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Facilitation of respiratory rhythm by a μ-opioid agonist in newborn rat pons–medulla–spinal cord preparations

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Abstract

We investigated the effect of a μ-opioid agonist, DAGO, on the respiratory frequency of pons–medulla–spinal cord preparations from newborn rats. Bath application of a low concentration of DAGO (0.2 μM) facilitated respiratory rhythm in pons–medulla–spinal cord preparations, whereas it induced respiratory depression in medulla–spinal cord preparations (without pons). At a higher concentration (1.0 μM), at which the inspiratory burst generation in the medulla was strongly depressed, the respiratory rhythm in half of the pons–medulla–spinal cord preparations increased and then decreased, thus showing a biphasic response. In the other half of these preparations, only the facilitatory effect was observed. The burst rate of pre-inspiratory neurons in the rostral ventrolateral medulla was also facilitated by DAGO application. Such facilitation of the respiratory rhythm is probably due to disinhibition of a pontine inhibitory system. Our findings also suggest the existence of a pontine excitatory system, which is depressed by the pontine inhibitory system under control conditions.

Keywords: Respiratory rhythm; μ-Opioid; Pons; Medulla; In vitro; Newborn rat

Activation of opioid receptors in the medulla induces respiratory depression. Recent studies of medulla–spinal cord preparations isolated from newborn rats [2] showed that subgroups of respiratory neurons in the medulla possess differential sensitivity to opioids [1,4,9,18]. Depression of the respiratory rhythm by opioids is possibly due to inhibition of inspiratory burst generation in the medullary inspiratory neuron network via pre- and postsynaptic activation of (μ)-opioid receptors in this preparation [1,9,18]. It is well-known that the respiratory rhythm of pons–medulla–spinal cord preparations is slower than that of medulla–spinal cord preparations [10,16], probably due to a tonic inhibitory influence from the pons [5]. Although it has been suggested that A5 noradrenergic neurons are involved in this inhibitory system [3,5], the detailed mechanisms are not well-understood. Herein, we report that the respiratory rhythm of pons–medulla–spinal cord preparations increases in response to opioid application. This opposite effect appears to be due to disinhibition of the pontine inhibitory system and also suggests the presence of a pontine excitatory system.

The brainstem and spinal cord of 0- to 2-day-old Wister rats were isolated together under deep ether anaesthesia as described previously [17]. The brainstem was decerebrated at the most rostral level of the pons for pons–medulla–spinal cord preparations and just rostral to the anterior inferior cerebellar artery for medulla–spinal cord preparations. The preparations were continuously superfused at 2.5–3.0 ml/min in a 2-ml chamber with the following standard solution (mM): NaCl, 124; KCl, 5.0; KH_2PO_4, 1.2; $CaCl_2$, 2.4; $MgCl_2$, 1.3; $NaHCO_3$, 26; glucose, 30; equilibrated with 95% O_2 and 5% CO_2 at 25–26 °C, pH 7.4. DAGO ([D-Ala2, *N*-Me-Phe4, Gly5-ol]-enkephalin; Sigma Chemical Co., St. Louis, MO, USA) as a μ-opioid agonist and naloxone hydrochloride dihydrate (Sigma Chemical Co.) as an opioid antagonist were dissolved in the standard solution and applied by superfusion for approximately 10 min. Inspiration-like activity was monitored at the C4 ventral root through a glass capillary suction electrode. Concentrations of the agonist were selected according to those used in previous studies from different

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laboratories: 0.2 μM was used to induce moderate depression of the respiratory rhythm, and 1.0 μM was used to induce the approximate maximum inhibition in medulla–spinal cord preparations [19] or slice preparations [8]. The respiratory rate (bursts/min) was calculated from the mean C4 burst activity for 3–5 min. Values are given as mean ± S.D. Significance of differences ($P < 0.05$) was determined with Students *t*-test for paired samples. Membrane potentials of pre-inspiratory (Pre-I) neurons in the rostral ventrolateral medulla were recorded by conventional whole-cell patch-clamp methods [11]. The electrodes, which had a tip inner diameter of 1.2–2.0 μm and resistance of 4–8 MΩ, were filled with the following pipette solution (mM): K-gluconate, 130; EGTA, 10; HEPES, 10; Na_2-ATP, 2; $CaCl_2$, 1; and $MgCl_2$, 1; with pH 7.2–7.3 adjusted by KOH. The membrane potentials were recorded with a single-electrode voltage-clamp amplifier (CEZ-3100, Nihon Kohden, Tokyo, Japan) after compensation of the series resistance (20–50 MΩ) and capacitance.

The mean respiratory rate was 6.4 ± 1.5/min ($n = 19$) for medulla–spinal cord and 2.6 ± 1.1/min ($n = 27$) for pons–medulla–spinal cord preparations. In medulla–spinal cord preparations, bath application of 0.2 μM DAGO for 10 min decreased the respiratory rate from 6.2 ± 1.6/min to 4.5 ± 2.0/min (73% of the control value, $n = 10$, $P < 0.001$) as shown in Figs. 1A-a and 2A. The respiratory rate partially recovered after a washing. Bath application of 1.0 μM DAGO for 10 min decreased the respiratory rate from 6.7 ± 1.4/min to 2.7 ± 1.6/min (40.3% of the control value, $n = 9$, $P < 0.001$) as shown in Figs. 1A-b and 2A. The respiratory rate after a 15-min washing was 4.8 ± 1.8/min, indicating partial recovery. To the contrary, in pons–medulla–spinal cord preparations, bath application of 0.2 μM DAGO for 10 min increased the respiratory rate from 3.1 ± 1.4/min to 6.7 ± 1.1/min (216% of the control value, $n = 7$, $P < 0.001$) as shown in Figs. 1B-a and 2B. After a 15-min washing, the respiratory rate remained at an increased level (6.6 ± 1.4/min). To eliminate the facilitatory effect, application of naloxone was required (Fig. 2B). Bath application of 1.0 μM DAGO for 10 min increased the respiratory rate, which reached a maximum in 5–10 min (from 2.4 ± 1.2/min to 6.4 ± 1.4/min; 267% of the control value, $n = 12$, $P < 0.001$; Fig. 2B). After reaching the maximum rate, the initial facilitation in 6 of 12 preparations turned into a sustained depression during the administration period of DAGO, from 6.4 ± 0.9/min to 2.8 ± 1.4/min (42.9% of the maximum value, $P < 0.001$). Thus, these preparations showed a biphasic response (Fig. 1B-b). The respiratory rate of these preparations 15 min after a washing was 3.8 ± 1.0/min (60% of the maximum value, $P < 0.01$). The other six preparations showed only a slight decrease in the respiratory rate, from 6.4 ± 1.9/min to 6.0 ± 1.3/min (94.5% of the maximum value, not statistically significant, Fig. 1B-c) after a 15-min washing. The average respiratory rate in all pons–medulla–spinal cord preparations was 4.0 ± 1.9/min (62.5% of the maximum value; $n = 12$) at the end of 1.0 μM DAGO application and was 5.0 ± 1.6/min (78% of the maximum value) 15 min after a washing. The respiratory rate

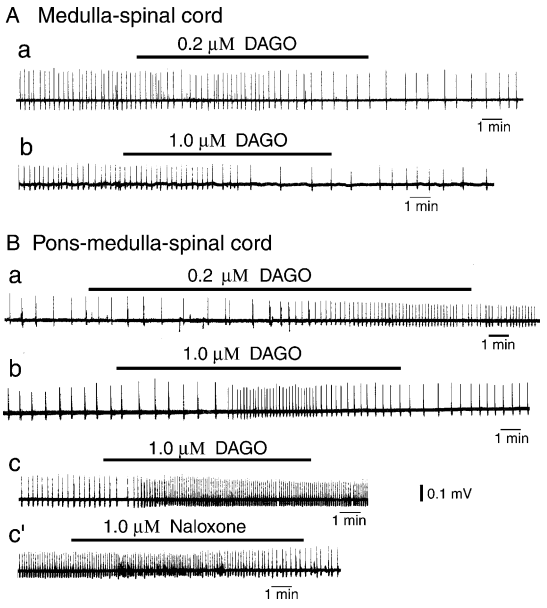

Fig. 1. Effects of DAGO on C4 respiratory rate of brainstem-spinal cord preparations with and without pons. (A) Medulla–spinal cord preparation: application of (a) 0.2 μM and (b) 1.0 μM DAGO decreased the respiratory rate. (B) Pons–medulla–spinal cord preparation: (a) application of 0.2 μM DAGO increased the respiratory rate, (b) application of 1.0 μM DAGO induced an initial increase of the respiratory rate, followed by a subsequent decrease, and (c) one micromole of DAGO induced a sustained increase of the respiratory rate, which was reduced by application of 1.0 μM naloxone (c′).

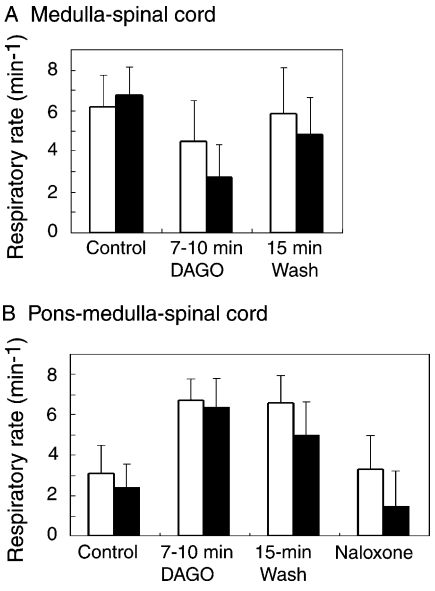

Fig. 2. Summary of effect of DAGO on respiratory rate of brainstem-spinal cord preparations with and without pons. (A) Medulla–spinal cord preparations and (B) pons–medulla–spinal cord preparations. White bars, effect of 0.2 μM DAGO. Black bars, effect of 1.0 μM DAGO. Respiratory rate was measured in the control, 7–10 min after start of DAGO application, and 15 min after washing. Naloxone (1.0 μM) was applied for 10 min.