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H-reflexes of different sizes exhibit differential sensitivity to low frequency depression

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Abstract

The amplitude of the H-reflex declines when activated repetitively. The magnitude of decline is greater when the amplitude of the H-reflex is small. To explore whether pre- or postsynaptic factors contribute to the differences observed in H-reflexes of different sizes, changes in the amplitude of H-reflexes of different sizes were measured during a train of stimulation in 10 normal subjects. Amplitudes of different sizes were obtained using differing stimulus intensities or during superimposed contraction, two manipulations which differently affect the number of active afferents and the excitation of the motoneuron pool. Small amplitude H-reflexes depressed to a lower plateau than larger H-reflexes and superimposed contraction did not alleviate the depression during each train. Nearly all the decline in larger amplitude H-reflexes occurred in a component that was in common with smaller amplitude H-reflexes. This suggests that the depressibility of the earliest activated units is greater than later activated units in H-reflexes and that the magnitude of decline is affected by prior activity as well as size. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

Stretch reflexes exhibit considerable variability. One factor that contributes to this variability is the history of recent activity. In animals, the amplitude of successive reflexes declines if elicited repeatedly, even at intervals as long as 10 s, a phenomenon termed low frequency depression (Lloyd and Wilson, 1957). In humans, the H-reflex, an electrically evoked homologue of the soleus stretch reflex, also displays low frequency depression with repetitive activation, at intervals similar to that seen in animals (Magladery and McDougal, 1951). This observation has led to the common practice in clinical neurophysiology of pausing for several seconds between successive stimuli when evaluating H-reflexes.

Low frequency depression is thought to result from changes localized to the presynaptic terminal, since animal studies have shown that the amplitude of composite excitatory postsynaptic potentials (EPSPs) decreases with repetition, at the same time that there is little change in the membrane conductance of the postsynaptic motoneuron (Curtis and Eccles, 1960; Hultborn et al., 1996). Until recently, the leading candidate mechanism for this depression was assumed to be presynaptic inhibition through GABA-ergic interneurons (Decandia et al., 1967; Delwaide, 1973). However, when low frequency depression of H-reflexes was compared to the more classical presynaptic inhibition elicited by antagonist afferents, the duration of presynaptic inhibition was an order of magnitude shorter than low frequency depression (Hultborn et al., 1996; Crone and Nielsen, 1989; Kohn et al., 1997). Such findings have led to the hypothesis that low frequency depression is distinct from presynaptic inhibition and may result from processes intrinsic to the presynaptic bouton which are excited by activation of the terminal (Hultborn et al., 1996).

While studying the phenomenon of low frequency depression, we noted that smaller H-reflexes, evoked by lower stimulus intensities, seemed more susceptible to low frequency depression than larger reflexes, evoked by higher

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stimulus intensities. Since the amplitude of an H-reflex reflects, in part, the number of motoneurons firing in response to the afferent volley, such differences could arise from several possible sites. For example, the characteristics of synapses distributed among early and late recruited units in the motoneuron pool could vary systematically. Size differences of muscle units and their electrical contribution to the H-reflex could also be a factor. To localize where the apparent differences arise, the decline in the amplitude of H-reflexes of different sizes was measured, using changes in stimulus intensity or tonic contraction to manipulate the initial size of the H-reflex. These manipulations change the reflex amplitude in different ways. Tonic, isometric voluntary contraction excites the motoneuron pool, with relatively less activation of stretch reflex afferents, whereas increasing stimulus intensities activate additional afferents and motoneurons.

2. Methods

Ten healthy normal volunteers, aged 23–56 years gave their written informed consent for the study protocol, which was approved by the institutional review board. Subjects were seated in a reclining chair with the hip flexed to about 120°. The thigh was loosely restrained with the knee slightly flexed to 160°, and with the ankle plantar flexed to 110°. The foot was strapped to a rigid frame with a metal foot plate activating a force transducer that permitted visual feedback of isometric plantar flexor force. Most subjects were able to produce 40–50 lb force in the apparatus. Soleus EMG was recorded with surface electrodes using a belly-tendon configuration. A Dantec Counterpoint electromyograph with amplifier filter settings of 2 Hz and 10 kHz was used to record EMG activity, which was digitized at 10 kHz using GWI boards and software and a Macintosh computer. The posterior tibial nerve was stimulated in the popliteal fossa with 1 ms duration pulses using an adjustable monopolar ball electrode held in place with a strap around the knee and a remote anode on the patella. Constant current stimulation was delivered through the electromyograph in parallel with a Grass S-11 stimulator and isolation unit.

At the beginning of each session, the stimulus intensities that evoked H-reflexes and M-waves of various sizes were determined, and for each subject, H-reflex size was quantified as a percentage of his/her maximal M-wave (M_{max}). Only stimulus intensities that evoked H-reflexes on the ascending limb of the recruitment curve were used. Two stimulus intensities were used to evoke H reflexes of differing amplitudes. Trains of 10 stimulus pulses at 1 s intervals were used to elicit low frequency depression, as described by Kohn et al., 1997. Intervals of 1 min or longer were allowed between stimulus trains, and 8–12 trials were averaged for each condition. Amplitudes of the last five H-reflexes in each train were averaged to compute a plateau value. A small M-wave was generally present for higher stimulus intensities, and its amplitude was observed not to change during the stimulus train (Fig. 1). For stimulus trials with voluntary contraction, the subject was instructed to produce and hold steady force at the target level when given a go signal. Once the subject indicated he/she had achieved a steady force, typically a few seconds, the train of stimulus pulses was given. Typically, this low level of contraction was maintained for about 30 s in each trial. Trials with different conditions (e.g. force and no force) were interleaved.

3. Results

3.1. Smaller reflexes have more marked low frequency depression

The mean H_{max} was 48% with a range of 34–82% M_{max} in this group of healthy volunteers. H-reflexes of two initial sizes, 20% M_{max} and approximately 50% M_{max} (or H_{max} if smaller than 50%) were elicited with trains of 10 stimulus pulses at a frequency of 1 per second in 8 subjects, using two intensities of stimulation. The first reflex in the pulse train was largest for reflexes of both sizes. Reflexes of initial amplitude equal to 20% M_{max} declined to a plateau of 5% M_{max}. Reflexes of initial amplitude equal to 50% M_{max}, also declined, but the plateau was much higher, at 22% M_{max} (Figs. 1 and 2A, B). In relative terms, the 75% decline of small reflexes was greater than the 56% decline of larger reflexes (paired $t = 3.0$, $P = 0.02$), although the absolute magnitude of depression was less.

To determine whether depression was distributed equally among the earlier and later recruited components of the H-reflex, a train of 20 pulses at 1 Hz was used. Pulses 1–10 were of the stimulus intensity that evoked reflexes of 20%

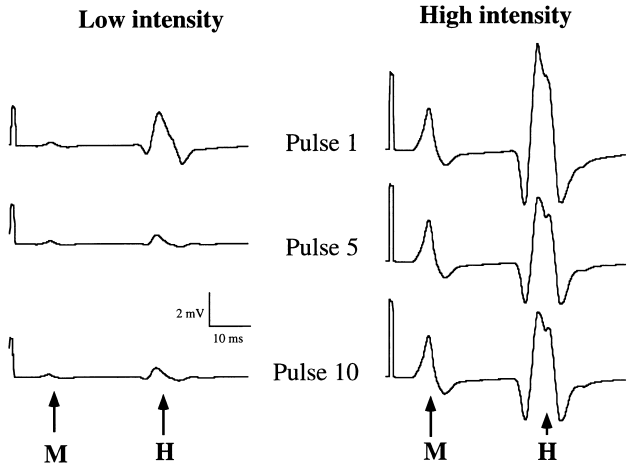

Fig. 1. H-reflex and M waves in response to first, fifth and tenth pulse in stimulus train of low intensity or high intensity in one subject. Each reflex is an average of 8 trials.

M_{max}, while pulses 11–20 were of the same intensity that evoked reflexes of 50% M_{max} in interleaved trials. The amplitude of the H-reflex elicited by the pulse 11 was approximately the same as the H-reflex elicited by the first pulse, which was of a lower stimulus intensity. The H-reflex declined from pulses 1 to 10, as expected, but showed very little change in amplitude from pulse 11 to 20 (Fig. 2C). The amplitude of the reflex elicited by pulse 11 did not differ from the plateau that had been obtained during the interleaved trials of H-reflex trains of initial amplitude 50% M_{max} (paired $t = 1.90$, $P = 0.10$). Thus, nearly all the depression of the larger H-reflexes could be accounted for by depression of the same components in the smaller amplitude H-reflexes.

3.2. Voluntary contraction does not relieve differences in depression

If the EMG output of the motoneuron pool were to increase in a non-linear manner with progressive activation of the motoneuron pool, low frequency depression could be masked during larger reflexes. To assess this possibility, H-reflexes were elicited during tonic contraction, which effectively 'clamps' the motoneuron pool at a higher level of excitation. In 6 subjects, stimulus trains consisting of 20 pulses (10 pulses at the low intensity followed by 10 pulses of higher intensity) were delivered with the leg relaxed or during the slight tonic contraction. Subjects applied 1 lb of plantar flexor force against a rigid foot plate using visual

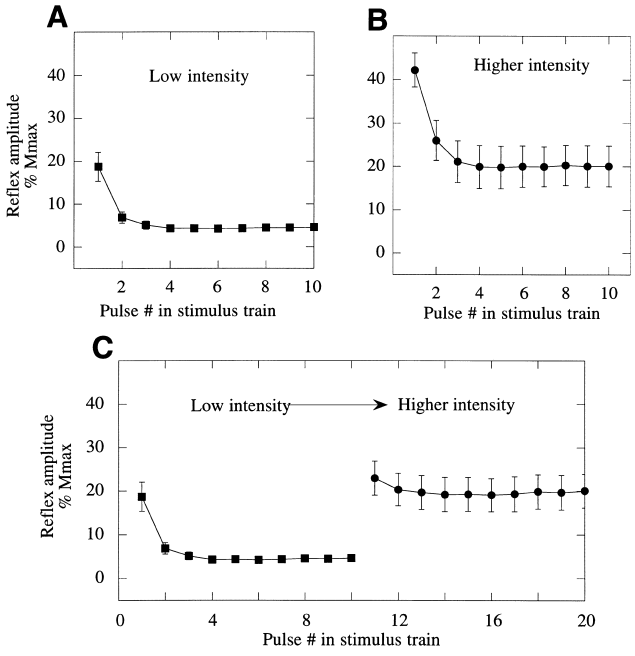

Fig. 2. Decline of the H-reflex amplitude with repetitive stimulation at 1/s in 8 subjects at rest using (A) stimulus intensity evoking initial H-reflex amplitude of 20% M_{max}, (B) stimulus intensity evoking initial H-reflex amplitude of 50% M_{max}, (C) sequential application of same stimulus intensities used in A and B in interleaved trials. Mean + standard error.

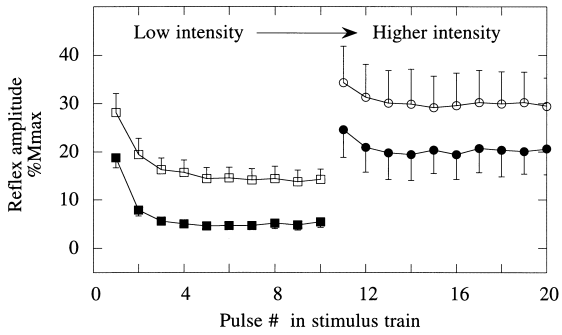

Fig. 3. Decline of the H-reflex amplitude with repetitive stimulation at 1/s in 6 subjects at rest (filled symbols) and during tonic isometric contraction (open symbols). Mean + standard error.

feedback of the force output to maintain a steady force level between stimuli in the train. This level was less than 5% of maximal voluntary force for all subjects, and was maintained for no more than 30 s at one time. The amplitude of the initial H-reflex obtained during tonic contraction was consistently larger than the initial H-reflex amplitude when the same intensity stimulus was delivered at rest. Stimuli that evoked a mean reflex amplitude of 19% M_{max} at rest evoked a mean reflex amplitude of 28% M_{max} during tonic contraction. H-reflexes declined 13.8% at rest and 13.9% during contraction. When plotted, the curves showed a parallel decline with a shift in the plateau from 5% M_{max} to 14% M_{max} (Fig. 3) during contraction for smaller reflexes and from 20% to 30% for larger reflexes. As at rest, there was little additional depression from pulse 11 to pulse 20. Thus low frequency depression is not fully accounted for by differing levels of excitation of the motoneuron pool.

3.3. Additional control experiments

To check the possibility that repeated stimuli induced abrupt changes in the excitability of the motoneuron pool, in one subject, a train of 20 pulses, all low intensity, was given. His reflex declined from 22% M_{max} initially to a plateau of 6% M_{max}, which was maintained throughout the train. In two other subjects, trains of 30 stimulus pulses were given: 1–10 low intensity, 11–20 high intensity, 21–30 low intensity. Reflex amplitudes during the last 10 pulses were comparable to the plateau obtained from the first set. It is unlikely, therefore, that induction of bistable states of motoneuron excitability (Kiehn, 1991) explains the lesser depression of larger reflexes.

During voluntary contraction, muscle spindles are also activated, which increases activity in the Ia afferents beyond the experimental stimulus trains. Naturally evoked activation could hasten the process of depression. To assess this possibility, the time constants of the decline of the first 10 reflexes were calculated at rest and during contraction. An exponential curve was fitted to the amplitude train and time

constants of the decline were calculated to be 1.94 s at rest and 1.98 s during tonic contraction. These were not significantly different (paired sample t test: $t = 1.71$, $P = 0.15$). This suggests that the level of activity in primary afferents produced by the contraction was insufficient to influence the depression detected.

4. Discussion

This study confirms the clinical impression that larger H-reflexes, evoked with higher stimulus intensities, exhibit relatively less low frequency depression than smaller reflexes and remain at a higher amplitude. However, size alone did not determine the magnitude of depression. Reflexes whose postactivation amplitude was adjusted by increasing the stimulus intensity exhibited less depression than reflexes of the same size without a recent history of activity. Conversely, reflexes made larger by tonic voluntary contraction depressed to a similar extent as the smaller reflexes elicited by the same intensity stimulus at rest. Nearly all of the depression occurred in a component of the reflex that was common to small and large reflexes. This suggests that there may be two populations of units within a reflex: depressible and non-depressible.

Earlier studies in cats (Lloyd and Wilson, 1957) as well as humans (Von Boxtel, 1986), also found size differences in sensitivity of reflexes to low frequency depression. In a simple monosynaptic reflex arc, such sensitivity could occur at either pre- or postsynaptic elements. In animals, motor units that generate small forces are recruited earlier during reflexes (Henneman, 1957; Zajac and Faden, 1985), and smaller reflexes have a proportionately greater contribution from this population of motor units. This relationship appears to hold true in human H-reflexes as well (Buchthal and Schmalbruch, 1970). Selective vulnerability of the early recruited motoneurons within the pool, or the synapses upon them, could theoretically give rise to the pattern observed. Because the differences in H-reflex depression persisted during voluntary contraction, when the state of postsynaptic excitability is increased, the source of this difference is likely to reside within the presynaptic terminals. In animal studies, Ia EPSPs onto different subtypes of motoneurons have different absolute amplitudes, different susceptibilities to posttetanic potentiation, and different responses to high frequency stimulation (Lev-Tov et al., 1983; Collins and Mendell, 1984; Collins et al., 1988). Type S motoneurons have large initial EPSPs which decline in amplitude during high frequency stimulation, whereas type F motoneurons have smaller initial EPSPs that tend to show facilitation. Individual motor units also may exhibit either an increased or decreased firing probability to high frequency stimulation of reflex afferents (Gossard et al., 1994). If such differences in EPSP behavior are also operative at lower frequencies, it is conceivable that they could underlie the observed differences in H-reflex depression.

The alternative explanation is that the apparent differences in sensitivity are explained by non-linearity in the output of the motoneuron pool, and this could occur in two ways. Differences in the number or size of muscle fibers in early and late recruited motor units recorded with surface EMG have been proposed to explain the initial sensitivity of small amplitude H-reflexes to facilitation and depression through interneuronal pathways (Crone et al., 1990). If muscle fiber innervation ratios of the early recruited motor units are smaller, the surface EMG would exhibit a greater than linear progressive increase for activation of successive units. The activation of the motoneuron pool has also been reported to have a non-linear relationship to the size of the input signal by Hultborn et al. (1996), who found that short stimulus trains to homonymous nerves produced a greater depression in the amplitude of ventral root reflexes than in the amplitudes of composite Ia EPSPs in individual cat motoneurons. They suggested that a high threshold for motoneuron firing allowed small changes in EPSP amplitude to have a disproportionate effect on reflexes. An extension of this argument is that smaller reflexes are more vulnerable than larger reflexes because the motoneuron pool is less securely activated. These alternative explanations all lie in the efferent limb of the reflex arc, and are best countered by our finding that voluntary contraction, which shifts the output of the motoneuron pool to a higher level, did not remove synaptic depression nor change the relative differences between small and larger H-reflexes.

In another study, voluntary contraction was reported to abolish frequency dependent depression (Burke et al., 1989), and its use was advocated for assessing H-reflexes for clinical evaluation in a variety of muscles in which they are difficult to elicit at rest. We fully agree that voluntary contraction can enhance detection of H-reflexes by increasing their amplitude. In that report, however, the mean amplitudes of reflexes at different frequencies were compared, corresponding to comparisons between the plateau amplitudes in the current study, rather than the depression that occurs following the first H-reflex after a period of non-activity. In that study a stronger degree of contraction may also have been used, since subjects were instructed to increase contraction until depression was relieved. It is possible that stronger contractions cause sufficient discharge of spindles to bring about some depression prior to stimulus delivery. That seems unlikely to be the case with the slight contractions used in this study, since the decline in reflex amplitude occurred with the same time constant at rest and during slight contraction.

During a voluntary ramp contraction, interneuronally mediated presynaptic inhibition declines immediately prior to and in the first few hundred milliseconds after movement onset, before returning to the baseline level obtained at rest (Meunier and Pierrot-Deseilligny, 1989). In our study, the typical interval of several seconds required to attain and verbalize the target force timed the onset of the stimulus train well beyond this period when dynamic

changes in presynaptic inhibition contribute to changes in reflex size. Weak autogenetic postsynaptic inhibition, such as Renshaw or Ib inhibition, would also be overcome by descending drive during voluntary contraction. Of note is that studies in humans (Hultborn et al., 1996; Kohn et al., 1997) have presented evidence that there is little postsynaptic inhibition during low frequency depression in reflexes elicited at rest, judged by the amplitude of transcranial magnetically evoked motor potentials.

The cellular processes producing 'postactivation' depression are still unknown, but it has now been shown for a variety of modes of activation, including single electrical pulses, tendon taps, and passive stretch (Nielsen et al., 1995). Depletion of transmitter following single stimuli seems a less likely explanation for depression than changes in the probability of neurotransmitter release. In animal preparations individual boutons have different probabilities of transmitter release (Redman and Walmsley, 1983; reviewed in Faber et al., 1991) and it may be that activity dependent phenomena represent changes between probability states among the population of boutons contributing to composite EPSPs. The dynamics of neurotransmitter release involve several steps in the cycling of synaptic vesicles, of which the whole cycle may take up to a minute. Vesicles are docked near the synaptic release sites, before becoming 'primed', a process that allows fast Ca^{2+} triggered membrane fusion (reviewed in Sudhof, 1995). Priming, which may be rate limiting for exocytosis, involves the formation of a complex between synaptic vesicle and plasma membrane proteins. The larger response to the first pulse may reflect the release of transmitters from a population of 'primed' vesicles with subsequent pulses engaging vesicles less ready for exocytosis. Synaptic vesicle proteins, such as synapsins, appear necessary for the expression of some simple activity dependent changes, such as paired pulse facilitation (Rosahl et al., 1995) and it is conceivable that differences in the content or phosphorylation of such proteins in individual boutons could allow for a variety of activity dependent behaviors.

Low frequency depression may be a mechanism to minimize afferent input from stretch receptors during sustained or repeated muscle stretch, while maximizing the reflex response to perturbation in resting muscle. This mechanism would decrease oscillations, and possibly prevent reflex clonus, as is suggested by the diminution of postactivation depression in patients with spasticity (Nielsen et al., 1995). Differences in the susceptibility of H-reflexes of different amplitudes to low frequency depression may serve to concentrate the damping effect upon those motor units that are most likely to be activated by small stretches or perturbations, without disabling the stretch responsiveness of higher threshold motoneurons. Differences in the susceptibility of different terminals of the same afferent could allow rapid adaptation of Ia inputs at motoneuron synapses while maintaining a constant signal to other targets such as in ascending pathways.

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