

Appendix

Electrophysiology (CMAP)

In the anesthetized rabbit, the peroneal nerve on the experimental side was fully exposed with the use of the same approach as used in the index procedure. A miniature bipolar stimulating electrode (Harvard Apparatus, Holliston, Massachusetts) was clamped around the exposed peroneal nerve proximal to the repair site, and a ground electrode was placed in the surrounding musculature. Two bipolar recording electrodes were placed through the skin on the tibialis anterior muscle surface, one proximally near the motor end plate and the other distally. The former was placed between the proximal and middle thirds of the tibialis anterior muscle, and the latter was placed between the middle and distal thirds of the muscle. With the use of a VikingQuest portable electromyography (EMG) system (Nicolet Biomedical, Madison, Wisconsin) and VikingQuest software on a personal computer (PC), the CMAP was measured. Stimulation duration was set at 0.02 ms, and the intensity was the minimum necessary to elicit a maximum CMAP signal. The responses were analyzed for CMAP amplitude. The skin was temporarily reapproximated with suture on completion of the CMAP testing until force measurements were conducted. Exposure and measurements were also repeated on the contralateral side, and the CMAP results were normalized with the use of data on the contralateral side.

Maximum Isometric Tetanic Muscle Force¹⁹

After the electrophysiology test, another skin incision was made anterior to the ankle to expose the tibialis anterior tendon. The anterior fascia and pulley were opened, and the tendon and distal part of the muscle were exposed. The tendon was cut distally, but the tibialis anterior muscle was kept in place without injuring the neurovascular pedicle or muscle fibers. The examined muscle was prevented from drying and was cooled with a constant warm (37°C) saline solution drip. The knee and ankle joints were rigidly secured to a testing platform by transfixation with Kirschner wires (Pfizer Howmedica, Rutherford, New Jersey), with the medial side of the leg facing down. The tibialis anterior tendon was horizontally oriented and was connected to a force transducer (MDB-50; Transducer Techniques, Temecula, California) with the use of a custom clamp. The clamp was positioned in such a way that the position of the tibialis anterior muscle was similar to its anatomical position. The force transducer was mounted on an adjustable lever arm, which regulated muscle tension during isometric contractions. The same miniature bipolar stimulating electrode used for CMAP testing was clamped around the exposed peroneal nerve distal to the repair site, and a ground electrode was placed in the nearby musculature. A bipolar stimulator (Grass SD9; Grass Instrument, Quincy, Massachusetts) was used to generate the stimulus, and the signal acquired from the force transducer was processed on a PC with the use of LabVIEW software (National Instruments, Austin, Texas).

Before the isometric tetanic muscle force was measured, the optimal values of initial muscle tension (preload) were determined. The value was considered optimal when maximum isometric force was yielded with use of twitch stimuli. First, the initial muscle tension was adjusted to equal 0 g, and this was followed by stimulation of the nerve by a series of two-pulse stimuli (2V, 0.4 ms). The initial muscle tension was increased passively by 50-g increments until no increase in active force was obtained. Then, at optimal initial muscle tension, load was determined. After that, the muscle rested in zero preload for five minutes before the tetanic contractions. With an optimal preload, the isometric muscle contraction force was measured with predetermined frequencies of 100, 125, and 150 Hz. All contractions were performed at 10 V and 0.4 ms of duration to ensure maximal activation of all tibialis anterior motor units. Stimulation was applied for a maximum of two seconds or until a force peak was clearly observed. The tibialis anterior was rested for five minutes between stimulations with no preload to avoid muscle fatigue. Once testing was complete and the three tetanic force measurements were determined, the skin was closed and the surgical exposure and testing process were repeated for the contralateral side. ■