Neuroactive Steroids Differ in Potency but Not in Intrinsic Efficacy at the GABA$_A$ Receptor in Vivo


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ABSTRACT

The objective of the present investigation was to characterize the in vivo EEG effects of (synthetic) neuroactive steroids on the basis of a recently proposed mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) model. After intravenous administration, the time course of the EEG effect of pregnanolone, 2β-3α-5α-3-hydroxy-2-(2,2-dimethylmorpholin-4-yl)-pregnan-11,20-dione (ORG 21465), 2β-3α-5α-21-chloro-3-hydroxy-2-(4-morpholinyl)-pregnan-20-one (ORG 20599), and alphaxalone was determined in conjunction with plasma concentrations in rats. For each neuroactive steroid the PK/PD correlation was described on the basis of a two-compartment pharmacokinetic model with an effect compartment to account for hysteresis. The observed concentration EEG effect relationships were biphasic and characterized with a mechanism-based pharmacodynamic model, which is based on a separation between the receptor activation process and the stimulus-response relationship. A single unique biphasic stimulus-response relationship could be identified for all neuroactive steroids, which was successfully described by a parabolic function. The receptor activation process was described by a hyperbolic function. Estimates for the maximum activation ($e_{max}$) were similar for the different neuroactive steroids but values of the potency estimate ($K_{pd}$) ranged from $157 \pm 16$ ng·ml$^{-1}$ for pregnanolone, $221 \pm 83$ ng·ml$^{-1}$ for ORG 20599, and $483 \pm 42$ ng·ml$^{-1}$ for alphaxalone to $1619 \pm 208$ ng·ml$^{-1}$ for ORG 21465. A statistically significant correlation was observed between the in vivo potency and the IC$_{50}$ in an in vitro [35S]-butilbicyclophosphorothionate binding assay ($r = 0.91$). It is concluded that the new PK/PD model constitutes a new mechanism-based approach to the quantification of the effects of (synthetic) neuroactive steroids in vivo effects. The results show that the neuroactive steroids differ in potency but not in intrinsic efficacy at the GABA$_A$ receptor in vivo.

Neuroactive steroids have long been known to produce anesthesia (Seyle, 1942), but the clinical development of neuromodulatory agents has been hampered by side effects that are in part related to the pharmacological formulation required for the intravenous administration (Anderson et al., 1997; Sear, 1998). At present, there is a renewed interest in the efficacy of neuroactive steroids for the management of epilepsy, anxiety, insomnia, migraine, drug dependence, depression, stress, and premenstrual syndrome (Gasier et al., 1997; Rupprecht and Holsboer, 1999). An important characteristic in this respect is the intrinsic efficacy at the GABA$_A$ receptor in vivo. It is known that both synthetic and endogenous neuroactive steroids are selective and potent modulators of GABA$_A$ receptor function, which are devoid of effects through activation of glucocorticoid and/or mineralocorticoid receptors upon acute administration (Paul and Purdy, 1992; for reviews, see Lambert et al., 1995; Rupprecht and Holsboer, 1999). Electrophysiological studies have demonstrated that neurosteroids have dual effects at the GABA$_A$ receptor upon binding. At nanomolar concentrations neuroactive steroids potentiate GABA-evoked currents, whereas at micromolar concentrations and in the absence of applied GABA, they can directly elicit membrane currents through activation of GABA$_A$ receptors (Harrison and Simmonds, 1984; Cottrell et al., 1987).

In vitro it has been shown that synthetic neuroactive steroids and other GABA$_A$ receptor modulators can differ in intrinsic efficacy, covering the entire spectrum from full agonists to inverse agonists (Sieghart, 1995; Anderson et al., 1997). It is well established that intrinsic efficacy is a major determinant of pharmacological actions in vivo (Kenakin, 1999). This underscores the importance of estimation of the intrinsic efficacy in vivo.

In recent years, quantitative EEG parameters have often been used as pharmacodynamic endpoint for the characterization of neuroactive steroids.
tion of PK/PD relationships of drugs acting at the GABA<sub>A</sub> receptor and the μ-opioid receptor (Danhof and Mandema, 1992; Cox et al., 1999). In addition, important progress has been made in the development of a new class of mechanism-based PK/PD models that use concepts from receptor theory (Van der Graaf and Danhof, 1997). A specific feature of these models is a separation between the characterization of the drug-receptor interaction on one hand and the stimulus-response relationship on the other hand. In the mean time, such mechanism-based PK/PD models have been successfully developed for drugs such as benzodiazepines (Tuk et al., 1999), synthetic opiates (Cox et al., 1998a), adenosine A<sub>1</sub> agonists (Van der Graaf et al., 1999), and 5-hydroxytryptamine<sub>1A</sub> agonists (Zuideveld et al., 2001). It has been shown that these mechanism-based PK/PD models constitute an excellent approach to the estimation of the in vivo intrinsic efficacy.

Recently, we have proposed a novel mechanism-based PK/PD modeling approach to describe the biphatic concentration-EfG effect relationship of the synthetic neuroactive steroid alphaxalone (Visser et al., 2002). In this approach, the initial receptor activation process is described by a monophasic and saturable function, whereas the stimulus-response function has a biphatic shape. In the model, the receptor activation was described on the basis of a hyperbolic function and the biphatic transducer on the basis of a parabolic function. In this manner, it has been possible to identify the biphatic stimulus-response relationship of alphaxalone.

An important question is, however, whether the biphatic stimulus-response relationship is only specific for alphaxalone or whether it applies to neuroactive steroids in general. In this respect, it is important that the stimulus-response relationship should be specific for the functioning of the biological system in vivo and independent of the administered drug. A second important question is whether quantitative differences in the EEG between neuroactive steroids are caused by differences in potency (i.e., affinity), intrinsic efficacy, or a combination of both.

In this investigation, we have characterized the in vivo PK/PD relationships of a series of neuroactive steroids (i.e., alphaxalone, ORG 20599, ORG 21465, and pregnanolone) with known differences in affinity at the GABA<sub>A</sub> receptor (Anderson et al., 1997) to determine whether a unique stimulus-response relationship can be identified for neuroactive steroids in general. A second objective was to estimate the intrinsic efficacy and intrinsic efficacy at the GABA<sub>A</sub> receptor.

Materials and Methods

Animals and Surgical Procedures. The protocol of this investigation was approved by the Committee on Animal Experimentation of Leiden University (Leiden University, The Netherlands). In this investigation, groups of six to eight male Wistar rats with a mean body weight of 297 ± 3 g (mean ± S.D., n = 42) were used (Charles River BV, Zeist, The Netherlands). After surgery, the rats were housed individually in standard plastic cages with a normal 12-h day/night schedule (lights on 7:00 AM) at a temperature of 21°C. The animals had access to standard laboratory chow (RMH-TM; Hope Farms, Woerden, The Netherlands) and acidified water ad libitum.

Nine days before the start of the experiments seven cortical electrodes were implanted into the skull as described previously (Visser et al., 2002). Briefly, the electrodes were placed at the locations 11 mm anterior and 2.5 mm lateral (F<sub>1</sub> and F<sub>2</sub>), 3 mm anterior and 3.5 mm lateral (C<sub>1</sub> and C<sub>2</sub>), and 3 mm posterior and 2.5 mm lateral (O<sub>1</sub> and O<sub>2</sub>) to lambda. A reference electrode was placed on lambda. Stainless steel screws were used as electrodes and connected to a miniature connector, which was insulated and fixed to the skull with dental acrylic cement. The surgical procedures were performed under anesthesia with 0.1 mg · kg<sup>−1</sup> i.m. medetomidine hydrochloride (Domitor; Pfizer, Capelle a/d IJssel, The Netherlands) and 1 mg · kg<sup>−1</sup> s.c. ketamine base (Ketalar; Parke-Davis, Hoofddorp, The Netherlands). After the first surgery, 4 mg of ampicillin (A.U.V., Cuijk, The Netherlands) was administered to aid recovery.

Three days before the start of the experiment indwelling cannulae were implanted in the right femoral artery for the serial collection of arterial blood samples and in the right jugular vein for drug administration. The cannulae were filled with heparinized 25% (g/ml) polyvinylpyrrolidone in saline (Brocacef, Maarssen, The Netherlands) and tunneled subcutaneously to the back of the neck where they were exteriorized and fixed with a rubber ring.

Drugs and Dosages. 2β,3α-5α-3-Hydroxy-2-(2,2-dimethylmorpholin-4-yl)-pregnan-11,20-dione (ORG 21465) and 2β,3α-5α-21-chloro-3-hydroxy-2-(4-morpholinyl)-pregnan-20-one (ORG 20599) were kindly donated by Organon Laboratories Ltd. (Newhouse, Scotland) (Fig. 1). Pregnanolone (5β-pregnan-3α-ol-20-one) and alphaxalone (5α-pregnan-3α-ol-11,20-dione) were purchased from Sigma-Alrich BV (Zwijndrecht, The Netherlands) (Fig. 1). Infusion solutions were prepared in DMSO (Baker, Deventer, The Netherlands). Per rat, 100 μl of the infusion solution was administered. ORG 21465 was administered in a dose of 8.7 ± 0.2 mg · kg<sup>−1</sup> in 5 min (n = 6). Pregnanolone was administered in two dosages of 4.0 mg · kg<sup>−1</sup> (n = 7) and 9.8 mg · kg<sup>−1</sup> (n = 6) in 5 min. ORG 20599 was administered in two dosages of 21 mg · kg<sup>−1</sup> (n = 8) in 5 min and 24 mg · kg<sup>−1</sup> (n = 6) in 15 min. Alphaxalone was administered in a dose of 4.8 mg · kg<sup>−1</sup> (n = 8), which was group C in a previous investigation (details in Visser et al., 2002). Control experiments with administration of the vehicle DMSO were also included.

Pharmacokinetic-Pharmacodynamic Experiments. The studies were conducted in accordance with the requirements of national legislation and appropriate guidelines for animal care. All experiments were started between 8:30 and 9:30 AM to exclude influences of circadian rhythms. The rats were placed in a rotating drum to control the level of vigilance, thereby avoiding the interference of sleep patterns. During the experiments, the rats were deprived of food and water. Two bipolar EEG leads (C<sub>1</sub>–O<sub>1</sub>) and (C<sub>2</sub>–O<sub>2</sub>) were continuously recorded using a Nihon-Kohden AB-621G bioel-

Fig. 1. Chemical structures of pregnanolone (A), ORG 21465 (B), alphaxalone (C), and ORG 20599 (D), which is a methane sulfonate salt (MeSO<sub>3</sub>H).
electric amplifier (Hoeckloos BV, Amsterdam, The Netherlands) and concurrently digitized at a rate of 256 Hz using a 1401plus interface (CED, Cambridge, UK). The digitized signal was fed into a Pentium III computer and stored on hard disk for off-line analysis. EEG baseline was recorded for 45 min. Thereafter, the neuroactive steroids were administered in a zero order intravenous infusion to the conscious and freely moving rats using an infusion pump (Biological Systems, West Lafayette, IN). The EEG recordings lasted until 250 min after the end of the infusion. For each 5-s epoch, quantitative EEG parameters were obtained off-line by Fast Fourier Transformation with a user-defined script within the data analysis software package Spike 2 for Windows, version 3.18 (CED). Amplitudes in the β-frequency band of the EEG (11.5–30 Hz) averaged over 25-s time intervals were used as a measure of drug-effect intensity. Serial arterial blood samples were collected at predefined time intervals in heparinized tubes and centrifuged at 5000 rpm for 15 min for plasma collection. Total volume of redrawn blood samples was kept equal to 2.1 ml during each experiment. Plasma samples were stored at −20°C until drug analysis.

**Drug Analysis.** Pregnanolone, ORG 21465, and alphaxalone plasma concentrations were determined by HPLC using the derivatization and fluorescence detection method described previously (Visser et al., 2000, 2002), which was slightly modified for ORG 21465. Briefly, to 50 μl of plasma, 50 μl of the internal standard (3 μg ml⁻¹ pregnenolone dissolved in acetonitrile) was added. Subsequently, 200 μl of acetonitrile was added to precipitate plasma proteins. After centrifugation, the supernatant was transferred to a clean tube, and 50 μl of 2 M NaOH and 25 μl of dansyl hydrazine solution (20 mg in methanol acidified with 40 μl of sulfuric acid) were added. The mixtures were stored in a dark place at room temperature for 20 h. Subsequently, 500 μl of 1 M NaOH (pregnanolone) or 500 μl of 0.2 M NaOH-glycine buffer pH 11 (ORG 21465) and 5 ml of dichloromethane were added, and the mixture was vortexed for 5 min. The phase system was centrifuged for 15 min at 4500g and the organic phase was transferred to a clean tube and evaporated under reduced pressure on a vortex vacuum evaporator (Buchler Instruments, Fort Lee, NJ) at 37°C. The residue was dissolved in 100 μl of mobile phase, of which a volume of 50 μl was injected into the HPLC system. The HPLC system consisted of a Spectroflow 400 solvent delivery system (Applied Biosystems, Ramsey, NJ), a 712 Autosampler (Waters, Milford, MA) and a PerkinElmer LC240 fluorescence detector (PerkinElmer Ltd., Beaconsfield, UK). Chromatography was performed on a C18, 3-μm cartridge column (100 × 4.6-mm i.d.; Chrompack, Bergen op Zoom, The Netherlands) equipped with a guard column. The mobile phase consisted of a mixture of 25 mM acetate buffer, pH 3.7, and acetonitrile (40:60, v/v, for pregnanolone; 45:55, v/v, for alphaxalone) or 17 mM phosphate buffer, pH 7.2, and acetonitrile (56:44, v/v, for ORG 21465). Flow rate was 1 ml min⁻¹. Fluorescence detection occurred at an excitation wavelength of 332 nm and an emission wavelength of 516 nm. Data acquisition and processing was performed using a Chromatopac C-R3A reporting integrator (Shimadzu, Kyoto Japan). Using 50 μl of plasma, the limit of quantification was 0.01 μg ml⁻¹ for pregnanolone, alphaxalone, and ORG 21465. Linear calibration curves were obtained in the range 0.025 to 25 μg ml⁻¹ (r > 0.995, n = 11) for pregnanolone, in the range 0.025 to 20 μg ml⁻¹ (r > 0.997, n = 9) for ORG 21465 and in the range 0.025 to 10 μg ml⁻¹ (r > 0.990, n = 17) for alphaxalone. For pregnanolone the intra-assay coefficients of variation at 0.5 and 5.0 μg ml⁻¹ were 9 and 12% (n = 15), for ORG 21465 the intra-assay coefficients of variation at 0.1 and 1.0 μg ml⁻¹ were 12 and 6% (n = 5), and for alphaxalone the intra-assay coefficients of variation for 0.25 and 2.5 μg ml⁻¹ were 6 and 8% (n = 10). The interassay coefficients of variation were 10 and 12% (n = 29) for pregnanolone, 16 and 5% (n = 23) for ORG 21465, and 16 and 12% (n = 28) for alphaxalone, respectively.

ORG 20599 plasma concentrations were determined using HPLC with mass spectrometry detection. To the plasma samples, 50 μl of 1.5 μg ml⁻¹ ORG 21465 as internal standard and 500 μl of 0.1 M acetic acid buffer, pH 4, were added. Extraction was performed with a mixture of 5 ml of petroleum/ether (40:60) and dichloromethane (55:45, v/v). The mixture was vortexed for 5 min and subsequently centrifuged for 15 min at 4500g. The samples were placed at −20°C to freeze the water phase. The organic phase was transferred to a clean tube and evaporated under reduced pressure at 37°C. The residue was dissolved in 100 μl of mobile phase of which 50 μl was injected into the liquid chromatography system with mass spectrometry detection. The mobile phase consisted of a mixture of methanol and water (80:20, v/v) and 1% (v/v) acetic acid. The system consisted of a Broma solvent delivery pump, set at a flow rate of 1.0 ml min⁻¹ (LKB, Upsala, Sweden); a 717plus Autosampler (Waters); and a triple state quadrupole (T.S.Q.) 700 mass spectrometer (Thermo Finnigan, San Jose, CA). Positive electrospray was used as ionization method with a sheath flow of 1 μl min⁻¹ with MeOH/H₂O (80:20, v/v) and 1% (v/v) acetic acid. Chromatography was performed on a lichroma Rosil NH2 column (156 × 4.6-mm i.d.; Alltech Associates, Deerfield, IL). Components of [M + H] at 438.5 and 446.5 Da were quantified. Run-time was 5 min. The limit of quantification was 0.05 μg ml⁻¹. Linear calibration curves were obtained in the range of 0.1 to 10 μg ml⁻¹ (r > 0.976, n = 5). The recovery of ORG 20599 was 90% at concentrations of 0.25 to 5 μg ml⁻¹. The intra-assay coefficients of variation at 0.5 and 5.0 μg ml⁻¹ were 7 and 6% (n = 5), whereas the interassay coefficients of variation were 13 and 11% (n = 18), respectively.

**Protein Binding.** Plasma protein binding was determined in vivo after administration of 5 mg kg⁻¹ pregnanolone and 10 mg kg⁻¹ ORG 21465. For each dose three rats were used. The protein binding for alphaxalone has been determined previously (Visser et al., 2002). At two time points after the administration of the neuroactive steroids 2-ml blood samples were drawn and collected in heparinized glass tubes. After the second sample the rats were directly sacrificed.

The tubes were centrifuged for 10 min at 5000 rpm to collect plasma. From each tube, two plasma samples of 50 μl were taken and the remaining plasma was centrifuged at 37°C (15 min, 2000 relative centrifugial field) using an ultrafiltration device (Centrifree; Millipore Corporation, Bedford, MA). Two samples of 100 to 400 μl of ultrafiltrate were taken.

Plasma protein binding of ORG 20599 was determined in vitro by adding 5, 15, 30, and 50 μg of ORG 20599 to 2 ml of plasma (n = 3/concentration). After equilibration of the mixtures for 30 min at 37°C, two plasma samples of 50 μl were taken from each tube, and the remaining plasma was centrifuged at 37°C using the ultrafiltrate device. Subsequently, two samples of 100 to 400 μl of ultrafiltrate were taken. After sample preparation, all plasma and ultrafiltrate samples were analyzed. The free fraction (fₚ) was calculated by dividing the free concentration in ultrafiltrate by the total (bound and free) concentration in plasma.

**Stability of ORG 20599 and ORG 21465 in Biological Fluids.** The stability of ORG 20599 and ORG 21465 in biological fluids was studied in an ex vivo experiment. Fresh rat blood was obtained by decapitation. ORG 20599 and ORG 21465 were added to 2 ml of plasma and 2 ml of blood. The decline of the concentration ORG 20599 and ORG 21465 was studied at 37 and 0°C. At fixed time points (0–30 min) samples of 100 μl were taken and stored at −20°C until analysis at the same day. In another experiment, ORG 20599 was added to 3 ml of blood for determination of the blood-plasma concentration ratio. Samples of 200 μl were taken and split. One sample was immediately hemolyzed and the second was heparinized for plasma collection. All samples were immediately assayed as described above.

**Pharmacokinetic Data Analysis.** In a population approach, the neuroactive steroid plasma concentration-time profiles of all individual rats in the different treatment groups were fitted simultaneously by explicitly taking into account both intraindividual variability in the model parameters as well as interindividual variability. A two-compartment model was selected for all compounds on the basis...
of the Akaike information criterion (Akaike, 1974). The concentration-time courses were modeled according to the following equations:

\[
\frac{dC_p}{dt} = \frac{input - Q \cdot C_p + Q \cdot C_i - CL \cdot C_p}{V_1} \tag{1}
\]

\[
\frac{dC_i}{dt} = \frac{Q \cdot C_p - Q \cdot C_i}{V_2} \tag{2}
\]

in which \(C_p\) and \(C_i\) represent the concentration of the neuroactive steroid in the compartments 1 and 2, respectively. The input = \(R_0\) for \(t < T\) and input = 0 for \(t > T\), where \(R_0\) and \(T\) are the zero order infusion rate and the duration of infusion. In these equations CL is the clearance, \(Q\) is the intracompartmental clearance, and \(V_1\) and \(V_2\) are the volumes of distribution of compartments 1 and 2.

The interindividual variability of these parameters was modeled according to an exponential equation:

\[
P_i = \theta_i \cdot \exp(\eta_i) \tag{3}
\]

where \(\theta\) is the population estimate for parameter \(P\), \(P_i\) is the individual estimate, and \(\eta_i\) the random deviation of \(P_i\) from \(P\). The values of \(\eta_i\) are assumed to be independently normally distributed with mean zero and variance \(\sigma^2\). For \(Q\) interindividual variability was fixed at zero. The residual error in the plasma drug concentration was characterized by a constant coefficient of variation error model:

\[
C_{\text{res}} = C_{\text{pi}} \cdot (1 + \varepsilon_i) \tag{4}
\]

where \(C_{\text{pi}}\) represents the \(i\)th plasma concentration for the \(i\)th individual predicted by the model. \(C_{\text{res}}\) represents the predicted concentration, and \(\varepsilon_i\) accounts for the residual deviation of the model-predicted value from the observed concentration. The value for \(\varepsilon\) was assumed to be independently normally distributed with mean zero and variance \(\sigma^2\).

The model was implemented in the ADVAN6 subroutine in NONMEM (version V, NONMEM project group, University of California, San Francisco, CA). The first-order conditional estimation method (first-order conditional estimation interaction) was used to estimate the population \(\theta, \omega^2\), and \(\sigma^2\). From individual Bayesian post hoc parameter estimates, CL, Q, V1, V2, V\text{dose}, and half-lives were calculated following standard procedures (Gibaldi and Perrier, 1982).

Subsequently, the individual Bayesian post hoc pharmacokinetic parameter estimates were used to calculate the individual neuroactive steroid plasma concentrations at the time points of EEG measurements. Hysteresis was characterized on the basis of a hypothetical effect-compartment model. In the effect compartment approach it is assumed that the rate of onset and offset of effect is governed by the rate of drug distribution to and from a hypothetical “effect site” (Sheiner et al., 1979). Under this interpretation the effect compartment model is linked to the plasma compartment by a first-order equilibration rate constant (\(k_{\text{eq}}\)) and with a first-order rate constant for drug loss (\(k_{\text{loss}}\)). The rate of change of the drug concentration in the effect compartment can then be expressed by the following differential equation:

\[
\frac{dC_e}{dt} = k_{\text{eq}} \cdot C_p - k_{\text{loss}} \cdot C_e \tag{5}
\]

where \(C_p\) represents the plasma concentration and \(C_e\) the effect-site concentration (see eqs. 1 and 2). Under the assumption that in equilibrium the effect site concentration equals the plasma concentration, this equation can be simplified to the following:

\[
\frac{dC_e}{dt} = k_{\text{loss}} \cdot (C_p - C_e) \tag{6}
\]

The \(k_{\text{eq}}\) was calculated nonparametrically using the program keo.obj.exe (S. J. Shafer, Palo Alto VA Medical Center, Stanford University, Palo Alto, CA). In the subsequent PK/PD analysis, the individual pharmacokinetic parameters (CL, Q, V1, and V2) and the \(k_{\text{eq}}\) were fixed at the estimated values, and effect-site concentrations were calculated at the time points of the pharmacodynamic measurements.

**Mechanism-Based Pharmacodynamic Analysis.** Concentration-effect data from the four neuroactive steroids alphaxalone, ORG 20599, ORG 21465, and pregnanolone served as input for the subsequent pharmacodynamic analysis. The data were analyzed by a recently proposed mechanism-based model in which the effect considered a function of a stimulus resulting from the drug-receptor binding (Tuk et al., 1999; Visser et al., 2002). In this theory, the drug at the effect site produces, upon binding to the receptor, a stimulus that is followed by a cascade of signal transduction processes, leading to the ultimate response (Fig. 2).

The definition of a drug-mediated response in terms of the classical occupation theory, as proposed by Stephenson and Furchgott (Kenakin, 1997), considers the drug effect to be the result of the interaction with a specific receptor followed by a stimulus to the biological system. Thus, the interaction with the receptor yields a stimulus \(S\) according to the following formula:

\[
S = \frac{\varepsilon \cdot [R] \cdot C}{C + K_a} \tag{7}
\]

where \(S\) is a function of the concentration (\(C\)), the parameter \(\varepsilon\) is a constant that measures the capacity of a drug to initiate a stimulus from one receptor and is a strictly drug-related parameter, \([R]\) is the total number of receptors, and \(K_a\) is the equilibrium dissociation constant. Subsequently this stimulus is propagated into the ultimate effect (\(E\)); its relation to the stimulus is given by an unknown function \(f\):

\[
E = f(S) \tag{8}
\]

Receptor mediated drug responses in any given tissue depend, therefore, on 1) two quantities determined by the drug: the intrinsic efficacy \(\varepsilon\) and the equilibrium dissociation constant \(K_a\); and 2) two quantities determined by the tissue: the constant \([R]\) and function \(f\). Two adjustments to this general model have to be made to apply it to in vivo systems. First, the total amount of receptors cannot easily be measured in vivo, thereby allowing only the product of \(\varepsilon\) and \([R]\) to be estimated. This can be defined as follows:

\[
e_{\text{PD}} = \varepsilon \cdot [R] \tag{9}
\]

where \(e_{\text{PD}}\) is the in vivo efficacy. Second, the maximal stimulus achieved by the drug must be set to 1 (\(e_{\text{PD}} = 1\)), to allow an inde-
pendent estimation of $f$ and $e_{PD}$ of other compounds. The relationship between effect-site-drug concentration and effect is thus characterized by the following equation:

$$E = f(S) = f \left[ \frac{e_{PD} \cdot C}{C + K_{PD}} \right]$$

(10)

where $K_{PD}$ is the in vivo potency and $e_{PD}$ the in vivo relative efficacy. In this investigation $K_{PD}$ is defined as in vivo potency instead of in vivo affinity, because for compounds exerting the maximal effect, differences in efficacy cannot be assessed, and because efficacy has no upper limit in principle, differences in efficacy are indistinguishable from differences in affinity when the efficacy is high (Colquhoun, 1998). In the analysis of low-efficacy compounds versus the drug reaching the highest stimulus, $K_{PD}$ can reflect the in vivo affinity.

Previously, we have shown that the neuroactive steroid alphaxalone showed biphasic EEG effects, which reached at high concentrations isoelectric EEG. This implied a physiological maximum of the stimulus (i.e., the maximal stimulus is observed at an EEG effect of 0 μV; Visser et al., 2002). Therefore, the value of $e_{PD}$ of alphaxalone was fixed at 1 in eq. 10. In the current investigation, pregnanolone also revealed isoelectric EEG at the highest dosage. Although ORG 20599 and ORG 21465 did not reach the isoelectric EEG, it cannot be excluded that higher dosages of ORG 20599 and ORG 21465 also give maximal EEG effects (i.e., isoelectric EEG).

The relationship $f$ between the initial stimulus ($S$) and the observed EEG effect was parameterized on the basis of a parabolic function:

$$E = E_{\text{sup}} - a \cdot (S^2 - b^2)$$

(11)

where $E_{\text{sup}}$ represents the top of the parabola, $a$ is a constant reflecting the slopes of the parabola, $b$ is the stimulus for which the top of the parabola (i.e., the maximal effect, $E_{\text{sup}}$) is reached, and the exponent $d$ results in an asymmetry of the parabola (Fig. 2). When no drug is present the EEG is equal to its baseline value ($E_0$). Equation 11 then reduces to the following:

$$E_0 = E_{\text{sup}} - a \cdot b^2$$

(12)

Substituting eq. 12 in eq. 11 and rearranging yields the following:

$$E = E_{\text{sup}} - a \cdot ((S^2) - 2 \cdot b \cdot S^2)$$

(13)

In the present analysis, the concentration-effect relationships of the different neuroactive steroids were fitted simultaneously to identify the drug-receptor interaction and the stimulus-response relationship. The $K_{PD}$ and the $e_{PD}$ (relative to alphaxalone) were estimated for each neuroactive steroid, whereas the parameters $a$ and $d$ were estimated for the whole population. Averaged amplitudes over 40 min of individual EEG recordings before infusion served as input for individual baseline values ($E_0$).

In the previous analysis (Visser et al., 2002), it was shown that the EEG effect dropped below baseline values and reached isoelectric EEG at maximal stimulus of alphaxalone. Because in the present investigation the effects did not drop under the baseline value except for the highest dose of pregnanolone, parameter $b$ could not be identified. Therefore, $b$ was fixed at the value obtained in the previous analysis ($0.44$ ± $0.1$ (7%); Visser et al., 2002). This did not influence the estimation of exponent $d$. In the previous analysis of the stimulus-effect relationship of alphaxalone it seemed impossible to estimate the value of parameter $d$. The only a priori information about the value of $d$ was that $d > 1$ was used to describe the observed asymmetry of the parabola. Based on numerical evaluation that revealed that the value of $d$ was likely to be between 2 and 4, $d$ was fixed at 3 (Visser et al., 2002). In the present analysis, due to the availability of information on a series of different neuroactive steroids, it became possible to estimate the value of $d$ in the simultaneous analysis of all neuroactive steroids.

It was observed that low baseline EEG ($E_0$) corresponded with low values for the visually determined $E_{\text{sup}}$. The relationship between $E_0$ and $E_{\text{sup}}$ is given in eq. 12. The variation in the $E_{\text{sup}}$ was not fully explained by the variation in $E_0$ when parameters $a$ and $b$ are kept constant. Parameter $b$ determines the location of the $E_{\text{sup}}$ whereas parameter $a$ determines the height of the parabola. Therefore, it was investigated whether parameter $a$ could be estimated in a fixed relationship to the baseline ($E_0$) in vivo:

$$a = A \cdot E_0$$

(14)

where $A$ represents a constant that amplifies variation in baseline into parameter $a$.

The interindividual variability in the pharmacodynamic parameter $K_{PD}$ was modeled according to the exponential eq. 3 and for the parameter $a$ according to a constant coefficient of variation error:

$$P_i = \theta_1 \cdot (1 + \eta_i)$$

(15)

Similar to the pharmacokinetic analysis, the residual variability in the pharmacodynamics was modeled as a coefficient of variation error according to eq. 4. The first-order estimation method was used to estimate the population $\theta$, $\omega^2$, and $\sigma^2$.

**Statistical Analysis.** Goodness of fit was evaluated on basis of visual inspection of the model fits and the value of the objective function. Model selection was based on the Akaike Information Criterion (Akaike, 1974) and assessment of parameter correlation. Statistical analysis was performed using one-way analysis of variance and a Tukey-Kramer multiple comparison test. In the case of non-homogeneity, as determined by Bartlett’s test, the nonparametric Kruskall-Wallis test was used. Statistical tests were performed using InStat version 3.0 for Windows (GraphPad Software, San Diego, CA). All data are represented as mean ± S.E., and $p < 0.05$ was considered significant.

**Results**

**Pharmacokinetics.** Fig. 3 shows the observed, the individual, and population-predicted plasma concentration-time profiles and the averaged EEG effect-time profiles for 8.7 mg · kg⁻¹ ORG 21465 (A), 21 and 24 mg · kg⁻¹ ORG 20599 (B and C), 4.0 and 9.8 mg · kg⁻¹ pregnanolone (D and E), and 4.8 mg · kg⁻¹ alphaxalone (F). For each neuroactive steroid, the indi-
individual pharmacokinetic profiles were best fitted to a two-compartment model. For alphaxalone, CL and Q were described as a function of body weight, which was described in detail previously (Visser et al., 2002). The population pharmacokinetic parameter estimates for each neuroactive steroid and the corresponding inter- and intra-individual variability are summarized in Table 1. The values of the distribution and elimination half-lives were $\pm 0.01$ and $22 \pm 2 \text{ min (n = 6)}$ for ORG 21465, $0.6 \pm 0.1$ and $33 \pm 2 \text{ min (n = 15)}$ for ORG 20599, and $2.0 \pm 0.8$ and $51 \pm 1 \text{ min (n = 15)}$ for alphaxalone, respectively. The values of the volume of distribution two times larger. This was associated with a larger inter- and intra-individual variability.

The stability of 40 $\mu$g of ORG 20599 and 40 $\mu$g of ORG 21465 was studied ex vivo in 2 ml of both blood and plasma at temperatures of 37 and 0°C. ORG 21465 seemed to be stable in blood and plasma at 37 and 0°C. In contrast, the concentration of ORG 20599 rapidly declined in blood at 37°C, but not in plasma, where it seemed to be stable during at least 30 min at 37°C (data not shown). The blood-plasma concentration ratio of ORG 20599 was determined by adding 50 $\mu$g to 3 ml of blood. Each time point, samples of 200 $\mu$l were taken and split. One sample was immediately hemolyzed and the second was heparinized for plasma collection.

The free fraction in plasma was 25.0 ± 0.5% for ORG 21465 (mean ± S.E., n = 11), 3.0 ± 0.8% for pregnanolone (mean ± S.E., n = 10), and 3.2 ± 0.3% (n = 18) for alphaxalone. Plasma protein binding of ORG 20599 was determined ex vivo in plasma. No concentrations ORG 20599 were measured in the ultrafiltrate despite large volumes (400 $\mu$l). The free fraction in plasma was less than 2% (n = 12) when taking the detection limit into account.

**Pharmacodynamics and Hysteresis.** The EEG effects of the neuroactive steroids, expressed as absolute amplitude in a 11.5- to 30-Hz band versus time, revealed a biphasic pattern as shown in Fig. 3. Upon start of the infusion, the amplitude immediately increased, followed by a partial decrease. After termination of the infusion, the effect increased again to the same height and then gradually returned to baseline. The partial decrease in amplitude was correlated to a state of unconsciousness of the rats and the decrease of the amplitude was deeper with higher dosages. For the highest dose of pregnanolone, isoelectric EEG was reached during this partial decrease. Not all rats in the treatment group of 24 mg·kg$^{-1}$ ORG 20599 showed a biphasic EEG effect, presumably as a result of the lower infusion rate and as a consequence, the lower maximal plasma concentrations ($C_{\text{max}}$). Duration of the effect (from the start of infusion until the return to baseline values of the effect) was 100 min for 8.7 mg·kg$^{-1}$ ORG 21465, 35 and 40 min for 21 and 24 mg·kg$^{-1}$ ORG 20599, and 130 and 200 min for 4.0 and 9.8 mg·kg$^{-1}$ pregnanolone, respectively. For 24 mg·kg$^{-1}$ ORG 20599 and 9.8 mg·kg$^{-1}$ pregnanolone the observed values of the baseline EEG were significantly lower than for the other groups (Table 3). In control experiments, the vehicle DMSO did not affect the EEG amplitudes (data not shown).

The individual pharmacokinetic parameter estimates were used to calculate the plasma concentrations at the time points of the individual effect measurements. The derived plasma concentration-effect relationships were biphasic and showed hysteresis. For each neuroactive steroid a representative plasma concentration-EEG effect profile is shown in Fig. 4. The individual dose is depicted in the graphs. Non-parametric hysteresis minimization yielded estimates for $k_{\text{eo}}$ of $0.53 \pm 0.1 \text{ min}^{-1}$ (mean ± S.E., n = 6) for ORG 21465, $0.17 \pm 0.03 \text{ min}^{-1}$ (mean ± S.E., n = 14) for pregnanolone, and $0.26 \pm 0.06 \text{ min}^{-1}$ (mean ± S.E., n = 14) for ORG 20599, corresponding to $k_{\text{eo}}$ half-lives of 1.3, 4.1, and 2.6 min, respectively.

**Mechanism-Based Pharmacodynamic Modeling.** The mechanism-based pharmacodynamic model was fitted to the biphasic effect-site concentration-effect relationships of all individual rats simultaneously. The individual profiles for all dosages could be successfully described using the mechanism-based pharmacodynamic model, yielding population estimates for the parameters $K_{\text{PD}}$ and $e_{\text{PD}}$ for each neuroactive steroid and estimates for the system parameters $a$ and $d$. Figure 5 shows the observed and predicted effect-site concentration versus EEG-effect relationship for representative

### Table 1

Population pharmacokinetic parameter estimates for CL, Q, $V_1$, and $V_2$ with the corresponding inter-individual coefficient of variation (%) and 95% confidence interval

<table>
<thead>
<tr>
<th></th>
<th>CL (ml min$^{-1}$ kg$^{-1}$)</th>
<th>Q</th>
<th>$V_1$</th>
<th>$V_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnanolone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>71.6 ± 5.0 (22%)</td>
<td>94.9 ± 8.2 (-)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Intra-individual variation was 18% for ORG 21465, 30% for pregnanolone, 49% for ORG 20599, and 23% for alphaxalone, respectively.

<table>
<thead>
<tr>
<th></th>
<th>CL (ml min$^{-1}$ kg$^{-1}$)</th>
<th>Q</th>
<th>$V_1$</th>
<th>$V_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ORG 20599</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>358 ± 84 (39%)</td>
<td>51.2 ± 41 (-)</td>
<td>0.15 ± 0.06 (62%)</td>
<td>1.83 ± 0.18 (27%)</td>
</tr>
<tr>
<td></td>
<td>189–526</td>
<td>0–133</td>
<td>0.34 ± 0.14 (132%)</td>
<td>4.03 ± 0.50 (12%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.06–0.61</td>
<td>3.04–5.02</td>
</tr>
<tr>
<td><strong>ORG 21465</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>48.8 ± 3.9 (17%)</td>
<td>134 ± 9 (-)</td>
<td>0.21 ± 0.05 (50%)</td>
<td>1.16 ± 0.14 (17%)</td>
</tr>
<tr>
<td></td>
<td>41–57</td>
<td>116–152</td>
<td>0.10–0.31</td>
<td>0.88–1.44</td>
</tr>
<tr>
<td><strong>Alphaxalone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>178 ± 10 (15%)</td>
<td>121 ± 16 (19%)</td>
<td>0.18 ± 0.01 (24%)</td>
<td>0.66 ± 0.06 (20%)</td>
</tr>
<tr>
<td></td>
<td>158–198</td>
<td>89–153</td>
<td>0.16–0.21</td>
<td>0.54–0.79</td>
</tr>
</tbody>
</table>

CI, confidence interval.

* Population pharmacokinetic analysis of group C taken from Visser et al. (2002).
rats of each neuroactive steroid (for the same rats as in Fig. 4). The population pharmacodynamic parameter estimates are shown in Table 2. The concentration-stimulus relationship (A) and the stimulus-effect relationship (B) for the representative rats are depicted in Fig. 6. It shows that the concentration-stimulus relationships of the neuroactive steroids differ in the potency (i.e., the value of $K_p$) but not in efficacy (i.e., the value of $e_p$), because the estimates of $e_p$ of the various neuroactive steroids were not different from 1.

A unique stimulus-effect relationship was observed for all neuroactive steroids. Population estimates for $a$ and $d$ were $103 \pm 25$ (52%) and $3.36 \pm 0.7$ (−), respectively. A large interindividual variability (52%) was found for parameter $a$. For pregnanolone (9.8 mg kg$^{-1}$), this parameter was significantly lower than for the other groups. It was investigated whether this large variability could be explained by the observed differences in baseline EEG effect ($E_0$) between the groups. In this respect, it is of interest that the calculated $E_{top}$ and the baseline EEG effect ($E_0$) was lower for both 24 mg kg$^{-1}$ ORG 20599 and 9.8 mg kg$^{-1}$ pregnanolone compared with the other treatment groups (Table 3). The relationship between the baseline ($E_0$) and the predicted $E_{top}$ and the visually determined top of the effect (measured $E_{top}$) are depicted in Fig. 7B. For comparison, all individual observed and predicted stimulus-response profiles are shown in Fig. 7A. The regression coefficient shows that the $E_{top}$ is approximately 3 times $E_0$. The measured values for $E_{top}$ are slightly higher due to the imperfection of visual determination. The ratio of $a$ and $E_0$ for each dosing group is $\sim 10$. When parameter $a$ is estimated as a function of the baseline, following the

### Discussion

**Pharmacokinetics.** The pharmacokinetics of pregnanolone, alphaxalone, and ORG 21465 was successfully described by a two-compartment model with linear elimination. The various neuroactive steroids exhibit similar pharmacokinetic characteristics, such as a very short distribution half-life and an elimination half-life between 20 and 50 min. The value of the clearance is around $\sim 20$ ml min$^{-1}$, which is equal to the rat liver blood flow, suggesting hepatic elimination with a high extraction ratio (Sear, 1996; Visser et al., 2000, 2002). The volume of distribution at steady state is larger for pregnanolone ($\sim 1.8$ l kg$^{-1}$) than for alphaxalone and ORG 21465 ($\sim 0.8$ l kg$^{-1}$). Interestingly, the pharmacokinetics in rats shows great similarity to that in humans. In humans, a clearance of $\sim 25$ ml min$^{-1}$ kg$^{-1}$ and a volume of distribution at steady state between 0.7 and 2.5 l kg$^{-1}$ have been reported for pregnanolone, alphaxalone, and ORG 21465 (Hering et al., 1996; Sear, 1996; Sneyd et al., 1997a,b).

To date, no pharmacokinetic information about ORG 20599 has been reported. In the present investigation, it was shown that the clearance of ORG 20599 is more than 5 times faster and that the volume of distribution was $\sim 2$ times larger compared with the other steroids. The fast clearance of ORG 20599, which is much higher than the hepatic blood flow and the large volume of distribution might be explained by the
rapid decline of ORG 20599 in blood due to fast metabolism in tissues other than the liver. The results of the ex vivo studies suggest that components of whole blood (e.g., red blood cells) might be an important factor in metabolism of ORG 20599, because the concentration of ORG 20599 declined rapidly in whole blood at 37°C and not in plasma, in contrast to ORG 21465, which was stable at 37°C in both blood and plasma. A possible explanation for this difference is the presence of an electron-drawing chloro-substitution in ORG 20599, which could make the keto-group more reactive and vulnerable to metabolism by keto-reductase in comparison with ORG 21465. The instability of ORG 20599 in blood has not been a confounding factor in the estimation of the pharmacokinetic parameters estimates, because ORG 20599 is stable at 0°C. In these in vivo experiments, it required maximally 20 s to take the blood sample and to put it on ice to stop the breakdown of ORG 20599. It was shown that in the in vitro experiments the breakdown of ORG 20599 was less than 5% within 20 s.

The plasma protein binding for each neuroactive steroid was at least ~97%, and to our knowledge no values have been reported previously for these compounds. The protein binding of ORG 20599 was determined in plasma. Even though, no ORG 20599 could be found in the ultrafiltrate, despite high plasma concentrations.

Values for $k_{\text{on}}$ half-life were between 1 and 5 min for each compound. The value of $k_{\text{on}}$ half-life for pregnanolone was in the same range as reported for human volunteers (Hering et al., 1996).

**Mechanism-Based Pharmacokinetic-Pharmacodynamic Modeling.** The recently proposed mechanism-based pharmacodynamic model was successfully applied in this investigation. It was shown that the neuroactive steroids differ in their in vivo potency and not in their in vivo efficacy. The biphasic stimulus-effect relationship $f$ was fully characterized on the basis of a parabolic function and was similar for each neuroactive steroid. An important aspect of the present analysis is that an estimate of the exponent $d$ could be obtained on the basis of a simultaneous analysis of the data from different neuroactive steroids. In the previous analysis (Visser et al., 2002), when only data of alphaxalone were considered, the value had to be fixed to 3, which seemed to be an appropriate approximation at that time. Interestingly, the population estimate

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**Table 2**

Averaged (mean ± S.E.) Bayesian post hoc parameter estimates for each neuroactive steroid for $K_{\text{PD}}$, $e_{\text{PD}}$, $a$, and $d$ with the corresponding interindividual coefficient of variation in parentheses and 95% confidence interval.

<table>
<thead>
<tr>
<th>Neuroactive Steroid</th>
<th>$K_{\text{PD}}$ (ng/ml$^{-1}$)</th>
<th>$e_{\text{PD}}$ (ng/ml$^{-1}$)</th>
<th>$a$ (%)</th>
<th>$d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnanolone</td>
<td>157 ± 16 (44%)</td>
<td>0.97 ± 0.01 (-)</td>
<td>90 ± 10 (52%)</td>
<td>3.36 ± 0.7 (-)</td>
</tr>
<tr>
<td>ORG 20599</td>
<td>221 ± 83 (187%)</td>
<td>0.91 ± 0.04 (-)</td>
<td>101 ± 10 (52%)</td>
<td>3.36 ± 0.7 (-)</td>
</tr>
<tr>
<td>ORG 21465</td>
<td>1619 ± 208 (56%)</td>
<td>1.04 ± 0.03 (-)</td>
<td>104 ± 7 (52%)</td>
<td>3.36 ± 0.7 (-)</td>
</tr>
<tr>
<td>Alphaxalone</td>
<td>483 ± 42 (40%)</td>
<td>1.0 (fixed)</td>
<td>116 ± 6 (52%)</td>
<td>3.36 ± 0.7 (-)</td>
</tr>
</tbody>
</table>

CI, confidence interval.
of \(d (3.36 \pm 0.7)\) is very close to the previously assumed value of 3, confirming the validity of the approach.

In the present investigation a fixed relationship \((a = A \cdot E_0,\) with \(A = 9.2)\) was found between the baseline values \((E_0)\) and values of \(a,\) and thereby explaining the large interindividual variation of \(a\) (and thus in \(E_{top}\)). This confirms that the parameters of the stimulus-response relationship are not drug-related but system-related parameters (Fig. 7). The maximal EEG effect \((E_{top})\) that can be observed in vivo, before the EEG starts to decrease, is \(-3\) times the baseline. This relationship is important for future investigations where variation in baseline is observed. In this investigation, the uniform and unique shape of the stimulus-effect relationship, which is determined for the all the neuroactive steroids studied so far, indicates that this relationship is indeed a unique system related property, reflecting the generation of the response upon GABA\(_A\) receptor activation.

The neuroactive steroids differed in their in vivo potency \((K_{PD})\) and not in their in vivo efficacy. Because for high-efficacy compounds differences in efficacy are indistinguishable from differences in affinity, the \(K_{PD}\) is herein defined as the in vivo potency. It was shown that neuroactive steroids all have a relative intrinsic efficacy that was not different from the value of the \(e_{PD}\) of alphaxalone. This is in agreement with the observations that all these neuroactive steroids were able to induce anesthesia in a similar way. Furthermore, in in vitro investigations on the modulation of human recombinant GABA\(_A\) receptors in oocytes, ORG 20599, and alphaxalone differed only little in the maximal enhancement and potency (Hill-Venning et al., 1996). It seems reasonable to assume that the efficacy of neuroactive steroids cannot be higher in vivo, because the isoelectric EEG at higher dosages indicates that the physiological limit has been reached. Furthermore, anesthetics such as propofol and pentobarbital have similar EEG patterns (Mandema and Danhof, 1990; Cox et al., 1998b). In this investigation, pregnanolone was the most potent neuroactive steroid, followed by ORG 20599, alphaxalone, and ORG 21465. It is of interest to compare the values of the \(K_{PD}\) obtained in the present in vivo investigation to the values obtained in vitro. Anderson et al. (1997) have studied the inhibition of \(^{[35]}\) STBPS binding of the neuroactive steroids in vivo. In Fig. 8, these in vitro IC\(_{50}\) estimates in the in vitro receptor assays are correlated to the in vivo \(K_{PD}\). The order of the in vivo potency was similar to the

![Fig. 7](image-url)
ranking in the in vitro IC₅₀ of inhibition of [³⁵S]-TBPS binding with the values for in vivo potency. Interestingly, the rank order of the potency in vitro and in vivo is similar. Estimates for in vivo Kᵦ are 2 to 6 times higher than the values for the in vitro IC₅₀. There are several mechanisms that might explain such a difference. One possible factor is the role of protein binding as a determinant in the pharmacodynamics of the neuroactive steroids. For benzodiazepines, it is well known that effects are correlated to the unbound concentrations (Mandema et al., 1991; Hoogerkamp et al., 1996). After correction for the degree of protein binding, values of the Kᵦ, unbound are obtained that are in the same potency order but 8 to 10 times lower than the in vitro IC₅₀ (Fig. 9). This might suggest a large receptor reserve, which is not uncommon for high-efficacy agonists in vivo (Cox et al., 1998a). In this respect, however, also the allosteric modulation of the GABA<sub>A</sub> receptor by GABA needs to be considered. The in vitro experiments were performed in the presence of 0.6 μM GABA, which enhances binding and thereby lowers the IC₅₀ in the in vitro investigations. At present, it is unknown to what extent such an effect occurs also in vivo. To date, no selective radioligand has been reported for the neuroactive steroid binding site; therefore, the affinity of an allosteric ligand cannot be determined directly. Recently, van Rijn et al. (1999) have described a method to assess the allosteric interactions between the binding of GABA and GABAergic anesthetics at the GABA<sub>A</sub> receptor in vitro using molecular modeling. It was shown that ORG 20599 had an in vitro K₄ of 214 ng · ml⁻¹ and that the presence of GABA enhances the affinity. This in vitro K₄ of ORG 20599 is remarkably similar to the in vivo Kᵦ.

The mechanism-based PK/PD model for neuroactive steroids presented in this investigation could be used for the prediction of the concentration-effect relationships of other modulators of the GABA<sub>A</sub> receptor. In Fig. 9, a simulation is shown for compounds varying in efficacy. It is predicted that monophasic concentration EEG effects profiles are produced by compounds that have a relative efficacy lower than 0.7. This shows that the biphasic effect contains specific information on the intrinsic efficacy of the drug under investigation. In this respect, it is of interest that benzodiazepines typically produce monophasic concentration-effect relationships (Mandema et al., 1991, 1992). In theory, according to the present mechanism-based pharmacodynamic model, this could be explained by the fact that benzodiazepines behave partial agonists (i.e., relative intrinsic efficacy <0.7) relative to synthetic neurosteroids.
In conclusion, a novel mechanism-based model has been successfully applied to characterize the biphasic concentration-effect relationships of neuroactive steroids. It was shown that the biphasic stimulus-effect relationship has a uniform and unique shape, which was similar for each neuroactive steroid. The exponent $d$ and the relationship between baseline and $E_{\text{top}}$ could be identified. All investigated neuroactive steroids are high-efficacy modulators and differ only in their potency. The endogenous neurosteroid pregnanolone was found to be the most potent neuroactive steroid. Interestingly, this novel mechanism-based model predicts monophasic concentration-EEG effects for compounds with a $e_{\text{PD}}$ lower than 0.7.

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References


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