Pharmacokinetic-Pharmacodynamic Characterization of the Cardiovascular, Hypnotic, EEG and Ventilatory Responses to Dexmedetomidine in the Rat

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Accepted for publication August 22, 1997

ABSTRACT

This study characterizes the pharmacokinetic-pharmacodynamic (PK-PD) relationships of the cardiovascular, EEG, hypnotic and ventilatory effects of the alpha-2 adrenergic agonist dexmedetomidine in rats. Dexmedetomidine was administered by a single rapid infusion (n = 6) and by an infusion regimen of gradually increasing rate (n = 8). HR, mean arterial pressure (MAP) and EEG signals were recorded continuously, as was the time at which the rats woke up spontaneously from drug-induced sleep, a measure of hypnosis. Arterial concentrations of dexmedetomidine and blood gases were determined regularly. A sigmoidal E\textsubscript{max} model was used to describe the HR, MAP and EEG concentration-effect relationships, with the EEG effect (activity in 0.5–3.5-Hz frequency band) linked to an effect-site model. The PK of dexmedetomidine could be described by a two-compartment model, with similar PK parameters for both infusion regimens. Plasma protein binding was 84.1[0.7]%\textsuperscript{1}. Because of complex cardiovascular homeostatic reflex mechanisms, HR and MAP could only be analyzed during gradually increasing infusions. The maximal decrease in HR was 35(2)%, and the maximal increase in MAP was 37(2)%. For both infusion regimens, similar PD parameters were found for the EEG and the hypnotic measure. These data suggest the absence of active metabolites or tolerance of the EEG and hypnotic effects. Judging on the basis of concentrations of dexmedetomidine (mean (S.E.M.)), HR decrease was the most sensitive response [EC\textsubscript{50} of 0.65(0.09) ng/ml], followed by increase in MAP [EC\textsubscript{50} of 2.01(0.14) ng/ml], change in EEG activity [EC\textsubscript{50} of 2.24(0.16) ng/ml] and the hypnotic measure [C\textsubscript{wake-up} of 2.64(0.10) ng/ml]. Ventilatory effects were minor.

Alpha-2 adrenergic agonists, such as clonidine, have been used in clinical practice as antihypertensive agents for almost 30 years. Recently, clonidine and a more selective alpha-2 adrenergic agonist, dexmedetomidine, have received considerable attention in anesthetic practice because of their analgesic, sedative, hypnotic and anxiolytic effects (Mizobe et al., 1995; Peden and Prys-Roberts, 1992). These drugs reduce requirements for opioids and anesthetic agents and attenuate the hemodynamic responses to tracheal intubation and surgical stimuli. Expected and potentially serious side effects after i.v. administration are an initial increase in arterial blood pressure accompanied by bradycardia. Other side effects include heart rhythm abnormalities (Bloor et al., 1992a), although studies in halothane-anesthetized dogs suggest an attenuating role for dexmedetomidine in epinephrine-induced arrhythmias (Hayashi et al., 1991). Dexmedetomidine is currently under phase III investigation for preanesthetic, intraoperative and postoperative use (Mizobe and Maze, 1995).

In order to develop a safe and rational dosing regimen for dexmedetomidine, most researchers have followed its effects over time as a function of dose in human subjects or in animals. An alternative approach is to study dexmedetomidine’s pharmacology on the basis of concentrations rather than dose to establish PK-PD relationships for its desired anesthetic effects, such as sedation and hypnosis, and its unwanted side effects, such as blood pressure increase and HR decrease. This would make it possible to predict the time-course of therapeutic and side effect profiles of dexmedetomidine for i.v. dosing strategies. However, the design of such PK-PD experiments is often restricted in human subjects, because they require the evaluation of multiple effect measures at a wide range of concentrations (e.g., Porchet et al., 1992; Scheinin et al., 1992). On the other hand, a PK-PD design of multiple effect measures is possible in the chronically instrumented rat model (Mandema and Danhof, 1990;
Ebling et al., 1991). In this animal model EEG, cardiovascular and ventilatory blood sampling to assay drug concentrations. PK-PD relationships with the EEG as a surrogate pharmacodynamic endpoint have been used to quantify the effects of opioids, barbiturates and benzodiazepines on the CNS. The EEG has been related to clinical measures of sedation and hypnosis and has been suggested as a measure of depth of anesthesia (Mandema and Danhof, 1992; Stanski, 1992; Gustafsson et al., 1996). The EEG, a noninvasive, continuous and objective measure, has not yet been applied to quantify the CNS drug effects of alpha-2 adrenergic agents.

The purpose of the present study was to characterize the PK-PD relationships of the cardiovascular, EEG, hypnotic and ventilatory effects of dexmedetomidine. Because the rate of drug administration can have an influence on the PK-PD relationships of these effects, we studied both a regimen of single rapid infusion (Study I) and a regimen of five consecutive infusions of gradually increasing rate (Study II). For instance, a fast infusion of dexmedetomidine could lead to a sudden increase in blood pressure, thereby triggering baroreceptor reflex mechanisms, which can lead to a reflex bradycardia. Different infusion regimens might also trigger the development of acute tolerance to the induced drug effects, and the presence of active drug metabolites might contribute to the measured effects. The design of Study II enabled us to separate dexmedetomidine's bradycardic actions from its blood-pressure-increasing actions during infusions of drug. During washout of the drug (Study I, II) the times at which the rats woke up spontaneously from drug-induced sleep were recorded and correlated with the EEG measure. This related the EEG as a continuous measure of CNS drug effect to a measure of sedation and hypnosis.

Materials and Methods

Animals and surgery. Fourteen male Wistar-derived rats (319–437 g, Harlan-Sprague-Dawley, Indianapolis, IN) were studied according to a protocol consistent with APS/NII guidelines and approved by the Stanford University IACUC. The animals were individually housed, in a 12-hr day/night schedule with lights on at 7 a.m. Both laboratory chow and water were available ad libitum. An acclimatization period of at least 5 days was allowed between arrival of the animals and surgery. For the measurement of EEG signals, cortical electrodes were implanted under isoflurane/O2 anesthesia and connected to a miniature plug, which was fixed with dental cement to the skull of the rats (Mandema and Danhof, 1990; Ebling et al., 1991). Postoperative pain relief was provided by a single injection of ketoprofen (Mandema and Danhof, 1992). Pain relief was provided by a single injection of ketoprofen (Mandema and Danhof, 1992). Pain relief was provided by a single injection of ketoprofen (Mandema and Danhof, 1992). Pain relief was provided by a single injection of ketoprofen (Mandema and Danhof, 1992). Pain relief was provided by a single injection of ketoprofen (Mandema and Danhof, 1992).

Animal handling and monitoring. To minimize the effect of stress during the PD procedures, the rats were handled and familiarized with the experimental setting on 3 or 4 occasions before the actual drug experiment. Because time-of-day-dependent PD profiles of dexmedetomidine and clonidine have been observed in the rat (Seidel et al., 1995), all experiments started between 9:30 and 11:00 a.m. The rats were placed in a nontransparent plastic cage, which allowed free but restricted movement. Decrease in body temperature of up to 6°C have been described after administration of dexmedetomidine to rodents (MacDonald et al., 1991). As temperature declines, HR and blood pressure decrease, and the EEG shifts to lower frequencies (DeBoer and Tobler, 1995). To minimize such interferences, we maintained the rectal body temperature, which was measured regularly, at 37–38°C by placing the plastic box on a water circulated heating pad. Experiments did not start before the animals' cardiovascular measures were normal (HR below 400 bpm and MAP pressure below 115 mm Hg). The rats were handled frequently during the studies to control their level of vigilance and to prevent them from falling asleep spontaneously. The animals' ventilatory status was assessed regularly by blood gas measurement in 40-μl arterial blood samples using a Ciba-Corning 178 pH/blood gas analyzer (Ciba-Corning, Pleasanton, CA). After drug administration, saline was infused continuously at a rate of 5 ml/hr to compensate for the diuretic actions of dexmedetomidine (Roman et al., 1979).

PK procedures. Rats received dexmedetomidine i.v. by a single 10-min infusion of 3 μg/kg/min (Study I, n = 6) or by five consecutive 10-min infusions of increasing rate: 0.1, 0.25, 0.5, 1.0 and 2.0 μg/kg/min (Study II, n = 8). Dexmedetomidine·HCl was administered in a saline solution. After the start of drug administration, arterial blood samples were collected in heparinized tubes at 0, 2, 5, 10, 12, 15, 25, 40, 70, 130 and 210 min (Study I) or 10, 20, 23, 30, 40, 43, 50, 53, 57, 65, 85, 110, 160 and 230 min (Study II). Different volumes of blood were withdrawn (100–600 μl) to provide sufficient drug for detection. Blood was replaced with an equal amount of heparinized saline. In each study the maximal amount of blood withdrawn was 3.2 ml for a typical rat of 400 g. At the end of the study, just before thionel euthanasia of the animals (70 mg/kg), a 1000-μl blood sample was drawn for protein binding determination of dexmedetomidine. The blood samples were transferred to heparinized tubes for centrifugation using a micro hematocrit centrifuge for determination of hematocrit and collection of plasma. The plasma samples were stored at −20°C until drug concentration analysis.

Drug assay. Dexmedetomidine·HCl concentrations were measured in triplicate by a sensitive radio receptor assay (Bol et al., 1997). The method is based on competition between the radioligand [3H]-clonidine and dexmedetomidine for high-affinity binding sites present in calf retina membranes. Nonspecific binding, determined with an excess of dexmedetomidine, was less than 3% of the total binding. The assay has a coefficient of variation of 8% in the range of 22.7 to 592 pg for a 200-μl plasma sample. When measured in triplicate, the lower limit of quantitation is 0.12 ng/ml for a 650-μl plasma sample.

Protein binding. The degree of protein binding of dexmedetomidine was determined by spiking 420 μl of rat plasma with 7 μl of a 1 μM dexmedetomidine solution, which resulted in drug concentrations of 4 ng/ml. Separation of drug from protein-bound drug was achieved by ultrafiltration at 37°C, using the Amicon Micropartition System (Amicon Division, Danvers, MA); the method was as described by Mandema et al. (1991). A 200-μl aliquot of ultrafiltrate was analyzed for dexmedetomidine by radioreceptor assay.

PD and data management. Cardiovascular and EEG signals were recorded continuously. Base-line values were established during a 15-min period before the start of the dexmedetomidine infusion. Calibration signals were run before the start of each experiment. The arterial catheter was connected to a Electromedics MS20 Transducer (Electromedics Inc., Englewood, CO) via a miniature low-dead-volume 22-gauge tee. The side arm of this tee permitted arterial blood sampling for drug concentration measurements. The transducer was connected to a Cardiomax-II interface (Grass, Quincy, MA), which derived arterial pressures and HR from the arterial wave. A flexible, shielded cable connection between the miniature plug on the head of the rat and the EEG machine allowed EEG signal recording from two left hemisphere cortical leads: fronto-central (F1-C4) and fronto-occip-
The signals were bandpass-filtered (0.5–50 Hz) and amplified. Cardiovascular and EEG signals were passed via an AD interface to an 80486 computer and managed by the BrainWave software package (BrainWave Systems Co., Thornton, CO). All signals were sampled at 256 Hz. HR, systolic blood pressure (SBP), and MAP were averaged on-line over epochs of 4 sec and stored. EEG signals were stored at 256 Hz. Epochs of EEG (4 sec) were analyzed off-line by Fast Fourier Transform to determine the power in four different frequency bands: 0.5 to 3.5 Hz (delta), 3.5 to 7.5 Hz (theta), 7.5 to 11.5 Hz (alpha) and 11.5 to 30 Hz (beta). Subsequently, after artifact removal, the cardiovascular and EEG data were averaged over predetermined intervals. The interval duration (0.5–15 min) depended on the rate of change of the signals. The resulting data points were used as effect measures for PK-PD modeling. The time at which the rats woke up spontaneously from dexmedetomidine-induced sleep, indicated by regain of the upright sitting position of the rat, was recorded as a measure of hypnosis. The time at which the rats lost consciousness was not determined, because this would have required that we test the righting reflex of the rats, which would have interfered with the EEG and cardiovascular recordings.

Data analysis. A two-compartment PK model, parameterized in clearance (CL), initial volume of distribution (V1), volume of distribution at steady state (Vss) and intercompartmental clearance (CLint), was fitted to the dexmedetomidine concentration vs. time profiles using the software program NONMEM (Beal et al., 1992). Residual error was modeled assuming a log-normal distribution of the concentration measurements. The Log Likelihood criterion and visual inspection of the fits were used for model selection. Distribution half-life (t1/2α) and terminal half-life (t1/2β) were calculated from the estimated PK parameters by standard procedures (Gibaldi and Perrier, 1982).

The observed EEG effect, the square root of the power in the 0.5 to 3.5-Hz frequency band, was linked via an effect-compartment model (Sheiner et al., 1979) and a sigmoidal E_{max} model to the predicted blood plasma concentrations of the individual rats:

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

where E is the predicted effect at effect-site concentration C_s, E_0 is the effect at base line, E_{max} is the maximal effect, EC_{50} is the effect-site plasma concentration that produces 50% of the maximal effect and n is a measure of curve steepness. The effect-compartment approach assumes a first-order equilibrium delay or hysteresis between plasma concentrations and concentrations at the site of action (effect site). The k_α is the first order-rate constant that quantifies this equilibrium delay (Sheiner et al., 1979), and t_{1/2,k_α} represents the time necessary to reach 50% of equilibrium between plasma and effect-site concentrations. The effect-site concentration-effect relationship derived by the effect-compartment approach is considered equivalent to a plasma concentration-effect relationship obtained under steady-state conditions.

It was not possible to characterize completely the cardiovascular responses after dexmedetomidine administrations (Study I, II), because homeostatic mechanisms complicated the induced cardiovascular responses, interfering with dexmedetomidine’s concentration-effect relationships. The pooled cardiovascular data (MAP, HR) during infusions of gradually increasing rate (Study II, n = 5) could be described using a sigmoidal E_{max} model.

All PD models were implemented in Matlab (MathWorks Inc., Natick, MA). An additive error model was used to characterize the residual error of the model fitted to the PD data. Estimates of asymptotic standard errors of the model parameters were obtained from the Fisher information matrix. All reported data are expressed as mean ± S.E.M. For statistical comparison between data sets, we used Student’s t test for paired or unpaired data, assuming equal variances (P < .05).

**Pharmacokinetics.** The upper graph of figure 1 shows the dexmedetomidine plasma concentration-time profile after a 10-min i.v. infusion of 3 μg/kg/min for all rats of Study I (n = 6). The lower graph of figure 1 shows the dexmedetomidine plasma concentration-time profile after five consecutive 10-min i.v. infusions of increasing rate (0.1, 0.25, 0.5, 1.0 and 2.0 μg/kg/min) for all rats of Study II (n = 8). The average concentrations of dexmedetomidine attained at the end of the infusion periods were 16.7 ± 1.3 ng/ml (Study I) and 15.4 ± 2.7 ng/ml (Study II). Each solid line in figure 1 represents the best fit of the PK model to the measured concentrations based on the means of the individual parameter estimates. A two-compartment model was chosen to describe the data of each study on the basis of the Log Likelihood criterion and visual inspection of the fits. The values of the pharmacokinetic parameters of both studies are summarized in table 1. No significant differences were found between the two administration schemes. The percentage of dexmedetomidine unbound in the plasma was similar for both studies and averaged 15.9 ± 0.7%.

**EEG and hypnotic effects.** Figure 2 shows the characteristic EEG changes derived from the F1-O1 lead with increasing concentrations of dexmedetomidine (Study II). EEG traces of 4 sec are shown at different time-points during the infusions.
infusion. The top trace displays cortical EEG activity typical for an awake rat during base-line recording. It is characterized by low-amplitude, high-frequency signals. Increasing concentrations of dexmedetomidine produce a progressive slowing of the EEG with increased amplitude, sometimes superimposed with sleep spindles (fig. 2, left part of middle trace). The increase in this slow-wave activity (0.5–3.5 Hz), which was accompanied by low-amplitude, high-frequency signals. Increasing concentrations of dexmedetomidine produce a progressive slowing of the EEG with increased amplitude. The concentrations indicated in the figure are predicted effect-site concentrations.

This phenomenon can be seen more clearly when the EEG effect in this rat is plotted against the measured plasma concentrations of dexmedetomidine change with time after drug administration (Study II). The arrow indicates when the rat wakes up from drug-induced sleep. The figure shows that the EEG measure continues to rise after termination of the infusions, which indicates hysteresis or a disequilibrium between plasma and effect-site concentrations.

This phenomenon can be seen more clearly when the EEG effect in this rat is plotted against the measured plasma concentrations of dexmedetomidine (fig. 4). The resulting concentration-effect loop indicates counterclockwise hysteresis. The disequilibrium between plasma and effect-site concentrations could be characterized with the first-order rate constant $k_{eq}$. The resulting effect-site or steady-state concentration-response relationship of the EEG could be described by a sigmoidal $E_{max}$ model (fig. 5). Estimated EEG parameters for both administrations (Study I, II) are listed in table

### Table 1

Estimates of PK parameters for i.v. dexmedetomidine after a single 10-min infusion of 3 $\mu$g/kg/min (Study I, $n = 6$) or five consecutive 10-min infusions of increasing rate (0.1, 0.25, 0.5, 0.1 and 2.0 $\mu$g/kg/min) (Study II, $n = 8$)

<table>
<thead>
<tr>
<th></th>
<th>CL (ml/kg/min)</th>
<th>$V_{ss}$ (l/kg)</th>
<th>$V_1$ (l/kg)</th>
<th>$CL_Q$ (ml/kg/min)</th>
<th>$t_{1/2a}$ (min)</th>
<th>$t_{1/2b}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>59.4</td>
<td>3.24</td>
<td>0.36</td>
<td>98.2</td>
<td>1.52</td>
<td>57.4</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>2.8</td>
<td>0.16</td>
<td>0.02</td>
<td>6.6</td>
<td>0.08</td>
<td>3.6</td>
</tr>
<tr>
<td>Study II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>61.4</td>
<td>3.50</td>
<td>0.52</td>
<td>120.8</td>
<td>1.91</td>
<td>56.2</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>2.7</td>
<td>0.23</td>
<td>0.07</td>
<td>17.6</td>
<td>0.26</td>
<td>3.3</td>
</tr>
</tbody>
</table>

CL, clearance; $V_{ss}$, volume of distribution at steady state; $V_1$, initial volume of distribution; $CL_Q$, intercompartmental clearance; $t_{1/2a}$, distribution half-life; $t_{1/2b}$, terminal half-life.
rats displayed 50% of their maximal EEG effect. The induced sleep coincided with the concentrations at which the rats woke up from drug-infusion. The concentrations at which the rats woke up from drug-infusion (0.1 μg/kg/min) in Study II. HR decrease was more pronounced in the infusion regimen of gradually increasing rate. MAP increase was more pronounced with the rapid-infusion regimen. In both studies, cardiovascular responses during dexmedetomidine infusions differed from those during the washout phase of the drug. For example, in Study II, at plasma concentrations of about 2 ng/ml, HR is 70% of base line during infusion of dexmedetomidine, but during washout of the drug, it has returned to base line at the same concentrations. These differences could not be explained by a disequilibrium between plasma and effect-site concentrations. The characterization of the time course of drug effect was also complicated by a disproportionate increase in HR around the time the rats spontaneously woke up from drug-induced sleep (arrows in Figure 6). However, plotting all HR and MAP responses against the individual predicted dexmedetomidine concentrations during multiple-rate infusions (Study II, n = 8) revealed consistent sigmoidal relationships for these effects. A sigmoid Eq model could be fitted to each of the pooled HR and MAP responses (fig. 7) with an Eq of 108 ± 1 mm Hg, Eq of 37 ± 2%, Eq of 2.01 ± 0.14 ng/ml and n of 2.58 ± 0.19 for the increase in MAP and an Eq of 405 ± 4 bpm, Eq of −35 ± 2%, Eq of 0.65 ± 0.09 ng/ml and n of 1.14 ± 0.21 for the decrease in HR.

Ventilatory effects. The effects of dexmedetomidine on the rats’ respiratory system were minor but significant for all ventilatory measures (P < .001). Base-line values of pH, pCO2, pO2 and O2 saturation changed from 7.50 ± 0.01, 31.1 ± 1.1 mm Hg, 99.3 ± 2.1 mm Hg and 97.9 ± 0.1% to 7.43 ± 0.01, 38.1 ± 0.8 mm Hg, 86.8 ± 0.7 mm Hg and 96.9 ± 0.1%, respectively, as measured immediately after termination of the dexmedetomidine infusions (Study I, II). These ventilatory depressant effects occurred when the rats were asleep. The withdrawal of arterial samples for drug analysis and blood gas determination had little effect on the blood cell volume. Blood hematocrit values changed from 36.0 ± 0.6

2. Only Eq differed significantly between the two studies. The concentrations at which the rats woke up from drug-induced sleep coincided with the concentrations at which the rats displayed 50% of their maximal EEG effect. The t1/2 of value calculated after combination of the data sets (Study I, II) was 8.57 ± 0.94 min. In both studies (though more pronounced in Study II), the induction of and recovery from the hypnotic effects of dexmedetomidine were accompanied by behavioral excitement in the rats. This behavioral excitement was most significant after the rats woke up spontaneously from drug-induced sleep, when most rats attempted escape jumps from the experimental setting and HR increased profoundly.

**Cardiovascular effects.** Figure 6 shows the average cardiovascular response-time profile of all rats (as percent change from base line) for the rapid single infusion of dexmedetomidine (Study I) and for the infusion regimen of gradually increasing rate (Study II). The upper graph of the figure shows that immediately after the start of the rapid single infusion, HR decreased concurrently with an increase in MAP. The bottom graph of the figure shows that these effects on HR and MAP could be separated by the lowest dexmedetomidine infusion (0.1 μg/kg/min) in Study II. HR decrease was more pronounced in the infusion regimen of gradually increasing rate. MAP increase was more pronounced with the rapid-infusion regimen. In both studies, cardiovascular responses during dexmedetomidine infusions differed from those during the washout phase of the drug. For example, in Study II, at plasma concentrations of about 2 ng/ml, HR is 70% of base line during infusion of dexmedetomidine, but during washout of the drug, it has returned to base line at the same concentrations. These differences could not be explained by a disequilibrium between plasma and effect-site concentrations. The characterization of the time course of drug effect was also complicated by a disproportionate increase in HR around the time the rats spontaneously woke up from drug-induced sleep (arrows in Figure 6). However, plotting all HR and MAP responses against the individual predicted dexmedetomidine concentrations during multiple-rate infusions (Study II, n = 8) revealed consistent sigmoidal relationships for these effects. A sigmoid model could be fitted to each of the pooled HR and MAP responses (fig. 7) with an Eq of 108 ± 1 mm Hg, Eq of 37 ± 2%, Eq of 2.01 ± 0.14 ng/ml and n of 2.58 ± 0.19 for the increase in MAP and an Eq of 405 ± 4 bpm, Eq of −35 ± 2%, Eq of 0.65 ± 0.09 ng/ml and n of 1.14 ± 0.21 for the decrease in HR.

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**Table 2**

<table>
<thead>
<tr>
<th>Study</th>
<th>t₁/₂k₀ (min)</th>
<th>E₀ (V)</th>
<th>Eq* (V)</th>
<th>n</th>
<th>Eq (ng/ml)</th>
<th>Ewake-up (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>10.9</td>
<td>0.049</td>
<td>0.170</td>
<td>2.65</td>
<td>2.34</td>
<td>2.62</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>2.6</td>
<td>0.002</td>
<td>0.007</td>
<td>0.35</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean</td>
<td>6.9</td>
<td>0.047</td>
<td>0.125</td>
<td>2.75</td>
<td>2.16</td>
<td>2.66</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>3.2</td>
<td>0.001</td>
<td>0.011</td>
<td>0.27</td>
<td>0.16</td>
<td>0.17</td>
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</table>
and $39.3 \pm 0.4$ at base line to $37.8 \pm 0.4$ and $35.1 \pm 0.6$ at the end of the study (Studies I and II, respectively).

**Discussion**

In this study, we quantified by PK-PD analysis the effects on EEG of dexmedetomidine, an alpha-2 adrenergic agonist. So far, the EEG has proved to be a useful measure of drug effect for benzodiazepines, opioids and hypnotic induction agents (Mandema and Danhof, 1992; Stanski, 1992; Gustafsson et al., 1996). Increasing concentrations of dexmedetomidine produce a progressive slowing of the EEG with increased amplitude (fig. 2). Because this effect mimics the effects of opioids (Scott et al., 1985; Mandema and Wada, 1995), the change in delta activity was chosen as the CNS measure. After termination of dexmedetomidine infusions, the rats woke up spontaneously from drug-induced sleep, in a concentration range similar to the EC$_{50}$ for the EEG effect. Another hypnotic measure in rats, the righting reflex, has been shown to be mediated by alpha-2 receptors in the nucleus locus ceruleus in the brain (Correa-Sales et al., 1992; Scheinin, 1992). Wakefulness, or vigilance, is associated with an increase in the firing rate of this nucleus. Using intracellular recordings from in vitro brain slices, Chiu et al. (1995) demonstrated a complete inhibition of cell firing at concentrations of dexmedetomidine up to 30 nM. A 50% inhibition in firing rate calculated from their results would approximate concentrations of 0.5 ng/ml. In similar experiments, Jorm and Stamford (1993) reported an EC$_{50}$ of 0.2 ng/ml for the same in vitro measure. The unbound plasma concentrations of dexmedetomidine that caused a 50% change in EEG activity, and at which the rats woke up spontaneously from drug-induced sleep, were in the same concentration range (0.4 ng/ml) as for this in vitro measure. These findings suggest that the EEG measure, the change in power in the 0.5 to 3.5-Hz frequency band, may be a valid surrogate measure of alpha-2 adrenergic hypnotic activity.

The plasma/effect-site equilibration half-life for the EEG measure averaged 8.6 min. Compared to other drugs with CNS-depressing activity in the rat—for example, thiopental (Ebling et al., 1991), heptabarbital (Mandema and Danhof, 1990), midazolam, flunitrazepam and other benzodiazepines (Mandema et al., 1991) and alfentanil (Mandema and Wada, 1993)—this $t_{1/2}$ is rather long, reflecting a slow onset of CNS effect. Scheinin and co-workers (1987) described similar findings in humans. They found that after a 5-min infusion of 100 $\mu$g of dexmedetomidine, sedation was still developing and was far from maximal. In fact, peak effect, as measured by visual analog scale, occurred approximately 40 min after termination of the infusion. It seems that dexmedetomidine lacks the properties required to induce a fast hypnotic onset in humans and rats.

Different values of $E_{\text{max}}$ for the EEG measure were observed in the two studies. No correlation between $E_{\text{max}}$ and weight of the rats ($r^2 = 0.16$), or between $E_{\text{max}}$ and rectal temperature of the rats ($r^2 = 0.48$) could be found, a result that eliminated skull size, age and body temperature of the rats as possible influencing factors. A data-fitting problem is also not likely, because $E_{\text{max}}$ could be well characterized in each animal. Dexmedetomidine is known to decrease cerebral blood flow in humans and dogs (Zornow et al., 1990; 1993). It is possible that the two distinct infusion regimens of dexmedetomidine led to differences in rat brain perfusion, causing differences in the $E_{\text{max}}$ of the EEG. Identical EC$_{50}$ values for the EEG measures and the concentrations at which the rats woke up from drug-induced sleep were found for the two different regimens. This in vitro finding would be highly unlikely in the presence of active metabolites or tolerance development and seems to support data from in vitro experiments where dexmedetomidine’s major metabolites...
were devoid of alpha-2 adrenergic activity (Salonen and Elo- ranta, 1990).

Biphasic vasoactive actions have been described in human and animal subjects for alpha-2 adrenergic agents such as clonidine and dexmedetomidine (Frisk-Holmberg et al., 1984; Paalzow and Edlund, 1979; Peden and Prys-Roberts, 1992). This effect is most apparent after i.v. administration, where higher initial peak levels are achieved (Dyck et al., 1993; Bloor et al., 1992b; Kallio et al., 1989). At low concentrations, binding to alpha-2 receptors located in vasomotor centers in the brain stem is thought to cause reduction of sympathetic tone, resulting in the decrease of HR and blood pressure (Van Zwieten and Chalmers, 1994). This effect, which may be due in part to additional binding to centrally located nonadrenergic imidazoline receptors, is responsible for the drugs’ favorable hemodynamic stabilizing properties when used as adjuncts in anesthesia. After administration of dexmedetomidine, blood concentrations of norepinephrine drop dramatically (Scheinin et al., 1992; Kallio et al., 1989; Bloor et al., 1992b). This leads to a reduced stimulation of peripheral alpha-1 adrenergic receptors in the vascular bed and of beta-1 adrenergic receptors located in the heart (Van Zwieten, 1988). This may contribute to the reduction in blood pressure and HR at low drug concentrations. At higher concentrations, binding to alpha-2 adrenergic receptors in the peripheral vascular bed results in vasoconstriction and an increase in blood pressure (Van Zwieten and Chalmers, 1994).

We were not able to characterize completely the complex cardiovascular effects of dexmedetomidine. Blood pressure and HR responses during infusions differed from those during the washout phase of the drug. These differences could not be explained by a disequilibrium between plasma and effect-site concentrations. Kleinbloesem et al. (1987) have shown, for the calcium channel blocker nifedipine, that the rate of increase in plasma concentrations is a major determinant of its hemodynamic effects in humans. Divergent hemodynamic responses of gradually vs. rapidly increasing infusion regimens could well be related to differences in baroreceptor activation. It seems likely that our findings are also the result of complex cardiovascular homeostatic mechanisms. Unfortunately, the data do not allow modeling of such complexities. However, in clinical practice, it is not likely that dexmedetomidine will be administered by bolus injection or rapid infusion (Dyck et al., 1993) because of concern about sudden increases in blood pressure and baroreflex-mediated bradycardia (Bloor et al., 1992b; Kallio et al., 1989). So far, slower rates of delivery, including p.o. (Gignone et al., 1987; Segal et al., 1991), i.m. (Dyck et al., 1993; Scheinin et al., 1992) and transdermal administrations (Kivistö et al., 1994; Segal et al., 1991), have been used to avoid these unwanted side effects. Unfortunately, the slower rates of delivery preclude a fast onset of hypnotic action.

Our study showed that the HR-decreasing and MAP-increasing effects of dexmedetomidine could be separated on the basis of plasma concentrations, decrease in HR being the most sensitive, followed by increase in blood pressure. Significant reductions in blood pressure, expected for clonidine-like ligands, were not seen during the drug infusions. One possible explanation is that the concentrations of dexmedetomidine achieved were too high, triggering the hypertensive effect. Another possibility is that we did not allow enough time for the development of this centrally mediated hypoten-