The influence of muscle spindle discharge on the human H reflex and the monosynaptic reflex in the cat

S. A. Wood, J. E. Gregory and U. Proske

Department of Physiology, Monash University, Clayton, Victoria 3168, Australia

1. Experiments were carried out to test the effect of changes in spindle resting discharge on the size of monosynaptic reflexes in the cat and on the H reflex in humans. Resting discharge was altered by contracting the triceps surae muscle at longer (hold-long) or shorter (hold-short) lengths than that at which the reflex was tested.

2. The reflex in the cat was larger after hold-long than after hold-short conditioning, and the difference, after an initial decline, was well maintained. For the human H reflex a similar pattern was observed except that 15 s after muscle conditioning the difference in reflex size had disappeared.

3. Monosynaptic reflex depression immediately after hold-long conditioning, when most of the muscle spindles are silent, was attributed to the high level of spindle discharge during the immediately preceding hold-long period. The time course of this inhibition was too long to be accounted for by presynaptic inhibition.

4. In the cat heteronymous muscle conditioning was used to test whether presynaptic inhibition could be responsible for reflex depression using the synergist muscle pair lateral gastrocnemius–soleus and medial gastrocnemius. Conditioning one of the pair did not affect the reflex in the other, the opposite result to that expected with presynaptic inhibition. A similar experiment in which the triceps H reflex in human subjects was facilitated by a quadriceps volley gave the same result.

5. Thus this study presents evidence that monosynaptic reflexes are depressed by the on-going discharge of muscle spindles in the homonymous muscle, but that this depression does not appear to involve 'classical' presynaptic inhibition.

Impulses from muscle spindles provide the afferent limb of the stretch reflex. In recent years it has become apparent that impulses from the primary endings of muscle spindles not only excite motoneurones monosynaptically but may exert inhibitory influences as well. The on-going discharge of muscle spindles, or resting discharge, has an important influence on the size of homonymous monosynaptic reflexes. In the cat, it has been shown that an increase in muscle spindle Ia activity evoked by vibration leads to depression of reflex amplitude (Gillies, Lance, Neilson & Tassinari, 1969). Similarly, in humans, Hoffman reflexes (H reflexes) are depressed under conditions where spindle discharge rate may be assumed to be high (Hugon & Paillard, 1955).

The levels of resting discharge of muscle spindles may be systematically altered by taking advantage of a muscle property known as thixotropy, which is believed to give rise to the after-effects that spindles exhibit (Gregory, Morgan & Proske, 1986). The current explanation of these effects in the passive muscle is that they result from the formation of a small number of relatively stable cross-bridges between actin and myosin myofilaments within intrafusal fibres. Stretch or contraction will break existing bridges that then re-form at whatever length the muscle is at.

When a muscle is shortened from a length at which stable cross-bridges have formed, the intrafusal fibres, stiffened by these cross-bridges, will tend to fall slack, thereby leading to shortening of the equatorial sensory region. Spindles in this condition would be expected to have a low resting discharge. They would also be relatively insensitive to stretch, as shown by a reduction in the size of the tendon jerk (Gregory, Morgan & Proske, 1987). If, on the other hand, the muscle is stretched rather than shortened from the length at which stable cross-bridges have formed, the intrafusal fibres will remain taut and the sensory region will be stretched by the stiffened intrafusal poles. Spindles in this condition would be expected to have a higher rate of resting discharge and would be sensitive to stretch, as shown by a large tendon jerk (Gregory *et al.* 1987).

Thus, at any particular muscle length, the resting discharge of the spindles will depend on the immediate history of length change and contraction, and can be altered systematically by means of different conditioning regimes. Other receptors in the muscle, like tendon organs, are thought not to show significant thixotropic effects and in any case they typically require stretch to long muscle lengths before they will develop a resting discharge (Houk &

Henneman, 1967). The ability to change spindle resting discharge without changing muscle length is particularly advantageous when working with human subjects, because surface electromyogram (EMG) recordings of H reflexes exhibit length-dependent effects (Gerilovsky, Tsvetinov & Trenkova, 1989).

It has been proposed that the relatively larger H reflex obtained in the presence of intrafusal slack is the result of a low level of spindle resting discharge (Gregory, Mark, Morgan, Patak, Polus & Proske, 1990). It has since been suggested that the diminished H reflex which accompanies the increase in resting discharge following muscle conditioning may be due to presynaptic inhibition of the Ia terminals initiated by the spindle impulses (Gregory *et al.* 1990). This hypothesis was based on earlier findings that monosynaptic reflexes and H reflexes are depressed following muscle stretch (Delwaide, 1973) or vibration (Gillies *et al.* 1969) and by chemical excitation of muscle spindles (Fujimori & Eldred, 1961).

We have extended our study of the effects of muscle conditioning-induced changes in spindle resting discharge on reflex amplitude both in the cat and in human subjects. We conclude that the reflex depression produced by on-going spindle discharge is not due to 'classical' presynaptic inhibition. Preliminary reports of this work have already been published (Wood, Gregory & Proske, 1994, 1995).

METHODS

Animal experiments

The experiments were carried out on nineteen adult cats, of both sexes, weighing between 2·4 and 4·0 kg. Usually anaesthesia was induced with an intramuscular injection of a mixture of ketamine (0·9 mg kg^{-1}) and xylazine (9 mg kg^{-1}) and was continued for the remainder of the experiment with α-chloralose (45 mg kg^{-1}) administered intravenously into a forelimb vein. In some experiments, anaesthesia was induced with sodium pentobarbitone (40 mg kg^{-1}) and maintained with additional doses given intravenously when necessary during the experiment. The trachea was cannulated and end-tidal CO_2 concentration was monitored. Rectal temperature was maintained at 37 °C by the use of a feedback-regulated heating blanket, and when supplementary heating was necessary an infrared heating lamp was used. A laminectomy was performed to expose spinal roots L6–S1.

The left hindlimb was dissected to expose the triceps surae muscle group which was then freed from surrounding connective tissue. Before any further dissection was done the maximum physiological length (L_{max}) was determined by maximally flexing the ankle joint with the knee and ankle in the position that they would occupy during the experiment. The distance between markers on the Achilles tendon and the tibia was noted, to be used later as a length reference. The calcaneum was severed, and a small piece left attached to the tendon was used to attach the muscle to a feedback-controlled electromagnetic muscle stretcher. An extensive denervation of the hip and lower limb, sparing the nerves supplying the triceps surae muscle, was performed. Exposed tissues were covered with mineral paraffin oil retained in baths fashioned from skin flaps.

Monosynaptic reflexes were recorded from the central cut ends of L7 and S1 ventral roots using platinum bipolar electrodes. Monosynaptic reflexes were evoked using triceps surae nerve stimulation at a strength submaximal for α-fibres. In five experiments, recordings were made from functionally single triceps surae Ia afferent fibres contained in fine filaments dissected from L7 and S1 dorsal roots.

In four experiments, evidence was sought for presynaptic inhibition acting on the intraspinal terminals of Ia afferent fibres. This was investigated using a method that tested the excitability of these terminals (Wall, 1958). A glass-coated tungsten microelectrode was inserted into the triceps motoneurone pool, making the impalement from the dorsomedial surface of the cord. Responses to current pulses delivered through the microelectrode were recorded in L7 and S1 ventral roots and antidromically from the muscle nerve. At the end of the experiment the microelectrode was left in position and the piece of spinal cord was removed and fixed for histological processing to confirm correct microelectrode placement. At the end of the experiment, animals were killed with 300 mg of sodium pentobarbitone.

Two muscle conditioning procedures were employed, one involving a stretch (hold-long) and the other a shortening (hold-short) from the test length, at a rate of 10 mm s^{-1}. Unless otherwise stated, the amplitude of these conditioning movements was 5 mm. At the conditioning length, the muscle nerve was stimulated at fusimotor strength for 1 s at 15 Hz. After the contraction, the muscle was kept at the conditioning length for a further 5 s before return to the initial or test length. The test length, the length at which the reflex measurements were made, was the same for both forms of conditioning. The test length for each experiment was selected by determining the length at which the largest difference in reflex response between hold-short and hold-long conditioning could be recorded. It typically lay in the range $L_{max} - 16$ mm to $L_{max} - 8$ mm.

Human experiments

The experiments were performed on ten healthy subjects aged 22–25 years. Subjects all gave informed consent to the experimental procedures, which had been approved by the relevant ethics committee. Subjects were seated in a reclining chair which was adjusted so that the right leg was flexed at the hip by approximately 120 deg and at the knee by approximately 130 deg. The right foot was strapped to a footplate so that the angle formed between the footplate and the lower leg was 110 deg. The footplate could be rotated about an axis coincident with the axis of the ankle joint and could be fixed in any position in both the plantarflexed and dorsiflexed directions.

Surface electrodes were used for both stimulation and recording. The triceps surae H reflex was evoked with a 1 ms duration constant current pulse applied though a cathodal ball (1 cm in diameter) positioned in the popliteal fossa. The anode was a rectangular plate (6 cm × 4 cm) strapped above the patella. The cathode was held in place by a frame firmly strapped to the leg. When the optimal position for eliciting the H reflex was found, the cathode was locked in place, where it remained for the duration of the experiment. In a few experiments the cathode was a Ag–AgCl adhesive electrode (3M 'Red Dot' paediatric electrodes) placed over the tibial nerve in the popliteal fossa. Stimulus strength was adjusted so that the size of the evoked H reflex (unconditioned) was approximately 25–50% of the maximal motor response (M_{max}). Stimulus conditions were adjusted so that a small direct response was visible together with each reflex. This provided an essential

control for electrode movement. Recording electrodes were placed over the soleus muscle. Reflexes were measured as the peak-to-peak amplitude of the response, and for each experimental sequence values were averaged over five trials.

As with experiments on the cat, two conditioning procedures were used, hold-short and hold-long, produced by dorsiflexing or plantarflexing the foot by 30 deg. With the foot rotated and locked in position, the subject was instructed to produce a large but not necessarily maximal contraction of the triceps surae muscle. After the contraction the foot was held in the conditioning position for a further 5 s, with the muscle relaxed, after which the experimenter rotated the footplate back to the test angle at which reflex testing was carried out. The test angle was always 110 deg. EMG activity was monitored by audio feedback to ensure that subjects remained relaxed, with no background muscle activity.

The stimulus for eliciting the H reflex was triggered by a magnetic switch which closed as soon as the foot returned to the test angle. Various conditioning–test intervals were obtained by incorporating a delay between the time of closure of the switch and the stimulus to the tibial nerve.

In five experiments the presence of presynaptic inhibitory action on triceps afferent terminals was sought using a method in which the triceps H reflex was conditioned with a femoral nerve volley (Hultborn, Meunier, Morin & Pierrot-Deseilligny, 1987). A 1 ms duration constant current stimulus was applied to the femoral nerve, in the femoral triangle. The cathode was a hemisphere, 2·5 cm in diameter, held in position by a weight placed over the electrode. The anode was a large plate electrode (15 cm × 8 cm), positioned on the upper, posterior aspect of the thigh. Stimulus strength was adjusted so that it was at motor threshold for quadriceps. Quadriceps EMG recordings were made with adhesive surface electrodes placed over the rectus femoris muscle. The timing of the femoral nerve volley was adjusted so that the interval between the two volleys led to a small facilitation of the triceps surae H reflex. Once this point had been reached an additional 400 μs was added to achieve maximal facilitation from the quadriceps Ia afferents. Intervals used for the five subjects ranged between −6·3 and −5·6 ms. A negative interval indicates that the triceps surae test volley preceded the quadriceps conditioning volley. After the correct facilitation interval had been determined, the hold-long reflex amplitude in triceps was adjusted for each subject to 25% of M_{max}. The H reflex was measured 5 s after the muscle was returned to the test angle. Mean values for each subject were taken from twenty individual trials carried out at a rate of one every 20 s. The hold-long reflex was then facilitated by the femoral nerve volley. Then the facilitation measurements were repeated, but after hold-short conditioning. To permit an estimate of the amount of facilitation the hold-short reflex was adjusted by increasing stimulus strength so that it was equal in size to the hold-long reflex. This was called the hold-short compensated reflex. The compensated reflex was then facilitated using the femoral nerve volley and the amount of facilitation was compared with that which occurred with the hold-long reflex. In every subject, the amount of facilitation observed with hold-long and hold-short compensated reflexes was remeasured several times and then averaged.

In both human and cat experiments, responses were displayed on either a Nicolet 2090 or 4000 digital oscilloscope and stored on floppy disks, or were recorded using an Apple Power Macintosh 7100/66 computer and a Maclab 8s system running Chart and Scope software (ADInstruments, Castle Hill, NSW, Australia). Data were analysed using the software package Igor Pro v2.02 (WaveMetrics, Portland, OR, USA).

RESULTS

Animal experiments

Figure 1 shows changes in the size of the triceps monosynaptic reflex following hold-long and hold-short muscle conditioning over a 29 s period after conditioning. It can be seen that, immediately following each form of conditioning, reflex amplitude was depressed, but then gradually recovered, reaching a nearly constant value at about 10 s. The final value, measured 29 s after conditioning, was less after hold-short, in this case being only 67% of that after hold-long conditioning.

Spindle discharges were recorded under the same conditions. The smaller reflex, obtained after hold-short conditioning, was accompanied by a higher level of resting discharge. For the spindle shown in Fig. 1 the discharge after hold-long conditioning was only 34% of that which occurred for hold-short conditioning, measured at 29 s.

The pooled reflex and spindle discharge data, from eight and five experiments, respectively, are shown in Fig. 2. Within each experiment the average peak-to-peak monosynaptic reflex after each conditioning procedure and for each interval was normalized with respect to the maximum reflex measured for that experiment. These values were then combined and averaged for the eight experiments. Normalization was necessary because absolute reflex amplitude varied considerably between animals.

The effects of conditioning on the monosynaptic reflex were relatively long lasting. At the longest conditioning–test interval used, 29 s, there was still a significant difference (Student's t test, $P < 0{\cdot}001$) between the hold-long conditioned reflex and the hold-short conditioned reflex. The average percentage difference in reflex size between the two conditioning procedures was 50·4 ± 21·6% (mean ± S.D.) at the 29 s conditioning–test interval. For the sixty-eight spindles sampled, the initial difference in discharge rate was large. This declined over the next 10 s to where it was maintained for the remainder of the test period. The mean percentage difference in resting discharge after the two forms of conditioning was 44 ± 34% (mean ± S.D.) at the 29 s time interval. This difference was highly significant (Student's t test, $P < 0{\cdot}001$).

It is proposed that the maintained difference in reflex size after the two forms of conditioning is related to the difference in spindle discharge rate observed at these long intervals. To investigate this further, spindle discharge rate was altered systematically by varying the conditioning procedure. Three types of muscle conditioning were used. These were hold-short and hold-long conditioning, using a 5 mm length step as before, and, additionally, hold-long conditioning with a small step (usually 1 or 0·5 mm) or no movement at all, just a fusimotor strength contraction at the test length. The purpose of the additional conditioning procedure was to generate muscle spindle resting discharges that were intermediate between those produced by the

standard conditioning procedures and in this way obtain a range of discharge rates. In Fig. 3, values for averaged normalized reflexes have been plotted against the mean change in spindle resting discharge, for five combined experiments. The change in spindle resting discharge was calculated by subtracting the hold-long discharge value, the lowest recorded discharge rate, from the average recorded discharge rate for each of the conditioning procedures used within each experiment. It is apparent that there is an inverse relation between the reflex amplitude and spindle discharge rate. This finding supports the idea, at least over the range 5–30 impulses s^{-1}, that the maintained discharge rate of spindle primary endings is a determinant of monosynaptic reflex size.

It is further proposed that the early inhibition of the monosynaptic reflex seen with both hold-long and hold-short conditioning immediately after returning to the test length (Fig. 1) is related to the immediately preceding level of spindle activity. After hold-short conditioning the inhibition results from the high frequency burst of impulses accompanying the dynamic response to stretch as the muscle is returned to the test length. After hold-long conditioning it results from the high rate of firing during the preceding hold-long phase. To test this idea, the effect of changing the resting discharge rate of muscle spindles during the hold-long phase was studied. The time course of inhibition was plotted following 5 and 1 mm hold-long conditioning steps. The combined results for six experiments are shown in Fig. 4. The 5 mm step produced a deeper and longer lasting inhibition of the monosynaptic reflex than the 1 mm step, again consistent with the idea that the immediately preceding discharge levels influence reflex size. At the earliest time interval used in these experiments, 100 ms, there was a 63% difference in monosynaptic reflex size between the 5 and 1 mm conditioning step sizes.

In a similar experiment, instead of altering the amplitude of the hold-long step, the duration of the hold time at the long length was altered. In six similar experiments, varying

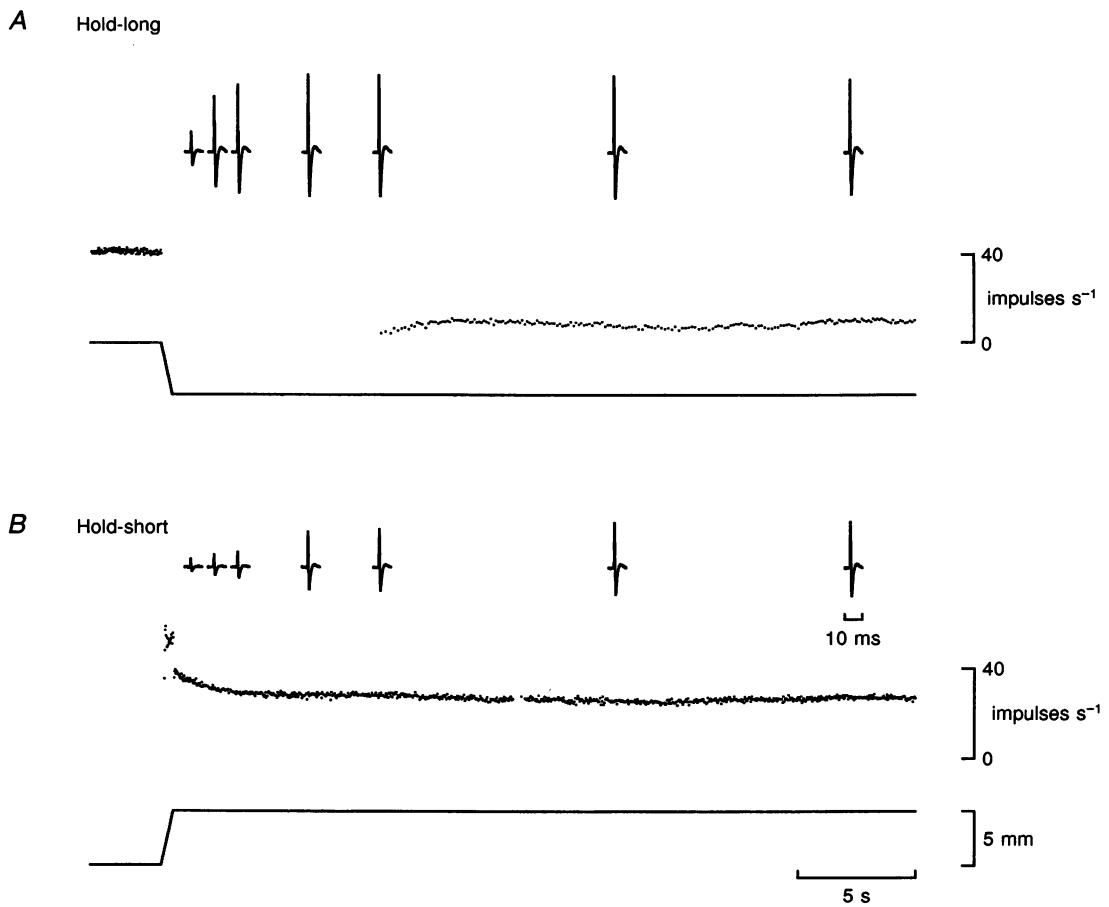


Figure 1. Monosynaptic reflex and muscle spindle responses following hold-long (*A*) and hold-short (*B*) triceps surae muscle conditioning in the cat

Not all of the conditioning sequence is shown. The lowest trace in each panel shows muscle length, an upward deflection representing an increase in length. The final length is the same in each case. The middle traces in each panel are instantaneous frequency records from the same single Ia afferent fibre. The top series of records in each panel show monosynaptic reflexes recorded at the times indicated by their position, from one animal. Note that only one monosynaptic reflex was elicited after each conditioning procedure and that each panel therefore represents a composite of 7 conditioning–test sequences.

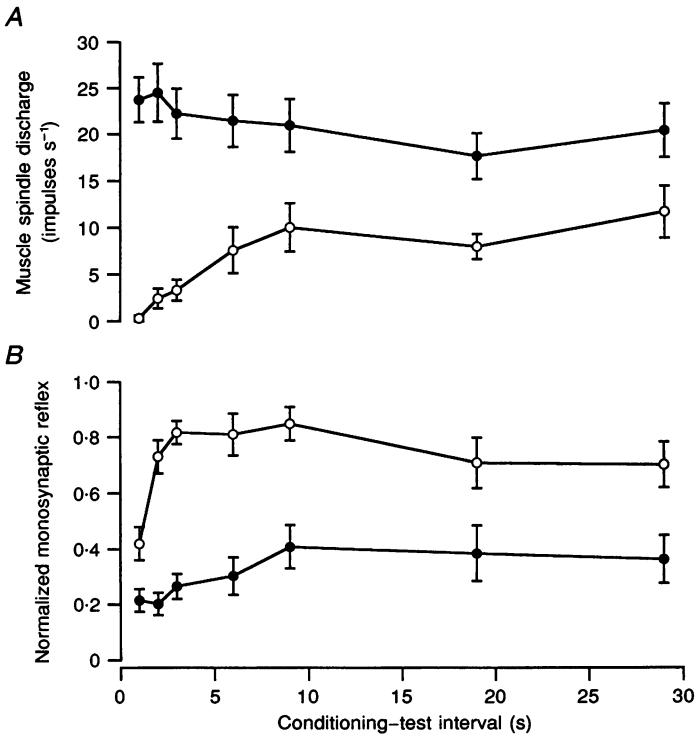


Figure 2. The time course of muscle conditioning effects on the monosynaptic reflex and spindle discharge rates in the cat

The conditioning–test interval is the time from the beginning of the movement back to the test length to the time when the monosynaptic reflex was elicited or spindle discharge rate measured. The reflex plot (*B*) shows mean data from 8 experiments after hold-long (○, mean ± S.E.M.) and hold-short conditioning(●, mean ± S.E.M.). The mean normalized monosynaptic reflex was calculated by first expressing the average monosynaptic reflex for each condition within each experiment as a fraction of the largest reflex recorded. These results were then averaged for each conditioning–test interval and for each conditioning procedure. Muscle spindle discharge rates (*A*) were calculated for 68 muscle spindle primary endings sampled from 5 experiments, again after hold-long (○, mean ± S.E.M.) and hold-short (●, mean ± S.E.M.) conditioning.

the duration of the hold time, over 1–20 s, produced no detectable differences either in the amount of initial reflex inhibition after conditioning or in the time taken to reach a steady level.

One further experiment was carried out to check that it was not the movement accompanying each conditioning procedure which was responsible for reducing the size of the reflex. First, the monosynaptic reflex was measured 100 ms after the start of the shortening movement returning the muscle to the test length. The conditioning procedure used in this instance was a 5 mm hold-long conditioning step of 10 s duration (Fig. 5*A*). Then, instead of using a single shortening movement, the reflex was measured again but this time after a double release conditioning movement (Fig. 5*B*). The purpose of the second shortening step was to lower the spindle firing rate in the period prior to returning to the test length. For both conditioning sequences the reflex was measured at the same time after the initial muscle stretch. Fusimotor strength contractions normally given during the hold phase of the conditioning procedures were, for the sake of clarity, not given in the example illustrated in Fig. 5. However, giving a conditioning fusimotor stimulus did not change the result. The reflex measured following the double release was significantly larger than after the single release. Once again, instantaneous frequency recordings made from single Ia afferent fibres

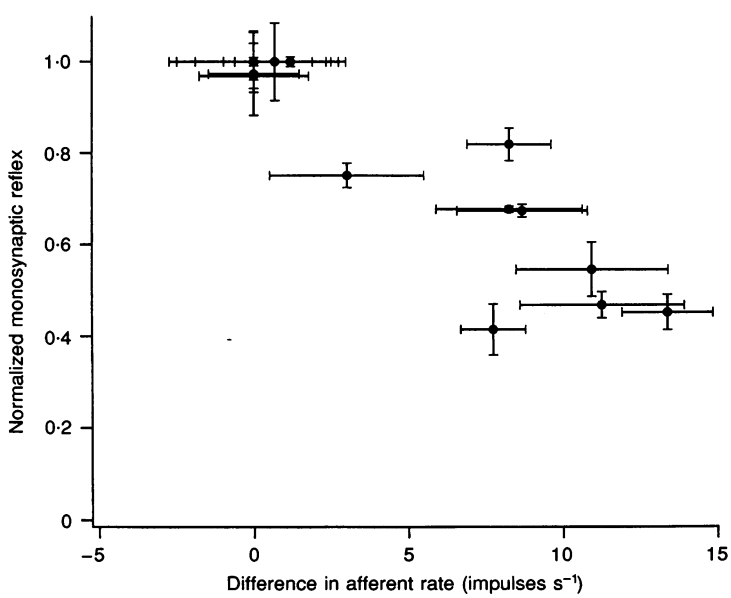


Figure 3. The relation between muscle spindle discharge rate and monosynaptic reflex amplitude in the cat

The mean normalized monosynaptic reflex has been plotted against the mean difference in afferent rate measured at a conditioning–test interval of 29 s for 5 experiments. For each experiment the difference in afferent rate was calculated by averaging the discharge rate recorded from a sample of muscle spindles for each of the three conditioning procedures used (see Methods). The mean value of hold-long discharge rate was then subtracted from the calculated values for all conditioning procedures. The normalized monosynaptic reflex for each experiment was calculated by expressing the mean reflex value, for each conditioning procedure, as a fraction of the largest monosynaptic reflex recorded for that particular experiment. Values are means ± S.E.M.

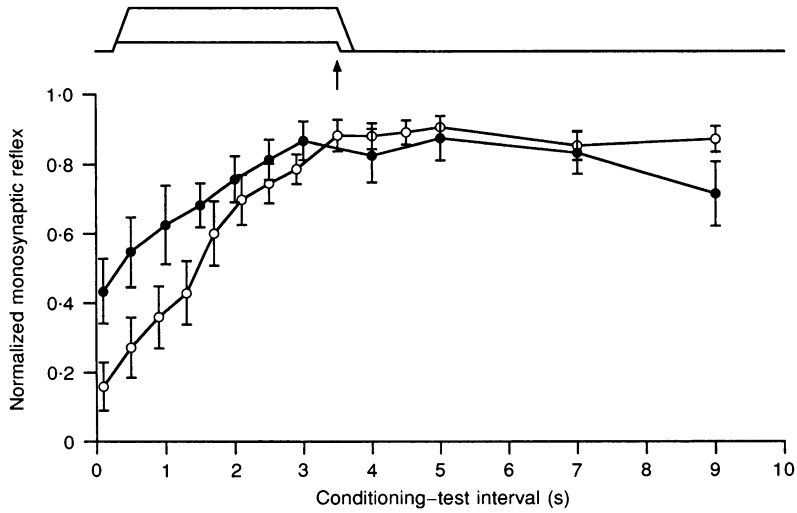


Figure 4. The effects of altering the preceding level of resting discharge on the monosynaptic reflex in the cat

Mean normalized reflex amplitude, from 6 experiments, is plotted for various conditioning–test intervals following hold-long conditioning using two different length steps, 1 mm (●, mean ± S.E.M.) and 5 mm (○, mean ± S.E.M.). The arrow beneath the upper length traces indicates the position corresponding to 0 s on the lower graph. Reflex amplitude following the 1 mm step was not depressed as much as when a 5 mm step was used.

showed that the frequency of firing was reduced to zero, in most cases, on shortening the muscle from the 10 mm to the 5 mm hold-long length. To summarize, the above results support the idea that the level of muscle spindle discharge during the period immediately before a monosynaptic reflex is measured is an important determinant of the size of the reflex.

At the time of these experiments the prevailing view was that inhibitory effects of Ia activity on the monosynaptic reflex of homonymous and synergist motoneurones were

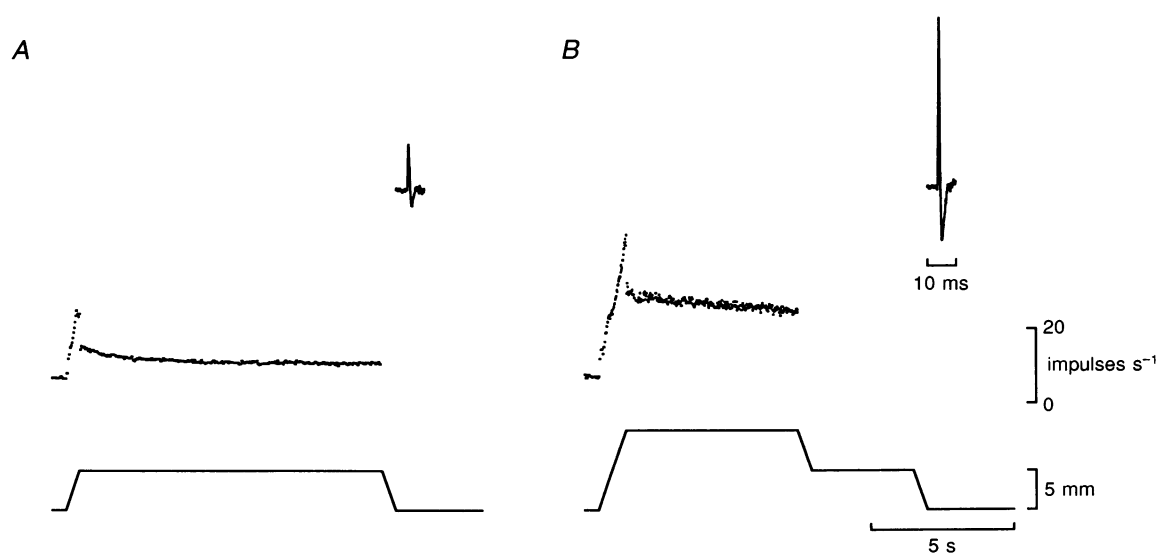


Figure 5. The preceding level of muscle spindle activity can alter the size of the monosynaptic reflex in the cat

A, monosynaptic reflex (uppermost trace) measured immediately after a 5 mm hold-long conditioning procedure. Lower trace is muscle length, while the middle trace is an instantaneous frequency recording made from a single muscle spindle primary ending. *B*, monosynaptic reflex measured after a double release conditioning procedure. The first release step was used to silence the muscle spindles, as shown by the record from a sample afferent in the middle trace. The reflex recorded immediately following the second release step is much larger than in *A*.

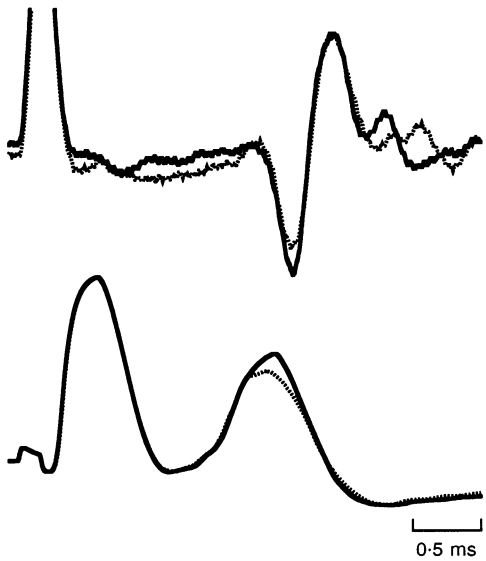


Figure 6. Antidromic nerve volleys and ventral root discharges in response to intraspinal stimulation of the triceps surae motoneurone pool in the cat

The upper traces show the superimposed hold-long conditioned (continuous line) and hold-short conditioned (dashed line) antidromic volleys recorded from the muscle nerve in response to an intraspinal stimulus. The lower traces are the superimposed ventral root discharges recorded in response to the same intraspinal stimulus. If presynaptic inhibition was responsible for the difference in reflex size then the antidromic volley recorded in the muscle nerve after hold-short muscle conditioning should have been larger than that recorded after hold-long muscle conditioning. This was not the case.

through a presynaptic inhibitory mechanism. Two methods were used to seek evidence for such a mechanism. The first method employed was that of Wall (1958). This involves testing the excitability of the afferent terminals that mediate the monosynaptic reflex by recording the size of antidromic volleys in the muscle nerve evoked by intraspinal stimulation through a microelectrode. With a microelectrode inserted into the triceps motoneurone pool a stimulus was applied, after both forms of conditioning, and the resulting antidromic volley recorded in the triceps surae muscle nerve. At the same time recordings were made from the ventral roots. Typically a double-peaked response was observed in the ventral roots, the first peak corresponding to the volley evoked by direct excitation of a population of motoneurones, the second being the monosynaptic reflex in response to stimulation of the Ia terminals (Fig. 6). A large direct response indicates that the tip of the microelectrode is within the motoneurone pool, in the region of the Ia terminals. Stimulation here, after hold-short conditioning, should have resulted in a larger antidromic response than

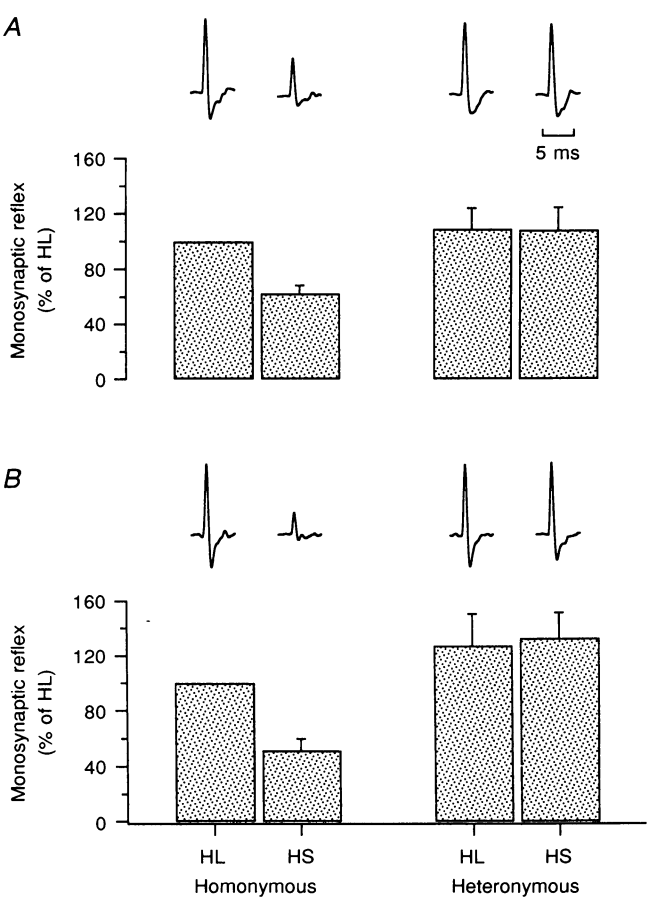


Figure 7. The effect of conditioning a close synergist muscle on the size of the monosynaptic reflex in the cat

The two histograms and the examples of recorded monosynaptic reflexes, shown above each column in the histograms, were obtained using the synergist medial gastrocnemius (MG) and lateral gastrocnemius–soleus (LG–S) muscle groups. In *A*, the reflex was elicited by stimulation of the MG nerve, after hold-long (HL) and hold-short (HS) conditioning applied either to the MG (homonymous) or LG–S (heteronymous) muscles, while in *B* the reflex was elicited from LG–S after conditioning LG–S or the synergist. Each column shows the average reflex amplitude, recorded from 4 experiments, expressed as a percentage of the value obtained when the homonymous muscle was conditioned as hold-long. In both cases, that is, whether the reflex was elicited from MG or LG–S, heteronymous muscle conditioning had no influence on the size of the homonymous reflex. Error bars are S.E.M.

that recorded after hold-long conditioning. If after hold-short conditioning the Ia terminals are depolarized, therefore having lower thresholds for excitation, the test stimulus should cause more Ia afferent fibres to be recruited, which should therefore result in a larger antidromic volley. However, there was no significant difference in the size of the antidromic volleys recorded following hold-long and hold-short conditioning. This procedure was repeated in four experiments and on no occasion was there a detectable difference between the size of the antidromic volleys measured after the two forms of conditioning.

In the second method, we observed the effect of conditioning a close synergist on the size of the homonymous test monosynaptic reflex. Presynaptic inhibition would be expected to exert its effects on both homonymous and heteronymous Ia terminals. By conditioning the synergist we wanted to see if it was possible to alter the size of the homonymous monosynaptic reflex. In these experiments the Achilles tendon was split longitudinally into two pieces so that the lateral gastrocnemius and soleus (LG–S) could be stretched separately from the medial gastrocnemius (MG). The nerve supplying the triceps muscle was also separated into two, the MG nerve and the LG–S nerve. Firstly, monosynaptic reflex recordings were made using homonymous muscle conditioning for both MG and LG–S separately. Then heteronymous muscle conditioning was performed for each muscle. LG–S was left attached to the muscle stretcher while MG was set at the test length. Then LG–S was subjected to hold-long and hold-short conditioning and the MG monosynaptic reflex was measured by applying a stimulus to the MG nerve. This procedure was then repeated, using MG for conditioning and LG–S for the test. Sometimes twin pulses, with a delay of 2 ms, were necessary as a single stimulus did not produce a measurable reflex. In each experiment and for each condition thirty-two trials were recorded and averaged. In all four experiments conditioning the homonymous muscle produced the

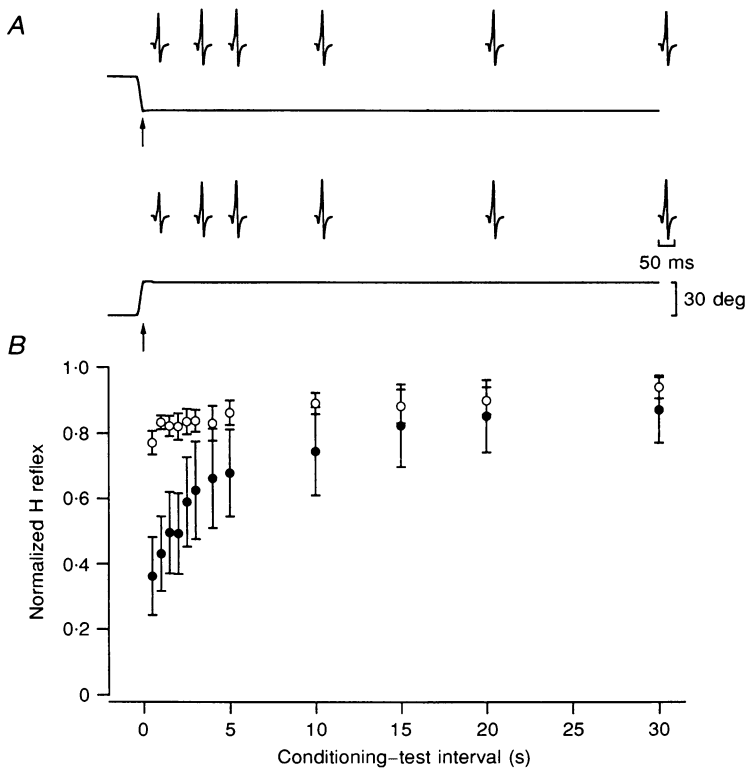


Figure 8. The H reflex measured at various times after muscle conditioning in human subjects

A, examples of H reflexes recorded after hold-long conditioning (upper traces) and hold-short conditioning (lower traces) in one subject. The continuous line indicates the ankle angle during the conditioning procedures. The test angle, the angle at which reflexes were elicited, was the same in each case. The stimulus artefact and direct response have, for clarity, been removed from the H reflex recordings, which are shown above the angle traces. Only one reflex was elicited in each conditioning–test sequence and each panel is therefore a composite of 6 sequences. The arrow beneath each angle trace indicates the position corresponding to 0 s on the graph in *B*. *B*, normalized mean H reflex amplitude plotted against conditioning–test interval. H reflexes were normalized within each experiment by expressing the H reflex averaged from 5 trials, for each conditioning–test interval, as a fraction of the largest H reflex recorded. The normalized H reflex for each condition and conditioning–test interval was averaged from 5 subjects. Reflexes recorded after hold-long conditioning (○, mean ± S.E.M.) were larger than after hold-short conditioning (●, mean ± S.E.M.).

expected muscle conditioning effects. That is, hold-short conditioning resulted in a smaller monosynaptic reflex than hold-long conditioning (Fig. 7). When the heteronymous muscle was conditioned there was no apparent change in the size of the homonymous test reflex, and this applied to both combinations of conditioning and test muscles.

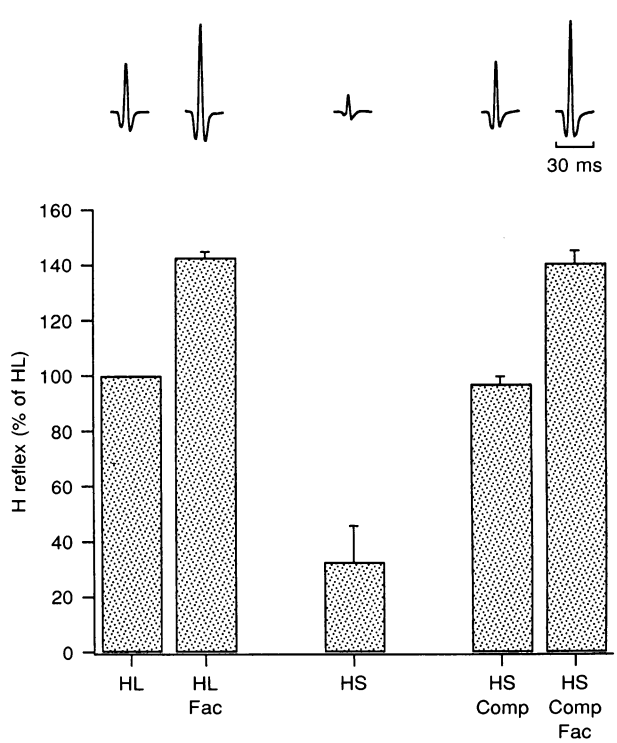
Human experiments

Muscle history effects have been described in human subjects, but a detailed study of effects on the human equivalent of the monosynaptic reflex, the H reflex, has not been carried out. The measurement of the time course of monosynaptic reflex changes following conditioning, described above for the cat, was repeated in human subjects, using the H reflex in triceps surae. The reflex was tested over a range of intervals following hold-short and hold-long conditioning. In each experiment, for each type of conditioning and for each conditioning–test interval, five trials were averaged. The averaged values were then normalized with respect to the maximum H reflex recorded for that particular experiment, and the results for the five experiments were combined and averaged. The pooled results are shown in Fig. 8*B*. Although, as in the cat, after hold-long conditioning the H reflex was larger than after hold-short conditioning, the maintained difference in reflex seen at long conditioning–test intervals for the cat was not present in the majority of subjects tested. In general, for human subjects the difference in H reflex size following the two forms of conditioning had disappeared within 15 s of return to the test length (Fig. 8*B*). The reflex measured after hold-long conditioning reached a constant amplitude within the first few seconds while for hold-short conditioning it reached its final value more slowly. This behaviour was different from that seen in the cat where a difference was maintained, at least over the range of conditioning–test intervals studied. Furthermore, for most of the human subjects studied there was little, if any, detectable inhibition of the H reflex immediately following hold-long conditioning. In the animal experiments the inhibition after hold-long was more profound and was present in all animals.

One way of testing for the presence of presynaptic inhibition in human subjects is to use the method of Hultborn *et al.* (1987), which is based on measuring the amount of heteronymous facilitation between quadriceps and triceps surae. This test was carried out with five human subjects. The method requires a conditioning stimulus at motor threshold for the quadriceps muscle group, which is used to facilitate the triceps H reflex. It has been shown previously that in humans the quadriceps Ia afferents project monosynaptically onto the triceps surae motoneurones (Hultborn *et al.* 1987). Presynaptic inhibition would be expected to influence all Ia terminals so that any stimulus causing presynaptic inhibition should also inhibit the synergist Ia terminals from quadriceps onto triceps motoneurones. Therefore any decrease in the amount of measured facilitation from quadriceps could be attributed to presynaptic inhibition. In our experiments, if presynaptic inhibition were responsible for the observed difference in triceps H reflexes after hold-long and hold-short conditioning then a difference in the amount of facilitation from quadriceps would be expected. Such a difference was not found (Fig. 9). Student's *t* test revealed no significant difference between the amount of facilitation caused by the conditioning quadriceps volley after hold-long conditioning and hold-short conditioning with compensation.

Figure 9. Heteronymous facilitation of the triceps surae H reflex in human subjects

Each column represents the size of the H reflex, expressed as a percentage of the hold-long H reflex size, averaged from 5 subjects after various conditioning procedures. Error bars are S.E.M. Hold-long (HL) conditioning was a standard 30 deg dorsiflexion movement followed by a triceps contraction, after which the ankle was returned to the test angle (see Methods). Hold-long facilitated (Fac) conditioning was when the H reflex was measured after hold-long conditioning and the test volley was accompanied by a facilitating quadriceps volley. The column labelled hold-short (HS) was a standard 30 deg plantarflexion followed by a voluntary triceps contraction, after which the ankle was rotated back to the test angle (see Methods). For hold-short compensated (Comp) conditioning the H reflex was measured after the hold-short conditioning procedure except that the stimulus was adjusted to make the H reflex approximately equal in size to that recorded after hold-long conditioning. With hold-short compensated facilitated conditioning, the compensated hold-short reflex was facilitated by a quadriceps volley. Above the columns are reflexes recorded from 1 subject under the corresponding conditions; for simplicity the stimulus artefact and the direct response have been removed.



DISCUSSION

It has been known for some time that stimuli which excite the primary endings of muscle spindles can also inhibit the phasic stretch reflex. Vibration (Gillies *et al.* 1969), muscle stretch (Delwaide, 1973) and the drug succinylcholine (Cook, Neilson & Brookhart, 1965), a depolarizing neuromuscular blocker, all stimulate the primary endings of muscle spindles and all depress the stretch reflex. In our experiments we were able to inhibit the monosynaptic reflex in the cat by using simple conditioning procedures to elevate muscle spindle resting discharge. From the data presented in Fig. 3 it was estimated for triceps surae of the cat that an increase in spindle resting discharge of 10 impulses s^{-1} was accompanied by an approximate fall of 40% in the amplitude of the monosynaptic reflex. It appears that the decrease in monosynaptic reflex amplitude brought about by the hold-short conditioning is maintained for as long as muscle spindle resting discharge remains elevated.

An interesting difference between the cat and human subjects was that in humans the difference in reflex size following the two forms of muscle conditioning disappeared at around 15 s in four out of five subjects, whereas in the cat the difference was maintained for the full period of measurement. A possible explanation is suggested by the observation that after a contraction muscle fibres tend to return to a preferred rest length (Ramsey & Street, 1940; González-Serratos, 1971). González-Serratos (1971) noted that myofibrils of single isolated frog fibres, set in gelatine, returned to a slack state following an isometric contraction that removed all previous slack. The forces acting to return the myofibrils to a slack state appeared to reside within the filaments themselves. If the intrafusal fibres of human muscle spindles have a similar preferred rest length they may not be able to remain taut for long periods following hold-short conditioning, and over time they may redevelop some slack. This would lead to a gradual lowering of resting discharge and increase in the size of the reflex. It is also of interest to note that microneurographic studies of single Ia afferent fibres in human subjects have shown that following a voluntary contraction only nineteen of fifty-five (34·5%) spindles sampled exhibited maintained increases in resting discharge (Wilson, Gandevia & Burke, 1995). In our animal experiments sixty-seven out of sixty-eight (98·5%) muscle spindles sampled showed a maintained increase in their discharge rate. This result may represent a fundamental difference between human and cat muscle spindles.

Another interesting difference to come out of the comparison between data for cats and humans is that the depression of the monosynaptic reflex immediately following hold-long conditioning is much less pronounced in human subjects than in the cat. One possible explanation is that the level of resting discharge reached during the hold phase of the hold-long conditioning for human muscles may not have been very different from that at the test length. It is known from microneurographic studies that spindle discharge rates recorded from human muscle spindles in response to muscle stretch are much lower that those recorded from the anaesthetized cat (Gandevia, Wilson, Cordo & Burke, 1994).

The mechanism proposed for the reflex inhibition in the experiments using vibration (Gillies *et al.* 1969), muscle stretch (Delwaide, 1973) and succinylcholine (Cook *et al.* 1965) was presynaptic inhibition resulting from an increase in homonymous muscle spindle activity. Indeed, Gillies *et al.* (1969) and Cook *et al.* (1965) described dorsal root potentials accompanying these stimuli, consistent with such an interpretation. In the present study we have presented evidence to show that the inhibition produced by increases in spindle resting discharge from muscle conditioning does not appear to involve 'classical' presynaptic inhibition. In the cat, antidromic testing of Ia terminal excitability in triceps provided no evidence of the expected increase in excitability following hold-short conditioning. In addition, heteronymous conditioning failed to alter the size of the homonymous monosynaptic reflex. The connections made by the interneurones serving the presynaptic inhibitory pathway are known to be widespread, that is, they are not restricted to the Ia afferent terminals of the homonymous muscle (Barnes & Pompeiano, 1970). Therefore if the elevated levels of resting discharge observed after hold-short conditioning resulted in reflex depression that was due to an increase in presynaptic inhibition, the terminals of heteronymous Ia fibres should also have been affected. This was not the case and effects remained strictly homonymous. Using heteronymous facilitation of the triceps surae H reflexes in human subjects we were similarly unable to show changes in facilitation by stimulating quadriceps afferents, this result being inconsistent with a presynaptic inhibitory action.

We claim that reflex inhibition is the result of an increase in spindle afferent activity. Immediately after hold-long conditioning the majority of spindles were silent, yet the reflex was also depressed. We have provided evidence (Figs 4 and 5) in support of the interpretation that during hold-long conditioning the high maintained levels of spindle activity, immediately before return to the test length, were responsible for this depression. This means that at the test length, the recovery time course of the reflex was initially determined largely by the fading effects of the previous period of high activity. This experiment therefore provides a useful method of measuring the time course of action of spindle activity-evoked inhibition.

Finally, the time course of monosynaptic reflex depression immediately following hold-long conditioning was also not consistent with a 'classical' presynaptic inhibitory effect. Experiments using electrical stimulation of flexor Ia afferents have revealed that extensor monosynaptic reflexes are inhibited for ~400 ms following the inhibitory stimulus (Eccles, Schmidt & Willis, 1962). In our experiments reflex depression following hold-long conditioning lasted for approximately 3 s, implying that the mechanism responsible for the inhibition operated over a much longer time course than 'classical' presynaptic inhibition.

It has recently been demonstrated that the human soleus H reflex is depressed for more that 10 s following a passive dorsiflexion (Hultborn, Illert, Nielsen, Paul, Ballegaard & Wiese, 1996). This effect was shown by nerve block to be due to large diameter afferents from the stretched muscle. The depression was not due to changes in motoneuronal excitability since motor evoked potentials by magnetic brain stimulation were not similarly depressed by the same passive dorsiflexion. The depression appeared to be confined to afferents of the stretched muscle. Similar experiments on decerebrate cats confirmed the presynaptic origin of the depression, which was attributed to post-activation transmitter depletion.

Long lasting depression of the H reflex in human subjects and the monosynaptic reflex in the cat produced by activation of the homonymous Ia afferent fibres has been reported previously. Electrical stimulation of Ia fibres (Táboříková & Sax, 1969; Crone & Nielsen, 1989), contraction of the test muscle (Schieppati & Crenna, 1984), a subliminal tendon tap (Katz, Morin, Pierrot-Deseilligny & Hibino, 1977; Crone & Nielsen, 1989) and stretch of the test muscle (Mark, Coquery & Paillard, 1968; Nielsen, Petersen, Ballegaard, Biering-Sørensen & Kiehn, 1993) are all conditioning procedures that have been used to demonstrate a long lasting reflex depression that occurs subsequent to the activation of the primary endings of muscle spindles. The inhibition has been shown to occur in the absence of any change in the excitability of the motoneurones and is therefore presynaptic (Nielsen *et al.* 1993; Hultborn *et al.* 1996). The most likely mechanism is a change in the probability of transmitter release. At frequencies of stimulation below 100 Hz, transmitter depletion and therefore excitatory postsynaptic potential (EPSP) depression appears to occur (Curtis & Eccles, 1960; Honig, Collins & Mendell, 1983). Given that hold-short conditioning resulted in an average resting discharge of 18 impulses s^{-1}, transmitter depletion may have been the dominating influence in our experiments. Curtis & Eccles (1960) also noted that the first EPSP in a train was relatively larger than ensuing EPSPs (see also Honig *et al.* 1983). If following hold-long conditioning most of the muscle spindles were silent, then the EPSP resulting from the test stimulus would be likely to be larger than if the stimulus was delivered during on-going repetitive muscle spindle activity as would be the case following hold-short conditioning.

To conclude, it would appear that a long term depression of the monosynaptic reflex results from raised levels of homonymous muscle spindle activity. This depression does not appear to involve 'classical' presynaptic inhibition, and some other presynaptic mechanism is believed to be responsible. It is of interest to reflect on the fact that, while Ia impulses evoke a powerful monosynaptic excitatory action on motoneurones, at the same time they give rise to presynaptic events which lead to long lasting depression of the reflex. Given that the kind of muscle conditioning used in these experiments is not unlike situations encountered during normal unrestricted movements, then the depression reported here is likely to be a common, everyday event. It will now be necessary to re-evaluate the various mechanisms of segmental regulation of movement and posture, incorporating this kind of push–pull mechanism into our thinking.

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Author's email address

S. Wood: Shane.Wood@med.monash.edu.au

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