Quantification of Pharmacodynamic Interactions between Dexmedetomidine and Midazolam in the Rat[1]

CORNELIS J. J. G. BOL,[2] JOHN P. W. VOGELAAR,[3] JIAN-PING TANG,[4] and JAAP W. MANDEMA[5]

Departments of Anesthesia, Stanford University School of Medicine, Stanford, CA (C.J.J.G.B, J.P.W.V, J.W.M., J.-P.T.); and Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University, Leiden, the Netherlands (C.J.J.G.B.)

Accepted for publication March 15, 2000   This paper is available online at http://www.jpet.org

ABSTRACT

The pharmacodynamic (PD) interaction between the benzodiazepine agonist midazolam and the α_2-adrenergic agonist dexmedetomidine was characterized for defined measures of anesthetic action and cardiovascular and ventilatory side effects in 33 rats. For various combinations of constant plasma concentrations of midazolam (0.1–20 μg/ml) and dexmedetomidine (0.3–19 ng/ml) obtained by target-controlled infusion, the whisker reflex (WR), righting reflex (RR), startle reflex to noise (SR), tail clamp response (TC), and corneal reflex (CR) were assessed. EEG (power in 0.5–3.5-Hz frequency band), mean arterial pressure, and heart rate were recorded continuously. Blood gas values and arterial drug concentrations were determined regularly. The nature and extent of PD interaction was quantified by the model parameter synergy ($SYN < 0$, antagonism; $SYN = 0$, additivity; and $SYN > 0$, synergy). With increasing drug concentrations WR was lost first, followed by RR, SR, TC, and CR. These effects were accompanied by an increase of the EEG measure. The drug interaction was synergistic for all stimulus-response measures and the degree of synergy increased with deeper levels of central nervous system depression (SYN was 7.3, 145, 560, 374, and 1490 for WR, RR, SR, TC, and CR, respectively). The cardiovascular side effects of dexmedetomidine, evaluated at similar PD endpoints, were reduced in the presence of midazolam. Ventilatory side effects were minor for all drug combinations. The nature and extent of the PD interactions were not reflected in the EEG measure.

In clinical anesthetic practice adequate general anesthesia requires a minimum of two different classes of anesthetic drugs. A hypnotic (inhalational anesthetic, i.v. anesthetic) and an analgesic (opiate) drug are titrated to achieve adequate CNS depression. A muscle relaxant can be used to facilitate surgical procedures. This anesthetic combination is termed "balanced anesthesia" (Hug, 1990; Lemmens, 1995). Drug combinations may lead to a reduction of the dose requirements of the individual drugs necessary to produce a specific therapeutic endpoint and hence reduce the unwanted side effects associated with either drug and improve the speed of recovery. Therefore, knowledge on drug interactions, evaluated for clinical measures of therapeutic effect and unwanted side effects, is fundamental for the understanding and optimization of clinical anesthetic practice.

Many variables influence the complex relationship between dosage, plasma concentration, and drug effect. To optimize the delivery of anesthetic drugs to individual patients, it is important to distinguish between pharmacokinetic (PK) and pharmacodynamic (PD) interactions. For example, Pavlin et al. (1996) demonstrated that in the combination of propofol and alfentanil, plasma concentrations of both drugs were elevated. Bührer et al. (1994) demonstrated that the reduction in thiopental dose requirements for EEG suppression by dexmedetomidine could be completely attributed to PK factors that altered thiopental drug distribution. However, drugs may display a pure PD interaction because they interact somewhere in the chain of events from receptor occupation to pharmacological effect.

Dexmedetomidine, a selective α_2-adrenergic agonist, is being studied for potential use in anesthetic practice because of its combined analgesic, sedative, hypnotic, and anxiolytic effects (Peden and Prys-Roberts, 1992; Mizobe and Maze, 1995). Dexmedetomidine reduces the dose requirements of opioids and anesthetic agents and attenuates the hemodynamic responses to tracheal intubation and surgical stimuli. Expected and potentially serious side effects after i.v. administrations of dexmedetomidine are an initial increase in ar-

ABBREVIATIONS: CNS, central nervous system; PK, pharmacokinetic; PD, pharmacodynamic; bpm, beats per minute; RR, righting reflex; MAP, mean arterial pressure; TCI, target-controlled infusion; WR, whisker reflex; SR, startle reflex to noise; TC, tail clamp response; CR, corneal reflex; SYN, synergy; N, steepness of the curves; GABA, γ-aminobutyric acid.

terial blood pressure accompanied by bradycardia. The cardiovascular and CNS depressant effects of dexmedetomidine after i.v. administration have been characterized in rats previously (Bol et al., 1997a, 1999).

In veterinary medicine, the racemic mixture of dexmedetomidine and medetomidine was combined with midazolam and provided a rapid induction of dogs and pigs with profound sedation, moderate analgesia, and excellent muscle relaxation (Nishimura et al., 1993; Hayashi et al., 1994, 1995). Also, a profound synergy was observed for loss of righting reflex (RR), a measure of hypnosis, for the combination of dexmedetomidine and midazolam in rats (Salonen et al., 1992). In clinical practice, midazolam is often given as premedication for anesthesia for its sedative and anxiolytic properties (Reves et al., 1985).

The purpose of the present investigation was to characterize the PD interaction between midazolam and dexmedetomidine for continuous- and stimulus-response measures of CNS depression, and cardiovascular and ventilatory side effects in rats. A mathematical model was used to quantify the nature and extent of the PD interactions on the level of plasma concentrations of the drugs. EEG, which was used as a continuous measure of CNS depression, was validated as a surrogate measure of the combined anesthetic action of dexmedetomidine and midazolam.

Materials and Methods

Animals and Surgery

Thirty-three male Wistar-derived rats (361–479 g; Harlan-Sprague-Dawley, Indianapolis, IN) were studied according a protocol adhering to APS/National Institutes of Health guidelines and approved by the Stanford University Institutional Animal Care and Use Committee. The animals were individually housed under a 12-h light/dark cycle with lights on at 7:00 AM. Both laboratory chow and water were available ad libitum. An acclimatization period of at least 5 days was allowed between surgery and arrival of the animals from the vendor. Surgical procedures and the handling and monitoring of the rats during the experiments were described elsewhere in detail (Bol et al., 1999). Briefly, EEG cortical electrodes were implanted under isoflurane/O_2 anesthesia and connected to a miniature plug, which was fixed with dental cement to the skull of the rats. After at least 1 week to allow recovery from surgery, and 1 day before the start of the experiments two vascular catheters were implanted under isoflurane/O_2 anesthesia. One catheter was inserted in the jugular vein for drug and saline administration. The other catheter was inserted into the right femoral artery for arterial blood sample collection and recording of the arterial pressure waveform. To minimize the influence of stress on the PD data recording, all rats were handled and familiarized with the experimental setting on three or four occasions before the actual drug experiments. The rats were placed in a nontransparent plastic cage that allowed free but restricted movement. The rectal body temperature of the rats was measured and maintained at 37–38°C by placing the cage on a water-circulated heating pad. Experiments did not start until the resting heart rate of the rats was below 400 beats per minute (bpm) and mean arterial blood pressure (MAP) was <115 mm Hg. During the studies the rats were handled frequently to control their level of vigilance and to prevent the animals from falling asleep spontaneously. The ventilatory status of the rats was assessed regularly by arterial blood gas measurement with a Ciba-Corning 178 pH/blood gas analyzer (Ciba-Corning, Pleasanton, CA). Additional saline was infused to compensate for the diuretic actions of dexmedetomidine in the rat.

PK Procedures

In four separate studies, combinations of constant plasma concentrations of dexmedetomidine and midazolam were rapidly achieved and maintained by target-controlled infusion (TCI). Eight concentrations of one drug were targeted in a stepwise fashion and kept constant for 30 min each, whereas the other drug was maintained at the same target concentration during the entire study. In study I, dexmedetomidine was targeted alone (0.6–19 ng/ml, $n = 9$). In study II, dexmedetomidine (0.5–7 ng/ml) was targeted in the presence of 0.1 μg/ml midazolam ($n = 8$). In study III, dexmedetomidine (0.3–5 ng/ml) was targeted in the presence of 0.3 μg/ml midazolam ($n = 8$). In study IV, midazolam was targeted alone (0.5–12 μg/ml, $n = 1$; 2.5–20 μg/ml, $n = 7$). The results of dexmedetomidine administered alone have been presented previously (Bol et al., 1999). The TCI of midazolam was started 30 min before the administration of dexmedetomidine (studies II and III). The STAN-PUMP TCI system (Shafer and Gregg, 1992) uses a laptop computer interfaced with a Harvard model 22 syringe infusion pump (Harvard Apparatus, South Natick, MA). PK parameters of dexmedetomidine and midazolam to drive the TCI systems were derived previously (Bol et al., 1997a; J. W. Mandema, unpublished observations). Dexmedetomidine·HCl (kindly provided by Farmos, Turku, Finland) was administered in a 0.9% saline solution, pH 7.4, and midazolam in a 0.9% saline solution, pH 4. One or two blood samples were taken at each plasma concentration targeted to determine the actual plasma concentration achieved for each drug and to document the stability of the plasma concentrations. The number and size of the blood samples taken were dependent on the expected measured concentrations and varied from 60 to 600 μl for dexmedetomidine and 100 μl for midazolam. Blood was replaced with an equal amount of heparinized saline. The maximal amount of blood withdrawn was 3.2 ml for a typical rat of 400 g. The blood samples were transferred to heparinized tubes for centrifugation with a micro hematocrit centrifuge to determine the hematocrit and to collect plasma. The plasma samples were stored at −20°C until drug concentration analysis. Dexmedetomidine·HCl plasma concentrations were measured in triplicate by a [³H]clonidine radio receptor assay (Bol et al., 1997b). This assay has a coefficient of variation of 7.8 to 8.4% in the range of 23.7 to 592 pg for 0.2 ml of plasma. Midazolam does not interfere with binding of dexmedetomidine to the α_2-adrenergic receptor (Salonen et al., 1992). Midazolam plasma concentrations were measured by a gas chromatography method developed by S. R. Harapat (personal communication). A brief description of the method has been given by Salonen et al. (1992). The assay has a coefficient of variation of 1.6 to 2.8% in the range of 8 to 320 ng for 0.5 ml of serum.

PD and Data Management

Cardiovascular and EEG Signals. Cardiovascular and EEG signals were recorded continuously and processed as previously described (Bol et al., 1997a). Briefly, a transducer was connected via a tee to the arterial catheter and connected to a Cardiomax-II interface (Grass, Quincy, MA) that derived arterial pressures and HR from the arterial waveform. A flexible, shielded cable connection between the miniature plug on the head of the rats and the EEG machine allowed EEG signal recording. Calibration signals were run before the start of each experiment. Baseline values for cardiovascular and EEG signals were established during a 15-min period before the start of the infusions. After manual signal artifact removal, all data were averaged over periods of 5 min. The cardiovascular and EEG data for the 15- to 20-min period after targeting a new concentration level were used for further data analysis. This time delay ensured a sufficient equilibrium between plasma and effect-site concentrations (Bol et al., 1997a). The results of dexmedetomidine administered alone have been presented previously (Bol et al., 1999).

Stimulus-Response Measures. The stimulus-response data were acquired in the 20- to 30-min period after the targeting of a new drug concentration to avoid interference with the recordings of EEG

and cardiovascular signals. The responses to the following defined stimuli were tested: whisker reflex (WR), RR, startle reflex to noise (SR), tail clamp response (TC), and corneal reflex (CR). Each stimulus-response was assessed twice per targeted drug concentration, allowing sufficient time between stimuli (1–1.5 min) for hemodynamics and EEG to return to prestimulus values. A third assessment was made when the two responses did not agree. A positive WR was defined as purposeful movement of the head toward the side where the whiskers were stroked. A positive RR was defined as a spontaneous return to the rat's previous position after being turned over on its back within 15 s. A noise stimulus (i.e., a hand clap) was used to assess the presence of the SR. A modified clipboard clamp was slowly released on the tail of the rat and the latency to respond with a forceful movement of any body part was assessed. All latency values between 0 and 15 s after application of the clamp were defined as a positive response, whereas values between 15 s and a 30-s cut-off were defined as a negative response. The location of the stimulus was marked to avoid previously used portions of the tail. The corneal reflex was defined as positive when blinking occurred immediately after stroking the cornea with the tip of a paper tissue. A positive response to a stimulus was assigned a value of one ($Y = 1$) and a negative response to a stimulus was assigned a value of zero ($Y = 0$). The responses to the stimuli were correlated with the EEG effect measured in the preceding 5-min period. A detailed description of the stimulus-response assessment procedures can be found elsewhere together with the results of dexmedetomidine administered alone (Bol et al., 1999).

Data Analysis

EEG. The square root of the power in the 0.5- to 3.5-Hz frequency band (F_1-O_1 lead) was chosen as the EEG drug effect measure. The EEG data averaged over the 15- to 20-min period after targeting a new drug concentration were pooled for each study and plot versus the corresponding measured concentrations. Blood plasma concentrations were averaged if two samples were taken at a targeted concentration. The effect of dexmedetomidine and midazolam on the EEG drug effect measure was characterized with the following sigmoidal E_{max} model:

$$E = E_0 + \frac{E_{max} \cdot C_p^{\ N}}{C_p^{\ N} + EC_{50}^{\ N}} \qquad (1)$$

where E is the predicted effect at measured steady-state plasma concentration C_p, E_0 is the effect at baseline, E_{max} is the maximal effect, EC_{50} is the steady-state plasma concentration that produces 50% of the maximal effect, and N is a measure of curve steepness. In the studies II and III the EEG effect after targeting 0.1 or 0.3 μg/ml midazolam was set as E_0. The model was implemented in Matlab (MathWorks Inc., Natick, MA). An additive error model was used to characterize the residual error of the model fit to the data. The parameters of the model were assumed to follow a normal distribution. The quality of the fit was evaluated by evaluation of the standard errors on the parameter estimates, by visual examination of the model fit to the raw data and visual examination of the residual plot.

Stimulus-Response Measures. The nature and extent of the PD interaction of each stimulus-response measure were quantitated by a method based on logistic regression, with an adapted version of the software program NONMEM for categorical data (Beal et al., 1992). All "response" ($Y = 1$) and "no-response" ($Y = 0$) data from studies I to IV were combined into one data set and the probability to measure no response to a certain stimulus was determined with the following equation:

$$P(Y = 0|C_A, C_B) = \frac{\left(\frac{C_A}{EC_{50A}} + \frac{C_B}{EC_{50B}} + SYN \cdot \frac{C_A}{EC_{50A}} \cdot \frac{C_B}{EC_{50B}} \right)^N}{\left(\frac{C_A}{EC_{50A}} + \frac{C_B}{EC_{50B}} + SYN \cdot \frac{C_A}{EC_{50A}} \cdot \frac{C_B}{EC_{50B}} \right)^N + 1} \qquad (2)$$

where, in the drug combination, P is the probability of no response to a particular stimulus ($Y = 0$) at the steady-state plasma concentrations C_A and C_B, EC_{50A} and EC_{50B} are the steady-state plasma concentrations of drug A and drug B when administered alone corresponding to a 50% probability of measuring no response to the stimulus, N is a measure of curve steepness, and SYN is a factor that expresses the nature and extent of the interaction. The interaction between drug A and drug B is additive when $SYN = 0$, antagonistic when $SYN < 0$, and synergistic when $SYN > 0$. The model is similar to the model proposed by Greco et al. (1990, 1995) but adjusted for categorical data. When the probabilities to measure a certain response are related to the concentration of only drug A, under the condition that the concentrations of drug B are kept constant during the experiment, eq. 2 can be simplified into eq. 3, the more familiar sigmoidal E_{max} model:

$$P(Y = 0|C_A, C_B) = \frac{C_A^{\ N}}{C_A^{\ N} + EC_{50A,\ app}^{\ N}} \qquad (3)$$

The observed EC_{50} of drug A will be the apparent EC_{50} of that drug. This model is equivalent to a logistic regression model dependent on log C_p. The model was used to estimate the (apparent) EC_{50} for each stimulus-response measure for each study separately. Equations 2 and 3 were fitted to the data by minimizing $-2 \times$ the sum of the log of the likelihoods of all individual measures. If the parameter SYN led to a decrease of >3.84 points in the objective function ($P < .05$, χ^2 distribution, $df = 1$), it was included into the model, otherwise it was set to zero. Equation 2 was evaluated at a 50% probability level ($P = .5$) to allow the construction of P_{50}-isoboles, which resulted in the following equation:

$$\left(\frac{C_A}{EC_{50A}} + \frac{C_B}{EC_{50B}} + SYN \cdot \frac{C_A}{EC_{50A}} \cdot \frac{C_B}{EC_{50B}} \right) = 1 \qquad (4)$$

Cardiovascular and Ventilatory Measurements. HR and MAP were normalized to the percentage change from the average pooled predrug baseline value within a study. For each rat individually, HR, MAP, and ventilatory measurements (pCO_2, pO_2, O_2 saturation, and pH) were plotted versus the drug plasma concentration of the corresponding targeted level. From these plots cardiovascular and ventilatory side effects were assessed by linear interpolation at a specific PD endpoint, i.e., the group estimate of the 50% probability level of no response to a particular stimulus. Subsequently, the data from all rats of a particular study were averaged and reported.

Results

Stimulus-Response Measures. With increasing concentrations of dexmedetomidine, midazolam, and their combinations the rats first lost the WR, followed by the RR, SR, TC, and finally CR. For each of these stimulus-response measures, and for each study separately (I–IV), the pooled response ($Y = 1$) and no-response ($Y = 0$) data were converted into a continuous probability-concentration relationship with eq. 3. The top of Fig. 1, A through C, shows the responses to the five different stimuli versus steady-state plasma concentrations of dexmedetomidine or midazolam for rats in studies II to IV. The corresponding curves of the probability to measure no response to a stimulus versus steady-state plasma concentration are depicted in the bottom of the figures. Each curve can be described by an (apparent) EC_{50}, and N, a measure of curve steepness.

The results of all four studies are summarized in Table 1. The mean $\pm$ S.E. concentrations of midazolam obtained after targeting 0.1 (study II) or 0.3 μg/ml (study II) were 0.097 ± 0.001 and 0.37 ± 0.01 μg/ml, respectively. The steepnesses of
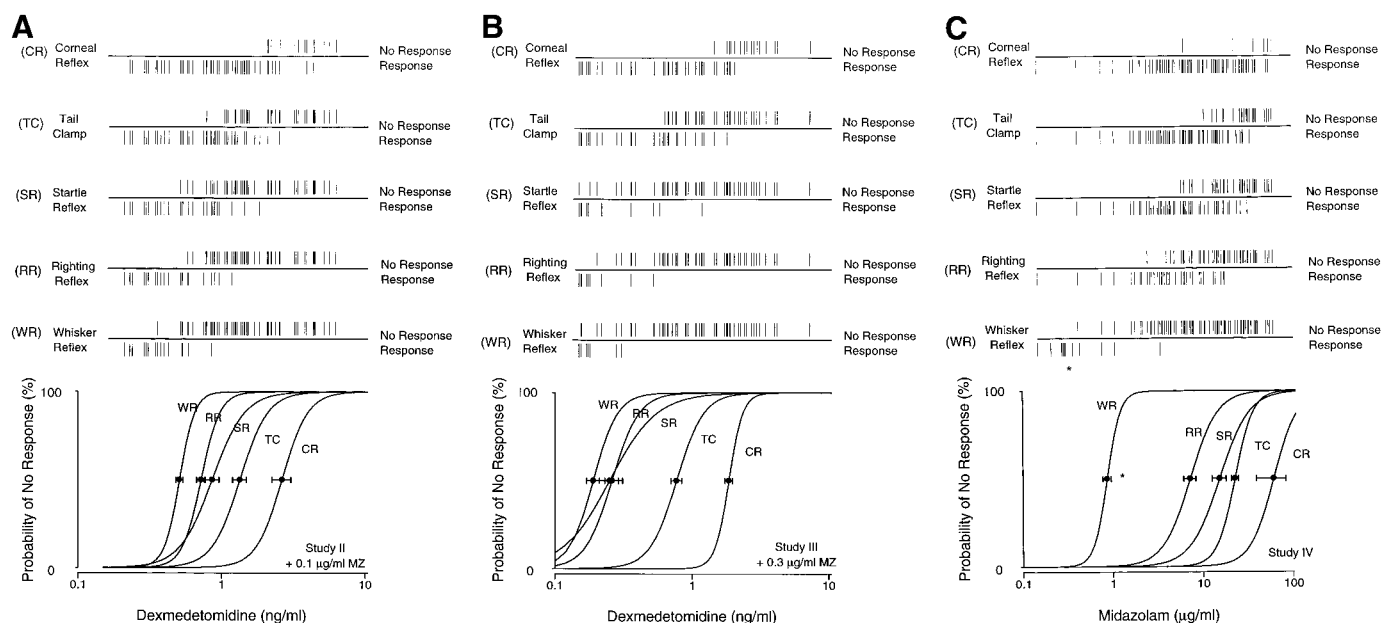
Fig. 1. A through C, relationships between steady-state plasma concentrations of dexmedetomidine and/or midazolam (MZ) and the responses to the five stimuli in the studies II to IV. The top of each figure depicts the pooled responses to the different stimuli (as indicated). A positive response to a stimulus is indicated by a tic below the horizontal lines ($Y = 1$), a negative response to a stimulus is indicated by a tic above the horizontal lines ($Y = 0$). The curves in the lower part of each figure represent the continuous relationships of the probability of no response to a particular stimulus and the steady-state plasma concentrations of dexmedetomidine and midazolam as obtained by logistic regression. The symbols indicate the estimated EC_{50} values $\pm$ S.E. for each measure. *, WR data from the midazolam baselines of the studies II and III were added to estimate the EC_{50} (C).

TABLE 1

Estimated steady-state plasma concentrations ($\pm$S.E.M.) of dexmedetomidine or midazolam in the studies I to IV, that correspond to a 50% probability of no response to a particular stimulus

	WR	RR	SR	TC	CR
Dexmedetomidine (ng/ml)					
Study I (no midazolam)	1.09 ± 0.10	2.13 ± 0.15	3.75 ± 0.64	5.49 ± 1.34	24.5 ± 12.3
Study II (0.097 ± 0.001 μg/ml midazolam)	0.51 ± 0.03	0.72 ± 0.05	0.86 ± 0.10	1.34 ± 0.15	2.63 ± 0.40
Study III (0.37 ± 0.01 μg/ml midazolam)	0.19 ± 0.02	0.26 ± 0.03	0.25 ± 0.06	0.77 ± 0.07	1.86 ± 0.12
Midazolam (μg/ml)					
Study IV	0.51 ± 0.19[a]	7.23 ± 1.12	15.3 ± 2.7	22.6 ± 2.0	60.7 ± 21.9

[a] Including midazolam baseline data from studies II and III.

the curves (N) ranged from 2.0 to 8.6 (Table 2). It was not possible to derive an EC_{50} for the WR in study IV because at the lowest targeted concentration of midazolam, most animals had already lost this reflex. However, when the WR data from the midazolam baselines of studies II and III were added (Fig. 1), an EC_{50} for this measure could be estimated.

Quantification of Drug Interactions. The nature and extent of the interaction between dexmedetomidine and midazolam were estimated for each stimulus-response measure by a pooled analysis of all response and no-response data obtained in the studies I to IV with eq. 2. The estimated parameter values are depicted in Table 3. Unlike previously

(Table 1), it was possible to reliably estimate an EC_{50} for the WR for midazolam by itself because of the additional information from the other studies, particularly the data obtained during the baselines of midazolam from the studies II and III, and from the interaction model itself. It can be seen from Table 3 that the amount of *SYN* increases with deeper levels of CNS depression.

A plot of the model-estimated probability of observing no response to a particular stimulus versus concentrations of dexmedetomidine and midazolam results in a three-dimensional response surface. A two-dimensional representation of the response surface can be obtained by an evaluation at a

TABLE 2

Estimated curve steepnesses N ($\pm$S.E.M.) for combinations of steady-state concentrations of dexmedetomidine and/or midazolam

	WR	RR	SR	TC	CR
Dexmedetomidine					
Study I (no midazolam)	7.63 ± 1.67	8.24 ± 0.99	2.62 ± 0.88	2.09 ± 0.28	1.95 ± 0.41
Study II (0.097 ± 0.001 μg/ml midazolam)	8.26 ± 1.57	6.66 ± 1.17	3.86 ± 1.01	4.49 ± 0.87	4.84 ± 1.10
Study III (0.37 ± 0.01 μg/ml midazolam)	4.93 ± 1.08	4.45 ± 1.54	2.55 ± 0.56	4.41 ± 0.62	8.58 ± 2.09
Midazolam					
Study IV	1.81 ± 0.52[a]	2.84 ± 0.22	2.64 ± 0.48	4.30 ± 0.80	3.27 ± 2.00

[a] Including midazolam baseline data from studies II and III.

TABLE 3
Synergy parameter estimates (±S.E.M.) for each stimulus-response measure determined by fitting all data of the studies I to IV simultaneously to eq. 2

	N	Dexmedetomidine EC_{50}	Midazolam EC_{50}	SYN
		ng/ml	$\mu g/ml$	
WR	3.85 ± 1.15	1.07 ± 0.10	0.86 ± 0.09	7.3 ± 1.9
RR	4.20 ± 0.42	2.12 ± 0.14	7.27 ± 1.08	145 ± 31
SR	2.69 ± 0.35	3.75 ± 0.52	15.3 ± 2.7	560 ± 175
TC	3.08 ± 0.37	4.51 ± 1.20	22.8 ± 2.1	374 ± 162
CR	3.06 ± 0.44	15.2 ± 4.0	63.1 ± 11.2	1490 ± 621

particular probability level. This is demonstrated for loss of the RR in Fig. 2. The figure shows how the interaction model fits the actual observed response and no-response data. The response surface was evaluated at the 10, 50, and 90% probability levels, indicated by the P_{10}-, P_{50}-, and P_{90}-isoboles. Data beyond midazolam concentrations of 0.6 μg/ml and dexmedetomidine concentrations of 4 ng/ml are omitted from display because of the wide range of concentrations studied. P_{50}-isoboles were constructed for all five stimulus-response measures with eq. 4 and the parameter values of Table 3, and are displayed in Fig. 3. Also indicated are the (apparent) EC_{50} values of the stimulus-response measures estimated by eq. 3 for each study separately (Table 1). The bottom right graph displays the five P_{50}-isoboles together in one graph (Fig. 3). The concentrations were scaled to units of EC_{50} of each drug when administered alone. The dashed straight line indicates an additive interaction ($SYN = 0$). The degree of deviation from the line of additivity corresponds to the degree of synergy of the interaction. The figure demonstrates that the fits of the interaction model are consistent with the results from the individual experiments.

EEG Effects. The EEG at predrug baseline recordings was characterized by low-amplitude, high-frequency signals. With increasing concentrations of dexmedetomidine administered alone (study I) or in combination with midazolam (studies II and III), an increase in slow-wave EEG activity (0.5–3.5 Hz) was observed accompanied by the loss of re-
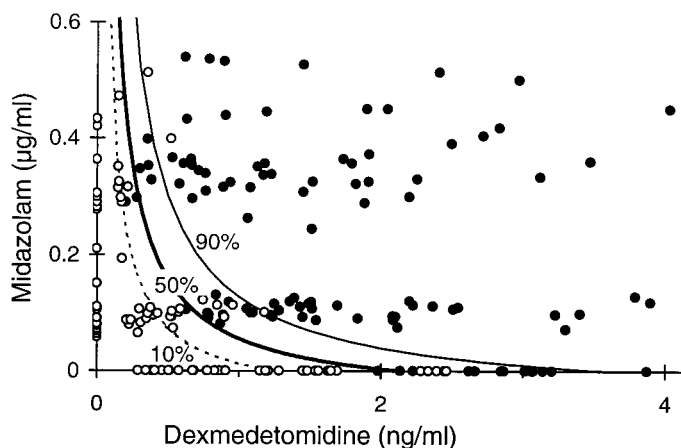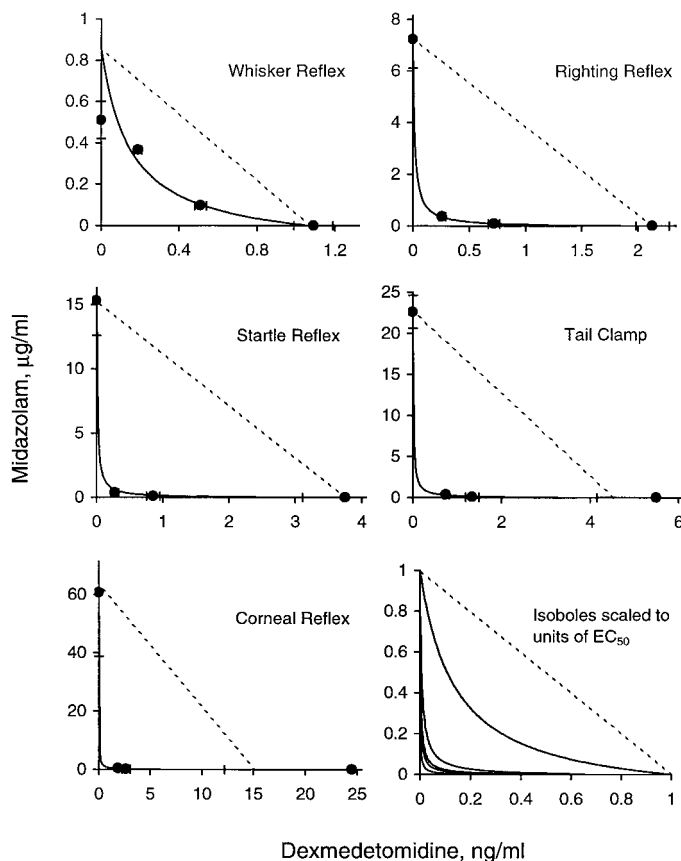

Fig. 3. Fits of the PD interaction model evaluated at the 50% probability level of no response to the five stimuli. Indicated are the (apparent) EC_{50} values ± S.E. (Table 1) of the stimulus-response measures estimated by eq. 3 for each study separately. The bottom right graph displays all fits together. The concentrations of dexmedetomidine and midazolam are scaled to units of EC_{50} of each drug when administered alone. The dashed straight line indicates an additive interaction ($SYN = 0$). The degree of deviation from the line of additivity corresponds to the degree of synergy of the interaction.

sponse to the stimulus-response measures. The changes in the EEG were similar to previously reported results on dexmedetomidine administered alone (Bol et al., 1997a). Midazolam administered alone (study IV) produced a typical increase in the high-frequency β-band (11.5–30 Hz) of the EEG (Mandema et al., 1991) in the nanograms per milliliter concentration range, accompanied by loss of response to WR. In the milligrams per milliliter concentration range midazolam induced slow-wave EEG activity that was accompanied with loss of response to RR, SR, TC, and CR. The increase in slow-wave EEG activity could be related to steady-state plasma concentrations of dexmedetomidine (studies I–III) or midazolam (study IV) with a sigmoidal E_{max} model (eq. 1). Figure 4 displays the model fits to the EEG data for the studies II to IV. Estimated parameters are depicted in Table 4. Compared with study I, significant differences ($P < .05$) were found for the EC_{50} of dexmedetomidine in the presence of 0.37 ± 0.01 μg/ml midazolam (study III) and for the maximal activity of the EEG (E_{max}) in study III. In Fig. 5, the EC_{50} values estimated for the stimulus-response measures (Table 1; except WR for midazolam, Table 3) are mapped on the predicted EEG curves.

Cardiovascular and Ventilatory Side Effects. The mean values of the pooled predrug baselines of the studies I

Fig. 2. Fit of the PD interaction model to the response (○) and no-response (●) data for loss of response to the RR for all combinations of dexmedetomidine (in nanograms per milliliter) and midazolam (in micrograms per milliliter). The data fit was evaluated at a 10% (dashed line), 50% (solid line), and 90% (thin line) probability level. Data beyond 0.6 μg/ml midazolam and 4 ng/ml dexmedetomidine are not displayed. The 10, 50, and 90% probability curves merge with the y-axis at concentrations of 4.3, 7.3, and 12.3 μg/ml.
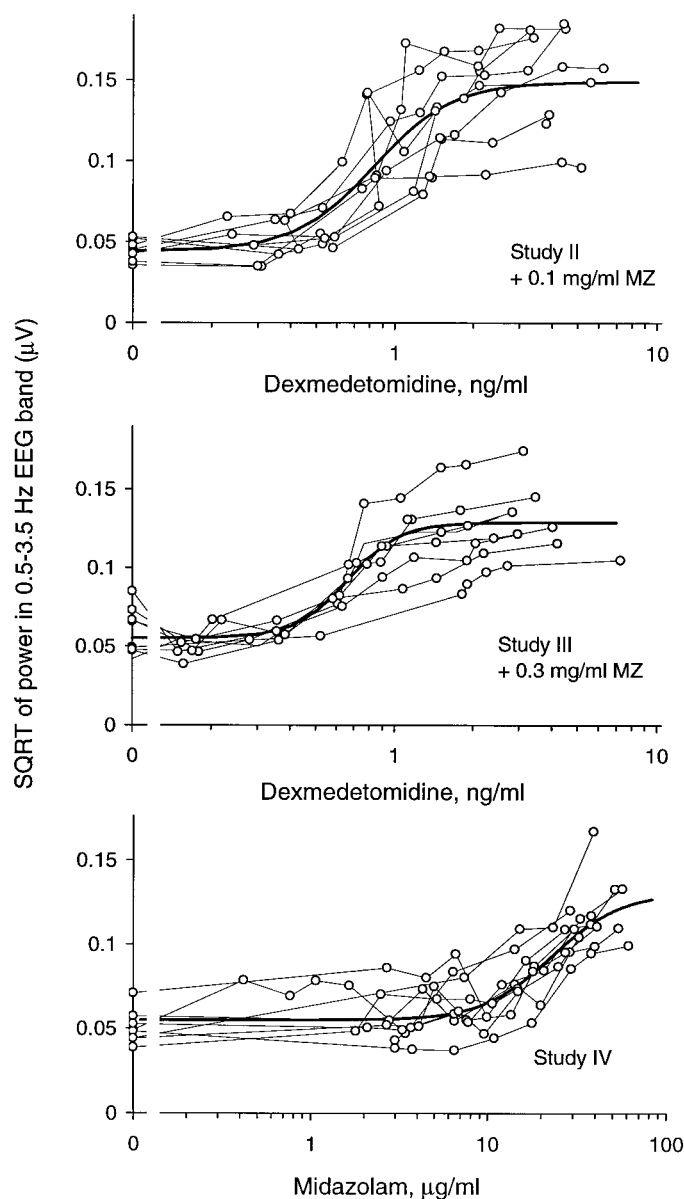
Fig. 4. EEG effect (change in square root of power in 0.5–3.5-Hz frequency band) versus steady-state plasma concentration of dexmedetomidine and/or midazolam (MZ) in the studies II to IV. The solid lines are the pooled fits to the measured EEG data. The thin lines and symbols represent the observed steady-state plasma concentration-EEG relationships for each individual rat, measured in the 15- to 20-min period after the start of a new concentration level.

to IV were 117 ± 2, 108 ± 2, 110 ± 1, and 113 ± 1 mm Hg, respectively, for MAP and 408 ± 6, 389 ± 14, 439 ± 14, and 397 ± 9 bpm, respectively, for HR. With increasing concentrations of dexmedetomidine either administered alone (study I) or in combination with midazolam (studies II and III), HR decreased and MAP increased. When midazolam was administered alone (study IV), cardiovascular side effects only occurred at the deeper levels of CNS depression. Figure 6 evaluates the cardiovascular side effects for each study, the percentage changes in HR and MAP from the mean of the pooled predrug baseline values, at the 50% probability level of no response to the WR and no response to the TC. The presence of low concentrations of midazolam in studies II and III reduced the EC_{50} of dexmedetomidine for

both stimulus-response measures (Table 1), leading to a reduction of the cardiovascular side effects. For example, at loss of response to TC for dexmedetomidine administered alone, HR was decreased by 28% and MAP was increased by 18% compared with baseline values. However, at the same behavioral endpoint, when dexmedetomidine was combined with 0.37 ± 0.01 μg/ml midazolam, HR was decreased by only 17% and MAP increased by only 8%.

Table 5 lists the ventilatory side effects for each study evaluated at the 50% probability level of no response to the TC. For all ventilatory measures changes from baseline were minor. The results of dexmedetomidine administered alone (study I) have been described in detail elsewhere (Bol et al., 1999).

Discussion

In this study, we characterized the nature and extent of the PD interaction between dexmedetomidine and midazolam for multiple pharmacological measures in the rat. It appeared that the interaction was synergistic for all stimulus-response measures (Table 3; *SYN* > 0) and the amount of synergy increased with deeper levels of CNS depression. Using the interaction model for continuous data, Greco and colleagues determined *SYN* values ranging from -0.39 to 227 (Greco et al., 1990; Gaumont et al., 1992; Faessel et al., 1996; Levasseur et al., 1996). Using the reduced interaction model (eq. 4), Vuyk et al. (1996) described for combinations of propofol and alfentanil *SYN* factors of 3.0, 3.1, and 26.5 for loss of the eyelash reflex, loss of consciousness, and response to opening of the peritoneum during surgery in patients. The *SYN* factors in our study estimated for SR, TC, and CR are beyond the highest reported value in the literature, suggesting a profound synergy for these measures. This reflects the observation that the combination of dexmedetomidine and midazolam (e.g., Table 1, study III) produces analgesia and deep levels of CNS depression at drug concentrations that only cause hypnosis (dexmedetomidine, 1.86 ng/ml) and sedation (midazolam, 0.37 μg/ml) when the drugs are administered alone.

The cardiovascular side effects of dexmedetomidine were reduced in the presence of midazolam when evaluated at two distinct PD endpoints, the 50% probability level of no response to the WR or TC (Fig. 6). Ventilatory side effects, evaluated at the 50% probability level of no response to TC, were minor in all the studied combinations (Table 5). In other species the combination of medetomidine, the racemic mixture of dexmedetomidine and levomedetomidine, and midazolam also has proven to be beneficial and is a commonly used approach in veterinary anesthesia (Nishimura et al., 1993; Hayashi et al., 1994, 1995). The combination might be useful in human clinical practice, especially with respect to the profound analgesic action and the reduction of the cardiovascular side effects of dexmedetomidine.

Many different methods have been developed to characterize the interaction between two drugs and have been reviewed by Greco et al. (1995). A widely used method is the construction of isoboles, in which the deviation from the line of additivity is a qualitative measure of the degree of interaction. Methods that quantify the degree of interaction between two pharmacologically active drugs are less frequently used. The mathematical method used in this study charac-

TABLE 4

EEG parameter estimates ($\pm$S.E.M.) for dexmedetomidine when administered alone (study I) in the presence of 0.097 ± 0.001 (study II) or 0.37 ± 0.01 μg/ml (study III) midazolam or for midazolam administered alone (study IV)

	Dexmedetomidine			Midazolam
	Study I	Study II	Study III	Study IV
E_0 (μV)	47 ± 4	44 ± 8	55 ± 4	66 ± 4
E_{max} (μV)	127 ± 7	105 ± 11	74 ± 6^a	61 ± 15
N	3.04 ± 0.64	2.87 ± 0.82	4.33 ± 1.43	3.22 ± 1.47
EC_{50} (ng/ml)	1.02 ± 0.08	0.83 ± 0.10	0.66 ± 0.05^a	23.4 ± 4.5^b

[a] Significantly different from study I.
[b] Expressed as micrograms per milliliter.

terizes the nature and extent of the PD interaction between two drugs, i.e., dexmedetomidine and midazolam, on the basis of drug concentrations rather than dose. As a consequence, changes in PK due to the drugs' mutual presence are accounted for, leading to an unbiased estimation of the degree of interaction between two drugs at the PD level.

A plot of the probability of observing no response to a particular stimulus versus concentrations of dexmedetomidine and midazolam requires a three-dimensional display. A two-dimensional representation can be obtained by an evaluation at a particular probability level (P). Figure 2 shows an evaluation at 10, 50, and 90% probability level for loss of the RR. At a 50% probability evaluation eq. 2 simplifies in eq. 4 and the steepness (N) of the underlying concentration-response relationship becomes unimportant. Figure 7 displays the P_{10}- ($P = .1$), P_{50}- ($P = .5$), and P_{90}-isoboles ($P = .9$) for loss of WR after scaling to units of EC_{10}, EC_{50}, and EC_{90}, respectively, of each drug when administered alone. The graph shows that the deviation from the line of additivity increases with increasing probability levels, indicating an increase in synergy. To increase the probability to lose a certain response a more intense CNS depression is required. A dependence of the degree of PD interaction on the intensity of drug effect was previously observed by Vuyk et al. (1996) for the interaction of propofol and alfentanil for measures of loss of consciousness and responsiveness to skin incision for surgical patients. Similarly, across the various measures of drug effect, we found the most synergy for the deepest levels of CNS depression (Table 3). Therefore, it can be postulated that the amount of synergy is related to the intensity of drug effect.

Furthermore, it can be hypothesized that if two drugs compete for the same intermediates in a shared pathway the interaction will be less effective. Thus, it seems that the degree of synergy also is related to the degree of disparity in the underlying signal transduction pathways. The profound interaction observed for dexmedetomidine and midazolam is in agreement with the known disparities in their signal transduction pathways. α_2-Adrenergic agonists can activate K^+ channels and inhibit voltage-sensitive Ca^{2+} channels (Maze and Regan, 1991), whereas benzodiazepines facilitate γ-aminobutyric acid (GABA)-mediated increase in CL^- currents through binding at the $GABA_A$-receptor complex (Haefely, 1989). Although these mechanisms are completely different, both systems should functionally converge in the CNS because α_2-adrenergic agonists and benzodiazepines, when administered alone, share similar pharmacological properties. Both compounds demonstrate sedative/hypnotic actions (Mandema and Danhof, 1992; Mizobe and Maze, 1995; Bol et al., 1997a, 1999), cause analgesia (present
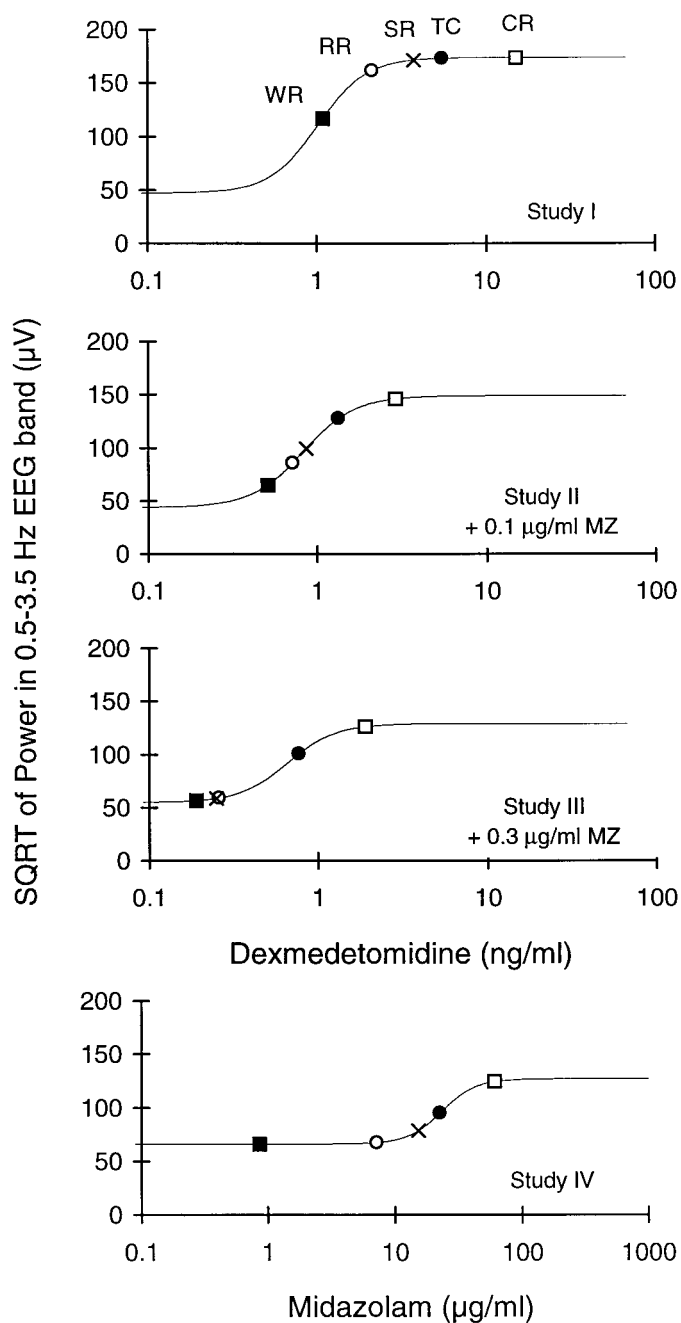

Fig. 5. EEG effect (change in square root of power in 0.5–3.5-Hz frequency band) versus steady-state plasma concentration of dexmedetomidine and/or midazolam in the studies I to IV. Lines are the fits to the pooled EEG data. For each study, (apparent) EC_{50} values estimated for the stimulus-response measures are mapped on the predicted EEG curves.
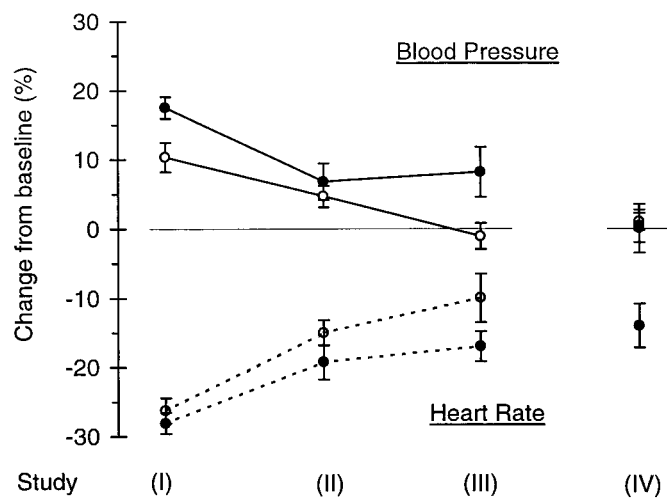
Fig. 6. Cardiovascular change from baseline (in percentages) evaluated at the 50% probability level of no response to the WR (○) and TC (●), for dexmedetomidine when administered alone (study I) or in the presence of 0.097 ± 0.001 μg/ml (study II) or 0.37 ± 0.01 μg/ml (study III) midazolam, or for midazolam administered alone (study IV).
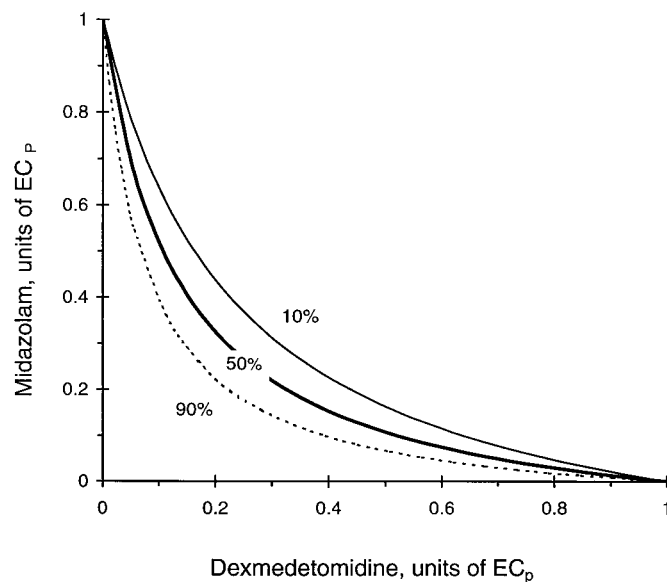

Fig. 7. Fits of the PD interaction model evaluated at a 10, 50, and 90% probability level for loss of the WR. The steady-state plasma concentrations of dexmedetomidine and midazolam are scaled to units of EC_{10}, EC_{50}, and EC_{90} (=EC_p) of each drug when administered alone.

study; Goodchild and Serrao, 1987; Mizobe and Maze, 1995), and reduce the firing rate of the nucleus locus ceruleus in vitro (Olpe et al., 1988; Berridge et al., 1993; Chiu et al., 1995).

We observed a clear separation between measures of sedation and hypnosis for midazolam (Figs. 1C and 5). RR was

lost at concentrations ~10 times higher than that of the WR. The loss of RR was followed by an increase in slow-wave EEG activity, emerging at micromolar concentrations of midazolam, which seemed associated with loss of SR, TC, and CR. Interestingly, two separate anticonvulsant effects seem to exist for midazolam in the direct cortical stimulation model (Hoogerkamp et al., 1996). The ability of midazolam to increase thresholds for localized seizure activity is much more profound in the micromolar concentration range. Micromolar binding of benzodiazepines has been reported and associated with the inhibition of voltage-sensitive Ca^{2+} uptake (Bowling and DeLorenzo, 1981; Taft and DeLorenzo, 1984; DeLorenzo, 1988). These observations could indicate that midazolam causes its more profound anticonvulsive activity, its slow-wave EEG activity, its deep levels of hypnosis, and other CNS depression activities when the GABA-sensitive-binding sites and the micromolar benzodiazepine-binding sites are activated simultaneously. However, in combination with dexmedetomidine (studies II and III) activation of the nanomolar GABA-sensitive-binding sites only is sufficient to cause such effects. The large shift in midazolam concentrations in the presence of dexmedetomidine from the microgram per milliliter range to the nanogram per milliliter range for the RR, SR, TC, and CR is expressed in the *SYN* factor. The results support the hypothesis that the degree of synergy is related to the degree of disparity in the underlying signal transduction pathways of the two drugs, i.e., the GABA-sensitive and α_2-adrenergic pathways.

Figure 5 relates the slow-wave EEG activity (0.5–3.5 Hz) to the various stimulus-response measures of CNS depression. Baseline EEG activity (E_0) increased slightly with increasing nanomolar concentrations of midazolam. Major EEG changes were observed in relation with the administration of dexmedetomidine or micromolar concentrations of midazolam. Interestingly, the E_{max} of the EEG measure seems to decrease with increasing concentration of midazolam. For dexmedetomidine by itself it appears that this EEG measure is more associated with measures of sedation and hypnosis than with deeper levels of CNS depression. However, in the presence of midazolam (studies II and III) this association with sedative and hypnotic measures seems to have changed. In study III, the rats lose their WR, RR, and SR before any appreciable EEG slow-wave activity occurs. For midazolam, the dissociation between sedative/hypnotic measures and EEG slow-wave activity was complete. For several classes of anesthetic drugs given alone, EEG-derived parameters have been related to clinical measures of sedation and hypnosis and have been suggested as surrogate measures of CNS depression (Mandema and Danhof, 1992; Stanski, 1992). However, the present data suggest that sedation, hypnosis, deeper levels of CNS depression, and EEG reflect different underlying mechanisms. The nature and extent of the interactions in the drug

TABLE 5

Ventilatory measurements (mean ± S.E.), evaluated at the 50% probability level of no response to the TC for dexmedetomidine when administered alone (study I) or in the presence of 0.097 ± 0.001 (study II) or 0.37 ± 0.01 μg/ml (study III) midazolam or for midazolam administered alone (study IV)

	Baseline	Study I	Study II	Study III	Study IV
pH	7.49 ± 0.01	7.43 ± 0.01	7.44 ± 0.01	7.46 ± 0.01	7.41 ± 0.01
pCO_2 (mm Hg)	28.8 ± 0.6	36.1 ± 0.8	38.1 ± 1.4	36.3 ± 0.8	37.2 ± 0.5
pO_2 (mm Hg)	93.1 ± 0.8	91.3 ± 1.2	86.6 ± 2.1	93.7 ± 1.0	91.0 ± 1.2
O_2 saturation (%)	97.5 ± 0.1	97.0 ± 0.1	96.5 ± 0.2	97.3 ± 0.1	96.8 ± 0.1

combinations were not reflected in the EEG measure used in this study. Based on this result, EEG slow-wave activity seems to be an inadequate measure to predict CNS depression for combinations of dexmedetomidine and midazolam. Possibly more complex EEG data analysis techniques are needed to obtain EEG measures that map to the different CNS components.

In summary, we used a mathematical model to quantitate the PD interaction between dexmedetomidine and midazolam for multiple measures of CNS depression in rats. It appeared that the interaction was synergistic for all stimulus-response measures and that the degree of synergy increased with deeper levels of CNS depression. The cardiovascular side effects of dexmedetomidine were reduced in the presence of midazolam, when evaluated as similar PD endpoints. For all combinations, the ventilatory side effects were minor. The nature and extent of the interactions were not reflected in the EEG measure. Based on this result, EEG slow-wave activity seems not an adequate measure to predict CNS depression for combinations of dexmedetomidine and midazolam.

Acknowledgments

References

Beal SL, Boeckmann AJ and Sheiner LB (1992) *NONMEM User's Guide Part I–VI*, NONMEM Project Group, University of California, San Francisco, CA.

Berridge CW, Page ME, Valentino RJ and Foote SL (1993) Effects of locus coeruleus inactivation on electroencephalographic activity in neocortex and hippocampus. *Neuroscience* **55:**381–393.

Bol CJJG, Danhof M, Stanski DR and Mandema JW (1997a) Pharmacokinetic-pharmacodynamic characterization of the cardiovascular, hypnotic, EEG and ventilatory responses of iv dexmedetomidine in the rat. *J Pharmacol Exp Ther* **283:** 1051–1058.

Bol CJJG, IJzerman AP, Danhof M, Mandema JW (1997b) Determination of dexmedetomidine in rat plasma by a sensitive [³H]-clonidine radioreceptor assay. *J Pharm Sci* **86:**822–826.

Bol CJJG, Vogelaar JPW and Mandema JW (1999) Anesthetic profile of dexmedetomidine identified by stimulus-response and continuous measurements in rats. *J Pharmacol Exp Ther* **291:**153–160.

Bowling AC and DeLorenzo RJ (1981) Micromolar affinity benzodiazepine receptors: Identification and characterization in central nervous system. *Science (Wash DC)* **216:**1247–1250.

Bührer M, Mappes A, Lauber R, Stanski DR and Maitre PO (1994) Dexmedetomidine decreases thiopental dose requirements and alters distribution pharmacokinetics. *Anesthesiology* **80:**1216–1227.

Chiu T-H, Chen M-J, Yang Y-R, Yang J-J and Tang F-I (1995) Action of dexmedetomidine on rat locus coeruleus neurons: Intracellular recording in vitro. *Eur J Pharmacol* **285:**261–268.

DeLorenzo RJ (1988) Mechanism of action of anticonvulsant drugs. *Epilepsia* **29:** S35–S47.

Faessel H, Slocum HK, Jackson RC, Boritzki T, Rustum YM and Greco WR (1996) Super *in vitro* synergy between trimetrexate and the polyglutamylatable antifolates AG2034, AG2032, AG2009 and Tomudex against human HCT-8 colon cells. *Proc Am Assoc Cancer Res Annu Meet* **37:**385.

Gaumont Y, Kisliuk RL, Parsons JC and Greco WR (1992) Quantitation of folic acid enhancement of antifolate synergism. *Cancer Res* **52:**2228–2235.

Goodchild CS and Serrao JM (1987) Intrathecal midazolam in the rat: Evidence for spinally-mediated analgesia. *Br J Anaesth* **59:**1563–1570.

Greco WR, Bravo G and Parsons JC (1995) The search for synergy: A critical review from a response surface perspective. *Pharm Rev* **47:**331–385.

Greco WR, Park HS and Rustum YM (1990) Application of a new approach for the quantitation of drug synergism of the combination of *cis*-diamminedichloroplatinum and 1-b-D-arabinofuranosylcytosine. *Cancer Res* **50:**5318–5327.

Haefely WE (1989) Pharmacology of the benzodiazepine receptor. *Eur Arch Psych Neurol Sci* **238:**294–301.

Hayashi K, Nishimura R, Yamaki A, Kim H-Y, Matsunaga S, Sasaki N and Takeuchi A (1994) Comparison of sedative effects induced by medetomidine, medetomidine-midazolam and medetomidine-butorphanol in dogs. *J Vet Med Sci* **56:**951–956.

Hayashi K, Nishimura R, Yamaki A, Kim H-Y, Matsunaga S, Sasaki N and Takeuchi A (1995) Cardiopulmonary effects of medetomidine, medetomidine-midazolam and medetomidine-midazolam-atipamezole in dogs. *J Vet Med Sci* **57:**99–104.

Hoogerkamp A, Arends RHGP, Bomers AM, Mandema JW, Voskuyl RA and Danhof M (1996) Pharmacokinetic/pharmacodynamic relationships of benzodiazepines in the direct cortical stimulation model of anticonvulsant effect. *J Pharmacol Exp Ther* **279:**803–812.

Hug CC (1990) Does opioid "anesthesia" exist? (Editorial). *Anesthesiology* **73:**1–4.

Lemmens H (1995) Pharmacokinetic-pharmacodynamic relationships for opioids in balanced anaesthesia. *Clin Pharmacokinet* **29:**231–242.

Levasseur L, Grecco WR, Rustum YM and Slocum HK (1996) Combined-action of paclitaxel and cisplatin against wildtype and resistant human ovarian carcinoma cells. *Proc Am Assoc Cancer Res Annu Meet* **37:**377.

Mandema JW and Danhof M (1992) Electroencephalogram effect measures and relationships between pharmacokinetics and pharmacodynamics of centrally acting drugs. *Clin Pharmacokinet* **23:**191–215.

Mandema JW, Tukker E and Danhof M (1991) Pharmacokinetic-pharmacodynamic modelling of the EEG effects of midazolam in individual rats: Influence of rate and route of administration. *Br J Pharmacol* **102:**663–668.

Maze M and Regan J (1991) Role of signal transduction in anesthetic action. *Ann NY Acad Sci* **625:**409–422.

Mizobe T and Maze M (1995) a2-Adrenoceptor agonists and anesthesia. *Int Anesthesiol Clin* **33:**81–102.

Nishimura R, Kim H-Y, Matsunaga S, Hayashi K, Tamura H, Sasaki N and Takeuchi A (1993) Sedative effect induced by a combination of medetomidine and midazolam in pigs. *J Vet Med Sci* **55:**717–722.

Olpe H-R, Steinmann MW, Hall RG, Brugger F and Pozza MF (1988) GABA_A and GABA_B receptors in locus coeruleus: Effects of blockers. *Eur J Pharm* **149:**183–185.

Pavlin DJ, Coda B, Shen DD, Tschanz J, Nguyen Q, Schaffer R, Donaldson G, Jacobson RC and Chapman CR (1996) Effect of combining propofol and alfentanil on ventilation, analgesia, sedation, and emisis in human volunteers. *Anesthesiology* **84:**23–37.

Peden CJ and Prys-Roberts C (1992) Dexmedetomidine: A powerful new adjunct to anaesthesia. *Br J Anaesth* **68:**123–125.

Reves JG, Fragen RJ, Vinik RH and Greenblatt DJ (1985) Midazolam. Pharmacology and uses. *Anesthesiology* **62:**310–324.

Salonen M, Reid K and Maze M (1992) Synergistic interaction between a2-adrenergic agonists and benzodiazepines in rats. *Anesthesiology* **76:**1004–1011.

Shafer SL and Gregg KM (1992) Algorithms to rapidly achieve and maintain stable drug concentrations at the site of drug effect with a computer-controlled infusion pump. *J Pharmacokinet Biopharm* **20:**147–169.

Stanski DR (1992) Pharmacodynamic measurement and modeling of anesthetic depth, in *The In Vivo Study of Drug Action* (van Boxtel CJ, Holford NHG and Danhof M eds) pp 155–179, Elsevier Publishing Co., Amsterdam.

Taft WC and DeLorenzo RJ (1984) Micromolar-affinity benzodiazepine receptors regulate voltage-sensitive calcium channels in nerve terminal preparations. *Proc Natl Acad Sci USA* **81:**3118–3122.

Vuyk J, Engbers FHM, Burm AGL, Vletter AA, Griever GER, Olofson E and Bovill JG (1996) Pharmacodynamic interaction between propofol and alfentanil when given for induction of anesthesia. *Anesthesiology* **84:**288–299.

Send reprint requests to: Cornelis J.J.G. Bol, Ph.D., Department of Clinical Pharmacokinetics, Janssen Pharmaceutica, B-2340 Beerse, Belgium. E-mail: kbol@janbe.jnj.com