Adv. Exerc. Sports Physiol., Vol.10, No.2 pp.49-53, 2004.

Electrophysiological Properties of Compensatory Activated Phrenic Motoneurons in Rats

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Abstract

MIYATA, H. and WADA, N., Electrophysiological Properties of Compensatory Activated Phrenic Motoneurons in Rats. Adv. Exerc. Sports Physiol., Vol.10, No.2 pp.49-53, 2004. To examine the activity- dependent plasticity of motoneuron function, male adult rats were divided into a control (CTL) group (n=17) and a denervation group (n=17) that had been subjected to unilateral phrenicotomy 4 weeks previously. Electrophysiological membrane properties (resting potential (mV), rheobase (nA) and input resistance (Mohm)) of the contralateral compensatory activated (CAC) phrenic motoneurons were measured. As compared to the CTL hemidiaphragm, the CAC hemidiaphragm showed increases in electrical activity by 51% one minute after the denervation, and increases in succinate dehydrogenase activity by 31% at the end of experiment. There were significant inverse relationships between the rheobase and input resistance of motoneurons in the both groups ($r=-0.48$, -0.62). Mean value of the rheobase in non-recruited motoneurons was significantly higher than that in recruited motoneurons in the both groups. Most of the motoneurons with low rheobase (<5nA) were recruited in the CAC, but not in the CTL motoneurons. As the results, the incidence of recruited motoneuron in the CAC (69%) was higher than that in the CTL motoneurons (48%). Mean interval of action potentials of the recruited motoneuron was identical between the CAC (38ms) and CTL (39ms) motoneurons. We concluded that a main strategy of the phrenic motoneurons to the CAC induced by contralateral phrenicotomy was additional recruitment of motoneurons with low rheobase.

Keywords: activation, diaphragm, phrenic motoneuron

Introduction

The diaphragm and phrenic motoneurons are the most important inspiratory motor system involved in mammalian ventilation. The major role in the motor system is changing gas in and out of the lungs to maintain arterial blood homeostasis, and the activity should be maintained through life. In this motor system, paralysis of the hemidiaphragm causes in a significant increase in the activation of contralateral phrenic motoneurons to sustain normal ventilation (9). Generally, the motoneuron has two strategies to adapt to an acute increase in activity demand, that is, increases in discharge frequency of recruited motoneurons and additional recruitment of non-recruited motoneurons. Although the pattern of motor unit recruitment of the cat diaphragm is speculated by measurements of transdiaphragmic force and fiber-type distribution (12), the alteration in discharge frequency and recruitment of motoneurons in response to chronic activity increases is not well addressed. Even under anesthesia, phrenic motoneurons received a descending excitatory drive from medullary premotor neurons. Therefore, by using intracellular recordings with glass microelectrodes for phrenic motoneurons, it is possible to distinguish recruited motoneurons with spike discharges from non-recruited motoneurons with only depolarization of membrane potential (6). In previous study (10), we demonstrated that phrenicotomy induced a de-differentiation of electrophysiological properties of the phrenic motoneurons, and that these changes were restricted to the non-recruited motoneurons. In this unilateral phrenicotomy model, to study the activity-dependent plasticity of motoneuron function, we examined the electrophysiological membrane properties of the compensatory activated (CAC) phrenic motoneurons.

Material and Method

Animal and treatment

Thirty-four young adult male Wistar rats (body weight at end of experiments, 250-350 g) were divided into CAC (n=17) and sham control (CTL, n=17) groups. All animals were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally (i.p.)) and prepared for aseptic surgery. Each rat was placed in the supine position on the operating table. A midline incision was made on the neck and superficial muscles. Under a surgical microscope, the main trunk of the unilateral phrenic nerve was dissected. After ensuring that there was no bleeding in the operating field, the layers of muscles and skin were sutured separately. In the CTL group, the same surgery without a section of the phrenic nerve was performed. The core temperature and heart rate remained stable, and were unaffected by surgery.

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During the denervation surgery, to confirm the compensatory activation of the contralateral diaphragm, the electromyographic (EMG) activity of the diaphragm was recorded in 7 rats using fine wire electrodes. The relative change in neuromuscular activity was represented by the relative value of total EMG activity (duty cycle *root mean square of 10 bursts) to that obtained before surgery.

All experimental and animal care procedures were approved by the Committee on Animal Care and Use at Yamaguchi University and followed Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences established by the American Physiological Society Animal Care Guidelines.

Intracellular recordings of phrenic motoneuron

Four weeks after the denervation surgery, the animals were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and artificially ventilated (frequency: 70-90 breaths/min).The main trunk of the intact phrenic nerve was separated from other tissues, and a micro cuff electrode was set around the nerve to stimulate phrenic motoneurons antidromically. Laminectomy was performed between C2 and C6. The animal was fixed in a stereotactic frame, and the exposed spinal cord was covered with warm mineral oil. The animal was paralyzed initially with pancronium bromide (1 mg, intravenously) and an additional dose (40-100% of the initial dose) was given every hour. The end-tidal CO_2 concentration was monitored and maintained at approximately 7.0% by adjusting the respiratory rate and tidal volume. Intracellular recordings from the phrenic motoneurons were made with a glass microelectrode filled with 3M potassium citrate solution (input resistance, 2-5 M ohm). The phrenic motoneurons were identified by the action potentials generated by antidromic stimulation (2 Hz). The motoneurons with spikes and without spikes during the depolarizing shift were defined as recruited and non-recruited, respectively. All electrophysiological measurements of the phrenic motoneurons were performed during the expiratory phase in the motoneurons with resting membrane potentials of at least -50 mV and an action potential of 50mV (peak value). The input resistance was determined by injecting current pulses (200 ms duration, 1-4 nA) through the microelectrode and recording the change in voltage. The rheobase was defined as the minimum magnitude of a depolarizing current pulse (300-500 ms, 1-16 nA) capable of eliciting spikes. In the recruited motoneurons, mean value of spike intervals (ms) were measured from several spikes of 10 bursts during the inspiratory phase.

Succinic dehydrogenase activity

To confirm a long-term compensatory activation on the intact diaphragm and phrenic motoneuron, the oxidative enzyme activity of diaphragm was evaluated by biochemical analysis. After the physiological measurements, the diaphragm muscle was removed from 5 rats in each group. The succinic dehydrogenase (SDH) activity of the diaphragm was evaluated spectrophotometrically in triplicate at 25℃, and results were expressed as μmoles per gram of noncollagenous protein (NCP) per minute (13). One piece of muscle was homogenized in ice-cold 33.3 mM phosphate buffer (pH 7.4). The SDH activity was determined on the basis of the technique of Cooperstein et al. (3). The electronic transfer system was blocked by the addition of cyanogen, and the extent of the reduction in cytochrome C was determined from the change in absorbance measured at 550 nm.

Statistics

The results obtained in this study were analyzed by a two-way ANOVA for all data with experimental groups (CTL or CAC) and motoneuron types (recruited or non-recruited) as grouping variables. Post hoc analysis was performed using the Scheffe method. In all cases, statistical significance was established at $p<0.05$. All values are reported as the mean + standard deviation.

Results

Model verification

One minute after the surgery, as compared to the value before surgery, the mean value of duty cycle and root mean square of the spontaneous inspiratory-related EMG activity were increased by 7 and 41%, respectively (Fig. 1). Totally, the EMG activity in the CAC diaphragm was 51% higher than the value before surgery. The increased activity continued for at least 30 minutes after the surgery. Furthermore, 4 weeks after the surgery, the SDH activity of the CAC dia-

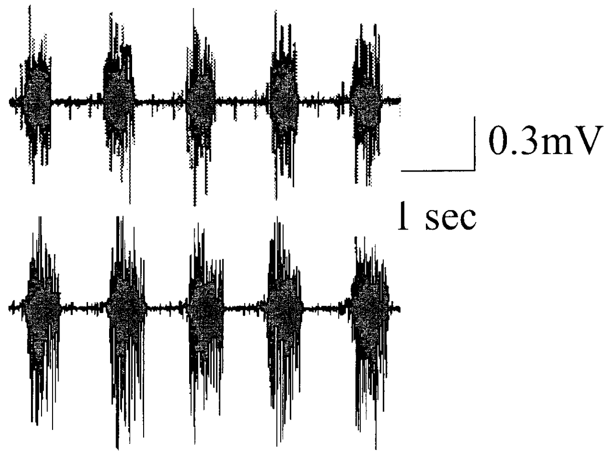

Fig. 1 Electromyographic (EMG) activity taken from the hemidiaphragm muscle of rat subjected to a contralateral denervation of phrenic. The spontaneous inspiratory-related EMG activity increased after the denervation. Upper trace: before denervation. Lower trace: after denervation.

phragm (62.6+8.5 μmol/g NCP/min) was significantly higher than that of the CTL diaphragm (47.7+6.0 μmol/g NCP/min) (Fig. 2).

Electrophysiological properties of the motoneurons

There was a significant relationship between the input resistance and rheobase of motoneurons in both the CTL and CAC groups (Fig. 3). In the CTL group, out of 54-recorded motoneurons, 26 were recruited types. While, out of 54-recorded motoneurons, 37 were recruited types in CAC motoneurons. A higher percentage of recruited motoneurons were found in CAC (69%) than CTL motoneurons (48%). There were few non-recruited motoneurons with a low rheobase ($<$5 nA) in CAC motoneurons, but not in CTL motoneurons.

In both groups, the mean values of resting potential in the non-recruited motoneurons were significantly lower than those in recruited motoneurons (Table 1). No significant differences between CTL and CAC motoneurons were found in the resting potentials of each type of motoneuron. The mean value of input resistance was significantly higher in the recruited motoneurons than the non-recruited motoneurons in each group (Table 1). The mean value of the rheobase was significantly lower in the recruited motoneurons than the non-recruited motoneurons in each group (Table 1). There were no significant differences in the electrophysiological membrane properties of motoneurons between the CTL and CAC groups.

The mean value of the spike interval in the CAC motoneurons (38.8+10.1 ms) was identical to that in the CTL (39.5+16.1 ms) motoneurons (Fig. 4). The range of spike intervals in the CAC motoneurons (13.9−75.3 ms) was also identical to that of the CTL motoneurons (17.0−78.9 ms).

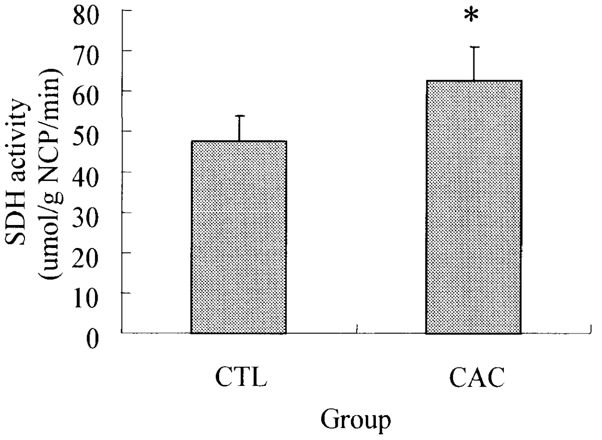

Fig. 2 Succinic dehydrogenase activities of the control (CTL) and compensatory activated (CAC) diaphragm muscle. NCP: non-collagenous protein. *: Significantly different from CTL at P$<$0.05.

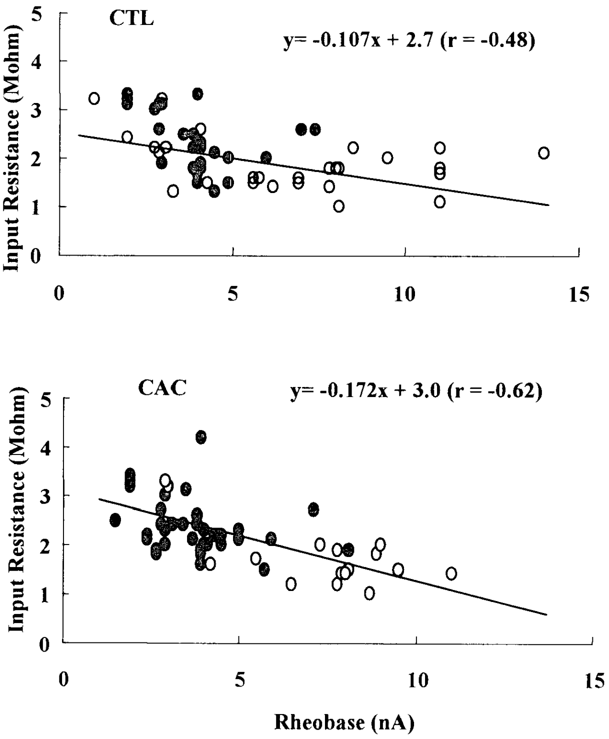

Fig. 3 Relationships between rheobase and input resistance of phrenic motoneurons in CTL (upper) and CAC (lower) groups. ●: recruited motoneurons. ○: non-recruited motoneurons. Most of the control data were already used in our previous study (10).

Table 1 Electrophysiological membrane properties (resting potential (RP), input resistance (IR), rheobase) of each type of motoneuron in control and compensatory activated groups[a].

Group	Control		Compensatory Activated	
Type (n)	Recruited (26)	Non-recruited (28)	Recruited (37)	Non-recruited (17)
RP (mV)	−56.1±3.7	−65.6±7.2*	−57.6±3.7	−68.4±6.5*
IR (Mohm)	2.4±0.6	1.9±0.6*	2.4±0.6	1.9±0.7*
Rheobase (nA)	4.0±1.3	6.5±3.3*	3.8±1.4	7.0±2.5*

Values are mean±standard deviation. * Significantly different from recruited motoneuron in each group at P$<$0.05.

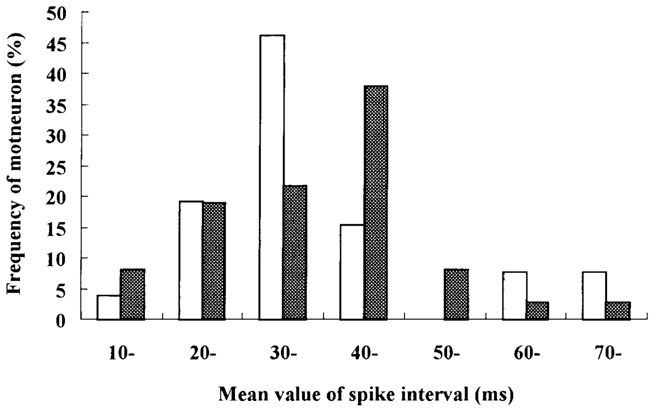

Fig. 4 Histograms of spike interval of recruited phrenic motoneurons in control (white bar) and CAC (black bar) groups.

Discussion

We confirmed the compensatory activation of phrenic motoneurons by a 51% increase in EMG activity one minute after contralateral phrenicotomy, and a 31% increase in the SDH activity of the diaphragm at the end of the experiment (4 weeks after the surgery). As a result, no significant differences were found in the electrophysiological properties of phrenic motoneurons between the CAC and CTL groups. Although no difference was found in the mean interval of spike discharge of recruited motoneurons between the CAC and CTL groups, the percentage of recruited motoneurons was higher in the CAC than CTL motoneurons. We concluded that a main strategy of the phrenic motoneurons to chronic increased activation was the additional recruitment of motoneurons with a low rheobase.

Recently, Beaumont & Gardiner (2) examined the electrophysiological properties of the motoneurons innervating hindlimb via tibial nerve in rats housed in wheel running cages. The study demonstrated that voluntary running rats had slow motoneurons with significantly deeper resting potentials than CTL rats. In a human study (1), motor units had lower initial firing rates and less discharge variability in the muscles of the dominant hands than non-dominant hands. The results suggest that the increased chronic activity might make the membrane properties of the motoneuron more slow type. The implications for these changes were considered to be a delay of the onset of membrane accommodation and late adaptation that would occur with repetitive firing (2). Although an extreme over-activation of phrenic motoneurons occurred in this study, the mean value of resting membrane potentials was non-significantly different in both the recruited and non-recruited motoneurons between the CAC and CTL groups. The difference in activity history between phrenic and other motoneurons might be a reason for the differential adaptation of the membrane properties. The activity level of phrenic motoneurons is extremely high as compared to that of the motoneurons innervating limb muscle. For example, the duty cycle of the rat diaphragm is about 40% (11), while in rat hindlimb muscles, duty cycles range from as low as 2% (extensor digitorum longus muscle) to 14% (soleus muscle) (7). Therefore, the adaptation toward slow type motoneurons might have occurred already in the phrenic motoneurons. Indeed, the rheobase values of recruited and non-recruited motoneurons in our CTL data are slightly lower than those of slow and fast type motoneurons in the CTL data from a previous study on the rat (2), respectively.

There is a longstanding argument over whether the motor unit number or discharge frequency increases for chronic activation (5), and much physiological evidence has been reported in several models (4, 5, 8). Regarding the diaphragm and phrenic motoneurons, the pattern of motor unit recruitment is speculated by measurements of transdiaphragmic force and a fiber-type distribution of the cat diaphragm (12). From the results, the non-recruited motoneurons would provide an emergency reserve for increasing diaphragmatic force. In our model of contralateral diaphragm paralysis, additional recruitment was induced in motoneurons with a relatively low rheobase. These motoneurons putatively innervate muscle fibers with slow-twitch contraction (Type I fiber) or fast-twitch contraction and high fatigue resistance (type II a) (12). In this emergency situation, there were still many non-recruited motoneurons with a high rheobase. This may indicate an adequate reserve for the phrenic-diaphragm motor system. On the other hand, the mean value of discharge interval in the recruited motoneurons was unchanged. These discharge intervals mean about 25 Hz of discharge frequency. The range of discharge frequency in the CAC motoneurons was 13—72 Hz, and the values were identical to the values in the CTL motoneurons. From these results, we concluded that at least in the hemi-diaphragm paralysis model, a main strategy of the phrenic motoneurons in response to chronic increased activation was additional recruitment of motoneurons, not by increases in discharge frequency of each recruited motoneuron.

Acknowledgements

This work was supported by a grant from The Japanese Ministry of Education, Science and Culture (No.12680035)

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(Received 5 January 2004, and in revised form 8 March 2004, accepted 7 April 2004)