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RESEARCH ARTICLE

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Effects in feline gastrocnemius-soleus motoneurones induced by muscle fatigue

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Abstract Responses of gastrocnemius-soleus (G-S) motoneurones to stretches of the homonymous muscles were recorded intracellularly in decerebrate cats before, during and after fatiguing stimulation (FST) of G-S muscles. Ventral roots (VR) L7 and S1 were cut, and FST was applied to VR S1, a single FST session including 4 to 5 repetitions of 12-s periods of regular 40 s^{-1} stimulation. Muscle stretches consisted of several phases of slow sinusoidal shortening-lengthening cycles and intermediate constant lengths. The maximal stretch of the muscles was 8.8 mm above the rest length. Effects of FST on excitatory postsynaptic potentials (EPSPs) and spikes evoked by the muscle stretches were studied in 12 motoneurones from ten experiments. Stretch-evoked EPSPs and firing were predominantly suppressed after FST, with the exception of a post-contraction increase of the first EPSP after FST, which was most likely due to after-effects in the activity of muscle spindle afferents. The post-fatigue suppression of EPSPs and spike activity was followed by restoration within 60–100 s. Additional bouts of FST augmented the intensity of post-fatigue suppression of EPSPs, with the spike activity sometimes disappearing completely. FST itself elicited EPSPs at latencies suggesting activation of muscle spindle group Ia afferents via stimulation of β-fibres. The suppression of the stretch-evoked responses most likely resulted from fatigue-evoked activity of group III and IV muscle afferents. Presynaptic inhibition could be one of the mechanisms involved, but homosynaptic depression in the FST-activated group Ia afferents may also have contributed.

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Introduction

In the narrow sense, the term "muscle fatigue" relates to a range of cellular processes that develop within the muscle tissue and at the neuromuscular junction during long-lasting muscular activity (Gandevia et al. 1995). However, in addition, the peripheral and central nervous systems become involved in fatigue processes. The activity of various types of sensory muscle afferents changes, and these fatigue-related changes affect widely distributed areas in the central nervous system at spinal and supraspinal levels (Gandevia et al. 1995, 1999; Gandevia 2001). Basic spinal reflexes change during muscle fatigue and related pain syndromes, which is crucially important for understanding work-related chronic neuromuscular pain syndromes and protective tonic reflexes that make movements slower. From indirect evidence in humans, it has been concluded that the decline of α-motoneurone firing rate during prolonged fatiguing muscle contractions results from an inhibitory reflex arising in group III and IV muscle afferents that are activated during muscle fatigue (Bigland-Ritchie et al. 1986; Woods et al. 1987; Garland et al. 1988). This inhibitory effect in turn could be due to several mechanisms, including depressive actions on other reflexes. Indeed, experimental data from humans revealed strong fatigue-related suppression of monosynaptic group Ia excitation in motoneurones supplying the exercising muscles. H-reflex amplitudes and short-latency responses to muscle stretch decreased after fatiguing contractions irrespective of whether fatigue was evoked by direct muscle or muscle nerve simulation (Garland and McComas 1990; Butler et al. 2003) or by voluntary contractions (Enoka et al. 1980; Balestra et al. 1992; Duchateau et al. 2002).

As pointed out by Gandevia (2001), it is difficult in human experiments to unequivocally determine the causes for the observed decreases in motoneurone excitability. One of the main limitations in non-invasive experiments on humans is the difficulty of dissecting fatigue- or pain-related changes in the activity of muscle afferents, pre-motoneuronal events and intrinsic motoneurone properties. The use of reduced animal preparations may circumvent some of the above difficulties, but even in this case, there are discrepancies between the data obtained on rats (Pettorossi et al. 1999) and decerebrate cats (Kalezic et al. 2004). In both studies, a strong inhibition of the monosynaptic reflex (MSR) was observed after fatiguing contractions of the parent muscles. Although clear effects were evoked after only 30–60 s of stimulation in the rat study, strong fatiguing contractions with durations exceeding 10–12 min were necessary to evoke depression of the gastrocnemius-soleus (G-S) MSRs in the cat study. Such differences may have been due to differences in experimental animals and in the type of reduced preparation used, in the frequency of fatiguing stimulation and in experimental setting.

The aims of the present study were as follows: 1) to complement the existing database on the effects of muscle fatigue on signal transmission from muscle afferents to homonymous and synergistic motoneurones from an intracellular perspective—intracellular recording enables a better distinction between pre- and postsynaptic events; and 2) to test the hypothesis that the effects of muscle stretch in homonymous/synergistic motoneurones are subject to essentially the same depression as are electrically evoked monosynaptic reflexes previously used as probes. This hypothesis is based on the supposition that, while muscle stretch, as a more natural stimulus, activates several types of mechanically sensitive muscle afferents, the effects of muscle stretch in homonymous/synergistic motoneurones are dominated by inputs from muscle spindle Ia afferents.

Materials and methods

Preparation

The experiments were carried out on 10 adult cats of either sex, weighing between 3.0 and 4.6 kg. The animals were purchased from state-controlled animal farms through the common animal facility of the University of Umeå. Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) were followed. The use of experimental animals was approved by the local Animal Ethics Committee (Umeå Djurförsöksetiska Nämnd, proj. 1997/0337). The animals were initially anaesthetised by inhalation of a mixture of oxygen and nitrous oxide (1:2) with halothane (2.5%). After insertion of a catheter into an external jugular vein, the inhalation anaesthesia was replaced with intravenous injections of pentobarbital sodium (Sigma); 1 ml of 10 mg/ml solution was administered intravenously every 15 min during the subsequent surgical procedures. For monitoring of the blood pressure, a catheter was inserted into a common carotid artery. A laminectomy was performed over the lumbar enlargement of the spinal cord. The animal was suspended within a firm frame, and additional pins were inserted into femur and tibia to rigidly fix the knee joint of the right leg. Then, an intercollicular decerebration was performed, and the anaesthesia was discontinued. The G-S muscles of the right hindlimb were separated from the surrounding tissues, with care taken to keep all supplying blood vessels intact. The Achilles tendon was detached at the distal insertion, leaving a small bone chip of the heel, which was used to hook up the tendon through a steel cable to the servo-controlled muscle stretcher. All right hindlimb nerves except for those innervating the G-S muscle were cut. The hindlimb muscles were placed in pools formed from surrounding skin flaps. A similar pool was formed above the exposed spinal cord. Both pools were filled with mineral oil whose temperature was maintained close to 37.5 C° by means of radiant heat. The rectal temperature was kept at a constant physiological level by heating the animal body with a controlled heating pad. If necessary, the animals were artificially ventilated, keeping the end-tidal CO_2 concentration at 3.8–4.5%. At the end of all experiments, the animals were sacrificed by the administration of a large overdose of pentobarbital sodium (5 ml of 60 mg ml^{-1} solution).

Stimulation and recording

An IBM personal computer was used to create patterns of stimulation. The DAC channels in the interface card had 12-bit resolution and 1-kHz sampling rate. The generated pulses synchronized the standard isolation units (model DS2A, Digitimer, UK) sending stimuli to nerves. The stimulus pulses were of 0.2-ms duration. Intracellular recordings from spinal motoneurones were made by use of glass microelectrodes filled with 1.5 M solution K_2SO_4 (resistance 1.5–4.5 MΩ) and of an Axoclamp 2A amplifier (Axon Instruments, USA). Only motoneurones exhibiting resting membrane potentials below −50 mV and amplitudes of action potentials above 70 mV were selected for analysis.

Fatiguing stimulation

The G-S muscles were connected through a steel cable to the servo-controlled muscle stretcher. Except during the stretches (see below), the muscles were held isometric near their rest length. Fatiguing contractions were evoked by stimulation of a part of, or the whole, ipsilateral VR S1 that was cut proximally. The current strength was adjusted to a level of 1.2–1.7 times the motor threshold (MT). A single stimulation period of 12-s duration consisted of a regular pulse train at a

rate of 40 s^{-1}. Several stimulation periods were applied to evoke the fatiguing muscle contractions, with rest intervals of various durations interposed between the stimulation periods. Because the fatiguing contractions were evoked during long-lasting intracellular recording from a motoneurone, the stimulation parameters were usually chosen in such a way as to avoid too strong muscle contractions (exceeding 14–16 N), which could lead to excessive mechanical oscillations of the spinal cord and deteriorate the recording conditions.

Test muscle stretches

The pattern of muscle stretching (see *upper traces* in Fig. 1) consisted of two sinusoidal waves with the same duration (1 s) but different amplitudes, followed by a double trapezoid with leading and trailing edges of sinusoidal form and equal duration (2 s). The amplitudes of the first wave and the first trapezoid were 4.9 mm, and the amplitude of the second sinusoidal wave and the whole amplitude of the double trapezoid were 8.8 mm. These rather complex muscle stretches allowed, first, to evoke excitatory postsynaptic potentials (EPSP) components of various amplitudes below or above threshold for spike generation, and, second, to compare postsynaptic responses at equal steady levels of muscle length but reached from different directions (shortening vs. lengthening). In each experiment, the muscle length at maximal stretch was several millimetres shorter than the length at a maximally flexed ankle joint.

Data acquisition and analysis

The AC and DC records of the motoneurone membrane potential, muscle tension and length, blood pressure, and stimulation marks were collected by CED Power 1401 (Cambridge Electronic Design, UK) connected to a personal computer. The program "Spike 2" (Cambridge Electronic Design, UK) was used for data acquisition and processing. The input signals were digitised with 16-bit resolution at rates of 1 kHz for the blood pressure, muscle length and tension signals, and 16 kHz for the membrane potential records. The data analysis was performed using the programs "Spike 2" and "Origin 7.0" (OriginLab Corporation, USA).

Statistical analysis

The statistical significance of the changes in motoneurone firing rate was determined by an analysis of variance (ANOVA) with repeated measures, the time-interval of the measurement being used as a within-subject factor. The tests before fatiguing stimulation (FST) were compared with the tests applied at various time intervals after FST. In case the sphericity assumption was not met, the Huynh-Feldt correction was applied. Differences were considered as significant at

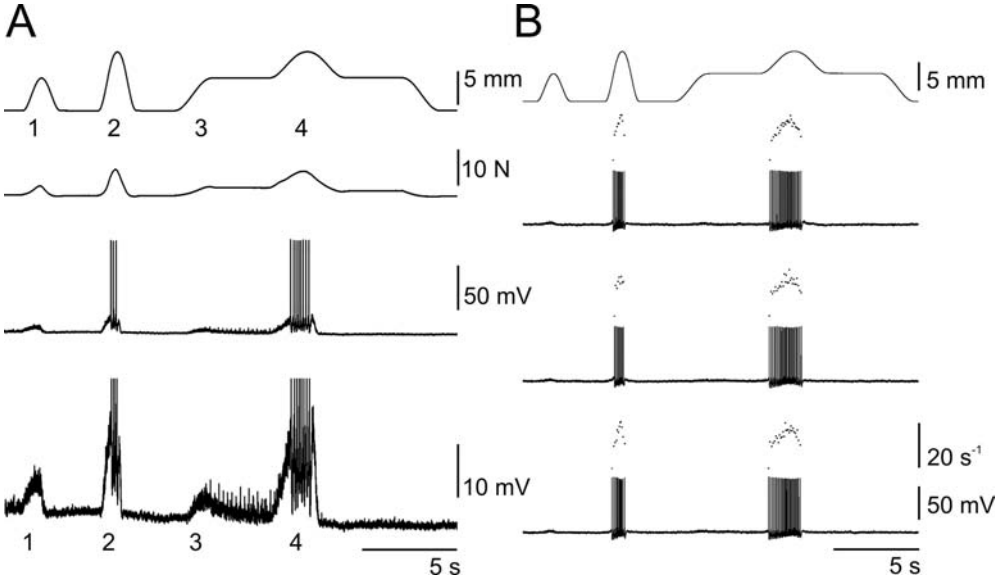

Fig. 1 Postsynaptic membrane potential changes in two G-S motoneurones (**a, b**) during standard series of muscle stretches. **a** Postsynaptic membrane potential changes of a G-S motoneurone, evoked by standard series of muscle stretches. *Traces* from top to bottom: muscle length, muscle tension, DC record of the membrane potential at two different amplifications. Action potentials are clipped in the *bottom trace* due to high amplification. Phases of the standard stretches and corresponding postsynaptic motoneurone responses are numbered 1–4 under the top and bottom records. **b** Postsynaptic membrane potential changes of a G-S motoneurone, evoked by the standard series of muscle stretches applied every 30 s over 15 min. The muscle length changes are shown at the *top*; DC records of the membrane potential and instantaneous firing rates of the evoked spike activity are shown for the first two (*middle panels*) and the last stretch sessions (*bottom panel*)

$P < 0.05$. Provided that changes in corresponding variables were significant in successive time intervals, the post-hoc comparisons were performed using the Bonferroni adjustment for multiple comparisons. The statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

General observations

Representative responses of two G-S motoneurones to homonymous muscle stretches are presented in Fig. 1. In Fig. 1a, muscle length, muscle tension, and DC records of the membrane potential at two amplifications are shown in the traces from top to bottom. The stretches (*upper trace*) were associated with membrane depolarizations, which—if large enough—triggered spikes (*third* and *fourth traces*). To simplify further analysis and comparisons of results from different experiments, the pronounced dynamic components of depolarization were numbered 1 through 4 (Fig. 1a, *lower trace*), corresponding to the dynamic phases of stretches (Fig. 1a, *upper trace*). The used pattern of muscle stretching provided the possibility to evoke EPSPs with amplitudes sub-threshold (components 1 and 3 in Fig. 1a) or suprathreshold (components 2 and 4 in Fig. 1a) for spike generation. In general, the stretch-evoked waves of membrane depolarization represented the temporal summation of a large number of low-amplitude EPSPs. However, sometimes individual EPSPs evoked by spikes in a single afferent fibre were discernable, as exemplified by the discrete high-amplitude EPSPs within component 3 in the *bottom row* of Fig. 1a. As far as membrane potential and action potential amplitude were stable, the excitation of motoneurones by the muscle stretches was highly reproducible; many repetitions of the stretches evoked almost identical EPSPs and patterns of spike activity. This is exemplified in Fig. 1b, where the standard series of muscle stretches was applied every 30 s over 15 min. The *lower three panels* show DC records of the membrane potential (*lower trace*) and instantaneous firing rates of the evoked spike activity (*dots* in *upper trace*) in response to the first two and the last muscle length changes (*top panel*).

Typical changes in motoneurone responses following muscle fatigue are shown in Fig. 2a, which displays intracellular recordings from another G-S motoneurone responding to a series of G-S muscle stretches before and after fatiguing muscle contractions. Muscle length, tension, instantaneous rates of evoked spike activity, and a DC record of the membrane potential are shown in the traces from top to bottom. Enlarged sections of the membrane potential records are also presented in Fig. 2c. A more detailed analysis of the stretch-evoked EPSPs will be done in the following sections. First some general features of the motoneurone responses will be considered.

Long-lasting intracellular recording was at times accompanied by slow membrane depolarization. In the experiment shown in Fig. 2, the drift in membrane potential was approximately 0.1 mV/min. Sometimes FST was associated with irregular slow waves of depolarization (marked by *open arrows* in Fig. 2a, *bottom trace*). At least partly, these waves could have resulted from deterioration of the recording conditions due to spinal cord movements. On the other hand, they could also have reflected synaptic inflow generated by the fatiguing stimulation. This supposition is supported by the results of averaging the membrane potential with respect to the VR stimuli. As shown in Fig. 2b, such averages revealed distinct EPSPs, whose amplitude did not exceed 0.1–0.15 mV in this experiment. These EPSPs were very stable, as shown by comparing averages obtained for different FST periods. The superimposed averages labelled c and d in Fig. 2b were obtained within two different periods of the fatiguing contractions marked by *thick horizontal bars* with the corresponding labels (*bottom trace* in Fig. 2a). The great stability of the EPSPs and their latency suggest a monosynaptic origin. Most likely, these EPSPs were evoked by volleys in group Ia spindle afferents, whose discharges were in part triggered by activations of β-axons during FST. Direct activations of γ-axons were unlikely at the low stimulus strength (1.4 MT) used in this experiment, this intensity being great enough to activate larger axons sending collaterals to both extra- and intrafusal muscle fibres (Kouchtir et al. 1995).

After cessation of the fatiguing muscle contractions, a constant-level depolarization associated with enhanced background membrane noise appeared, as seen in Fig. 2c, which reproduces enlarged pieces of records from Fig. 2a. The *vertical arrows* at the *bottom traces* of Fig. 2a–c indicate the end of the FST periods (marked by one to three *asterisks* in Fig. 2a). Component 1 of the stretch-evoked depolarization wave, which did not elicit spikes, also increased in the first stretch sequences after FST. This is appreciated by comparing components 1 in the pre- and post-contraction stretches a1 and b1 (Fig. 2c). In response to the subsequent stretch sequences (a2 and b2), component 1 was restored to its usual smaller size (Fig. 2c). The sensory inflow associated with the first low-amplitude wave of stretch apparently diminished the post-contraction synaptic activity and small membrane depolarization (component 1 in a1 and b1, Fig. 2c).

Fatigue-induced changes in stretch-evoked firing rate

Dynamic stretches of the G-S muscles evoked membrane depolarizations, which—if large enough—elicited spike activity (phases 2 and 4 in Figs. 1 and 2). This discharge changed following muscle fatigue, as exemplified in Fig. 2a. The instantaneous firing rates in response to the first stretches decreased after the second application of FST (marked by two *asterisks*). This depression was most

conspicuous after the long fatiguing contraction marked by three *asterisks*. To quantify and statistically evaluate such changes, the following procedure was adopted.

In most cases of high-quality intracellular recordings from G-S motoneurones, spikes were evoked only by high-amplitude stretch waves (phases 2 and 4; Fig. 1a). For the experiment presented in Fig. 2, the number of spikes (*N*) and the means + S.E.M. of the instantaneous firing rates were determined for each discharge response to a standard stretch sequence, and plotted in Fig. 3a versus the time elapsed from the beginning of the recording. The *solid dots* represent the spike counts *N*, and the *black bars* represent the means + S.E.M. For the calculation of the mean instantaneous rates, the long intervals between spike bursts in phases 2 and 4 were not taken into account.

Statistical comparison of the mean instantaneous rates (*columns* in Fig. 3a) quantitatively confirmed the impression that firing rates in the stretch responses after each FST were temporarily depressed. Consecutive repetitions of FST periods or their prolongation (by using sequences of standard 12-s periods of stimulation; Fig. 2a) increased the intensity of post-fatigue suppression. A subsequent recovery was indicated by the fact that the increase from this initial depressed state was also statistically significant. Similar results were obtained for three more G-S motoneurones recorded in different experiments (Fig. 3b–d).

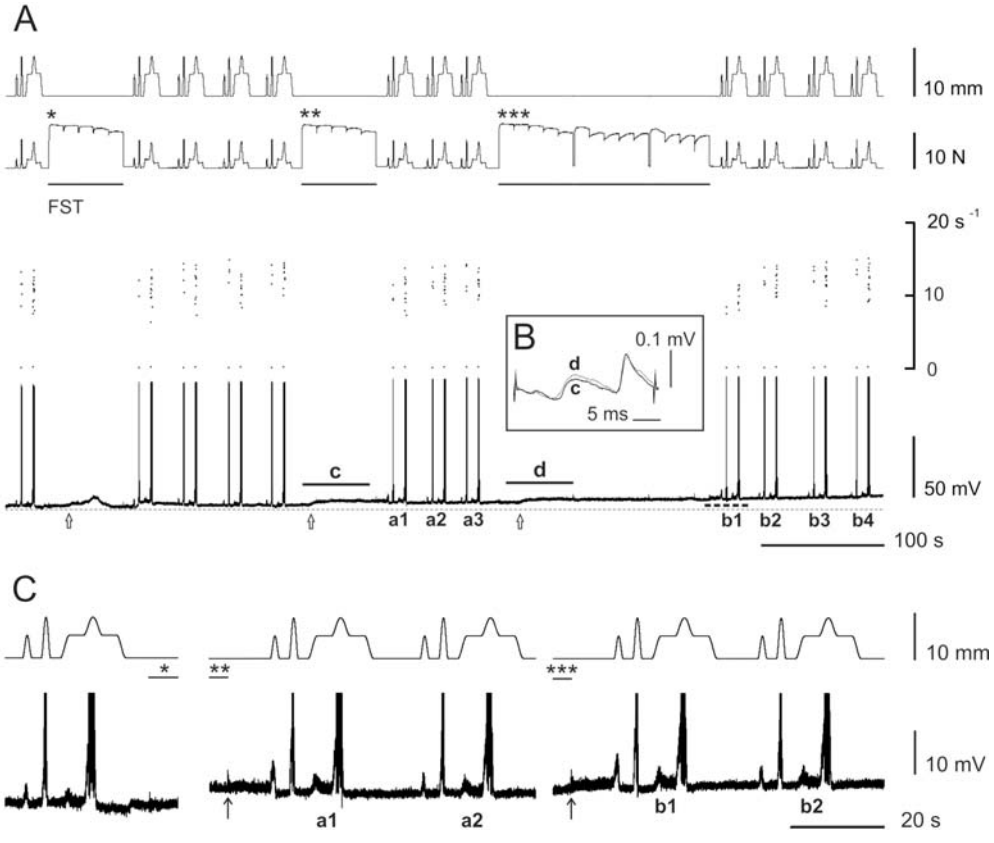

Fig. 2 Changes in stretch-evoked excitation of a G-S motoneurone after fatiguing stimulation of G-S muscles. Motoneurone different from the two in Fig. 1. **a** Postsynaptic membrane potential changes of a G-S motoneurone during standard series of muscle stretches and fatiguing muscle contractions. *Traces* from top to bottom: muscle length, tension, instantaneous rate of evoked spike activity, and DC record of the membrane potential. The *horizontal bars* below the tension record indicate the sessions of fatiguing stimulation (labelled FST). The first two sessions of FST (denoted by one and two *asterisks* at the tension record) included sequences of five 12-s periods of regular stimuli at a rate 40 s^{-1}, with 70-ms intervals between periods, which evoked small phasic drops of tension. The third bout of FST (labelled by three *asterisks*) was three times longer, with 1-s intervals interposed between consecutive FST sessions. The current strength of the stimuli was 1.4 MT. Below the intracellular DC record (*bottom trace*), *open arrows* indicate the beginning of slow waves of depolarization during muscle contractions. Several sections of stretch responses are shown at larger scales in **b**, averaged DC membrane potential records triggered by the pulses within FST. The time spans, over which the averaging was performed, are indicated by the *thick horizontal lines* (labelled *c* and *d*) above the DC membrane potential record (*bottom trace* in **a**). The averaged membrane potentials are represented by *thick* and *thin lines*, labelled *c* and *d*, respectively. The labels a1–a3 and b1–b4 are used for marking different stretch sessions after second and third bouts of FST. **c** Enlarged sections of records reproduced from **a**, displaying consecutively three segments of the record: before the first, after the second and third FST sessions; the same labels a1–a2 and b1–b2 as in **a**. Muscle length and DC record of membrane potential are shown on *top* and *bottom rows*. Periods of FST are indicated below the length records as in panel **a**; moments of the stimulation cessation are additionally marked by *asterisks*. Action potentials are clipped off on the membrane potential records due to a high amplification

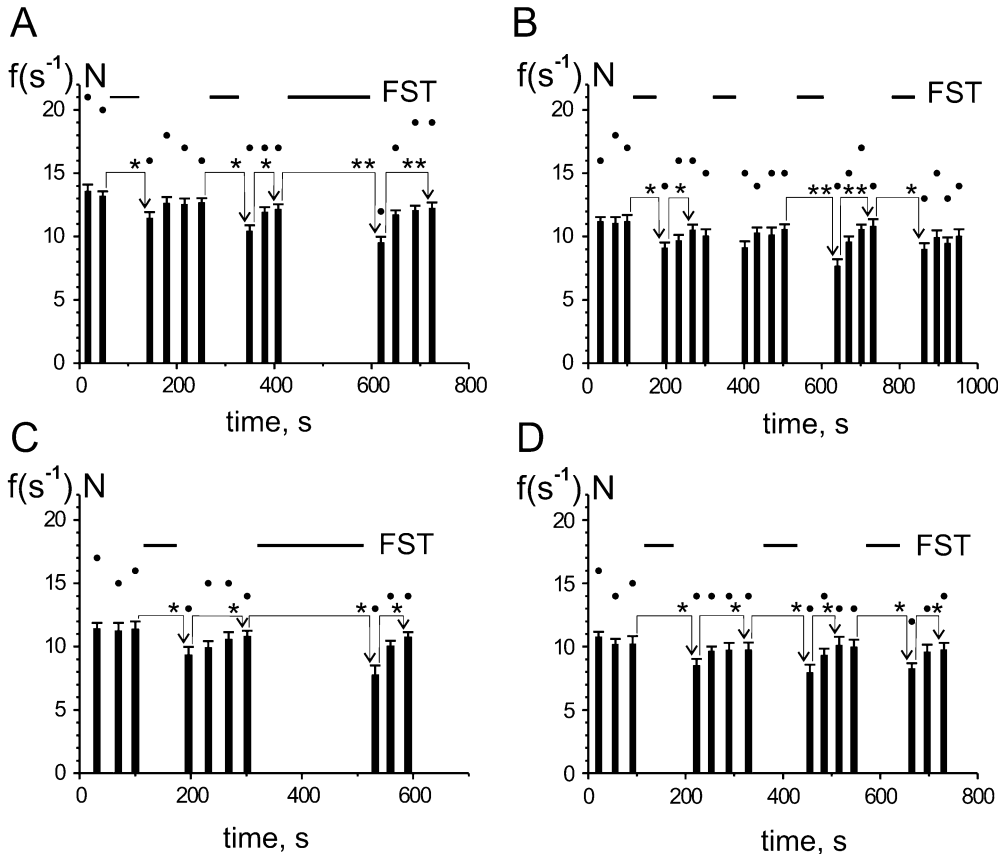

Fig. 3 Fatigue-dependent changes in statistical characteristics of spike activity (firing rates) in different sessions of the muscle stretches. **a–d** Statistical parameters of stretch-evoked spike activity of four (**a–d**) G-S motoneurones from four different experiments. The *bar graphs* plot the means + S.E.M. of the instantaneous firing rate defined for each stretch sequence (see text); *closed circles* denote the number of spikes (*N*). Data from the motoneurone presented in Figs. 1 and 2 are shown in **a**. In all cells (**a–d**), spikes were discharged on the 2nd and 4th stretch phases (definition of the phases in Fig. 2a), and instantaneous rates were defined only for the interspike intervals within each of the above stretch phases, while the intervals between boundary spikes in these phases were not taken into account. Repeated measures ANOVA with post-hoc Bonferroni adjustment for multiple comparisons revealed significant differences as indicated (*$P < 0.05$, **$P < 0.01$). See Methods for a more detailed description of the statistical procedure

Strong fatigue-induced suppression of stretch-evoked postsynaptic responses in G-S motoneurones

An example of a G-S motoneurone displaying a particularly strong suppression of stretch-evoked discharges is presented in Fig. 4. The first application of a series of six successive 12-s fatiguing contractions (Fig. 4a) eliminated spikes (*lower trace*) in the first post-FST stretch sequence (a1), thereafter the instantaneous firing rate (*middle trace*) gradually increased again during the following stretches (a2–a4). However, at the same time, the number of spikes per stretch sequence diminished from 5 (a2) to 3 (a3, a4), with discharges being absent at the apexes of component 4 of the stretches. After approximately 130 s of recovery, the second application of a similar FST sequence (Fig. 4b) led to full suppression of firing (b1–b4). The disappearance of stretch-evoked spike activity cannot be accounted for by deterioration of the recording conditions since the membrane potential and amplitude of the action potentials remained virtually unchanged. In this experiment, the strength of FST was 1.5 MT, and such a stimulus intensity evoked high-amplitude EPSPs that could be clearly seen even on the raw membrane potential records (Fig. 4a and b, *lower traces*). Stimulus-triggered averages (Fig. 4c) of these EPSPs revealed their extreme stability (compare the averages labelled c and d, computed over the time spans indicated by *horizontal bars* and labelled respectively at the beginning and end of the second FST session). The amplitudes of the EPSPs exceeded 2 mV.

Dynamics of post-fatigue changes in stretch-evoked EPSPs

Since the motoneurone shown in Fig. 4 did not discharge after FST in response to some stretch sequences (a1 in Fig. 4a, and b1–b4 in Fig. 4b), the evoked EPSPs lent themselves to further analysis as to fatigue-dependent changes in their amplitude and time course. The membrane potential records in Fig. 4 were subjected to off-line smoothing with a time constant of 0.05 s, the effect being illustrated in Fig. 5a (*lower trace*). This trace shows the original record (*black band*) and the smoothed record

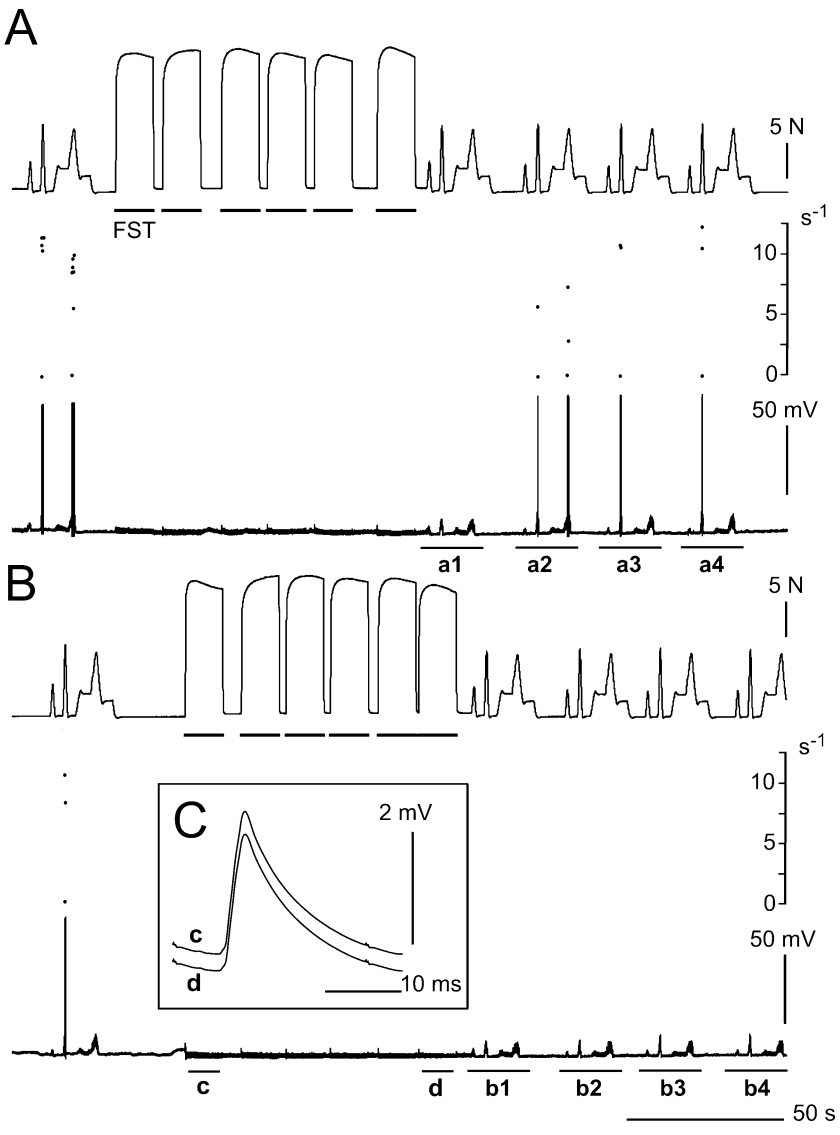

Fig. 4 Example of a G-S motoneurone with strong suppression of stretch-evoked excitation after fatiguing muscle contractions. **a**, **b** Responses to two repetitions of FST, each including six 12-s periods of regular stimuli (rate 40/s), at a stimulation strength of 1.5 MT. The interval between the end of record **a** and the beginning of record **b** was about 130 s. *Upper traces:* tension records. *Middle traces:* instantaneous firing rate. *Lower traces:* intracellular DC recording. These recordings are further analyzed in Figs. 5 and 6c. Superimposed averages of membrane potential triggered by the fatiguing stimuli. The intervals, over which averaging was performed, are indicated by the *horizontal lines* labelled *c* and *d* below the membrane potential record in **b**, the averages in **c** are marked correspondingly (*c* and *d*)

(*white line within black band*). Figure 5b (*upper traces*) shows the superposition of the first EPSP sequences in response to the first stretch sequences just after cessation of the first and second FST sessions (a1, b1 in Figs. 4a, b and 5b). These two traces were very similar, except that component 2 was reduced in amplitude after the second FST (compare records a1 and b1 enlarged in Fig. 5b, *lower traces*). Similarly, Fig. 5c shows that component 4 in b4 was reduced relative to a4. Thus, although the membrane potential and amplitudes of spikes remained constant in parts a and b of Fig. 4, repeated fatiguing muscle contractions led to both suppression of spike activity and parallel reduction of stretch-evoked EPSPs.

As noted above, fatiguing contractions of the muscles left after-effects in their motoneurones. The most consistent after-effect was the post-contraction steady membrane depolarization (indicated by the *dashed horizontal line* in Fig. 5b, *upper traces*), which most likely resulted from post-contraction increases in muscle-spindle discharge. This depolarization almost disappeared after cessation of the first stretch wave (compare *upper traces* in panels b and c). In the *upper left panel* of Fig. 5d, the stretch sequences b1 through b4 (Fig. 4b) were superimposed, and individual components (1, 2, and 4) of the superposition are shown enlarged in three other panels. Component 1 (*upper right panel* of Fig. 5d) of the EPSP sequence in response to the first muscle stretch after FST (*thick line* labelled b1) was substantially increased (also compare components 1 in Fig. 2a–c), and its latency was reduced. In addition, component 1 from b1 exhibited a fast wave of depolarization at its beginning, reflecting most probably a postsynaptic response to synchronous afferent discharge. This wave disappeared in the following stretch sessions (b2–b4), and the postsynaptic responses evoked by the first low-amplitude muscle stretches became almost identical.

The "positive" after-effects of fatiguing contractions described above extended beyond component 1. Component 2 (*lower left panel* in Fig. 5d) also had a

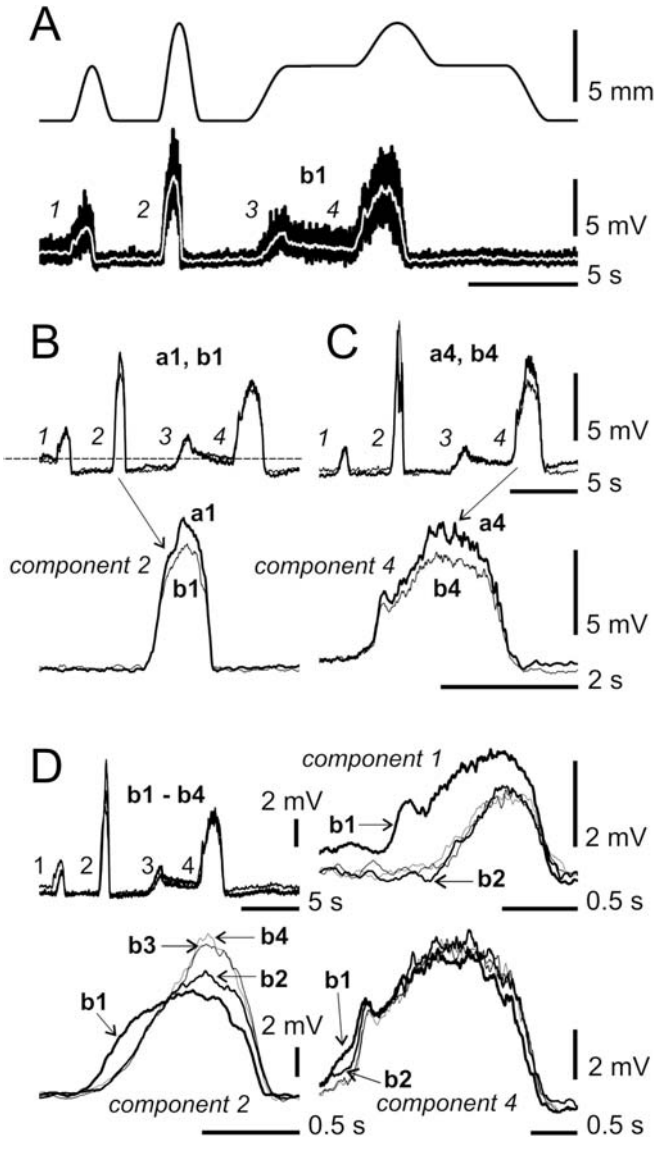

Fig. 5 Post-fatigue changes in different components of stretch-evoked EPSPs. Same cell as in Fig. 4a, stretch-evoked changes of the membrane potential extracted from the record displayed in Fig. 4b within the interval labelled b1 (*upper trace:*muscle length; *bottom trace:* membrane potential). The original membrane potential record (*black band*) was subjected to off-line low-pass smoothing with a time constant of 0.05 s, the result of which is represented by the *white line* within the *black band*. **b–d** Superposition of different stretch-evoked EPSPs after smoothing. The labels (e.g. *a1, b1* in **b**) indicate the origins of the data in Fig. 4. Sometimes component 2 produced spikes (*upper row* in **c**, see also Fig. 4a), which were distorted after the smoothing procedure, and such cases were not used for analysis. Enlarged sections of the record in *upper rows* of **b** and **c** are shown in the *bottom rows*, as indicated by *arrows*

shorter latency in b1 than in the following stretch sessions. It also had a faster leading edge, although this edge quickly subsided into a faster drop. Slight "positive" after-effects were even preserved in component 4 (*lower right panel* in Fig. 5d), for which the growth of depolarization in b1 was faster than in b2–b4 stretch sessions. On the other hand, the amplitude of component 2 (*lower right panel* in Fig. 5d) gradually increased in consecutive stretch sessions from b1 to b4, demonstrating a tendency for recovery of the stretch-evoked postsynaptic responses from their maximal suppression. These "negative" after-effects following FST developed in parallel with the post-fatigue suppression of the stretch-evoked firing described earlier.

The "positive" after-effects are best explained by linking them to corresponding properties of muscle spindle responses within FST-activated muscle. As mentioned before, some muscle spindles were most likely activated by stimulation of β-axons sending terminals to both extra- and intrafusal muscle fibres. Assuming that extrafusal and intrafusal mechanics show some resemblance and that intrafusal mechanics are reflected in spindle afferent discharge and post-synaptic motoneurone responses, there might be some similarity between post-contraction changes seen in the stretch-evoked EPSPs and in extrafusal mechanical properties. To test this hypothesis, EPSP trajectories and muscle tension records were plotted against muscle length, as illustrated in Fig. 6a, b. In Fig. 6a (*bottom panel*), the length-tension loop labelled as b1 (recorded just after FST) was very different from the loops b2 and b3 in the following stretch sequences. Loop b1 signified a greatly increased muscle stiffness (defined as steepness of the curve at any moment) at the very beginning of the muscle stretch, and demonstrated an enlarged tension throughout the subsequent muscle elongation. Assuming analogous increases in stiffness and tension of intrafusal muscle fibres after β-activation and domination of the membrane potential changes by spindle input would explain the earlier and stronger depolarization just after FST (*thick curves* labelled b1 in Fig. 6a, *upper panel*). Such after-effects in muscle spindle responses would evoke stronger postsynaptic depolarization of G-S motoneurones at shorter length values (compare corresponding length-membrane potential loops b1, b2, and b3 in *upper panel* of Fig. 6a). The post-stimulation effects were not completely abolished by the first low-amplitude stretch, as the length-tension loop for component 2 (Fig. 6b) in the first stretch sequence after FST was also slightly above the loops for the following sequences (compare b1 with b2 and b3 in *bottom panel* of Fig. 6b). In this case, though, the muscle stiffness was not increased as much anymore as for component 1 (compare *bottom panels* in Fig. 6a, b), and the depolarization started almost at the same length in b1, b2 and b3 (Fig. 6b, *upper panel*). With repetition of the stretch sequences, the EPSPs of component 2 (Fig. 6b, *upper panel*) did not correlate so well with the corresponding muscular mechanical events as for component 1, but some clear differences between length-tension loops b1 vs. b2, b3 were rather well preserved. The above reactions were typical as could be seen from the responses in another motoneurone shown in Fig. 6c and d.

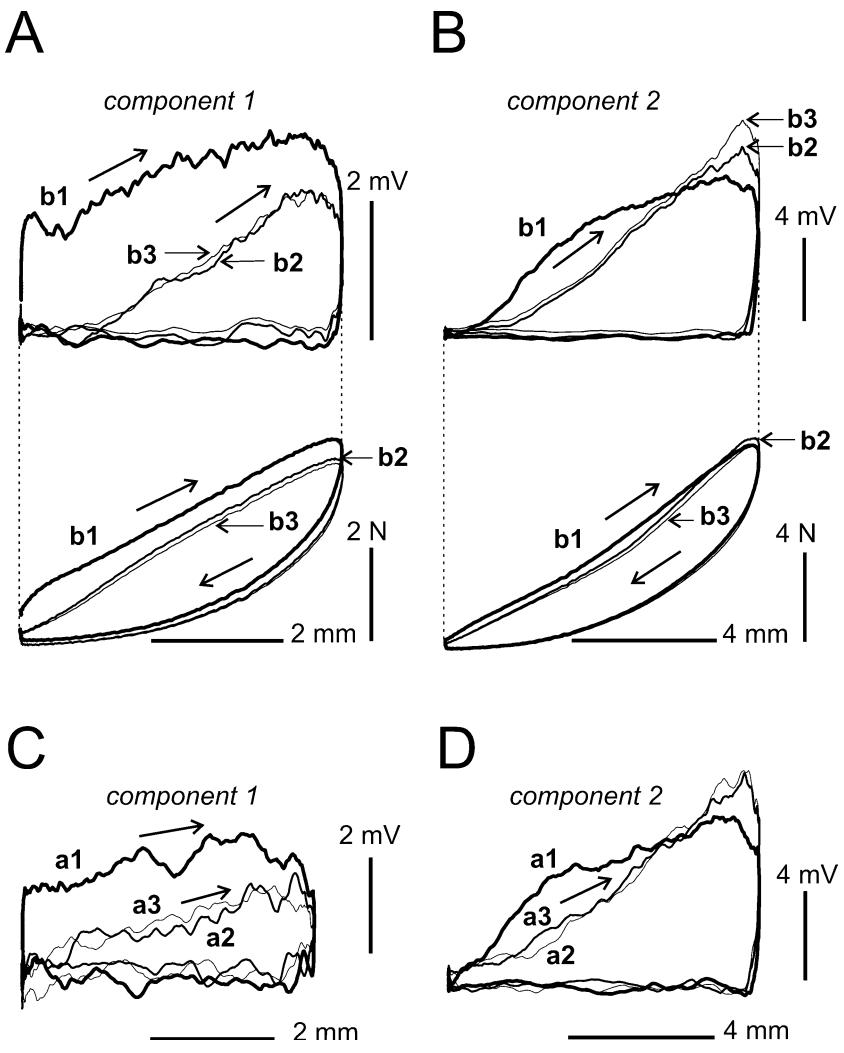

Fig. 6 Post-fatigue changes in dependencies of membrane potential on muscle length. **a–d** Records from two different G-S motoneurones (the same cell as in Figs. 4 and 5 is shown in **a, b**). EPSP component 1 (*upper panel* in **a** and **c**) and EPSP component 2 (*upper panel* in **b** and **d**) are plotted vs. length changes. For the first cell (**a, b**), the respective tension records are plotted vs. length in the *lower rows* in **a** and **b**, for comparison. Designations b1–b3 in **a, b** are the same as in Figs. 4 and 5; a1–a3 in **c, d** similarly correspond to the first three stretch sessions applied after FST. The *arrows* on the loops show the time direction of the length changes

Discussion

In the present work, changes in stretch-evoked excitation of G-S motoneurones were studied after fatiguing G-S contractions over long time spans. Intracellular recordings were also used by a French group who studied contraction-induced excitation of cat peroneal motoneurones (Kouchtir et al. 1995; Perrier et al. 2000). However, only weak and short-lasting muscle contractions with maximal isometric forces of 2–7 N were used, so that fatigue-evoked changes in motoneuronal excitability during and after sufficiently strong and long-lasting muscle contractions were not studied. Even though in our experiments the forces compatible with stable intracellular recordings did also not reach maximal tetanic G-S forces, they were strong enough to evoke prominent fatigue effects.

Even a single FST session of 12-s duration could decrease the firing rates of stretch-evoked spike activity, but these effects were usually unstable. Four to six times longer stimulation periods more reliably evoked post-contraction suppression. Repetition of the fatiguing contractions resulted in more profound suppression of firing as well as longer recovery. Sometimes, the spike activity was fully suppressed. In general, a similar post-contraction depression was observed in the postsynaptic components of the stretch responses. This suppression then gradually faded away during the following stretch sequences. A rough estimation of the recovery time yielded 60–100 s (this period of time comprised 3 to 4 standard stretch sequences).

Several factors could have contributed to the depression of stretch-evoked motoneurone excitation. Postsynaptic inhibition was likely not involved because there were no signs of related membrane hyperpolarizations (Figs. 2, 4). Post-fatigue reduction in muscle spindle discharge rate could have played a role. At least in the rat masseter muscle, muscle spindle afferent activity has been shown to decrease during muscle fatigue (Brunetti et al. 2003). But in our experiments possible motoneurone disfacilitation was not reflected in hyperpolarization. Second, high-rate afferent activity evoked by activation of skeletal muscles could have led to neurotransmitter exhaustion in some afferent terminals, thus evoking

homosynaptic depression in the monosynaptic group Ia-motoneurone pathway (Capek and Esplin 1977). However, this would probably have happened only in those group Ia afferents that were activated by β-axons; all other Ia afferents were likely unloaded and, hence, silenced by muscle contraction. Third, presynaptic inhibition could have played a substantial role in suppressing transmitter release from group Ia afferent terminals. It is known that the background presynaptic inhibition of group Ia fibres can be enhanced by the discharge of other Ia fibres (Brink et al. 1984). Thus, the long-lasting activation of group Ia afferents via β-axons during fatiguing contractions could have been a possible suppression mechanism. Moreover, presynaptic inhibition is modulated by inputs from group III and IV muscle afferents. Indeed, presynaptic inhibition increased upon activation of high-threshold afferents of group III and IV, after muscle fatigue and after neurochemical stimulation (Pettorossi et al. 1999; Rossi et al. 1999; Brunetti et al. 2003; Kalezic et al. 2004). At least partly, this process could have contributed to the continuous drop in amplitude of the stimulus-evoked EPSPs upon repetition of FST sessions as seen in Fig. 5. Both neurotransmitter depletion and presynaptic inhibition could have contributed to the short-term suppression of the stretch-evoked postsynaptic responses.

In the present experiments, we deliberately used muscle stretch as a fairly natural stimulus for muscle afferents, in order to see to what extent the results would be comparable to the findings of post-fatigue changes in G-S MSRs evoked by synchronous stimulation of group Ia afferents (Pettorossi et al. 1999; Kalezic et al. 2004). Despite the differences in afferent stimulation, the basic phenomenon of post-fatigue suppression was the same, although the precise mechanisms may have been different to some extent. After all, group Ia afferents very likely contributed significantly to the stretch-evoked EPSPs recorded in the present study. Therefore, at least in part, the long-lasting suppression of these EPSPs could have resulted from processes similar to those underlying the prolonged inhibition of the MSRs. Kalezic et al. (2004) presented evidence for increased presynaptic inhibition during and after muscle fatigue as a factor decreasing the transmission of group Ia excitatory effects to homonymous and synergistic motoneurones. The suppression of Ia excitatory effects could be also associated with the reduction of muscle spindle afferent activity during muscle fatigue (Brunetti et al. 2003).

The larger spectrum of afferents excited by muscle stretch (this study) certainly activated additional pathways to motoneurones, which would be modifiable by afferent signals set up by muscle fatigue (e.g. see Windhorst and Boorman 1995). In addition to group Ia afferents, muscle stretch may also activate group II muscle spindle afferents, group Ib afferents from Golgi tendon organs, as well as high-threshold, mechanically sensitive afferents especially of group III, and these afferents may have complex effects on motoneurones (for reviews see Schomburg 1990; Jami 1992; Clarac et al. 2000).

In particular, the likely activation of Golgi tendon organs during FST could have evoked di- or trisynaptic IPSPs in synergistic extensor motoneurones (reviews: Schomburg 1990; Jami 1992; Jankowska 1992). However, the inhibitory effects of contraction-evoked Ib afferent activation on motoneurones appear to be restricted to a phasic inhibition at the beginning of contraction (Perrier et al. 2000). In our experiments, there was no sign of postsynaptic inhibition. In general, however, Ib-evoked inhibition underlies modulation by inputs from group III and IV muscle afferents. In experiments on decerebrate and spinalized cats, chemical activation of group III and IV afferents from calf muscles facilitated the non-reciprocal group I inhibition exerted by gastrocnemius-soleus Ib afferents onto extensor motoneurones (Schomburg et al. 1999). By contrast, in man, inhibition evoked in soleus α-motoneurones by electrical stimulation of group I fibres of the medial gastrocnemius nerve was inhibited by nociceptive afferents of the dorsal foot (Rossi and Decchi 1997; Rossi et al. 1999). These differences probably were due to differences in animals, preparations and experimental sets. In our experiments, group Ib inhibition and its modulation by group III and IV muscle afferents appear to have played no significant role.

Finally, the stretch-evoked firing of motoneurones should activate recurrent inhibition via Renshaw cells, whose discharge can be modulated by activity of group III and IV muscle afferents. In fact, in decerebrate and spinalized cats, Renshaw cell firing was mostly reduced after intramuscular injection of bradykinin and serotonin (Windhorst et al. 1997). Furthermore, in experiments similar to the present ones, the intensity of recurrent inhibition has been shown to be reduced after muscle fatigue (Kalezic et al. 2004). However, while this suppression of recurrent inhibition should have disinhibited the related motoneurones, in our experiments, the disinhibition was apparently overpowered by suppressive processes, such as enhanced presynaptic inhibition.

While FST predominantly suppressed stretch-evoked motoneurone responses, the responses to the first stretch after each fatiguing contraction increased. This increase was probably caused by after-effects in the activity of muscle spindles due to thixotropic properties of intrafusal and extrafusal muscle fibres (Kostyukov and Cherkassky 1992; Proske et al. 1993; Kostyukov 1998). These after-effects could have come about by passive stretching of the spindles during relaxation of the extrafusal muscle after cessation of FST and/or by a post-activation increase in mechanical stiffness of the intrafusal muscle fibres due to their activation via β-axons during FST. The present study and previous investigations (Kouchtir et al. 1995; Perrier et al. 2000) have adduced evidence that low-intensity electrical stimulation of the G-S muscle nerve also activated β-axons, which would activate muscle spindles and via this route

exert reflex effects on α- and γ-motoneurones. That the discharge of γ-motoneurones may be increased by muscle fatigue via afferent feedback has been shown earlier (Ljubisavljevic et al. 1992), but in our preparations with cut ventral roots, the rate increase would not in turn affect the discharge rate and sensitivity of muscle spindles, which it would in intact preparations.

Conclusions

Stretch-evoked EPSPs and spike firing in G-S motoneurones were predominantly suppressed after fatiguing contractions of the muscles they innervate, with the exception of a post-contraction increase of the first EPSP after FST, which was most likely due to after-effects in the activity of muscle spindle afferents. The post-fatigue suppression of the EPSPs and spike activity was followed by restoration within 60–100 s. Additional applications of FST augmented the intensity of the post-fatigue suppression of EPSPs, with the spike activity sometimes disappearing completely. Fatiguing stimulation was accompanied by EPSPs at latencies suggesting activation of muscle spindle group Ia afferents via stimulation of β-fibres. The suppression of the stretch-evoked motoneurone responses most likely resulted from fatigue-induced activity of group III and IV muscle afferents. Presynaptic inhibition could have been one of the mechanisms involved, but homosynaptic depression in the FST-activated group Ia afferents may also have contributed.

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