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Research article

Effects of α2-adorenoceptor agonist dexmedetomidine on respiratory rhythm generation of newborn rats

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HIGHLIGHTS

- Dexmedetomidine is clinically used as an analgesic and sedative agent.
- We examined the effects of this drug on respiratory activity and spinal reflex responses.
- Dexmedetomidine (0.1–10 μM) dose-dependently depressed respiratory rhythm.
- This drug depressed spinal reflex response at a lower concentration range (0.1–1 nM).
- These depressions were reversed by α2-adorenoceptor antagonist atipamezole.

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ABSTRACT

Dexmedetomidine, an α2-adrenoceptor agonist which has a slight side effect on breathing, is clinically used as an analgesic and sedative agent. Previous studies have shown depressing or modest effects of α2-adorenoceptor agonists on respiratory rhythm generation in newborn rat preparation in vitro. In contrast, it was recently reported that dexmedetomidine induced long-lasting activation of respiratory rhythm in brainstem–spinal cord preparation isolated from neonatal mice. In the present study, we examined whether dexmedetomidine induces any effects on respiratory rhythm in brainstem–spinal cord preparation isolated from newborn rats. We also examined the effects of dexmedetomidine on reflex response in the spinal cord, which is presumed to be an indication of nociceptive response. We found that the administration of dexmedetomidine, at the range of 0.1–10 μM, dose-dependently depressed respiratory rhythm and that the inhibitory effect was reversed by atipamezole, an α2-adorenoceptor antagonist. Spinal cord reflex responses were depressed by the application of dexmedetomidine at the range of 0.1–1 nM, a lower concentration than that affecting respiratory rhythm. The inhibitory effect was also reversed by atipamezole. Our findings provide neuronal mechanisms that support the clinical use of dexmedetomidine, which shows sedative and antinociceptive effects with minimal side effects on breathing.

1. Introduction

Dexmedetomidine, an α2-adrenoceptor agonist, is widely used as an analgesic and sedative agent, which is known to have slight side effects on breathing [1–4]. Some studies have shown respiratory depression or short-term apnea induced by dexmedetomidine [5–9]. The inhibitory effects of dexmedetomidine, however, are thought to be minimal and patients are easily awakened under the sedation [10]. Brainstem–spinal cord preparations from the newborn rat or mouse provide an established

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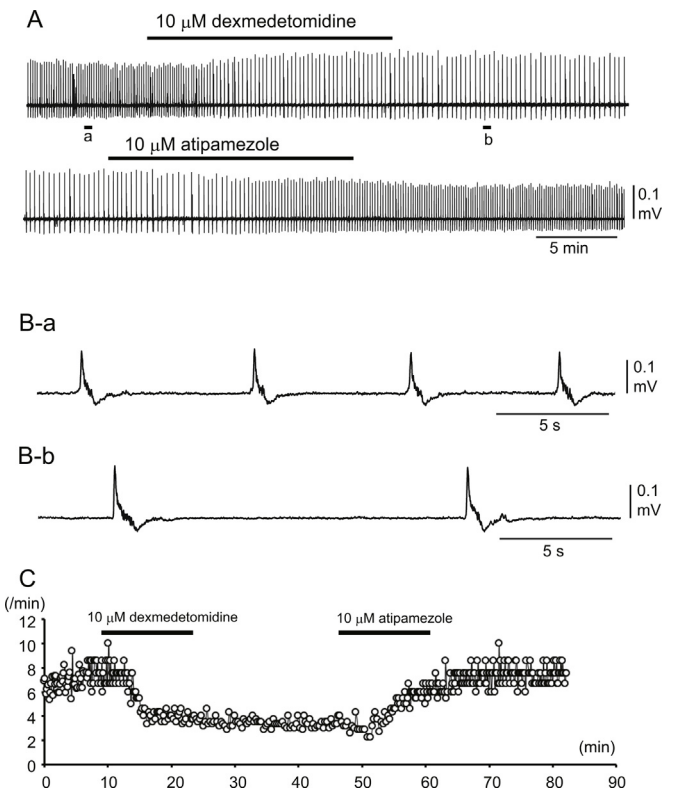

Fig. 1. Typical examples of dexmedetomidine effects on C4 activity. Change in C4 trace in response to application of 10 μM dexmedetomidine (15 min) (A). Lower panel shows a continuous recording following the upper panel. After 20 min washout of dexmedetomidine, 10 μM atipamezole (α2-antagonist) was applied. Note that the decrease in burst rate by dexmedetomidine was reversed with treatment of atipamezole. Faster sweep representation of C4 activity (B). B-a and B-b correspond to a and b in (A). Time course of C4 burst rate following 10 μM dexmedetomidine and 10 μM atipamezole application (C). Each circle denotes burst rate (bursts/min) calculated from the recording in (A).

model for the basic analysis of neurophysiological and pharmacological properties of the respiratory center [11,12]. Previous studies have shown depressing or modest effects of α2-adrenoceptor agonist on respiratory rhythm generation in newborn rat preparation in vitro [13–15]. However, Voituron et al. [16] reported the long-lasting activation of respiratory rhythm generation by dexmedetomidine and clonidine in brainstem–spinal cord preparation isolated from neonatal mice and noted that the effects were not antagonized by α2 antagonist, yohimbine. In contrast, Tamiya et al. [17] recently demonstrated that the administration of dexmedetomidine induced depression of respiratory rhythm via α2 adrenoceptors in newborn rats in vivo. Regarding antinociceptive effects, α2-adrenoceptors and opioid receptors have important roles at the spinal cord level [18–24]. It has been established that slow ventral root potential induced by ipsilateral dorsal root stimulation in the isolated (typically lumbar) spinal cord of newborn rats reflects nociceptive reflex. This in vitro experimental model is useful for assessing the actions of analgesics [25–28]. Dexmedetomidine and morphine inhibited slow ventral root potentials following ipsilateral dorsal root stimulation in newborn rat spinal cord preparation in vitro [29].

In consideration of the discrepancies regarding the effects of dexmedetomidine on respiratory activity reported in the above-mentioned studies, we examined whether the drug induces any effects on respiratory rhythm in brainstem–spinal cord preparation isolated from newborn rat, which has been used for in vitro analyses of respiratory control in a number of previous studies [12]. We also examined the effects of dexmedetomidine on reflex response in the spinal cord, which are presumed to be an indication of nociceptive response.

2. Materials and methods

The Animal Research Committee of Showa University approved the experimental protocols of the present study. Experiments were performed with brainstem–spinal cord preparations from 0 to 2 day old Wistar rats. Newborn rats were anesthetized with isoflurane, and the brainstem and spinal cord were isolated according to previously described methods [11,30]. The brainstem was decerebrated rostrally, between the roots of the sixth cranial nerve and the lower border of the trapezoid body; thus, most of pons was removed. The preparation was placed in a perfusion chamber (2 ml), ventral side upwards, and superfused at a rate of 3.0 ml/min with the following artificial cerebrospinal fluid (ACSF) [11] (in mM): 124 NaCl, 5.0 KCl, 1.2 KH_2PO_4, 2.4$CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$, and 30 glucose, equilibrated with 95% O_2 and 5% CO_2, pH 7.4, at 25–26 °C. Rhythmic inspiratory burst activity was continuously recorded from the fourth or fifth cervical ventral root (C4 or C5), which contains the phrenic nerve fibers, using a glass suction electrode. The burst rate (bursts/min) was calculated from the mean rate for 3–5 min.

To simultaneously evaluate the effects of dexmedetomidine on respiratory activity and putative nociceptive responses, the ipsilateral dorsal root of C7 or C8 was stimulated using a glass suction electrode and induced reflex response was recorded from C4/C5 together with rhythmic inspiratory activity through a 0.5 Hz high pass filter. The dorsal root was stimulated every 10 s with a 5–20 V, 200 μs square pulse. In some experiments, spinal cord preparations, including the C4 to Th2 level, were isolated (thus, not including the medulla) and used for the test of the above reflex response. We measured the peak amplitude and area of the reflex response; the former represents the amplitude of short latency oligosynaptic response and the latter may indicate total magnitude of the synaptic response. Regarding the effects of drugs on the reflex response, there is no significant difference between preparations with and without the medulla. Therefore, data from both preparations were accumulated and analyzed.

The preparations were superfused with ACSF for at least 15–30 min until inspiratory rhythm or spinal reflex response became stable. Then, dexmedetomidine was bath-applied for 15 min and washed out. In some preparations, α2-adorenoceptor antagonist, atipamezole hydrochloride (Orien Co., Espoo, Finland) was also examined after dexmedetomidine was washed out. We used clinical grade dexmedetomidine hydrochloride (200 μg/2 ml physiological saline stock solution), which was supplied as Precedex (for intravenous injection) from Hospira Japan Co., Ltd. (Osaka, Japan).

All data analyses were performed by LabChart 7 Pro (ADInstruments, Castle Hill, Australia). Data are presented as mean and standard deviation (SD). The significance of values was analysed by one-way ANOVA followed by a Bonferroni Multiple Comparison Test (GraphPad InStat; GraphPad Software Inc., CA, USA), at a confidence level of $P < 0.05$.

3. Results

3.1. Effects of dexmedetomidine on the C4 nerve activity

The effects of dexmedetomidine (0.001–10 μM) on C4 nerve activity were examined in 34 preparations. The control C4 burst rate was 6.17 ± 1.46 /min. C4 nerve activity was dose-dependently inhibited by 15 min application of dexmedetomidine in the range of 0.1–10 μM. A typical example is shown in Fig. 1. The dose-dependent effects of dexmedetomidine on C4 burst rate are