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CIRCUMFERENTIAL PRESSURE'S INHIBITORY EFFECTS ON SOLEUS H-REFLEX

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Abstract

Background: Circumferential pressure (CP) applied to the lower leg reduces soleus motor neuron reflex excitability (MNRE); however, the mechanism of control is unknown. Aim: To investigate the effect that CP has on disynaptic reciprocal inhibition (DSRI) and on Ia presynaptic inhibition (IaPI) of the soleus H-reflex in healthy subjects. Methods: DSRI of soleus motoneurons and presynaptic control of soleus group Ia afferents were measured before, during and after CP was applied to the calf. Pressure was set to 40-45 mmHg. DSRI was evaluated by observing changes in the H-reflex amplitude after a conditioning stimulus was applied to the common peroneal nerve. IaPI was assessed using two separate protocols involving conditioning of the soleus H-reflex: femoral nerve facilitation (FNS) (heteronymous) and D1 and D2 inhibition (homonymous). A change in DSRI and IaPI was determined by comparing the $H_{pressure}$, $H_{post\text{-}pressure}$ phases to the $H_{pre\text{-}pressure}$ phase of the conditioned H-reflexes. Results: A mean 12% decrease in FNS was observed during CP ($p < 0.05$). D1 and D2 inhibition decreased slightly. CP did not affect DSRI. Conclusion: The results show that CP applied to the calf significantly increased heteronymous soleus IaPI, but affected homonymous IaPI less. It was concluded the CP does increase IaPI of soleus motoneurons but only modestly. The change was not large enough to explain the dramatic inhibition that occurs in the (unconditioned) H-reflex amplitude when CP is applied. Therefore, IaPI is not the primary inhibitory mechanism that CP uses to lower MNRE.

Keywords

• Circumferential pressure • H-reflex • Soleus muscle • Presynaptic inhibition • Disynaptic reciprocal inhibition • Motoneuron reflex excitability

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Background

Circumferential pressure (CP) application and its effects on muscle activity in both the upper and lower limbs has been extensively studied [1-8]. Initial investigations examined the effect CP had on soleus monosynaptic reflexes in subjects without neurological deficits and compared them to subjects with cerebral vascular accidents [2,6]. A significant decrease in the soleus H-reflex amplitude and the soleus stretch reflex occurred throughout the pressure application. In a related study involving subjects with complete traumatic spinal cord injuries (SCI), CP around the calf resulted in a similar soleus H-reflex inhibition [7]. From these results it was suggested that the mechanism responsible for the decrease in H-reflex amplitude was spinal in origin [7].

Many spinal cord mechanisms may account for the decrease in H-reflex amplitudes during pressure application [1-5,9-12]. Tissue ischemia and decreases in nerve conduction velocity were shown not to be involved [5,6]. Investigations on presynaptic inhibition of Ia afferents have not been formally conducted; however, results from an investigation on F-waves suggest that it may have a role [1].

Studies have described a spinal cord control system that may be partly mediated through a Ia presynaptic inhibitory mechanism [13-20]. Hultborn *et al.* and Guissard *et al.* observed soleus H-reflex amplitude depression after passive dorsiflexion of the ankle and hypothesized the inhibition was the result of Ia afferent activation [15,21]. Other investigators described H-reflex inhibition evoked by Ia, group II and Ib afferents after muscle contraction [13,16]. Cutaneous inputs have also been shown to effect transmission in presynaptic inhibitory pathways [22-24]. It is therefore hypothesized that the decrease in motoneuron reflex excitability (MNRE) observed during CP may use a similar mechanism.

Movement, the fundamental component of behavior, is produced when skeletal muscles contract and relax in a regulated manner. Every movement, no matter how simple, is controlled by the precise firing pattern of motoneurons populations within the motoneuron pool (excluding the contributions from muscle's viscoelastic properties). The processes that influence motoneuron behavior (excitability) therefore are important. This is especially true for clinicians who are continually looking for ways to intervene with the motor system to treat neuromuscular disorders.

The purpose of this investigation was to determine the effect that CP has on pre-synaptic inhibition of the soleus H-reflex in healthy subjects. Disynaptic reciprocal inhibition (DSRI) was also assessed to determine if CP had any affect on the Ia inhibitory interneuron exerted at a postsynaptic level. It was hypothesized that CP will cause a decrease in H-reflex amplitude mediated through a presynaptic inhibitory mechanism. The results of this study will increase our understanding of the physiological mechanisms that regulate muscle

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activity and may also provide evidence to the extent in which sensory afferents affect the excitability of the lower limb's spinal reflex arc.

Subjects and methods

Subjects

Thirty-eight subjects (26 women, 12 men) volunteered for this study. The subjects had no history of neurological disease or lower extremity musculoskeletal disorders, and ranged in age from 19 to 65 years old (mean=30.3, SD=10.0). Subjects were asked to refrain from caffeine, aspirin, alcohol, and exercise 12 hours prior to data acquisition as these factors have been shown to alter MNRE [25]. All subjects signed informed consent forms approved by the University of Rhode Island Institutional Review Board before participating in this study.

Electromyography (EMG)

To prepare for EMG electrode placement the skin of the subject's dominant lower limb was shaved and cleaned with alcohol. Coupling gel was used on all surface electrodes to ensure proper conductance. Two 10 mm disc-recording electrodes were placed 3 cm apart on the posterior lower leg, inferior to the gastrocnemius muscle belly, and in alignment with the Achilles tendon. Two more sets of 10 mm disc recording electrodes were placed 3 cm apart on the skin over the tibialis anterior (TA) and vastus lateralis muscle bellies to monitor their activity during the study. A 5x5 cm metal plate acted as the ground and was fixed to the anterior lateral calf, between the fibular head and lateral malleolus. EMG activity was amplified 1000x and recorded using a bandwidth of 10-10,000 Hz. Data was digitized at a sample frequency of 10K using the Powerlab ADInstruments Chart 4 Windows Data Acquisition and Analysis software and was stored on a computer's hard drive for future analysis. The EMG's amplifier had an input impedance of 1MΩ (<47pf), a common-mode rejection ratio of 96 dB at 50 Hz and a signal to noise ratio of <1 μV r.m.s. (root mean sqare of voltage amplitude).

Soleus H-reflex

Surface electrodes were used for both stimulation of the tibial nerve and recording H-reflex data. The H-reflex was elicited by a 2.5 cm monopolar stimulating ball electrode placed on the skin over the tibial nerve in the popliteal fossa. A 10 x10 cm sponge reference electrode was fixed to the distal anterior thigh.

The H-reflex was evoked using a rectangular 1ms pulse at 0.17 Hz (1 pulse every 6 seconds). The size of the maximal motor response (M_{max}) and the maximal H-reflex (H_{max}) were measured at the beginning and randomly throughout the experiment. Three criteria were used to determine proper electrode placement: 1) the H-reflex was evoked at a lower intensity than the soleus M-wave, 2) the least amount of intensity was required to elicit a maximum H-reflex and 3) the soleus M-wave and H-reflex displayed a similar wave configuration. The stimulus strength was then adjusted to give an unconditioned H-reflex that also evoked a small M-wave and this H-reflex was used as the experimental control ($H_{unconditioned}$) [26,27]. The H-unconditioned reflex was approximately 25% M_{max}. $H_{unconditioned}$ was randomly monitored throughout the experiment. If a deviation in amplitude occurred (±1 SD), $H_{unconditioned}$ was readjusted back to initial baseline values ($H_{baseline}$)[28].

Pressure

A 16-21 cm air splint was applied to the lower leg depending upon leg length proximal to the recording electrodes and distal to the fibular head. This location allowed room for the conditioning electrode to be placed on the skin over the Common Peroneal Nerve (CPN). Caution was also taken not to compress the CPN by the air- splint. Prior to starting the experiment the air splint was inflated and then passively deflated. This procedure allowed the pressure cuff to conform to the subject's leg and minimized recording electrode displacement during data acquisition. During the pressure phase of the experiment, the air splint was inflated to 40.0 – 45.0 mmHg with the aid of a pressure transducer that monitored backflow from the splint. To decrease the chance of ischemia:

1. The subject's blood pressure was taken before the beginning of data recording. If diastolic pressure was below 45 mmHg, the experiment was terminated.
2. Skin color distal to the splint was closely monitored during the pressure phase of the experiment.
3. Pressure values were continually monitored and adjusted during the experiment to maintain pressures that remained within a 40-45 mmHg window.
4. M-waves were monitored throughout the experiment to ensure reflex configuration did not change.

Design

The subjects were seated comfortably in a reclined chair with their dominant lower extremity positioned in 60° of hip flexion, 20° of knee flexion, and 20° of ankle plantar flexion. The subject's ankle was placed in an adjustable ankle rest and a moveable footrest supported the foot. To diminish any descending influence on spinal motoneurons during the experiment, subjects were instructed to remain still and quiet during testing. In addition, EMG activity from soleus, tibialis anterior and quadriceps muscles were monitored to assure no ongoing muscle activity occurred during the experiment. Figure 1 illustrates the general experimental setup.

The experiment consisted of three-test phases: pre-pressure, pressure, and post-pressure. Within each phase conditioned and unconditioned reflexes were elicited. Unconditioned H-reflexes were randomly elicited throughout the experiments to maintain consistency within the experiment. Any change in its amplitude resulted in immediate adjustment of $H_{unconditioned}$ back to $H_{baseline}$.

The pre-pressure phase consisted of eliciting and recording $H_{conditioned}$ reflexes. The air splint was then inflated and maintained at the desired pressure. The increase in pressure caused $H_{unconditioned}$ to decrease substantially in every subject. It was therefore necessary to readjust $H_{unconditiioned}$ back to $H_{baseline}$. After one minute of inflation, a second set of $H_{conditioned}$ reflexes were elicited and recorded. The air splint was then passively deflated and $H_{unconditioned}$ was again readjusted back to $H_{baseline}$. After a one-minute delay, another post-pressure $H_{conditioned}$ reflex set of recordings were taken.

Experimental techniques used to assess inhibition

Presynaptic inhibition was examined using two separate protocols: 1) the technique of heteronymous Ia facilitation of the femoral nerve (FN) terminating on the soleus motor neurons described by Hultborn [15] and 2) common peroneal nerve (CPN) stimulation (D1 and D2 inhibition) described by Lundbye-Jensen and Nielsen [29] and Mizuno *et al.* [30] (Figures 2a and 2b). In general, D1 and D2 inhibition measures presynaptic inhibition elicited by peripheral nerve stimulation while FN facilitation is a reflection of the amount of ongoing presynaptic inhibition of FN Ia afferents [29]. Disynaptic reciprocal inhibition initially described by Crone *et al.* [31] was also assessed to evaluate the effect that the antagonist muscle nerves have on the soleus Ia inhibitory interneurons (Figure 2c).

Conditioning femoral nerve simulation (FNS) of the soleus H-reflex

FNS was applied to the skin over the femoral nerve in the femoral triangle using a 10 mm

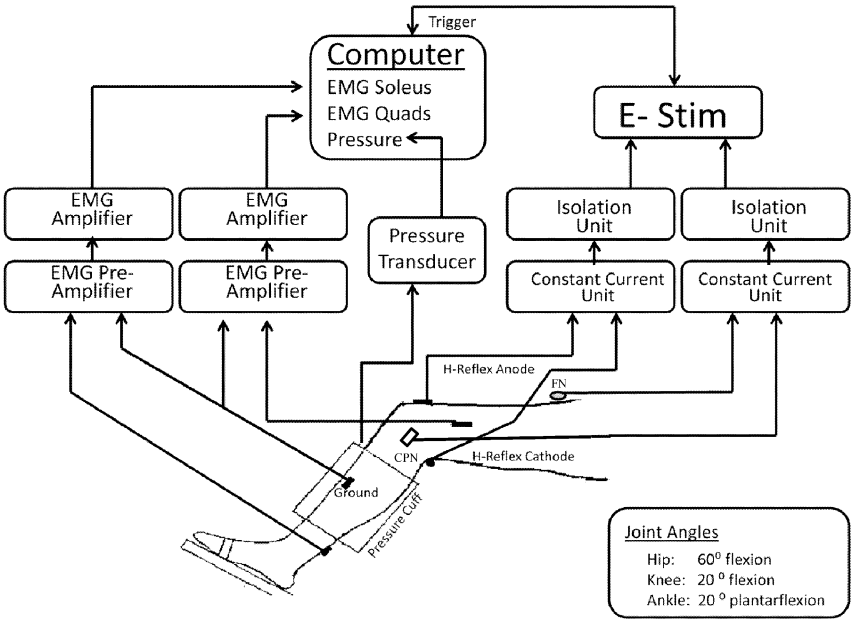

Figure 1. Experimental set-up. EMG monitoring electrodes and wiring schematic are not shown for the tibialis anterior muscle.

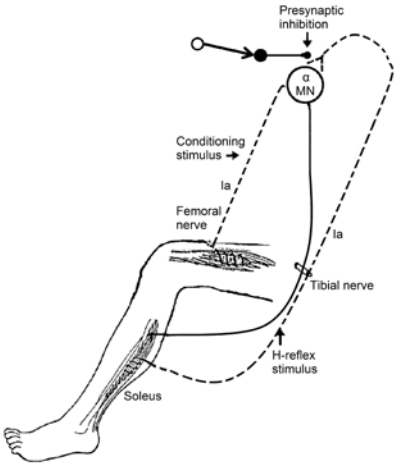

Figure 2a. Heteronymous Ia presynaptic inhibition of Ia afferents elicited by a condition stimulus to the femoral nerve. Figure illustrates the spinal circuit during which femoral nerve stimulation at 1.1x motor threshold intensity delivered after tibial nerve stimulation elicits monosynaptic excitation of the soleus motoneurons. The mean ratio's between $H_{conditioned}/M_{max}$ relative to the $H_{uncondtioned}/M_{max}$ reflex amplitude were compared among the experiment phases. A decrease in the mean $H_{unconditoned}/M_{max}$ would indicate an increase in Ia presynaptic inhibition.

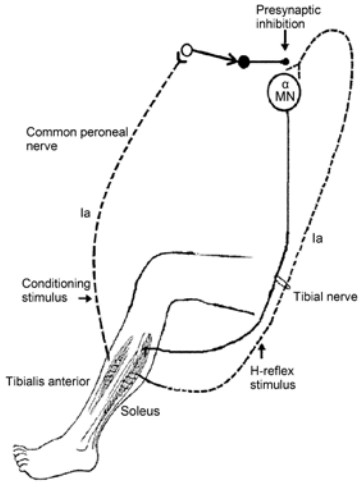

Figure 2b. Homonymous presynaptic inhibition of Ia afferents elicited by a conditioning stimulus to the common peroneal nerve. Figure illustrates the spinal circuit during which a 1.1x motor threshold conditioning stimulus was applied at interstimulus intervals of 10 ms and 25 ms for D1 inhibition and at 50 ms, 75 ms and 100 ms for D2 inhibition. The level of homonymous presynapitc inhibition was measured by calculating the change in the mean ratio's between $H_{conditioned}/M_{max}$ relative to the $U_{uncondtioned}/M_{max}$ reflex amplitude among the experiment phases.

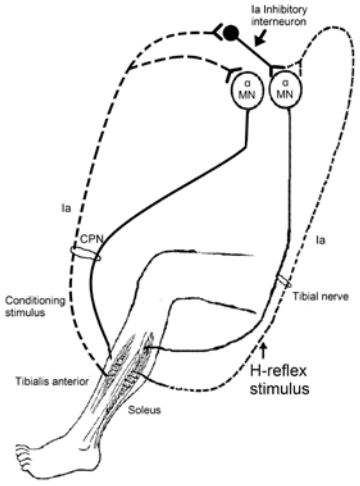

Figure 2c. Disynaptic Reciprocal inhibition (DSRI). Figure illustrates the spinal circuit of DSRI exerted on ankle plantar flexors following a 1ms common peroneal nerve conditioning stimulus at 1.1x motor threshold. DSRI involves the Ia inhibitory interneuron that is exerted at a postsynaptic level. Conditioning stimuli were given at 1, 3 and 10ms before tibial nerve stimulation eliciting the H-reflex. DSRI was measured by calculating the change in the mean ratio's between $H_{conditioned}/M_{max}$ relative to the $U_{uncondtioned}/M_{max}$ reflex amplitude among the experiment phases.

monopolar surface electrode. A 3x3 cm anodal sponge fixed to the posterior proximal thigh served as the reference electrode. A bipolar recording electrode (10 mm discs) was placed on the skin over the belly of the vastus lateralis muscle 6-8 cm proximal and lateral to the patella to monitor myoelectric activity from the quadriceps muscle.

FNS consisted of a 1 ms rectangular pulse given at a frequency of 0.17 Hz. Stimulus strength was adjusted to 1.1 x the quadriceps motor threshold (MT) and the afferent volley was used as the conditioning stimulus that facilitated $H_{unconditioned}$. This facilitation occurred between a conditioning-test interval of -3.0 to -7.5 ms. The negative conditioning-test interstimulus intervals designate that the conditioning simulation was applied after the test stimulation. The delay was adjusted according to the height of the subject until the H-reflex was consistently larger than $H_{unconditioned}$, as observed on the digital storage oscilloscope. Hultborn *et al*. [15] have shown that during the first 0.5 ms the heteronymous Ia facilitation is only mediated through a monosynaptic pathway and not contaminated by other input. Ia presynaptic inhibition was measured by calculating the change in the mean ratio's between $H_{conditioned}$ /M_{max} relative to the $H_{unconditioned}$ /M_{max} reflex amplitudes among the experimental phases. Twenty-five $H_{conditioned}$ reflexes were elicited and averaged in each phase. A decrease in the mean $H_{conditioned}$ /M_{max} would indicate an increase in Ia presynaptic inhibition.

Before reaching any conclusions that a change in H-reflex facilitation was due to on-going presynaptic inhibition of Ia afferents mediating the conditioning volley, a change in the reflex recruitment gain must be ruled out [32]. To ensure that any effects were not due to these changes D1 and D2 inhibition was also analyzed. FNS and D1/D2 inhibition provide autonomous information concerning presynaptic inhibition and they assist in excluding changes in recruitment gain as a cause for changes in H-reflex size [33,34]. In addition, all experiments were performed in a reclined position at rest [35] and the M-wave amplitude was closely monitored throughout the experiment [26]. Any observable change (±1 SD) in its amplitude or configuration resulted in data omission.

CPN conditioning stimulus of the Soleus H-reflex

A bipolar electrode (10 mm disks) was placed on the skin of the fibular head over the CPN. This stimulus was used as a conditioning stimulus to evoke reciprocal, D1 and D2 presynaptic inhibition of the soleus muscle. The optimal stimulation site was selected based on the following criteria: the Tibialis Anterior (TA) motor threshold was lower than that of the peroneal muscles and at increased levels of stimulation intensities, ankle eversion and peroneal muscle activity were absent. Stimulation to the CPN was delivered with a 1ms constant current pulse at 1.1 x TA MT. H-reflexes were then elicited following conditioning stimulation to the CPN at eight predetermined interstimulus delays. The delays were set at 1, 3, 5 10, 25, 50, 75 and 100 ms and randomly delivered. For each interstimulus delay, peak to peak amplitudes of 5 H/M_{max} ratios were averaged and expressed as a percent of $H_{unconditioned}$/$M_{max.}$

Throughout all phases of this experiment $H_{unconditioned}$ amplitudes were randomly checked to ensure it remained unchanged. If $H_{unconditioned}$ amplitude increased or decreased, tibial nerve stimulation intensity was adjusted back to original $H_{baseline}$ value [28].

Statistical analysis

The StigmaStat version 2.0 statistical software program was used for all data analysis. $H_{conditioned}$ reflex amplitudes were averaged for the pre-pressure phase and for each of the two test-phases (pressure and post-pressure). Peak-to-peak amplitudes were then measured.

Friedman repeated measures analysis of variance on ranks tests were used to analyze the change from pre-pressure values in the average $H_{conditioned}$ reflex amplitudes during and after pressure application. Parametric testing was not performed because the data was not normally distributed. Dunnett's multiple comparison tests were used when significant F values were found. The level of significance for all post hoc tests was designated $p<0.05$.

Results

Statistical analysis was performed on 19 of the 38 initial subjects, ranging in age from 22-34 years old (mean=26.8, SD=3.4). Inclusion criteria were: 1) Subject had a consistent facilitation of $H_{unconditioned}$ with the conditioning stimulus, 2) No change in soleus, TA and/or quadriceps M-wave configurations, and 3) a consistent $H_{unconditioned}$ that could be re-established before, during and after pressure application. Figure 3 shows a typical H-reflex from a representative subject.

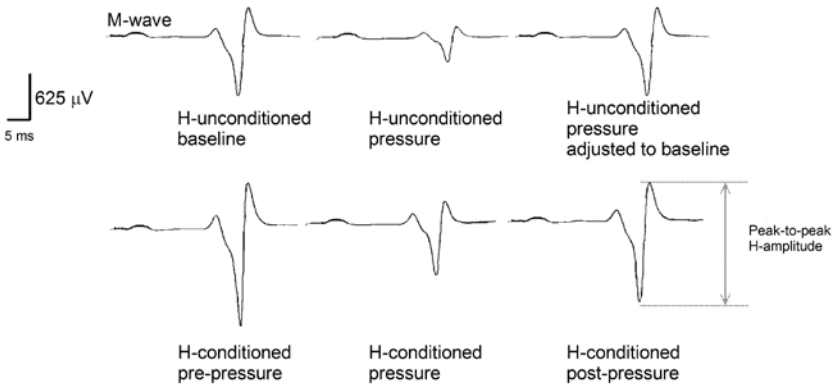

Figure 3. Demonstration of the H-reflex peak-to-peak amplitude changes for heteronymous Ia presynaptic inhibition that takes place throughout the experiment in a typical subject. Note the pre-pressure H-reflex facilitation from baseline levels that results from femoral nerve stimulation and the inhibition that occurs in the conditioned H-reflex during CP when compared to pre-pressure values. Each line represents an average of 20 recordings.

Nineteen subjects were omitted from the analysis because they failed to meet inclusion criteria. When CP was applied, $H_{unconditioned}$ amplitude dropped dramatically in every subject (55% mean decrease; SD 27.45). To accommodate for this significant drop, $H_{unconditioned}$ was increased to restore it to initial $H_{baseline}$ levels [28]. The re-establishment of $H_{unconditioned}$ proved challenging and was the primary criteria for excluding subjects from the study (n=11). The other eight subject's data were omitted due to changes in reflex configurations of the M wave, H-reflex or both when the air splint was inflated.

When compared to pre-pressure values, a significant (p = 0.013) mean decrease of 12.5% was observed in FNS during CP application (Figure 4). This decrease in the $H_{conditioned}$ reflex amplitude is believed to be due to an increase in Ia Presynaptic Inhibition (IaPI) [15]. Figure 5 shows the individual data from the 19 subjects whose date were analyzed. As can be seen from the figure, 15 subjects exhibited an increase in IaPI during the pressure phase. Subjects 2, 5 and 13 showed an increase in the $H_{conditioned}$ reflex amplitude, indicating large decreases in IaPI of 98%, 44%, and 28% respectively. Subject 19 was the only one who did not show any change in IaPI. If the three subjects who showed decreases in IaPI were treated as outliers and had their results excluded from data analysis, IaPI would have had a mean increase of 26% when compared to pre-pressure levels. Because their $H_{unconditioned}$ values dramatically decreased similarly to the other 16 subjects when CP was initially applied, it was considered the decrease in FNS observed was an accurate representation of their response and therefore their data were included in the analysis.

Post-pressure data was not significantly different from pre-pressure levels. Most subjects showed a decrease in IaPI toward baseline pre-pressure levels. Individual responses however varied. For example, five subjects showed a phase reversal demonstrating a substantial decrease in IaPI above baseline levels (subjects 3, 6, 8, 14, 19) and in one subject (subject 18) IaPI increased beyond pressure levels (Figure 5).

Figure 6 illustrates the DSRI, D1 and D2 inhibition between pressure and pre-pressure measurements that were observed at CPN conditioning interstimulus intervals of 1, 3, 5, 10, 25, 40 75 and 100 ms. As can be seen from the figure, CP caused a slight mean increase in D1 and D2 inhibition for all the interstimulus intervals tested in this experiment. However, only the 100 ms interval reached significance ($p<0.05$). Post-pressure values recorded at

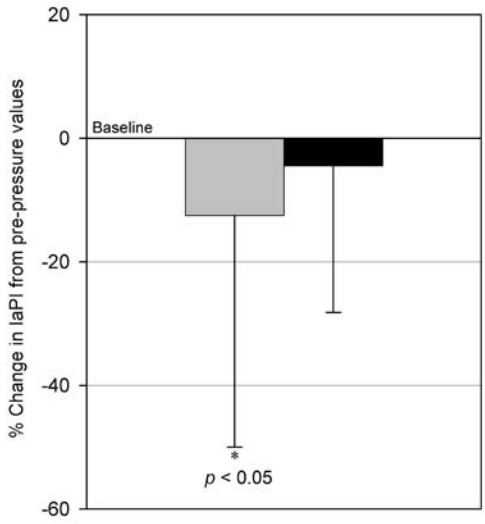

Figure 4. Mean percent change *(n = 19)* in the $H_{conditioned}$ reflex during CP after FNS. A mean 12.5% decrease was observed. Standard deviations are also shown. Decreases in the $H_{conditioned}$ reflex amplitudes represent an increase in IaPI. (0% = $H_{conditioned}$ pre-pressure values) = pressure, = post-pressure

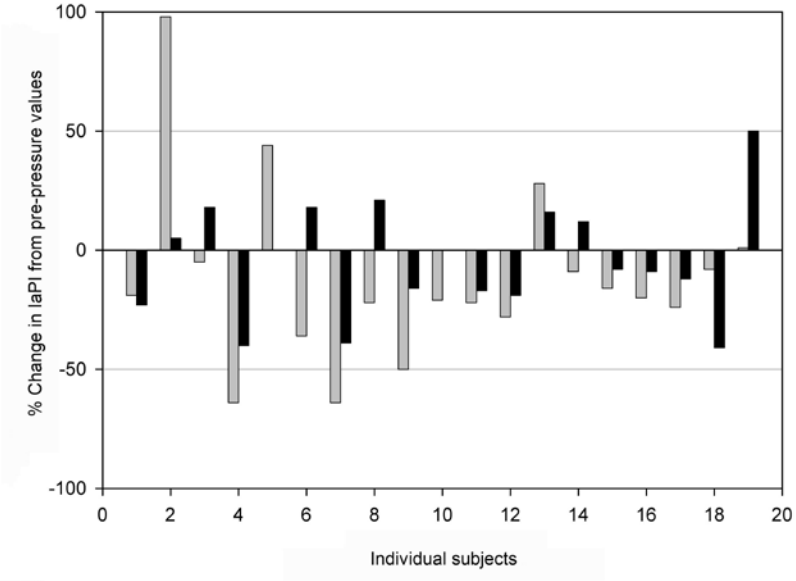

Figure 5. Heteronmous Ia presynaptic inhibition individual subject data showing the percent change in the $H_{conditioned}$ reflex amplitudes during and after pressure inhibition when compared to pre-pressure values. Note: subjects 2, 5, and 13 exhibited a decrease in presynaptic inhibition as shown by an increase in the $H_{conditioned}$ reflex amplitudes. Each bar represents an average of 20 recordings. (0% = $H_{conditioned}$ pre-pressure). = pressure, = post-pressure

one minute after pressure release returned to baseline pre-pressure levels in all subjects ($p>0.05$). This rapid return to pre-pressure levels is likely due to CP's short-lasting effect [6,7].

Discussion

The main finding of this study showed that CP to the calf significantly decreased soleus H-reflex heteronymous Ia facilitation when compared to pre-pressure values. Two distinct methods involving conditioning the soleus H-reflex, FNS and D1/D2 inhibition were used to assess presynaptic inhibition. FNS reflects the level of ongoing presynaptic inhibition of the Ia afferents onto motoneurons while D1/D2 inhibition gives an indication of presynaptic inhibition elicited by peripheral nerve stimulation. Additionally, the two techniques provide autonomous information about presynaptic inhibition that help in determining if changes in the recruitment gain within the soleus motoneuron pool is responsible for any of the observable changes in H-reflex amplitude [32]. For example, a change in the level of presynaptic inhibition should induce similar changes in the amplitude of the conditioned H-reflexes recorded with the two methods as long as the conditioning stimulus elicits a monosynaptic excitatory postsynaptic potential of constant size in primary afferent depolarization interneurons (PAD) or motor neurons. Thus, an increase in presynaptic inhibition would result in a decrease in the amplitude of the conditioned H-reflex for both D1/D2 inhibition and FNS. Since this was the case, it was believed that CP caused a true increase in presynaptic inhibition and not a decrease in reflex gain.

Previous studies investigating CP around the calf suggested that muscle stretch is a likely cause [5-7]. The compressive forces created by the air splint would seemingly cause a minimal amount of stretch to all of the muscle fibers beneath the splint. Studies have shown that small amplitude passive stretches to the soleus causes significant reductions in alpha MNRE [36,37]. Guissard *et al*. further showed that this decrease in MNRE following small passive stretch was due to an increase in Ia presynaptic inhibition [21]. If muscle stretch is the novel stimulus that initiates the increase in IaPI, group II muscle afferents are likely responsible. They have been shown to discharge as long as muscle stretch is sustained [38,39], to have a low threshold of activation [38], to inhibit extensor muscles [40,41], and to generate primary afferent depolarization by the activation of GABAergic interneurons [42].

Cutaneous receptors that specifically sense stretch and pressure (Merkel and Ruffini receptors) also have been indicated in increasing IaPI during pressure [23,43]. These receptors are stimulated by lightly stretching the skin [44], continue to discharge for the entire duration of stimulus [45], and may also have a role in modulating presynaptic inhibition of Ia afferents [23,42,43]. How much they function in inhibiting MNRE through a presynaptic mechanism is unknown. Further research is needed to assess the effect of these cutaneous receptors on MNRE when CP is applied to a limb.

Descending input onto spinal motoneurons from cortical and brain stem areas may also have contributed to the lowering of H-reflex amplitudes observed in this study. It is well known that these descending pathways converge onto dorsal horn neurons and have a gating and filtering affect on PAD through a presynaptic mechanism [46-48]. Recent studies have shown that short-latency afferent inhibition is mediated though a spinal rather than a cortical circuit in the lower extremity [49,50]. This aspect coupled with the finding that CP significantly decreased the soleus H-reflex in people with complete traumatic spinal cord injury [7] strongly suggests that any influence from supraspinal systems would be negligible.

Air splint inflation dramatically decreased the unconditioned H-reflex amplitude in every subject tested in this investigation. Sometimes (n=11), the extent of the inhibition was so significant that H-baseline levels could not be re-established. For CP to inhibit the H-reflex to this level, a greater than 12.5% increase in IaPI would be necessary. In addition, three subjects displayed decreases in IaPI during pressure, yet their unconditioned H-reflex was strongly inhibited. If IaPI was the sole mechanism of

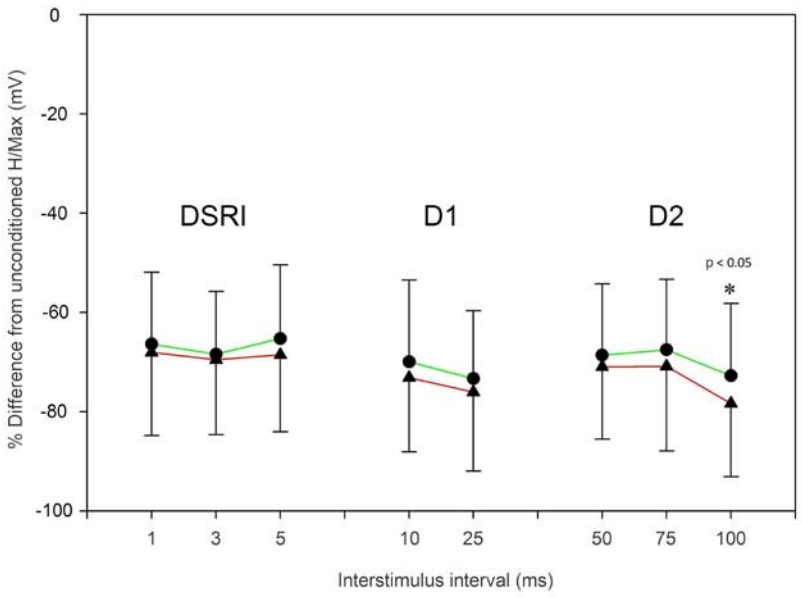

Figure 6. Mean percent ratio change *(n = 19)* in the $H_{conditioned}$ reflexes following stimulation to the common peroneal nerve at interstimulus intervals of 1, 3, 5, 10, 25, 50, 75, 100 ms. Interstimulus intervals represent Disynaptic Reciprocal Inhibition (1, 3, 5), D1 (10, 25) and D2 inhibition (50, 75, 100 ms) respectively. Interstimulus interval at 100 ms was the only significant interstimulus interval ($p<0.05$). SDs are also shown.

neuronal control during CP application, all subjects would have shown inhibition of the conditioned H-reflex and to a much greater extent. Thus, the increase in IaPI observed in this study cannot fully explain the dramatic decrease in the unconditioned H-reflex during the application of CP.

It is a difficult task to hypothesize what other spinal mechanisms may participate in lowering MNRE besides Ia presynaptic inhibition. Previous studies show that tissue ischemia [6-8] and changes to the input/output properties of the reflex arc are not responsible [5]. Ib non-reciprocal inhibition is unlikely involved because the soleus tendon receptors are not sensitive enough to detect the forces produced by the minimal passive stretch created by the air splint [51,52]. Finally, CP did not affect DSRI. These findings imply that another mechanism, possibly musculo-skeletal in origin, may be involved.

Leukel *et al*. [5] conducted a study that investigated the effect pressure applied around the calf had on spinal cord reflexes similar to the methods used in this study. The only difference was that pressures of 240-250 mmHg were used compared with the 40-45 mmHg used in our study. Leukel *et al*. [5] showed a significant H-reflex depression similar to what we observed. Since CP did not cause any change in the input/output properties of the reflex arc, they hypothesized, that the viscous-elastic components of the musculo-tendonous junction distal to the cuff were responsible for the decrease in reflex amplitude. They argued that the inflated cuff "clamps" the muscle thereby preventing the underlying tissue from naturally moving. It was further shown that this damping effect occurs only when the muscle tensions were low. The decrease in H-reflex amplitude observed in this study may have been mediated through a similar mechanism caused by air splint compression. Whether the low pressures used in this study were sufficient to induce the mechanical change necessary to affect H-reflex amplitude still requires further investigation.

Finally, little attention has been given to the effects that group III and IV muscle afferents have on the motoneuron pool. Usually these afferents are concerned with relaying information to the central nervous system regarding the metabolic state and mechanical activity of exercising muscle [53-55]. Substantial evidence exists, however, that group III and IV muscle afferents also play an important role in regulating spinal motoneuron excitability [56-60]. The discharge properties of these afferents were shown to affect the motoneurons in a flexor-reflex pattern of excitation; increasing excitability of ipsilateral flexors and inhibition of ipsilateral extensors [59]. It is quite possible that CP may elicit group III and IV afferents that inhibit the soleus muscle motoneurons. Evidence demonstrates that pressure application [59] and muscle stretch [59,60] activate these afferents. Whether CP reaches the critical level to elicit these afferents remains speculative and requires further research. It does, however, provide an additional explanation for the dramatic decrease observed in the soleus H-reflex amplitude during CP application to the calf.

Clinical implications

Results from previous pressure studies applied by an air splint to the calf showed that MNRE decreased in all subjects and patients tested [6,7]. Due to these results, these authors advocate using CP in treating hypertonia resulting from upper motor neuron syndrome. It is always an arduous task for therapists to choose a therapeutic modality that is efficacious without knowing how it works. The results of this study were unable to detect fully how CP lowers MNRE except that Ia presynaptic inhibition plays a small role. Clinicians therefore should be aware of the many spinal and peripheral mechanisms that CP may use in modulating MNRE and routinely monitor their treatment effects to assure if the functional outcomes in their patients are what was expected. This is especially true when muscle contraction and movement are a condition [3,5].

Summary

The unconditioned H-reflex amplitude dramatically decreased in every subject during pressure application. Previous research conducted on people with complete traumatic spinal cord injury has suggested that this decrease in H-reflex amplitude was spinal in origin [7]. In an attempt to elucidate the spinal mechanism that is responsible for this inhibition, two segmental inhibitory mechanisms were investigated: disynaptic reciprocal inhibition and Ia presynaptic inhibition. Results showed that Ia presynaptic inhibition increased in 79% of our subjects but only modestly. No change was seen in DSRI. It was concluded that IaPI contributed to the H-reflex inhibition during CP but could not account for the total observed reflex inhibition. Additional mechanism(s) of control must also be involved [1]. The possible mechanisms that may participate are discussed.

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Abbreviations

CP	– Circumferential Pressure
CPN	– Common Peroneal Nerve
DSRI	– Disynaptic Reciprocal Inhibition
FN	– Femoral Nerve
FNS	– Femoral Nerve Stimulation
EMG	– Electromyography
$H_{baseline}$	– Unconditioned H-reflex (25% of M_{max})
H_{max}	– Maximal H-Reflex
$H_{conditioned}$	– Conditioned H-Reflex
$H_{unconditioned}$	– Unconditioned H-reflex
H-Reflex	– Hoffmann Reflex
IaPI	– Ia Presynaptic Inhibition
M_{max}	– Maximal Motor response
MNRE	– Motoneuron Reflex Excitability
PAD	– Primary Afferent Depolarization
SCI	– Spinal Cord Injury
SD	– Standard Deviation
TA	– Tibialis Anterior muscle

References

[1] Agostinucci J., The effects of circumferential pressure on the soleus muscle F-wave in healthy subjects, J. Electromyiogr. Kinesiol., 2012, 22, 223-227

[2] Agostinucci J., Inhibitory effects of circumferential pressure on flexor carpi radialis H-reflex in adults with neurological deficits, Percept. Mot. Skills, 2010, 110, 89-103

[3] Agostinucci J., Effect of air-splint pressure on the soleus stretch reflex during a voluntary ramp plantar flexion. Neurophysiol., 2010, 12, 31-38

[4] Agostinucci J., Holmberg A., Mushen M., Plisko J., Gofman M., The effects of circumferential air-splint pressure on flexor carpi radialis H-reflex in subjects without neurological deficits, Percept. Mot. Skills, 2006, 103, 565-579

[5] Leukel C., Lundbye-Jensen J., Gruber M, Zuur A.T., Goohofer A., Taube W., Short-term pressure induced suppression of the short-latency response: a new methodology for investigating stretch reflexes, J. Appl. Physiol., 2009, 107, 1051-1058

[6] Robichaud J. A., Agostinucci J., VanderLinden D. W., Effect of air-splint application on soleus muscle motoneuron reflex excitabililty in nondisabled subjects and subjects with cerebrovascular accidents, Phys. Ther., 1992, 72, 176-185

[7] Robichaud J. A., Agostinucci J., Air-splint pressure effect on soleus muscle alpha motoneuron reflex excitability in subjects with spinal cord injury, Arch. Phys. Med. Rehabil., 1996, 77, 778-782

[8] Robichaud J. A., Brunt D., Effect of circumferential pressure on response parameters during ballistic ankle plantar flexion in healthy subjects, Percept. Mot. Skills, 1994, 78, 427-434

[9] Enriquez-Denton M., Nielsen J., Perreault M. C., Morita H., Petersen N., Hultborn H., Presynaptic control of transmission along the pathway mediating disynaptic reciprocal inhibition in the cat, J. Physiol., 2000, 526, 23-637

[10] Gottlieb G. L., Myklbust B. M., Penn R. D., Agarwal G. C., Reciprocal Excitation of Muscle Antagonists by the primary afferent pathway, Exp. Brain. Res., 1982, 46, 454-456

[11] Levin M., Chapman C. E., Inhibitory and facillitatory effects from the peroneal nerve onto the soleus H-reflex in normal and spinal man, Electroencephalo. Clin. Neurophysiol.,1987, 67, 468-478

[12] Milanov I. G., Flexor reflex for assessment of common interneuron activity in spasticity, Electromyogr. Clin. Neurophysiol., 1992, 32, 621-629

[13] Abbruzzese M., Reni L., Minatel C., Favale E., Presynaptic and postsynaptic mechanisms underlying H-reflex changes produced by a selective voluntary contraction, Muscle Nerve, 1998, 21, 439-453

[14] Calancie B., Broton J. G., Klose K. J., Traad M., Difini J., Ayyar D. R., Evidence that alterations in presynaptic inhibition contribute to segmental hypo- and hyperexcitabililty after spinal cord injury in man, Electroecphalogr. Clin. Neurophysiol., 1993, 89, 177-186

[15] Hultborn H., Meunier S., Morin M. C., Pierrot-Deseilligny E., Assessing changes in presynaptic inhibition of Ia fibres: a study in man and the cat, J. Physiol., 1987, 389, 757-772.

[16] Iles J. F., Roberts R. C., Inhibition of monosynaptic reflexes in human lower limb. J. Physiol., 1987, 108, 450-462

[17] Inghilleri M., Lorenzano C., Gilio F., Pedace F., Romeo S., Manfredi M., et al. Ia presynaptic inhibition after muscle twitch in the arm, Muscle Nerve, 2000, 23, 748-752

[18] Kohn A. F., Floeter M. K., Hallett M., Presynaptic inhibition compared with homosynaptic depression as an explanation for soleus H-reflex depression in humans, Exp. Brain Res., 1987, 116, 375-380

[19] Morin C., Pierrot-Deseilligny E., Hultborn H., Evidence for presynaptic inhibition of muscle spindle Ia afferents in man, Neurosci. Lett., 1984, 44, 137-142

[20] Nielsen J., Petersen N., Is presynaptic inhibition distributed to corticospinal fibers in man?, J. Physiol., 1994, 477, 147-158

[21] Guissard N., Duchateau J., Hainaut K., Mechanisms of decreased motoneuron excitation during passive muscle stretching, Exp. Brain Res., 2001, 137, 163-169

[22] Aimonetti J. M., Vedel J.P., Schmied A., Pagni S., Inhibition versus facilitation of the reflex responsiveness of identified wrist extensor motor units by antagonist flexor afferent inputs in humans, Exp. Brain Res., 2000, 133, 391-401

[23] Nakashim K., Rothwell J. C., Day B. L., Thompson P.D., Marsden C. D., Cutaneous Effects of Presynaptic Inhibition of flexor Ia afferents in the human forearm, J. Physiol., 1990, 426, 369-380

[24] Rudomin P., Schmidt R. F., A presynaptic inhibition in the vertebrate spinal cord revisited, Exp. Brain Res., 1999, 129, 1-37

[25] Eke-Okoro S. T., The H-reflex studied in the presence of alcohol, aspirin, caffeine, force, and fatigue, Electromyogr. Clin. Neurophysiol., 1982, 22, 579-589

[26] Knikou M., The H-reflex as a probe: Pathways and pitfalls, J. Neurosci. Methods, 2008, 171,1-12

[27] Palmieri R. M., Ingersoll C. D., Hoffman M. A., The Hoffman reflex: methodologic considerations and applications for use in sports medicine and athletic training research, J. Athl. Train., 2004, 39, 268-277

[28] Crone C., Hultborn H., Mazieres L., Morin C., Nielsen J., Pierrot-Deseilligny E., Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat, Exp. Brain Res., 1990, 81: 35-45

[29] Lundbye-Jensen J., Nielsen J. B., Immobilization induces changes in presynaptic control of group Ia afferents in healthy humans, J. Physiol., 2008, 586, 4121-4135

[30] Mizuno Y., Tanaka R., Yanagisawa N., Reciprocal group I inhibition on triceps surae motoneurons in man, J. Neurophysiol., 1971, 34, 1010-1017

[31] Crone C., Hultborn H., Jespersen B., Nielsen J., Reciprocal Ia inhibition between ankle flexors and extensors in man, J. Physiol., 1987, 389, 163-185

[32] Kernell D, Hultborn H. Synaptic effects on recruitment gain: a mechanism of importance for the input-output relations of motoneurone pools?, Brain Res., 1990, 507, 76-179

[33] Nielsen J., Kagamihara Y., Differential projection of the sural nerve to early and late recruited human tibialis anterior motor units: change of recruitment gain, Acta. Physiol. Scand., 1993, 147, 385-401

[34] Nielsen J., Kagamihara Y., The regulation of presynaptic inhibition during co-contraction of antagonistic muscles in man, J. Physiol., 1993, 464, 575-593

[35] Pierrot-Deseilligny E., Assessing changes in presynaptic inhibition of Ia afferents during movement in humans, J. Neurosci. Methods, 1997, 74, 189-199

[36] Pinniger G. J., Nordlund M., Steele J. R., Cresswell A. G., H-reflex modulation during passive lengthening and shortening of the human triceps surae, J. Physiol., 2001, 534, 913-923

[37] Romano C., Schieppati M., Reflex excitability of human soleus motoneurons during voluntary shortening or lengthening contractions, J. Physiol., 1987, 390, 271-284

[38] Carpenter D. O., Henneman E., A relation between the threshold of stretch receptors in skeletal muscle and the diameter of their axons, J. Neurophysiol., 1966, 29, 353-368

[39] Somjen G., Sensory coding in the mammalian nervous system, Plenum Publishing Corp., New York, 1975

[40] Lundberg A., Malmgren K., Schomburg E. D., Reflex pathways from group II muscle afferents 2: functional characteristics of reflex pathways to alpha motoneurons, Exp. Brain Res., 1987, 65, 282-293

[41] Ushiba J., Masakado Y., Komune Y., Muraoka Y., Chino N., Tomita Y., Changes of reflex size in upper limbs using wrist splint in hemiplegic patients, Electromyogr. Clin. Neurophysiol., 2004, 44, 175-182

[42] Rudomin P., In search of lost presynaptic inhibition, Exp. Brain Res., 2009, 196, 139-151

[43] Iles J.F., Reciprocal inhibition during agonist and antagonist contraction, Exp. Brain Res., 1986, 62, 212-214

[44] Chambers K. H., Monika A., Duering V., Iggo, A., The structure and function of the slowly adapting type II mechanoreceptor in hairy skin, Q. J. Exp. Physiol. Cogn. Med. Sci., 1972, 57, 417-445

[45] Boulais N., Misery L., The epidermis: a sensory tissue, Eur. J. Dermatol., 2008, 18, 119-127

[46] Jacobs J. V., Horak F. B., Review: Cortical control of postural responses, J. Neural Transm., 2007, 114, 1339-1348

[47] Brouwer B, Ashby P., Corticospinal projections to lower limb motoneurons in man, Exp. Brain Res., 1992, 89, 649-654

[48] Wall, P. D., The laminar organization of dorsal horn and effects of descending impulses, J. Physiol., 1967, 403-423

[49] Roy F. D, Gorassini M. A., Peripheral sensory activation of cortical circuits in the leg motor cortex of man, J. Physiol., 2008, 4091-4105

[50] Grey M. J., Ladouceur M., Andersen J. B., Nielsen J. B., Sinkjaer T., Group II muscle afferents probably contribute to the medium latency soleus stretch reflex during walking in humans, J. Physiol., 2001, 25-933

[51] Jami L., Golgi tendon organs in mammalian skeletal muscle: functional properties and central actions, Physiol. Rev., 1992, 72, 623-666

[52] Gregory J. E., Proske U., The responses of Golgi tendon organs to stimulation of different combinations of motor units, J. Physiol., London, 1979, 295, 251-262.

[53] Adreani C. M., Hill J. M., Kaufman M. P., Responses of group III and IV muscle afferents to dynamic exercise, J. Appl. Physiol., 1997, 82, 1811-1117

[54] Kaufman M. P., Hayes S. G., The exercise pressor reflex, Clin. Auton. Res., 2000, 12, 429-439

[55] Mitchell J. H., Kaufman M. P., Iwamoto G. A., The exercise pressor reflex: its cardiovascular effects, afferent mechanisms and central pathways, Annu. Rev. Physiol., 1983, 45, 229-242

[56] Garland S. J., Kaufman M. P., Role of muscle afferents in the inhibition of motoneurons during fatigue, In: Fatigue: neural and muscular mechanism, Gandevia S.C., Enoka R.M., McComas A.J., Stuart D.G., Thomas C.K. (Eds.), Plenum Press, New York, 1995

[57] Martin P. G., Smith J. L., Butler J. E., Gandevia S. C., Taylor J. L., Fatigue-sensitive afferents inhibit extensor but not flexor motoneurons in humans, J. Neurosci., 2006, 26, 4796-4802

[58] Martin P. G., Weerakkody N., Gandevia S. C., Talor J. L., Group III and IV muscle afferents differentially affect the motor cortex and motoneurons in human, J. Physiol., 2008, 586, 1277-1289

[59] Kniffki D. D., Schomburg E. D., Steffens H., Synaptic effects from chemically activated fine muscle afferents upon α-motoneurones in decerebrate and spinal cats, Brain Res., 1981, 206, 361-370

[60] Abrahams V. C., Lynn B., Richmond F. J., Organization and sensory properties of small myelinated fibres in the dorsal cervical rami of the cat, J. Physiol., 1984, 347, 177-187