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Blockade of phosphodiesterase 4 reverses morphine-induced ventilatory disturbance without loss of analgesia

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ABSTRACT

Aims: Ventilatory disturbance is a fatal side-effect of opioid analgesics. Separation of analgesia from ventilatory depression is important for therapeutic use of opioids. It has been suggested that opioid-induced ventilatory depression results from a decrease in adenosine 3′,5′-cyclic monophosphate content in the respiratory-related neurons. Therefore, we examined the effects of caffeine, a methylxanthine non-selective phosphodiesterase (PDE) inhibitor with adenosine antagonistic activity, and rolipram, a racetam selective PDE4 inhibitor, on ventilatory depression induced by morphine.

Main methods: Spontaneous ventilation and paw withdrawal responses to nociceptive thermal stimulation were measured in anesthetized rats simultaneously. The efferent discharge of the phrenic nerve was recorded in anesthetized, vagotomized, paralyzed and artificially ventilated rats.

Key findings: Rolipram (0.1 and 0.3 mg/kg, i.v.) and caffeine (3.0 and 10.0 mg/kg, i.v.) relieved morphine (1.0 mg/kg, i.v.)-induced ventilatory depression but had no discernible effect on its analgesic action. Rolipram (0.3 and 1.0 mg/kg, i.v.) and caffeine (10.0 and 20.0 mg/kg, i.v.) recovered morphine (3.0 mg/kg, i.v.)-induced prolongation and flattening of inspiratory discharge in the phrenic nerve.

Significance: Inhibition of PDE4 may be a possible approach for overcoming morphine-induced ventilatory depression without loss of analgesia.

Introduction

Opioids exhibit a narrow therapeutic window between doses of analgesia and respiratory disturbance [7,8,18]. Opioids slowed breathing frequency and reduced its depth, and also dampen ventilatory responses to hypercapnia and hypoxia principally through activation of μ opioid receptors [11,19,29,36]. Naloxone, an antagonist of μ opioid receptors, naloxone effectively and rapidly reverses ventilatory depression induced by opioids, however it attenuates opioid analgesia concomitantly [7]. Overcoming opioid-induced ventilatory depression with leaving its analgesic efficacy would be a therapeutic benefit for patients in pain and reduce death from opioid poisoning.

It has been suggested that ventilatory depression results from decreasing adenosine 3′,5′-cyclic monophosphate (cAMP) content in the respiratory-related neurons by opioids [2]. Therefore, offset of a decrease in cAMP may be a potential pharmacological approach for overcoming opioid-induced ventilatory disturbances. Several drugs that elevate intracellular cAMP content such as 5-HT_{4a} and dopamine D_1 agonists restore opioid-induced respiratory depression in anesthetized rats without any effect on analgesia [21,22,24]. In an earlier human study, caffeine antagonized the respiratory depressant effects of morphine and codeine [4]. Furthermore, caffeine restored morphine-induced apneustic (prolonged plateau-like inspiratory) discharge of the phrenic nerve in anesthetized rats [17] and a μ agonist (DAMGO)-induced depression of respiratory-related rhythm in the rat brainstem slices [33]. Additionally, another methylxanthine theophylline restored DAMGO-induced depression of the phrenic inspiratory discharge in in situ heart–brainstem preparation of neonatal rats [26]. These results may be derived from the blocking actions of methylxanthines on phosphodiesterases (PDEs) and also on A-type adenosine receptors [9]. On the other hand, when combined with morphine, caffeine had potentiating, attenuating or no effect on morphine analgesia depending on experimental conditions such as the dose, route of administration, nociceptive test and animal species [35]. Moreover, it is unknown whether caffeine at doses that restore the opioid-induced ventilatory depression affects the analgesic action of opioid. Therefore, its usefulness in countering respiratory depression should be carefully assessed. PDE inhibitors, rolipram and isobutylmethylxanthine, recovered respiratory depression after treatment of fentanyl, DAMGO or prostaglandin E_1 in in vitro newborn rat models of the neuronal control of respiration [2,33,42]. Currently, the effects of systemic PDE inhibitors on opioid-induced ventilatory depression and analgesia remain to be unsolved in vivo.

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The present study was performed to explore the possibility of therapeutic usefulness of caffeine and rolipram on ventilatory disturbances in patients with opioid treatment. We examined the effects of these drugs on morphine-induced ventilatory depression and analgesia simultaneously in spontaneously breathing anesthetized rats. In addition, to assess the neuronal mechanisms of caffeine and rolipram, we investigated their effects on apneustic discharges of the phrenic nerve induced by morphine in anesthetized, paralyzed and artificially ventilated rats with vagotomy.

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.

Experiment 1: Simultaneous measurements of spontaneous ventilation and nociceptive response in anesthetized rats

The method was similar to that reported previously [18]. Male Wistar rats (6–7 weeks, 120–200 g) were anesthetized intraperitoneally with urethane (1.1–1.3 g/kg) and placed on a cloth holder in the prone position. For measurement of tracheal airflow, a catheter was inserted into the trachea and fitted to a respiratory flow head (MLT1L; AD Instruments Pty Ltd., Castle Hill, Australia) connected to a spirometer (ML141; ADInstruments). 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, Tokyo) for detection of the withdrawal response of hind leg to noxious thermal stimulation. The paw was immersed in a 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 via a venous cannula after a 15-min control recording, and other drugs were injected 25 min after the morphine injection. The tracheal airflow and withdrawal response were displayed on a computer display using a signal processing software and stored in a hard disk for off-line analyses (Macintosh-PowerLab; AD Instruments Pty, Castle Hill, Australia).

Experiment 2: Measurement of the phrenic nerve discharge in anesthetized, paralyzed and artificially ventilated rats with vagotomy

The experiment was performed according to our previous report [28]. Male Wistar rats (300–550 g) were anesthetized intraperitoneally with a mixture of urethane (1.0 g/kg) and α-chloralose (25.0 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. The animals were paralyzed with intravenous injection of pancuronium bromide (2.0 mg/kg) and artificially ventilated. Tracheal pressure was kept between 1 and 8 cmH_2O and end-tidal CO_2 was maintained at 5–6%. The right phrenic nerve was dissected and cut distally. The proximal end of the 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 after a stable recording of the phrenic nerve discharge, and other drugs were injected 15 min after the morphine injection. All recordings were displayed on the computer display using a signal processing software and stored in the hard disk for off-line analyses (PowerLab; AD Instruments).

Drugs

Morphine hydrochloride (Shionogi & Co. Ltd., Osaka, Japan) and caffeine (Nacalai Tesque, Kyoto, Japan) were dissolved in physiological saline. Urethane (Sigma, St. Louis, MO, USA) and α-chloralose (Tokyo Kasei Kogyo, Tokyo, Japan) were dissolved in distilled water. Rolipram (Cayman Chemical Co., Ann Arbor, MI, USA) was dissolved in 0.3% and 2.0% ethanol in distilled water for experiment 1 and experiment 2, respectively. According to the previous reports [1,20,38,40,44], sufficient doses of rolipram (0.1–1.0 mg/kg) and caffeine (3.0–20.0 mg/kg) were used to block PDE4.

Data acquisition and statistical analysis

In experiment 1, the latency (Latency) of withdrawal response to the thermal stimulation was measured. Tracheal airflow (tidal volume) and ventilatory rate were measured and averaged for 10 s, and minute ventilation (V_E) was calculated. In experiment 2, the peak amplitudes (Amplitude) of integrated phrenic nerve discharge were measured. The inspiratory (Ti) and expiratory periods (Te), 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 ± SEM (n = number of animals). The statistical differences were evaluated using two-tailed multiple *t*-test with Bonferroni correction following one-way analysis of variance (ANOVA). Differences with $P < 0.05$ were considered significant.

Results

Experiment 1: Effects of rolipram and caffeine on morphine-induced ventilatory disturbance and analgesia

We have previously demonstrated the efficacy of morphine on breathing and analgesia in anesthetized rats [18], where morphine (1.0 and 3.0 mg/kg, i.v.) suppressed V_E and prolonged the paw withdrawal latency in a dose-dependent manner. Referring to those results, a dose of 1.0 mg/kg, which produced significant but not too much effect on ventilation and analgesia, was chosen for experiment 1 in this study.

Intravenous injection of morphine (1.0 mg/kg) slowed the ventilatory rate together with a decrease in the magnitude of tracheal airflow and also produced analgesia (Fig. 1). The V_E decreased to 80.8 ± 1.8% (n = 7) of the control and withdrawal latency against the thermal stimulation increased from 1.9 ± 0.2 s to 3.2 ± 0.2 s (n = 7) after 15 min. Additional administration of rolipram (0.1 and 0.3 mg/kg, i.v.) counteracted the ventilatory depression induced by morphine (Fig. 1). The higher dose of rolipram restored the V_E to 117.9 ± 3.1% (n = 7) of the control 5 min after injection, which was significantly different from that in the vehicle group (81.8 ± 2.5%, n = 7, $P < 0.01$). Both doses of rolipram did not interfere with morphine analgesia.

Caffeine (3.0 and 10.0 mg/kg, i.v.) also antagonized the morphine-induced ventilatory depression (Fig. 2). The V_E recovered to 101.8 ± 3.1% (n = 6) of the control 5 min after 10.0 mg/kg of caffeine, which was significantly different from that in the vehicle group (83.6 ± 2.0%, n = 9, $P < 0.01$). Caffeine had no discernable effect on morphine analgesia.

Experiment 2: Effects of rolipram and caffeine on morphine-induced apneustic discharge of the phrenic nerve

The phrenic nerve discharge was characterized by an augmenting discharge during inspiration and a small decrementing discharge followed by complete silence during expiration in eupnea (Fig. 3A). Ti and Te were 0.39 ± 0.01 s and 1.82 ± 0.06 s, respectively (n = 20). Intravenous morphine (1.0–10.0 mg/kg) exerted inhibitory effects on the phrenic nerve discharge. The augmenting discharge during