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

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Iranian Journal of Neurology, Vol.8, No.28 ,Winter 2010, 588-596

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 testing15 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±5.34µv), II (38±3.27µv), and γd (62±4.29 µv) were calculated in the RD. The obtained mean SPs amplitude difference was21% for Ia and7.7% 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 controling 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 (γd) and γ-static (γs) 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 mammalians.[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 γd-axon in rat.

Methods and Materials

This experimental study was carried out on the caudate spindle Ia, II, and γd axons of 15 normal adult male *Sprague-Dawley* rats, weighing 270±51gr. Anesthesia with a urethane (1.7g/ kg, I.P) supplementary doses (o.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 γd 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 γd 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 duc; 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).

SNFs group	Number of testing fibers	RD-SPs Amplitude values (μv)	PR-SPs Amplitude values (μv)	DS-SPs Amplitude values (μv)
γd fiber	15	56-70	85-105	10-17
Ia fiber	15	65-80	180-200	235-255
II fiber	15	30-42	52-66	55-70

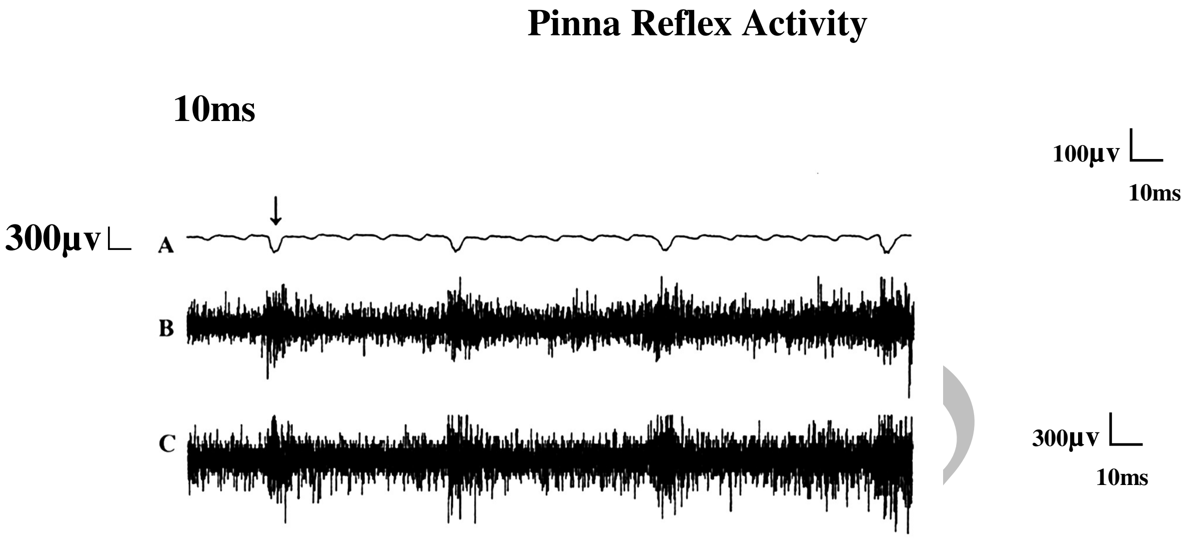

Figure1. Trace A marked wave of ear's movement by arrow, trace B and C show the bursting spindle SPs of γd and afferents, respectively, during applied PR.

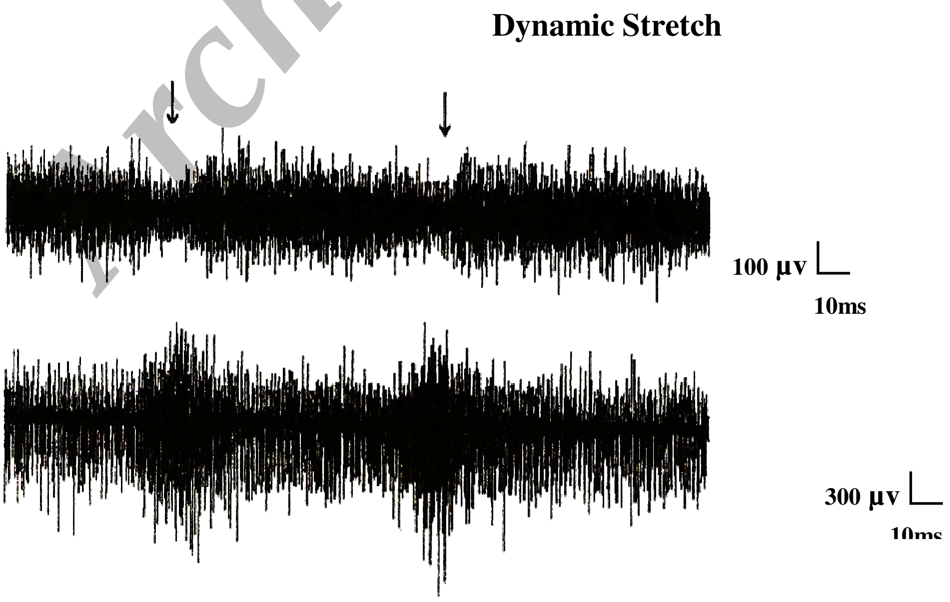

Figure2. Top trace the γd spike potentials are depressed in marked arrows, but in bottom trace spindle Ia and II fibers show bunches of firing spike potentials during DS.

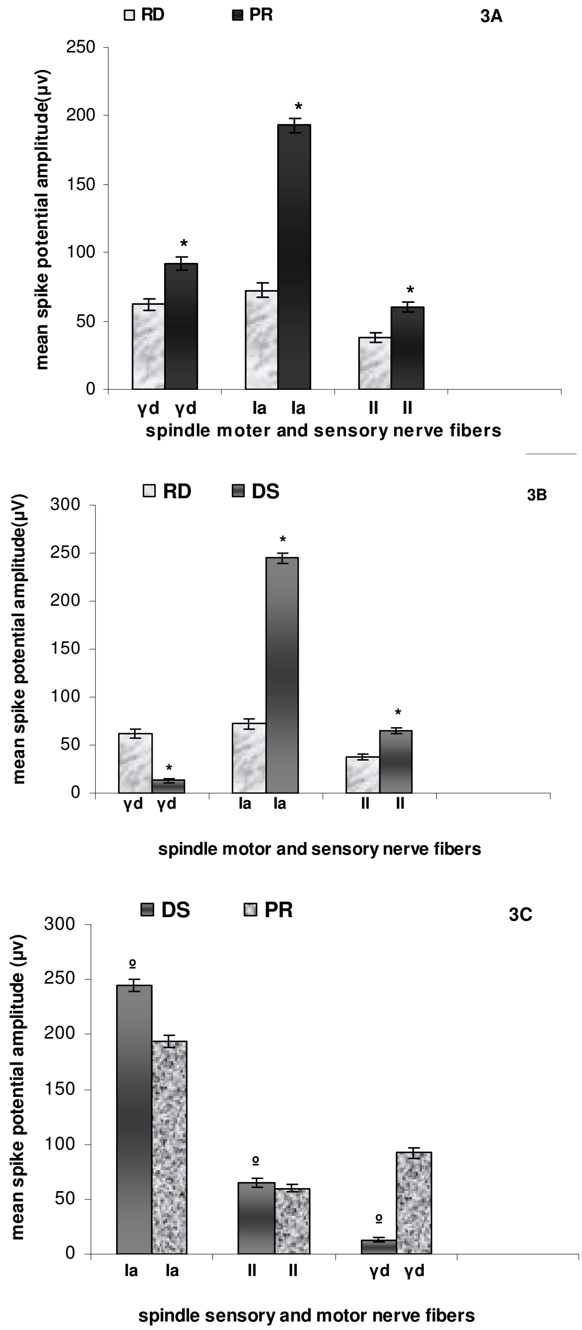

Figure 3. (A) Comparison of spike potentials (SPs) amplitudes histogram of gamma dynamic (γd), Ia, and II spindle nerve fibers in the resting discharge (RD) group with in the pinna reflex (PR) group. *Significant difference with RD group. (B) Comparison of SPs amplitudes histogram of γd, Ia, and II spindle 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 γd discharged before the SPs of Ia and II SPs (figure 1 trace B). The mean SPs amplitude values of γd, 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 γd 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 γd SPs amplitude than the γd 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 γd in the DS was 85% less than in the PR. Comparing of means SPs amplitudes of Ia, II, and γd are illustrated in figure3C.

Discussion

The γd-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 γd 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 γd SPs acted only like a transit way between the γd-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 γd SPs in despite of the fact that γd 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 γ and beta axons.[6, 20] The beta axon was excluded in this study. The γd 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 γd 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 γd 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 γd 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 γd 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.

Acknowledgment

I'm grateful to the valuable suggestions of Dr. M .Gladden ; the previous faculty member of Glasgow University.

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اثرات رفلکس pinna و کشش دینامیکی بر روی تخلیه spikes اعصاب دوک عضلانی ناحیه دم موش صحرایی

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چکیده

سابقه و هدف: آکسونهای حرکتی γd که عصب دهی فیبرهای دوک عضلانی را بر عهده دارند می‌توانند تحریک اعصاب دوک عضلانی را سبب شوند. هدف از این مطالعه مقایسه کیفیت اثر رفلکس pinna (PR) و کشش دینامیکی (DS) در چگونگی تخلیه و آمپلی تیود پتانسیل های spike اعصاب Ia , II و γd دوک عضلانی ناحیه دم موش صحرایی بوده است.

روش بررسی: از پانزده موش صحرایی نر بالغ , چهل و پنج عدد فیبرهای افرنت و γd (بطور جداگانه برای هر نوع فیبر) در طرف چپ L 5, تخلیه پتانسیل های spike در حالت استراحت و در هنگام اجرای PR و DS جهت مطالعه ثبت گردید. مقایسه بین میانگین‌های آمپلی تیود پتانسیل spike فیبرهای Ia ,II , و γ d در حالت استراحت با PR و DS انجام گردید.

یافته ها: آمپلی تیودهای پتانسیل های spike در حالت استراحت برای فیبرهای Ia (۷۲ ± ۵,۳۴ μv), II (۳۸ ± ۳,۲۷ μv), و γd (۶۲ ± ۴,۲۹μv) محاسبه گردید. مقایسه اختلاف میانگین های آمپلی تیودها پتانسیل spike برای Ia , II در حالت DS با حالت PR به ترتیب ٪۲۱ و ٪۷,۷ بیشتر بدست آمده است. پتانسیل‌های spikes برای Ia و II در هر دو حالت DS وPR, overlap نشان داده‌اند. در موقعیت PR پتانسیل‌های spike متعلق به Ia و II بعد از پتانسیل های γd ظاهر شدند در حالیکه این وضعیت در DS مشاهده نگردید. اختلاف بین میانگین‌های آمپلی تیود پتانسیل spike متعلق به γd در حالت DS با حالت PR ٪۸۵ کمتر بوده است.

نتیجه گیری: DS (غیر وابسته به پتانسیل spike , γd) یک محرک برتر برای تحریک فیبرهای Ia و II نسبت به PR (وابسته به پتانسیل spike , γd) بوده است. γd با DS قویا غیر فعال بوده است. این نتایج می‌توانند نقشی در کنترل اختلالات حرکتی بر عهده داشته باشند.

واژگان کلیدی : spindle II, Ia و آکسون γd , آمپلی تیود پتانسیل spike , رفلکس PR , کشش دینامیکی