5-HT_{2c} receptors modulate the discharge activities of inspiratory neurons in the medial region of Nucleus Retrofacialis of neonatal rats in vitro

QIAN Zhibin[1] , SONG Xiaorong[1] , JI Mingli[2] , LIU Chunxia[1] , LIU Xiaoli[1]

1 Department of Functional Lab of Xinxiang Medical University, 2 Department of Pathophysiology of Xinxiang Medical University, 453003, Xinxiang, Henan, China
Corresponding author: Qian Zhibin, qianzhibin@yahoo.cn

Abstract: Objective To investigate whether 5-HT_{2C} receptors modulate the discharge activities of inspiratory neurons in the medial region of Nucleus Retrofacialis (mNRF) of neonatal rats. **Methods** Experiments were performed in in vitro brainstem slice preparations from neonatal rats. These preparations included the mNRF with the hypoglossal nerve (XII nerve) rootlets retained. The rhythmic discharge activities of the inspiratory neurons (I neurons) and respiratory-related rhythmic discharge activities (RRDA) were simultaneously recorded by using microelectrodes in the mNRF and suction electrodes at the XII nerve rootlets, respectively. Roles of 5-HT_{2C} receptors on the discharge activities of I neurons were investigated by administration of the 5-HT_{2C} receptor agonist 2-Chloro-6-(1-piperazinyl)-pyrazine hydrochloride, 6-Chloro-2-(1-piperazinyl)pyrazine hydrochloride (MK212), and its specific antagonist 4-dionehydrochloridehydrate,8-[5-(2,4-Dimethoxy-5-(4-trifluoromethylphenylsulphonamido)phenyl-5-oxopentyl]-1, 3,8-triazaspiro[4.5]decane-2 (RS102221) dissolved in modified Kreb's solution for perfused slices. **Results** MK212 prolonged inspiratory time (TI), shortened respiratory cycle (RC), enhanced integral amplitude (IA) and the spike frequency (SF) of I neurons. By contrast, RS102221 produced opposite effects. **Conclusions** 5-HT_{2C} receptors take part in modulate the discharge activities of I neurons in mNRF of neonatal rat.
[QIAN Zhibin, SONG Xiaorong, JI Mingli, LIU Chunxia, LIU Xiaoli. **5-HT_{2c} receptors modulate the discharging activities of inspiratory neurons in the medial region of Nucleus Retrofacialis of neonatal rats in vitro.** Life Sci J 2012;9(3):183-186]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 27

Key words: the medial region of Nucleus Retrofacialis; 5-HT_{2C} receptors; inspiratory neuron; brainstem slices

Introduction

Neuronal networks in the medulla oblongata generate respiratory rhythm in mammals. The previous experiments have demonstrated that the medial region of Nucleus Retrofacialis (mNRF) is the site of respiratory rhythmogenesis[1,2]. The mNRF overlaps the preBötzinger complex partly, a region proposed to be the core site for respiratory rhythm generation[3].

In rats, serotonergic projections from the caudal raphe nuclei to the hypoglossal nucleus to regulate respiration[4]. 5-HT receptors have been found in the ventral respiratory group (VRG) from neonatal mice and rat where they are essential for the modulation of respiratory rhythm[5]. 5-HT receptors are involved in generation and modulation of basic respiratory rhythm in mammals[6,7]. The 5-HT membrane receptors include seven subtypes, besides 5-HT_3 is ligand-gated ion channel receptor, others are G-protein coupled receptors[8]. To investigate the effects of 5-HT_{2C} receptors on the inspiratory neurons (I neurons) and to further elucidate the role of the 5-HT_{2C} receptors are involved in the respiratory network, the present study was designed and performed.

Materials and methods

Materials Neonatal Sprague–Dawley rats, both sexes, 0-3 days, n=7, were supplied by Experimental Animal Center of Xinxiang Medical University. MK212 and RS102221 were bought from Sigma.

Methods Rats were deeply anesthetized with ether and quickly decapitated at the C3-C4 spinal level. The brainstem was dissected in ice-cold modified Kreb's solution (MKS in mmol/L: NaCl 124, KCl 5, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26, Glucose 30 and pH 7.35–7.45) that was equilibrated with carbogen (95% O_2 and 5% CO_2). This operation must be finished within 3 mins. The brainstem was glued rostral end up onto an agar block, mounted into a vibratome and serially sliced until the rostral boundary of the mNRF was identified by anatomical landmarks such as disappearance of the facial nucleus and appearance of the inferior olive, the nucleus ambiguus, and the hypoglossal nucleus. A 750μm transverse slice was cut, which contained the mNRF with the hypoglossal nerve rootlets retained. The brainstem slice was transferred to a recording chamber (3ml) and continuously perfused with oxygen-saturated MKS at a rate of 7–9 ml/min at 25–27℃. The activities of I neurons in mNRF and the discharge of hypoglossal nerve (XII nerve) rootlets were simultaneously recorded by using

microelectrodes and suction electrodes, respectively. The RRDA were amplified by a DC preamplifier; the activities of I neurons were amplified by a microelectrode amplifier. Signals were band-pass filtered (0.1–3.3 kHz), data were sampled (5 kHz) and stored in the computer via BL-420F biological signal processing system after being amplified.

MK212 and RS102221 were dissolved in DMSO and diluted to 10μmol/L, keeping the concentration of DMSO less than 0.1%. In this concentration DMSO has no effects on the discharge of inspiratory neuron[9,10].

Statistical analysis

All data were expressed as means±S.E.M, and repeated measure was used to compare the values obtained before and after drug application. Differences were considered statistically significant as $p < 0.05$.

Results

7 I neurons were recorded, respiratory related neurons were identified by correlating their on-going activities with the hypoglossal rhythmic discharges[2,7]. As shown in Tab.1 and Fig.1, application of MK212 significantly prolonged TI by 15.94%, shortened RC by 17.86%, enhanced IA and SF by 32.79% and 41.31% of I neurons, respectively. After washout of MK212, the discharge activities of I neuron were recovered to the control level. By comparison with MK212, RS10221 produced opposite effects, it shortened TI by 20.22%, prolonged RC by 23.46%, decreased IA and SF by 14.51% and 30.05% of I neurons.

Tab.1 Effect of MK212 and RS102221 on the discharge activities of I neurons

Group	TI(s)	IA(μV.s)	RC(s)	SF(Hz)
Control	0.69±0.06	674.27±60.21	18.54±2.85	16.34±2.61
MK212	0.80±0.05 **	895.33±70.21 **	15.73±2.52 **	23.09±3.07 **
Washout	0.67±0.06	694.57±57.03	17.11±2.66	17.22±3.11
RS102221	0.55±0.04 $^{**\#\#}$	576.44±34.67 $^{**\#\#}$	22.89±3.14 $^{**\#\#}$	11.43 ±2.48 $^{**\#\#}$
F	12.593	33.205	15.123	3.746
P	0.001	0.000	0.000	0.000

$^{**}p < 0.01$ vs control group $^{\#\#}p < 0.01$ vs MK212 group

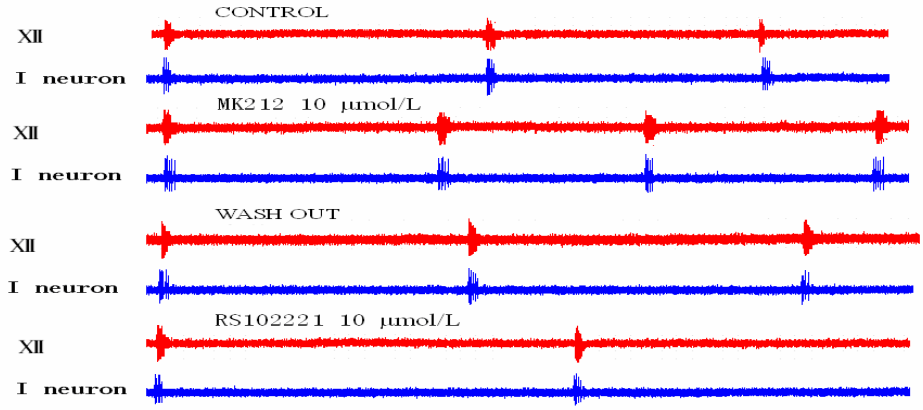

Fig.1 Effect of DOI and ketanserine on the discharge activity of I neurons

Discussions

This study was performed in in vitro brainstem slices containing the neurons critical for integration of respiratory drive. The respiratory frequency of this preparation was markedly slower than that in vivo due to the isolation of nervous system from mechanosensory afferent inputs and the removal of vagal mechanosensory afferent inputs[11]. However, the discharge patterns of respiratory motor neurons in vitro were similar to that in the intact mammal but different from gasping[12,13]. The I neurons, which appear to be fundamental components of the inspiratory pattern generation, have been proposed to be responsible for respiratory rhythm.

Our experiment found that RS102221 significantly shortened TI, prolonged RC, decreased

IA and SF of I neurons, confirming that there are serotonergic neuron releasing endogenous 5-HT which modulate the discharge activities of I neurons in slice. Blocking 5-HT_{2C} receptors by RS102221 depress the excitability of I neuron. On the other hand, MK212 prolonged TI, shortened RC, enhanced IA and SF suggesting that there are 5-HT_{2C} receptors in membrane of I neuron. 5-HT_{2C} receptors modulate the excitability of I neurons, RRDA was changed to follow I neurons discharge activity. RC was shortened significantly by MK212 which indicated that activating 5-HT_{2C} receptors increased interneuronal activities and inhibit expiratory neurons, further suggesting that 5-HT_{2C} receptors are involved in the phase-switching between expiration and inspiration.

After being activated, 5-HT_{2C} receptors activate phospholipase C, it catalyzed phosphatidylinositol diphosphate transforming into inositol triphosphate and diacylglycerol[14]. Phosphatidylinositol diphosphate increase [Ca^{2+}] of cytolymph from endocytoplasmic reticulum[15]; protein kinase C was actived by diacylglycerol[16]. Protein kinase C increased open probability of Na^+ channel through phosphorylation and also increased concentration of reactive oxygen species[17,18]. Reactive oxygen species can increase open probability of Na^+ channel too[2,19]. The increasing open probability of Na^+ channel in turn increased the excitability of inspiratory neurons and respiratory center. We presume this is the mechanism 5-HT_{2C} receptors effect on inspiratory neurons.

In conclusion, the present study suggests that in medullary respiratory center, 5-HT_{2C} receptors modulate the basic rhythmic respiration through modulate the excitability of medullary respiratory neurons.

Acknowledge

This work was supported by the Henan Natural Science Foundation (No. 102102310156), Foundation of Henan Department of Education (No. 2010B180024, No. 2010GGJS-118, No. 2011HASTIT023) to Qian Zhibin. Corresponding author: Qian Zhibin, male, associate professor, PhD, e-mail: qianzhibin@yahoo.cn, tel: +86-13462339596.

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5/2/2012