British Journal of Anaesthesia **95 (3)**: 367–71 (2005)
doi:10.1093/bja/aei179 Advance Access publication June 24, 2005

BJA

Development of acute tolerance to the EEG effect of propofol in rats†

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Background. A previous study in rats with propofol suggested the development of acute tolerance to the EEG effect. The aim of this study was to evaluate acute tolerance by means of EEG-controlled closed-loop anaesthesia as this approach allows precise determination of drug requirement to maintain a defined drug effect.

Methods. Ten male Sprague–Dawley rats [weight 402 (40) g, mean (SD)] were included in the study. The EEG was recorded with occipito-occipital needle electrodes and a modified median frequency (mMEF) of the EEG power spectrum was used as a pharmacodynamic control parameter. The propofol infusion rate was controlled by a model-based adaptive algorithm to maintain a set point of mMEF=3 (0.5) Hz for 90 min. The performance of the closed-loop system was characterized by the prediction error PE=(mMEF–set point)/set point. Plasma propofol concentrations were determined from arterial samples by HPLC.

Results. The chosen set point was successfully maintained in all rats. The median (SE) and absolute median values of PE were −5.0 (0.3) and 11.3 (0.2)% respectively. Propofol concentration increased significantly from 2.9 (2.2) $\mu g\,ml^{-1}$ at the beginning to 5.8 (3.8) $\mu g\,ml^{-1}$ at 90 min [mean (SD), $P<0.05$]. The cumulative dose increased linearly, with a mean infusion rate of 0.60 (0.16) mg kg^{-1} min^{-1}. The minimum value of the mean arterial pressure during closed-loop administration of propofol was 130 (24) mm Hg, compared with a baseline value of 141 (12) mm Hg.

Conclusions. The increase in propofol concentration at constant EEG effect indicates development of acute tolerance to the hypnotic effect of propofol.

Br J Anaesth 2005; **95**: 367–71

Keywords: anaesthetic techniques, closed-loop controlled infusion; anaesthetics i.v., propofol; model, rat; monitoring, electroencephalography, median frequency; pharmacology, acute tolerance

Accepted for publication: April 12, 2005

In an earlier study in rats with propofol, we observed development of acute tolerance to the EEG effects, even after a relatively short infusion of 90 min.[1] However, this finding was limited by the fact that ketamine was used for instrumentation of the animals, so that an interaction of ketamine and propofol could not be excluded. Furthermore, development of tolerance was observed indirectly, as the concentration–effect relationship could not be modelled with a standard pharmacodynamic model. In the present study we chose a different design to evaluate development of acute tolerance. First, on the day of the experiment, anaesthesia for instrumentation was done with propofol instead of ketamine. Secondly, we determined the propofol requirement necessary to maintain a constant EEG. For this purpose we used a closed-loop system presented in a recent publication.[2] With this device, propofol can be administered automatically to maintain a defined EEG effect. An increase in propofol concentration together with a constant EEG effect could be interpreted as an indication for development of tolerance.

Methods

Animals

After approval by the appropriate animal investigation committee, 10 adult male Sprague–Dawley rats, weighing 402

†Presented in part at the Euroanaesthesia meeting, Lisbon, Portugal, June 5–8, 2004.

(40) g [mean (SD)], were included in the study. Animals were delivered by Charles River Wiga, Sulzfeld, Germany at least 7 days before the experiments for quarantine and acclimatization. Animals were healthy with respect to serology, bacteriology, parasitology and pathology. The rats were housed in pairs in polycarbonate cages type III (Uno, Zevenaar, The Netherlands) on standard research bedding (soft wood fibre, Altromin, Lage, Germany) at 21.0 (0.5)°C, 60% humidity, 12 h light/12 h dark cycle, with pelleted standard rodent diet (No. 1320, Altromin) and tap water *ad libitum*.

Instrumentation

Two days before starting the experiments, rats were anaesthetized with ketamine 76 (7) mg (Ketavet®, 100 mg ml^{-1}; Pharmacia, Erlangen, Germany) intraperitoneally. Incision sites were infiltrated with 2% lidocaine. A jugular vein catheter was placed for drug infusion, tunnelled subcutaneously and externalized on the dorsal surface of the neck. On the day of the experiment, rats were anaesthetized with propofol 5 mg i.v. (Diprivan®, 10 mg ml^{-1}; AstraZeneca, Wedel, Germany) followed by target-controlled infusion with target concentrations of 2–4 μg ml^{-1}. A second catheter was placed into the femoral artery for blood sampling, blood gas analysis and blood pressure monitoring. Stainless steel EEG needle electrodes were placed occipito-occipitally. The trachea was intubated for artificial ventilation to maintain stable blood gas status. During artificial ventilation the rats were paralysed with repetitive doses of pancuronium. The animals' temperature was maintained with a heating pad.

EEG processing and pharmacodynamic analysis

A one-channel EEG was continuously recorded with an Aspect A1000 monitor (Aspect Medical Systems™, Natick, MA, USA). The digitized EEG signal was processed on-line with own EEG analysis software (sampling rate 128 Hz, epoch length 8 s) and the median frequency (MEF) of the power spectrum (0.5–49 Hz) was determined using a fast Fourier transform. In previous studies we found that the EEG of rats under propofol anaesthesia showed burst suppressions and spike-like patterns with high-frequency components, so that the MEF first decreased with increasing propofol concentration and then paradoxically increased.[1 3] We therefore introduced a modified median frequency (mMEF), which takes into account the occurrence of burst suppressions and spikes. The mMEF algorithm uses pattern recognition to identify spikes, and modifies the MEF if burst suppressions and/or spikes are detected.[1] mMEF decreases continuously with increasing propofol concentration, and this parameter was also used in the present study. In addition, two alternative EEG parameters, the spectral edge frequency (SEF90) and the approximate entropy, were determined in an off-line analysis to give more evidence that the EEG effect remained constant during closed-loop control. SEF90 was determined as the 90% quantile of the EEG power spectrum. The approximate entropy is a statistical parameter which quantifies the amount of regularity in data, and was introduced some years ago as an EEG measure of anaesthetic drug effect, based on the hypothesis that the EEG during higher anaesthetic concentrations would be more ordered and less random than at lower anaesthetic concentrations.[4] Approximate entropy was determined by the algorithm given by Bruhn and colleagues.[4]

Drug administration

Propofol was administered using a closed-loop system which was developed in our department (IvFeed 4.7; Klinik für Anästhesiologie, Universität Erlangen-Nürnberg, Germany). The system allows administration of propofol either as target-controlled infusion (TCI) to achieve a defined propofol plasma concentration which is set by the user, or as closed-loop infusion with a defined mMEF as the set point. Closed-loop control was realized using an adaptive control algorithm combining a pharmacokinetic and a pharmacodynamic model to relate dose with effect. During closed-loop control, propofol is administered also as TCI, but the target concentration is now determined by the closed-loop algorithm based on the pharmacodynamic model and the difference between the set point and actually measured EEG effect. A detailed description of the system can be found in a previous publication.[2] As the EEG set point we chose a mMEF of 3 (0.5) Hz, based on previous experience with propofol.[2] At this level, a relatively deep anaesthesia is seen and the EEG is characterized by spike-like patterns, but the incidence of burst suppression is low and propofol-induced blood pressure decrease is not too profound. As the mMEF can decrease further to a minimum value of 0 Hz, which will be reached if the EEG is completely suppressed, a set point of 3 Hz avoids a ceiling effect where the mMEF is virtually independent of drug concentration. During instrumentation and at the beginning of the experiment, propofol was administered to target constant propofol blood concentrations. When mMEF was close to the chosen EEG set point of 3.0 (0.5) Hz, the EEG-controlled closed-loop administration was started and maintained for 90 min. At 90 min, the propofol infusion was stopped. To provide arousal stimuli and avoid natural sleep during closed-loop controlled drug administration, rats received noxious stimuli (tail squeeze) which were randomized with respect to time and intensity.

Drug sampling and propofol assay

Arterial blood samples of 300 μl each were collected immediately before the start, every 15 min during closed-loop control, and 10 min after ending closed-loop control. Maintenance fluids (sodium Ringer's lactate 600 μl) were given after each blood sample. Samples were collected into heparinized microcapillaries and centrifuged in Eppendorf tubes, and the plasma was stored at −20°C until analysis. Propofol plasma concentration was determined

by high performance liquid chromatography (HPLC) as described earlier.[1]

Statistics

Analysis of variance for repeated measurements and the Tukey test were used to test the propofol concentrations for differences compared with the value at the start of closed-loop control. Performance of the closed-loop system was assessed by the prediction error PE=(mMEF–set point)/set point and the absolute prediction error APE=abs(PE). Performance in the population was characterized by the median prediction error (MDPE), the median absolute prediction error (MDAPE) and the wobble, as defined by Varvel.[5] Data are presented as mean (SD) unless stated otherwise. For propofol concentrations the 95% confidence interval (CI) is also given. Statistical analysis was performed with Statistica 6.0 (StatSoft, Tulsa, OK, USA).

Results

Propofol closed-loop infusion of 90 min could be performed in all rats. The closed-loop control was started 48 (9) min after the start of propofol administration. Figure 1 shows the time courses of mMEF and propofol concentration for a typical case. The mean mMEF for all animals was 3.0 (0.7) Hz at the beginning and 2.8 (0.5) Hz at the end of closed-loop control (Fig. 2). After stopping propofol infusion, the mMEF increased to 7.9 (3.2) Hz. MDPE, MDAPE and wobble during closed-loop control were −5.0 (0.3), 11.3 (0.2) and 10.0 (0.3%) respectively [mean (SE)]. The EEG parameters spectral edge frequency and approximate entropy also remained constant during closed-loop control and increased after stopping infusion (Fig. 2). The cumulative doses increased linearly with a mean infusion rate of 0.60 (0.16) mg kg^{-1} min^{-1} during closed-loop control (Fig. 3). The measured propofol concentration increased significantly from 2.9 (2.2) (95% CI 1.3–4.5) μg ml^{-1} at the beginning to 5.8 (3.8) (95% CI 3.1–8.6) μg ml^{-1} at the end of closed-loop infusion ($P<0.05$, Fig. 4). Ten minutes after stopping infusion, the propofol concentration decreased to 2.8 (1.3) (95% CI 1.8–3.8) μg ml^{-1}. Mean arterial pressure dropped slightly from 141 (12) mm Hg to a minimum of

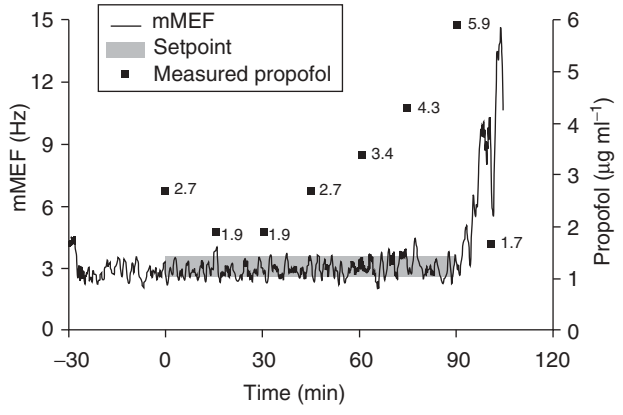

Fig 1 Modified EEG median frequency (mMEF) and measured propofol plasma concentrations in a representative case. Time is given in minutes after start of closed-loop control. The set point of 3.0 (0.5) Hz is indicated by the grey bar.

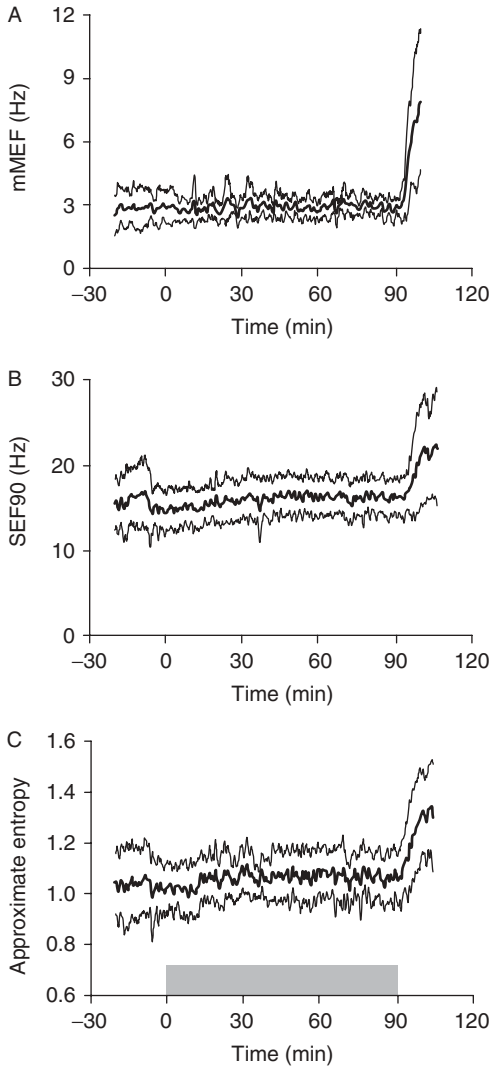

Fig 2 Modified EEG median frequency (mMEF), spectral edge frequency (SEF90) and approximate entropy for all animals (mean and SD). The grey bar at the bottom indicates the time of closed-loop control.

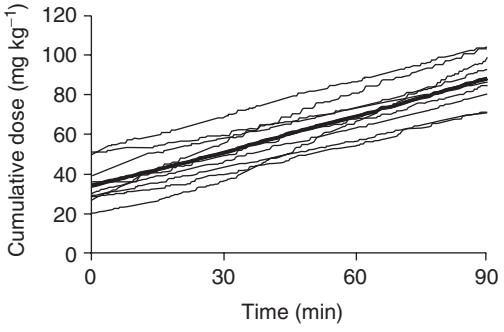

Fig 3 Cumulative doses for each animal during closed-loop infusion of propofol. The bold line represents the mean for all rats.

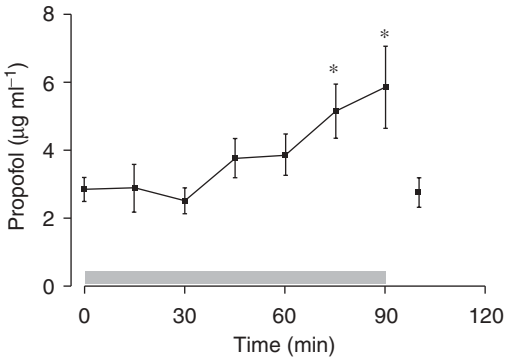

Fig 4 Measured propofol concentrations for all animals (mean and SE). The grey bar at the bottom indicates the time of closed-loop control. *P<0.05 *vs* concentration at t=0.

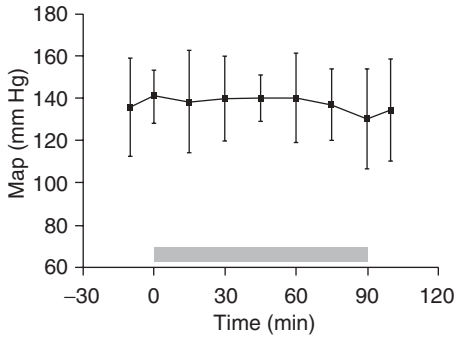

Fig 5 Mean arterial pressure (MAP) for all animals (mean and SD). The grey bar at the bottom indicates the time of closed-loop control.

130 (24) mm Hg at the end of the closed-loop infusion (Fig. 5). Blood gases remained stable throughout the study.

Discussion

The aim of this study was to investigate the development of acute tolerance to propofol in rats. In an earlier study[1] we found some evidence for acute tolerance but, as drug interactions could not be ruled out, we performed the present study with a refined design, using a closed-loop system to determine automatically the propofol requirement for a defined EEG effect. When discussing development of tolerance, one has to discriminate between tolerance with respect to dose and tolerance with respect to concentration. Tolerance is sometimes defined as an increase in the dose that is necessary to maintain a defined effect. However, one has to consider that the dose–effect relationship is determined by the pharmacokinetics and the pharmacodynamics of the drug. An increase in clearance by enzyme induction also leads to an increase in dose requirement as the concentration decreases on continuous infusion with a constant rate. This type of tolerance is therefore called 'pharmacokinetic' or 'metabolic' tolerance and must be distinguished from 'pharmacodynamic' or 'receptor-site' tolerance.[6] These two types of tolerance are indistinguishable if one measures only the effect as a function of dosing.

In the present study the use of a closed-loop system facilitated determination of the dose required to maintain a defined EEG effect, whereas measurement of propofol plasma concentrations allowed discrimination between pharmacokinetic and pharmacodynamic tolerance. The cumulative dose increased linearly during closed-loop control, indicating that the EEG effect of mMEF=3.0 (0.5) Hz could be maintained with an average constant infusion rate of 0.60 (0.16) mg kg^{-1} min^{-1}. Thus, the dose–effect relationship did not show any signs of development of tolerance. However, the measured propofol concentration increased significantly, particularly after the first 60 min of infusion. This can be interpreted as development of pharmacodynamic tolerance. As the chosen set point of mMEF=3.0 (0.5) Hz allowed a further decrease in mMEF until complete suppression of EEG activity, a ceiling effect where the effect does not further increase despite increasing concentration can be ruled out. From blood gas analysis and haemodynamic monitoring, we also excluded a change in the general physiological state of the animals. The observation that two other EEG parameters, spectral edge frequency and approximate entropy, also remained constant during closed-loop control gives additional evidence that at least the electroencephalographic state of anaesthesia remained constant during closed-loop control. The increase in propofol concentrations at nearly constant infusion rates may indicate either that steady state was not yet reached, so that the compartments were not in equilibration, or that there was a kind of non-linearity in propofol pharmacokinetics, which was also observed in an earlier study.[1]

Development of acute tolerance to the hypnotic effect of propofol is controversial. In an early study of propofol in animals, Cockshott and colleagues[7] reported acute tolerance to propofol in dogs within 4–6 h with respect to the propofol concentration at wakening. Fassoulaki and colleagues[8] investigated sleeping time in rats after repetitive propofol bolus doses and found that sleeping time decreased significantly. However, there was no significant difference between wakening blood concentrations and it was thus concluded that this observation was an example of metabolic tolerance. However, as acute tolerance is defined as altered sensitivity to a drug within the duration of a continuous exposure to the drug,[9] the study design by Fassoulaki might not be appropriate for detecting acute tolerance. Larsson and colleagues[9] found that rats receiving propofol infusions of 1 h with an EEG suppression of at least 1 s as the pharmacodynamic end-point showed significantly greater propofol concentrations compared with the concentration at induction.

The relevance of these findings for the application of propofol in man must be interpreted with caution. As rats have a much higher rate of metabolism, development of tolerance in man should not occur as fast as in animals. Whereas development of tolerance to propofol in man was not seen in some studies,[10 11] there was one case report detailing the development of tolerance,[12] and tolerance was

also found in studies with propofol infusion over some days.[13 14] However, these studies were conducted in intensive care patients, so that interaction with other drugs and a change of the general physiological state could not be ruled out.

It should also be mentioned that the present findings have no consequences for propofol dosing in rats as the EEG effect could be maintained with a nearly constant infusion rate. The finding that the apparent pharmacokinetic non-linearity and the apparent development of tolerance do compensate for each other raises the question of whether these effects result from the specific circumstances of the present study. We have not determined unbound propofol, and a constant effect together with increasing total propofol concentrations could be explained by a decrease in the fraction of free propofol over time, so that the unbound active drug would remain constant. Because of the withdrawal of blood and the substitution with Ringer's lactate one would expect a decrease in protein binding and thus an increase in the fraction of free propofol. One has, however, to consider the large amount of fat which was also administered using Diprivan® 1% and which may also change the equilibrium between free and bound propofol. Either the dilution caused by the substitution with Ringer's lactate is negligible, given that the total volume injected was 4.2 ml compared with a central volume of at least 130 ml for propofol,[1] or it should also lead to lower propofol concentrations. As the propofol concentration declined rapidly after stopping infusion and the blood pressure showed only a slight decrease during closed-loop control, a change in propofol metabolism by markedly reduced liver function seems unlikely.

For other drugs, different mechanisms for development of tolerance have been discussed, such as decrease in receptor number or a change in binding affinity.[15] As the mechanism(s) of anaesthesia are still unclear, metabolic as well as receptor site tolerance to propofol merits further investigation.

Acknowledgement

The authors acknowledge the technical assistance of Mr Rainer Knoll in performing the propofol assay.

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