Short-term pressure induced suppression of the short latency response – a new methodology for investigating stretch reflexes

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Abstract

During experiments involving ischemic nerve block we noticed that the short latency response (SLR) of evoked stretches in m. soleus decreased immediately following inflation of a pneumatic cuff surrounding the lower leg. The present study aimed to investigate this short-term effect of pressure application in more detail.

58 healthy subjects were divided into 7 protocols. Unilateral stretches were applied to the calf muscles to elicit an SLR, bilateral stretches to evoke a subsequent medium latency response (MLR). Furthermore, H-reflexes and sensory nerve action potentials (SNAPs) were recorded. Additionally, stretches were applied with different velocities and amplitudes. Finally, the SLR was investigated during hopping and in 2 protocols that modified the ability of the muscle-tendon complex distal to the cuff to stretch. All measurements were performed with deflated and inflated cuff.

Results of the protocols were: a) inflation of the cuff reduced the SLR but not the MLR, b) the H-reflex, the M-wave, and, c) SNAPs of n. tibialis remained unchanged with deflated and inflated cuff, d) the SLR was dependent on the stretch velocity with deflated and also inflated cuff, e-f) the reduction of the SLR by the cuff was dependent on the elastic properties of the muscle-tendon complex distal to the cuff, and g) the cuff reduced the SLR during hopping.

The present results suggest that the cuff did not affect the reflex arc per se. It is proposed that inflation restricted stretch of the muscles underlying the cuff so that most of the length change occurred in the muscle-tendon complex distal to the cuff. As a consequence, the muscle spindles lying within the muscle may be less excited resulting in a reduced SLR. Due to its applicability in functional tasks, the introduced method can be a useful tool to study afferent feedback in motor control.

Introduction

Movement is brought about by efferent and afferent neural activity driving the spinal α-motoneurons. The role of afferent feedback in motor control has been studied by using an ischemic nerve blockage (e.g. 21; 27). With this technique, a pneumatic cuff is encompassed and inflated around a limb to stop blood circulation distal to the cuff. Thicker nerve fibres are more susceptible to ischemia than smaller ones. Therefore, it has been argued that the conductivity of the large diameter Ia afferent fibres is diminished before that of efferent motoaxons (14; 19). Previously, ischemia has been used to assess the contribution of (Ia) afferent feedback in relation to descending motor commands (5), in motor responses to painful stimuli (27), and in postural tasks as well as walking (9; 10; 14). However, in functional movements like walking, ischemia is associated with several methodological limitations: first, there is the limited time for investigation. It exists only a short time window of a few minutes where solely the (Ia) afferents rather than the motoaxons are thought to be blocked (19). Second, the delayed effect of the Ia afferent ischemic nerve block means that subjects have to wait for approximately 15 - 20 minutes while held ischemic before the measurement can start. Given the short time window and the delayed effect, repeated measures are very time consuming. Third, ischemia causes pain in the affected limb. It is therefore both uncomfortable and demanding for subjects to perform dynamic tasks naturally. Fourth, ischemia increases the risk of vascular diseases (e.g. thromboses) caused by the prolonged blockage of blood flow.

The present study introduces a new tool to investigate afferent feedback in functional tasks which inherits none of the abovementioned limitations. This tool was discovered in a recent study involving ischemic nerve blockage. Here we noticed that the earliest peak of the soleus (SOL) stretch reflex in the EMG, termed short latency response (SLR), was markedly reduced following inflation of a pneumatic cuff surrounding the lower leg. The SLR, which is considered to be mediated by fast conducting Ia afferent fibres (2; 13; 20), was reduced immediately after inflation. The aim of the present study was twofold: first, to detect the underlying mechanism of the reduction of the SLR by short-term pressure application and second, to test the applicability of this method to investigate afferent feedback in functional tasks. To clarify which mechanism was responsible for the reduction of the SLR we elucidated whether the inflated cuff affects solely the SLR or also other reflex peaks with longer latencies. Therefore, a subsequent reflex peak to the SLR, termed medium latency response (MLR), was elicited while inflating the cuff. The MLR is attributed to oligosynaptic excitation of spinal motoneurons via group II afferents (3; 8; 14; 22-24) and possibly by group

Ib afferents (7). In addition, the afferent and efferent paths of the (Ia afferent) reflex arc were investigated by using H-reflexes and sensory nerve action potentials (SNAPs). This should clarify whether the reduction of the SLR was caused by manipulation of the neurophysiological components of the reflex arc. Furthermore, it was assessed whether the cuff changed the mechanical characteristics of the initiated stretch.

To investigate the second issue of the present study – the applicability of the introduced method in functional tasks – short-term pressure to the calf muscles was applied by inflating the cuff during hopping. Hopping has previously been shown to comprise a strong SLR (17) and we therefore hypothesized that inflation of the cuff would decrease the SLR if the method was applicable in functional tasks.

Materials and Methods

A total of 58 subjects without orthopaedic and neurological disorders were divided into 7 groups with 7 different protocols. Group 1, containing 8 subjects (aged 23 ± 3 years) participated in protocol 1 *(effect on the SLR and MLR)*. Group 2 (10 subjects, aged 27 ± 2 years) participated in protocol 2 *(H-reflex recruitment)*. Group 3 (6 subjects, aged 24 ± 2 years) participated in protocol 3 *(sensory nerve action potential – SNAP)*. Group 4 (8 subjects, aged 25 ± 3 years) participated in protocol 4 *(different stretch velocities and amplitudes)*. Group 5 (9 subjects, aged 24 ± 4 years) participated in protocol 5 *(high tension)*. Group 6 (5 subjects, aged 24 ± 2 years) participated in protocol 6 (*taping*) and group 7 (12 subjects, aged 27 ± 3 years) participated in protocol 7 *(hopping)* (see also Tab. 1). The study was approved by the ethics committee of the Albert-Ludwigs-University in Freiburg and experimental procedures were performed in accordance with the Declaration of Helsinki. All subjects gave written informed consent prior to participation.

EMG recordings and kinematics

EMG recordings were obtained from the SOL and tibialis anterior (TA) muscles of the right leg. After preparation, bipolar surface electrodes (Blue sensor P, Ambu®, Bad Nauheim, Germany) were attached to the skin (2 cm inter-electrode distance). The reference electrode was placed on the tibial plateau. EMG signals were amplified (×1000), bandpass-filtered (10 – 1000 Hz) and sampled at 4 kHz. The EMGs were stored for offline analysis with custom built software (LabView® based, National Instruments®, Austin, Texas).

In protocol 7 *(hopping)*, ankle angles (dorsiflexion and plantarflexion) and knee angles (knee flexion and extension) were recorded (sampling frequency 4 kHz) by custom built goniometers. The goniometer axis of rotation was aligned with rotational axes of the ankle and knee joint, respectively.

Cuff

A conventional blood pressure cuff (Bosch & Sohn®, Jungingen, Germany) was used to compress the right calf muscles. The cuff was placed just above the Achilles tendon. SOL and TA EMG electrodes were placed distal to the cuff (see also Fig. 1). In trials with inflated cuff, pressure was always between 220 – 240 mmHg. In all protocols, the measurements started immediately (5 seconds) after the cuff was inflated.

H-reflexes

H-reflexes were elicited with an electrical stimulator (constant current stimulator AS100, Alea Solutions®, Zürich, Switzerland) in the right SOL by stimulating the posterior tibial nerve. Stimuli consisted of square-wave pulses of 1 ms duration. The anode, a rubber pad of 10×5 cm, was fixed on the anterior aspect of the knee just underneath the patella. During upright stance, the cathode (2 cm in diameter) was placed in the popliteal fossa, moved stepwise until the optimum position for eliciting an H-reflex was found and then fixed with tape (stimulation intensities in this condition ranged between 5 and 15 mA). Care was taken that the stimulation did not activate TA.

Experimental protocols

Protocol 1 – effect on the SLR and MLR

This protocol aimed to elucidate the effect of the inflated cuff on the SLR and MLR, respectively. All measurements were conducted in a custom made ankle ergometer. Subjects stood upright with their feet strapped to the left and right platform of the ergometer. They had the knees extended and the ankles at 90°. Arms had to be held akimbo. The rotation axis of the upper ankle joint coincided with the rotation axis of the torque platform. Torque of the rotation platform was assessed using a torque transducer (Burster®, Gernsbach, Germany) and displacement was measured with a goniometer (Megatron®, München, Germany). Both devices were mounted between the servomotor of the ankle ergometer and the platform. Unilateral stretches were applied to the right leg plantarflexor muscles by rotating the platform with a maximal velocity of 250°/sec (time from the onset of the stretch to the maximal velocity was set to 10 ms) and a displacement of 10° dorsiflexion (toe up rotation). The initiation of the dorsiflexion movement of the platform was defined as the onset of the stretch (t = 0). Fifteen stretches were evoked with deflated cuff, followed by 15 stretches with inflated cuff. Immediately (5 seconds) after the cuff was deflated again, 15 stretches were elicited to monitor longer lasting effects on the SLR. The inter-stimulus interval between the stretches was set to 4 s.

Unilateral stretches evoked solely an SLR. In order to elicit an additional MLR, the right and left platform of the ergometer were synchronously rotated while subjects were standing with their eyes closed. Fifteen stretches were applied with the cuff deflated and 15 with the cuff inflated in a randomized order.

Protocol 2 – H-reflex recruitment

This protocol was conducted to investigate whether the Ia afferent pathway proximal to the cuff, the motor axonal properties and/or the recording conditions were affected by inflation of the cuff. Subjects received unilateral stretches to their right leg plantar flexor muscles as in protocol 1. The stretches were applied to ensure the depressive effect of the cuff on the SLR. Thereafter, H/M recruitment curves were recorded with deflated and inflated cuff, respectively, during quiet upright stance. The inter-stimulus interval between the electrical stimuli was 4 s. Each H/M recruitment curve contained approximately 20 – 30 stimuli ranging from the intra-individual stimulation intensity to evoke the H-reflex threshold (500 µV peak to peak amplitude) to the intensity to evoke the maximum M-wave ($M_{max)}$. Stimulation intensity was progressively increased until H_{max} was obtained and further increased until M_{max} was recorded. To exclude that changes in the SOL H-reflex originated from alterations in the excitability of the homonymous (postsynaptic modulation) and antagonistic (reciprocal inhibition) motoneuron pool, the background EMG activity of the rectified SOL and TA were calculated as mean values in a 100 ms time window prior to the electrical stimulation for all sampled H-reflexes/M-waves. Intra-individual means were calculated for all sampled sweeps.

Protocol 3 – sensory nerve action potential – SNAP

This protocol was conducted to test whether the afferent fibres of n. tibialis were compressed by the overlying cuff which could have resulted in an altered conductivity of the nerve. Therefore, the size and the latency of sensory nerve action potentials (SNAPs) of n. tibialis, stimulated distal to malleolus medialis and recorded in the popliteal fossa (1), were compared between deflated and inflated cuff. Before starting with the SNAP recordings, the inhibitory effect on the SLR by the inflation of the cuff was tested in the ankle ergometer while applying unilateral stretches as in protocol 1. When the cuff was able to successfully reduce the SLR, its placement was fixed with tape. The SNAP measurements were performed at rest in a supine position. At first, the best placement for recording n. tibialis SNAPs was determined by stimulating the nerve in the popliteal fossa using an electrical stimulator (constant current stimulator DS7, Digitimer®, Welwyn Garden City / Hertfordshire, UK) with the cathode proximal (pulse width was 0.2 ms). The best placement was defined as the spot at which motor responses in SOL using the lowest stimulation threshold could be obtained. At this spot, a monopolar recording electrode (Blue sensor P, Ambu®, Bad Nauheim, Germany) was fixed (signals were amplified (×10000), bandpass-filtered (10 – 1000 Hz) and sampled at 4

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kHz). A reference electrode was placed on the tibial plateau. Somatosensory stimulation of n. tibialis afferents distal to malleolus medials was obtained by using the same stimulator with the same electrode configuration (pulse width 0.2 ms, cathode proximal) as for stimulation in the popliteal fossa. The stimulation intensity was adjusted to evoke slight motor responses in the toe flexor muscles. During the SNAP recordings, subjects were asked to relax and close their eyes. In conditions with deflated and inflated cuff, 170 SNAPs were recorded and averaged using a stimulation frequency of 2 Hz.

Protocol 4 – different stretch velocities and amplitudes

Protocol 4 elucidated whether the SLR with inflated cuff was dependent on the velocity of the stretch. Subjects received unilateral stretches to their leg plantarflexor muscles as in protocol 1. The stretches were evoked with 3 different amplitudes (5°, 10°, 14°) and 3 different maximal velocities (50°/s, 150°/s, 250°/s). The protocol was executed with deflated and inflated cuff. The time from the onset of the stretch to the maximal velocity was set to 10 ms. The chronological order of the 9 conditions was randomized. In total, 15 stretches were recorded for each condition.

Protocol 5 – high tension

Protocol 5 elucidated the effect of the inflated cuff when the tension of the muscle-tendon complex was high. Subjects again received unilateral stretches to their right leg plantarflexor muscles as in protocol 1. Thereafter, during upright stance, subjects were asked to lean forward close to the feeling of falling over. This caused a high pre-tension of the leg plantarflexor muscles. In this posture, subjects received 15 unilateral stretches with deflated and inflated cuff, respectively.

Protocol 6 – taping

In this protocol, the ability of the muscle-tendon unit distal to the cuff to stretch was artificially modulated. This protocol is – in its main aim – analogue to protocol 5, in which the elastic properties were changed by a higher tension of the muscle-tendon unit (leaning forward). In protocol 6, the muscle-tendon complex distal to the cuff was taped longitudinally down the back of the lower leg (Leukotape®, BSN medical GmbH, Hamburg, Germany). The rigidity of the tape restricted the tendomuscular system distal to the cuff from moving in response to the stretch. Taping was performed in a supine position while subjects were asked to fixate their ankle joint at 95° plantarflexion. Distal, the tape was fixed at the heel and

proximal directly at the insertion of the Achilles tendon. After taping, the cuff was fixed so that its lower border overlaid the proximal end of the tape. With the tape, subjects received 15 unilateral stretches with deflated and inflated cuff in the ankle ergometer as in protocol 1. Then, the tape was cut in the middle (but still covered the skin) to regain the natural ability of the muscle-tendon complex distal to the cuff to stretch. Again, 15 stretches were applied with deflated and inflated cuff.

Protocol 7 – hopping

In this protocol the cuff was applied during dynamic hopping movements. Subjects performed low height hopping on a three dimensional force platform (Kistler®, Winterthur, Switzerland) with an individually chosen hopping frequency for 2 minutes (warm up). The subjects were instructed to hop with "stiff" knees and perform the hops primarily by ankle joint movement. Arms had to be held akimbo. After the warm up, subjects were asked to hop submaximally at approximately 60 – 80 % of their maximal individual hopping height with an individually preferred frequency for the rest of the experiment. 20 hops were performed with deflated cuff and 20 hops with the cuff inflated. The order of these measurements (hopping with deflated and inflated cuff) was randomized, e.g. some subjects started hopping with the cuff inflated and others with deflated cuff. After this set, the order was reversed, so that a total number of 40 hops was recorded for each condition (cuff deflated and inflated).

To investigate whether the Ia afferent pathway proximal to the cuff, the motor axonal properties and/or the recording conditions were affected by inflation of the cuff, H/M recruitment curves were recorded during hopping with deflated and inflated cuff in 10 out of the 12 tested subjects. Like in a previous study (18), H-reflexes were elicited to occur at the peak of the SLR during hopping. The peak of the SLR during hopping (first peak in SOL after 35 ms with respect to ground contact) was determined from the rectified and averaged SOL EMG of the 40 jumps of the previous hopping condition in which the cuff was deflated. A light barrier (Optojump, Microgate®, Bolzano, Italy), mounted 5 cm above the force plate, served as a trigger to elicit the H-reflex (for details see (18)). Again, the chronological order of the conditions (hopping with deflated and inflated cuff) was randomized. The procedure of the H/M recordings was the same as in protocol 2 *(H-reflex recruitment)*. In order to avoid fatigue, the inter-stimulus interval between the electrical stimuli during hopping was 1 sec (e.g., given an average time of the hopping cycle of 300 ms the subject was stimulated at every third hop).

Data analysis and statistics

H-reflexes, M-waves and SNAPs were expressed as peak to peak amplitudes. H_{max} and M_{max} were calculated from peak to peak amplitudes of the maximum H-reflexes and the maximum M-waves of the unrectified SOL EMG. M_{max} was determined from the largest single response. H_{max} was determined from the largest single peak to peak response and additionally calculated as the mean of the three largest single responses. The latter accounted for possible outliers of H_{max}.

The peak of the SLR (peak of the first maximal deflection in the EMG caused by the stretch) was detected from the rectified SOL EMG (see also Figs. 1 and 4). The peak of the MLR was defined as the second maximal deflection in the EMG occurring at least 10 ms later than the peak of the SLR. A 20 ms time window was set around the peak of the SLR and the mean was calculated for each trial. After analyzing each trial the mean was calculated for all trials. The MLR in SOL was calculated as a mean of the rectified EMG in a 20 ms time window which followed the time window of the SLR. For instance, when a subject showed a peak of the SLR at 40 ms the SLR was calculated from 30 to 50 ms. The MLR in this example was calculated from 50 to 70 ms.

In protocol 4 *(different stretch velocities and amplitudes)*, a total of 9 conditions with different maximal stretch velocities and amplitudes were tested. The size of the SLR of these conditions was intra-individually normalized to the basic stretching condition of the present study, which is: 10° stretch amplitude and 250°/s maximal stretch velocity.

During hopping, the ankle and knee angles at the SLR were calculated as root mean square (RMS) values in a 20 ms time window around the peak of the SLR. Thereby, 180° knee angle refers to knee extension. An angle of 90° between the fifth metatarsal and the fibula is defined as 90° ankle angle.

To account for changes in the motor axonal properties and/or changes in the recording conditions between deflated versus inflated cuff, the SLR during stance and the H_{max} during stance and hopping were normalized to M_{max} (SLR / M_{max} × 100 and H_{max} / M_{max}, respectively). This normalization procedure could only be obtained in protocol 2 *(H-reflex recruitment)* and 7 *(hopping)*, in which electrical nerve stimulation was applied and thereby M_{max} was determined. All values are expressed as group mean ± standard deviation (S.D.) if not indicated differently.

The SLR obtained with deflated and inflated cuff in protocol 4 *(different stretch velocities and amplitudes)* was analysed by means of repeated-measures ANOVA with the within-subject

factors amplitude (5°, 10°, 14°) and maximal velocity (50°/s, 150°/s, 250°/s). Paired Student's T-tests were used for all other statistical analyses.

Differences were regarded significant at $p < 0.05$ for all tests. SPSS software 15.0 (SPSS®, Chicago, Illinois) was used for the statistical analyses.

Results

Protocol 1 – effect on the SLR and MLR

During unilateral stretches, the SLR was significantly higher with deflated (0.34 ± 0.17 mV) than with inflated cuff (0.17 ± 0.12 mV, $p < 0.01$) but recovered immediately after the cuff was deflated again (0.34 ± 0.17, $p = 0.95$). During bilateral stretches while the subjects closed their eyes, the SLR was again higher with deflated (0.30 ± 0.13 mV) than with inflated cuff (0.24 ± 0.13 mV, $p < 0.001$). However, the MLR, which was observed in all subjects, was not different between deflated and inflated cuff (0.18 ± 0.07 versus 0.17 ± 0.07 mV, $p = 0.53$, Fig. 2). The peak of the SLR showed a latency of 42 ± 4 ms and the peak of the MLR of 61 ± 3 ms.

Protocol 2 – H-reflex recruitment

Normalized to M_{max}, the SLR with unilateral stretches was significantly different between deflated ($6.0 \pm 4.9\%$) and inflated cuff ($4.1 \pm 4.6\%$, $p < 0.05$) but recovered immediately after the cuff was deflated again ($6.3 \pm 4.5\%$, $p = 0.71$). The peak of the SLR showed a latency of 43 ± 3 ms.

With respect to the normalized H_{max}, there was no difference between deflated and inflated cuff, neither when the largest single response was compared (0.42 ± 0.21 versus 0.43 ± 0.2, $p = 0.71$) nor the mean calculated out of the three largest single responses (0.39 ± 0.21 versus 0.40 ± 0.19, $p = 0.93$). The H-reflex latency (peak) during stance was 39 ± 3 ms. M_{max} amplitude was 9.1 ± 2.6 mV with deflated cuff. This was not statistically different from M_{max} with inflated cuff (8.4 ± 2.4 mV, $p = 0.10$, Fig. 3). The background EMG activity of SOL and TA were not different between deflated (SOL: 38 ± 2 µV; TA: 18 ± 5 µV) and inflated cuff (SOL: 36 ± 3 µV, $p = 0.37$; TA: 17 ± 5 µV, $p = 0.18$), indicating that the excitability of the homonymous and antagonistic motoneuron pool were unaltered between the conditions.

Protocol 3 – sensory nerve action potential – SNAP

In order to investigate whether inflation of the cuff affected (compressed) the underlying afferent fibres of n. tibialis, the amplitude and the latency (peak) of SNAPs were compared between deflated and inflated cuff. There was no change in the SNAP amplitude between deflated (9.3 ± 0.2 µV) and inflated cuff (10.4 ± 0.2 µV, $p = 0.10$). The latency of the SNAPs was the same between deflated and inflated cuff (9 ± 1 ms versus 9 ± 1 ms, $p = 0.89$).

Protocol 4 – different stretch velocities and amplitudes

With deflated cuff, the maximal stretch velocity but not the stretch amplitude showed a significant effect on the size of the SLR (stretch velocity: $F_{(2, 14)} = 34.06$; $P < 0.001$; stretch amplitude: $F_{(2, 14)} = 2.03$; $P = 0.17$, Fig. 4). This indicates that with deflated cuff the size of the SLR was dependent on the stretch velocity but not on the amplitude of the stretch. This was also true for the SLR with inflated cuff: the stretch velocity but not the stretch amplitude had an significant effect on the size of the SLR (stretch velocity: $F_{(2, 14)} = 14.26$; $P < 0.01$; stretch amplitude: $F_{(2, 14)} = 0.12$; $P = 0.89$, Fig. 4). The peak of the SLR showed a latency of 40 ± 4 ms.

Protocol 5 – high tension

During quiet upright stance, subjects again showed a significantly higher SLR with deflated cuff (0.27 ± 0.16 mV) than with inflated cuff (0.16 ± 0.13 mV, $p < 0.01$). However, when subjects were instructed to lean forward in order to pretense their leg plantarflexors, the SLR was no longer decreased with inflated compared to deflated cuff (0.27 ± 0.11 mV versus 0.27 ± 0.13 mV, $p = 0.95$, Fig. 5). The SLR with deflated cuff showed no difference when posture was altered, e.g. subjects standing relaxed versus leaning forward ($p = 0.94$). The latency of the peak of the SLR in this protocol was 41 ± 3 ms.

Protocol 6 – taping

In the conditions without tape, subjects again showed a significantly higher SLR with deflated cuff (0.30 ± 0.08 mV) than with inflated cuff (0.16 ± 0.09 mV, $p < 0.001$). Based on the mean values, this represented a reduction of the SLR of 46%. In the conditions with tape, there was no longer a significant reduction of the SLR between deflated (0.28 ± 0.11 mV) and inflated cuff (0.23 ± 0.09 mV, $p = 0.10$). Based on the mean values, the reduction of the SLR in the taping condition was only about 18%. The peak of the SLR in protocol 6 showed a latency of 40 ± 2 ms.

Protocol 7 – hopping

During hopping, we observed a significantly reduced SLR with inflated cuff compared with deflated cuff ($p < 0.01$, Fig. 6). Thereby, inflation of the cuff decreased the SLR from 0.60 ± 0.28 mV (deflated cuff) to 0.49 ± 0.28 mV. The latency of the peak of the SLR was 51 ± 3 ms with respect to the instant of ground contact. H/M recruitment curves recorded at the SLR showed no changes in M_{max} between deflated (8.5 ± 2.2 mV) and inflated cuff (7.9 ± 1.9 mV,

p = 0.18). Normalized H_{max} was also not different between deflated and inflated cuff, neither when the largest single response was compared (0.56 ± 0.20 versus 0.60 ± 0.11, p = 0.58) nor when the mean was calculated out of the three largest single responses (0.53 ± 0.21 versus 0.57 ± 0.1, p = 0.60). Ankle and knee joint angles did not differ between deflated and inflated cuff at the SLR (ankle: $94 \pm 7\,°$ versus $95 \pm 6\,°$, p = 0.35; knee: $159 \pm 12\,°$ versus $158 \pm 12\,°$, p = 0.37).

Discussion

Mechanism of the cuff

The present study showed several interesting findings. First, it was demonstrated that solely the SLR but not the MLR was depressed by inflation of the cuff (protocol 1). The MLR is ascribed to group II (3; 8; 22-24) and possibly Ib afferents (7) whereas the SLR is attributed to the activation of fast conducting Ia afferent fibres (2; 4; 6; 11; 13; 16; 20). In the present study, it might have been possible that the inflation of the cuff changed the excitability of the Ia afferent pathway proximal to the cuff and thereby reduced the SLR. Therefore, we conducted protocol 2 in which H-reflexes were elicited. The H-reflex is mainly transmitted via Ia afferent fibres (26). The comparability of H_{max} between deflated and inflated cuff suggests that the Ia afferent pathway proximal to the cuff was unaffected by the mechanical pressure. Furthermore, comparable M_{max} amplitudes between deflated and inflated cuff indicate that the motor axonal properties were not significantly altered by the cuff and that the recording conditions did not change. Based on these observations, there remain 2 components of the reflex arc which could have been influenced by the inflated cuff: the afferent nerve fibres underneath the cuff might have been compressed and/or the muscle spindles receiving the stretch could have been affected. The results of protocol 3 make it unlikely that the transmission of afferents was altered by the cuff. In this protocol, SNAPs – which are propagated mainly via large (Ia) afferent fibres – were elicited distal to the cuff and recorded in the popliteal fossa (1). The amplitude and the latency of the SNAPs were the same between deflated and inflated cuff. Therefore, as the conductivity of afferent nerve fibres underlying the cuff was not altered by inflation of the cuff, the decrement of the SLR seems to be associated with the muscle spindles. The results of protocol 4 support this assumption. Here, different stretch velocities and amplitudes were applied. The modulation of the size of the SLR with deflated and also inflated cuff was related to the stretch velocity but not the amplitude of the stretch. Primary spindle endings of Ia afferent fibres, which are thought to be responsible for eliciting at least the first part of the SLR, were argued to signal the very initiation of a length change of a muscle (25). The strength of the initial length change of a muscle can be modulated by the stretch velocity. The dependency of the SLR on the stretch velocity but not the stretch amplitude was shown in previous studies (12) and confirmed in the present study. As the reduction of the SLR was dependent on the stretch velocity not only with deflated but also with inflated cuff, it is argued that the spindle endings responsible for the generation of the SLR were affected by the pneumatic pressure. The muscle spindles could have been affected in at least two ways: either via a kind of "unloading" by direct

mechanical pressure of the cuff and/or by the possibility that the cuff transformed the initiated stretch mechanically. In the following, we provide evidence for the second possibility. The hypothesis of the transformation of the stretch is based on the concept that the cuff clamps the muscle and thereby prevents the underlying tissue from natural movement to the stretch. Thereby, in the condition with inflated cuff, it is argued that the muscle-tendon unit distal to the cuff dampens the velocity of the stretch (transforms the velocity) based on its viscoelastic properties. With deflated cuff, when the muscle underneath the cuff is able to stretch naturally, the velocity of the initiated stretch is argued to be directly transferred to the muscle. Therefore, the ability of the muscle underneath the cuff and the ability of the muscle-tendon complex distal to the cuff to move naturally in response to the induced stretch determines the physical behaviour of the whole muscle-tendon unit to the stretch (see also Fig. 7). That implies that, when the tension of the muscle-tendon complex distal to the cuff and therefore its stiffness is too high, the dampening effect is lost and the velocity of the "original" stretch is directly transferred to the clamped muscle. This loss of dampening was seen in protocol 5 where subjects were instructed to lean forward and thereby cause a high pre-tension to the muscle-tendon units of the leg plantarflexor muscles. In this posture, the SLR was no longer reduced by cuff inflation. Complementary to protocol 5, the results of protocol 6 provide further evidence that the ability of the muscle-tendon unit distal to the cuff to stretch determines the effectiveness of the cuff to reduce the SLR. Taping in this protocol aimed to prevent the muscle-tendon complex distal to the cuff from moving. It is important to note that the muscle-tendon unit distal to the cuff was prevented from moving because the endpoints of the tape were aligned with the underlying muscle-tendon unit. The inflated cuff was argued to clamp the muscle. Thereby, the proximal end of the tape placed underneath the cuff was also connected to the muscle. The distal end of the tape was fixed at the heel, thus, at the same site as the connection of the muscle-tendon unit to the bone. Using these two fixations of the rigid tape, the mechanical behaviour of the muscle-tendon unit distal to the cuff to stretch was altered when the cuff was inflated. In line with this hypothesis, the SLR was no longer significantly different between deflated and inflated cuff in the condition with tape. However, as soon as the tape was cut, the SLR was significantly reduced with inflated compared to with deflated cuff. An unanswered question related to the abovementioned hypothesis of the effect of the cuff is why the SLR was affected and the MLR not. The size of the MLR, in contrast to the SLR, was shown to be influenced by the amplitude of the stretch (the muscle length) and less by the velocity of the stretch (12; 15). Therefore, it remains to be elucidated if/why

information about the muscle length was normally transmitted to the muscle but the velocity of the stretch transformed.

Application in functional tasks

The introduced method can be of functional significance. An immediate, completely reversible and easily repeatable depression of the SLR by short-term inflation of a cuff surrounding the muscle can be used to gain a deeper understanding of afferent feedback mechanisms in motor control. In the present study, the new technique was used to investigate afferent feedback during hopping (protocol 7). With ischemia, a selective blockage of the SLR during hopping is difficult because of the limited time window allowed for testing, the associated pain and therefore the restricted movement behaviour. During hopping, short-term inflation of the cuff was able to reduce the SLR. However, it has to be noted that although the group mean values were significantly different, there remained a few subjects showing no changes of the SLR between deflated and inflated cuff during hopping. Interestingly, in these subjects, it was possible to reduce the SLR by cuff inflation during upright stance in the ankle ergometer. There are at least 2 implications associated with this finding: one is that there is no SLR during hopping (in these subjects) and the other is that the cuff did not work in these subjects. Since other studies provided evidence that the SLR plays a role during hopping (17) it is argued that – based on the abovementioned explanatory model of the mechanism of the cuff – the failure to reduce the SLR in these subjects may be due to a high tension of the muscle-tendon unit during ground contact (when the stretch occurred). Therefore, caution may be advised when interpreting effects of the introduced methodology, e.g. in conditions in which excessive tendomuscular tension is produced.

Besides this methodological constraint, it is concluded that due to its immediate, reversible and easily repeatable effect, accompanied with a lower risk of vascular diseases (e.g. thromboses) than ischemia, and its applicability in functional tasks, the introduced method bears the potential to investigate stretch reflex contributions in highly dynamic tasks. In this context, it is important to note that changes in the movement execution (e.g. the mechanics) cannot be attributed to the depression of the SLR. This is not possible as the mechanics between deflated and inflated cuff differ fundamentally and not just because of the depressive effect on the SLR. Finally, it is important to note that the results of all protocols do not restrict the effect of the cuff to a selective blockage of Ia afferents. The cuff may affect other afferents as well.

Acknowledgements

Figure legends

Fig. 1 Schematic diagram of the placement of the EMG electrodes and the cuff covering the calf muscles.

Fig. 2 The graph shows stretch reflex responses of protocol 1 *(effect on the SLR and MLR)* in a single subject (mean of 15 trials). Bilateral stretches were applied while the subject closed the eyes. This procedure caused not only an SLR but also an MLR in the EMG. Inflation of the cuff decreased only the SLR, the MLR remained unaffected.

Fig. 3 H/M recruitment curves with deflated and inflated cuff during quiet stance in a single subject. Grey vertical lines indicate M_{max} and H_{max}, respectively.

Fig. 4 Shown are grand mean values of the SLR of protocol 4, in which stretches were applied with 3 different maximal velocities (250°/s: high; 150°/s: middle; 50°/s: low) and 3 different amplitudes (5°, 10° and 14°, respectively). Values were normalized to the basic stretching condition of the present study: 10° amplitude, 250°/s maximal stretch velocity. The solid horizontal bar indicates 100%. Black vertical bars show S.D. Asterisks mark significant effects.

Fig. 5 A: The SLR of the stretch reflex (mean of 15 trials) in protocol 5 *(high tension)* was markedly reduced during quiet upright stance when the cuff was inflated (shown is a single subject). However, in graph B, this reduction was no longer visible when the subject was asked to lean forward and thereby induced a high tension of the leg plantarflexor muscles.

Fig. 6 Hopping with deflated (dashed line) and inflated (solid line) cuff in a single subject. Shown are rectified and averaged (40 sweeps for each condition) EMGs of SOL and TA. The ground reaction force is also averaged but unrectified. The grey vertical line indicates the peak of the SLR in SOL. Zero refers to ground contact.

Fig. 7 Schematic diagram of the postulated mechanism of the cuff. A) Resting state of the standing subject with relaxed muscle-tendon complex. B) Application of a stretch with deflated cuff. The stretch causes a lengthening of the tendon and also the muscle. C) Application of a stretch with inflated cuff. Compared to B) the stretch causes augmented lengthening of the tendon but only minor changes in muscle length due to its

restriction. The muscle-tendon complex distal to the cuff acts as a damper which transforms the velocity of the stretch.

Tab. 1 Overview of the different experimental protocols conducted in the present study.

Tab. 1

Protocol Name	Content
1) Effect on the SLR and MLR	Bilateral stretches to evoke an SLR and an additional MLR, influence of the cuff on both reflex responses.
2) H-reflex recruitment	Recording of H/M recruitment curves, influence of the cuff on Hmax and Mmax.
3) Sensory nerve action potential - SNAP	Electrical stimulation of n. tibialis distal to the cuff and recording of SNAPs proximal to the cuff, effect of the cuff on the SNAPs.
4) Different stretch velocities and amplitudes	Unilateral stretches with 3 different velocities and 3 amplitudes, dependency of the SLR with inflated cuff on the stretch velocity and amplitude.
5) High tension	Unilateral stretches while subjects were leaning forward to pretense the leg extensor muscles, effect of high tendomuscular tension on the SLR.
6) Taping	Unilateral stretches while taping the muscle-tendon unit distal to the cuff to prevent it from moving to the stretch, effect of taping on the SLR.
7) Hopping	Hopping movements, effect of the cuff on the SLR during hopping.

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m. gastrocnemius lateralis

m. gastrocnemius medialis

cuff

m. soleus EMG electrodes

m. soleus

Achilles tendon

25

26

A

B

C

muscle

stretch
dorsalflexion

tendon

foot

cuff