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Influence of Hypovolemia on the Pharmacokinetics and Electroencephalographic Effect of γ-Hydroxybutyrate in the Rat

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Background: **Hypovolemia alters the effect of propofol in the rat by influencing the pharmacokinetics and the end organ sensitivity. We now studied the effect of hypovolemia on the anesthetic γ-hydroxybutyrate (GHB) because in contrast with propofol it increases blood pressure.**

Methods: **Thirty-two rats were randomly assigned to undergo moderate hypovolemia or a control procedure. Each rat received either an infusion of sodium–GHB (390 mg · kg^{-1} · 5 min^{-1}) or the same volume of an equimolar solution of sodium chloride (6.9%). Plasma samples were taken for GHB assay (high-performance liquid chromatography) and the electroencephalography and blood pressure values were recorded. A two-compartment model with Michaelis–Menten elimination was fitted to the concentration-time data and a sigmoid E_{max} model to the electroencephalographic effect *versus* effect site concentration curve allowing the study of the end organ sensitivity.**

Results: **Plasma concentration–time curves and the total volume of distribution in hypovolemic and normovolemic rats were comparable with only small but significant differences in central volume of distribution and the intercompartmental clearance. There was no significant difference either in the distribution from the plasma to the brain (k_{e0}) or in the end organ sensitivity (EC_{50} = 335 ± 76 μg/ml in control *vs.* 341 ± 89 μg/ml in hypovolemic rats). GHB temporarily increased mean arterial pressure in both groups, which cannot be explained by the sodium salt alone.**

Conclusions: **Hypovolemia does not influence the overall concentration–time curve of GHB and induces no changes in the electroencephalographic effect of GHB in the rat. This difference with propofol may be due to the fact that it increases blood pressure but also due to its different pharmacokinetic properties.**

IT is well known that doses of anesthetics must be reduced in hypovolemic patients.[1] Animal experiments have also shown that during hypovolemia the effect of anesthetic drugs can increase, which has mainly been explained by pharmacokinetic changes in distribution, metabolism, and/or elimination of drugs.[2–5] Using a model in the rat, which allows the study of the anesthetic effect by means of the electroencephalogram, we have shown that the increased effect of propofol in hypovolemic rats may be explained by a reduction in the volume of distribution and/or the systemic clearance and by an increase in end organ sensitivity.[6]

In the present study, we investigated the pharmacokinetics and pharmacodynamics of γ-hydroxybutyrate (GHB) in this hypovolemic rat model because, in contrast with the hypotensive effects of propofol and etomidate,[6,7] GHB restores the blood pressure in a hemorrhagic shock model in the rat.[8] In this context it is interesting to note that, because of this hypertensive property, GHB is still used as an adjunct to the anesthesia of hemodynamically unstable patients[9] and recently also in the sedation of postoperative intensive care patients.[10] We assumed that this increase in blood pressure might counteract a possible influence of hypovolemia on the pharmacokinetics and pharmacodynamics of GHB. In these experiments, the pharmacokinetics and the electroencephalographic effect of GHB are studied together with the blood pressure and the heart rate in hypovolemic and control rats. GHB was compared with an equimolar sodium chloride solution to find out whether the increase in blood pressure might be due to salt loading.[8]

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 6A.

Materials and Methods

Animal Instrumentation

The study protocol was approved by the Ethics Committee for Animal Research of the Faculty of Medicine of the University of Ghent, Belgium. Male Wistar rats (280–450 g) were purchased from Janvier (Bagneux, France) and kept at 21°C with a 12-h light–dark cycle. Five days before the start of the experiment, the electroencephalographic electrodes were applied to the rats as described previously.[6] In brief, five epidural electroencephalographic electrodes were implanted in frontal and central positions at both sides of the skull, and a reference electrode was placed on λ. After a 5-day recuperation period, polyethylene catheters (PE 10) were inserted into the femoral artery of all rats, used for the acquisition of blood samples and the registration of the

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blood pressure and heart rate, and into the vein for the infusion of GHB. All surgery was done during pentobarbital anesthesia (60 mg/kg intraperitoneally). Arterial blood pressure was recorded on a Beckman recorder and heart rate 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). After a 24-h recuperation period and overnight fasting, all rats were loosely restrained in a cage. The core temperature was measured every hour with a flexible thermistor probe inserted rectally to a depth of 5 cm and the animal was externally warmed when the body temperature decreased to less than 37°C.

Experimental Protocol

All experiments started between 8:00 and 9:00 AM. Thirty-two rats were randomly assigned to undergo hypovolemia (n = 16) or a control (n = 16) procedure and, after a 30-min recuperation period from hypovolemia, rats were subdivided into two groups receiving either GHB (n = 8) or saline (n = 8), resulting in four treatment groups. Three rats died before the end of the experiment: one in each of the two groups treated with GHB and one in the hypovolemia group treated with saline.

Hypovolemia was induced by removing 30% of the initial blood volume (assumed to be 60 ml/kg) in six increments over 30 min through the arterial line,[11] while in the 16 control animals, no blood was removed. During the 30-min recuperation period, all animals received an intravenous bolus of heparin (1 unit/g body weight, dissolved in 300 μl saline).

The sodium salt of GHB (390 mg/kg) was dissolved in water and the GHB concentration of the injection solution was calculated in order to administer a total volume of 0.3 ml/100 g body weight. A sodium chloride solution (6.9%) containing the same amount of sodium as the GHB 390 mg/kg dose was used as a control. A dose of 390 mg/kg GHB was chosen as preliminary experiments have shown that this induced the maximal attainable electroencephalographic effect in all animals. All solutions were infused during a 5-min period using an IVAC P4000 infusion pump (IVAC, Hampshire, UK).

Arterial blood samples of 100 μl were taken at regular time points for determination of GHB plasma concentrations. Sampled blood was replaced with the same volume of isotonic saline solution.

Electroencephalography was measured for the duration of the experiment from the right frontocentral lead using a D/EEG Lite digital electroencephalogram recorder (Telefactor®, Zwolle, The Netherlands) at a sampling rate of 200 Hz. The low-pass and high-pass filters were set at 1 Hz and 70 Hz, respectively. The depth of sedation was assessed clinically by means of four reflexes[12]: the startle reflex to noise, the righting reflex, the cornea reflex, and a forceful response by any body part to a tail clamp were assessed every 30 s during the first 15 min after the start of the infusion and every 5 min thereafter. As preliminary experiments showed that, in some rats, the startle reflex already disappeared following the induction of hypovolemia, this reflex was also assessed every minute after the induction of hypovolemia. The time of the disappearance and the return of the reflexes were recorded.

At 330 min after the start of the infusion, an arterial blood sample was drawn for measurement of hematocrit, blood gases, osmolarity, sodium, and total protein concentrations. In order to assess the maximal attainable electroencephalographic effect, all rats received at 360 min after the start of the first infusion of GHB, a second infusion of 90 $mg \cdot kg^{-1} \cdot min^{-1}$, until a period of electrical electroencephalographic suppression lasting 1 s or longer was seen. Analysis of previous experiments has shown that the E_{max} of the electroencephalographic parameter corresponds in the raw electroencephalogram to a burst-suppression pattern with the isoelectric period lasting 1 s or longer and that the effect of the second infusion is independent of the effect of the first infusion.[13]

Blood pressure and heart rate were recorded throughout the experiment.

Drug Assay

The GHB concentration was determined in rat plasma (20 μl) by a validated high-pressure liquid chromatography method.[14] As the GHB protein binding is less than 1%, total concentrations were measured instead of free concentration.[15] The calibration curve ranged from 10 to 750 μg/ml GHB. Quality control samples at low (20 μg/ml), medium (300 μg/ml), and high (700 μg/ml) concentrations were analyzed in duplicate together with the samples. For each quality control sample, the coefficient of variation was less than 15% (n = 14) and the accuracy was between 96 and 107% (n = 14). The lower limit of quantitation was 10 μg/ml.

Analysis of Data

The pharmacokinetics and pharmacodynamics of GHB were quantified as described previously.[13] In brief, a two-compartmental model with Michaelis–Menten elimination kinetics with a weight factor of y^{-2} was fitted to the plasma concentration–time profiles for each individual rat using Winnonlin version 1.5 (Pharsight Corporation, Mountain View, CA):

$$\frac{dC_1}{dt} = \frac{R}{V_C} - \frac{Cl_d \cdot C_1}{V_C} + \frac{Cl_d \cdot C_2}{V_T} - \frac{V_{max} \cdot C_1}{(K_m + C_1) \cdot V_C}$$

where dC_1/dt is the rate of decrease of drug concentration over time t, V_C is the distribution volume of the central compartment, V_T is the distribution volume of the peripheral compartment, R is the infusion rate, Cl_d is the intercompartmental clearance, C_1 is the concentration in the central compartment, C_2 is the concentration

Table 1. Effect of Hypovolemia on Some Physiologic Characteristics in Rats Treated With γ-Hydroxybutyric Acid or an Equimolar Solution of Sodium Chloride

	GHB		NaCl	
	Hypovolemia (n = 7)	Control (n = 7)	Hypovolemia (n = 7)	Control (n = 8)
pH	7.51 ± 0.04*	7.52 ± 0.02*	7.47 ± 0.02	7.46 ± 0.02
$Paco_2$ (mmHg)	32.2 ± 4.8	32.1 ± 4.3	29.9 ± 3.7	29.5 ± 3.0
HCO_3^- (mM)	25.9 ± 4.0*	26.0 ± 2.8*	21.5 ± 2.3	21.5 ± 2.5
Base excess (mM)	3.6 ± 3.9*	4.1 ± 2.1*	−1.2 ± 1.6	−0.7 ± 2.3
Hematocrit (%)	23 ± 1†	33 ± 3	22 ± 3†	34 ± 6
Total plasma protein (g/100 ml)	4.04 ± 0.27†	4.81 ± 0.25	3.91 ± 0.33†	4.61 ± 0.20
Osmolarity (mOsm/kg)	313 ± 9†	303 ± 5	319 ± 4†	304 ± 9
Plasma sodium (mEq/l)	146 ± 2	146 ± 2	147 ± 1	146 ± 1

Results are expressed as mean ± SD.

* $P < 0.05$, γ-hydroxybutyric acid compared with sodium chloride. †$P < 0.05$, hypovolemia compared with the control group; one-way analysis of variance, followed by Newman–Keuls test if appropriate.

GHB = γ-hydroxybutyric acid (390 mg · kg^{-1} · 5 min^{-1}); NaCl = sodium chloride; $Paco_2$ = arterial carbon dioxide tension; HCO_3^- = hydrogen carbonate.

in the peripheral compartment, V_{max} is the theoretical maximum rate of the elimination, and K_M is the Michaelis–Menten constant.

The volume of distribution at steady state (V_{SS}) was defined as

$$V_{SS} = V_C + V_T$$

This was the best fitting model based on the Akaike information criterion,[16] the visual inspection of the curve and the residual plots.[17] Using these estimated pharmacokinetic parameters, plasma concentration-time curves were constructed from time 0 to a common final time point of 420 min, because the time of the last measurable sampling point varied and at 420 min, all extrapolated concentrations were less than the detection limit for GHB. The area under the curve (AUC) from time 0 to 420 min was then calculated using the trapezoidal rule (Kinetica 2000, Innaphase Co., Philadelphia, PA).

The electroencephalographic effect of GHB was continuously recorded in each individual rat and the amplitude per second parameter of the 15.5–30 Hz band was calculated with aperiodic analysis.[18] As previously described, this parameter is suitable for the PK/PD modeling of GHB in the rat.[13] The electroencephalographic effects were linked to corresponding GHB plasma concentrations using the pharmacokinetic parameters of each individual rat. Hysteresis in the electroencephalographic effect *versus* the plasma concentration relation was minimized in a parametric way and the effect-site concentration of GHB was calculated by the following link model[19,20]:

$$\frac{dC_e}{dt} = k_{e,0}C_1 - k_{e,0}C_e$$

where C_e is the effect site concentration, k_{e0} is the first-order rate constant for the distribution from the central compartment to the effect site, and C_1 is the concentration in the central compartment. The $T1/2k_{e0}$ is the equilibration half-life for distribution between the central compartment and the effect site.

A sigmoid inhibitory E_{max} model was used to describe the relation between the effect site concentration and the effect:

$$E = E_0 - \frac{E_{max} \cdot C_e^n}{EC_{50}^n + C_e^n}$$

where E_0 is the baseline effect, E_{max} is the maximal inhibition of the electroencephalographic effect measured after the GHB infusion of 90 ml · kg^{-1} · min^{-1} at 360 min, EC_{50} is the concentration required to obtain 50% depression of the baseline effect, and n is a constant expressing the slope of the concentration effect relation.

The effect site concentration of GHB at the time of disappearance and return of righting reflex were interpolated from the effect *versus* time curve and the effect *versus* effect-site concentration curve.

Statistical Analysis

The results are expressed as mean ± SD. Comparison of physiologic parameters and hemodynamic data between the four treatment groups were made using one-way analysis of variance followed by a Newman–Keuls test, if appropriate. Pharmacokinetic and pharmacodynamic parameters and the time points of the reflexes were compared between the GHB-treated groups by a Mann–Whitney U test and within groups using the Wilcoxon test (Statistica version '99, Statsoft Inc., Tulsa, OK). $P < 0.05$ was considered statistically significant.

Results

Hypovolemia caused significantly lower hematocrit and total plasma protein concentrations at the end of the experiment as shown in table 1. In the GHB-treated rats, the pH was higher than in the saline group with an

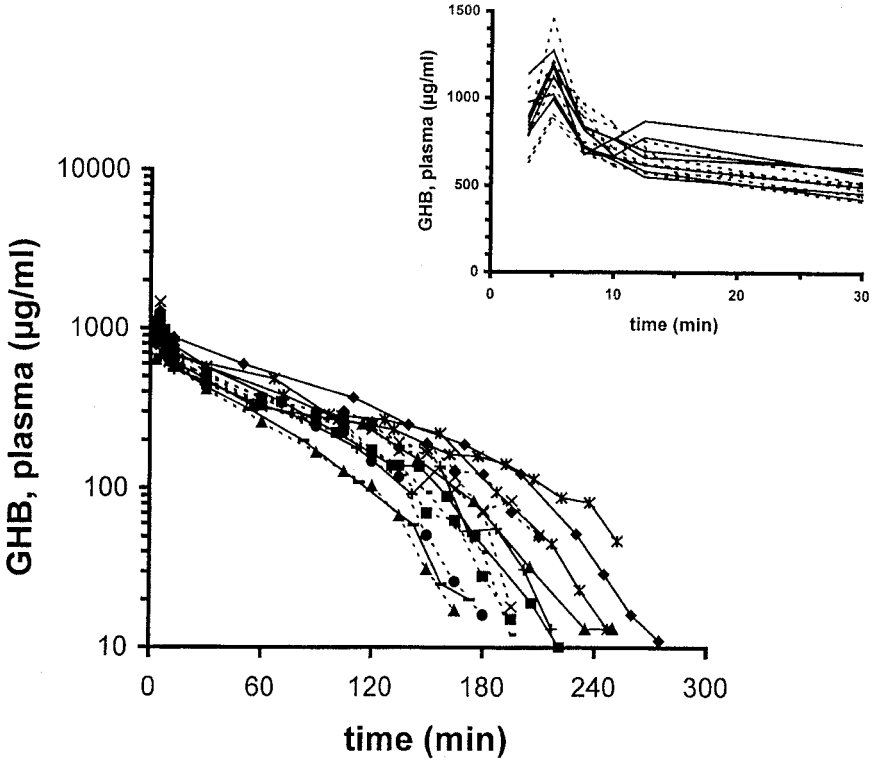

Fig. 1. Plasma concentration of γ-hydroxybutyrate (GHB) as a function of time in control (dotted lines; n = 7) and hypovolemic (solid lines; n = 7) rats during and after an intravenous infusion of γ-hydroxybutyrate (390 mg/kg during 5 min). (*Insert*) The same curves on a linear scale during the first 30 min after the start of the infusion.

elevated HCO_3^- concentration and a normal $Paco_2$, indicating metabolic alkalosis. The sodium plasma concentration at the end of the experiment was not significantly different between the four groups and the osmolarity was slightly higher in the hypovolemic than in the control rats.

The individual plasma concentration–time curves of GHB in the hypovolemic rats and the control rats are shown in figure 1. The model that best fitted to the data of each individual rat was a two-compartment model with Michaelis–Menten elimination kinetics, and the resulting pharmacokinetic parameters are given in table 2. Between the hypovolemic and the control group, there was no significant difference in V_{max}, K_M, V_T,V_{SS}, and the AUC_{0-420}, but a small statistically significant difference in V_c and Cl_d was observed between both groups.

The time course of the amplitude per second in the 15.5–30 Hz frequency band of the electroencephalogram after the first infusion (390 mg · kg^{-1} · 5 min^{-1}) and the second infusion (90 mg · kg^{-1} · min^{-1}) of GHB, given at the end of the experiment to estimate the maximal attainable electroencephalographic effect (E_{max}), is shown for both groups in figure 2. There was no significant difference between hypovolemic and control rats, neither for the E_{max} (table 3) nor for the time needed to reach the E_{max} (8.2 ± 0.8 min in hypovolemic and 9.2 ± 0.8 min in control rats, $P = 0.65$). There was also no significant difference between the two groups for the electroencephalographic depression reached after the first infusion of 390 mg/kg GHB during 5 min (E_1 = 447 ± 25 μV/s in hypovolemic and 499 ± 47 μV/s in control rats, $P = 0.48$), and for both groups, the effect reached by this first infusion was not significantly smaller than the maximal attainable effect E_{max} ($P = 0.2$ in both groups). The total amplitude per second in the 15.5–30 Hz frequency band of the electroencephalogram was neither influenced by the induction of hypovolemia nor by the administration of the equimolar solution of sodium chloride. Furthermore, this electroencephalographic parameter remained stable in the sodium chloride–treated rats for the duration of the experiment.

The electroencephalographic amplitude *versus* plasma concentration curve after the first infusion showed hys-

Table 2. Pharmacokinetic Parameters after Intravenous Infusion of γ-Hydroxybutyric Acid in Hypovolemic and Control Groups

	GHB	
	Hypovolemia (n = 7)	Control (n = 7)
V_{max} (mg · min^{-1} · kg^{-1})	2.55 ± 0.50	2.52 ± 0.45
K_M (μg/ml)	89 ± 40	60 ± 55
V_C (ml/kg)	180 ± 61*	248 ± 48
V_T (ml/kg)	437 ± 50	415 ± 46
V_{SS} (ml/kg)	617 ± 87	672 ± 73
Cl_d (ml · min^{-1} · kg^{-1})	61 ± 22*	39 ± 10
AUC_{0-420} (mg · min^{-1} · ml^{-1})	64 ± 17	55 ± 9

Results are expressed as mean ± SD.

* $P < 0.05$, Mann–Whitney U test.

GHB = γ-hydroxybutyric acid (390 mg · kg^{-1} · 5 min^{-1}); V_{max} = theoretical maximum rate of the elimination; K_M = Michaelis–Menten constant; V_C = volume of distribution of the central compartment; V_T = volume of distribution of the peripheral compartment; V_{SS} = total volume at steady-state; Cl_d = intercompartmental clearance; AUC_{0-420} = area under the curve extrapolated from time 0 to 420 min.

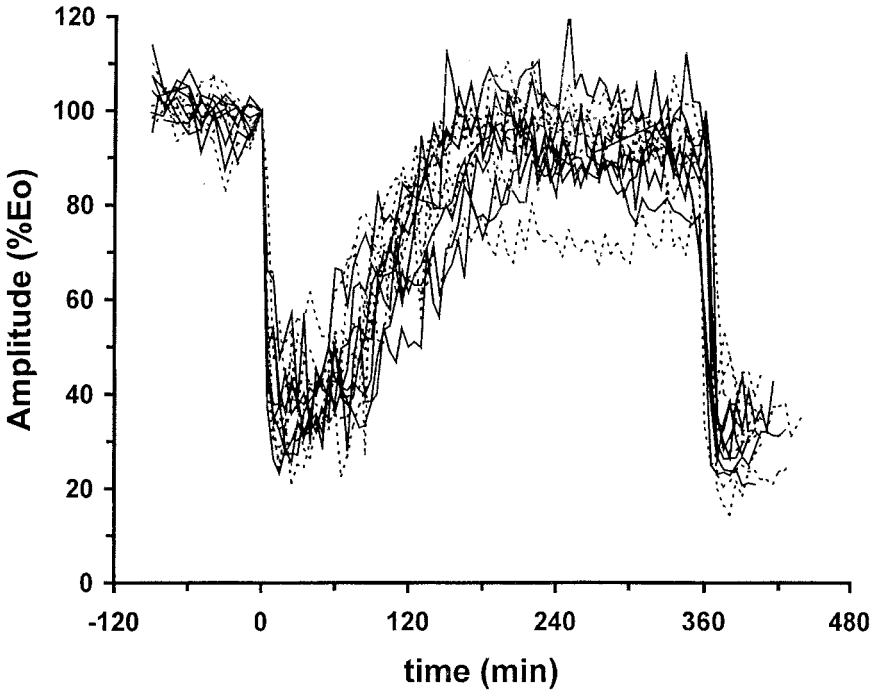

Fig. 2. Individual time course of the electroencephalographic amplitude in the 15.5–30 Hz frequency band, expressed as percentage change of baseline activity (E_0), after intravenous infusion of γ-hydroxybutyrate (GHB) (390 mg/kg during 5 min) in individual hypovolemic (solid lines; n = 7) and control rats (dotted lines; n = 7). The infusion started at time = 0 and a second infusion of γ-hydroxybutyrate (90 mg · kg^{-1} · min^{-1} until 1 s of isoelectric electroencephalography) started at time 360 min.

teresis. Using a link model, apparent effect site concentrations were calculated. An inhibitory E_{max} model was fitted to the electroencephalographic effect *versus* GHB effect site concentrations, and individual curves are shown in figure 3. Pharmacodynamic parameters were derived from these curves and are shown in table 3. No significant difference in the EC_{50}, E_0, E_{max}, n, k_{e0}, and $T1/2k_{e0}$ was observed between the hypovolemic and the control rats.

With regard to the reflexes, the cornea reflex, and the reaction to a tail clamp could not be reliably assessed, as only a minority of the rats lost these reflexes after GHB. The startle reflex could also not be compared between the hypovolemic and the control groups since hypovolemia by itself already induced a loss of this reflex in three animals. With regard to the time of loss and return of the righting reflex, no significant differences were observed (9 ± 3 min *vs.* 10 ± 4 min at loss and 60 ± 10 min *vs.* 47 ± 22 min at return for the hypovolemic and the control rats, respectively). Neither was there a difference in the GHB effect site concentration at loss and return of righting reflex (412 ± 101 μg/ml *vs.* 429 ± 87 μg/ml at loss and 378 ± 88 μg/ml *vs.* 395 ± 67 μg/ml at return for the hypovolemic and the control rats, respectively).

Table 3. Electroencephalographic Parameters after Intravenous Infusion of γ-Hydroxybutyric Acid for the Hypovolemic and Control Groups

	GHB	
	Hypovolemia (n = 7)	Control (n = 7)
E_0 (μV/sec)	583 ± 67	650 ± 138
E_{max} (μV/sec)	460 ± 64	525 ± 154
EC_{50} (μg/ml)	322 ± 74	335 ± 76
n	2.66 ± 0.8	2.51 ± 0.56
k_{e0} (min^{-1})	0.10 ± 0.02	0.11 ± 0.04
$T^{1/2}_{ke0}$ (min)	7.19 ± 1.46	6.74 ± 1.96

Results are expressed as mean ± SD.

GHB = γ-hydroxybutyric acid (390 mg · kg^{-1} · 5 min^{-1}); E_0 = baseline value of the electroencephalographic parameter; E_{max} = maximal attainable depression, measured after the second infusion of 90 mg · kg^{-1} · min^{-1} until 1 s of isoelectric electroencephalogram; EC_{50} = GHB effect site concentration when 50% of maximum is reached; n = shape factor; k_{e0} = first-order rate constant; $t^{1/2}$ k_{e0} = half-life of the distribution from the central compartment to the effect site.

The mean arterial blood pressure at different time points is shown in table 4 for the four groups. The induction of hypovolemia caused a significant decrease in blood pressure, with partial recuperation at the time of the start of the GHB infusion. The GHB induced an increase in blood pressure, and the maximum reached was significantly higher than with the saline infusion in both hypovolemic and control rats. The time until the blood pressure returned to the preinfusion values was significantly longer in the GHB-treated rats. Only a transient decrease in heart rate was observed during the first minutes of the GHB infusion.

Discussion

The present study investigates the influence of hypovolemia on the pharmacokinetics and the electroencephalographic effect of GHB in a rat model because, in contrast with propofol, it has other pharmacokinetic characteristics and increases blood pressure.

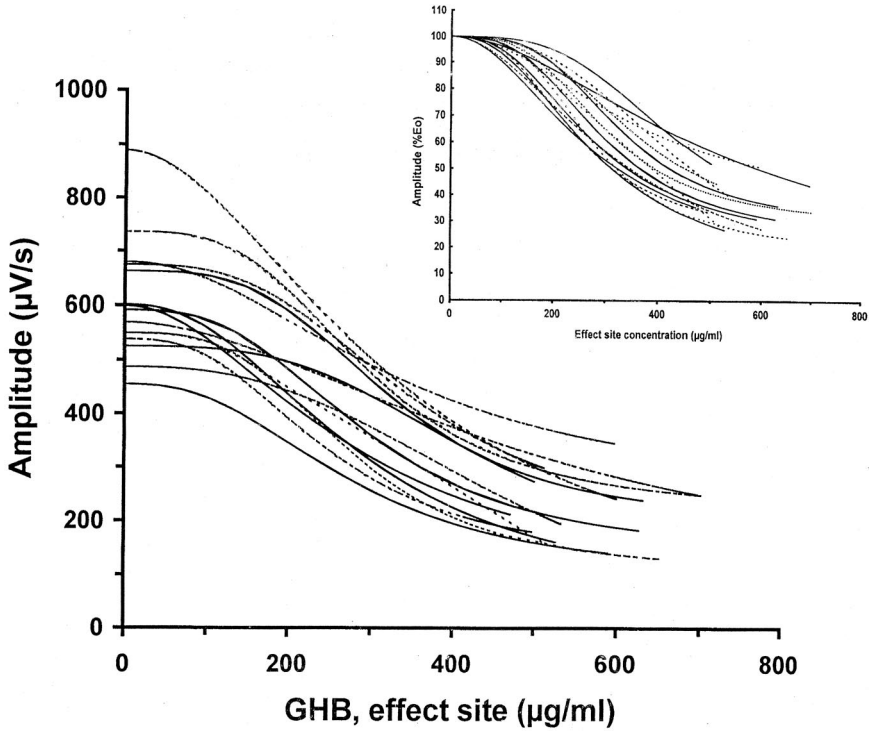

Fig. 3. Electroencephalographic effect in the 15.5–30 Hz frequency band *versus* γ-hydroxybutyrate effect-site concentration of individual control (dotted lines, n = 7) and hypovolemic (full lines, n = 7) rats after an intravenous infusion of γ-hydroxybutyrate (390 mg/kg during 5 min). A sigmoid E_{max} model was fitted to the data. (*Insert*) Same relation expressing the effect as a percentage of the E_0 (100%).

Moderate hypovolemia was induced in unanesthetized rats by removing 30% of the blood volume.[21] As previously described, this induces a decrease in blood pressure, which partially recuperates before the start of the infusion and is accompanied by a decrease in hematocrit and total protein concentrations at the end of the experiment.[11] In contrast with experiments in which etomidate and propofol were studied during hypovolemia,[6,7] metabolic alkalosis was observed. Metabolic alkalosis was also described during the sedation of patients in the intensive care unit with the sodium salt of GHB[10] and has been ascribed to the metabolism of GHB in the Krebs cycle during which two acid ions per metabolized GHB molecule are necessary.[22]

The GHB induced an increase in blood pressure in both control and hypovolemic rats, as already described by others.[8,23] In our experiments, the increase in blood pressure was sustained and remained higher than the preinfusion level for more than 2 h. This increase contrasts with previous experiments in which the anesthetics etomidate and propofol consistently caused a further decrease in blood pressure in hypovolemic animals.[6,7] The increase in blood pressure cannot solely be ascribed to the sodium component of sodium-GHB, as it was significantly higher and longer lasting than the increase induced by an equimolar solution of sodium chloride. This equimolar solution of sodium chloride (NaCl 6.9%) is comparable to the 7.5% hypertonic saline solutions used in experimental trials for the small-volume hypertonic resuscitation of trauma patients.[24] The mechanism underlying the increase in blood pressure is unclear. A sustained increase in central neurologic sympathetic activity has been proposed.[23] Others have observed *in vitro* an increase in norepinephrine release from the

Table 4. Mean Arterial Pressure and Time for Return to Baseline in Control and Hypovolemic Rats after Intravenous Infusion of γ-Hydroxybutyric Acid or Equimolar Solution of Sodium Chloride

	GHB		NaCl	
	Hypovolemia (n = 7)	Control (n = 7)	Hypovolemia (n = 7)	Control (n = 8)
Before hypovolemia	113 ± 10	121 ± 11	121 ± 13	116 ± 8
Immediately after induction of hypovolemia	68 ± 24*	120 ± 11	79 ± 16*	113 ± 12
At start of infusion	91 ± 10*	117 ± 11	98 ± 11*	109 ± 7
Maximum blood pressure (mm Hg)	138 ± 13*†	158 ± 9†	110 ± 12	120 ± 14
Time for return to preinfusion level (min)	160 ± 58†	147 ± 57†	36 ± 22	21 ± 18

Results are expressed as mean ± SD.

*$P < 0.05$, compared with the control group and †$P < 0.05$, comparison between rats treated with GHB and rats treated with NaCl; one-way analysis of variance for repeated measures.

GHB = γ-hydroxybutyric acid (390 mg · kg^{-1} · 5 min^{-1}); NaCl = sodium chloride.

heart and the brown adipose tissue following GHB administration, suggesting a short-time depleting effect on heart and adipose tissue catecholamines.[25] Boyd *et al.*[8] found no increase in total peripheral resistance and hypothesized that this increase was due to a combination of an increase in venous return, an increase in cardiac contractility, and the hypertonicity of the solution.

The pharmacokinetics of GHB were studied using a dose provoking the maximal attainable electroencephalographic depression. The dose needed in the present experiments was slightly higher than in a previous study,[13] which is probably due to the use of a different substrain of Wistar rats (Wistar–Hannover *vs.* Wistar–Wistar, previously). A difference between rat strains and substrains in brain sensitivity to other drugs like apomorphine, ethanol, barbital, and pentobarbital has been reported.[26,27] In this context, the difference in sensitivity to GHB and baclofen of rats within one strain was used to selectively breed two rat lines differing in sensitivity.[28]

A two-compartment model with Michaelis–Menten elimination kinetics was fitted to the GHB plasma concentration–time data. The nonlinear elimination phase, corresponding with the concave phase following the distribution phase (fig. 1), is due to saturation of the GHB metabolism when relatively high doses are used. The volume of the central compartment (V_C) was smaller and the intercompartmental clearance (Cl_d) was greater in the hypovolemic rats. The reduction in V_C can be explained by the hypovolemic state and was also observed for propofol[6] and fentanyl.[4] The increase observed in intercompartmental clearance contrasts with the results of Egan *et al.*[4] who showed for fentanyl a decrease in intercompartmental clearance during hypovolemia. These authors attributed this to the fact that a decrease in cardiac output induces a decrease in intercompartmental clearance. Indeed, a direct relation between the cardiac output and the intercompartmental clearance of small or lipophilic substances like alfentanil has been shown.[29,30] It is tempting to speculate that the increase in intercompartmental clearance observed with GHB in our experiments may be explained by an increase in cardiac output since Boyd *et al.*[8] showed that the administration of GHB to hypovolemic rats induces threefold increases in cardiac output compared to GHB treated normovolemic controls. It should however be emphasized that we based the estimates of the V_C and Cl_d on a small number of sampling points, in contrast with others who sampled mainly during the first minutes after the start of the infusion,[31] and that we did not measure cardiac output. Furthermore, also keep in mind that the observed changes in V_C and Cl_d have only a small impact on the time course of the GHB concentration (fig. 1) and therefore may have only little influence on the hypnotic effect of GHB in hypovolemic rats. In this context, it is interesting to note that Johnson *et al.* also showed for remifentanil in an isobaric hemorrhage model in the pig that the reduction of the V_C and the intercompartmental clearance by 50% has only minimal impact on the overall pharmacokinetic curve.[32]

The steady state volume of distribution (V_{SS}) of GHB did not change during hypovolemia, which contrasts with our previous results obtained with propofol. This may be explained by the increase in blood pressure induced by GHB in the hypovolemic rats, which may counteract the effects of hemorrhagic hypovolemia. However, another explanation may be that the volume of distribution of GHB is much smaller than for propofol. It has indeed been reported that hypovolemia does not change the volume of distribution of drugs like inulin and antipyrine, which also have a small volume of distribution.[33] For indocyanine green, another substance with a small volume of distribution and high plasma binding, only a minor decrease has been described.[33] In contrast, hypovolemia produces a marked decrease for drugs with a large volume of distribution, like morphine,[34] etomidate,[7] and lidocaine.[2]

No significant differences were observed in the metabolism (V_{max} and K_M) of GHB between the hypovolemic and the control rats, which contrasts with the marked decrease in clearance observed for propofol.[6] This discrepancy may be due to the previously mentioned difference in blood pressure, but also to the fact that the extraction ratio of GHB is lower than for propofol. Indeed, using the values for V_{max} and K_M obtained in our experiments and assuming a liver plasma flow[35] in the rat of 30 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}, the extraction ratio during the linear elimination phase is 0.47, which is intermediate. Moreover, this extraction ratio will be even lower during the nonlinear phase and will change continuously because it depends on the plasma concentration.[36] It is conceivable that this lower extraction ratio will render GHB less susceptible than propofol and other high extraction drugs (like morphine,[37] lidocaine,[2] and fentanyl[4]) to a decrease in clearance when liver blood flow is reduced during hypovolemia.[38]

The rather large variability in the estimation of the K_M can be explained by the K_M value lying near the lower limit of quantitation, as a result of which the precision of the estimated K_M value depends on the sampling times. Indeed, when the concentration decreases very rapidly at the end of the concentration–time curve, it is possible that between two sampling points the concentration is already beyond the concentration corresponding to the K_M and the lower limit of quantitation. As can be derived from figure 1, this was the case in two control rats and one hypovolemic rat.

A possible influence of hypovolemia on brain sensitivity to the hypnotic effect of GHB was investigated by means of testing reflexes and the analysis of the changes observed in the electroencephalogram.[13,39] The righting reflex, in contrast to the other reflexes, proved to be a valuable tool since there was no significant difference between the GHB effect site concentration at loss and

return of righting reflex within each rat, which pleads against the possible occurrence of acute tolerance during the experiment.[40] Moreover, the effect site concentration at loss and return of righting reflex of about 400 μg/ml was slightly higher than the EC_{50}, which suggests that the electroencephalographic parameter used might be a valid surrogate measure for the hypnotic effect of GHB.[41] The times at the loss and return of the righting reflex and the corresponding effect site concentration were not significantly different between the hypovolemic and the control group, which pleads against a difference in end organ sensitivity.

Although only a minority of the rats lost the cornea and tail clamp reflex, which are associated with deep hypnosis, we are confident that the maximal electroencephalographic effect was reached by the first infusion of GHB because for both groups this effect was not significantly smaller than the maximal attainable effect, E_{max}, reached by the second infusion ($P = 0.2$ in both groups). This implies that the electroencephalographic parameter might be a surrogate parameter for the sedative effect rather than for the hypnotic effect of GHB.[12]

Plotting the electroencephalographic parameter *versus* GHB plasma concentration showed hysteresis. After the minimization of this hysteresis, no difference in k_{e0} was observed,[20] which suggests that the distribution between central compartment and effect site was not influenced by the induction of the hypovolemia. This was to be expected since the GHB induced hypertension does not induce a change in cerebral blood flow[23] and the blood pressure remained above the level of cerebral blood flow autoregulation.[42] Also, during hypovolemia with etomidate[7] and propofol,[6] no changes in the k_{e0} were observed. Pharmacodynamic parameters (E_0, EC_{50}, and n) were obtained after minimization of the hysteresis, and no differences were observed, which is in contrast with the increased sensitivity to the hypnotic effect of propofol observed in hypovolemic rats.[6]

It is concluded that the induction of hypovolemia in the rat does not influence the overall pharmacokinetics or the pharmacodynamics of GHB. This contrasts with our previous findings with etomidate and propofol, and this might be explained not only by the increase in blood pressure induced by the infusion of GHB, but also by the different pharmacokinetic properties of GHB. These results may be of interest in the context of the recent use of GHB as a sedative drug in the intensive care unit.

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