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MUSCLE BUT NOT CUTANEOUS C-AFFERENT INPUT PRODUCES PROLONGED INCREASES IN THE EXCITABILITY OF THE FLEXION REFLEX IN THE RAT

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SUMMARY

1. Stimulation of cutaneous afferent fibres in the sural nerve and muscle afferent fibres in the gastrocnemius–soleus nerve at a strength that excites C axons produces a delayed and long-lasting burst of activity in posterior biceps femoris/semitendinosus flexor motoneurones.

2. Following a 20 s stimulation at 1 Hz to the sural nerve the flexor motoneurones continue to fire for 20 s while a similar stimulus to gastrocnemius–soleus nerve results in an after-discharge lasting three times longer.

3. Using stimuli to the sural and gastrocnemius–soleus nerves as conditioning stimuli (20 s, 1 Hz) changes in the excitability of the flexor reflex were measured by recording the discharge evoked by a test sural nerve stimulus or by a standard pinch to the ipsilateral and contralateral toes.

4. Prior to any conditioning stimulus the flexor reflex remained stable for prolonged periods. Conditioning stimuli at strengths that activated large myelinated afferent fibres only, or large and small myelinated afferent fibres, failed to produce more than a very transient alteration in the reflex excitability.

5. Conditioning stimuli at C-fibre strength to the sural nerve produced a marked increase in the excitability of the reflex for 10 min. C-fibre strength gastrocnemius–soleus nerve conditioning stimuli resulted in a similar increase in excitability followed by a second phase of facilitation peaking at 20–30 min and lasting for up to 90 min.

6. The afferent barrage initiated by cutting the sural and gastrocnemius–soleus nerves resulted in similar patterns of reflex excitability increases with the muscle nerve resulting in a more prolonged effect than the cutaneous nerve.

7. The results show that a brief C-afferent fibre input into the spinal cord can produce a prolonged increase in the excitability of the flexion reflex and that muscle C-afferent fibres evoke longer-lasting changes than cutaneous C fibres. The differences in the time course of the post-conditioning effects may be related to the well-described differences in the sensory consequences of injury to skin *versus* deep tissue.

INTRODUCTION

It is a common clinical observation that minor deep injury in a limb results in a prolonged tenderness which spreads far from the site of the injury while apparently equivalent cutaneous injuries result in more spatially and temporally restricted sensory disorders. Similar differences have been noted by comparing the effects of experimental noxious stimuli to skin and to deep tissue in man (Lewis & Kellgren, 1939; Lewis, 1942; Hockaday & Whitty, 1967). Because the deep tissue tenderness is so widespread it is more likely to be mediated by central changes than peripheral ones. A recent study in our laboratory has shown that a peripheral thermal injury in decerebrate rats results in a prolonged increase in the excitability of the flexor reflex with decreases in threshold and increases in responsiveness (Woolf, 1983*a*). Part of this increase has to be attributed to changes within the spinal cord because a sensory blockade of the injury does not reverse it. We have now investigated the central consequences of cutaneous and muscle afferent inputs that may mediate the injury-induced increases in the excitability of the spinal cord and which may also underlie the clinical and human experimental data.

Tissue-damaging stimuli are carried by thin myelinated (Aδ, group III) and by non-myelinated (C, group IV) afferent fibres in cutaneous and muscle nerves. In both types of nerve the non-myelinated afferent fibres outnumber the myelinated afferents (McLachlan & Janig, 1983). Cutaneous C-evoked responses in the spinal cord have been well-studied (see Perl, 1984) but little is known of muscle C-evoked activity (Foreman, Schmidt & Willis, 1979). There does appear to be a difference in the site of termination of the two groups of C fibres, with the cutaneous C fibres terminating in lamina II of the dorsal horn (Grant, Arvidsson, Robertson & Ygge, 1979; Swett & Woolf, 1983) and the muscle C fibres terminating in lamina I and V (Brushart & Mesulam, 1980; Bakker, Richmond & Abrahams, 1982; Craig & Mense, 1983; Swett 1983). This difference may have important implications for the type of activity evoked in the spinal cord by these two different groups of afferent fibres.

We report here that a brief low frequency input from cutaneous and muscle non-myelinated but not myelinated afferent fibres results in a prolonged increase in the excitability of the flexion reflex in the decerebrate spinal rat and that the muscle C fibres are more effective in producing such changes than cutaneous C fibres.

METHODS

The experiments were performed on sixteen Sprague–Dawley rats weighing between 200 and 300 g. Under ether anaesthesia the trachea and a carotid artery were cannulated. The rats were then maintained under Althesin (alphadolone/alphaxolone) anaesthesia with small I.A. doses at 10 min intervals during the surgical preparations. They were then decerebrated precollicularly by aspiration, the Althesin discontinued and the animals ventilated under gallamine paralysis. The sural nerve, the medial and lateral nerves to gastrocnemius–soleus and the nerve to the posterior head of biceps femoris and semitendinosus were exposed in the left popliteal fossa. The sural and medial and lateral gastrocnemius–soleus nerves were placed on bipolar stimulating electrodes. Small filaments of the nerve to posterior biceps femoris/semitendinosus were placed on a recording electrode. The nerves were covered in a pool of warm mineral oil. Spinalization was performed at T8–T10 via a small laminectomy. Recordings were only commenced at least 1 h post-spinalization. Blood pressure, heart rate, end-tidal P_{CO_2} and rectal temperature were monitored and maintained within normal limits.

Recordings were made from either single or small numbers of α-motoneurone efferent fibres per filament. The techniques used to characterize the motoneurones were as previously described (Woolf & Swett, 1984). Stimulation of the sural and gastrocnemius–soleus nerves were performed at three strengths: 100 μA, 50 μs, which is sufficient to activate Aβ cutaneous afferent fibres and group I

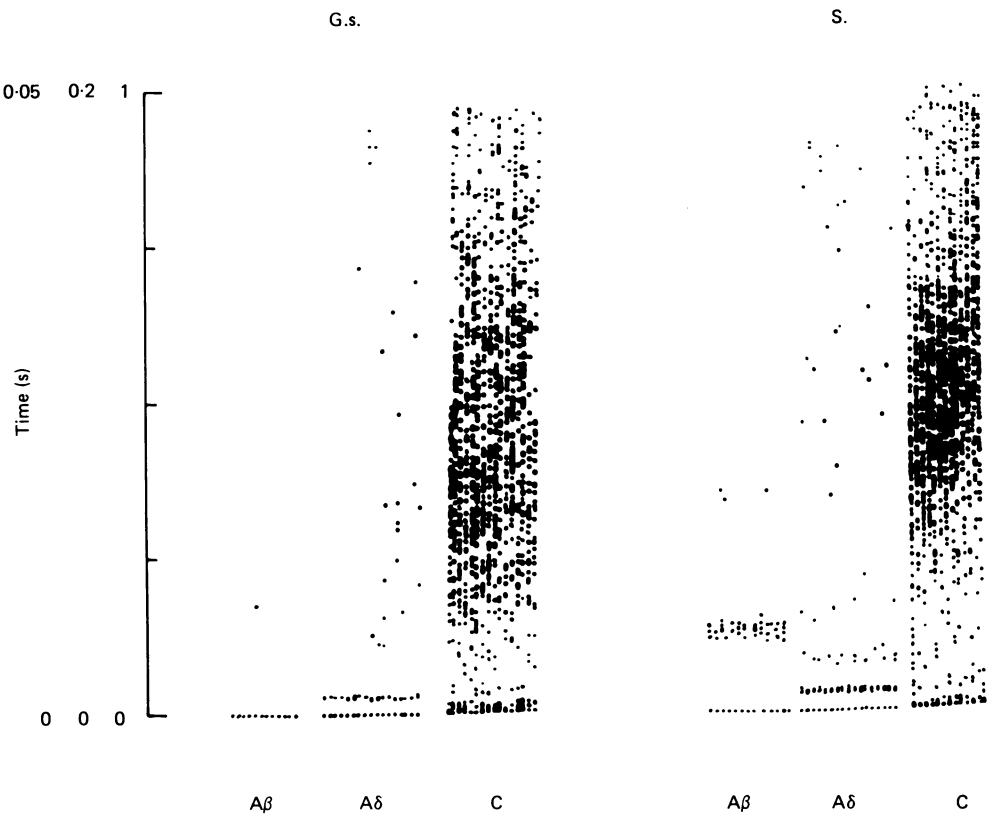

Fig. 1. A raster dot display illustrating the activity evoked in a posterior biceps femoris/semitendinosus motoneurone by stimulating the sural nerve (s.) and the gastrocnemius–soleus nerve (g.s.). Each dot represents an action potential. The vertical scale represents the latency of response with the stimulus artifact at time 0. The horizontal scale represents real time with each stimulus repeated every 2 s. Three strengths of stimulation were used: 100 μA at 50 μs (Aβ); 500 μA at 50 μs (Aδ); and 5 mA at 500 ms (C). The vertical time scale is greatly expanded for the Aβ stimulus (extreme left scale), the middle scale refers to the time scale for the Aδ stimulus and the right scale to that used to record the late, prolonged C-evoked discharges.

and II muscle afferent fibres; 500 μA, 50 μs which in addition activates Aδ cutaneous afferent fibres and group III fibres; and 5 mA, 500 μs which is sufficient to stimulate C fibres and group IV fibres. The suitability of these strengths was tested in pilot experiments by recording compound action potentials on the appropriate dorsal roots following stimulation of the nerves. For convenience we will call group I and II afferent fibres, Aβ; group III, Aδ; and group IV, C and then specify whether cutaneous or muscle afferent fibres were stimulated. Natural stimulation of the cutaneous receptive fields of the motoneurones was performed by applying for 3 s a standard, suprathreshold pinch to the middle three toes across their dorsal and plantar surfaces.

RESULTS

Cutaneous and muscle C-afferent-fibre-evoked flexion reflexes

Stimulation of the sural nerve at a strength that only activates Aβ afferent fibres evokes a short latency (5–8 ms) reflex in posterior biceps femoris/semitendinosus motoneurones. Increasing the stimulus strength to recruit Aδ afferent fibres recruits

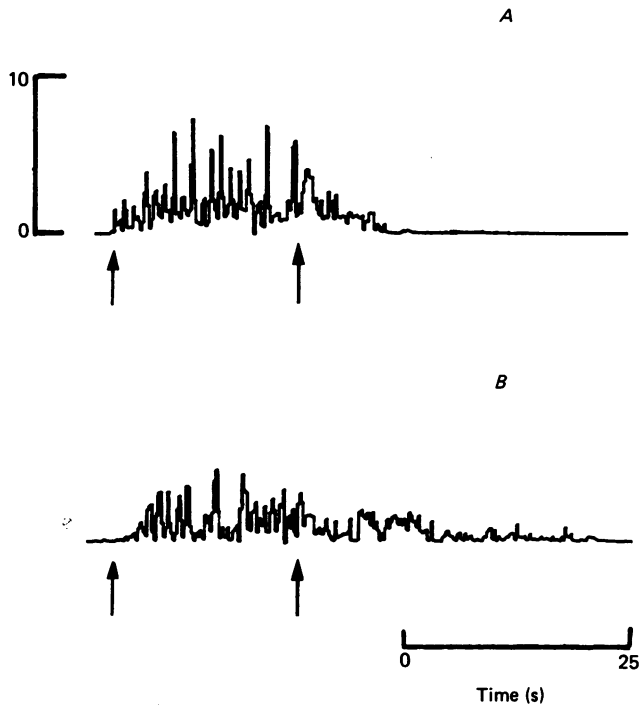

Fig. 2. A rate-meter record (number of action potentials per 200 μs bin; vertical scale) of the activity evoked in a posterior biceps femoris/semitendinosus motoneurone by stimulating the sural nerve (*A*) and the gastrocnemius–soleus nerve (*B*) at C-fibre strength for 20 s at 1 Hz. The onset and offset of stimulation is indicated by the arrows. Note the differences in the amplitude of the evoked response and the post-stimulus after-discharge.

a further response at a longer latency (20–100 ms) with an associated after-discharge, while stimulation at a strength that activates the cutaneous C fibres results in an even longer-latency reflex response (150–500 ms) with a very long after-discharge (Fig. 1) (Woolf & Swett, 1984). Stimulation of the myelinated afferent fibres in the gastrocnemius–soleus nerve fails to produce a short-latency low threshold reflex (Fig. 1) but when the non-myelinated afferent fibres are activated a long-latency prolonged discharge is evoked from posterior biceps femoris/semitendinosus motoneurones (Fig. 1). A comparison of the reflex responses evoked by muscle and cutaneous C-afferent fibres (Figs. 1 and 2) showed that typically a greater response was generated during the stimulus by stimulation of the sural nerve, but that the post-stimulus after-discharge was longer following stimulation of C afferents in the

gastrocnemius–soleus, than in the sural nerve. In Fig. 2, for example, following a 1 Hz C-fibre strength stimulus to the sural for 20 s, a posterior biceps femoris/semitendinosus motoneurone fires for 10 s, while a similar stimulus to the gastrocnemius–soleus nerve results in a 30 s after-discharge. The mean post-stimulation after-discharge was 21 ± 10 s (S.E. of mean = 8) for the sural and 77 ± 29 s ($n = 10$) for the gastrocnemius–soleus nerve.

Muscle and cutaneous C-afferent-fibre-induced excitability increases in posterior biceps femoris/semitendinosus motoneurones

Apart from the differences in the duration of the actual discharge in posterior biceps femoris/semitendinosus motoneurones evoked by brief sural and gastrocnemius–soleus nerve C-fibre strength stimuli, there is also a marked difference in the duration of the excitability increase of the reflex that follows such stimuli. The excitability of the flexor reflex was tested in two ways, by measuring the response to a test sural nerve C-fibre stimulus and by measuring the reflex discharge evoked by stimulation of the cutaneous receptive fields of the posterior biceps femoris/semitendinosus motoneurones.

(*1*) *Sural test stimuli.* Fig. 3 illustrates the change in the flexion reflex activity evoked by a test sural C-fibre stimulus (0·5 Hz) following a 1 Hz, 20 s C-fibre conditioning stimulus to the gastrocnemius–soleus nerve. Prior to the conditioning stimulus the sural nerve C-fibre strength test stimulus at 0·5 Hz evoked a uniform early and late response when repeated at 10 min intervals. At shorter intervals though, such test stimuli could alter subsequent responses. Ten minutes following the gastrocnemius–soleus conditioning stimulus the flexion reflex discharge evoked by the sural stimulus was greatly increased (Fig. 3). This increased test-evoked response remained present for 50 min before it returned to the preconditioning level at 60 min after the conditioning stimulus.

In Fig. 4 a similar increase in sural test C-evoked responses following a gastrocnemius–soleus conditioning stimulus is illustrated. This Figure which is from another animal shows not the actual action potentials evoked by the test stimulus, which were displayed in raster form in Fig. 3, but integrals of the number of action potentials evoked during and after the test stimulus. The number of impulses evoked by the test sural nerve stimulus (0·5 Hz for 20 s) increase dramatically following the gastrocnemius–soleus nerve conditioning stimulus and remain elevated for 40 min. The post-test sural stimulus after-discharge which lasted 20 s prior to the conditioning stimulus both increases in amplitude and duration (35 s) for up to 50 min after the conditioning stimulus (Fig. 4). In the same animal the effect of a 1 Hz, 20 s sural C-fibre strength conditioning stimulus on the sural test stimuli was also studied. Fig. 5 shows that a sural conditioning stimulus also increases the excitability of the reflex but the effect although large (see the wind-up in the test response 5 min post-conditioning stimulus), is short-lived, lasting less than 10 min.

(*2*) *Pinch-evoked responses.* Posterior biceps femoris/semitendinosus motoneurones in the decerebrate spinal rat have, in the absence of any conditioning stimuli, distinct and stable cutaneous receptive fields (Woolf & Swett, 1984). These motoneurones respond to high intensity mechanical stimuli (firm pressure or pinch) with a maximal response from the ipsilateral toes. At least 50% of these motoneurones also have a

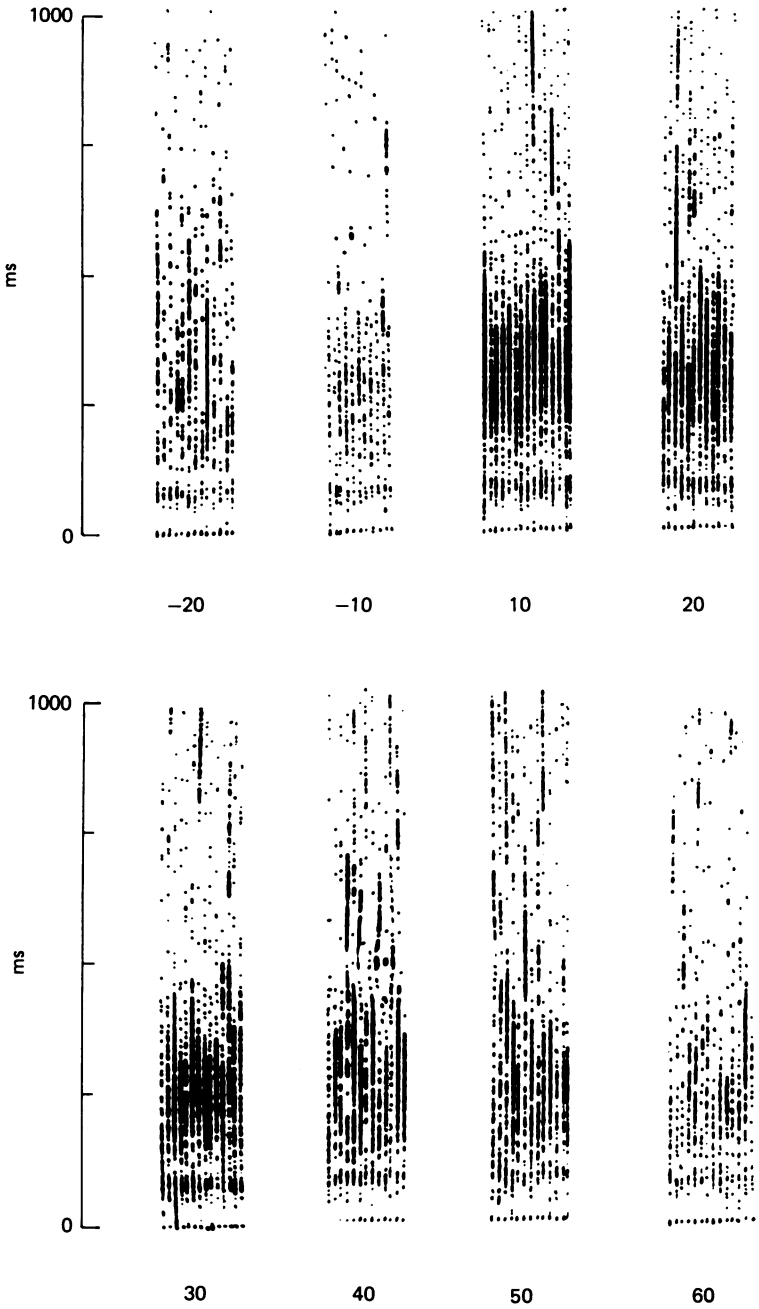

Fig. 3. Raster dot displays of the activity evoked in a posterior biceps femoris/semitendinosus motoneurone by a test stimulus to the sural nerve (0·5 Hz, C-fibre strength). At time 0 a conditioning stimulus to the gastrocnemius–soleus nerve (20 s, 1 Hz) at C strength was applied. Note the increase in the sural nerve test-evoked discharge lasting for 40 min post-conditioning. The number under each display refers to the time in minutes relative to the onset of the conditioning stimulus.

contralateral receptive field which does have however, a higher threshold and produces a smaller response (Woolf & Swett, 1984). A sustained suprathreshold pinch to the middle three toes both ipsi- and contralaterally produces a high frequency, relatively short-duration response (0·5–1·5 s) which is very stable, the total number of impulses evoked by such a stimulus varying by only $\pm 10\%$ over several hours.

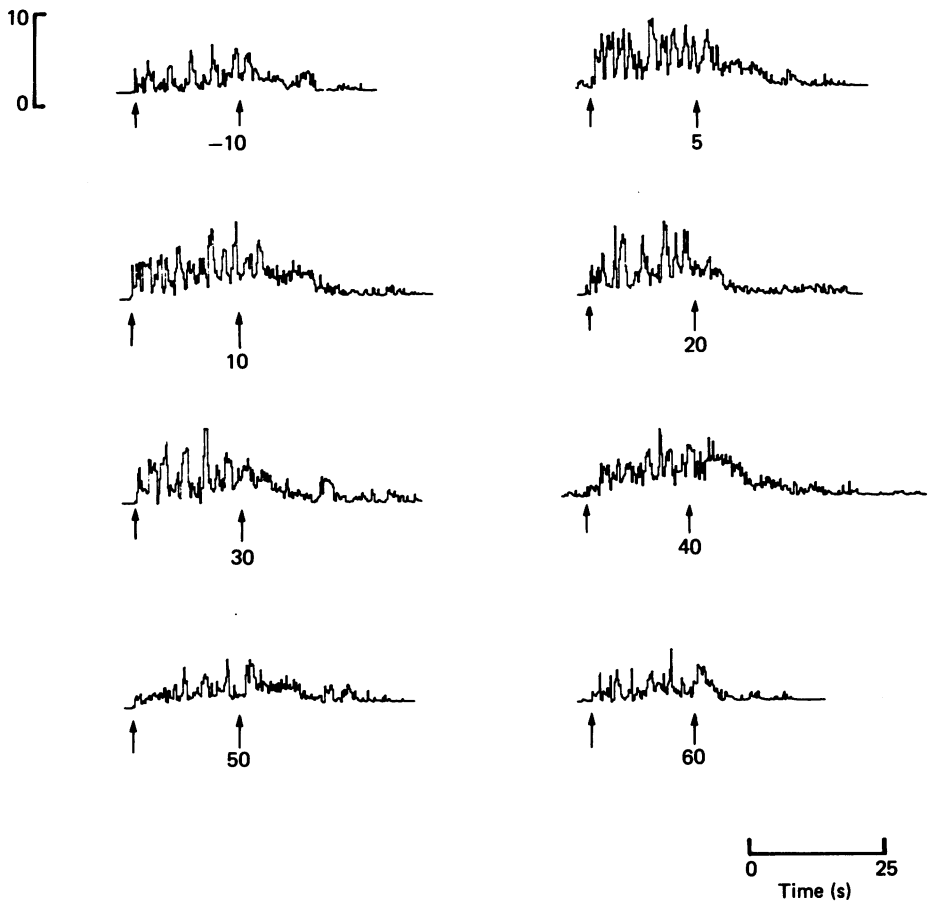

Fig. 4. Rate-meter recordings of the activity evoked in a posterior biceps femoris/semitendinosus motoneurone by a test stimulus to the sural nerve (0·5 Hz, 20 s, C-fibre strength) applied at the time indicated by the arrows. At time 0 a 20 s 1 Hz C-strength conditioning stimulus was applied to the gastrocnemius–soleus nerve. Note the increased test-evoked activity and the longer post-test stimulus after-discharge that occurred for 40 min after the conditioning stimulus. (Vertical scale = number of action potentials per 200 μs bin.)

Conditioning stimuli to the sural and the gastrocnemius–soleus nerves alters the pinch-evoked reflex response in the posterior biceps femoris/semitendinosus motoneurones. Fig. 6 illustrates the ipsilateral and contralateral pinch-evoked response in a motoneurone prior to and following a 20 s, 1 Hz C-fibre strength conditioning stimulus to the gastrocnemius–soleus nerve. The conditioning stimulus resulted both in an increase in the number of action potentials evoked by the pinch

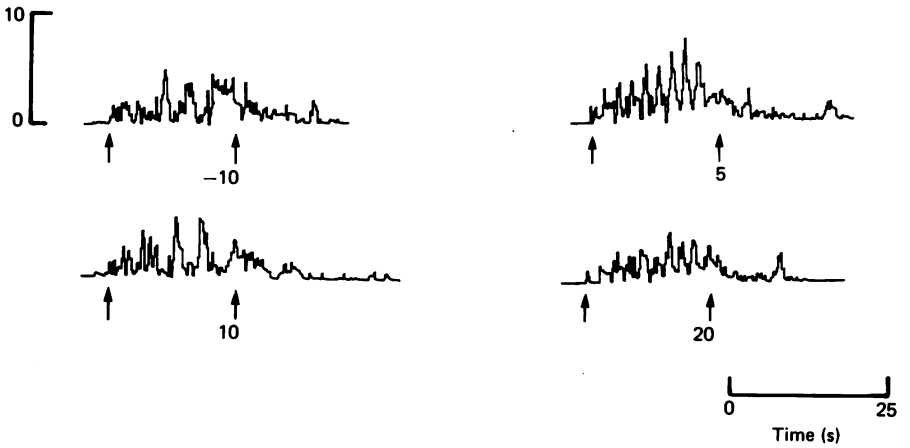

Fig. 5. Rate-meter recordings of the activity evoked in the same motoneurone as illustrated in Fig. 4 by a test stimulus to the sural nerve (0·5 Hz, 20 s, C-fibre strength) applied at the time indicated by the arrows. At time 0 a 20 s 1 Hz C-strength conditioning stimulus was applied to the sural nerve. This produced a much shorter facilitation of the reflex than the conditioning stimulus to the gastrocnemius–soleus nerve. (Vertical scale = number of action potentials per 200 μs bin.)

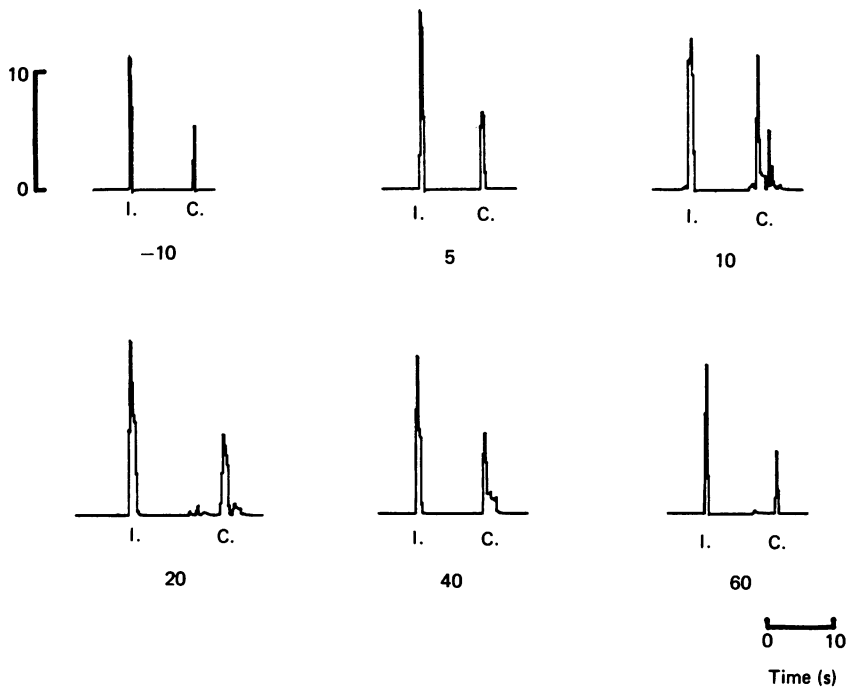

Fig. 6. Rate-meter recording of the response evoked in a posterior biceps femoris/semitendinosus motoneurone by a standard pinch applied to the ipsilateral middle three toes (I.) and the contralateral middle three toes (C.) for 3 s. At time 0, a 20 s 1 Hz C-strength conditioning stimulus was applied to the gastrocnemius–soleus nerve. Note the increase in the ipsi- and contralateral pinch-evoked responses from 5 to 40 min post-conditioning relative to the 10 min preconditioning response. (Vertical scale = number of action potentials per 200 μs bin.)

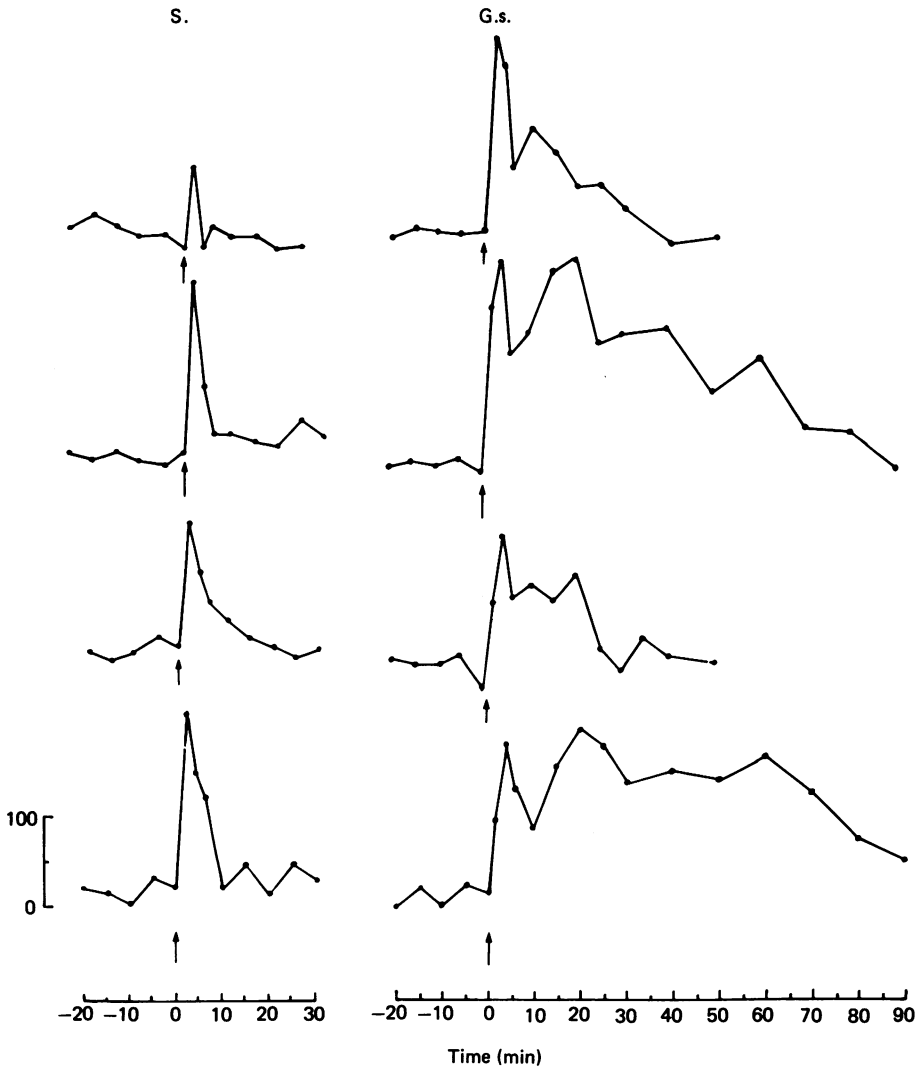

Fig. 7. A diagram illustrating the relative change in the total number of action potentials evoked by an ipsilateral pinch following sural (s.) and gastrocnemius–soleus (g.s.) nerve conditioning stimuli for 20 s at C-fibre strength in four posterior biceps femoris/semitendinosus motoneurones. In the upper three traces the conditioning stimuli were applied at 1 Hz, in the lower trace a 10 Hz stimulus was applied to the sural and gastrocnemius–soleus nerves. Each point on each graph is the integral of the total number of action potentials evoked by a pinch at that time. The base-line preconditioning absolute responses have not been indicated but were similar for all motoneurones. Note the single phase of facilitation following the sural nerve and two phases following the gastrocnemius–soleus nerve conditioning stimuli. (Vertical scale represents number of action potentials per pinch.)

and in the duration of the pinch-evoked response. In this instance this increased excitability of the reflex lasted for 40 min.

In Fig. 7 the change in the total number of action potentials evoked by the standard ipsilateral pinch before and after sural and gastrocnemius–soleus C-fibre strength

conditioning stimuli (20 s) are shown for four different motoneurones in four animals. The sural conditioning stimulus produces a marked but brief increase in the pinch-evoked reflex while the gastrocnemius–soleus conditioning stimulus results in a much more prolonged increase in the reflex excitability. That this difference is unlikely to be due to any difference in the amount of activity evoked in the spinal

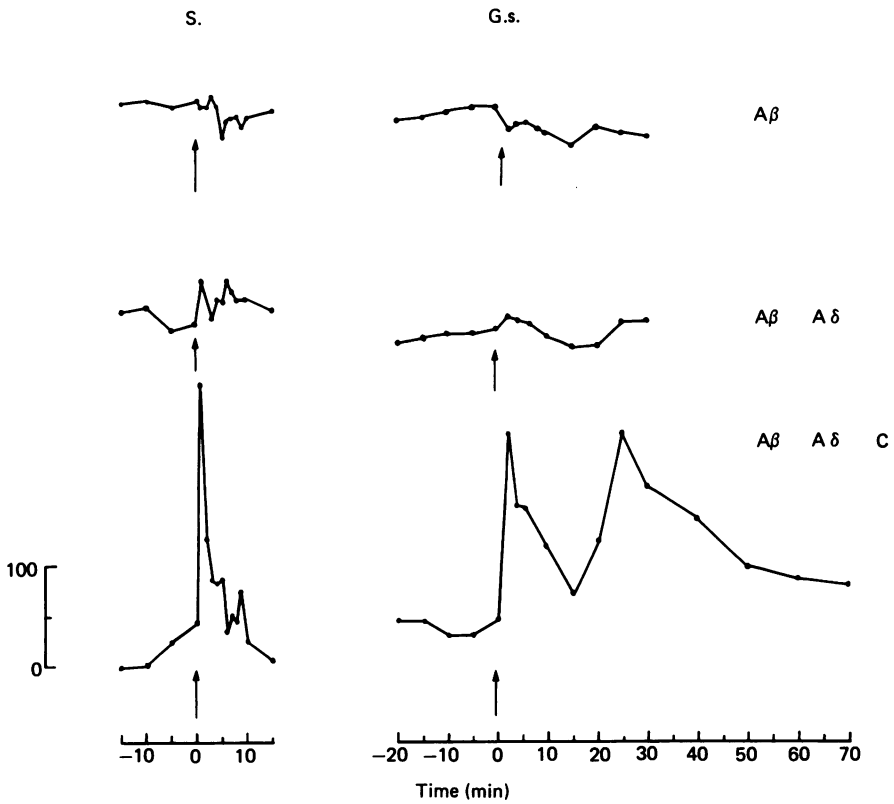

Fig. 8. Changes in the total number of action potentials elicited by an ipsilateral pinch in a posterior biceps femoris/semitendinosus motoneurone following conditioning stimuli to the sural (s.) and gastrocnemius–soleus (g.s.) nerves at strengths sufficient to activate large myelinated afferent fibres only (Aβ), small and large myelinated afferent fibres (Aβ and Aδ) and non-myelinated afferents (Aβ, Aδ and C). Only the latter conditioning stimulus produced a large change in the pinch-evoked responses, with a more prolonged effect from the gastrocnemius–soleus nerve. (Vertical scale represents number of action potentials per pinch.)

cord by the sural and gastrocnemius–soleus inputs is shown by the bottom trace in Fig. 7 where the conditioning stimulus for both the sural and gastrocnemius–soleus nerve was at 10 Hz rather than at 1 Hz for the upper three traces. Even at 10 Hz a sural nerve conditioning stimulus only produces a relatively short-lived effect. In five animals a 1 Hz 20 s C-strength sural conditioning stimulus produced an increase in the reflex excitability lasting 10 ± 3 min (S.E. of mean) while similar gastrocnemius–soleus conditioning stimuli resulted in excitability increases lasting 48 ± 8 min (S.E. of mean, $n = 9$), the longest lasting 90 min.

That the increase in the flexor reflex excitability was due to C afferents in the sural and gastrocnemius–soleus nerves was established by performing the conditioning stimuli at graded strengths, to activate selectively the large myelinated afferents, small myelinated afferents and the non-myelinated afferents in four animals. Fig. 8

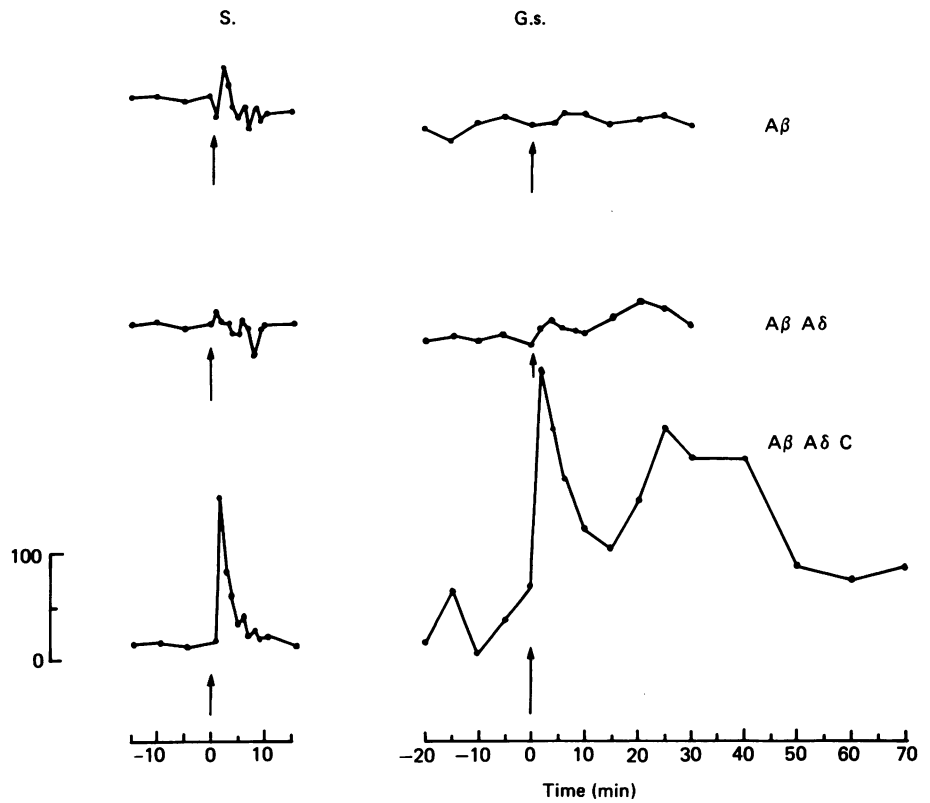

Fig. 9. Changes in the total number of action potentials evoked by a contralateral pinch in the same motoneurone as that illustrated in Fig. 8, following conditioning stimuli to the sural and gastrocnemius–soleus nerves at graded strengths. The C-strength sural and gastrocnemius–soleus nerve conditioning stimuli increase contralateral-evoked pinch responses as well as the ipsilateral ones (Fig. 8). (Vertical scale represents number of action potentials per pinch.)

illustrates such an experiment and shows that unless the conditioning stimulus was of an intensity sufficient to activate the non-myelinated afferents in the sural and gastrocnemius–soleus there was no significant increase in the reflex excitability. In Fig. 9 the changes in the contralateral pinch-evoked responses are shown to be very similar to that of the ipsilateral. The increase in the excitability particularly following the sural conditioning stimulus cannot be due therefore to some local factor resulting from the antidromic invasion of the C terminals in the skin.

A distinct feature of the increased excitability resulting from the gastrocnemius–soleus conditioning stimuli was that it consists of two phases (Figs. 7, 8 and 9): an early phase that resembles the duration of the sural response (<10 min) and a later

phase peaking at 15–25 min post-conditioning stimulus which was unique to the gastrocnemius–soleus conditioning stimuli.

Afferent injury barrage and the flexor reflex

An incidental observation made early during the course of these experiments was that section of either the sural or gastrocnemius–soleus nerves resulted in long-lasting

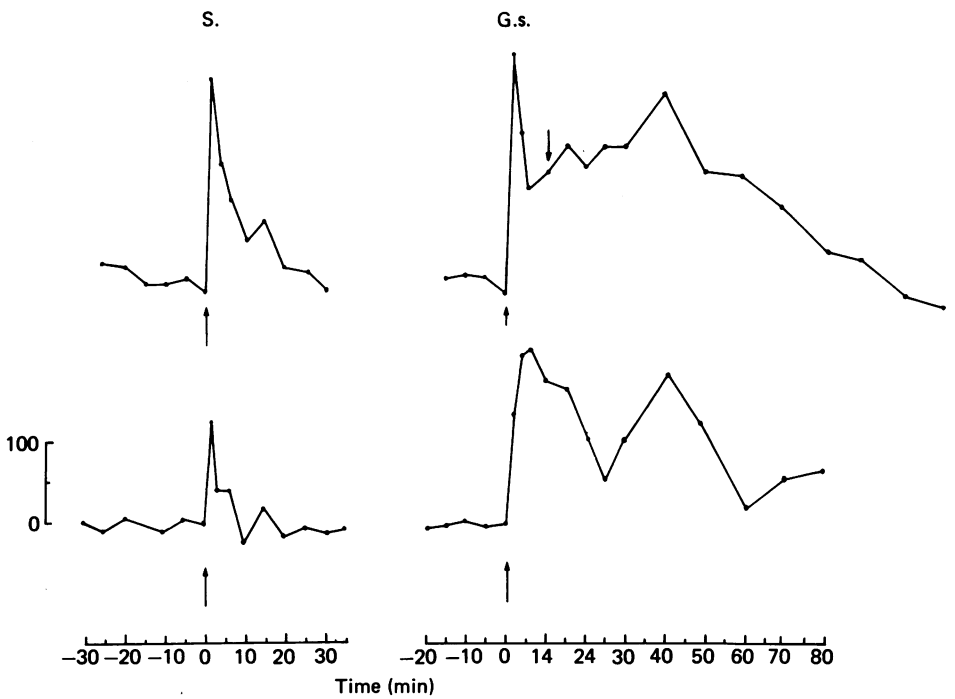

Fig. 10. Changes in the total number of action potentials evoked by an ipsilateral pinch in two posterior biceps femoris/semitendinosus motoneurones after section of either the sural (s.) or gastrocnemius–soleus (g.s.) nerves at the times indicated by the arrows. Section of the sural nerve produced a fairly short facilitation of the reflex while section of the gastrocnemius–soleus nerve caused a more prolonged increase in the reflex excitability. In the upper right trace the arrow indicates the time at which the cut end of the gastrocnemius–soleus nerve was immersed in 2 % xylocaine to abolish any on-going activity. (Vertical scale represents number of action potentials per pinch.)

changes in the excitability of the flexor reflex. Fig. 10 demonstrates the change in the ipsilateral pinch responses in posterior biceps femoris/semitendinosus motoneurones that occurred following sections of the sural and gastrocnemius nerves. Following sural nerve section the pinch-evoked responses increased for 15 ± 3 min (S.E. of mean, $n = 5$), following gastrocnemius–soleus nerve section the responses were elevated for 57 ± 13 min (S.E. of mean, $n = 6$).

To test whether the prolonged change in the flexor reflex excitability was the result of a prolonged injury discharge or whether the injury discharge triggered a prolonged central change, xylocaine (2 %) was applied to the cut end of the gastrocnemius–soleus

nerve 10 min post-section (Fig. 10). This treatment which would be expected to stop all afferent activity from the axotomized nerve fibres did not decrease the excitability increase.

DISCUSSION

The results show that a conditioning tetanus at C-fibre strength to the sural nerve produces an exaggeration of the flexor reflex which lasts about 10 min whereas a similar tetanus to the gastrocnemius–soleus nerve enhances the reflex for a period lasting five times longer. An inspection of the time course of the post-conditioning exaggerated reflex (Figs. 7–10) suggests that it may occur in at least two phases. The time course of the reflex excitability in the first 10 min appears to be similar for both nerves but only when the gastrocnemius–soleus nerve had been used for the conditioning stimulus did a second later phase of increase appear as the first declined. Whether these two phases represent different mechanisms operating at different times and why the second phase is restricted to muscle C-afferent fibres will be the subject of subsequent research.

Two types of conditioning stimuli were used on both nerves; electrical stimulation and nerve section. Only electrical conditioning stimuli at strengths which excited C-afferent fibres were adequate to produce the prolonged reflex facilitation. These high strength stimuli evoked a delayed long-latency response of the motoneurones consistent with an afferent conduction velocity of 1 m/s or less repeating for the gastrocnemius–soleus what has already been seen for the sural nerve (Woolf & Swett, 1984). Chemical activation of fine muscle afferent fibres has previously been shown to excite flexor motoneurones (Kniffki, Schomburg & Steffens, 1981). The onset of delayed central responses both in the dorsal horn and in motoneurones coincides with the appearance of the C compound action potential recorded in peripheral nerve or dorsal roots (Fitzgerald & Wall, 1980; Woolf & Swett, 1984).

There is no doubt that both the sural nerve and gastrocnemius–soleus nerve in the rat contain many small fibres. Horseradish peroxidase labelling of these nerves results in many small dorsal root ganglion cells containing the enzyme (C. J. Woolf, personal observation). In the cat, McLachlan & Janig (1983) find the gastrocnemius–soleus nerve to contain 700 myelinated afferent and 1100 C-afferent fibres while the sural contains 800 myelinated afferent and 3900 C-afferent fibres. There is no reason to think that cat and rat differ substantially in the ratio of myelinated to non-myelinated afferents. It makes it the more remarkable therefore that the conditioning volley in the gastrocnemius–soleus nerve should have a much more marked effect than that from sural when presumably the C volley is smaller from the muscle nerve than from the cutaneous one.

The conditioning effect produced by section of the nerves presumably depends on the generation of an injury discharge. Section of nerves produces a repetitive afferent volley in all types of nerve fibres but it dies down after a short time (Wall, Waxman & Basbaum, 1974). We provide evidence here that the central effects were initiated by the injury discharge but not sustained by any on-going afferent barrage by showing that local anaesthesia of the cut nerve after 10 min did not alter the subsequent prolonged central facilitation. The fact that we could imitate the effects of an electrical conditioning tetanus by simply cutting the nerve shows that the

electrical effects were not caused by current spread to distant structures and underlines the different central effects of cutaneous and muscle nerves. It is salutory to recognize that we, like many physiologists, have in the past cut nerves in the course of preparing for an acute physiological experiment without realizing that we are inducing prolonged central changes.

Two types of test stimuli were used to measure changes in the excitability of the reflex; electrical stimulation of the sural nerve and pinch to the foot. The results in terms of the time course of facilitation were identical for both methods. This may seem surprising in view of the fixed repetition of the electrically evoked afferent volley *versus* the inevitably variable response to a natural stimulus. The reason for the similarity is contained in the nature of the phasic flexion reflex which is a self-limited burst of activity in flexor motoneurones set off by a suprathreshold stimulus. It is a 'flick' response which does not vary substantially in amplitude or duration once the input has achieved threshold. The stability of the manually evoked reflex can be judged by the stability of the base line measured for 20–30 min before the conditioning stimulus in Figs. 7–10.

A peripheral change in the pinch test stimulus could be proposed to explain the reflex excitability changes if the conditioning stimulus had antidromically produced a sensitization of high threshold afferents by the axon reflex (Fitzgerald, 1979). A peripheral explanation cannot, however, be valid for three reasons. First the contralateral responses increased as much after the conditioning stimulus as the ipsilateral ones. Next the phenomena reported here occurred just as well if both the test nerve and the conditioning nerve were cut in the periphery. Finally it is most unlikely that antidromic activation of afferent fibres in the gastrocnemius–soleus would alter the sensitivity of toe afferents.

It is therefore apparent that we must turn to the spinal cord as the site of the mechanism of the prolonged reflex facilitation since the periphery is not the source and these animals had a spinal transection. Primary afferent input into the spinal cord evokes excitatory and inhibitory effects on dorsal horn neurones. The output from the spinal cord generated by a given input reflects the balance of the excitatory and inhibitory influences on the output neurones. We have demonstrated here that a brief C-fibre strength conditioning stimulus produces a prolonged excitability increase in motoneurones. The same kind of input has previously been shown to produce excitation of deep dorsal horn cells (wind-up) (Mendell, 1966) and also an inhibition of similar cells (Woolf, 1983*b*). That flexor motoneurones are also subject to afferent-mediated inhibitions has been repeatedly shown and recently a conditioning tetanus at C-fibre strength for 30 min at 2 Hz has been shown to produce a long post-conditioning flexor reflex inhibition (Chung, Fang, Cargill & Willis, 1983). The difference between this result and that which we have found here clearly relates to the duration of the conditioning stimulus. Brief C inputs produce a predominant excitation while sustained inputs (ninety times longer) ultimately result in inhibition.

Excitation of dorsal horn neurones following muscle nociceptor activation has been studied much less extensively than the excitation following cutaneous stimuli. One important study of primate spinothalamic tract neurones though showed that intra-arterial injections of bradykinin directed at muscle produced firing lasting for several minutes and that a hypertonic saline injection into the Achilles tendon

produced an enhanced response lasting for over 15 min (Foreman *et al.* 1979). The longer after-discharge that we observed immediately after the conditioning stimulus to the gastrocnemius–soleus nerve compared to the sural nerve stimulus may reflect a similar phenomenon, elicited though, by electrical stimuli.

At present we know nothing of the reason for the difference in the central excitability increase elicited by muscle and cutaneous nerves which is regrettable because it might be related to the important clinical phenomenon of widespread and prolonged tenderness following tissue injury in deep tissue. The difference between the two inputs could be related to an unknown difference in the chemical constituents of the two types of non-myelinated fibre. Peptides such as substance P which are contained in C fibres have been suggested as the source of prolonged changes in the isolated spinal cord (Konishi, Akagi, Yanagisawa & Otsuka, 1983) and in spinal cord slices (Urban & Randic, 1984). The possible difference of central termination of the two groups of fibres mentioned in the introduction might suggest that different post-synaptic groups of cells mediate the effects of the different afferents on reflex excitability. It will be necessary to search along the spinal linkage from afferents to motoneurones to locate the source and mechanisms of the prolonged changes observed here and those observed after thermal injury (Woolf, 1983*a*).

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