Short communication

Age-dependent chemosensitive pontine inhibition of medullary respiratory rhythm generation in the isolated brainstem of the neonatal rat

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Abstract

Age-dependence and chemosensitivity of the pontine inhibitory effect on medullary respiratory rhythm generation were examined in the isolated brainstem–spinal cord of the neonatal rat. In early preparations (days 1–2), the increase in RR (ΔRR) induced by the pons resection was larger in 8% CO₂ (pH 7.2) than in 2% CO₂ (pH 7.8). That difference was not found in late preparations (days 3–4). Under a given pH, the ΔRR was larger in early preparations than in late preparations.

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In the isolated brainstem–spinal cord preparation of the neonatal rat, the pons is thought to exert an inhibitory effect on the medullary respiratory rhythm generator [2,3,5]. In a previous study, this inhibition was attributed to noradrenergic output from area A5 in the ventrolateral pons [5]. Recent studies have demonstrated that the membrane potential of neurones in the locus coeruleus (LC), another noradrenergic nucleus located in the dorsal pons, was respiration-modulated, indicating that the LC may also play an important role in the control of breathing [6,7]. Furthermore, several recent studies suggest that the noradrenergic areas described above are sensitive to changes in extracellular pH/pCO₂ (i.e., chemosensitive) [1,4,6–9]. This indicates that the pontine effect on the medulla could be modified by shifts in extracellular pH/pCO₂. The objective of the present study was to determine how the chemosensitivity of the pons modifies its inhibitory effect on the medulla and whether the pontine effect and its modification exhibit any developmental changes in the isolated brainstem–spinal cord of the neonatal rat.

The brainstem and spinal cord were isolated en bloc from 80 neonatal (1–4 days old) Sprague–Dawley rats, deeply anesthetized with ether, according to a previously described procedure [6,7]. The reason for the use of neonatal rats is that the preparation needs to be small enough to obtain adequate tissue perfusion and oxygenation. The preparation was placed in the recording chamber (2.5 ml) with its ventral surface up and was superfused with artificial cerebrospinal fluid (ACSF) at a constant rate of 7 ml/min using a peristaltic pump (312 MP1, Gilson, WI), at a temperature of 26–28°C. The ACSF contained 125 mM NaCl, 4.0 mM KCl, 2.0 mM CaCl₂, 1.0 mM MgSO₄, 0.5 mM NaH₂PO₄, 26 mM NaHCO₃, and 30 mM d-glucose. The ACSF was equilibrated with a gas mixture consisting of either 2% CO₂, 8% N₂, 90% O₂ (control; pH 7.8) or 8% CO₂, 2% N₂, 90% O₂ (hypercapnic acidosis; pH 7.2). Periodic respiratory bursts from hypoglossal nerve roots or ventral roots of a higher cervical nerve (C1–4) were recorded using a glass suction electrode. The signals were filtered (50 Hz–1 kHz), amplified (AB 651J, Nihon-Kohden, Tokyo, Japan), rectified and integrated (El-601G, Nihon-Kohden; time constant, 100 ms). Both the raw signals and the integrated signals were charted using a brush recorder (WT-685G, Nihon-Kohden) (Fig. 1A).
frequency of the respiratory bursts (respiratory rate, RR) was determined before and after resection of the pons. The determination was made by averaging RR values for at least several minutes after the rate had stabilized. During the measurement, there was no decline in RR or the amplitude of the respiratory bursts. The difference in RR (ΔRR) before and after resection was used as a quantitative index in assessing the pontine effect on medullary respiratory rhythm generation. All resections were performed by the same person, using microscissors, at the ponto-medullary junction. The resected pons was left in its original position to exclude any possible changes in tissue perfusion and oxygenation. In most cases, the RR was determined using the same nerve throughout the experiment. The preparations were divided into two groups according to the age of the rats (early (days 1–2) versus late (days 3–4)). Each group was further divided into two subgroups based on the superfusate used (control versus hypercapnic acidosis). In the initial phase of the experiment, 33 preparations were fixed in 4% formaldehyde after the above measurements were made. Transverse sections (100 μm) of the caudal pieces were then made using a microtome (VSL, WPI, FL). These sections were stained with cresyl violet and screened under a light microscope. This revealed that the pons resections were made almost exclusively at a level between 1.1 and 1.3 mm rostral to the obex, verifying the reproducibility of the resections, so no further histological examinations were made.

All values are expressed as the mean±standard error. P values of less than 5% (P<0.05) were considered to be statistically significant.

**Intact pons preparations (Fig. 1B):** under control conditions (2% CO₂), the RR was 4.7±0.5 min⁻¹ in the early (days 1–2) preparations (n=20) and 4.7±0.3 min⁻¹ in the late (days 3–4) preparations (n=24). This was inconsistent with a previous study showing that RR was faster on days 3–4 than on days 1–2 [7]. This difference may be attributed to a smaller sample size and the superfusate at a higher temperature in the present study. In the late preparations, the RR was significantly higher under hy-
Recessed pons preparations (Fig. 1C): resection of the pons induced a significant increase in the RR in all four groups (early, control: from 4.7 ± 0.5 min⁻¹ before resection to 7.6 ± 0.4 min⁻¹ after resection; early, hypercapnic acidosis: from 4.8 ± 0.6 to 9.3 ± 0.7 min⁻¹; late, control: from 4.7 ± 0.3 to 6.2 ± 0.3 min⁻¹; late, hypercapnic acidosis: from 6.0 ± 0.4 to 7.2 ± 0.3 min⁻¹). The RR increased and stabilized immediately after the pons resection. In the absence of the pons, the RR for a given developmental stage (i.e., early or late) was significantly higher under hypercapnic acidosis than under control conditions. Under either of the conditions (i.e., control or hypercapnic acidosis), the RR was significantly higher in the early preparations than in the late preparations.

Difference in RR (ΔRR) (Fig. 1D): in the early preparations, the difference in RR (ΔRR) before and after resection of the pons was significantly larger under hypercapnic acidosis than under control conditions. In the late preparations, there was no significant difference in ΔRR between the conditions. Under either of the conditions, the ΔRR was significantly larger in the early preparations than in the late preparations.

There are three major findings regarding ΔRR. First, ΔRR was larger under hypercapnic acidosis than under control conditions in the early preparations. Secondly, the preceding phenomenon was not observed in the late preparations. Finally, ΔRR was larger in the early preparations than in the late preparations under either control conditions or hypercapnic acidosis. These findings indicate that the inhibitory effect of the pons on the medulla is augmented by hypercapnic acidosis in the preparations isolated on days 1–2, but not those isolated on days 3–4. Furthermore, pontine inhibition appears to weaken during the postnatal period. The augmentation of the pontine inhibition by hypercapnic acidosis which was observed in the early preparations might be explained solely by activation of neurones in area A5, a possible main source of the inhibition [5]. It has been demonstrated that these neurones express the Fos protein, a marker of neuronal activation, in rats exposed to hypercapnic conditions [4]. In the late preparations, however, other explanations are definitely required, since the augmentation of the pontine inhibition by hypercapnic acidosis was eliminated in those preparations. We now assume that LC could play an important role in causing the different phenomena, between the early and the late preparations. In the isolated brainstem–spinal cord preparation, the membrane potential of LC neurones shows an excitatory response to hypercapnic acidosis even in the absence of chemical and electrical synaptic transmission [6, 7]. Fos protein was found to be expressed also in LC of rats exposed to hypercapnic conditions [4]. Furthermore, in contrast to the case of area A5, stimulation of LC by local acidification through microinjection of acetazolamide induced respiratory augmentation in anesthetized, vagotomized and ventilated animals [1]. These findings suggest that the activation of LC by hypercapnic acidosis might counterbalance the inhibition from area A5 in the late preparations. Weaker pontine inhibition under either of the conditions (i.e., control or hypercapnic acidosis) in the late preparations might also suggest the relative dominance of LC over area A5 in that particular neonatal period.

Although the physiological significance of these findings remains to be determined, this study is the first to demonstrate that, in the isolated brainstem–spinal cord preparation of the neonatal rat, the pons is responsible for an age-dependent chemosensitive inhibition of medullary respiratory rhythm generation.

References