Anesthetic Profile of Dexmedetomidine Identified by Stimulus-Response and Continuous Measurements in Rats

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ABSTRACT

This study characterizes the anesthetic profile of dexmedetomidine on the basis of steady-state plasma concentrations using defined stimulus-response, ventilatory, and continuous electroencephalographic (EEG) and cardiovascular effect measures in rats. At constant plasma concentrations of dexmedetomidine (range, 0.5–19 ng/ml), targeted and maintained by target-controlled infusion, the whisker reflex, righting reflex, tail reflex (to noise), tail clamp response, hot water tail-flick latency, and attenuation of heart rate (HR) increase associated with tail-flick (sympathoadrenal block) and corneal reflex, were assessed in 22 rats. EEG (power in 0.5- to 3.5-Hz frequency band), mean arterial pressure, and HR were recorded continuously. Blood gas values and arterial drug concentrations were determined regularly. The following steady-state plasma EC50 values of dexmedetomidine (mean ± S.E. nanograms per milliter) were estimated: HR decrease (0.51 ± 0.04), EEG (1.02 ± 0.08), whisker reflex (1.09 ± 0.10), sympathoadrenal block (1.85 ± 0.80), mean arterial blood pressure increase (1.99 ± 0.44), righting reflex (2.13 ± 0.15), tail-flick latency (3.65 ± 0.87), startle reflex (3.75 ± 0.64), tail clamp (5.49 ± 1.34), and corneal reflex (24.5 ± 12.3). At the EC50 value of tail clamp, ventilatory depression was minor. In rats, dexmedetomidine creates bradycardia, sedation/hypnosis, sympathoadrenal blocking effects, and blood pressure-increasing effects at plasma concentrations below 2.5 ng/ml. Higher plasma concentrations are needed to lose the startle reflex, tail-flick, tail clamp, and corneal reflex responses. Ventilatory depressant effects are minor. The applied EEG measure seems to reflect sedation/hypnosis but seems to have limited value to predict the deeper levels of analgesia and anesthesia of dexmedetomidine.

Traditionally, when inhalational anesthetic agents were used alone, depth of anesthesia has been regarded as a passage through well-defined stages where one endpoint comes before or after another (Stanski, 1990; Kissin, 1993). With the use of opioids, i.v. anesthetic agents, and their combinations, this rank order of effects can change and may even be deliberately altered in accordance with the variable goals of anesthesia. A “balanced anesthetic” is the result (Hug, 1990; Lemmens, 1995). It therefore seems impossible to determine the potency of different anesthetic actions with one measure, and so far, a general measure of “depth” of anesthesia has not been accepted (Prys-Roberts, 1987; Stanski, 1990; Hug, 1990; Kissin, 1993; Thornton and Gareth Jones, 1993). Instead, specific defined stimuli and specific responses are needed to assess the particular anesthetic state (Stanski, 1990). This approach has been used, for instance, to characterize the plasma concentrations of alfentanil required to supplement nitrous oxide anesthesia in patients (Ausems et al., 1986) and to assess the pharmacodynamics (PD) of thiopental in patients (Hung et al., 1992) and in rats (Gustafsson et al., 1996).

Recently, α2-adrenergic agonists, like dexmedetomidine, are being studied for potential use in anesthetic practice. Dexmedetomidine has analgesic, sedative/hypnotic, and anxiolytic properties (Peden and Prys-Roberts, 1992; Mizobe and Maze, 1995). As an adjuvant, it reduces anesthetic requirements and attenuates the hemodynamic responses to tracheal intubation and surgical stimuli, providing cardiovascular stability during surgery. Expected and potentially serious side effects after i.v. administration are an initial increase in arterial blood pressure accompanied by bradycardia. To date, the anesthetic profile of dexmedetomidine has not been quantified on the basis of drug concentrations. This would require an exploration of the relative potencies of...
multiple therapeutic and side effect measures of dexmedetomidine at a wide range of steady-state drug concentrations. Such a design is restricted in human subjects for safety reasons because the cardiovascular side effects of dexmedetomidine would limit the targeting of high concentrations. As an alternative, we studied the pharmacological effects of dexmedetomidine in rats.

The purpose of this investigation was to characterize the pharmacological effects of dexmedetomidine on the basis of steady-state plasma concentrations using defined stimulus-response and electroencephalographic (EEG), cardiovascular, and ventilatory effect measures in rats.

Materials and Methods

Animals and Surgery

Twenty-two male Wistar-derived rats (Harlan-Sprague-Dawley, Indianapolis, IN) were divided into two groups (study 1: \( n = 9 \), b.wt., 418 ± 13 g; study 2: \( n = 13 \), b.wt., 413 ± 12 g) and studied according to a protocol adhering to American Physiological Society/National Institutes of Health guidelines and approved by the Stanford University Institutional Animal Care and Use Committee. The animals were individually housed and maintained on a 12-h day/night schedule 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 arrival of the animals from the vendor and surgery.

One day before the start of the experiments (studies 1 and 2), two vascular catheters were implanted under isoflurane/O\(_2\) anesthesia. One catheter was inserted in the jugular vein and forwarded into the superior vena cava 0.5 cm above the right atrium for drug and saline administration. The other catheter was inserted into the right femoral artery and forwarded into the aorta about 0.5 cm from the bifurcation of the common iliac arteries for blood sample collection and recording of the arterial pressure wave. The catheters were tunneled s.c. to exit on the dorsal surface of the neck.

For the rats involved in the EEG experiments (study 1), cortical electrodes were implanted under isoflurane/O\(_2\) anesthesia at least 1 week before catheter implementation. These electrodes were connected to a miniature plug, which was affixed 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 administration of 0.1 mg/kg buprenorphine.

Animal Handling and Monitoring

All rats (studies 1 and 2) were handled and familiarized with the experimental setting on three or four occasions before the actual drug experiments to minimize the effect of stress on the PD data recording. If the rats were to be included in the tail-flick experiments (study 2), three tail-flick latencies (Jansen et al., 1963) were also determined on each occasion, to stabilize the nociceptive responses. All experiments started between 9:30 and 11:00 AM because time-of-day-dependent PD profiles of dexmedetomidine and clonidine have been observed in rats (Seidel et al., 1995). The rats were each placed in a nontransparent plastic cage, which allowed free but restricted movement. Rodent body temperature decreases up to 6\(^\circ\)C have been described after the administration of dexmedetomidine (MacDonald et al., 1991). As temperature lowers, heart rate (HR) and blood pressure decrease and the EEG shifts to lower frequencies (DeBoer and Tobler, 1995). To minimize such interferences, the rat's rectal body temperature, which was measured regularly, was maintained at 37–38\(^\circ\)C by placing the plastic cage on a water-circulating heating pad. Experiments did not start until the HR of the rats was below 400 beats/min and mean arterial blood pressure (MAP) was below 115 mm Hg. During the studies, the rats were handled frequently to control their level of vigilance and to prevent the rats from falling asleep spontaneously. The ventilatory status of the rats was assessed regularly by blood gas measurement in small (40-\(\mu\)l) arterial blood samples using a Ciba-Corning 178 pH/blood gas analyzer (Ciba-Corning, Pleasanton, CA). Additional saline was infused to compensate for the diuretic actions of dexmedetomidine (Roman et al., 1979).

Pharmacokinetic Procedures

In two separate studies, rats received dexmedetomidine i.v. by target-controlled infusion (TCI) to rapidly achieve and maintain constant plasma concentrations of dexmedetomidine. In study 1 (\( n = 9 \)), the dexmedetomidine plasma concentrations of: 0.6, 1.2, 1.8, 2.4, 3.6, 4.8, 9.6, and 19 ng/ml were targeted sequentially in each animal. Each concentration level was maintained for a period of 30 min. In study 2, rats were randomly assigned to two groups. One group of rats (\( n = 9 \)) was subdivided into three groups and targeted sequentially one of the following set of plasma concentrations: 0.5, 1, 2, and 4 ng/ml (\( n = 3 \)); 1, 3, 5, and 8 ng/ml (\( n = 2 \)); or 2, 5, 8, and 12 ng/ml (\( n = 4 \)). Each concentration level was maintained for a period of 35 min. The other group of rats (\( n = 4 \)) was administered saline over five periods of 35 min at a rate of 2 ml/h. 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). Pharmacokinetic parameters to drive the TCI system were derived previously (Bol et al., 1997a). Dexmedetomidine·HCl (kindly provided by Farmos, Finland) was administered in a 0.9% saline solution. Two arterial blood samples were taken at each targeted concentration to determine the actual achieved plasma concentrations of dexmedetomidine. To provide sufficient drug for detection the blood sample volumes ranged from 600 \(\mu\)l at the lowest targeted concentrations to 60 \(\mu\)l at the highest targeted concentrations of dexmedetomidine. The maximum amount of blood withdrawn was 3.2 ml for a typical rat of 400 g. The blood was replaced with an equal amount of heparinized saline. The blood samples were transferred to heparinized tubes for centrifugation using a microhematocrit centrifuge to determine the hematocrit and to collect the plasma. The plasma samples were stored at −20\(^\circ\)C until drug concentration analysis. Dexmedetomidine·HCl plasma concentrations were measured in triplicate with a sensitive [\(^3\)H]clonidine radioreceptor 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 a 0.2-ml plasma sample. Because two blood samples were taken at each targeted concentration level, the average of the corresponding plasma concentrations was used for correlation with the PD measures.

PD and Data Management

Study 1: Cardiovascular and EEG. Cardiovascular and EEG signals were recorded continuously. Baseline 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 an 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 rats and the EEG machine allowed EEG signal recording from two left hemisphere cortical leads: fronto-central (F\(_3\)–C\(_1\)) and fronto-occipital (F\(_7\)–O\(_1\)). The signals were band-pass 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 (SBP) and diastolic blood pressures, and MAP were averaged on-line over epochs of 4 s, and EEG signals were stored at 256 Hz. Epochs of EEG (4 s) were analyzed off-line by fast Fourier transform to deter-
mine 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). The raw digitized EEG signal was replayed on the computer screen, and epochs of EEG containing artifacts, mostly clipped EEG signals due to movement of the rats, were removed. Subsequently, the data were averaged over 5-min periods. The effect-site equilibration half-life time for the EEG effect of dexmedetomidine was previously estimated at 8.6 min (Bol et al., 1997a). To ensure sufficient equilibration between plasma and effect-site concentrations, the cardiovascular and EEG data of the 15- to 20-min period after targeting a new concentration level were used for further analysis.

**Study 1: Stimulus-Response Measures.** The stimulus-response data were acquired in the 20- to 30-min period after targeting a new concentration level to avoid interference with the EEG and cardiovascular signals. In analogy to Gustafsson et al. (1996), several of the following defined stimulus-response measures were tested sequentially: whisker reflex, loss of righting reflex, startle reflex to noise, tail clamp response, and corneal reflex. To minimize excessive stimulation, only two or three of these stimulus-response measures were physically tested at each drug concentration level. At the lowest plasma concentrations of dexmedetomidine, hand clamp, tail clamp, and corneal reflex were not tested and were assumed to be positive. Once a response was lost, it was confirmed at the next higher concentration level. It was assumed to be negative at subsequent higher concentration levels. In practice, a kind of window of two or three stimulus-response measures was moved along the concentration curve. This procedure led to a total of 16 to 24 concentration-response pairs for each rat. A positive whisker reflex was defined as purposeful movement of the head toward the side where the whiskers were stroked. A positive righting reflex 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 startle reflex. The tail of the rat was lifted with one hand, and a modified clipboard clamp was slowly released on the tail with the other hand. The latency to respond with a forceful movement of any body part was assessed. We did allow for a 30-s measuring period (i.e., a cut-off time was set at 30 s). After the completion of the experiment, all latency values between 0 and 15 s after the application of the clamp were defined as a positive response, and all latency values between 15 s and the cut-off time were defined as negative. 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. To ensure that the eyes did not dry, we applied grease to each eye after the rats had lost the corneal reflex with the tip of a paper tissue. To ensure that the eyes did not dry, we applied grease to each eye after the rats had lost the corneal reflex with the tip of a paper tissue. To ensure that the eyes did not dry, we applied grease to each eye after the rats had lost the corneal reflex with the tip of a paper tissue. To ensure that the eyes did not dry, we applied grease to each eye after the rats had lost the corneal reflex with the tip of a paper tissue. To ensure that the eyes did not dry, we applied grease to each eye after the rats had lost the corneal reflex with the tip of a paper tissue.

**TABLE 1**

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**Study 2: Tail-Flick Latencies.** The rats were lifted out of their cage and the distal two thirds of their tail was immersed in a 55°C water bath (Janssen et al., 1963). Nociception was determined as the rats’ latency (in seconds) to flick their tail or vocalize on exposure. A cut-off latency of 10 s was used to prevent damage to the tail. Before dexmedetomidine (n = 9) or saline (n = 4) administrations, tail-flick latencies were determined at 2, 12, and 22 min of a 30-min period to establish baseline values for each rat. In the four (dexmedetomidine) or five (saline) 35-min periods after baseline, tail-flick latencies were determined at 22 and 32 min. This delay ensured a sufficient equilibrium between plasma and effect-site concentrations. The total number of tail-flick measurements was 11 for the rats receiving dexmedetomidine and 13 for the rats receiving saline.

**Study 2: Sympathoadrenal Block.** Cardiovascular signals were recorded continuously during the tail-flick experiments. Procedures and data management were the same as for study 1, except that the data were not averaged over 5-min periods. When the rats’ tails were exposed to the 55°C water, MAP and HR increased, followed by tail-flick or vocalization. It was assumed that at the moment of tail-flick, the rat experienced the same degree of pain, and therefore the noxious stimulus was considered to be of equal magnitude. The ability of dexmedetomidine to block or attenuate the HR response associated with this constant stimulus was assumed to reflect the drug’s sympathoadrenal blocking actions, providing cardiovascular stability during surgery. The degree of sympathoadrenal block was defined by the percentile difference between the average HR in the 30-s period before the start of the tail-flick procedure and the HR measured 20 s after the actual flick of the tail. An overview of all the PD measures is given in Table 1.

**Data Analysis**

**Study 1: EEG and Cardiovascular.** The square root in power of the 0.5- to 3.5-Hz frequency band was chosen as the EEG measure; HR and MAP were chosen as cardiovascular measures. Data averaged over the 15- to 20-min period after targeting a new concentration level of dexmedetomidine were pooled for all animals of study 1 and plotted versus the measured plasma concentrations of those levels. The effect of dexmedetomidine on the EEG (F₁-O₁ lead), HR, or MAP was characterized using the sigmoidal E₅₀ model:

\[
E = E_{0} + \frac{E_{\text{max}} - E_{0}}{1 + \left(\frac{C_{p}}{EC_{50}}\right)^{n}}
\]

where \( E \) is the predicted effect at measured steady-state plasma concentration \( C_{p} \), \( E_{0} \) is the effect at baseline, \( E_{\text{max}} \) 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. HR

**TABLE 1**

Overview of all PD measures

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**Anesthetic Profile of Dexmedetomidine**

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and MAP were expressed as the percentage change from the mean of the pooled baseline values of all rats, leaving $E_o$ set at 0. An additive error model was used to characterize the residual error of the model fit to the data.

**Study 1: Stimulus-Response Measures.** The pooled “response” ($Y = 1$) and “no response” ($Y = 0$) data for each stimulus-response measure were converted into a continuous probability versus drug-concentration relationship via logistic regression. The following equation was used (see Appendix):

$$P(Y = 0|C_p) = \frac{C_p^n}{C_p^n + EC_{50}^n}$$  

(2)

where $P$ is the probability of no response to the stimulus ($Y = 0$) at the steady-state plasma concentration $C_p$, $EC_{50}$ is the steady-state plasma concentration with a 50% probability of no response, and $n$ is a measure of curve steepness, reflecting the interindividual variability in the response measure. The model was fitted to the data by minimizing $-2$ times the log of the sum of the likelihoods of all individual measures.

**Study 1: Ventilatory Measurements.** Blood gas values were plotted versus the measured dexmedetomidine plasma concentrations of each rat. From these curves, the individual $pCO_2$, $pO_2$, $O_2$ saturation, and pH values were determined by linear interpolation at the group estimate of the $EC_{50}$ for loss of response to the tail clamp.

**Study 2: Tail-Flick Latencies.** The tail-flick latencies (in seconds) of all animals were pooled and plotted versus the measured plasma concentrations of each animal. The antinociceptive effect of dexmedetomidine was characterized using eq. 1, in which $E_{\text{max}}$ was substituted by the cut-off latency (10 s) minus the latency at baseline ($E_o$). An additive error model was used to characterize the residual error of the model fitted to the data.

**Study 2: Sympathoadrenal Block.** The sympathoadrenal blocking actions of dexmedetomidine, expressed as the percentile difference between the average HR in the 30-s period before the start of the tail-flick procedure and the HR measured 20 s after the actual flick of the tail, could be described by eq. 1, in which $E_{\text{max}}$ was substituted by $-E_o$. An additive error model was appropriate to characterize the residual error of the model fit to the pooled data.

**Studies 1 and 2: General Statistics and Methods.** The models were fitted to the data using the software program Matlab (MathWorks Inc., Natick, MA) or NONMEM (Beal et al., 1992). The data in the manuscript are expressed as mean ± S.E. A two-tailed paired or unpaired Student's $t$ test assuming equal variances was used ($p < .05$) for statistical comparison between data sets.

**Results**

**Study 1: Pharmacokinetic, EEG, and Cardiovascular Measurements.** Figure 1 displays the actually achieved plasma concentrations of dexmedetomidine after targeting plasma levels with the TCI system. The concentrations were measured in the 15- to 20-min period after targeting a new concentration level. Increasing plasma concentrations of dexmedetomidine lowered the HR of the rats, followed by a progressive slowing of the EEG with increased amplitude and an increase in MAP. The sympathoadrenal block-
reflex), \(2.62 \pm 0.88\) (startle reflex to noise), \(2.62 \pm 0.88\) (tail clamp), and \(1.95 \pm 0.41\) (corneal reflex), respectively.

**Study 1: Ventilatory Measurements.** At baseline, \(pCO_2, pO_2, O_2\) saturation, and pH were \(28.7 \pm 0.6\) mm Hg, \(93.2 \pm 1.6\) mm Hg, \(97.5 \pm 0.1\)%, and \(7.48 \pm 0.01\), respectively. At loss of response to tail clamp, these measures were \(36.1 \pm 0.8\) mm Hg, \(91.3 \pm 1.2\) mm Hg, \(97.0 \pm 0.1\)%, and \(7.43 \pm 0.01\). \(pCO_2, O_2\) saturation, and pH responses were significantly different (\(p < .05; n = 9\)) from baseline.

**Study 2: Tail-Flick Latencies.** One group of four rats was administered saline during five consecutive periods of 35 min after baseline. The average tail-flick latency within each period did not differ from baseline (\(p < .05\)). During the fifth period after baseline, three of four rats licked their tail after exposure to the 55°C water. Because this change in behavior, it was decided to target only four drug levels in the dexmedetomidine-receiving group of rats. Figure 5 shows the measured tail-flick latencies versus steady-state dexmedetomidine plasma concentrations for this group (\(n = 9\)). The maximal response was restricted to a latency of 10 s. The data could be fitted to a sigmoidal \(E_{\text{max}}\) model with \(E_0 = 4.14 \pm 0.27\) s, \(N = 1.52 \pm 0.28\), and \(EC_{50} = 3.65 \pm 0.87\) ng/ml.

**Study 2: Sympathoadrenal Block.** Figure 6 displays pre- and post-tail-flick HR recordings for the rats receiving saline. The increase in HR observed after each tail-flick test was consistent for repeated measurements. Figure 7 shows the percentage difference between pre- and post-tail-flick HR recordings with increasing plasma concentrations of dexmedetomidine. These data could be fitted versus dexmedetomidine plasma concentrations with eq. 1, in which \(E_{\text{max}}\) was substituted by minus \(E_0\): \(E_0 = 35.9 \pm 1.9\%\), \(EC_{50} = 1.85 \pm 0.80\) ng/ml, and \(N = 0.73 \pm 0.22\).

The results of the studies 1 and 2 are summarized in Table 2.

**Discussion**

In this study, we characterized the pharmacological effects of dexmedetomidine on the basis of increasing steady-state plasma concentrations using defined stimulus-response and continuous effect measurements in rats. Table 2 demonstrates that dexmedetomidine, when administered alone, can be typified as a drug that creates bradycardia, sedation/
The sympathoadrenal blocking activity of dexmedetomidine was calculated by taking the difference of the average HR in the 30-s period before the start of the tail-flick procedure and the HR measured 20 s after the actual tail-flick. The underlying assumption of this measure was that at the moment of tail-flick, the rat experienced the same degree of pain, and therefore the noxious stimulus was considered to be of equal magnitude, resulting in an equal HR response. The HR responses observed after saline administration were consistent over a range of multiple assessments (Fig. 6) and seem to support this assumption. The current study confirms the ability of dexmedetomidine to attenuate HR responses to noxious stimuli (Peden and Pryse-Roberts, 1992; Mizobe and Maze, 1995). In rats, the sympathoadrenal blocking activity occurs in a plasma concentration range in which bradycardia, hypertension, and sedation, with the exception that a significant decrease in blood pressure did not occur at low plasma concentrations, as would be expected for clonidine-like drugs. This may suggest that there are differences in the sensitivity of humans and rats toward the blood pressure-decreasing effect of dexmedetomidine or in their blood pressure control mechanisms.

Potential differences in the protein binding of dexmedetomidine could also contribute to this discrepancy. Kivistö et al. (1994) reported a 32% decrease in HR and a 25% decrease in SBP around peak plasma concentrations of 0.5 ng/ml after i.m. administration. Most subjects were sedated or asleep but arousable. Data abstracted from a study of Scheinin et al. (1992), also after i.m. administration, indicated a 20% decrease in HR and SBP at plasma concentrations around 0.4 ng/ml. Volunteers were also sedated or asleep, but arousable. Dyck et al. (1993) describe biphasic changes in blood pressure after an i.v. infusion. During the 5-min infusion, dexmedetomidine plasma concentrations increased to about 10 ng/ml, MAP increased by 22%, and HR decreased by 27%. Over the 4 h after infusion, dexmedetomidine plasma concentration decreased to about 0.3 ng/ml, MAP declined by 20%, and HR rose to 5% below baseline. Dyck et al. (1993) recommend, based on their observations, that plasma concentrations less than 1.0 ng/ml be maintained to avoid peripheral vasoconstriction that creates hypertension. These clinical observations in humans occurred at similar plasma concentrations to what we found in rats to develop bradycardia, hypertension, and sedation, with the exception that a significant decrease in blood pressure did not occur at low plasma concentrations, as would be expected for clonidine-like drugs. This may suggest that there are differences in the sensitivity of humans and rats toward the blood pressure-decreasing effect of dexmedetomidine or in their blood pressure control mechanisms.

So far, only limited concentration-effect data are available from human dexmedetomidine studies. The sympathoadrenal blocking activity of dexmedetomidine was calculated by taking the difference of the average HR in the 30-s period before the start of the tail-flick procedure and the HR measured 20 s after the actual tail-flick. The underlying assumption of this measure was that at the moment of tail-flick, the rat experienced the same degree of pain, and therefore the noxious stimulus was considered to be of equal magnitude, resulting in an equal HR response. The HR responses observed after saline administration were consistent over a range of multiple assessments (Fig. 6) and seem to support this assumption. The current study confirms the ability of dexmedetomidine to attenuate HR responses to noxious stimuli (Peden and Pryse-Roberts, 1992; Mizobe and Maze, 1995). In rats, the sympathoadrenal blocking activity occurs in a plasma concentration range in which bradycardia, hypertension, and sedation, with the exception that a significant decrease in blood pressure did not occur at low plasma concentrations, as would be expected for clonidine-like drugs. This may suggest that there are differences in the sensitivity of humans and rats toward the blood pressure-decreasing effect of dexmedetomidine or in their blood pressure control mechanisms.

Potential differences in the protein binding of dexmedetomidine could also contribute to this discrepancy.
small i.v. doses of dexmedetomidine were ineffective in relieving thresholds for experimentally induced dental pain and cutaneous heat pain, and miscellaneous effects were observed for relieving tourniquet-induced ischemic pain (Jaakkola et al., 1991; Kauppila et al., 1991). In patients, small doses of dexmedetomidine were effective in relieving pain after laparoscopic tubal ligation (Aho et al., 1991). In these studies, side effects included sedation, bradycardia, and hypotension. No increase in MAP was reported in these studies, probably because only low plasma concentrations were obtained. It seems that the pain-relieving actions of dexmedetomidine are dependent on the type and intensity of the noxious stimulus. However, it seems that to obtain sufficient pain relief, in both humans and rats, sedative and cardiovascular side effects cannot be avoided. Like in rats, only minor ventilatory effects have been reported after the administration of dexmedetomidine to humans.

In general, it seems that the PD actions of dexmedetomidine are similar for rats and humans; therefore, the steady-state concentration-effect relationships for the various measures obtained in this rat study might be predictive for the human clinical situation (Belleville et al., 1992).

Increasing concentrations of dexmedetomidine produce a progressive slowing of the EEG with increased amplitude (Bol et al., 1997a). Because this effect mimics the effects of opioids (Scott et al., 1985; Mandema and Wada, 1995), the change in delta activity (power in 0.5–3.5-Hz frequency band) was chosen as EEG effect measure. Previously, we reported that rats spontaneously woke up from dexmedetomidine-induced sleep at plasma concentrations close to the EC50 value for the EEG effect (Bol et al., 1997a) and that the (unbound) drug concentrations were in the same range (0.2 to 0.5 ng/ml) as the concentrations necessary to inhibit the firing rate of the nucleus locus ceruleus (LC) in vitro (Jorm and Stamford, 1993; Chiu et al., 1995). The activity of this nucleus in the brain is associated with wakefulness or vigilance (Scheinin, 1992). The righting reflex, a widely used measure of hypnosis, has been shown to be mediated by α2 receptors in the LC (Correa-Sales et al., 1992). Also in the present study, the (unbound) EC50 value for the EEG effect was in the concentration range necessary to inhibit the firing rate of the LC in vitro, but here it coincided with loss of the whisker reflex. The righting reflex was lost at higher drug concentrations. The data suggest that the whisker reflex and righting reflex reflect different degrees of sedation and hypnosis.

The concentration-effect relationship of the EEG effect of dexmedetomidine was characterized on the basis of multiple steady-state plasma concentrations. This design has the advantage that other measures can be measured concurrently at identical concentrations in the same subject, allowing a validation of the applied EEG measure (power in 0.5–3.5-Hz frequency band). In Fig. 8, the EC50 values for the five different stimulus-response measures are mapped on the continuous concentration-EEG curve. It can be observed that when the EEG measure reaches its maximal value, successively higher concentrations of dexmedetomidine are needed to abolish the startle reflex, the tail clamp response, and corneal reflex. Potential relationships between anesthetic state and EEG-derived measures have been suggested (Mandema and Danhof, 1992; Stanski, 1992). It seems that for the α2 agonist dexmedetomidine, the applied EEG measure has no discriminative power to predict different clinical states of anesthesia. Combined with previous results (Bol et al., 1997a), the data suggest that the proposed EEG measure may better reflect different degrees of sedation and hypnosis.

In conclusion, we have characterized the concentration-effect relationships for various measures of the pharmacological effect of dexmedetomidine, including whisker reflex, righting reflex, startle reflex to noise, tail clamp, tail-flick, corneal reflex, ventilatory depression, decrease in HR, increase in MAP, sympathoadrenal block, and EEG activity. It was demonstrated on the basis of increasing steady-state plasma concentrations that i.v. dexmedetomidine primarily exerts bradycardic, sympathetic depressant, and sedative/hypnotic actions. Only at higher plasma concentrations, at increased MAP and when the startle reflex is lost, are analgesic actions and loss of the corneal reflex observed. Ventilatory depressant effects were minor. The applied EEG measure seems to reflect sedation/hypnosis but seems to have limited value to predict the deeper dexmedetomidine levels of analgesia and anesthesia.

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Appendix: Equation for Logistic Regression

More familiar is the following equation for logistic regression:

\[ P(Y = 0 | x) = \frac{e^{\beta_0 + \beta_1 x}}{1 + e^{\beta_0 + \beta_1 x}} \]

If \( \beta_0 = -N \cdot \ln(E_{50}) \), \( \beta_1 = n \), and \( x = \ln(C) \), eq. 2 results. This equation is similar to the sigmoidal \( E_{\text{max}} \) eq. 1 used in the analysis.

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