Effect of dexmedetomidine on cardiorespiratory regulation in spontaneously breathing adult rats

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Abstract

Purpose
We examined the cardiorespiratory effect of dexmedetomidine, an α2-adrenoceptor/imidazoline 1 (I1) receptor agonist, in spontaneously breathing adult rats.

Methods
Male rats (226–301 g, n = 49) under isoflurane anesthesia had their tail vein cannulated for drug administration and their tail artery cannulated for analysis of mean arterial pressure (MAP), pulse rate (PR), and arterial blood gases (PaO2, PaCO2, pH). After recovery, one set of rats received normal saline for control recording and was then divided into three experimental groups, two receiving dexmedetomidine (5 or 50 μg·kg⁻¹) and one receiving normal saline (n = 7 per group). Another set of rats was divided into four groups receiving dexmedetomidine (50 μg·kg⁻¹) followed 5 min later by 0.5 or 1 mg·kg⁻¹ atipamezole (selective α2-adrenoceptor antagonist) or efaroxan (α2-adrenoceptor/I1 receptor antagonist) (n = 6 or 8 per group). Recordings were performed 15 min after normal saline or dexmedetomidine administration.

Results
Compared with normal saline, dexmedetomidine (5 and 50 μg·kg⁻¹) decreased respiratory frequency (fR, p = 0.04 and < 0.01, respectively), PR (both p < 0.01), and PaO2 (p = 0.04 and < 0.01), and increased tidal volume (both p = 0.049). Dexmedetomidine at 5 μg·kg⁻¹ did not significantly change minute ventilation (VE) (p = 0.87) or MAP (p = 0.24), whereas dexmedetomidine at 50 μg·kg⁻¹ significantly decreased VE (p = 0.03) and increased MAP (p < 0.01). Only dexmedetomidine at 50 μg·kg⁻¹ increased PaCO2 (p < 0.01). Dexmedetomidine (5 and 50 μg·kg⁻¹) significantly increased blood glucose (p < 0.01), and dexmedetomidine at 50 μg·kg⁻¹ increased hemoglobin (p = 0.04). Supplemental atipamezole or efaroxan administration similarly prevented the 50 μg·kg⁻¹ dexmedetomidine-related cardiorespiratory changes.
Principal conclusion
These results suggest that dexmedetomidine-related hypoventilation and hypertension are observed simultaneously and occur predominantly through activation of \( \alpha_2 \)-adrenoceptors, but not \( I_1 \) receptors, in spontaneously breathing adult rats.

Introduction
Dexmedetomidine provides its sedative and analgesic effects through its stimulant effect on \( \alpha_2 \)-adrenoceptors. Its use, which was originally restricted to adult patients in intensive care units during mechanical ventilation [1, 2], has expanded, for example, to patients undergoing surgical operations (e.g. dental therapy) [3], or to infants and children undergoing nuclear medicine imaging examination [4]. In the clinical setting, it has been indicated that dexmedetomidine preserves ventilation and may be useful in patients with COVID-19 [5], but hypertension, hypotension and bradycardia are major complications limiting its use [2].

Dexmedetomidine is an \( \alpha_2 \)-adrenoceptor/imidazoline 1 (\( I_1 \)) receptor agonist, and it has been suggested that activation of \( I_1 \) receptors [6], as well as activation of \( \alpha_2 \)-adrenoceptors [2], inhibits sympathetic outflow in the central nervous system and causes hypotension and bradycardia. However, there has been little attention paid to dexmedetomidine-related decrease in minute ventilation (\( V_{\text{E}} \)) [7, 8], because dexmedetomidine-related hypotension stimulates ventilation by activating chemoreceptors [9] and can minimize dexmedetomidine-related respiratory suppression.

Recently, we examined the cardiorespiratory effects of intraperitoneal injection of dexmedetomidine (50 \( \mu \)g-kg\(^{-1}\)) in spontaneously breathing newborn rats (2–5 days old) [10, 11]. Our findings suggested that, in newborns, dexmedetomidine suppresses respiratory frequency and heart rate predominantly through \( \alpha_2 \)-adrenoceptor activation [10, 11], whereas mean inspiratory flow (\( V_T/T_I \), where \( V_T \) is tidal volume and \( T_I \) is inspiratory time) was stimulated by \( I_1 \) receptor activation [11]. Hence, to extend our knowledge of respiratory regulation during dexmedetomidine administration, we examined cardiorespiratory indices in spontaneously breathing adult rats (8 weeks old), including \( V_T/T_I \), mean arterial blood pressure (MAP), and arterial blood gases (ABGs). We also examined whether activation of \( I_1 \) receptors, together with activation of \( \alpha_2 \)-adrenoceptors, is involved in dexmedetomidine-related respiratory suppression by using two different antagonists, i.e. atipamezole (selective \( \alpha_2 \)-adrenoceptor antagonist) and efaroxan (\( \alpha_2 \)-adrenoceptor/\( I_1 \) receptor antagonist) [11].

Materials and methods
The experimental protocol was reviewed and approved by the Animal Research Committee of the Nippon Dental University School of Life Dentistry at Tokyo, Japan (Protocol Approved Numbers: 18-02-1 and 19-14). The animals were treated in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (The Physiological Society of Japan), and we complied with the ARRIVE guidelines (https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1000412).

All efforts were made to minimize animal suffering and the number of animals used.

Animals
Adult male Wistar rats were obtained from CLEA Japan Inc. (Tokyo, Japan) and maintained in the Nippon Dental University’s animal center at 22˚C–25˚C under a 12–hour:12–hour dark:light cycle with ad libitum access to food and water. On the day of the experiment, male rats (8 weeks
old, 226–301 g; n = 49) were randomly assigned to the experimental groups. In the current study we used only male rats to exclude possible effects of gender difference on ventilation [12] and hypotension [13]; we plan a follow-up study with female animals in future.

**Drugs**

We used the $\alpha_2$-adrenoceptor/I$_1$ receptor agonist dexmedetomidine hydrochloride (Precedex; Maruishi Pharmaceutical Co., Osaka, Japan), the $\alpha_2$-adrenoceptor antagonist atipamezole (Antisedan; Orion Pharmaceutical Group Ltd., Espoo, Finland), and the $\alpha_2$-adrenoceptor/I$_1$ receptor antagonist efaroxan hydrochloride (Sigma-Aldrich, St. Louis, MO, USA). Dexmedetomidine (at 100 $\mu$g·mL$^{-1}$ in normal saline), and atipamezole and efaroxan (each at 2.5 mg·mL$^{-1}$ in normal saline) were stored in a freezer ($-20^\circ$C). In the experiments, a single dose the drugs (i.e. normal saline or 5 or 50 $\mu$g·kg$^{-1}$ dexmedetomidine, and 0.5 or 1.0 mg·kg$^{-1}$ atipamezole or efaroxan), was administered at a volume of 0.4–0.5 mL·kg$^{-1}$ with a 250–μL syringe (Gastight Syringe; Hamilton Company, Reno, NV, USA).

**Surgical preparations**

On the day of the experiment, the rats were and anesthetized with 1–3% isoflurane (Forane Inhalant Liquid; Abbott Japan Co., Ltd., Tokyo, Japan). Surgical preparations were performed under a surgical microscope (SZ60; Olympus, Tokyo, Japan). Briefly, local anesthetic (Xylocaine 2%; Aspen Japan K.K., Tokyo, Japan) was applied subcutaneously, and two incisions (each 5–10 mm long) were made in the ventral surface at the proximal end of the animal’s tail. At each incision, a catheter (24G × 1", Nipro, Tokyo, Japan, and PE-50, Intramedic, Becton Dickinson and Company, Sparks, MD, USA) was gently inserted: one into a tail vein to admin-ister the drug intravenously and one into the tail artery to monitor the pulse rate (PR) and mean arterial pressure (MAP) and to sample arterial blood for analysis of ABGs (PaO$_2$, PaCO$_2$, and pH), hemoglobin, hematocrit, electrolytes (Na$^+$, K$^+$, and Ca$^{2+}$), and glucose. Each catheter was filled with saline-heparin solution (100 U·mL$^{-1}$, a total volume of 0.1 mL). The rat was then given aspoxicillin (Doyle; Sawai Pharmaceutical Co., Ltd, Osaka, Japan) and an analgesic (flurbiprofen axetil, 0.25 mg) intravenously, the catheters were fixed, and the incision was closed with instant adhesive. No bleeding or blood reflux was observed during the prepara-tions. After returning the rat to its cage, we monitored its behavior carefully. We observed that all animals recovered consciousness within 5 min and could access water and laboratory chow by themselves. About 3 hours later, we started the recording.

The animals were placed individually in a loose and flexible custom-made cylindrical container made of soft stainless-steel netting, in which the animal was able to roll and move back and forth. We used that container to avoid the risk of the animal turning back to bite or pull out the indwelling temperature probe or catheters during the measurements in the chamber (Fig 1). Chamber and body temperatures (°C) were monitored by means of fine chromel-alu-mel thermocouples (Omega Model 871a; Omega Engineering, Stamford, CT, USA). During the experiment, ambient temperature in the chamber was controlled at 25℃ ± 2℃ with the help of a circulating water bath (NCB-2510B; Tokyo Rikakikai Co. Ltd, Tokyo, Japan). To measure body temperature, the probe was inserted about 5 mm into the rectum with the aid of lidocaine jelly (Xylocaine Jelly; AstraZeneca K.K., Osaka, Japan) and lightly attached to the tail with a Band-Aid (Johnson & Johnson Services, Inc., New Brunswick, NJ, USA).

**Measurement of ventilation**

Ventilation was measured by using a barometric technique [14, 15]. Briefly, we put the rat in the container into a cylindrical acrylic resin chamber (2300 mL) (Fig 1), and continuously delivered
air through the chamber from the front (i.e. the inlet) to the back (i.e. the outlet) at a steady flow of 1400 mL min\(^{-1}\) at STPD (standard temperature and pressure, dry) controlled by an adjustable flowmeter. To prevent the animal getting wet from urination during the recording, we laid a thick paper towel (Kim Towel, Nippon Paper Crecia Co., Ltd., Tokyo, Japan) beneath the container. Gas concentrations were monitored with a calibrated polarographic O\(_2\) analyzer and an infrared CO\(_2\) analyzer (Fox Box; Sable Systems International, North Las Vegas, NV, USA). To measure spontaneous breathing, the chamber inlet and outlet were temporarily closed, and the pressure oscillations in the recording chamber were monitored with a differential pressure transducer (DP45 \(\pm\) 5.6 cm H\(_2\)O; Validyne Engineering, Northridge, CA, USA) connected to a pre-amplifier (Model 1253A; San-ei Instruments, Tokyo Japan); these readings were displayed on a computer screen and recorded. The chamber was sealed for less than 1 min (mean 30 s), and, when it was reopened, the CO\(_2\) concentration at the outflow did not exceed 1%. We analyzed 20 to 50 regular breaths (mean 41 breaths), excluding spontaneous augmented breaths, to determine respiratory frequency (\(f_R\)) and tidal volume (\(V_T\)), from which we calculated minute ventilation (\(V_E = f_R \cdot V_T\)), total respiratory duration (\(T_{TOT}\)), and inspiratory and expiratory time (\(T_I\) and \(T_E\)). The volume was computed at BTPS (body temperature and pressure, saturated) and normalized by the weight of the animal in kilograms. The signal was calibrated for volume by injecting a known amount of air (e.g. 0.5 mL) when blood sampling was terminated at the end of the measurement.

**Measurement of pulse rate and mean arterial pressure**

The arterial catheter was connected via a three-way stopcock to a liquid-filled pressure transducer (MLT0699; AD Instruments, Bella Vista, Australia) for the measurement of PR and
MAP. The zero signal to the transducer was set to correspond to the chest level of the animal. The output was amplified (Model 2238; San-ei Instruments, Tokyo, Japan) and the signal was displayed on a computer screen and recorded.

Data storage and analysis
The signals of ventilation, MAP, PR, temperatures, and in-and out-flowing gas concentrations (O\textsubscript{2} and CO\textsubscript{2}) were monitored simultaneously and stored on a personal computer at a sampling frequency of 1 kHz for subsequent data analysis (PowerLab 4/25 and LabChart 8.0; AD Instruments).

Arterial blood analysis (ABGs, electrolytes, glucose, hemoglobin, and hematocrit)
By using the arterial catheter, a 0.2 mL blood sample was collected anaerobically and immediately transferred into a disposable cartridge (CG8\textsuperscript{+}; Abbot Japan) designed for the automated blood analyzer (i-STAT; Abbot Japan). Blood electrolytes (Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+} [mmol L\textsuperscript{-1}]), blood glucose (mg dL\textsuperscript{-1}), hemoglobin (g dL\textsuperscript{-1}), and hematocrit (%), as well as ABGs (PaO\textsubscript{2} and PaCO\textsubscript{2} [mmHg]; pH), were measured by the automated blood analyzer. The values for ABGs were corrected with respect to body temperature, as proposed by Severinghaus [16]. Any blood remaining after the analysis was then returned to the animal, with a small amount of normal saline added to it to minimize loss in blood mass. In all groups, at each recording, the blood sampling was performed at the end (i.e. approximately 20 min after the administration of normal saline or dexmedetomidine).

Protocols
The protocols used were based on those of our previous studies on newborn rats [10, 11]. Each intravenous administration (of normal saline, dexmedetomidine, atipamezole, or efaroxan), which was followed by flushing with normal saline (0.2 mL, corresponding to the liquid capacity of the venous catheter), was performed gently over a period of 1 min.

**Protocol 1:** After recovery from anesthesia in the recording chamber, a set of rats (249−300 g, n = 21) received normal saline for control recording and was then randomly divided into three groups to receive normal saline (NS), dexmedetomidine (5 μg kg\textsuperscript{-1}) (DEXMD-5), or dexmedetomidine (50 μg kg\textsuperscript{-1}) (DEXMD-50) (n = 7 in each group) for experimental recording (Fig 2A).

**Protocol 2:** After recovery from anesthesia in the recording chamber, a set of rats (226−297 g, n = 28) was randomly divided into four groups to receive 50 μg kg\textsuperscript{-1} of dexmedetomidine followed 5 min later by 0.5 mg kg\textsuperscript{-1} of atipamezole or efaroxan (DEXMD-50+ATI-0.5 or DEXMD-50+EFA-0.5; n = 8 in each group), or 1.0 mg kg\textsuperscript{-1} of atipamezole or efaroxan (DEXMD-50+ATI-1.0 or DEXMD-50+EFA-1.0) (n = 6 in each group) for experimental recording (Fig 2B). Basically, 0.5 mg kg\textsuperscript{-1} of atipamezole [17] or efaroxan [18] was selected to prevent the effect of 50 μg kg\textsuperscript{-1} of dexmedetomidine in rats, in vivo or in vitro, and further examination at a higher dose (i.e. 1.0 mg kg\textsuperscript{-1}) [10, 11] was added for each drug in this study. In Protocol 2, control recording (i.e. normal saline administration) was skipped, because intravenous volume loading could be excessive compared with that in Protocol 1. The summed data (n = 21) obtained at control recording in Protocol 1 were used as the data for NS in Protocol 2.

Statistical analysis
Values are expressed as means and SD. The sample size for each cardiorespiratory index was determined by power analysis (α = 0.05 and β = 0.20) based on our previous study on newborn rats [11]; it was estimated that 6 to 7 animals per group were required.
(a) Protocol 1

NS

**Control**
- Normal saline administration

**Experimental**
- Normal saline administration

**DEXMD (5 or 50 μg·kg⁻¹)**
- Normal saline administration
- Dexmedetomidine administration

Respiratory measurement
Blood sampling

Monitoring blood pressure and pulse rate

(b) Protocol 2

**DEXMD-50 plus ATI**
- Dexmedetomidine administration (50 μg·kg⁻¹)
- Atipamezole administration (0.5 or 1 mg·kg⁻¹)

**DEXMD-50 plus EFA**
- Dexmedetomidine administration (50 μg·kg⁻¹)
- Efaroxan administration (0.5 or 1 mg·kg⁻¹)

Respiratory measurement
Blood sampling

Monitoring blood pressure and pulse rate
For Protocol 1, the significance of differences among the three groups (NS, DEXMD-5, and DEXMD-50) was evaluated by one-way analysis of variance (ANOVA), and differences between groups in ANOVA were evaluated by Turkey-Kramer test. For Protocol 2, the summed data (n = 21) obtained at control recording in Protocol 1 were used as the data for the NS. The significance of differences among the three groups (NS, DEXMD-50+ATI-0.5, and DEXMD-50+EFA-0.5; or NS, DEXMD-50+ATI-1.0, and DEXMD-50+EFA-1.0) was evaluated by one-way analysis of variance (ANOVA), and differences between groups in the ANOVA were evaluated by Turkey-Kramer test. A p-value of less than 0.05 was considered significant.

Statistical analyses were performed by using BellCurve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

Protocol 1

The absolute values obtained at control recording are summarized in Table 1. Normal saline administration did not result in any difference among the groups, and we considered that all animals (n = 7 + 7 + 7 = 21) were basically from the same population.

Table 2 summarizes the absolute values obtained at experimental recording. Compared with the NS, the DEXMD-5 and DEXMD-50 had decreased cardiorespiratory frequencies, i.e. $f_R$ and PR, ($f_R$, p = 0.04 and < 0.01, respectively; PR, both p < 0.01), and increased $V_T$ (both p = 0.049); $V_E$ of DEXMD-5 was not significantly different (p = 0.87), whereas that of DEXMD-50 was significantly decreased (p = 0.03). In the analysis of breathing pattern, $T_{TOT}$ in DEXMD-5 was not significantly different (p = 0.34) owing to significant $T_I$ prolongation (p < 0.01) without $T_E$ prolongation (p = 0.94), and $T_{TOT}$ in DEXMD-50 was significantly prolonged owing to significant $T_I$ prolongation with $T_E$ prolongation (each p < 0.01). $V_T/T_I$ of the DEXMD-5 and DEXMD-50 was not significantly different (p = 0.45 and 0.60, respectively); $T_I/T_{TOT}$ of DEXMD-5 was significantly higher (p = 0.02), whereas that of DEXMD-50 was not significantly different (p = 0.20) (Fig 3A). In the analysis of circulation, MAP did not decrease in DEXMD-5 (p = 0.24), whereas it significantly increased in DEXMD-50 (p < 0.01). PR was significantly decreased in both DEXMD-5 and DEXMD-50 (both p < 0.01). Analysis of ABGs revealed that, although PaO$_2$ decreased in both DEXMD-5 and DEXMD-50 (p = 0.04 and p < 0.01, respectively), PaCO$_2$ increased (p < 0.01) and pH decreased (p = 0.01) only in DEXMD-50. Arterial blood glucose increased in both DEXMD-5 and DEXMD-50 (both p < 0.01), and hemoglobin increased in DEXMD-50 (p = 0.04). In both DEXMD-5 and DEXMD-50, no significant change was observed in electrolytes, hematocrit, or body temperature.

Differences between DEXMD-5 and DEXMD-50 were significant in $f_R$, $V_E$, $T_E$, $T_I/T_{TOT}$, MAP, and arterial PaCO$_2$, pH, hemoglobin and glucose.

Protocol 2

The absolute values obtained in the DEXMD-50+ATI-0.5 or DEXMD-0.5+EFA-0.5 are summarized in Table 3, and the absolute values obtained in the DEXMD-50+ATI-1.0 and
DEXMD+EFA-1.0 are summarized in Table 4. In both tables, the summed data obtained at control recording in Protocol 1 (n = 21) were used as the data for the NS.

Table 1. Absolute values at control recording (Protocol 1).

|                              | NS (n = 7) | DEXMD-5 (n = 7) | DEXMD-50 (n = 7) |
|------------------------------|------------|----------------|-----------------|
|                              | mean | SD     | mean | SD     | mean | SD     |
| Respiratory indices          |     |        |      |        |      |        |
| $f_R$ (breaths min$^{-1}$)   | 125  | 8      | 118  | 9      | 124  | 20     |
| $V_T$ (mL kg$^{-1}$)         | 6.00 | 1.03   | 6.50 | 0.93   | 5.95 | 1.06   |
| $V_E$ (mL min$^{-1}$ kg$^{-1}$) | 749  | 121    | 744  | 93     | 732  | 124    |
| $T_{TOT}$ (s)                | 0.48 | 0.03   | 0.49 | 0.08   | 0.49 | 0.06   |
| $T_I$ (s)                    | 0.19 | 0.01   | 0.20 | 0.02   | 0.19 | 0.02   |
| $T_E$ (s)                    | 0.29 | 0.03   | 0.33 | 0.04   | 0.31 | 0.03   |
| $V_T/T_I$ (mL s$^{-1}$ kg$^{-1}$) | 31.2  | 5.5    | 33.6 | 5.5    | 31.1 | 5.7    |
| $T_I/T_{TOT}$                | 0.41 | 0.03   | 0.38 | 0.03   | 0.40 | 0.02   |
| Circulatory indices          |     |        |      |        |      |        |
| PR (beats min$^{-1}$)        | 378  | 28     | 391  | 35     | 376  | 31     |
| MAP (mmHg)                   | 135  | 7      | 140  | 19     | 140  | 16     |
| Arterial blood data          |     |        |      |        |      |        |
| $P_{aO_2}$ (mmHg)            | 95   | 4      | 91   | 4      | 92   | 3      |
| $P_{aCO_2}$ (mmHg)           | 38.2 | 4.4    | 40.1 | 2.9    | 41.3 | 3.4    |
| pH                           | 7.40 | 0.02   | 7.43 | 0.03   | 7.42 | 0.01   |
| $Ca^{2+}$ (mMol L$^{-1}$)    | 1.27 | 0.09   | 1.33 | 0.07   | 1.35 | 0.08   |
| $Na^+$ (mMol L$^{-1}$)       | 147  | 4      | 144  | 3      | 143  | 2      |
| $K^+$ (mMol L$^{-1}$)        | 3.21 | 0.4    | 3.45 | 0.33   | 3.50 | 0.11   |
| Hemoglobin (g DL$^{-1}$)     | 11.4 | 1.8    | 11.5 | 0.9    | 12.7 | 1.2    |
| Hematocrit (%)               | 33   | 5      | 34   | 3      | 37   | 3      |
| Glucose (mg DL$^{-1}$)       | 134  | 24     | 153  | 19     | 144  | 14     |
| Body temperature (˚C)        | 38.1 | 0.3    | 38.0 | 0.4    | 38.3 | 0.4    |

Values were obtained 15 and 20 min after a single dose administration of normal saline (vehicle, control) in three animal groups: NS, DEXMD-5, and DEXMD-50. At the control recording, all groups received only normal saline. There was no significant difference among the three groups at control recording. Hence, we considered the animals (in total, 7 + 7 + 7 = 21) to have been randomly selected from a single population.

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As shown in Table 3, most of the results in the DEXMD-50+ATI-0.5 and DEXMD-50+EFA-0.5 were not significantly different from those in the NS, with the exception of $T_I$ (p = 0.03, only in DEXMD-50+EFA-0.5), PR (both p < 0.01), and arterial blood glucose (both p < 0.01) (Table 3, Fig 3B and 3C). No significant difference was observed between any parameter in the DEXMD-50+ATI-0.5 and the DEXMD-50+EFA-0.5.

As shown in Table 4, in DEXMD-50+ATI-1.0 and DEXMD-50+EFA-1.0, $f_R$ (both p < 0.01) and $V_T/T_I$ (p = 0.02 and 0.03, respectively) were higher than in the NS owing to significantly shortened $T_{TOT}$ (both, p = 0.01) and $T_I$ (both p < 0.01), respectively (Fig 3D and 3E). In the analysis of circulation, MAP increased (both p < 0.01), whereas PR decreased (both p < 0.01), compared with those in the NS (Table 4). In addition, arterial blood glucose increased compared with that in the NS (p = 0.04 and < 0.01, respectively). No significant difference was observed between any parameter in the DEXMD-50+ATI-1.0 and the DEXMD-50+EFA-1.0.

Fig 3 graphically shows the respiratory pattern of NS, DEXMD-5, and DEXMD-50 (Protocol 1) (Fig 3A) and those of the DEXMD-50+ATI-0.5, DEXMD-50+EFA-0.5, DEXMD-50+ATI-1.0, or DEXMD-50+EFA-1.0 (Protocol 2) (Fig 3B–3E). In Protocol 1, DEXMD-5 and
Table 2. Effect of dexmedetomidine (5 μg·kg⁻¹ or 50 μg·kg⁻¹) at experimental recording (Protocol 1).

|                      | NS (n = 7) | DEXMD-5 (n = 7) | DEXMD-50 (n = 7) |
|----------------------|------------|----------------|-----------------|
| Respiratory indices  |            |                |                 |
| F(_b_)(breaths·min⁻¹) | 126 9      | 102 16         | *0.04 78 23     | <0.01 104 |
| V_T (mL·kga⁻¹)       | 5.94 0.88  | 7.92 1.59      | *0.049 8.00 2.36 | *0.049 0.99 |
| V_E (mL·min⁻¹·kg⁻¹)  | 750 123    | 777 106        | 0.87 587 86     | *0.03 1.01 |
| T_TOT (s)            | 0.48 0.03  | 0.60 0.08      | 0.34 0.82 0.27  | <0.01 0.06 |
| T_I (s)              | 0.19 0.03  | 0.29 0.04      | *<0.01 0.27 0.05 | <0.01 0.76 |
| T_E (s)              | 0.29 0.01  | 0.31 0.08      | 0.94 0.55 0.23  | <0.01 0.02 |
| V_T/T_I(mL·s⁻¹·kg⁻¹) | 33.4 9.0   | 28.3 7.2       | 0.45 29.3 7.0   | 0.60 0.96 |
| T_I/T_TOT            | 0.40 0.03  | 0.50 0.07      | *0.02 0.35 0.07 | 0.20 <0.01 |
| Circulatory indices  |            |                |                 |
| PR (beats·min⁻¹)     | 384 42     | 324 25         | *<0.01 286 14   | <0.01 0.06 |
| MAP (mmHg)           | 137 13     | 121 21         | 0.24 182 14     | <0.01 <0.01 |
| Arterial blood data  |            |                |                 |
| PaO₂ (mmHg)          | 91.6 5.5   | 83.0 6.0       | *0.04 78.9 7.4  | <0.01 0.55 |
| PaCO₂ (mmHg)         | 39.1 3.8   | 38.8 3.0       | 0.99 47.1 4.4   | *<0.01 0.04 |
| pH                   | 7.40 0.02  | 7.42 0.02      | 0.46 7.37 0.03  | *0.01 <0.01 |
| Ca²⁺ (mmoll⁻¹)       | 1.29 0.12  | 1.35 0.03      | 0.30 1.37 0.06  | 0.12 0.89 |
| Na⁺ (mmoll⁻¹)        | 146 3      | 143 2.5        | 0.054 147 2     | 0.09 0.96 |
| K⁺ (mmoll⁻¹)         | 3.14 0.41  | 3.43 0.24      | 0.39 3.37 0.11  | 0.34 0.99 |
| Hemoglobin (g·dl⁻¹)  | 11.8 1.4   | 11.6 1.2       | 0.92 13.7 1.6   | *0.04 0.02 |
| Hematocrit (%)       | 35 4       | 34 3           | 0.95 40 5       | 0.17 0.11 |
| Glucose (mg·dl⁻¹)    | 143 14     | 249 26         | *<0.01 329 29   | <0.01 <0.01 |
| Body temperature (˚C)| 38.1 0.5   | 37.8 0.8       | 0.78 37.8 0.9   | 0.99 0.75 |

*Significant difference (p < 0.05) between NS and DEXMD-5 or DEXMD-50.
†Significant difference (p < 0.05) between DEXMD-5 and DEXMD-50.

NS, DEXMD-5, and DEXMD-50 (n = 7 in each group) received normal saline, dexmedetomidine 5 μg·kg⁻¹, and dexmedetomidine 50 μg·kg⁻¹, respectively, at experimental recording.

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DEXMD-50 prolonged T_I (both p < 0.01) compared with NS and increased V_T (both p = 0.049), and these significant changes resulted in there being no change in V_T/T_I (p = 0.45 and p = 0.60, respectively), which is the slope at inspiration and an index of respiratory drive. In Protocol 2, in the DEXMD-50+ATI-0.5 (Fig 3B) or DEXMD-50+EFA-0.5 (Fig 3C), V_T/T_I remained comparable to that of the NS (n = 21) (p = 0.77 and p = 0.79, respectively), without changes in V_T (p = 0.93 and p = 0.49, respectively) and T_I/T_TO (p = 0.43 and p = 0.29, respectively). In the DEXMD-50+ATI-1.0 (Fig 3D) and DEXMD-50+EFA-1.0 (Fig 3E), V_T/T_I significantly increased (p = 0.02 and p = 0.03, respectively) without changes in V_T (p = 0.20 and 0.45, respectively) and T_I/T_TO (p = 0.95 and p = 0.99, respectively).

**Discussion**

In previous studies, we examined the cardiorespiratory effects of an intraperitoneal injection of dexmedetomidine (50 μg·kg⁻¹) on spontaneously breathing newborn rats [10, 11]. We found that the cardiorespiratory suppression that occurred following administration of dexmedetomidine was reversed by the addition of atipamezole (a selective α₂-adrenoceptor antagonist) [10]. Similar dexmedetomidine-mediated changes in respiration-related activities have...
Protocol 1 Respiratory pattern

![Graph](image)

(a) Effect of DEXMD-5 and DEXMD-50
- □ NS (n = 7)
- ○ DEXMD-5 (n = 7)
- ● DEXMD-50 (n = 7)

NS vs DEXMD-5 or DEXMD-50

$V_T$ (both $p = 0.049$)

$V_T/T_i$ ($p = 0.45$, $p = 0.60$)

$T_F/T_{TOT}$ ($p = 0.02$, $p = 0.20$)

Time (s)

Protocol 2 Respiratory pattern

![Graph](image)

(b) DEXMD-50+ATI-0.5
- □ NS (protocol 1, n = 21)
- ○ DEXMD-50+ATI-0.5 (n = 8)

$V_T$ ($p = 0.93$)

$V_T/T_i$ ($p = 0.77$)

$T_F/T_{TOT}$ ($p = 0.43$)

Time (s)

d) DEXMD-50+ATI-1.0
- □ NS (protocol 1, n = 21)
- ○ DEXMD-50+ATI-1.0 (n = 6)

$V_T$ ($p = 0.20$)

$V_T/T_i$ ($p = 0.02$)

$T_F/T_{TOT}$ ($p = 0.95$)

Time (s)

e) DEXMD-50+EFA-1.0
- □ NS (protocol 1, n = 21)
- ○ DEXMD-50+EFA-1.0 (n = 6)

$V_T$ ($p = 0.45$)

$V_T/T_i$ ($p = 0.03$)

$T_F/T_{TOT}$ ($p = 0.99$)

Time (s)
Table 3. Effect of atipamezole or efaroxan (0.5 mg kg\(^{-1}\)) (Protocol 2).

| Respiratory indices | NS (n = 21) | DEXMD-50+ATI-0.5 (n = 8) | DEXMD-50+EFA-0.5 (n = 8) | p values (vs. NS) | p values (ATI-0.5 vs. EFA-0.5) |
|---------------------|-------------|--------------------------|--------------------------|-------------------|-------------------------------|
| \(f_b\) (breath min\(^{-1}\)) | 123 \(\pm\) 13 | 127 \(\pm\) 14 | 0.73 | 115 \(\pm\) 31 | 0.42 | 0.22 |
| \(V_T\) (mL kg\(^{-1}\)) | 6.15 \(\pm\) 0.99 | 6.00 \(\pm\) 1.15 | 0.93 | 6.63 \(\pm\) 0.93 | 0.49 | 0.43 |
| \(V_E\) (mL min\(^{-1}\) kg\(^{-1}\)) | 742 \(\pm\) 108 | 728 \(\pm\) 179 | 0.96 | 746 \(\pm\) 86 | 0.99 | 0.95 |
| \(T_{TOT}\) (s) | 0.49 \(\pm\) 0.06 | 0.50 \(\pm\) 0.04 | 0.94 | 0.54 \(\pm\) 0.10 | 0.22 | 0.48 |
| \(T_1\) (s) | 0.19 \(\pm\) 0.02 | 0.20 \(\pm\) 0.03 | 0.67 | 0.23 \(\pm\) 0.05 | 0.03 | 0.27 |
| \(T_4\) (s) | 0.31 \(\pm\) 0.04 | 0.29 \(\pm\) 0.04 | 0.74 | 0.28 \(\pm\) 0.10 | 0.97 | 0.70 |
| \(V_T/T_1\) (mL s\(^{-1}\) kg\(^{-1}\)) | 32.0 \(\pm\) 5.4 | 30.3 \(\pm\) 7.9 | 0.77 | 30.4 \(\pm\) 4.8 | 0.79 | 0.99 |
| \(T_1/T_{TOT}\) | 0.40 \(\pm\) 0.03 | 0.42 \(\pm\) 0.05 | 0.43 | 0.42 \(\pm\) 0.05 | 0.29 | 0.97 |

| Circulatory indices | NS (n = 21) | DEXMD-50+ATI-0.5 (n = 8) | DEXMD-50+EFA-0.5 (n = 8) | p values (vs. NS) | p values (ATI-0.5 vs. EFA-0.5) |
|---------------------|-------------|--------------------------|--------------------------|-------------------|-------------------------------|
| PR (beat min\(^{-1}\)) | 384 \(\pm\) 31 | 309 \(\pm\) 15 | *< 0.01 | 316 \(\pm\) 17 | *< 0.01 | 0.80 |
| MAP (mmHg) | 138 \(\pm\) 14 | 143 \(\pm\) 20 | 0.44 | 131 \(\pm\) 15 | 0.25 | 0.36 |

| Arterial blood data | NS (n = 21) | DEXMD-50+ATI-0.5 (n = 8) | DEXMD-50+EFA-0.5 (n = 8) | p values (vs. NS) | p values (ATI-0.5 vs. EFA-0.5) |
|---------------------|-------------|--------------------------|--------------------------|-------------------|-------------------------------|
| \(PaO_2\) (mmHg) | 92.9 \(\pm\) 3.8 | 95.0 \(\pm\) 5.5 | 0.55 | 92.5 \(\pm\) 6.7 | 0.98 | 0.57 |
| \(PaCO_2\) (mmHg) | 37.8 \(\pm\) 9.6 | 39.4 \(\pm\) 8.5 | 0.89 | 35.6 \(\pm\) 6.8 | 0.83 | 0.67 |
| \(pH\) | 7.41 \(\pm\) 0.02 | 7.41 \(\pm\) 0.02 | 0.81 | 7.42 \(\pm\) 0.03 | 0.58 | 0.39 |
| \(Ca^{2+}\) (mmol L\(^{-1}\)) | 1.32 \(\pm\) 0.08 | 1.34 \(\pm\) 0.14 | 0.88 | 1.28 \(\pm\) 0.13 | 0.63 | 0.50 |
| \(Na^+\) (mmol L\(^{-1}\)) | 145 \(\pm\) 3 | 143 \(\pm\) 4 | 0.67 | 146 \(\pm\) 4 | 0.64 | 0.32 |
| \(K^+\) (mmol L\(^{-1}\)) | 3.4 \(\pm\) 0.3 | 3.4 \(\pm\) 0.6 | 0.99 | 3.2 \(\pm\) 0.5 | 0.53 | 0.60 |
| Hemoglobin (g dL\(^{-1}\)) | 11.9 \(\pm\) 1.4 | 12.3 \(\pm\) 2.0 | 0.80 | 10.9 \(\pm\) 1.8 | 0.35 | 0.22 |
| Hematocrit (%) | 35 \(\pm\) 4 | 36 \(\pm\) 6 | 0.75 | 32 \(\pm\) 5 | 0.38 | 0.21 |
| Glucose (mg dL\(^{-1}\)) | 143 \(\pm\) 21 | 201 \(\pm\) 31 | *< 0.01 | 192 \(\pm\) 35 | *< 0.01 | 0.80 |
| Body temperature (°C) | 38.1 \(\pm\) 0.4 | 38.1 \(\pm\) 0.5 | 0.78 | 38.1 \(\pm\) 0.4 | 0.77 | 0.99 |

*Significant difference (p < 0.05) between the NS and the DEXMD-50+ATI-0.5 or DEXMD-50+EFA-0.5.
No significant difference in any parameter was observed between DEXMD-50+ATI-0.5 and DEXMD-50+EFA-0.5.
Data for the NS are the summed data (n = 21) obtained at control recording in Protocol 1. Rats in the DEXMD-50+ATI-0.5 or DEXMD-50+EFA-0.5 (n = 8 in each group) were given dexmedetomidine (50 μg kg\(^{-1}\)) followed 5 min later by 0.5 mg kg\(^{-1}\) atipamezole or efaroxan.

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patterns, and lung volumes per bodyweight differ from those in newborn rats [21, 22] and may influence the values of \( V_f/T \) [23]. Hence, to add information related to maturity, we examined spontaneously breathing adult rats in this study by following essentially the same protocol as that which we applied to newborn rats [10, 11]. The difference was that two different doses (5 and 50 \( \mu \text{g} \cdot \text{kg}^{-1} \)) were prepared (Protocol 1) in consideration of possible age-related differences in drug sensitivity [24] and in pharmacokinetics and pharmacodynamics [2], as well as of the possible effect of dexmedetomidine on MAP [25], which is unmeasurable in newborn rats [10, 11].

Compared with the mean values of NS (= 100%) (Table 2), administration of 5 \( \mu \text{g} \cdot \text{kg}^{-1} \) of dexmedetomidine decreased \( f_R \) to approximately 81% and PR to approximately 84% (\( p = 0.04 \) and \( p < 0.01 \), respectively), and 50 \( \mu \text{g} \cdot \text{kg}^{-1} \) of dexmedetomidine decreased \( f_R \) to 62% and PR to 74% (both \( p < 0.01 \)). \( V_f \) was increased to 133% and 135% by administration of 5 and 50 \( \mu \text{g} \cdot \text{kg}^{-1} \) dexmedetomidine, respectively (\( p = 0.049 \)), but \( V_T \) (the product of \( f_R \) and \( V_f \)) was increased to 78% (\( p = 0.03 \)) only by 50 \( \mu \text{g} \cdot \text{kg}^{-1} \) dexmedetomidine. In taking this information together with the results obtained in previous studies of newborn rats given 50 \( \mu \text{g} \cdot \text{kg}^{-1} \) of dexmedetomidine [10, 11], we found that administration of 50 \( \mu \text{g} \cdot \text{kg}^{-1} \) of dexmedetomidine consistently resulted in more severe suppression of \( f_R \) relative to heart rate and increased \( V_T \).

### Table 4. Effect of atipamezole or efaroxan (1 \( \mu \text{g} \cdot \text{kg}^{-1} \)) (Protocol 2).

| NS (n = 21) | DEXM-50+ATI-1.0 (n = 6) | DEXM-50+EFA-1.0 (n = 6) |
|-------------|-------------------------|-------------------------|
| **Respiratory indices** | | |
| \( f_R \) (breaths.min\(^{-1}\)) | 123 ± 13 | 160 ± 41 | \( < 0.01 \) | 154 ± 22 | \( < 0.01 \) | 0.85 |
| \( V_T \) (mL.kg\(^{-1}\)) | 6.15 ± 0.99 | 5.41 ± 0.50 | 0.20 | 5.64 ± 0.80 | 0.45 | 0.90 |
| \( V_f \) (mL.min\(^{-1}\).kg\(^{-1}\)) | 742 ± 108 | 850 ± 257 | 0.29 | 861 ± 160 | 0.22 | 0.99 |
| \( T_R \) (s) | 0.49 ± 0.06 | 0.41 ± 0.08 | \( < 0.01 \) | 0.40 ± 0.05 | \( < 0.01 \) | 0.96 |
| \( T_I \) (s) | 0.19 ± 0.02 | 0.14 ± 0.01 | \( < 0.01 \) | 0.15 ± 0.02 | \( < 0.01 \) | 0.76 |
| \( T_I/T \) (mL.s\(^{-1}\).kg\(^{-1}\)) | 32.0 ± 5.4 | 39.1 ± 4.3 | \( < 0.02 \) | 38.6 ± 6.5 | \( < 0.03 \) | 0.98 |
| **Circulatory indices** | | |
| PR (beats.min\(^{-1}\)) | 384 ± 31 | 321 ± 14 | \( < 0.01 \) | 317 ± 37 | \( < 0.01 \) | 0.98 |
| MAP (mmHg) | 138 ± 14 | 154 ± 6 | \( < 0.01 \) | 156 ± 14 | \( < 0.01 \) | 0.96 |
| **Arterial blood data** | | |
| \( \text{PaO}_2 \) (mmHg) | 92.9 ± 3.8 | 96.7 ± 9.0 | 0.29 | 94.3 ± 5.6 | 0.82 | 0.74 |
| \( \text{PaCO}_2 \) (mmHg) | 37.8 ± 9.6 | 33.8 ± 3.9 | 0.56 | 37.2 ± 6.1 | 0.98 | 0.76 |
| pH | 7.41 ± 0.02 | 7.40 ± 0.01 | 0.17 | 7.42 ± 0.01 | 0.94 | 0.20 |
| \( \text{Ca}^{2+} \) (mmol.L\(^{-1}\)) | 1.32 ± 0.08 | 1.25 ± 0.05 | 0.19 | 1.29 ± 0.11 | 0.78 | 0.66 |
| \( \text{Na}^+ \) (mmol.L\(^{-1}\)) | 145 ± 3 | 147 ± 5 | 0.49 | 145 ± 2 | 0.98 | 0.72 |
| \( \text{K}^+ \) (mmol.L\(^{-1}\)) | 3.4 ± 0.3 | 3.1 ± 0.3 | 0.14 | 3.2 ± 0.3 | 0.48 | 0.81 |
| Hemoglobin (g.dL\(^{-1}\)) | 11.9 ± 1.4 | 10.8 ± 1.0 | 0.20 | 11.7 ± 1.4 | 0.93 | 0.51 |
| Hematocrit (%) | 35 ± 4 | 32 ± 3 | 0.24 | 34 ± 4 | 0.96 | 0.51 |
| Glucose (mg.dL\(^{-1}\)) | 143 ± 21 | 164 ± 19 | \( < 0.04 \) | 193 ± 30 | \( < 0.01 \) | 0.06 |
| Body temperature (°C) | 38.1 ± 0.4 | 37.9 ± 0.4 | 0.87 | 38.2 ± 0.5 | 0.82 | 0.99 |

\(^*\) Significant difference (\( p < 0.05 \)) between the NS and the DEXM-50+ATI-1.0 or the DEXM-50+EFA-1.0.

No significant difference in any parameter was observed between DEXM-50+ATI-1.0 and DEXM-50+EFA-1.0.

Data for the NS are the summed data (\( n = 21 \)) obtained at control recording in Protocol 1. Rats in the DEXM-50+ATI-1.0 and DEXM-50+EFA-1.0 (\( n = 6 \) in each group) were given dexmedetomidine (50 \( \mu \text{g} \cdot \text{kg}^{-1} \)), followed 5 min later by 1.0 mg \( \text{kg}^{-1} \) atipamezole or efaroxan.

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irrespective of whether the animal was mature or newborn. In addition, rats administered 5 or 50 μg·kg⁻¹ dexmedetomidine showed hypoxemia (i.e. decrease in PaO₂) (p = 0.04 and p < 0.01, respectively), hyperglycemia (i.e. increase in blood glucose) (both p < 0.01), and increase in hemoglobin (p = 0.92 and p = 0.04). In rats given 50 μg·kg⁻¹ of dexmedetomidine, the significantly decreased V′E (p = 0.03) was consistent with the results of ABGs, which indicated hypoventilation (i.e. increase in PaCO₂) (p < 0.01) and acidemia (i.e. decrease in pH) (p = 0.01). MAP was not changed significantly by 5 μg·kg⁻¹ of dexmedetomidine (p = 0.24) but was increased to 133% by 50 μg·kg⁻¹ dexmedetomidine (p < 0.01). In adult male human volunteers, incrementally administered dexmedetomidine gradually increases MAP (+ 12% from baseline) after transient hypotension (~13%), and the increased MAP coincides with a drop in heart rate and stroke volume (and hence cardiac output), increases in pulmonary and systemic vascular resistance, and a slight increase in PaCO₂ [25]. Hence, in our rats, it is possible that the increased PaCO₂ (Table 2) was induced by suppression of both ventilation and cardiac output upon administration of 50 μg·kg⁻¹ of dexmedetomidine. The results of Protocol 1 (Tables 1 and 2), suggest that the effects of 50 μg·kg⁻¹ dexmedetomidine are not merely suppressive (e.g. on the V′E and PR), but also stimulatory (e.g. on the V₁, MAP, hemoglobin, and glucose), in spontaneously breathing adult rats.

In Protocol 2, the summed data (n = 21) obtained at control recording in Protocol 1 were used as the data for the NS. All animals were administered 50 μg·kg⁻¹ of dexmedetomidine (Fig 2B). Compared with the mean values of NS (n = 21), administration of 0.5 mg·kg⁻¹ atipamezole or efaroxan in addition to 50 μg·kg⁻¹ of dexmedetomidine prevented changes in most of the cardiorespiratory indices affected by administration of 50 μg·kg⁻¹ dexmedetomidine alone (Table 3). In earlier studies, almost complete prevention of the dexmedetomidine-related physiological changes was obtained when dexmedetomidine and atipamezole were used in a ratio of 1 to 10 (i.e. 100 μg·kg⁻¹ dexmedetomidine and 1.0 mg·kg⁻¹ atipamezole) in fentanyl/nitrous oxide-anesthetized adult rats [17], or when dexmedetomidine and efaroxan were used in a ratio of 1 to 10 (i.e. 10⁻⁶ M dexmedetomidine and 10⁻⁵ M efaroxan) in adult rat hippocampal slices [18]. Similarly, we found no significant difference in any parameter, except PR and glucose (both p < 0.01) in rats administered 0.5 mg·kg⁻¹ atipamezole in addition to 50 μg·kg⁻¹ dexmedetomidine, and T₁, PR, and blood glucose (p = 0.03, p < 0.01, p < 0.01, respectively) in rats administrated 0.5 mg·kg⁻¹ efaroxan in addition to 50 μg·kg⁻¹ dexmedetomidine. Moreover, in this experiment, no significant difference in any parameter, including V₁/T₁ (Fig 3B–3E), was observed between rats given 0.5 mg·kg⁻¹ of atipamezole and efaroxan (Table 3) or 1.0 mg·kg⁻¹ of atipamezole and efaroxan (Table 4) in addition to 50 μg·kg⁻¹ dexmedetomidine.

Dexmedetomidine activates both α₂-adrenoceptors and I₁ receptors, and, in theory, supplemental administration of atipamezole (a selective α₂-adrenoceptor antagonist) would block only α₂-adrenoceptor activation, whereas supplemental administration of efaroxan (an α₂-adrenoceptor/I₁ receptor antagonist) would block the activation of both α₂-adrenoceptors and I₁ receptors. Hence, the similarity in the effects of supplemental atipamezole and efaroxan administration suggests that dexmedetomidine-related cardiorespiratory changes in spontaneously breathing adult rats occur predominantly through α₂-adrenoceptor activation, not I₁ receptor activation.

In our previous study on spontaneously breathing newborn rats, V₁/T₁ was not affected by dexmedetomidine (50 μg·kg⁻¹) alone or by dexmedetomidine (50 μg·kg⁻¹) plus 1, 5, or 10 mg·kg⁻¹ of atipamezole, but it was significantly decreased by dexmedetomidine (50 μg·kg⁻¹) plus 1, 5, or 10 mg·kg⁻¹ of efaroxan; therefore, we concluded that it is I₁ receptor activation that maintains V₁/T₁ (i.e. an index of respiratory drive) in newborn rats [11]. In contrast, in the present study, the distinct effect of I₁ receptor activation on V₁/T₁ was not apparent in
spontaneously breathing adult rats (Fig 3B–3E). Together, these results on adult and newborn rats suggest that the functional roles of $\alpha_2$-adrenoceptors and $I_1$ receptors on the cardiorespiratory system differ between immature and mature animals.

As limitations of the study, we cannot exclude the possible influences of isoflurane anesthesia and flurbiprofen axetil, which we administered for surgical preparation before the recordings. In addition, we did not directly measure the flow signals but instead used a barometric method to measure the fluctuations caused in chamber pressure by respiratory movement. Therefore, for example, $V_T$ can be overestimated in cases where the respiratory flow resistance is high [26]. However, this seems unlikely, because an earlier study on adult rats under mechanical ventilation reported that dexmedetomidine (250 $\mu$g $\cdot$ kg$^{-1}$ intraperitoneal injection followed by intravenous infusion of 0.5 $\mu$g $\cdot$ kg$^{-1}$) did not significantly change respiratory mechanical parameters in comparison with those measured in animals that received diazepam (5 mg) and pentobarbital (20 mg $\cdot$ kg$^{-1}$), intraperitoneally [7]. Ventilation is under the influence of circadian rhythm [27] and $V_T/T_1$ is reported to increase with age. In human infants, “on-switching” and “off-switching” of inspiratory activity may depend on the sleep state [28], and the lengths of time spent in different sleep states (i.e. rapid-eye-movement (REM) sleep (or active sleep) [29] and quiet sleep) change with postnatal development [30]. REM sleep can be suppressed by clonidine [31], which is another clinically used $\alpha_2$-adrenoceptor/$I_1$ receptor agonist [1, 6]. In this study, although we restricted our measurements to the afternoon (approximately 14:00–16:00), it was not clear how dexmedetomidine administration affected sleep state of the spontaneously breathing animal during the measurements.

We observed significantly increased hemoglobin after administration of 50 $\mu$g $\cdot$ kg$^{-1}$ dexmedetomidine (Table 2) and the effect was prevented by atipamezole or efaroxan (Tables 3 and 4); this might have due to dexmedetomidine-related diuretic [32] and hyperglycemic effects [33]. The hemocoencentration increases viscosity [34] and may obstruct circulatory $O_2$ and $CO_2$ transport and secondarily stimulate sympathetic outflow. However, unlike the effect on MAP, blockade of the $\alpha_2$-adrenoceptor or the $\alpha_2$-adrenoceptor/$I_1$ receptor by atipamezole and efaroxan could not completely prevent the dexmedetomidine-related decrease in PR (Tables 3 and 4), and the results were similar to those observed previously in newborn rats [10, 11]. It is possible that PR is influenced by a reflex bradycardia, either with possible enhancement of the reflex bradycardia through the $\alpha_2$-adrenoceptor (i.e. by administration of dexmedetomidine alone) or without this enhancement (i.e. by administration of dexmedetomidine plus atipamezole or efaroxan) [35]. Further investigation of the mechanisms underlying the persistent drop in the PR is warranted. In other words, from a practical point of view, the results suggest that $f_R$ is a better indicator than heart rate or PR for monitoring the cardiorespiratory effects of dexmedetomidine or its antagonists (e.g. atipamezole or efaroxan).

Conclusions

The results of this study suggest that not all cardiorespiratory indices are suppressed by dexmedetomidine in spontaneously breathing adult rats: some ($V_T$ and MAP) are stimulated to increase. The similarity in the effects of supplemental administration of atipamezole and efaroxan suggests that dexmedetomidine-related decrease in $V_E$ and increase in MAP are observed simultaneously and occur predominantly through activation of $\alpha_2$-adrenoceptors, but not $I_1$ receptors, in the spontaneously breathing adult rats.

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