Effects of Single Pinna Reflex and Dynamic Stretch on Spike Discharges of Caudate Muscle Spindle Nerves in Rat

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Abstract

Introduction: The γd motor axons that innervate the muscle spindle fibers could induce the excitation of spindle nerves. The purpose of this study was comparing the quality effects of pinna reflex (PR) and dynamic stretch (DS) on the discharges and amplitudes of spike potentials (SPs) of spindle Ia, II, and γd axons in rat.

Methods and Materials: The experimental study on the SPs discharges of 45 single caudate muscle spindle afferents and γd axons from the left side L5 roots were recorded at rest and during testing 15 adult normal rats by PR and by DS. The comparison among the means SPs amplitudes of Ia, II, and γd axons in the resting discharge (RD) and after applying the PR and the DS were investigated by measuring their amplitudes.

Results: The SPs amplitudes of Ia (72±5.34 µv), II (38±3.27 µv), and γd (62±4.29 µv) were calculated in the RD. The obtained mean SPs amplitude difference was 21% for Ia and 7.7% for II; in the DS more than in the PR. The SPs of Ia and II made some overlaps by using the two stimula. The γd SPs followed the SPs of Ia and II during the PR whereas this case didn’t occur for the spindle axons during the DS. The mean SPs amplitude difference of γd in the DS was 85% less than in the PR.

Conclusion: The DS (independent to γd SPs) was as distinctive stimulus for exciting of single Ia and II fibers as the PR (dependent to γd SPs). The γd showed strong inactivity by the DS. These results would be playing role in controlling the movement disorders.

Key words: Spindle Ia, II, and γd axons, Spike Potential Amplitude, Pinna Reflex, Dynamic Stretch

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Introduction
Acute stimulation of motor nuclei in brain stem can cause to excite the gamma (γ) motoneurons in spinal cord and then may be the spike potentials (SPs) of γ axons appeared in the resting position and during body’s dynamic state.\(^{(1,2)}\) The γ motor axons including γ-dynamic (γ\(d\)) and γ-static (γ\(s\)) innervate the muscle spindle fibers.\(^{(3)}\) The impulse of γ-axon could discharge the SPs of primary (Ia) and secondary (II) spindle afferents in a conscious and in anesthetized mammalian.\(^{(4,5)}\) There are a few studies about the anatomy and histology of spindle nerves in rat; however, these parameters generally have a slight difference with those of other mammals.\(^{(6,7)}\) Direct dynamic stimulus on the alpha motor axon could create an acute isometric contraction in extrafusal fibers and it also causes the synchronized activity of muscle spindle fibers; the discharging of spindle afferents are produced afterward.\(^{(8,9)}\) In this trial, the Ia is excited, but the II fiber doesn’t have any activity.\(^{(10)}\) In addition, by applying the dynamic stretch (DS) on the intrafusal fibers, unexpected firing SPs of Ia are evoked with the high amplitude but the II fiber did not discharge any SPs despite the deformation of the concerned muscle spindle fiber.\(^{(11,12,13,14)}\) Thus, this study was conducted to find out the quality effects of single phasic PR and single active DS on the actions of single spindle afferents and γ\(d\)-axon in rat.

Methods and Materials
This experimental study was carried out on the caudate spindle Ia, II, and γ\(d\) axons of 15 normal adult male Sprague-Dawley rats, weighing 270±51gr. Anesthesia with a urethane (1.7g/kg, I.P) supplementary doses (0.85g/kg, I.P) was provided when necessary.\(^{(15)}\) Deep anesthesia was checked by absence of the pinch reflex. Body temperature was controlled between 36°C-37°C by machine control (Harvard Apparatus Limited, USA). At the end of each experiment, the rat was killed with overdose of anesthetic drug. A lumbar laminectomy was performed from L3 to L6. In left side of L5, the dorsal and ventral roots were separated from each other for performing the test. Afferent fibers were identified by conduction velocity. Motor axons included alpha, beta, and γ with different diameters, which make synapses with extrafusal and intrafusal fibers.\(^{(16)}\) Under the stereomicroscope (PzMIII-BS, WPI, SarasolaF lUSA) the alpha and beta axons were cut but the γ-axon remained intact. All isolated spindle nerves were kept warm within the mineral oil during testing them.

Stimulation and recordings Spindle nerve fibers and data collection
The SPs of spindle Ia, II, and γ\(d\) axons were recorded by the Power lab set (ML866, 4 channels, AD Instruments comp. Australia) with the high filter1KHz, 10 ms/Div sweep, 300µv/Div for afferents, and 100µv/Div for γ\(d\) axon. The analysis of data was done with a computer (LG
comp. korea) that was connected to a Power lab set. The SPs were obtained using a monopolar silver hook electrode that was located under each nerve fiber during testing by the mechanical stimuli; PR and DS and also at rest discharge (RD). The single phasic PR stimulus with 5mv sensitivity was done inside the left external ear duct; after that with 2-min interval, the single active DS was displayed by bending the tail with 60° toward the right side. Finally, the recordings of SPs discharges of 45 single spindle Ia, II, and γd axons were collected in 3-group whereas each one of the spindle SPs has three different voltage due to three different kinds of positions. Amplitude of spike potential was measured from peak-to-peak.\(^{(7)}\)

**Statistical analysis**

Comparison of means differences of SPs amplitudes were based on the use of paired student t-test; a value of \(P<0.05\) was considered statistically significant.

**Results**

Totally, the 135 amplitudes of spindle nerves SPs were measured the ranges of which in the RD, PR, and DS positions are shown in the table 1. Figures 1 and 2 present examples of SPs of single Ia, II, γd axons.

Table 1. Ranging of spike potentials (SPs) amplitudes of spindle nerve fibers (SNFs) of normal male rats are presented in resting discharge (RD), in pinna reflex (PR), and in dynamic stretch (DS).

<table>
<thead>
<tr>
<th>SNFs group</th>
<th>Number of testing fibers</th>
<th>RD-SPs Amplitude values (µv)</th>
<th>PR-SPs Amplitude values (µv)</th>
<th>DS-SPs Amplitude values (µv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>γd fiber</td>
<td>15</td>
<td>56-70</td>
<td>85-105</td>
<td>10-17</td>
</tr>
<tr>
<td>Ia fiber</td>
<td>15</td>
<td>65-80</td>
<td>180-200</td>
<td>235-255</td>
</tr>
<tr>
<td>II fiber</td>
<td>15</td>
<td>30-42</td>
<td>52-66</td>
<td>55-70</td>
</tr>
</tbody>
</table>
Pinna Reflex Activity

10ms

300µv

A

B

C

100µv

10ms

100µv

10ms

300µv

10ms

Figure 1. Trace A marked wave of ear’s movement by arrow, trace B and C show the bursting spindle SPs of γd and afferents, respectively, during applied PR.

Dynamic Stretch

100 µV

10ms

300 µV

10ms

Figure 2. Top trace the γd spike potentials are depressed in marked arrows, but in bottom trace spindle Ia and II fibers show bunches of firing spike potentials during DS.
Figure 3. (A) Comparison of spike potentials (SPs) amplitudes histogram of gamma dynamic (γd), Ia, and II spindle nerve fibers in the resting discharge (RD) group with in the pinna reflex (PR) group. *Significant difference with RD group. (B) Comparison of SPs amplitudes histogram of γd, Ia, and II spindle nerve fibers in the RD group with in the dynamic stretch (DS) group. *Significant difference with RD group. (C) Comparison of SPs amplitudes histogram of Ia, II, and γd in the DS group with in the PR group. ºSignificant difference with the PR group. Data are presented as means ± SD.
Effect of single phasic PR on spindle SPs amplitudes and its comparison with the RD
Figure 1 trace C is the record SPs of Ia and II during PR. The II SPs was discharged clearly just the same as discharging of Ia SPs, but the II discharge was not synchronized with the discharging of Ia. The Ia SPs were followed by the II SPs and they showed a higher amplitudes than the amplitude of II SPs. The SPs of Ia and II made some overlaps. The SPs of $\gamma$d discharged before the SPs of Ia and II SPs (figure 1 trace B). The mean SPs amplitude values of $\gamma$d, Ia, and II were significant ($P<0.001$) and were increased by the PR stimulus compared with the means amplitudes of the same fibers in the RD position (figure 3A).

Effect of single DS on spindle SPs amplitudes and comparison with the RD
Figure 2 top trace shows the strong inhibition of $\gamma$d SPs by the DS. During the DS trial, the bursting discharge of SPs of Ia was appeared with the high point amplitude whereas the II SPs had less marked amplitude and they were emerged after the SPs of Ia (figure 2 bottom trace). The mean values of SPs amplitude of Ia and II were significant ($P<0.001$) and were increased by the DS stimulus compared with the means amplitudes of Ia and II in the RD position, but the DS produced a significant decrease in the $\gamma$d SPs amplitude than the $\gamma$d SPs amplitude in the RD (figure 3B).

Comparison of DS and PR effects on the means spindle SPs amplitudes
The percentages of mean amplitude differences of Ia and II in the DS were 21% and 7.7%, respectively, more than in the PR. The mean amplitude difference between the Ia and the II in the DS was calculated 73% while the difference between the Ia and the II was obtained 69% in the PR. As the final result, the DS was determined as an affective stimulus for discharging and promoting SPs amplitudes of spindle Ia and II rather than the PR stimulus. The mean difference of $\gamma$d in the DS was 85% less than in the PR. Comparing of means SPs amplitudes of Ia, II, and $\gamma$d are illustrated in figure 3C.

Discussion
The $\gamma$d-motoneuron was excited via vestibulo-spinal pathway fiber while an adequate strength stimulus was applied to the inside of the left external ear duct of rat. The $\gamma$d impulse caused the contraction of muscle spindle fibers and incessantly the SPs of afferents were appeared.\(^{8,19}\) The discharging SPs magnitudes of Ia and II were due to the adjustment of deforming muscle spindle fibers. It seemed that the $\gamma$d SPs acted only like a transit way between the $\gamma$d-motoneuron signal and the contractile elements of muscle spindle fibers. This process was completely under the control of the Deiter’s nucleus activity in the brain stem. By testing the PR, increasing of amplitude of Ia SPs was exactly dependant to the aid and the level intensity of electrical
power of $\gamma_d$ SPs in despite of the fact that $\gamma_d$ SPs amplitude was shorter than the SPs amplitude of Ia. Scott (1995) and Maltenfort (2003) reported that the discharging of SPs of spindle afferents were controlled by the both $\gamma$ and beta axons.$^{(6, 20)}$ The beta axon was excluded in this study. The $\gamma_d$ axon displayed only as a reflector of firing signal of Deiter's nucleus to the Ia fiber. According the above findings many other factors besides the impulse of $\gamma_d$ may have an influence on the high activity of Ia fiber; some of which are: the quality of viscoelasticity nature within spindle bag1,$^{(21, 22)}$ Ia diameter, and anatomical location of Ia along in the muscle spindle fiber.$^{(23)}$ However the same voltage of $\gamma_d$ couldn't create any remarkable SPs amplitude in II fiber. The spindle II afferent was excited only by static stimulus.$^{(24)}$. This study shown that the spindle II afferent was excited by both single phasic PR and DS, though the sizes of SPs amplitudes were different. Furthermore, there were two different types of voltage for amplitudes of spindle SPs in recordings. It didn’t seem that the both types of amplitude voltage were related to Ia SPs; the low voltage of amplitude was definitely for II and the high one for Ia. Concerning the effect of stretch reflex on muscle spindle, It was reported by Ellaway (2002) that this stimulus could be modulated synaptically within the spinal cord by the activation of fusimotor system.$^{(25)}$ If so, how could the excitations of afferents be justified by the $\gamma_d$ impulse during testing by DS whereas all the spindle nerve fibers were tested at ipsilateral in this study.

**Conclusion**

These findings demonstrated that the active DS (independent to signals of brain stem) was as distinctive stimulus for exciting of spindle Ia and II fibers as the phasic PR (depending on the signals of the brain stem) The activity of $\gamma_d$ axon was inhibited by the DS. During testing by the both DS and PR, the first SPs discharge corresponded to the Ia and the second discharge was due to II SPs. These data would be effective in controlling the movement disorders.

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**References**

انرث زلفکس و کشش دینامیکی بر روی تخیه عصب دوک spikes و عصبانی ناحیه دم موش صحرایی

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چکیده
سابقه و هدف: آکسون‌های حرکتی (PR) یک عصب دوم فیبر‌های دوک عضلانی را بر عهده دارند. می‌توانند تحريك‌های اعصاب دوک عضلانی را سپس شوند. هدف از این مطالعه مقایسه کیفیت اثر زلفکس (PR) pinna و کشش دینامیکی (DS) در چگونگی تخیه و آمپلی تیود پتیانسیل های spike عصب دوک عضلانی ناحیه دم موش صحرایی بوده است.

روش بررسی: از یانزده موش صحرایی نر بالغ، چهل و پنج عدد فیبرهای افرن و دو (بطور جدایانه برای هر نوع DS و PR) در طرف چپ 5 L تخلیه پتیانسیل های spike در حال استراحت و در هنگام اجرای پتیانسیل های spike مطالعه ثبت گردید. مقایسه بین میانگین‌های آمپلی تیود پتیانسیل فیبر‌های دوک عصب (PR و DS) در حال استراحت با II Ia spikes استقامه گردید.

یافته‌ها: آمپلی تیودهای پتیانسیل های II Ia spikes در حالت استراحت برای فیبرهای دوک (PR و DS) یافته‌ها (μV و μV) (34.2 μV و 34.2 μV) محاسبه گردید. مقایسه اختلاف میانگین های آمپلی تیودهای پتیانسیل II Ia با حالت DS در حالت II Ia spikes در حالت استراحت دو DS و PR با حالت DS نشان داده‌اند. در موقعیت پتیانسیل های II Ia spikes با حالت DS و II Ia spikes با حالت DS از این دو حالت II Ia spikes بعد از پتیانسیل هایIII و IV ظاهر شدند در حالیکه این وضعیت درPD مشاهده گردید. اختلاف DS پتیانسیل های II Ia spikes با حالت DS و II Ia spikes با حالت DS بین میانگین‌های آمپلی تیود پتیانسیل II Ia spikes با حالت DS و II Ia spikes با حالت DS نتیجه‌گیری (PR و DS) گرفتن برای پتیانسیل های II Ia spikes با حالت DS و II Ia spikes با حالت DS بوده است. این این نتایج می‌تواند نقشی در کنترل اختلالات حرکتی بر عهده داشته باشد.

واژگان کلیدی: ناکسانITO،کشش دینامیکی،PR،زلفکس،spindle II Ia