closest points. The propofol effect–site concentration at the return of righting reflex was also derived by linear interpolation.

Statistical Analysis

The results are expressed as mean ± SD. Comparison of physiologic parameters and pharmacokinetic and pharmacodynamic estimates between hypovolemic and control animals were made using multivariate multiple regression. Hemodynamic and propofol blood binding data were compared using a two-way analysis of variance for repeated measures. A *P* value less than 0.05 was considered statistically significant.

Results

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

In the hypovolemic animals, a significantly lower dose of propofol was needed to reach the end point of 5-s isoelectric electroencephalogram (11.6 ± 1.5 mg/kg *vs.* 29.5 ± 7.2 mg/kg; $P < 0.01$), corresponding to a mean infusion duration of 4.6 ± 0.6 and 11.8 ± 3.0 min, respectively. Although we aimed to stop the infusion at a similar end point of 5-s isoelectric electroencephalogram, the duration of the isoelectric period was significantly longer in the hypovolemic animals compared with the controls (6.2 ± 0.6 s *vs.* 5.4 ± 0.6 s; $P < 0.01$).

Table 1 shows the mean arterial pressure (MAP) and HR in control and hypovolemic rats before, during, and after infusion of propofol. Baseline MAP and HR 1 h before the start of infusion were not different between the hypovolemia and control groups (129 ± 9 mmHg *vs.* 130 ± 9 mmHg and 457 ± 42 beats/min *vs.* 465 ± 51 beats/min). In the hypovolemic animals, MAP decreased to a minimum of 87 ± 21 mmHg at the end of the hypovolemia procedure, and then gradually increased again to 106 ± 15 mmHg in the period preceding the infusion of propofol. HR remained stable during this period. In the control group, MAP and HR remained stable during the predrug period. Immediately after the start of the propofol infusion, a decrease in MAP was observed both in hypovolemic and control animals, becoming maximal at the end of the infusion. This was accompanied by a decrease in HR in both

Table 1. Mean Arterial Blood Pressure (MAP) and Heart Rate (HR) in Control and Hypovolemic Animals before, during, and after Intravenous Infusion of Propofol

	Control (n = 9)		Hypovolemia (n = 9)	
	MAP (mmHg)	HR (beats/min)	MAP (mmHg)	HR (beats/min)
At start of infusion	133 ± 11	473 ± 52	106 ± 15*	468 ± 28
At end of infusion	78 ± 12	343 ± 35	49 ± 6*	340 ± 27
3 h after end of infusion	116 ± 9	478 ± 33	85 ± 9*	438 ± 34

Results are expressed as mean ± SD.

* $P < 0.01$ compared with the control group; two-way analysis of variance for repeated measures.

groups. At the end of the experiment, MAP was significantly lower in the hypovolemic animals compared with controls. In the control animals, HR remained stable until the end of the experiment, whereas in the hypovolemic animals, HR tended to decrease by the end of the experiment.

Hypovolemia caused a significant reduction in body temperature, arterial carbon dioxide partial pressure, plasma HCO_3^-, hematocrit, and plasma total protein compared with the control group; in contrast, the arterial oxygen partial pressure was significantly higher (table 2). Figure 1 shows the time course of both propofol blood concentrations and the electroencephalographic parameter, expressed in amplitude per second in the frequency range of 11.5–30 Hz, for a typical control rat during and after propofol infusion. Shortly after the start of infusion, at relatively low concentrations, an increase in electroencephalographic amplitude was observed. However, as the infusion continued and propofol concentrations further increased, a decrease in electroencephalographic amplitude occurred, reaching a minimum shortly after the end of infusion. After the end of infusion, propofol concentrations rapidly declined, and a second activation phase in electroencephalographic amplitude occurred that was followed by a return to baseline values.

Table 2. Effect of Hypovolemia on Some Physiologic Characteristics

	Control (n = 9)	Hypovolemia (n = 9)
Body weight (g)	345 ± 28	358 ± 21
Temperature (°C)		
Prestudy	37.6 ± 1.8	37.8 ± 1.2
Poststudy	37.3 ± 0.6	35.3 ± 0.9*
pH	7.44 ± 0.03	7.42 ± 0.06
P_{CO_2} (mmHg)	35.8 ± 2.4	31.4 ± 3.7†
P_{O_2} (mmHg)	95.6 ± 7.2	122.8 ± 12.4*
HCO_3^- (mEq/l)	24.5 ± 1.5	20.5 ± 2.9*
Hematocrit (%)	36.0 ± 2.1	27.6 ± 0.9*
Plasma albumin (g/100 ml)	2.98 ± 0.33	2.71 ± 0.27
Plasma total protein (g/100 ml)	5.74 ± 0.43	4.92 ± 0.33*

Results are expressed as mean ± SD.

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

P_{CO_2} = partial pressure of carbon dioxide; P_{O_2} = partial pressure of oxygen; HCO_3^- = bicarbonate.

The individual concentration-*versus*-time profiles of propofol in control and hypovolemic rats are shown in figure 2. In each rat, data were best fitted by a three-exponential model. The infusion time needed to reach the end point of 5-s isoelectric electroencephalogram was significantly shorter in the hypovolemic animals than in the controls, which is also clear from the inserted figure showing the time course of the propofol blood concentrations during the first 30 min after the start of the infusion.

The pharmacokinetic parameters of propofol for both groups of animals are shown in table 3. The maximal propofol concentration at the end of the infusion was similar in both groups. Systemic clearance and volume of distribution of the central compartment and at steady state were significantly lower in the hypovolemic animals. The initial half-life was significantly lower in the hypovolemic group. Mean residence time and intermediate and terminal half-lives were not significantly different between the two groups.

The possibility of differences in propofol blood binding between control and hypovolemic animals was investigated in additional experiments in which the free blood fraction of propofol was determined during the course of the experiment. These experiments showed that the free blood fraction of propofol during the experiment was slightly but not significantly higher in the hypovolemic animals (n = 6) compared with the controls (n = 6; 2.0 ± 0.0 *vs.* 1.8 ± 0.4, 2.1 ± 0.3 *vs.* 1.8 ± 0.3, 2.2 ± 0.3 *vs.* 1.9 ± 0.3, 2.2 ± 0.3 *vs.* 2.0 ± 0.4 before the

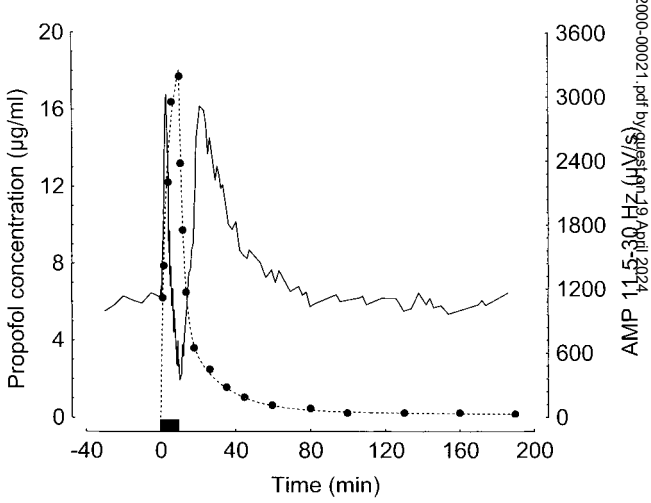

Fig. 1. Propofol blood concentration (circles) and the electroencephalographic effect expressed as amplitude per second in the 11.5–30-Hz frequency band (solid line) over time in a typical control rat during and after propofol infusion (filled bar). The dashed line constitutes the best fit to the concentration data. AMP = amplitude.

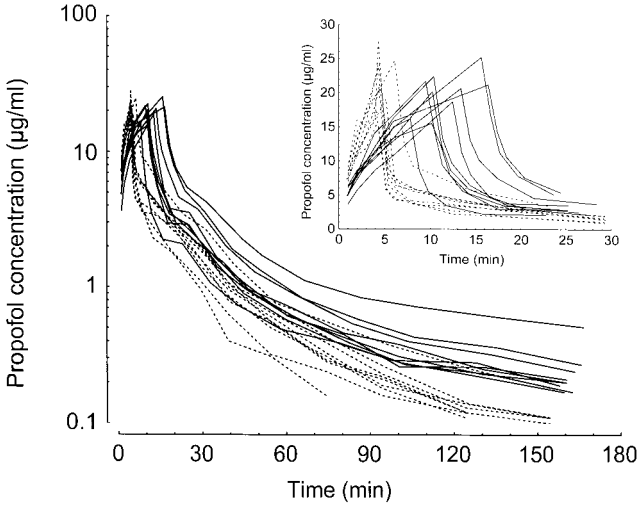

Fig. 2. Blood concentration of propofol as a function of time in control (solid lines; n = 9) and hypovolemic (dotted lines; n = 9) rats during and after an intravenous infusion of propofol (150 mg · kg^{-1} · h^{-1}). Infusion is started at time 0 and terminated when the electroencephalogram indicated burst suppression with isoelectric periods of 5 s or longer. The inserted figure shows the time course of the propofol blood concentration during the first 30 min after the start of the infusion.

start of the solvent infusion and at 1, 2, and 3 h after the end of the infusion, respectively).

The pharmacodynamics of propofol were studied by correlating the electroencephalographic effect with the propofol concentrations. When the electroencephalographic amplitude was plotted against the blood concentrations of propofol, profound hysteresis was observed in both groups as illustrated in figure 3A for the same control animal shown in figure 1. This hysteresis was collapsed for both control and hypovolemic animals by estimating k_{eo} using the hysteresis minimization program, resulting in a biphasic effect–site concentration–electroencephalographic effect relation of propofol (fig. 3B). The individual biphasic curves for both groups are shown in figure 4. This biphasic relation was characterized by descriptors, which are shown in table 4 for both control and hypovolemic animals. The equilibration constant, k_{eo}, and the equilibration half-life, $t^{1/2}_{keo}$, were similar in both groups, as were the E_0 and E_{max}. The EC_{50}, EC_m, EC_b, $EC_{i,50}$, and $EC_{i,90}$ were lower in the hypovolemia group compared with the controls; except for EC_{50}, these differences were statistically significant. The effect–site concentration at the return of righting reflex was significantly lower in the hypovolemic animals.

Table 3. Effect of Hypovolemia on the Pharmacokinetic Parameters of Propofol

	Control (n = 9)	Hypovolemia (n = 9)
Dose (mg/kg)	29.5 ± 7.2	11.6 ± 1.5*
Maximal concentration (μg/ml)	16.9 ± 2.1	16.5 ± 2.2
Systemic clearance (ml · min^{-1} · kg^{-1})	85 ± 9	59 ± 9*
Volume of distribution of the central compartment (l/kg)	0.40 ± 0.09	0.16 ± 0.03*
Volume of distribution at steady state (l/kg)	4.01 ± 0.89	2.11 ± 0.75*
Mean residence time (min)	47.3 ± 10.2	36.9 ± 12.1
Initial half-life (min)	1.3 ± 0.3	0.5 ± 0.1*
Intermediate half-life (min)	11.2 ± 1.4	11.5 ± 1.7
Terminal half-life (min)	100.4 ± 26.2	84.6 ± 35.2

Results are expressed as mean ± SD.

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

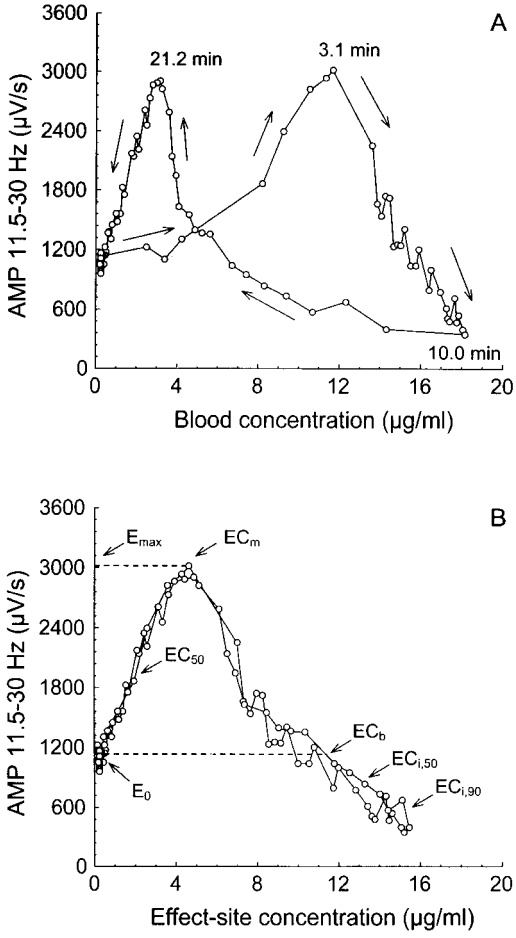

Fig. 3. (*A*) Electroencephalographic amplitude (AMP) *versus* propofol blood concentration in a typical control rat that received 25.0 mg/kg propofol. (*B*) Electroencephalographic amplitude *versus* propofol effect–site concentration after hysteresis minimization. Descriptors of the biphasic relation are indicated.

Discussion

A volume-controlled hypovolemia model in the awake rat was used to investigate the influence of hypovolemia on the pharmacokinetics and electroencephalographic effect of propofol. Removal of 30% of the estimated blood volume was used, which is considered as moderate hypovolemia.[17] This is reflected by the moderate physiologic changes at the end of the experiment. pH

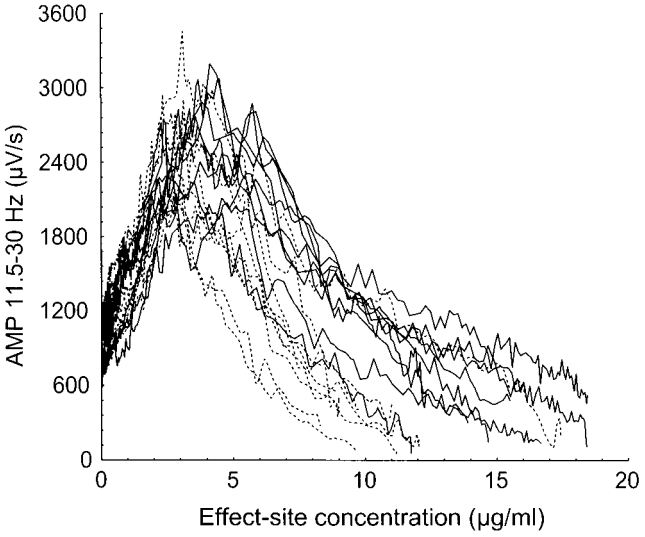

Fig. 4. Biphasic relation between electroencephalographic effect and apparent effect–site concentration for control (solid lines; n = 9) and hypovolemic (dotted lines; n = 9) rats. AMP = amplitude.

was well maintained in the hypovolemic animals as the decrease in HCO_3^- was compensated by a decrease in carbon dioxide partial pressure caused by hyperventilation, resulting in an increase of oxygen partial pressure. The reduced hematocrit and protein concentrations caused by hypovolemia can be explained by the dilutional effects of transvascular fluid shifts. Body temperature was slightly reduced in the hypovolemic animals. Blood pressure values at the start and at the end of the experiment are in agreement with the relatively mild degree of hypovolemia.

Table 4. Pharmacodynamic Descriptors of the Biphasic Electroencephalographic Effect–Concentration Relation in Control and Hypovolemic Animals at Intravenous Infusion of Propofol

	Control (n = 9)	Hypovolemia (n = 9)
E_0 (μV/s)	910 ± 210	849 ± 182
EC_{50} (μg/ml)	1.7 ± 0.3	1.5 ± 0.4
E_{max} (μV/s)	2,705 ± 384	2,588 ± 453
EC_m (μg/ml)	4.2 ± 1.2	2.8 ± 0.3*
EC_b (μg/ml)	11.2 ± 2.3	7.7 ± 1.9*
$EC_{i,50}$ (μg/ml)	13.2 ± 2.2	9.3 ± 2.7*
$EC_{i,90}$ (μg/ml)	15.0 ± 2.3	10.5 ± 2.6*
Concentration at return of righting reflex (μg/ml)	2.8 ± 0.5	2.2 ± 0.3*
k_{eo} (min^{-1})	0.39 ± 0.06	0.33 ± 0.06
$t^{1/2}_{k_{eo}}$ (min)	1.82 ± 0.33	2.14 ± 0.39

Results are expressed as mean ± SD.

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

E_0 = electroencephalographic effect at baseline; EC_{50} = concentration required to obtain 50% activation of the electroencephalographic effect; E_{max} = maximal activation of the electroencephalographic effect; EC_m = concentration required to produce maximal electroencephalographic activation; EC_b = concentration required to produce the baseline effect between maximal electroencephalographic activation and maximal electroencephalographic inhibition; $EC_{i,50}$ = concentration required to obtain 50% reduction of the electroencephalographic effect below baseline; $EC_{i,90}$ = concentration required to obtain 90% reduction of the electroencephalographic effect below baseline; k_{eo} = first-order rate equilibration constant; $t^{1/2}_{k_{eo}}$ = equilibration half-life.

Propofol infusion was started at a fixed time interval after the induction of hypovolemia. It could be argued that equivalent levels of hypovolemia are better confirmed by pathophysiologic indices, but the model has been extensively used and showed metabolic and hemodynamic changes reproducible with time.[17]

After administration of propofol in control and hypovolemic animals until electroencephalographic burst suppression with isoelectric periods of 5 s or longer, MAP gradually decreased, reaching a maximum reduction at the end of the infusion that was significantly higher in the hypovolemic animals (54%) compared with the controls (41%; $P < 0.01$). This hypotensive effect of propofol was also demonstrated by Yang *et al.*,[18] who showed that burst suppression of the electroencephalographic signal was invariably accompanied by discernible hypotension in the rat. The low blood pressure levels observed in our study were short-lasting as MAP started to increase shortly after the end of infusion in both groups. The percentage reduction in heart rate (± 30%) was identical in both groups, and recuperation occurred shortly after the end of infusion. These data are in agreement with the experience that hypovolemia potentiates the hypotensive effects of propofol.[19] In a study by Hoka *et al.*[20] after propofol administration in intact and hypovolemic rats, MAP was reduced to the same extent as in our study and was attributed mainly to an increase in vascular capacitance caused by inhibition of sympathetic vasoconstrictive activity. In a study in which we investigated the pharmacology of etomidate in hypovolemic rats,[8] a reduction in blood pressure of comparable magnitude occurred as with propofol in the present study. Obviously, caution should be taken when comparing these two studies as they were not conducted simultaneously and they were not intended to make a comparison between the hemodynamic effects of etomidate and propofol. Care should also be taken when extrapolating these animal data to the hypovolemic patient who often suffers from underlying diseases. It is interesting to note in this context that the use of propofol in hypovolemic patients is generally discouraged because of its pronounced hypotensive effects in these patients,[21] whereas etomidate is generally accepted as the drug of choice because of its more stable hemodynamic profile. However, it should be mentioned that the results of the studies comparing the hemodynamic effects of propofol with etomidate are not unequivocal.[22–24] No studies are currently available that compare the hemodynamic effects of propofol and etomidate in hypovolemic patients using equianesthetic doses as measured objectively by, for example, electroencephalography.

The propofol dose needed to reach approximately the same degree of electroencephalographic effect in the

hypovolemic rats as compared with the controls was reduced by 60%. A first explanation for this increased effect may be that pharmacokinetic changes occurred during hypovolemia, resulting in higher effect–site concentrations for a given dose of propofol. A second explanation is that the pharmacodynamics may be altered, resulting in an increased end organ sensitivity for propofol.

Pharmacokinetic analysis revealed that both systemic clearance and distribution volume were significantly decreased in the hypovolemic animals. The reduction in propofol clearance may be explained by a decreased hepatic blood flow during hypovolemia, as propofol is extensively cleared by the liver.[9] Seyde and Longnecker[25] showed that withdrawal of 30% of the estimated blood volume in rats resulted in a 30% reduction in liver blood flow. A reduced liver blood flow during hypovolemia was also proposed in other studies to partially explain the reduced clearance for, *e.g.*, midazolam,[6] methylprednisolone succinate,[26] lidocaine,[26] prednisolone,[27] morphine,[7] etomidate,[8] and fentanyl.[28] The decreased clearance in the hypovolemic animals may also be explained by the reduction in body temperature, as hypothermia was shown to decrease the clearance of drugs such as remifentanil and pentobarbital.[29] However, the decrease in body temperature in the latter studies was more pronounced than that in our experiments. Moreover, Leslie *et al.*[30] found that induction of mild hypothermia (34°C) in human volunteers did not change total body clearance of propofol. Hypothermia induced changes in systemic clearance are therefore unlikely to have occurred in our experiments.

The decrease in distribution volume in hypovolemic rats may be explained by a reduced organ perfusion with preservation of blood flow to more essential organs, *i.e.*, heart and brain.[25] The same mechanism was suggested to explain the reduced distribution volume during hypovolemia in animal experiments with lidocaine,[31] atropine,[32] prednisolone,[27] morphine,[7] etomidate,[8] and antipyrine.[33]

The hypovolemia-induced reduction in plasma albumin and formed elements of blood may also have altered the distribution of propofol, which is extensively bound to albumin and these elements.[9] Reduction in plasma albumin and formed elements of blood may theoretically lead to an increased free fraction of propofol, resulting in higher free concentrations and ensuing in an increased distribution volume. This possibility was investigated in experiments in which the free propofol blood fraction was measured in both control and hypovolemic rats. These experiments revealed that the free fraction was slightly but not significantly higher in the hypovolemic animals compared with the controls. Therefore, changes in free fraction do not seem to account for the increased anesthetic effect. In this context, it should be noted that in humans, propofol free fraction in blood is in the same range as that observed in our study, but in contrast with the rat, it distributes less extensively in the formed blood elements.[34]

The aforementioned pharmacokinetic changes may explain the increased hypnotic effect of propofol during hypovolemia. However, this does not preclude that, in addition to the pharmacokinetic changes, alterations in end organ sensitivity may also contribute to the increased anesthetic effect of propofol. To examine this possibility, the pharmacodynamics of propofol were investigated in the present experiment by quantifying the changes in electroencephalographic amplitude in the 11.5–30-Hz frequency band, as this was shown to provide a continuous, sensitive, and objective measure of drug effect on the brain.[13] The applicability of the electroencephalogram in our hypovolemic animal model has been demonstrated in previous experiments,[8] showing that neither hypovolemia nor its accompanying hypothermia influenced the electroencephalogram. An influence of propofol-induced hypotension on the electroencephalogram was also found to be unlikely in that preliminary experiments in which the time course and degree of the hypotension was mimicked by the infusion of sodium nitroprusside showed no effect on the electroencephalogram. Consequently, the electroencephalographic changes can be considered as caused by the drug.

Relating the propofol-induced electroencephalographic effect to the propofol blood concentrations revealed a biphasic relation with profound hysteresis. A similar relation between electroencephalographic effect and propofol concentration has been demonstrated in humans.[35] After hysteresis minimization, the equilibration rate constant, k_{eo}, and the equilibration half-life, $t^{1/2}_{keo}$, both measures of the equilibration delay between blood and effect–site, were obtained. These values were not different between control and hypovolemic animals, which is an indirect proof that cerebral blood flow is similar in both groups as the rate of brain uptake of propofol is believed to be determined by the brain blood flow.[36] This was confirmed in a study by Werner *et al.*[37] that showed that propofol did not alter cerebral blood flow autoregulation within a pressure range of 50–140 mmHg, and therefore, cerebral blood flow is unlikely to be depressed to a greater extent in the hypovolemic animals compared with the controls. Similar results were observed with etomidate[8] and other substances such as lidocaine.[31] The k_{eo} and $t^{1/2}_{keo}$ values observed in our study were comparable to those found by other investigators for propofol in humans[38] and rats.[39]

After minimizing the hysteresis, the biphasic effect *versus* effect–site relation was quantified by several descriptors that allowed estimation of the potency and the intrinsic efficacy of propofol in both groups. EC_{50}, EC_m, EC_b, $EC_{i,50}$, and $EC_{i,90}$ were systematically lower in the hypovolemia group, indicating an increased potency of propofol during hypovolemia. E_{max} was not different between control and hypovolemic animals, indicating

that hypovolemia had no influence on the intrinsic efficacy of propofol. The fact that the more pronounced electroencephalographic effect was observed with similar peak blood concentrations is also in line with an increased end organ sensitivity in hypovolemic animals. Indeed, it should be remembered that although we aimed to stop the infusion at a similar end point of 5-s isoelectric electroencephalogram, the duration of the isoelectric period was significantly longer in the hypovolemic animals compared with the controls. This overshooting was caused by the more rapid electroencephalographic changes in the former group.

In addition to the electroencephalographic effect, the righting reflex was used as a clinical measure of depth of anesthesia. The propofol effect–site concentrations at the return of righting reflex were significantly lower in the hypovolemic animals compared with the controls, which is another argument in favor of an increased end organ sensitivity. The wake-up concentrations observed in our study were in the same range as those reported by other investigators in humans[40] and rats.[13] We therefore conclude that end organ sensitivity increased during hypovolemia. This has also been suggested for barbiturates,[3] benzodiazepines,[5,6] and etomidate.[8] For the first two drugs, end organ sensitivity was investigated by measuring cerebrospinal fluid concentrations at the loss of righting reflex, whereas for etomidate the same methodology was applied as in the present study. The increased end organ sensitivity may be explained by a change in physiologic processes or a release of endogenous substances that enhance the anesthetic effects of propofol during hypovolemia. β-Endorphins, for instance, have been shown to increase in response to hemorrhage.[41] Because endorphins may interfere with the γ-aminobutyric acid (GABA)ergic system[42] and because it is suggested that propofol produces anesthesia by acting at $GABA_A$ receptors,[43] an interaction between endorphins and propofol may be hypothesized as a possible mechanism for the increased end organ sensitivity. Hypothermia arising from hypovolemia may also influence end organ sensitivity, as hypothermia was shown to affect the pharmacodynamics of pentylenetetrazol[44] and the effect-*versus*-concentration relation of alfentanil.[45] However, drug sensitivity during hypothermia did not increase, but rather decreased,[44] and hypothermia was more pronounced than in our experiments. Cerebral ischemia, which may result from propofol-induced cerebral hypoperfusion in the hypovolemic rats, cannot be excluded as a possible cause for the increased end organ sensitivity. However, this is unlikely because cerebral blood flow autoregulation during propofol anesthesia was shown to be preserved within a large pressure range, as stated earlier.

The results of this study are in line with the clinical experience that the dose of anesthetics should be reduced in hypovolemic patients. Our animal study demonstrates that the decreased anesthetic requirement of propofol during hypovolemia is caused by changes in both pharmacokinetics and pharmacodynamics as reflected in the electroencephalographic parameter. These changes were observed in a relatively mild degree of hypovolemia, and care should be taken for extrapolation to hypovolemic conditions with more serious hemodynamic compromise. Obviously, caution should be taken when extrapolating our animal data to the clinical situation. Our experiments differ from the clinical situation in which fluid resuscitation is often instituted before or simultaneously with the induction of anesthesia. However, fluid resuscitation does not necessarily reverse the pharmacologic changes. Indeed, Klockowski and Levy showed that the increased end organ sensitivity of hypovolemic rats to desmethyldiazepam was not reversed by prompt replacement of blood. Our data suggest that the electroencephalogram may be an interesting tool to monitor anesthetic depth in critically ill patients as it indirectly reflects changes in both pharmacokinetics and end organ sensitivity.

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