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Effect of α_1-adrenergic receptor antagonist on the noradrenaline-induced facilitation in respiratory rhythm in newborn rat pons-medulla-spinal cord preparations

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This study is dedicated to the late Professor Yoshiyuki Ohide, Department of Pediatric Dentistry, Nippon Dental University, School of Dentistry at Tokyo.

Abstract

We hypothesized that facilitation of respiratory rhythm by noradrenaline (NA) in rat pons-medulla-spinal cord preparations is mediated through α_1-adrenergic receptors. In 0- to 4-day-old rats, the respiratory frequency (f_R) was monitored at the C4 ventral root and trigeminal motor (V_{MO}) outputs. f_R at temperature (Te) = 23 °C was lower than that at a higher Te (27 °C) and was increased by NA. At 23 °C, lower concentrations of NA were needed to produce the same increases in f_R seen at 27 °C. With highest NA concentration we tested (50 μM), activity at C4 was maintained in all preparations at both Te, whereas that at V_{MO} was maintained in 50% (27 °C) or 88% (23 °C) of the preparations. Particularly, tonic activity at C4 appeared in all preparations at both Te, but that at the V_{MO} occurred in 0% (27 °C) or 18% (23 °C) of the preparations. Based on these results, we used the lower Te (23 °C) and applied a low concentration of NA (3 μM) to the preparations. We found that: (1) with the addition of NA, f_R was increased without the occurrence of tonic activity and (2) NA-related f_R facilitation was inhibited by pre-treatment with the α_1-adrenergic receptor antagonist prazosin (2 μM). f_R was increased by application of the α_1-adrenergic receptor agonist phenylephrine (4 μM), and this response was inhibited by prazosin (4 μM). At Te = 23 °C, f_R facilitation by NA in newborn rat pons-medulla-spinal cord preparations was obtained by activation of α_1-adrenergic receptors.

Keywords: Noradrenaline; Pons-medulla-spinal cord preparation; Respiratory frequency; Prazosin; Phenylephrine; Temperature

Systemic administration of noradrenaline (NA) increases ventilation in human volunteers [20], whereas its direct injection into the bulbar respiratory center depresses neuronal activity in decerebrated cats [3]. These results suggest that NA and activation of adrenergic receptors may influence ventilation, but the effects are conflicting in in vivo experiments. In recent in vitro studies of newborn rat brainstem-spinal cord preparations, the frequency of spontaneous inspiration-like activity (f_R), which was monitored at the fourth cervical spinal vertebral roots (C4), was faster in medulla-spinal cord preparations than in pons-medulla-spinal cord preparations [2,4,5,8]. It has been suggested that the pontine A5 area releases NA endogenously and exerts a tonic inhibition on the medullary respiratory rhythm generator (RRG) through the activation of medullary α_2-adrenergic receptors [4,8]. Moreover, exogenous NA application (in particular, to the pontine component) accelerates the f_R in newborn rat pons-medulla-spinal cord preparations [5], and this effect (i.e., withdrawal of the inhibition on the RRG) is thought to be mediated by the activation of α_2-adrenergic receptors on the pontine A5 neurons [5,9]. Taken together, these results suggest that NA and adrenergic receptors are involved in the regulation of breathing and play important roles in control of the RRG. They also suggest that the pons has a mainly inhibitory action on the RRG through α_2-adrenergic receptors, at least in newborn rat brainstem-spinal cord preparations.

In contrast, although β-adrenergic receptor seems to have little influence on the f_R regulation in rat brainstem-spinal cord preparations [1,5], another α-adrenergic receptor, i.e., α_1-adrenergic receptors, have been suggested to mediate

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excitatory activities in respiration-related neurons, including the pre-inspiratory (Pre-I) neurons in the rostral ventrolateral medulla (RVLM) [1], the inspiration-related motoneurons of the XIIth cranial [7] and cervical spinal [13] nerves, when NA or adrenaline is applied to medulla-spinal cord preparations or medullary slices in newborn rats. However, the f_R facilitation role played by α_1-adrenergic receptors, which is opposite to the inhibitory role played by α_2-adrenergic receptors, has never been demonstrated in rat pons-medulla-spinal cord preparations. We therefore, applied the α_1-adrenergic receptor antagonist prazosin at low environmental temperature (Te) and tested whether activation of α_1-adrenergic receptors is indeed involved in NA-induced f_R facilitation in newborn rat pons-medulla-spinal cord preparations.

Newborn rats (0- to 4-day-old, Wistar, $n = 54$) were deeply anesthetized with ether and decerebrated at the intercollicular level, as referred to in earlier studies [4,25]. The cerebellum was removed, and the spinal cord was transected at the C7–C8 level. The preparation was superfused at a rate of 3–5 mL/min in a 3-mL recording chamber with a solution composed of (in mM) KCl, 3.0; NaCl, 128; $MgSO_4$, 1.0; $NaHCO_3$, 24; NaH_2PO_4, 0.5; $CaCl_2$, 1.5; D-glucose, 30, equilibrated with 95% O_2–5% CO_2 at 23 °C (22.7–23.1 °C), pH 7.4, i.e., artificial cerebrospinal fluid (aCSF) [11]. The experiments were approved by the Animal Ethics Committee of the School of Dentistry, Nippon Dental University, Tokyo. The preparation was placed ventral surface upward in the chamber (Fig. 1A). To monitor inspiratory-like activity and to obtain the respiratory rate (f_R, min^{-1}), the dissected C4 ventral root and trigeminal motor (V_{MO}) outputs were recorded with glass suction electrodes connected to amplifiers (DAM-50, World Precision Instruments, Inc., Sarasota, FL, USA) in which the signals were amplified and band-pass-filtered (0.3–3 kHz). Data on each signal were recorded on paper (OMNIACE 8100, NEC, Tokyo, Japan) and stored on a computer, using an interface (ML820 PowerLab2/20, ADInstruments Japan, Tokyo, Japan) at a sampling frequency of 10 kHz for subsequent data analysis. Norepinephrine bitartrate salt hydrate (Sigma–Aldrich Co., St. Louis, Mo., USA), prazosin hydrochloride (α_1-adrenergic receptor antagonist, Sigma-Aldrich Co.), and phenylephrine hydrochloride (α_1-adrenergic receptor agonist, Wako Pure Chemical, Osaka, Japan) were dissolved in the aCSF at known concentrations (0.1–50 μM) equilibrated with 95% O_2–5% CO_2. They were then applied to the preparation by superfusion through flow pipes placed over the chamber. f_R facilitation was usually shown as the ratio (%) of the f_R obtained 2–5 min after drug application versus the control f_R (=100%) obtained 0–3 min before drug application at each Te (i.e., 23 or 27 °C). The chamber Te was monitored continuously throughout the experiments. To see the effect of Te (23, 27, or 31 °C) on the f_R, Te was changed at a rate of 2 °C/min by circulating warm or cold water in a coiled flexible plastic Tygon tube (Saint-Gobain, Tokyo, Japan), which surrounded the chamber's 40-cm-long aCSF inflow tubes. Then, with the Te controlling system, Te was maintained warm (27 °C) or cold (23 °C) to see the effect of drug applications.

All presented values are means ± S.E.M. Comparisons were made by one-way repeated-measures ANOVA followed by the Bonferroni t-test, or two-tailed paired t-test as appropriate ($p < 0.05$).

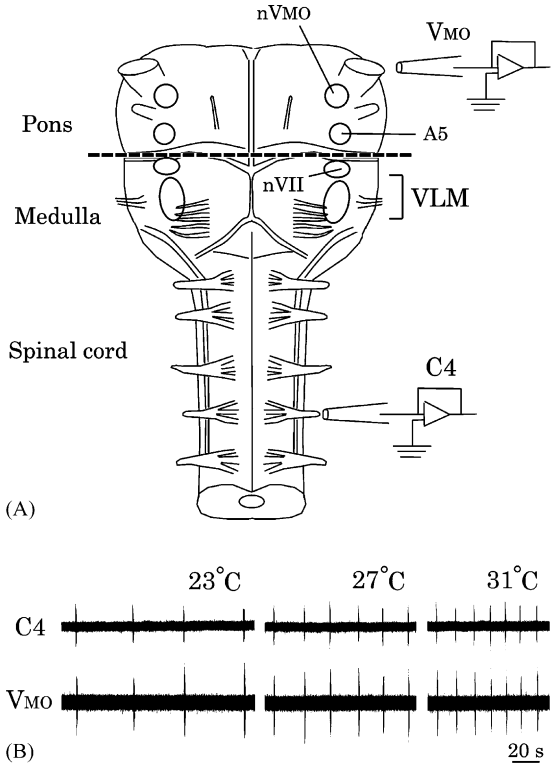

Fig. 1. (A) Pons-medulla-spinal cord preparation. Drawn with the ventral surface upward. Dashed line indicates approximate boundary between pons and medulla. (B) Effects of Te on the f_R. Within 23–31 °C, the f_R was the lowest in the lowest Te (i.e., 23 °C). Abbreviations: A5, area of A5 noradrenergic neurons; C4, 4th cervical spinal ventral roots; nV_{MO}, motor nucleus of the trigeminal nerve; nVII, facial nucleus; V_{MO}, the trigeminal motor nerves; VLM, ventrolateral medulla.

Firstly, we examined the effects of Te (23, 27, or 31 °C) on the f_R. At each Te, synchronous inspiration-like activities were obtained at C4 and V_{MO}, and as shown in Fig. 1B, The lowest f_R (min^{-1}) was obtained at 23 °C, and the highest f_R (min^{-1}) was obtained at 31 °C. At least, within Te = 23–27 °C, the stimulant effects of temperature on f_R were similar to those documented in earlier studies [14,18], one of which shows the highest f_R at Te = 27–28 °C [14].

To see whether the f_R response (i.e., % changes from control at each of the test Te) was significant at a lower Te (i.e., 23 °C) as well as at the higher Te (27 °C, i.e., the standard Te used in studies of such preparations [4,5,10,11,15,21,22]), we applied NA (0.1, 1, 2, 5, 10, 20, or 50 μM) to the preparations to compare the NA dose–f_R response curves at Te = 23 and 27 °C. Each concentration of NA was applied for 5–7 min and interposed with the inflow of normal aCSF for 10–15 min. Fig. 2A is an example of the f_R response at C4 before (i.e., control) and after NA (5 μM) application at Te = 23 or 27 °C. Fig. 2B shows the NA dose–f_R response curves at Te = 23 and 27 °C, measured at C4. Before NA application, the mean control value (min^{-1}) of f_R was 1.7 (23 °C) or 2.6 (27 °C). In particular, at lower concentrations of NA (up to 10 μM), the % increase in f_R at Te = 23 °C was similar