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Reciprocal Inhibition of Soleus Motor Output in Humans During Walking and Voluntary Tonic Activity

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SUMMARY AND CONCLUSIONS

1. The extent to which an active, human motoneuron pool can be inhibited via short-latency inhibitory pathways was studied by stimulating the common peroneal nerve and recording the inhibition of on-going soleus electromyographic (EMG) activity. The responses were compared at the same EMG level during walking and tonic voluntary activity to determine whether the inhibition was task dependent.

2. In both tasks the amount of inhibition (measured as the depression in rectified, filtered, and averaged EMG activity) increased approximately linearly with the amount of motor activity, as determined from the mean EMG level before stimulation (correlation coefficient ≥ 0.9). No difference in the amount of inhibition was found between the two tasks at the same stimulus and EMG levels.

3. Previously published studies based on the H-reflex method have reported that the amount of inhibition decreases with the amount of motor activity. On the contrary, single-unit studies and the present results suggest that segmental inhibitory reflexes retain their capacity to mediate a rapid reduction of motoneuronal discharge during voluntary activity. This inhibition may be important in regulating the amount of activity early in the stance phase of walking and during the transition from stance to the swing phase.

4. Analytic results are derived in an APPENDIX that should be of general interest in interpreting the inhibition of motor units from a peristimulus time histogram (PSTH). The linear correlation between inhibition and level of voluntary activity can be explained if newly recruited units are strongly inhibited by the stimulus, whereas previously active motor units are inhibited relatively less, as their firing rate increases with increasing background activity.

INTRODUCTION

In contrast to the many studies of autogenic excitatory reflexes from group Ia afferents in humans, corresponding inhibitory reflexes to antagonist α-motoneurons have been studied far less. The most widely held view, based on H-reflex studies, is that the inhibition mediated by Ia interneurons decreases when the motoneuron pool is active and increases when the antagonist motoneuron pool is active (e.g., Cavallari et al. 1984; Day et al. 1984; Shindo et al. 1984). These alterations in the efficacy of inhibitory action are attributed to a parallel convergence of descending inputs onto Ia interneurons and α-motoneurons (Hultborn 1972; Lundberg 1966, 1970).

However, more recent H-reflex studies of the human ankle musculature (Crone et al. 1985; Iles 1986) failed to confirm an increase in the potency of Ia inhibition acting on an extensor (soleus) during progressively more forceful flexor (tibialis anterior) contractions. The discrepancy may be due to differences in the details of applying the H-reflex methodology. In particular, small test reflexes are more prone to inhibition than larger test reflexes (Crone et al. 1985; see also Mazières et al. 1984; Meinck 1980). Thus a failure to maintain the same test-reflex amplitude throughout the range of muscle activity investigated may lead to erroneous conclusions (Crone et al. 1985).

A more basic concern arises from the method itself. The H-reflex represents the synchronous discharge of α-motoneurons in response to a very large excitatory postsynaptic potential (EPSP) induced by the electrical stimulation of Ia afferents in the muscle nerve. Summation of small or moderately sized inhibitory postsynaptic pulentials (IPSPs) resulting from stimulation of Ia afferents in the antagonist nerve may be incapable of significantly shunting the very large EPSPs and so preventing depolarization of the membrane to threshold. Therefore inhibitory action on motoneurons may go undetected when it is measured by its effect in reducing a large synchronous discharge.

More recently, Ashby and his colleagues (Ashby and Wiens 1989; Bayoumi and Ashby 1989; Mao et al. 1984) have examined the effects of group I stimulation on the tonic activity of single motoneurons. Evidence of inhibition is often observed in a peristimulus time histogram (PSTH), although the pattern of projections differs somewhat from that observed in studies on experimental animals (summarized in Fig. 4 of Bayoumi and Ashby 1989). Both H-reflex and single-unit–inhibition studies have only been attempted with tonic contractions, and their relevance to movements such as walking is therefore unknown.

We have tried to improve understanding of segmental inhibition in the extensor musculature of the human ankle in two main respects. First, we have reappraised the relation between the amount of inhibition and the amount of tonic voluntary motor activity in the inhibited muscle by modifying a technique used by Agarwal and Gottlieb (1972). An IPSP may not be capable of preventing the membrane of a tonically firing α-motoneuron from depolarizing to threshold, but it will delay the interspike interval. This can be seen as a depression in the PSTH of single motor units (Ashby and LaBelle 1977; Kudina 1980). Similarly, the inhibition will produce a depression in the mean rectified EMG activity that represents a weighted average of the PSTHs of all discharging motor units. The method, therefore, has the merit of assessing inhibition by its effect

in decreasing the asynchronous motor output under natural conditions.

Second, we compared the amount of inhibition of soleus muscle during a tonic activity (standing) with that during locomotion in the same subject. This was done to determine whether there was a task-dependent modulation of the transmission efficacy of Ia interneurons, as has been shown for the autogenic Ia excitatory reflex on soleus motoneurons (Capaday and Stein 1986, 1987a). The characteristics of this reflex (threshold and gain) vary systematically in going from standing to walking and running and are specifically adapted to the functional requirements of each motor task (Stein and Capaday 1988). A preliminary account of some of these results has been presented (Capaday and Stein 1987b).

METHODS

The experiments reported in this paper were done on 15 normal human subjects ranging in age between 21 and 49 y. All subjects gave their consent after being fully informed of the purpose and procedures of the experiments.

Experimental procedures

Reciprocal inhibition of the ankle extensor muscle, soleus, elicited by electrical stimulation of the common peroneal (CP) nerve, was investigated at several levels of tonic contraction and throughout the stance phase of walking. For the tonic contractions subjects stood upright and maintained a required level of soleus electromyogram (EMG) by generating plantar flexion. An analog voltmeter, calibrated so that a full-scale deflection of the needle corresponded to the subject's maximum voluntary soleus EMG (high-pass filtered 10 Hz, rectified, low-pass filtered 20 Hz), provided visual feedback of the level of soleus activity. During each test subjects were instructed to maintain a given percentage (up to 80%) of their maximum EMG for a period of ~30–60 s during which 25–30 stimuli were applied to the CP nerve in a pseudorandom sequence.

Similarly, inhibition of the soleus muscle during the stance phase of walking (i.e., the phase of the walking cycle during which it is active) was investigated by applying stimuli to the CP nerve in a pseudorandom sequence throughout the locomotor cycle. The subjects walked on a treadmill at a speed of 4 km/h. The average step-cycle time was 1.1 s, of which the stance phase comprised ~0.6 s. Walking was studied first to allow the experimenters to determine the range of activation of soleus during locomotion. The subject was then asked to approximately reproduce these levels tonically. Furthermore, the same effective stimulus strength (see below) was used during tonic activity as during walking.

EMG recordings

Bipolar EMG recordings were obtained from fine stainless steel wires, each inserted with appropriate aseptic precautions into the soleus muscle by a hypodermic needle. The wires were multistranded, teflon coated, and bared at their tip for ~2–3 mm. Such intramuscular electrodes were used to reduce recording of the very large and broad M-wave of the ankle flexors and foot everters produced by stimulation of the CP nerve. This method of recording also reduced contamination of on-going soleus EMG activity by that from other nearby ankle extensors such as gastrocnemius. In addition, bipolar recordings were obtained from surface Ag-AgCl disc electrodes (diameter 0.7 cm) placed over the soleus and tibialis anterior (TA) muscles.

Electrical stimulation

Electrical stimuli were applied to the CP nerve by a surface disc electrode of the same type as that used for surface EMG recordings. The electrode was placed near the head of the fibula, attached to the skin with adhesive tape, and secured by an elastic rubber strap around the leg. The stimulus return electrode, which consisted of a large metal plate covered by gauze and wetted by saline, was attached just below the knee by a rubber strap. Stimulus pulses were 1 ms in duration and delivered in both tasks in an equiprobable pseudorandom sequence with a minimum interstimulus interval of 0.4 s and a maximum of 4 s.

M-waves were recorded over the TA muscle and were used as a measure of effective stimulus strength. The M-wave threshold was determined in the quietly standing subject and a value close to 1.5 times motor threshold (MT) was used during walking and tonic activity. In many subjects a stimulus of this strength produced an M-wave of approximately constant amplitude throughout the stance phase of locomotion. This is in marked contrast to stimulation of the tibial nerve in the popliteal fossa during locomotion (see Capaday and Stein 1986, 1987a), where the effective stimulus strength varies widely throughout the locomotor cycle. Because of the large angular displacements at the knee during walking, the distance between the stimulating electrode and the tibial nerve changes.

In those subjects with >20% variation of the M-wave at a fixed stimulus strength, the experiment was repeated at several stimulus intensities (up to 2 × MT). The records of soleus inhibition at various times during the stance phase could then be compared using trials that produced nearly equal TA M-waves.

Data analysis

During tonic activity the effects of the stimuli applied to the CP nerve were determined by averaging (Digital Equipment, PDP 11/40 computer) the high-pass (10-Hz) filtered, rectified, and low-pass filtered (100-Hz) intramuscular EMG responses of the soleus. Filtering at 100 Hz produces some smoothing of the rec-

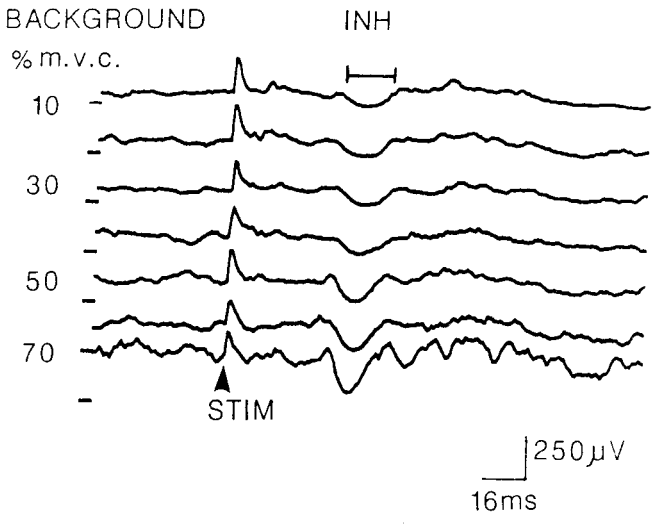

FIG. 1. Inhibition of tonic voluntary EMG activity in soleus by a stimulus of 1.5 × MT to the common peroneal nerve. EMG activity was rectified, filtered, and averaged (n = 25) as described in METHODS. Strength of contractions increases from *top* to *bottom*. Contraction strengths, as indicated, varied from ~10 to 70% of the maximum voluntary contraction (m.v.c.), respectively. Short dash at the *left* of each record represents the 0 DC level of the record above it. Mean level of activity in the background period before the stimulus artifact was compared with the mean level during the period of inhibition indicated by the bar.

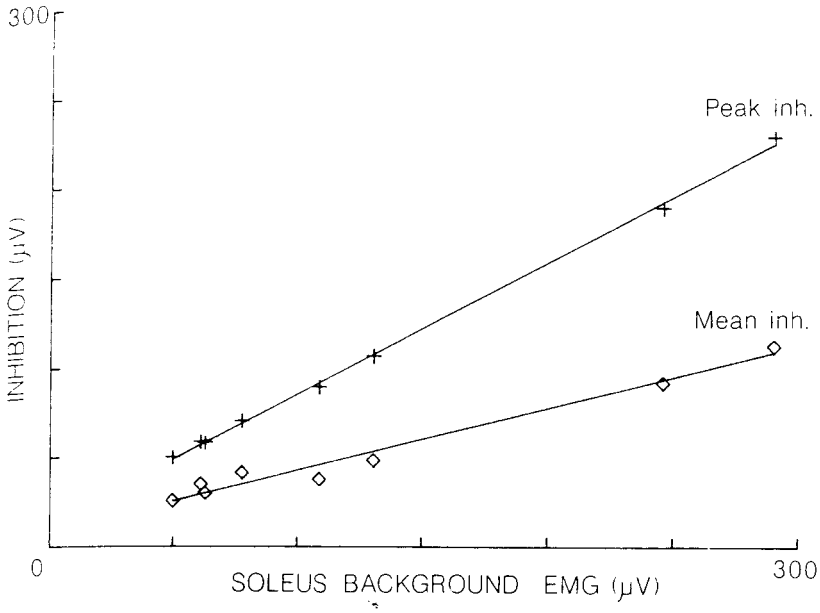

FIG. 2. Relation between the amount of inhibition and the background activation level as measured by the mean value of the rectified and filtered EMG. Both the mean decrease in the EMG (◇) during the inhibitory period (see Fig. 1) and the peak decrease (+) are plotted against the background EMG level. Slopes of lines were 0.35 and 0.73, and linear regression coefficients were $r = 0.99$. Thus transiently nearly ¾ of the ongoing activity ceased and was delayed for ~20 ms.

ords, but the corresponding time constant $T = 1/200\pi = 1.6$ ms is short compared to the time course of the events recorded here.

The total duration of each average was 150–250 ms including a prestimulus period of 50–100 ms. This prestimulus (background) period was used to calculate the average level of EMG activity preceding the inhibitory stimulus. To quantify the effects of the stimulus at each of the tonic levels used, the mean level of the depression in the EMG over an interval determined by visual inspection of each record (Fig. 1) was computed. The amount of inhibition was measured as the difference between the mean background level and the mean level of the depression (referred to as the mean amount of inhibition). The mean amount of inhibition was then plotted versus the background activity level. A similar method of measurement was used by Matthews (1986) to study the inhibitory effects of vibrating the triceps on voluntary motor activity in the biceps.

The data were processed on-line for two purposes. First, to determine the amplitude of the TA M-wave in various phases of the step cycle, the cycle was divided into 16 parts, each ~70-ms in duration. The computer used the latency between the step marker and the stimulus marker to determine in which of these 16 phases of the step cycle the stimulus occurred. Responses that occurred in the same phase were averaged together. Second, the same method was used to obtain the responses of the soleus to stimulation of the CP nerve at various phases of the step cycle. Further details of the method of "phase-dependent" averaging can be found in previous publications from this laboratory (Akazawa et al. 1982; Capaday and Stein 1986, 1987a).

The on-line facility allowed the experimenter to determine *1*) whether the stimulus strength initially chosen was producing comparable M-waves throughout the step cycle and *2*) whether the stimulus to the CP nerve was producing inhibition of the soleus during the various phases of the step cycle (i.e., whether the stimulus was sufficiently strong or properly located over the nerve). The data obtained during walking (soleus and TA EMGs as well as the step marker and stimulus marker) were also recorded on FM magnetic tape for off-line analysis. The tape-recorded data were used, when necessary, to check the on-line analysis and to obtain a measure of the average EMG patterns during unperturbed step cycles. The computer selected and averaged step cycles in which no stimulus occurred to provide a representative measure of the motor output during normal step cycles of an experimental trial that typically lasted ~6–7 min.

RESULTS

Inhibition of tonic soleus activity

Stimulation of the CP nerve inhibited soleus EMG activity at all levels of voluntary activity tested (Fig. 1). The

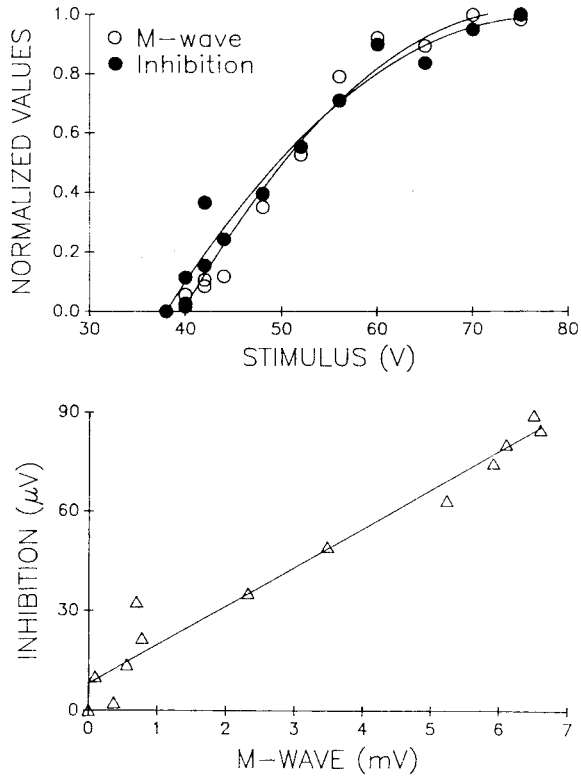

FIG. 3. As the stimulus to the CP nerve was increased, both the M-wave in the TA muscle (○) and the inhibition of ongoing activity in the soleus muscle (●) increased in parallel. Both are plotted after normalization to the maximum value in the *top* of the figure and in unnormalized form against one another in the *bottom*. Parabolic curves, *top*, and the straight line, *bottom*, were fitted to minimize the mean square error, and all had correlation coefficients >0.98.

records are from one subject and are arranged in order of increasing activity level from *top* to *bottom*. The amount of inhibition (measured as the difference between the background level and the mean level of the depression in the rectified EMG) always increased in proportion to the activity level (Fig. 2). In the 15 subjects studied the linear correlation coefficient (r) between the average amount of inhibition and the background activity level was at least 0.9. The inhibitory phenomenon always began at nearly the same latency as the H-reflex in the same muscle, which is strong evidence that it is of segmental origin.

The inhibition in Fig. 2 decreased the EMG activity by ~35% on average over the period indicated by the horizontal bar. The peak value of the inhibition (maximum depression) is also plotted in Fig. 2 (×'s) against the background EMG level. The slope of the least-squares fitted line is nearly 0.75. These results clearly show that most of the discharging motor units can be momentarily inhibited (i.e., their firing time is delayed) at all levels of motor activity. Thus an inhibitory pathway to the soleus α-motoneuron pool is not turned off as the activity in the pool increases.

To analyze what receptors are responsible for the inhibition, the level of the stimulus was varied at a fixed level of background activity. The M-wave produced by the stimulus in the TA muscle and the inhibition of voluntary activity in the soleus muscle increased in parallel as the stimulus level was increased (*top*, Fig. 3), which suggests that group I fibers were responsible for both effects. The M-wave and the inhibition were normalized for ease of comparison. In the *bottom* part of Fig. 3 the unnormalized data are plotted against one another, and the parallel increase is quite striking (correlation coefficient = 0.98). The intercept of the fitted straight line has a slightly positive value, which suggests that the inhibition was activated at a slightly lower threshold than the M-wave. Similarly, the best-fitting parabolic curve for the inhibition in the *top* of Fig. 3 intercepts the stimulus axis at a slightly lower value than that of the M-wave. The interpretation of these findings in regard to

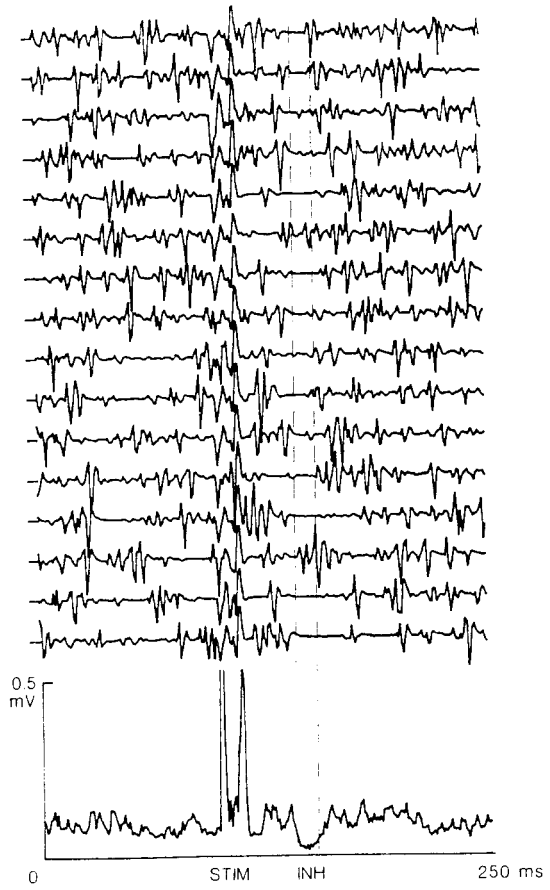

FIG. 4. Raster of 16 sweeps of unrectified EMG is shown together with the average rectified EMG (n = 30 sweeps) on the same time scale. Although EMG activity is clearly less common during the period of inhibition after the stimulus, there is no interval when activity is completely absent on all traces. Vertical scale represents 0.5 mV for the average and 2 mV for the unrectified traces. As a well as a stimulus artifact, there was some pickup of the M-wave from the dorsiflexors in this experiment. Same experiment as Fig. 3; stimulus = 1.3 MT.

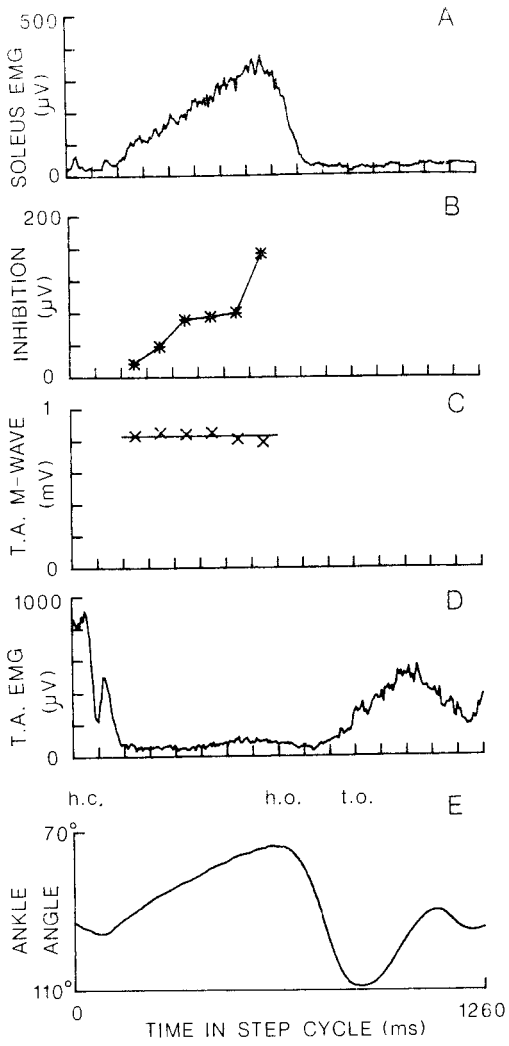

FIG. 5. As the EMG activity in the soleus muscle increases during the step cycle (A) so too does the inhibition (B) from stimulating the common peroneal nerve, even though the M-wave (C) elicited by the stimulus in the tibialis anterior (TA) muscle does not change. From the EMG pattern in (D) the inhibition of soleus clearly occurs when the TA muscle is essentially inactive. Finally, changes in ankle angle (E) are shown together with an indication of when the heel contacts the ground (h.c.), when it comes off the ground (h.o.), and when the toe comes off the ground (t.o.) to end the stance phase.

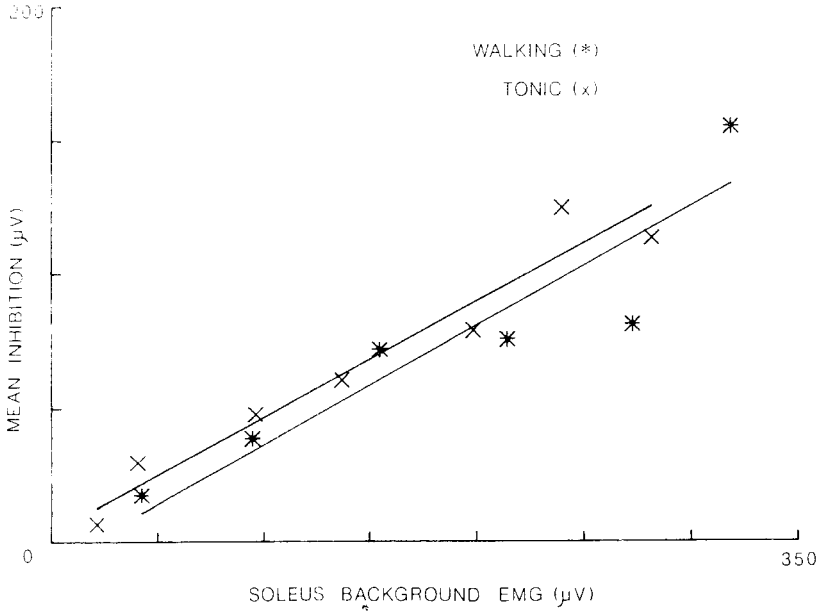

FIG. 6. Comparison of the mean amount of inhibition as a function of the background EMG level during walking (*) and tonic activity (×) in the same subject. Slopes of lines were 0.40 ± 0.09 (mean ± SE) for walking and 0.47 ± 0.05 for tonic activity. Neither slopes nor intercepts were significantly different between the 2 tasks.

the neural pathway responsible for the inhibition will be discussed later.

Figure 3 also serves to justify the use of 1.3–1.5 MT in other experiments. With a stimulus of 52 V (~1.3 MT in Fig. 3) a strong inhibition is observed, but both the level of inhibition and the M-wave are still sensitive to changes. Even at this high level of inhibition, the voluntary EMG activity never ceased completely but merely showed a decreased spike density. Figure 4 displays a raster of 16 individual sweeps without rectification together with the usual rectified and filtered average. Gaps in the activity of motor units are observed more frequently during the period of inhibition than elsewhere in the individual sweeps, but motor-unit firing can occur at all latencies within this period. Thus the gradation of inhibition observed in Fig. 3 and elsewhere in the paper represents a modulation in the probability of single-unit activity that should be sensitive to changes in the reflex pathways subserving this inhibition.

Inhibition of soleus activity during walking

The EMG pattern of the soleus during walking (Fig. 5*A*) consists of a ramplike increase of activity after heel contact (h.c. in Fig. 5*E*), reaching a maximum just before the heel comes off the ground (h.o.), and then ceasing abruptly just before the toe leaves the ground (t.o.). Therefore the EMG activity of soleus increases throughout most of the stance phase of walking. Stimulation of the CP nerve produced inhibition of the soleus muscle at all times in the stance phase of the walking cycle with a time course similar to that shown in Fig. 1. Moreover, as can be seen in Fig. 6*B*, the amount of inhibition increases from the beginning to the end of stance, even though the M-wave is quite constant (Fig. 5*C*).

As during tonic activity, a strong linear correlation is observed between the amount of inhibition and the mean value of the EMG at the time in the step cycle when the inhibitory stimulus occurred (Fig. 6). One can also directly compare the amount of inhibition during walking (*) and during tonic activity (×) in the same subject. The slopes of the two regression lines were not significantly different, indicating that the efficacy of transmission in the pathway mediating the inhibition was essentially the same in the two tasks.

Two other points are evident from Fig. 5. First, the EMG pattern of TA consists of two bursts of activity. One burst occurs at the time of h.c. and serves to stiffen the ankle, whereas the other burst occurs just after t.o. and dorsiflexes the ankle. In between, the TA is essentially silent. Second, the measurement of ankle angle (Fig. 5*E*) reveals that the soleus, which acts only at the ankle, is stretched during most of the stance phase. Equivalently, TA must shorten during most of this phase. On the assumption that the spindles in a shortening muscle during walking are unloaded in human as in the cat (Prochazka et al. 1976), the TA muscle afferents will be relatively inactive during most of the stance phase unless a perturbation unexpectedly stretches the muscle. However, late in the stance phase the soleus shortens for a period of ~200 ms (Fig. 5*E*) as it lifts the body off the ground and consequently stretches the quiescent TA. In this part of stance the TA muscle afferents will become intensely active and may inhibit the soleus motor output via the reciprocal inhibitory pathway, as will be discussed under the heading *Functional significance.*

DISCUSSION

Three central questions warrant further discussion. *1*) What neural pathway(s) are responsible for the observed inhibition? *2*) What are the functional implications of this inhibition? *3*) What, if any, neural mechanisms regulate this reflex? These questions will be discussed in turn below.

Neural pathways

At least five different pathways might be suggested for the inhibition observed in the voluntary activity of soleus EMG by stimulating the CP nerve, although admittedly

some of these would be contrary to conventional models of the spinal circuitry: *1*) reciprocal inhibition from Ia afferents; *2*) Golgi inhibition from Ib afferents; *3*) inhibition of extensors associated with a flexor reflex arising from cutaneous and other afferents; *4*) Renshaw inhibition from activating α-motoneurons, and *5*) presynaptic inhibition of soleus Ia terminals by activation of primary afferents in the CP nerve.

All the studies using either the H-wave method or single-unit recordings that were mentioned in the INTRODUCTION have concluded that the inhibition was produced by the well-known disynaptic inhibition from group Ia muscle spindle afferents to antagonistic motoneurons. This is also the simplest explanation of our results. The parallel increase in the M-wave in the TA muscle and the inhibition of ongoing activity in soleus (Fig. 3) confirm that group I afferents are responsible. Based on H-reflex studies in soleus muscle, one might expect group Ia muscle spindle afferents to be excited at a slightly lower threshold than α-motoneurons, but little or no difference in threshold was observed.

The small difference in threshold between the M-wave and the inhibition could arise in several ways. First, soleus is the prime example of a slow twitch muscle (Ariano et al. 1973). Thus many of its motoneurons are likely to be smaller and have a higher threshold to electrical stimulation than motoneurons to faster twitch muscles such as TA. In other words, motor threshold is a relative rather than an absolute measure of the fraction of sensory axons that are being stimulated. Second, soleus is well endowed with muscle spindles (Matthews, 1972), so the number of group Ia afferents stimulated at any level may be much higher when stimulating the soleus (or the whole tibial) nerve than for the TA (or the CP) nerve. The Ia interneurons require a certain amount of convergence to fire (Hultborn, 1972), and the convergence may occur at lower threshold in one direction than the other. Even when stimulating the tibial nerve and observing the inhibition of single TA motoneurons, little or no difference between motor threshold and the threshold for inhibition was observed in normal subjects. A lower threshold was observed for spinal cord patients in whom this pathway was facilitated (Ashby and Wiens 1989).

The higher-threshold stimuli used here will also activate afferents from Golgi tendon organs (group Ib) and cutaneous afferents. The reflex effects of Ib afferents are classically inhibitory to agonists and excitatory to antagonists (Eccles 1964; Powers and Binder 1985), but they have also been grouped with cutaneous and other afferents under the term flexor-reflex afferents or FRA (reviewed, for example, by Baldissera et al. 1981). The FRA are well known to produce excitation of flexor muscles and inhibition of extensors. The presence of the inhibition at a low threshold ($1 \times$ MT) and at short latency (about equal to that of the H-reflex in this muscle) are contrary to this explanation. Furthermore, under the conditions of these experiments we never observed a flexor reflex in the TA (an ankle flexor).

A stimulus above the motor threshold will antidromically activate Renshaw cells via motor axon collaterals (Baldissera et al. 1987). Classically, Renshaw inhibition is not seen between strict antagonists, but stimulation of the peroneal nerve in the cat did produce Renshaw inhibition in the gastrocnemius-soleus muscles (Baldissera et al. 1981). The time course of the inhibition is similar to that in a PSTH calculated by Kirkwood et al. (1981) for Renshaw inhibition between external and internal intercostal muscles. The parallel increase in the M-wave and the inhibition observed in some experiments (Fig. 4) may also be used to support the idea of Renshaw inhibition. Thus it cannot be ruled out categorically as an explanation, albeit an unconventional one, for the effects observed here.

Finally, the inhibition might have resulted from presynaptic inhibition of the soleus Ia afferents by activation of group I afferents in the CP nerve, as first described by Eccles et al. (1962). However, such presynaptic inhibition typically requires a short train of high-frequency stimuli that precedes the test stimulus to the soleus motoneurons by 60–90 ms. Furthermore, presynaptic inhibition should simply reduce the firing rate of motoneurons rather than producing gaps of activity such as is observed experimentally (Fig. 4). As explained in the APPENDIX, gaps are due to hyperpolarization of the membrane potential by sufficiently strong IPSPs.

Functional significance

As shown in Fig. 5 the TA muscle is active for two periods during the step cycle, and the muscle is also stretched twice during the cycle. The first period of activity corresponds in time to the lengthening that occurs briefly after heel contact. The TA activity prevents the ankle from rapidly extending, which would cause the foot to slap against the ground. Limiting soleus activity to a low level by Ia inhibition (or by Renshaw inhibition for that matter) will aid in the functional role of TA during this period.

The second period of stretch for the TA muscle (roughly between heel off and toe off in the step cycle of Fig. 5) precedes the TA activity during the swing phase. The activity in Ia afferents from TA muscles at that time would help to terminate the soleus activity and to activate TA motoneurons. In conjunction with a decrease of the central excitatory drive to motoneurons of the ankle extensors, this reflex would contribute to the transition from stance to swing. A peripheral input that depends on the exact kinematic events at the ankle would be useful. For example, if raising the body off the ground is somewhat delayed, the inhibition from the ankle flexors would also be delayed, thus allowing for a prolongation of extensor activity.

We have previously hypothesized, based on the modulation of the H-reflex and measurements of muscle length (Capaday and Stein 1986, 1987a), that part of the motor output of soleus during locomotion (walking, running) is due to the autogenic-Ia–afferent feedback. We propose here that Ia afferents from its antagonists, the ankle flexors, may also contribute to the rapid and timely cessation of this activity via the Ia inhibitory pathway.

Regulation of the pathway

Our results clearly show that inhibition from antagonists remains functional during voluntary activity in a muscle group. They extend work with single units beginning with Ashby and Labelle (1977) and Kudina (1980) and continu-

ing to the present. These findings in the intact human are fully consistent with direct recordings of IPSPs from Ia interneurons to active motoneurons during fictive locomotion in the cat (Pratt and Jordan 1987). These workers found that IPSPs could be evoked in knee-flexor motoneurons during their active phase and that the IPSPs increased with membrane depolarization, as would be expected on the basis of the increasing electrical gradient for the chloride ions.

Nonetheless, these results were surprising in that several neural pathways might be expected to shut down the Ia inhibitory pathway: *1*) the convergence of common descending inputs onto Ia interneurons and α-motoneurons was already mentioned in the INTRODUCTION. Thus in the phases of the step cycle when CP motoneurons are inhibited, one might expect that transmission of reciprocal inhibition from Ia afferents to soleus might also be inhibited; *2*) a stimulus to α-motoneurons in the CP nerve would also activate Renshaw cells, which are known to inhibit Ia interneurons projecting to antagonist motoneurons (Hultborn et al. 1971), and *3*) Ia interneurons of antagonistic muscles mutually inhibit each other (Baldissera et al. 1981, 1987). Thus activation of soleus Ia interneurons, either by descending input or Ia-afferent feedback, would inhibit Ia interneurons from muscles innervated by the CP nerve.

The earlier H-reflex studies suggested that reciprocal inhibition was not present once a motor pool becomes active, and its absence could have been explained by any of these pathways. However, this suggestion has been weakened by inconclusive results from other laboratories with the use of the H-reflex method and by methodological concerns (see INTRODUCTION). Other methodological issues applicable to these H-reflex studies can be raised. Classically, the conditioning/test method was developed and used to test the interaction between two variables (excitation and inhibition) on a quiescent motoneuron pool (Lloyd 1941; Renshaw 1941) in which the membrane potential of motoneurons was assumed constant. Extending the approach to an active pool introduces a third variable, namely the increased depolarization of motoneurons as the recruitment level increases. This may change the interaction between the other two variables in unknown ways.

Not only did we find that the inhibitory pathways remained functional when the soleus motor pool became active, but we found a proportional increase in the amount of inhibition with the level of activity (Fig. 2). The relationship was also unchanged in going from standing to walking (Fig. 6). Similarly, Matthews (1986) found that biceps brachii could be inhibited by the application of a few cycles of a vibratory stimulus to its antagonists (triceps brachii) at each of the contraction strengths investigated (not exceeding 50% of maximum). His method of measuring the amount of inhibition was similar to ours, and the relation between amount of inhibition and background activity (measured in Matthews' study as force) was also linear. Therefore inhibitory pathways of the arm muscles appear to behave similarly to those of the ankle.

Possible neural mechanisms for the proportional increase in background activity and measured inhibition are considered in the APPENDIX but will be briefly outlined here. Perhaps the simplest explanation would be that the synchronous inhibitory volley, produced by the stimulation of the CP nerve, is sufficient to briefly silence all the motoneurons in the soleus pool. The variation in the timing of the inhibition and the filtering produced by the surface EMG may be sufficient that the apparent activity never reaches zero during the peak of inhibition. If the EMG were increased mainly by recruiting more motor units, which were also transiently silenced by the stimulus,

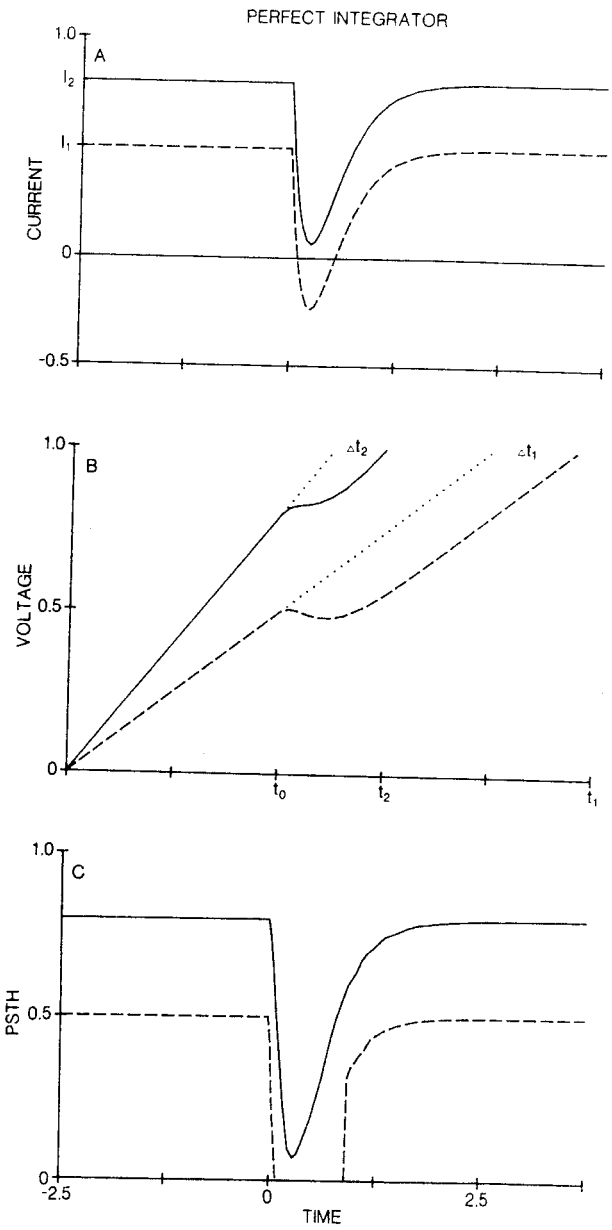

FIG. 7. *A*: same synaptic inhibition (*Eq. 3*) is superimposed on 2 different levels of steady excitatory current (I_1 and I_2) that are indicated by dashed and solid lines, respectively. *B*: voltage change produced in a perfect integrator (*Eq. 1*) is shown with and without (dotted line) the inhibition. Times of firing in the 2 examples (t_1 and t_2) are changed by amounts indicated (Δt_1 and Δt_2). *C*: corresponding changes in the peristimulus time histogram (PSTH) have been calculated. With the larger current (I_2) the form of the synaptic current is preserved. With the smaller current (I_1) that goes transiently negative, a gap is observed in the PSTH. Further explanation in the APPENDIX. Values used in this example are A = 4, C = 1, Q_i = 0.2, I_1 = 0.5, I_2 = 0.8, and V_t = 1.

the inhibition might remain a roughly constant fraction of the background level of activity. A similar pattern of recruitment in standing and walking might also explain the constancy of the inhibition between the two tasks.

Several experimental observations and theoretical arguments can be brought against this simple explanation. For example, in the rasters such as shown in Fig. 4, no period of complete silence is observed but rather a decreased probability of motor units firing for some period of time. If a unit is not silenced, then the calculations in the APPENDIX indicate that the amount of inhibition should be largely independent of the firing rate. If rate coding as well as recruitment is used to increase the EMG, a strict proportionality may not be expected. Even if a unit is silenced, the duration of the inhibition should decrease as the firing rate increases. The shortening of the duration is such that the area of inhibition is constant (Fig. 7) in the perfect integrator model. Although the area is not constant in another model (the leaky integrator, Fig. 8), a marked shortening of the gap is still observed as the firing rate increases.

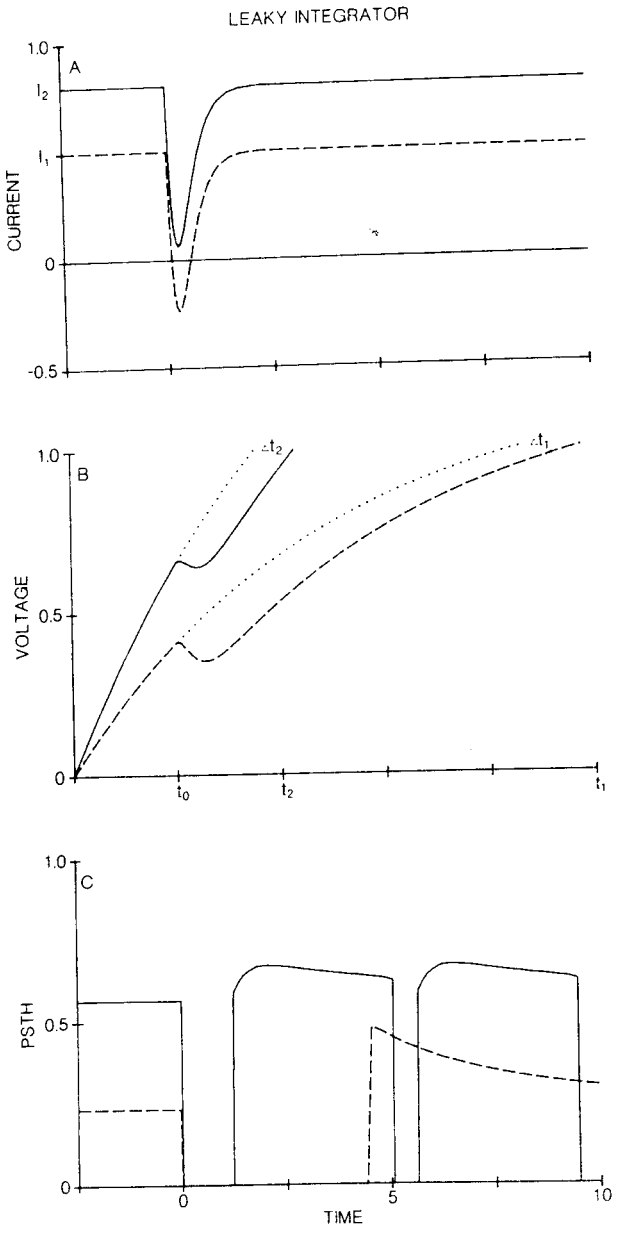

FIG. 8. Format is similar to that of Fig. 7 except that a leaky integrator with a time constant T = 6 ms has been used. A longer time scale has been used to show the decay of the synaptic potential in *B*. PSTHs in *C* are more complex with increases as well as decreases in firing density and gaps that continue with the period of the interspike intervals. Further explanation in the APPENDIX.

Thus the proportionality between inhibition and background activity in walking and standing probably arises in a more complex way. At low levels of activity most but not all units are inhibited. As the level of activity increases, the firing rate of these units increases and a smaller fraction of their impulses may be delayed for a shorter period of time. However, other larger units are recruited that are more profoundly inhibited, so approximately the same percentage of inhibition is observed overall. Single-unit EMG studies would be helpful to test this hypothesis, if recording could be maintained stably and selectively under all conditions studied here. Recording from single units during human walking has been extremely difficult but has been done in selected muscles of a few subjects (Grimby, 1984).

Whatever the neural mechanisms prove to be, our results with the use of natural activation of motoneurons in human demonstrate that inhibitory pathways remain functional during voluntary actions. Indeed, the amount of inhibition increases in proportion to the level of motor output, both in walking and standing. Finally, a functional role can be suggested for this inhibition in regulating the motor output during natural activities such as walking.

APPENDIX

There have been a number of papers (see Midroni and Ashby 1989 and their references to earlier work) that used computer simulations to model the effect of an EPSP or an IPSP on a PSTH. In this APPENDIX we will only consider a few simple examples that can be solved analytically and serve to provide some insight into the results obtained in the text. As indicated earlier, records such as shown in Fig. 1 represent weighted sums of the PSTHs of all the active motoneurons. Specifically, we will consider here various possible effects of an IPSP on the membrane potential of a repetitively firing neuron and its consequences on the PSTH of that neuron. Although equations have been derived here for inhibitory potentials, the sign can generally be reversed to describe the effects of excitatory potentials.

Perfect integrator

The simplest example arises if the nerve membrane behaves as a perfect integrator over some period of time t. The voltage $v(t)$ can then be calculated by

$$v(t) = \frac{1}{C}\int_0^t i(u)du \qquad (A1)$$

where C represents the capacitance of the membrane, which is assumed constant, and i the current crossing the membrane, which is a function of the variable of integration u. We assume that the membrane starts at a voltage $v = 0$ at time $t = 0$ and that an action potential is fired at time t_f when the membrane reaches a threshold voltage V_t. If there is a constant excitatory current I_e, then

$$V_t = I_e t_f / C \qquad (A2)$$

The time of firing will be $t_f = V_tC/I_e$, and the rate at which action potentials will be generated will be $I_e/(V_tC)$. We now add to the excitatory current an inhibitory synaptic current that delivers a charge Q_i beginning at time t_i with the form shown in Fig. 7*A* (Jack et al. 1975). The total current is given by

$$i(u) = \begin{cases} I_e & , u \le t_i \\ I_e - Q_iA^2(u - t_i)\exp[-A(u - t_i)], & u > t_i \end{cases} \quad (A3)$$

where A is a rate constant for the decay of the synaptic current. *Equation A3* can be substituted into *Eq. A1* and integrated. After some manipulations one finds that

$$v(t) - \frac{I_e t}{C} = \frac{Q_i}{C}\{1 - [1 + A(t - t_i)]\exp[-A(t - t_i)]\} \quad (A4)$$

The second term on the right represents the deviation in voltage Δv produced by the inhibitory current, and its effect on the voltage trajectory is illustrated in Fig. 7*B*. It is easily shown that $\Delta v = 0$, when $t = t_i$ and $\Delta v = Q_i/C$ when t is sufficiently large. Because of the fact that the membrane was assumed to be a perfect integrator, Δv simply represents the integral of the current waveform in between these two limits and any other integrable current waveform could be substituted as required. The delay in time to firing Δt_f can also be shown to vary between 0 and Q_i/I_e.

The effect of the inhibition on the PSTH is illustrated for two levels of excitatory current in Fig. 7*C*. For the higher level I_2 the total current is always positive (Fig. 7*A*); the voltage increases monotonically (Fig. 7*B*), and the PSTH has the form of the inhibitory current. The reason for the correspondence in form is that Δv and Δt_f are both integrals of the synaptic current under these conditions. The PSTH can be shown to be proportional to the differential of Δv with respect to the time of stimulation t_i, so the original waveform is retrieved. The result applies to any arbitrary current waveform as long as the total current does not go negative (Fig. 7*A*).

For the lower level I_1, the total current is transiently negative; the slope of the membrane voltage becomes negative (hyperpolarization), and a gap is observed in the PSTH. The IPSP waveform is no longer preserved in the PSTH, but it can be shown that the area of the inhibition remains unchanged at $Q_i/(CV_t)$. The constant area of inhibition is a specific property of the perfect integrator, as we shall see below. However, the finding that gaps will be present in a PSTH whenever the total current goes negative and the membrane begins to hyperpolarize is a general result that applies to a broad class of models.

Leaky integrator

A perfect integrator is clearly an oversimplification of a nerve membrane, but a leaky integrator is a better approximation (Stein 1980). For a leaky integrator *Eq. A1* becomes

$$v(t) = \frac{1}{C}\int_0^t i(u)\exp[-B(t - u)]du \quad (A5)$$

where B is the inverse of the time constant T = RC, and R is the membrane resistance, which will be assumed constant for subthreshold voltages. The threshold voltage V_t will be reached with an excitatory current I_e at a time t_f, where

$$V_t = I_eR(1 - \exp[-Bt_f]) \quad (A6)$$

and so

$$t_f = T\ln[(I_eR)/(I_eR - V_t)] \quad (A7)$$

If $I_eR \gg V_t$, $t_f = V_tC/I_e$, as for the perfect integrator. If the current again has the form of *Eq. 3*, the change in voltage Δv is

$$\Delta v = \frac{A^2Q_i}{(B - A)^2C}\{\exp[-A(t - t_i)][(B - A)(t - t_i) - 1] + \exp[-B(t - t_i)]\} \quad (A8)$$

as shown in Fig. 8*B*. The change in voltage is initially similar to that of the perfect integrator, but a longer time scale has been used to show the decay of voltage with the time constant T.

If a realistic value for the time constant of cat motoneurons is used (T = 6 ms) (Eccles 1964), the whole time scale of Fig. 8 is 12.5 ms. The rate constant A = 4 ms^{-1} gives a peak current at 0.7 ms, and the current is essentially over by 2.5 ms, values that are very close to those measured for cat motoneurons under voltage clamp (Araki and Terzuolo 1962). The times to fire an action potential in Fig. 8 (5–10 ms) are short compared with those observed in motoneurons, because the currents underlying the afterhyperpolarization have not been included. They could easily be added to *Eq. A3*, which would give more realistic firing rates. However, this would not change any of the arguments presented below so these currents have been omitted for simplicity.

Figure 8*C* gives the PSTHs for the two levels of excitatory current (I_1 and I_2). The rates are lower than in Fig. 7*C* because the ionic current across the resistance of the membrane slows the voltage trajectory and hence delays the time when threshold is reached. Both histograms show a gap after the application of the inhibitory current pulse. However, the gap depends markedly on the firing rate. With the smaller excitatory current it is much longer than the duration of the inhibitory current. With the higher excitatory current it is comparable in duration with the inhibitory current waveform. The gap disappears completely if the current is increased further (not shown) so that the slope of $v(t)$ remains positive. This result is a generalization of the finding for the perfect integrator (see also DISCUSSION).

Three further points are worth noting in Fig. 8*C*. First, after the gap the PSTH shows a rebound increase that was not seen in the perfect integrator. This increase results from the decay of the IPSP with time. If a spike occurs long after a stimulus, it is delayed less than one occurring soon after the stimulus. Thus the density of spikes will be higher in the PSTH after the gap.

Second, this increased density must be balanced by a decreased density in the form of a shorter gap that will occur, for example, with the current I_2, at a time $t_i + t_2$. Another way of understanding the secondary (and tertiary) gaps is that, if there are no spikes occurring shortly after t_i, no units will be starting their interval so as to fire at times t_2 (and $2t_2$) later. In the absence of neuronal variability, this pattern would repeat indefinitely. However, in a real neuron with variability the PSTH will revert to its original flat level sooner or later (Midroni and Ashby 1988).

Finally, the area of the gaps is not the same for the two currents, as was true in the leaky integrator. The area is quite a bit larger with the smaller excitatory current, and the rebound increase is larger as well. The implications of these theoretical results for our experimental data are considered in the DISCUSSION.

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