Relationship Between Etomidate Plasma Concentration and EEG Effect in the Rat

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Purpose. The effect-plasma concentration relationship of etomidate was studied in the rat using electroencephalographic changes as a pharmacodynamic parameter.

Methods. Etomidate was infused (50 mg/kg/h) in chronically instrumented rats (n = 6) until isoelectric periods of 5 s or longer were observed in the electroencephalogram (EEG). The EEG was continuously recorded during the experiment and frequent arterial blood samples were taken for determination of etomidate plasma concentrations. The changes observed in the raw EEG signal were quantified using aperiodic analysis in the 2.5–7.5 Hz frequency band. The return of the righting reflex was used as another parameter of anesthesia.

Results. A mean dose of 8.58 ± 0.41 mg/kg needed to be infused to reach the end point of 5 s isoelectric EEG. The plasma concentration time profiles were most adequately fitted using a three-exponential model. Systemic clearance, volume of distribution at steady-state and elimination half-life averaged 93 ± 6 ml/min/kg, 4.03 ± 0.24 l/kg and 59.4 ± 10.7 min respectively. The EEG effect-plasma concentration relationship was biphasic exhibiting profound hysteresis. Semi-parametric minimization of this hysteresis revealed an equilibration half-life of 2.65 ± 0.15 min, and the biphasic effect-concentration relationship was characterized nonparametrically by descriptors. The effect-site concentration at the return of the righting reflex was 0.44 ± 0.03 μg/ml.

Conclusions. The results of the present study show that the concentration-effect relationship of etomidate can be characterized in individual rats using aperiodic analysis in the 2.5–7.5 Hz frequency band of the EEG. This characterization can be very useful for studying the influence of diseases on the pharmacodynamics of etomidate *in vivo*.

KEY WORDS: etomidate; pharmacokinetics; pharmacodynamics; rat; electroencephalogram.

INTRODUCTION

Etomidate is frequently used in hemodynamically compromised patients for induction of anesthesia, as it is claimed to have an interesting cardiovascular profile. Etomidate anesthesia causes relatively minor changes in arterial blood pressure and cardiac output (1–3), which is explained by preservation of both sympathetic outflow and autonomic reflexes (1). Due to pathophysiological changes induced by shock, one may expect alterations in the hypnotic effect of etomidate in this group of patients. It would be almost unpracticable to study this in a clinical setting, and therefore we intend to study etomidate in a shock model in the rat. However, no data are available on the concentration-effect relationship of etomidate in the intact rat. In the present study, therefore, we decided to characterize the pharmacokinetic/pharmacodynamic correlation of etomidate in an intact rat model using the electroencephalogram (EEG). The loss of the righting reflex was used as a clinically more relevant effect parameter of the hypnotic effect of etomidate. However, as this only provides a single pharmacodynamic endpoint, the EEG was used allowing a continuous measure of drug effect. Etomidate was infused and the EEG changes were registered and quantified thus allowing characterization of the pharmacokinetic/pharmacodynamic relationship of etomidate.

MATERIALS AND METHODS

Animal Instrumentation

The study protocol was approved by the Ethics Committee for animals of the Medical School.

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

One week before the experiment, epidural EEG electrodes were implanted (4). During atraumatic fixation in a stereotaxic device, seven holes were drilled into the skull without penetration of the dura, at the locations 11 mm anterior and 2.5 mm lateral (F_l and F_r), 3 mm anterior and 3.5 mm lateral (C_l and C_r), and 3 mm posterior and 2.5 mm lateral (O_l and O_r) 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 heparine solution (100 IU/ml) were inserted into the femoral artery and vein through a small incision in the groin also under pentobarbital anesthesia. The catheters were threaded under the skin of the back and exteriorized at the nape of the neck. Interference from the pentobarbital anesthesia with the EEG recordings on the day of the experiment is highly unlikely in view of the short elimination half-life of pentobarbital (37 min) (5).

In order 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 and data were saved on a hard disk using a hemodynamic data acquisition software system (HDAS, University of Maastricht, the Netherlands). Heart rate was directly derived from the pulse signal.

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 digital EEG recorder (Telefactor®) at a sampling rate of 200 Hz. The high-pass and low-pass filter cutoff frequencies were set at 1 Hz and 70 Hz, respectively.

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The return of the righting reflex was used as another parameter of depth of anesthesia. The loss of righting reflex was not determined since it occurs so rapidly that it would require frequent manipulation of the animals, which would interfere with the registration of the EEG.

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. After 20 minutes of baseline hemodynamic and EEG recording, the animals received an intravenous infusion of etomidate, dissolved in propylene glycol (Hypnomidate®, Janssen Pharmaceutica, Beerse, Belgium). Etomidate was given at a rate of 50 mg/kg/h. The infusion was terminated when burst suppression with isoelectric periods of 5 s or longer was observed in the EEG. 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 amount 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. The animals were subsequently euthanized by an overdose of pentobarbital.

Control animals underwent the same experimental protocol with infusion of the vehicle, propylene glycol.

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 *et al.* (6). Detection was performed by UV at 240 nm. Coefficient of variation for the determination of etomidate at concentrations of 400, 800, and 4000 ng/ml ranged from 4.6% to 11.7%; overall accuracy (analytical recovery) ranged from 86.7% to 113.1% (n = 10 for each concentration, 5 assays). The lower limit of quantitation 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 hours at 37°C as described previously (7). Two hundred µl of plasma spiked with 1 µg/ml etomidate and adjusted to pH 7.4, were dialysed against 200 µl phosphate buffer (0.15 M, pH 7.4). After dialysis etomidate concentration was determined in 100 µl aliquots of dialysate as described above.

Analysis of Data

The pharmacokinetics and pharmacodynamics of etomidate were quantified for each individual rat. The plasma concentration time profiles during and after infusion were described by a poly-exponential equation using WinNonlin version 1.5 (Scientific Consulting, Inc.). 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 (8). Calculation of the pharmacokinetic parameters was done according to Gibaldi and Perrier (9).

Etomidate drug effect was assessed from the EEG signal processed by aperiodic analysis (10). 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 (TNW) and the amplitude per second (AMP), the two basic parameters derived from aperiodic EEG analysis, can be calculated in several freely selectable frequency bands (4). The amplitude per second from 2.5 Hz to 7.5 Hz in the left fronto-occipital lead was used as measure of the effect of etomidate as etomidate produced the most robust effect in this frequency range compared to the other EEG frequency bands. The EEG data were averaged over predetermined intervals. The interval duration (10 s–2 min) depended on the rate of change of the signals.

Hysteresis in the EEG effect versus plasma concentration curve was minimized by a semi-parametric approach using a FORTRAN written program (11) to reveal the apparent effect-site concentration-effect relationship and for estimation of the first order-rate equilibration constant k_{e0}. Etomidate plasma concentrations were calculated based on the compartmental model obtained from each individual rat.

After hysteresis minimization, without invoking a pharmacodynamic model, the EEG effect versus effect-site concentration curve was characterized nonparametrically with the use of descriptors (12). The descriptors used are: the baseline effect (E_0), the maximal activation of the EEG effect (E_{max}), the concentration required to produce the maximal activation (EC_m), the concentration required to obtain 50% activation of the EEG effect (EC_{50}) and the concentration required to produce the baseline effect between maximal activation and maximal inhibition (EC_b). E_0, E_{max}, and EC_m were directly obtained from the data and EC_{50} and EC_b were derived by linear interpolation between the two closest points. The effect-site concentration corresponding with the start of 5 s EEG suppression (C_{5s}) and with the return of righting reflex ($C_{wake\text{-}up}$) were also derived by linear interpolation. The results are expressed as means ± s.e. mean.

RESULTS

In six animals, a mean dose of 8.58 ± 0.41 mg/kg etomidate was needed to be infused to reach the end point of five seconds isoelectric EEG. This corresponded with a mean infusion duration of 10.3 ± 0.5 minutes. All animals fell asleep with a loss of righting reflex within the first minutes after the start of the infusion.

Mean arterial blood pressure decreased during etomidate infusion from a mean baseline value of 116 ± 3 mmHg to a minimum of 93 ± 5 mmHg near the end of the infusion. Blood pressure then gradually returned to baseline values within a mean interval of 24.9 ± 0.3 min. Heart rate decreased from 410 ± 24 beats per minute (bpm) to 356 ± 16 bpm at the end of the infusion, and recuperated simultaneously with the blood pressure. Body temperature decreased from 37.7 ± 0.1°C at the beginning to 36.1 ± 0.3°C at the end of the experiment.

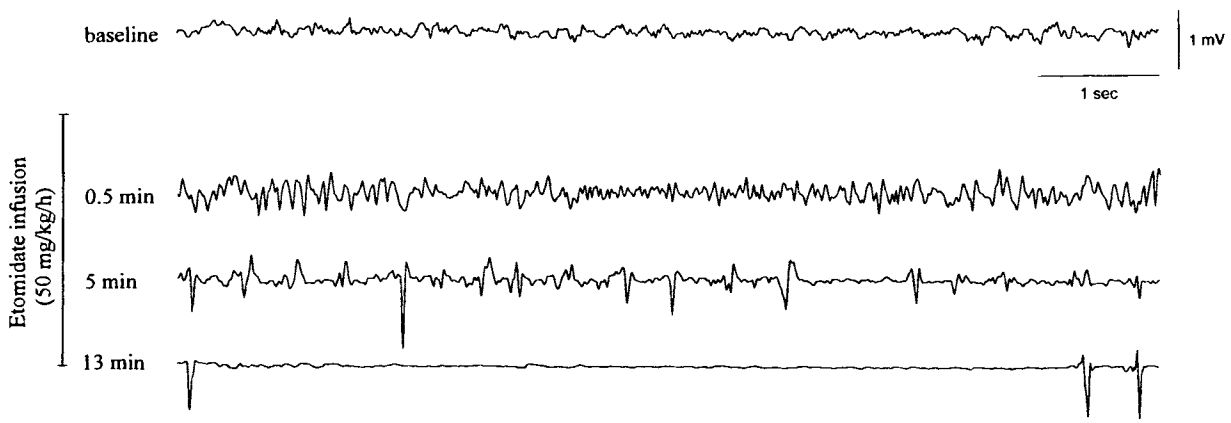

Fig. 1. EEG changes in the left fronto-occipital lead before and after infusion of etomidate in a typical rat. The infusion is stopped when an isoelectric EEG period of five seconds or more occurs (13 min).

Hematocrit, blood gases, and protein concentration were all within the physiological range at the end of the experiment.

The changes in EEG morphology during etomidate infusion in a typical rat are shown in Fig. 1. The baseline EEG in the awake rat was characterized by low-voltage high-frequency activity. Shortly after the start of the infusion (0.5 min), an increase in amplitude and a decrease in frequency were observed. Five minutes after the start of the infusion, bursts of EEG occurred between periods of electrical suppression, i.e. burst-suppression.

Fig. 2 shows the time course of the plasma concentration of etomidate and the EEG amplitude for a typical rat during and after the 10 min infusion of etomidate. Plasma concentrations rose rapidly during infusion and reached a maximum by the end of the infusion, followed by a rapid exponential decline. The plasma concentration time profile was most adequately fitted using a three-exponential model. The calculated pharmacokinetic parameters for all animals are shown in Table 1. The unbound fraction of etomidate in plasma was 19.1 ± 1.1%. Preliminary experiments showed that protein binding remained stable during the experiment (data not shown).

The EEG effect was characterized by an increase in the amplitude per second (AMP) at the beginning of the infusion reaching a peak activity. This increase was followed by a decrease in amplitude reaching a minimum shortly after the end of the infusion. A second activation phase subsequently occurred which was followed by a return to baseline values. The animal regained consciousness with a return of righting reflex shortly after the second activation phase.

In control animals (n = 3) the EEG effect versus time profile remained stable during and after the infusion of the solvent propylene glycol (data not shown). When the EEG amplitude was plotted against the plasma concentrations of etomidate, an "eight shaped" figure was revealed as shown for a typical rat in Fig. 3A. The observed hysteresis was minimized by estimating k_{e0} using the hysteresis minimization program. This resulted in a biphasic effect-site concentration-EEG effect relationship of etomidate (Fig. 3B). Concentration-effect profiles were similar in all six animals and were characterized by nonparametric descriptors. The pharmacodynamic parameter estimates of etomidate are given in Table 2.

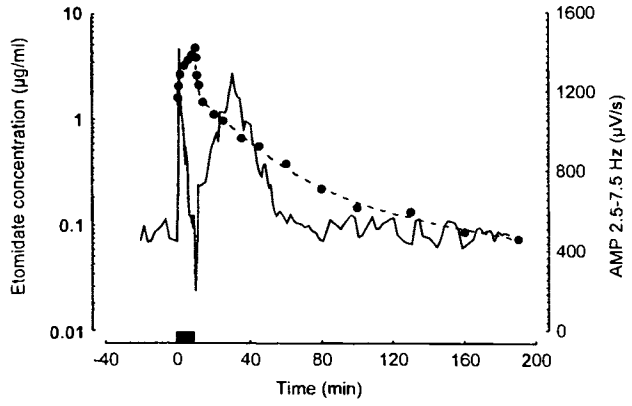

Fig. 2. The plasma concentration of etomidate (•) and the EEG effect expressed as amplitude per second in the 2.5–7.5 Hz frequency band (—) over time for a typical rat during and after etomidate infusion (represented by the filled bar). The dashed line constitutes the best fit to the concentration data.

DISCUSSION

In the present study, the effect versus concentration relationship of etomidate in the rat was characterized using the EEG changes as the pharmacodynamic effect.

During etomidate infusion, blood pressure and heart rate decreased by 20% and 13%, respectively. Analogous changes in blood pressure and heart rate were observed by De Wildt *et al.* (13), in which etomidate was infused (10 mg/kg/h) in spontaneously breathing rats. In humans, data about the effect

Table 1. Pharmacokinetic Parameters of Etomidate After an Intravenous Infusion of 50 mg/kg/h Until 5 Seconds EEG Suppression

Maximal concentration (µg/ml)	4.06 ± 0.22
Systemic clearance (ml/min/kg)	93 ± 6
Volume of distribution of the central compartment (l/kg)	0.25 ± 0.02
Volume of distribution at steady state (l/kg)	4.03 ± 0.24
Mean residence time (min)	44.3 ± 4.0
Initial half-life (min)	0.5 ± 0.0
Intermediate half-life (min)	7.3 ± 2.1
Terminal half-life (min)	59.4 ± 10.7

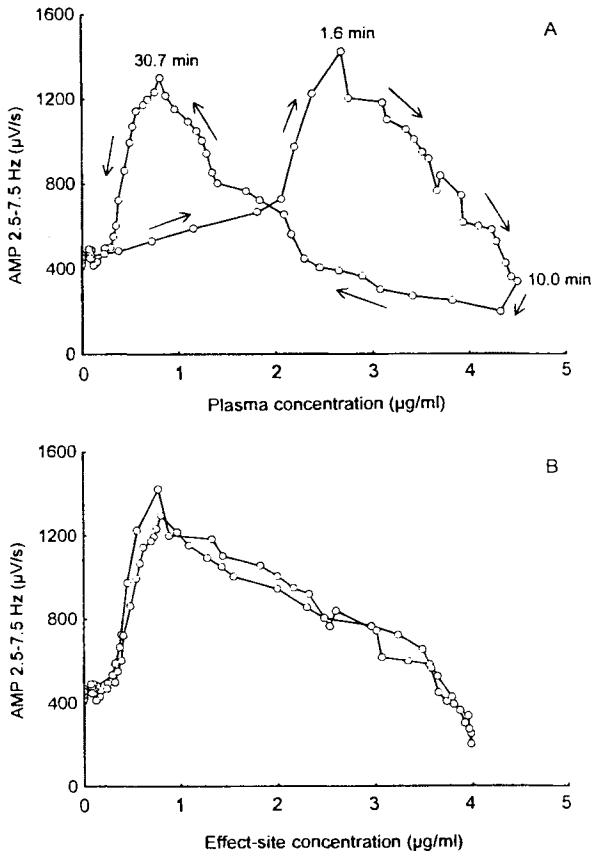

Fig. 3. (A) AMP versus etomidate plasma concentration in a typical rat. (B) AMP versus etomidate effect-site concentration after hysteresis minimization.

of etomidate on cardiovascular parameters range from hemodynamic stability (1,14,15) towards depression of heart rate and blood pressure (16). A possible explanation for this discrepancy may be the differences in the use of premedication and dosage between the studies.

Hematocrit, blood gases, protein concentration and body temperature were in the physiological range at the end of the experiment, indicating that the animals remained in good condition during the experiment. Preliminary experiments showed that during etomidate infusion, blood gases experienced only minor changes i.e. a small decrease in arterial pO_2 and a minimal increase in arterial pCO_2.

Table 2. Parmacodynamic Parameter Estimates of Etomidate After an Intravenous Infusion of 50 mg/kg/h Until 5 Seconds EEG Suppression

E_0 (μV/s)	495 ± 37
EC_{50} (μg/ml)	0.36 ± 0.05
E_{max} (μV/s)	1509 ± 105
EC_m (μg/ml)	0.78 ± 0.04
EC_h (μg/ml)	3.06 ± 0.22
$C_{wake\text{-}up}$ (μg/ml)	0.44 ± 0.03
C_{5s} (μg/ml)	3.30 ± 0.19
k_{eo} (min^{-1})	0.27 ± 0.02
$t^{1/2}_{keo}$ (min)	2.65 ± 0.15

Upon etomidate administration the EEG pattern changed from a low-voltage high-frequency pattern to high-voltage multiple slow-wave complexes in the 2.5–8 Hz frequency band, followed by patterns of burst-suppression. It has been shown for other anesthetics like thiopental that this burst-suppression corresponds with deep anesthesia (17). Similar changes of EEG pattern were seen after etomidate administration in dogs (18), whereas in humans, additional activity in the 0.5–2.5 Hz frequency band was observed (19–21). In our experiments, a change of activity in the latter frequency band could not be observed. This difference might be related to species differences.

The changes observed in the raw EEG signal were quantified using aperiodic analysis in the 2.5–7.5 Hz frequency band instead of the more frequently used Fourier analysis as the latter assumes the stationarity of the examined EEG signal and may therefore turn unstable and give erratic results during burst suppression and isoelectric periods.

The EEG parameter showed a biphasic time course during and after etomidate infusion. This biphasic EEG effect has also been described for other general anesthetics like thiopental (12), heptabarbital (4) and propofol (22–24).

With regard to the pharmacokinetics, distribution was very fast following etomidate infusion with initial and intermediate half-lives of 0.5 and 7.3 min, respectively. Elimination half-life was 59.4 min which closely corresponds with the elimination blood half-life of 61.7 min, found also in the rat by others (25). Etomidate plasma clearance was 93 ml/min/kg. Assuming a liver plasma flow of 51 ml/min/kg (26), it can be calculated that at least 45% of etomidate present in the plasma is cleared extrahepatically. This substantial extrahepatic clearance was also observed by others who found an etomidate plasma clearance of 123 ml/kg/min following intraarterial administration of etomidate to rats (25). It was indeed demonstrated that elimination of etomidate occurs by ester-hydrolysis in plasma and in the liver with approximately equal rate constants (27). The volume of distribution at steady state was 4.03 l/kg or 4 times the relative body weight. The central volume of distribution was small; 0.25 l/kg or about 6% of the total distribution volume for etomidate.

We observed that in rat plasma 80.9% of etomidate was bound to plasma proteins, which is similar to the reported value of 79.5% found by others (28). The latter study showed that the percentage of etomidate bound to serum albumine was independent of the drug concentration, within a large concentration range.

A plot of the EEG amplitude versus etomidate plasma concentrations revealed an "eight shaped" hysteresis curve (Fig. 3A). This hysteresis was collapsed using the minimization algorithm which includes a first order monoexponential equilibrium model. In order to evaluate the appropriateness of this model and the adequacy of the minimization of the hysteresis, individual EEG-effect versus effect-site concentration plots were evaluated by visual inspection and by calculating the EC's (EC_{50}, EC_m, EC_h) from the ascending and descending limb of the hysteresis curve (29). The latter analysis revealed that there was no significant and systematic difference between the values of the EC's when calculated from the ascending or descending limb of the hysteresis curve and that the difference between the EC's averaged zero. Based on these criteria, it was concluded

that the hysteresis was adequately minimized and that the first order monoexponential equilibrium model is appropriate.

Hysteresis minimization revealed a k_{e0} of 0.27 min^{-1} ($t^{1/2}_{ke0}$ = 2.65 min). This constant is a measure estimate of the equilibration delay between plasma and effect-site, provided that no other factors like acute tolerance, sensitization, active metabolites, and enantiomers affect this hysteresis. Acute tolerance and sensitization have not been documented for etomidate. Etomidate metabolites, following ester hydrolysis, are pharmacologically inactive (30). Enantiomers are probably not a problem as in our experiments only the R(+)-isomer of etomidate was administered. However this is only an assumption as the occurrence of *in vivo* isomerization cannot be excluded.

In humans, Arden *et al.* found a monophasic correlation between etomidate plasma concentrations and the median EEG frequency, which showed an equilibration half-life of 1.6 min (21). Using the same EEG parameter, Schwilden *et al.* observed no equilibration delay (31). This discrepancy is possibly explained by the much longer duration of infusion and the lower infusion rate used in the latter study.

After minimizing the hysteresis, the biphasic effect versus effect-site concentration relationship was quantified by using nonparametric descriptors because parameters of biphasic pharmacodynamic models are not estimable (22). This relationship is considered equivalent to an effect-plasma concentration relationship under steady-state conditions. The concentration at 50% activation, at maximal activation and at baseline effect between maximal activation and burst-suppression are inversely related to potency. The EEG effect at maximal activation is a unique descriptor of the intrinsic efficacy of etomidate. The inhibitory part of the curve was not included in the description of the concentration-effect relationship as this part represented only a small portion, and data were consequently insufficient to describe this inhibitory part reliably.

The average concentration at which the animals regained return of the righting reflex was 0.44 μg/ml. Humans became awake at comparable concentrations ranging from 0.3 to 0.5 μg/ml (31). The start of 5s EEG suppression occurred at an estimated effect-site concentration of 3.30 μg/ml or an unbound concentration of 0.63 μg/ml. At this point, bursts of EEG occurred between periods of 5s or more electrical silence, which is considered as a state of deep hypnosis.

From the results of the present study, it can be concluded that the *in vivo* pharmacodynamics of etomidate can be investigated in an experimental rat model. This will allow us to study the influence of several disease states like hemorrhagic shock on the pharmacodynamics and effect-site distribution of etomidate.

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REFERENCES

1. T. J. Ebert, M. M. Muzi, R. Berens, D. Goff, and J. P. Kampine. Sympathetic responses to induction of anesthesia in humans with propofol or etomidate. *Anesthesiology* **76**:725–733 (1992).
2. M. P. Colvin, T. M. Savege, P. E. Newland, E. J. Weaver, A. F. Waters, J. M. Brookes, and R. Inniss. Cardiorespiratory changes following induction of anaesthesia with etomidate in patients with cardiac disease. *Br. J. Anaesth.* **51**:551–556 (1979).
3. B. Simon and G. P. Young. Emergency airway management. *Acad. Emerg. Med.* **1**:154–157 (1994).
4. J. W. Mandema and M. Danhof. Pharmacokinetic-pharmacodynamic modeling of the central nervous system effects of heptabarbital using aperiodic EEG analysis. *J. Pharmacokin. Biopharm.* **18**:459–481 (1990).
5. F. W. Ossenberg, M. Peignoux, D. Bourdiau, and J. P. Benhamou. Pentobarbital pharmacokinetics in the normal and in the hepatectomized rat. *J. Pharmacol. Exp. Ther.* **194**:111–116 (1975).
6. P. Le Moing and J. C. Levron. High-performance liquid chromatographic determination of etomidate in plasma. *J. Chromatogr.* **529**:217–222 (1990).
7. F. M. Belpaire and M. G. Bogaert. Binding of diltiazem to albumin, α_1-acid glycoprotein and to serum in man. *J. Clin. Pharmacol.* **30**:311–317 (1990).
8. H. Akaike. A new look at the statistical model identification. *IEEE Trans. Automat. Control AC* **19**:716–723 (1974).
9. M. Gibaldi and D. Perrier. *Pharmacokinetics*, Marcel Dekker, New York, 1982.
10. T. K. Gregory and D. C. Pettus. An electroencephalographic processing algorithm specifically intended for analysis of cerebral electrical activity. *J. Clin. Monit.* **2**:190–197 (1986).
11. D. Verotta and L. B. Sheiner. Simultaneous modeling of pharmacokinetics and pharmacodynamics: An improved algorithm. *CABIOS* **3**:345–349 (1987).
12. W. F. Ebling, M. Danhof, and D. R. Stanski. Pharmacodynamic characterization of the electroencephalographic effects of thiopental in rats. *J. Pharmacokin. Biopharm.* **19**:123–143 (1991).
13. D. J. De Wildt, F. C. Hillen, A. G. Rauws, and B. Sangster. Etomidate-anaesthesia, with and without fentanyl, compared with urethane-anaesthesia in the rat. *Br. J. Pharmacol.* **79**:461–469 (1983).
14. J. M. Gooding and G. Corssen. Effect of etomidate on the cardiovascular system. *Anesth. Analg.* **56**:717–719 (1977).
15. J. Schüttler, H. Schwilden, and H. Stoeckel. Infusion strategies to investigate the pharmacokinetics and pharmacodynamics of hypnotic drugs: Etomidate as an example. *Eur. J. Anaesth.* **2**:133–142 (1985).
16. K. Fruergaard, M. Jenstrup, J. Schierbeck, and F. Wiberg-Jorgensen. Total intravenous anaesthesia with propofol or etomidate. *Eur. J. Anaesth.* **8**:385–391 (1991).
17. L. L. Gustafsson, W. F. Ebling, E. Osaki, and D. R. Stanski. Quantitation of depth of thiopental anesthesia in the rat. *Anesthesiology* **84**:415–427 (1996).
18. A. Wauquier, W. A. E. Van Den Broeck, J. L. Verheyen, and P. A. J. Janssen. Electroencephalographic study of the short-acting hypnotics etomidate and methohexital in dogs. *Eur. J. Anaesth.* **47**:367–377 (1978).
19. M. M. Ghoneim and T. Yamada. Etomidate: A clinical and electroencephalographic comparison with thiopental. *Anesth. Analg.* **56**:479–485 (1977).
20. A. Doenicke, B. Löffler, J. Kugler, H. Suttmann, and B. Grote. Plasma concentration and EEG after various regimens of etomidate. *Br. J. Anaesth.* **54**:393–400 (1982).
21. J. R. Arden, F. O. Holley, and D. R. Stanski. Increased sensitivity to etomidate in the elderly: Initial distribution versus altered brain response. *Anesthesiology* **65**:19–27 (1986).
22. S. Dutta, Y. Matsumoto, N. U. Gothgen, and W. F. Ebling. Concentration-EEG effect relationship of propofol in rats. *J. Pharm. Sci.* **86**:37–43 (1997).
23. S. Dutta and W. F. Ebling. Formulation-dependent pharmacokinetics and pharmacodynamics of propofol in rats. *J. Pharm. Pharmacol.* **50**:37–42 (1998).
24. E. H. Cox, C. A. J. Knibbe, V. S. Koster, M. W. E. Langemeijer, E. E. Tukker, R. Lange, P. F. M. Kuks, H. J. M. Langemeijer,

L. Lie-A-Huen, and M. Danhof. Influence of different fat emulsion-based intravenous formulations on the pharmacokinetics and pharmacodynamics of propofol. *Pharm. Res.* **15**:442–448 (1998).

25. A. G. De Boer and D. D. Breimer. Rectal, oral and I.A. administration of etomidate to rats: Significant avoidance of hepatic first-pass elimination following rectal administration. *Arch. Int. Pharmacodyn. Ther.* **270**:180–191 (1984).
26. M. S. Yates, C. R. Hiley, P. J. Roberts, D. J. Back, and F. E. Crawford. Differential effects of hepatic microsomal enzyme inducing agents on liver blood flow. *Biochem. Pharmacol.* **27**:2617–2621 (1978).
27. P. J. Lewi, J. J. P. Heykants, and P. A. J. Janssen. Intravenous pharmacokinetic profile in rats of etomidate, a short-acting hypnotic drug. *Arch. Int. Pharmacodyn. Ther.* **220**:72–85 (1976).
28. W. E. G. Meuldermans and J. J. P. Heykants. The plasma protein binding and distribution of etomidate in dog, rat and human blood. *Arch. Int. Pharmacodyn. Ther.* **221**:150–162 (1976).
29. J. W. Mandema, P. Veng-Pedersen, and M. Danhof. Estimation of amobarbital plasma-effect site equilibration kinetics. Relevance of polyexponential conductance functions. *J. Pharmacokin. Biopharm.* **19**:617–634 (1991).
30. J. J. P. Heykants, W. E. G. Meuldermans, L. J. M. Michiels, P. J. Lewi, and P. A. J. Janssen. Distribution, metabolism and excretion of etomidate, a short-acting hypnotic drug, in the rat. Comparative study of R-(+) and S-(−)-etomidate. *Arch. Int. Pharmacodyn. Ther.* **216**:113–129 (1975).
31. H. Schwilden, J. Schüttler, and H. Stoeckel. Quantitation of the EEG and pharmacodynamic modelling of hypnotic drugs: Etomidate as an example. *Eur. J. Anaesth.* **2**:121–131 (1985).