Intraoperative Direct Mechanical Stimulation of the Anterior Cruciate Ligament Elicits Short- and Medium-Latency Hamstring Reflexes

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Submitted 21 April 2005; accepted in final form 19 August 2005

Friemert, B., M. Faist, C. Spengler, H. Gerngross, L. Claes, and M. Melnyk. Intraoperative direct mechanical stimulation of the anterior cruciate ligament elicits short- and medium-latency hamstring reflexes. J Neurophysiol 94: 3996–4001, 2005. First published August 31, 2005; doi:10.1152/jn.00410.2005. The anterior cruciate ligament (ACL) has not only a mechanical but also a sensorimotor function. Patients with injuries of the ACL frequently complain of knee instability despite good mechanical stabilization after surgical reconstruction. Compared with healthy subjects, their latencies of hamstring reflexes after anterior tibia translation are considerably increased. There is evidence for the existence of a reflex arc between the ACL and the hamstrings. The aim of this study was to determine if there is a direct reflex response after an isolated mechanical stimulation of the ACL in humans. In 10 patients who underwent arthroscopy, hamstring electromyographic (EMG) responses were assessed intraoperatively after applying an isolated load on the ACL. Latencies, amplitudes, and integrals of the EMG responses were analyzed. In four patients, the measurements were repeated after injection of local anesthetics into the ACL. In all subjects, responses with mean latencies of 42 ± 4.4 (SD) ms corresponding to a medium latency response (MLR) were found. In seven subjects, they were preceded by responses with a short-latency (SLR) of 24 ± 2.7 ms. The maximum amplitude was 8.6 ± 7 mV, the integral 0.064 ± 0.05 mV·s. The injection of local anesthetics reduced the amplitude by 34 ± 12% and the integral by 50 ± 20%. Direct mechanical stimulation of the ACL evokes considerably smaller SLRs and MLRs than anterior tibia translation during standing. It is argued that latency changes observed in patients with ACL ruptures may be rather due to changes in the sensorimotor integration of the afferent input from the knee joint than to the absence of the direct ACL reflex.

INTRODUCTION

The anterior cruciate ligament (ACL) has not only a mechanical but also a sensorimotor function. The ACL contains mechanoreceptors (Biedert et al. 1992; Raunest et al. 1998; Zimny et al. 1986), which belong to a complex proprioceptive system of the knee joint (Hogervorst and Brand 1998; Johannsson et al. 1991; Krauspe et al. 1995; Miyatsu et al. 1993; Solomonow and Krosggaard 2001). Recently it was shown that external perturbations applied to the knee evoked reflexes that significantly increased knee joint stiffness (Dhaher et al. 2005). Proprioception is impaired in the knees of patients with ACL rupture (Friden et al. 1996; Jerosch and Prymka 1996). Clinically, these patients may have symptoms of knee instability (giving-way phenomenon) despite good mechanical stabilization after the surgical reconstruction of the ACL. Because most of the afferents arising from the ACL and other ligamentous structures play a role in reflexes that are integrated at the spinal level, oligosynaptic or polysynaptic pathways are involved (Nardone and Schiappati 1998; Solomonow and Krosggaard 2001). An example of such an integrated reflex is the ACL-hamstring reflex, which is a sensorimotor unit combining the mechanical function of the ACL (to prevent anterior tibia translation) and the agonistic function of the hamstrings (to pull the tibia backward) (Liu and Maitland 2000; Miyatsu et al. 1993; More et al. 1993). The existence of this reflex arc was postulated for the first time by Gruber et al. (1986) and was later confirmed in experimental studies on cats (Solomonow et al. 1987) and humans (Krosggaard et al. 2002).

Beard et al. (1994) measured the latency of hamstring reflex contractions after mechanically induced tibia translation in healthy control subjects and patients. They found that the latency was nearly twice as long in ACL-deficient legs. They postulated that the longer latency was caused by the loss of the ACL-hamstring reflex. Similar results were reported by Bruhn (1999), although they measured an increase in latency (~6 ms) between injured and healthy subjects that was far less marked. However, not only the receptors in the ACL but also the other receptors in the ligaments, the capsule and the knee-stabilizing muscles, are excited by tibia translation. Friemert et al. (2005) were for the first time able to show that mechanically induced tibia translation in standing subjects evokes a complex multiphasic reflex response that consists of a short-latency response (SLR) and a medium-latency response (MLR). The SLR with a latency of ~20 ms was interpreted as the hamstring stretch reflex and, therefore, is mediated by Ia afferents (Faist et al. 1999; Friemert et al. 2005). The mechanisms underlying the generation of the MLR component of these complex signal responses have thus far not been studied in knee joints. Corna et al. (1995) were the first to provide evidence that at least part of the MLR is mediated by secondary muscle spindle (group II) afferents. Similar findings have been reported by Nardone and Schiappati (1998), Bove et al. (2003), and Grey et al. (2001), who investigated MLRs during locomotion. As far as we know, there are no studies looking at the influence of knee joint afferents on the SLR and the MLR.

In particular, it is unclear whether SLR and/or MLR components can be ascribed to the direct reflex pathways that link the ACL and the hamstrings. Therefore the first aim of this...
study was to determine if there is a direct reflex response after an isolated mechanical stimulation of the ACL. The second question is whether such a reflex response consists of different components corresponding to SLR and MLR.

**Methods**

**Patient population**

Our study included 13 men with no history of neurological disorders (mean age: 24.6 ± 5.5 yr, height: 179 ± 5 cm). All patients underwent arthroscopy for knee problems (7 × plica syndrome, 1 × meniscus, 2 × cartilage defects, and 3 × for purely diagnostic purposes due to anterior knee pain syndrome). No subject had a history of additional orthopedic disorders. In three cases, it was impossible to analyze the data due to a defective trigger in one case and massive artifacts in two cases. We were thus able to analyze the data obtained from 10 patients. The patients gave their written informed consent in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of the University of Ulm (No. 216/2002).

**Anesthesia**

All surgical procedures were performed under general anesthesia (total intravenous anesthesia, TIVA, with remifentanil 0.5–1.0 μg/kg and propofol 50–150 μg·kg⁻¹·min⁻¹, intubation). In the doses used here, it is unlikely that the excitability of the monosynaptic reflex arc or neuromuscular function was affected (for details, see Kerz et al. 2001). Muscle relaxants (precurarization with rocuronium bromide 5 mg, then suxamethonium chloride 1–1.5 mg/kg) were used only at the induction of anesthesia. The half-time of this relaxation was 10 min. The time between the induction of anesthesia and the beginning of measurements was 54.5 ± 9 min on average (positioning of the leg and the electrodes, sterile cleaning of the operating field, installation of the arthroscopy instruments, arthroscopic procedure, and preparation of the measurement system). No further relaxants were used during anesthesia. Because they were only used in the initial part of the test, it is unlikely that they influenced neuromuscular excitation at the time when the measurements were performed (for details, see Beers and Camporesi 2004; Chang et al. 1994; Shiraiishi et al. 1992; Suzuki et al. 1998). Last but not least neuromuscular function was assessed before the tests were started [2-Hz stimulation frequency, train-of-four ratio >0.8 (Gatke et al. 2002)]. Therefore medication effects on muscular responses are unlikely.

**Measurement system**

A commercially available hook probe (Wolf, Knittlingen, Germany) with a modified (rounded) tip was attached posteriorly and distally to the ACL (Fig. 1). In a few cases, little synovia between the ACL and the posterior cruciate ligament had to be removed, to allow the hook to be placed correctly. No patient had severe adhesions or hyperplasia of the synovial membrane, so that it was possible in all cases to place the hook in the appropriate position. A sterile thin wire rope was attached to the hook probe. A dynamic force of 300 N (see Preparatory work) was applied to the ACL. A load cell (0–2,000 N, Biovision, Wehrheim, Germany) was placed between the wire rope and the weight to control the force applied to the ACL. Furthermore, it was used as a trigger signal (onset of force application to the ACL) to determine the onset latency of the electromyographic (EMG) signals. Video recordings were made of all measurements.

**Measurements**

The patients were positioned for arthroscopic knee surgery on the operating table. A commercially available tourniquet (Ulrich Com-
Preparatory work

As part of the preparatory work for this study, two sheep knee joints with intact capsulo-ligamentous structures were used to determine the forces to be applied. A miniarthrotomy was performed to mimic the experimental setup described in the preceding text for the human experiment. To compare the applied force used in the present study to previous studies, it was shown that a weight of 1.5 kg and a height of 30 cm was required to generate a force of 300 N. Solomonow et al. (1987) suggested that a minimum force of 130 N was needed to elicit a hamstring reflex. The tensile strength of an intact cruciate ligament has been reported to be 1,725 ± 660 N in humans (Noyes and Grood 1976) and 1,118 ± 244 N in sheep (Durselen et al. 1996). The two types of cruciate ligaments thus have similar tensile strengths. Once the two measurement series had been completed, the cruciate ligaments of the sheep knee joints were exposed, and no damage to the ACLs could be seen. Thus the intraoperative measurements are far below the thresholds to cause damage to the ACL.

Data analysis

The EMGs of both the medial and the lateral hamstring muscles were used for analysis. Latencies were assessed as the time from the beginning of force application (trigger) to the first onset of the reflex response. The onset of the SLR was determined semi-automatically using the trigger signal from the load cell as the beginning of ACL stimulation. The first significant rise in EMG activity (defined as average amplitude plus ±5 SD of the preloading baseline activity) was identified as the SLR. Then the exact onset of this SLR was assessed by visual inspection on the computer screen using a cursor which was set at the beginning of the EMG deflection. Earliest responses with a latency of >30 ms (medial hamstrings: 3 cases; lateral hamstrings: 2 cases) were defined as pure MLRs. In case of superimposed signal configuration, we used our evaluation algorithm to differentiate MLR from SLR. Briefly, the duration of the hamstring SLR can be estimated quite accurately by multiplying the time between the onset of the SLR and the first SLR peak with the empirical factor of 3.28 as shown in detail in our previous paper (Friemert et al. 2005). The end of the MLR was defined 30 ms after the onset of the MLR. The maximum amplitude and the integral from the SLR and MLR were determined. In addition, the total duration of the reflex response was assessed. The end of the reflex response was defined as the point where the EMG returned to baseline for ≥10 ms. Due to artifacts that frequently occurred after the MLR response, the total reflex duration could not be assessed reliably in all cases. An example of such artifacts is illustrated in Fig. 2A in the single sweep of the lateral hamstrings. The nature of these artifacts is most probably mechanically due to the weight connected with the rope and the leg holder used. In the LA group, we also measured the integrals and maximum amplitudes over a time window of 100 ms after the trigger signal (with and without local anesthetic) to assess the effects of local anesthesia. Strong suppression after local anesthesia made a separate analysis of SLRs and MLRs impossible. For this reason, only overall EMG activity was quantified and compared. Mean values, SDs and the range of values were calculated using SPSS software.
Reflex responses

All patients showed reflex responses after mechanical stimulation of the ACL. In seven patients, the first onset was observed after 23.9 ± 2.7 ms (range: 18–26 ms) in the medial hamstrings and was classified as an SLR. Detailed individual results of all subjects are given in Table 1. All these seven patients showed a second reflex response (MLR) with an average latency of 40.9 ms (range: 35–46 ms) after the end of the first reflex response. A typical raw EMG response is shown in Fig. 2A, the averaged data of 50 responses are shown in B. In three patients, the first onset was detected after 44 ± 1.2 ms (range: 43–45 ms), which corresponds to an MLR. These latencies are similar to the MLR latencies of those patients with a preceding SLR. The mean MLR latency of all subjects was 41.5 ± 4.4 ms (range: 35–45). Similar results were obtained for the lateral hamstring muscles, although it was possible to analyze only 8 of 10 subjects for technical reasons (see Tables 1 and 2).

TABLE 1. Individual results & mean values

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Latency, ms</th>
<th>Integral, mV*s</th>
<th>Amplitude, mV</th>
<th>Duration SLR, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SLR</td>
<td>MLR SLR</td>
<td>MLR</td>
<td>SLR</td>
</tr>
<tr>
<td>Medial hamstring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23.9 ± 2.7</td>
<td>41.5 ± 4.4</td>
<td>0.064 ± 0.054</td>
<td>0.166 ± 0.222</td>
</tr>
<tr>
<td>Maximum</td>
<td>25.6</td>
<td>45.8</td>
<td>0.161</td>
<td>0.677</td>
</tr>
<tr>
<td>Minimum</td>
<td>18.0</td>
<td>35.0</td>
<td>0.001</td>
<td>0.014</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Lateral hamstring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>23.4 ± 3.3</td>
<td>41.9 ± 3.5</td>
<td>0.091 ± 0.158</td>
<td>0.092 ± 0.156</td>
</tr>
<tr>
<td>Maximum</td>
<td>26.6</td>
<td>46.0</td>
<td>0.373</td>
<td>0.442</td>
</tr>
<tr>
<td>Minimum</td>
<td>18.0</td>
<td>36.6</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>8</td>
</tr>
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</table>

Amplitude/duration/integral

The mean amplitude of the SLR was 8.6 ± 7.1 mV (range: 1.9–16.8 mV) and of the MLR 8.9 ± 11.2 mV (range: 1.2–36.4 mV). The mean duration of the SLR was 17.1 ± 3.7 ms (range: 10.8–21.1 ms). The MLR integral was calculated on the basis of a 30-ms interval. The integral of the SLR showed a value of 0.064 ± 0.05 mV*s (range: 0.011–0.16 mV*s), the integral of the MLR was 0.17 ± 0.22 mV*ms (range: 0.014–0.68 mV*s). The mean duration of the overall reflex response was 74.4 ± 15 ms (range: 55–112 ms). There were no significant differences between the mean results for the lateral hamstrings and those for the medial hamstrings (Table 1).

Local anesthesia

A 34 ± 12% (range: 30–54%) decrease in the EMG amplitude and a 50 ± 20% (range: 35–76%) decrease in the integral was observed in the LA group (Fig. 3).
individual sensory threshold. However, it remains unclear which receptors or neural pathways were stimulated by this technique, although the authors concluded that the muscular responses had been elicited by the stimulation of group II and III afferents. It should be kept in mind that group III fibers also transmit the sensation of pain. In addition in their study, five of eight patients received a spinal anesthetic. Although the measurements were performed 6–8 h after the induction of anesthesia, an effect of this procedure on latency cannot be entirely ruled out. In addition, most patients complained of postoperative pain in the knee joint, which may have additionally influenced the reflex responses with longer latencies as was shown by Andersen et al. (2000). The anesthesia used in the present study is unlikely to have relevantly affected neuromuscular transmission or central excitability at least as far as the SLR is concerned (see METHODS). Tsuda et al. (2003) examined cruciate ligament grafts in patients who had undergone surgery. After electrical stimulation, they were able to show latencies of 120 ms. This value approximately corresponds to the time window described by Dyhre-Poulsen and Krogsgaard (2000). These long latencies now raise the question of whether the short latencies measured in the present study are realistic.

In the cat, Solomonow et al. (1987) reported an SLR that was attributed to the high mechanical load according to Dyhre-Poulsen and Krogsgaard (2000). The fact that the mechanical stimulus evokes a considerably larger response indicates that this stimulus is more effective than the one applied electrically. In accordance with the recommendations of Solomonow et al., we used such a high mechanical load (300 N). As described by Freeman and Wyke (1967), the ACL contains Golgi corpuscles with afferents showing a conduction velocity of 75 m/s, which approximately corresponds to the conduction velocity of group Ia afferents. In a subject with a height of 185 cm, the distance from the knee joint to the lumbar segments L₃–L₄ and back to the mid-thigh is ~120 cm (Gomez-Barrena et al. 1996). Accordingly, the conduction time can be estimated at ~16 ms. Assuming that a synaptic connection accounts for a delay of 1 and 3 ms at the motor end-plate (Solomonow and Krogsgaard 2001), our reflex latencies appear to be realistic. The injection of lidocaine into the ACL led to a 50% decrease in the integral and a 34% decrease in the amplitudes. These findings support the view that the ACL is involved in these muscular responses. In a recently published study, Friemert et al. (2005) reported a latency of 21 ms for the SLR after mechanical anterior tibia translation. In accordance with the results obtained by Faist et al. (1999) for the tendon jerk reflex, this finding was interpreted as a reaction associated with the hamstring stretch reflex mediated by group Ia muscle spindle afferents.

Our intraoperative measurements showed a mean maximum amplitude of 8.6 mV and a mean integral of 0.064 mV*s. To compare the present data with effects observed after the stimulation of all knee structures, we analyzed the data obtained in the experiments for our previous study (Friemert et al. 2005) in the same way using an eighth-order Butterworth high-pass filter (35 Hz). We found a mean maximum amplitude of 234 mV and a mean integral of 7 mV*s for those experiments performed in a standing position. This implies that the responses measured during the standing position are 30-fold larger than those obtained intraoperatively during lying (or 100-fold larger if the integrals are considered). At the same time, the background EMG only shows a fivefold increase. It is unlikely that the different positions alone induce such a strong suppression of the SLR and MLR responses. Thus the results of the present study strongly suggest that only a minor part of the reflex response is mediated by the direct ACL-hamstring reflex pathway. In patients with ACL ruptures, the latencies of hamstring responses after anterior tibia translation were nearly twice as long as in normal subjects (Beard et al. 1994; Bonfim et al. 2003). According to the present results, it appears unlikely that these results are directly attributable to the impaired ACL-hamstring reflex. This view was already suggested by Hог-верст and Brand (1998). A possible explanation is changes in the sensory-motor integration, i.e., that the longer latencies are secondary to a change in afferent information, which may cause inhibitory or excitatory effects on different interneurons or changes in the activity of afferents. Johansson et al. (1991) suggested that the gamma motor neurons may play a crucial role in transmitting information from the cruciate ligament, which may alter the entire regulation of muscular activity and consequently could also affect the latency. Johansson et al. (1990) and Sojka et al. (1989) were able to show that afferents from the anterior/posterior cruciate ligament have a direct effect on the gamma motoneuron system. They recorded electrical potentials from primary muscle spindle afferents under various stretch load conditions. The study also included tests in which loads of 5–40 N
were applied to cruciate ligaments. Compared with the unloaded legs, the loaded legs showed a marked increase in the activity of the muscle spindle afferents.

Lundberg et al. (1978) found that alpha motoneurones responded only weakly to very high electrical stimulations in the posterior articular nerve (PAN), whereas 93% of the gamma cells reacted to similar stimulations (Johansson et al. 1986). This could explain why a cruciate ligament rupture is not associated with the absence but with a latency shift of the reflex response (Beard et al. 1994). The large differences in reflex size suggest that the direct ACL-hamstring reflex after intra-operative isolated stimulation is only a small component of the overall response after tibia translation during standing (Friedert et al. 2005).

In conclusion, the direct ACL-hamstring reflex pathway seems to be comparably weak and should not cause relevant latency shifts after ACL ruptures. Therefore the latency changes that were observed in patients with ACL ruptures may be the result of changes in the sensorimotor integration of the afferent input from the knee joint.

GRANTS

This work was supported by the Fraunhofer Grant FV InSan I 0102-V-1105.

REFERENCES


