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Reversal of morphine-induced respiratory depression by doxapram in anesthetized rats

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ABSTRACT

The present study was undertaken to investigate whether doxapram, a blocker of tandem pore K^+ (TASK-1/−3) channels, is a useful tool for recovery from morphine-induced ventilatory disturbances. Spontaneous ventilation and the hind leg withdrawal response against noxious thermal stimulation were recorded simultaneously in anesthetized rats. Morphine (1.0 mg/kg, i.v.) decreased the minute volume resulting from depression of the ventilatory rate and tracheal airflow. Concomitantly, it prolonged the latency of withdrawal response against the thermal stimulation. Subsequent intravenous injection of doxapram recovered the morphine-induced ventilatory depression. This effect of doxapram declined rapidly after a single injection (1.0–3.0 mg/kg, i.v.) but persisted with a continuous infusion (0.33 mg/kg/min). Neither single injection nor continuous infusion of doxapram had any detectable effect on the analgesic potency of morphine. The central respiratory activity was recorded from the phrenic nerve in anesthetized, vagotomized, paralyzed and artificially ventilated rats. Morphine (3.0 mg/kg, i.v.) induced respiratory depression, characterized by a prolonged plateau-like inspiratory discharge (apneustic discharge) in the phrenic nerve. Doxapram (10 mg/kg, i.v.) restored the morphine-induced apneustic discharge to an augmenting inspiratory discharge. This study demonstrated that doxapram counteracted morphine-induced respiratory depression by stimulating the central respiratory network without compromising morphine antinociception. These results support the clinical use of doxapram for amelioration of ventilatory disturbances in patients treated with opioids.

1. Introduction

Opioids are effective for treatment of acute pain such as postoperative pain and also important for palliative care to relief severe and chronic pain. However, all opioids in clinical use have serious adverse reactions, of which respiratory depression is potentially lethal (Dahan et al., 2010; Kimura and Haji, 2014). Opioids directly depress the central respiratory activity by activating opioid receptors expressed in the bulbar respiratory neurons (Haji et al., 2003; Lalley, 2003; Mansour et al., 1988), leading to weakening of ventilation, depression of ventilatory responses to hypercapnia and hypoxia, and induction of abnormal breathing (Pattinson, 2008; Shook et al., 1990). Naloxone, an antagonist of opioid receptors, is able to reverse the opioid-induced respiratory depression, but it reduces or abolishes opioid analgesia as well. Therefore, it is important to prevent/reverse the ventilatory disturbances without loss of analgesic action. One solution would be to add a respiratory stimulant, which through activation of a non-opioidergic mechanism will stimulate breathing and consequently counteract the opioid-induced ventilatory disturbances. There are some centrally acting candidates that either increase the intracellular cAMP content, activate the 5-HT_{1A} function, or facilitate the muscarinic mechanism in the central respiratory network (Boom et al., 2012; Dahan et al., 2010; Kimura and Haji, 2014; Kimura et al., 2013, 2015). However, almost all have not been developed to treat ventilatory disturbances and come with side effects that limit their clinical use.

Agents that block tandem pore K^+(TASK-1/−3) channels are suggested to antagonize the opioid-induced respiratory depression (Golder et al., 2013; van der Schier et al., 2014). Among them, doxapram has been demonstrated to inhibit such K^+ channels in the type 1 carotid body cells (Cotten, 2013; Lahiri et al., 2006; Peers, 1991) and in the medullary respiratory neurons (Bayliss et al., 2001; Osaka et al., 2014; Yamamoto et al., 2002), resulting in stimulation of respiration. Indeed, doxapram has been prescribed for expecting a facilitating effect on ventilation in postoperative patients (Gairola et al., 1980; Golder et al., 2013; Yost, 2006). However, effects of doxapram on opioid analgesia were not fully evaluated, although involvement of a variety of K^+ channels in nociception has been proposed (Ocaña et al., 2004; Tsantoulas and

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McMahon, 2014). Furthermore, it was reported that doxapram-induced increase in cardiac output was associated with reduced plasma concentration of alfentanil (Roozekrans et al., 2013). This result raised concern that doxapram might attenuate both opioid-induced ventilatory depression and analgesia.

Therefore, we recorded spontaneous ventilation and nociceptive response simultaneously in anesthetized rats and evaluated the effectiveness of doxapram on morphine-induced ventilatory depression and analgesia in the same animal. Next, we examined effects of doxapram on a prolonged, plateau-like inspiratory (apneustic) discharge in the phrenic nerve induced by morphine (Kimura et al., 2015; Niwa and Haji, 2011).

2. Materials and methods

The present study was approved by the Animal Care Committee at Aichi Gakuin University and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

2.1. General surgery and measurements

2.1.1. Experiment 1: Measurements of spontaneous ventilation and blood pressure in anesthetized rats

Male Wistar rats (20–25 weeks, 400–500 g body weight) were anesthetized intraperitoneally with a mixture of urethane (1.0 g/kg) and α-chloralose (25 mg/kg). The head of the animal was fixed in the supine position. For measurement of tracheal airflow, the trachea was intubated and fitted to a respiratory flow head (MLT1L, AD Instruments, Castle Hill, Australia) connected to a spirometer (ML141, AD Instruments). An arterial cannula was inserted into the carotid artery for recording blood pressure and a venous cannula into the femoral vein for administration of drugs. The arterial cannula was connected with a pressure transducer and the blood pressure signal was amplified (AP-601G; Nihon Kohden, Tokyo, Japan). Doxapram was administered intravenously after a stable recording. The tracheal airflow and blood pressure were displayed on computer display using a signal processing software and stored on hard disk for off-line analyses (PowerLab, AD Instruments).

2.1.2. Experiment 2: Simultaneous measurements of spontaneous ventilation and nociceptive response in anesthetized rats

The method was similar to that reported previously (Kimura et al., 2013, 2015). Younger male Wistar rats (6–7 weeks, 130–200 g body weight) were used, since ageing decreased the anti-nociceptive effect of morphine in adult rats (Jourdan et al., 2002) and since the similar size of rats were used in the previous reports that examined the nociceptive response against thermal stimulation (Hamura et al., 2000; Kimura et al., 2013). The animal was anesthetized lightly with urethane (1.1–1.3 g/kg, i.p.) to retain the nociceptive response. For measurement of tracheal airflow, a catheter was inserted into the trachea and fitted to a respiratory flow head (MLT1L; AD Instruments) connected to a spirometer (ML141; AD Instruments). Nociceptive reflex was assessed by a paw immersion test in parallel with measurement of breathing. The left hind leg was suspended through a hole in the holder. The ankle was connected to a force-displacement transducer (SB-1T; Nihon Kohden) for detection of the withdrawal response of hind leg to noxious thermal stimulation. The paw was immersed in water bath (48.0 ± 0.2 °C) every 15 min. A 10 s cut-off time was imposed to avoid tissue damage. Morphine was administered intravenously after a stable recording. Doxapram was administered by a single injection 29 min after the morphine injection or by a continuous infusion 15 min after the morphine injection. The tracheal airflow and hind leg withdrawal response were displayed on computer display using a signal processing software and stored in hard disk (PowerLab, AD Instruments).

2.1.3. Experiment 3: Measurement of the phrenic nerve discharge in anesthetized, paralyzed and artificially ventilated rats with vagotomy

The experiment was performed according to our previous reports (Kimura et al., 2015; Niwa and Haji, 2011). Male Wistar rats (20–25 weeks, 400–500 g body weight) were anesthetized intraperitoneally with a mixture of urethane (1.0 g/kg) and α-chloralose (25 mg/kg). Catheters were inserted into the trachea and femoral vein. The head of the animal was fixed in a stereotaxic frame in the supine position. The vagus nerve was cut bilaterally to avoid synchronization of the central respiratory rhythm of the phrenic nerve discharge with the artificial stroke rhythm of a ventilator. The animals were paralyzed with intravenous injection of pancuronium (1.0 mg/kg) and artificially ventilated. Tracheal pressure was kept between 1 and 8 cm H_2O and end-tidal CO_2 was maintained at 4.5–5.5%. The right phrenic nerve was dissected and cut distally. The proximal end of phrenic nerve was mounted on bipolar Ag-AgCl electrodes and covered with liquid paraffin. Efferent discharges of the phrenic nerve were recorded as a hallmark of the central respiratory activity. Signals were amplified, rectified, filtered (30–3000 Hz) and integrated (0.1 s time constant) with a leaky integrator (San-ei 7747, Tokyo, Japan). Morphine was administered intravenously after a stable recording of the phrenic nerve discharge, and doxapram was injected 15 min after the morphine injection. Raw and integrated phrenic neurograms were displayed on computer display using a signal processing software and stored on hard disk (PowerLab, AD Instruments).

2.2. Drugs

Morphine hydrochloride (Shionogi Co. Ltd., Osaka, Japan) was dissolved in saline. Doxapram hydrochloride (DOPRAM Injectable, Kissei Pharmaceutical Co., Tokyo, Japan), which was supplied as 20 mg/ml in water with chlorobutanol (5 mg/ml) added as a preservative, was diluted with saline. Urethane (Sigma, St. Louis, MO, USA) and α-chloralose (Tokyo Kasei Kogyo, Tokyo, Japan) were dissolved in distilled water.

2.3. Data acquisitions and statistical analyses

In experiment 1, the tidal volume, ventilatory rate and minute volume were calculated from the tracheal airflow signal and averaged for 10 s. Mean blood pressure was measured. In experiment 2, the latency of withdrawal response to the thermal stimulation was measured. The minute volume was calculated and averaged for 10 s. In experiment 3, the peak amplitude of integrated phrenic nerve discharge was measured. The inspiratory and expiratory periods, which were distinguished referring to the phrenic discharge, were measured. Data were taken at 5 consecutive respiratory cycles and averaged. All variables were obtained before and at several points after the drug application. Data are expressed as the mean ± S. E. M. (n = number of animals). The statistical differences were evaluated using two-tailed multiple *t*-test with Bonferroni correction or Student's *t*-test following one-way analysis of variance (ANOVA). Differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Experiment 1: Effects of doxapram on ventilation and blood pressure

At first, effects of doxapram (0.3–10 mg/kg, i.v.) alone on ventilation and blood pressure were examined in spontaneously