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NERVOUS OUTFLOW FROM SKELETAL MUSCLE FOLLOWING CHEMICAL NOXIOUS STIMULATION

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SUMMARY

1. In order to determine the nervous outflow from skeletal muscle during chemically induced muscle pain, the impulse activity of various types of muscle afferents in response to close intra-arterial injections of pain-producing substances (bradykinin, 5-hydroxytryptamine, histamine and potassium) was studied in anaesthetized cats using a single fibre recording technique.

2. By administration of algesic agents in doses which produce pain in man and pain reactions in animals, about half of the group IV and two thirds of the group III muscle afferents could be activated. In contrast, group II and group I afferent units were usually not excited by chemical noxious stimulation. If effects at all occurred in the thick myelinated afferents, they consisted of a depression of the fibre activity rather than of an activation.

3. The qualitative features of the discharges of group III muscle afferents induced by chemical stimulation resembled those of the group IV units very closely. The group III units differed from the group IV afferents in that their responses to a given dose of bradykinin were of greater magnitude.

4. It is concluded that the chemically induced muscle pain is probably mediated by certain portions of the group IV and group III afferents, whereas the reactions of group II and group I units to algesic agents are such that a contribution to muscular chemo-nociception seems improbable.

INTRODUCTION

Muscle receptors with non-myelinated (group IV) afferent fibres have been shown to be excited by intra-arterial injection of small doses of endogenous algesic agents such as bradykinin, 5-hydroxytryptamine, histamine and potassium (Mense & Schmidt, 1974; Franz & Mense, 1975; Fock & Mense, 1976). As it is known that these chemical stimuli produce

pseudo-affective responses in animals (Guzman, Braun & Lim, 1962) and pain in man (Lindahl, 1961; Keele & Armstrong, 1964), they were considered to be noxious, the resulting sensation of muscle pain being mediated, at least partly, by group IV afferent units. Studies on cutaneous nerves have demonstrated, however, that intra-arterial injections of pain-producing substances elicit discharges not only in non-myelinated and thin myelinated (group III) fibres, but also in part of the thicker myelinated ones (group II) (Fjällbrant & Iggo, 1961; Beck & Handwerker, 1974).

The present experiments were designed to find out whether the excitatory effects of pain-producing substances are confined to muscle receptors with group IV afferent fibres, or whether myelinated afferents contribute to the nervous outflow from skeletal muscle during chemical noxious stimulation. Various types of muscle afferents were tested with intra-arterial injections of amounts of bradykinin which were distinctly supra-threshold for group IV fibres, or with equieffective doses of other algesic agents.

The results demonstrate that considerable portions of both group IV and group III muscle afferents respond with raised activity to painful chemical stimulation, whereas in group II and group I units no comparable reaction is present.

METHODS

Details of the methods employed have been described elsewhere (Franz & Mense, 1975). The experiments were performed on anaesthetized cats (70 mg/kg chloralose i.p.), part of which were identical with those used in previous investigations (Franz & Mense, 1975; Fock & Mense, 1976).

Recordings of single afferent units from the gastrocnemius-soleus (GS) muscle were made extracellularly from dissected strands of the sciatic nerve or the corresponding dorsal rootlets. The impulse activity was processed by a special purpose computer and plotted as a time histogram. Group IV and group III units were identified by their conduction velocity following electrical stimulation of the GS muscle nerves, fibres conducting at less than 2·5 m/s being accepted as group IV (Paintal, 1967) and those having a conduction velocity between 4 and 30 m/s as group III afferents (Burgess & Perl, 1973).

With few exceptions the group II (conduction velocity 30–72 m/s) and group I units (conduction velocity 72–120 m/s, Matthews, 1972) of this study were recorded from dorsal root filaments. For identification of the receptive endings, contractions of the GS muscle were induced by electrical stimulation of the ventral roots L7/S1. Units showing a decrease in discharge frequency during muscular contraction were regarded as muscle spindle afferents; they were assigned to primary endings if they showed a marked dynamic behaviour on sudden changes of muscle length and an activation on tapping the tendon. Secondary endings were identified by their less dynamic response behaviour and the ineffectiveness of tendon tap. For identification of afferents from Golgi organs their activation during muscular contraction was used (cf. Matthews, 1972).

Chemical stimulation of the GS muscle was performed by injecting bradykinin triacetate (Brad.), 5-hydroxytryptamine creatinine sulphate (5-HT), histamine

dihydrochloride (Hist.), potassium chloride (K^+) and succinylcholine chloride (SCh) into a branch of the sural artery. The injection doses of Brad., 5-HT, Hist. and SCh are expressed in terms of the free base; for K^+ the amount of potassium is indicated. All injections consisted of 0·3 ml. of the stimulating solution which was introduced into the circulation with 1 ml. Tyrode.

RESULTS

Group IV afferent units

The activation of muscular group IV afferent units by algesic substances has been described in previous publications (Mense & Schmidt, 1974; Franz & Mense, 1975; Fock & Mense, 1976). For the purpose of the present study, doses of the algesic agents had to be administered which were suprathreshold for chemosensitive units. Therefore, dose–response curves for the effects of bradykinin on the impulse activity of group IV afferents were determined by injecting the stimulant in increasing doses at intervals of 2 min. (Fig. 1). Following injection of higher doses of bradykinin, separate afterdischarges appeared in some of the units (Fig. 1*A*). Usually the increase in the effects of bradykinin was steep, and saturation in the magnitude of response occurred at a higher dosage (Fig. 1*B*). The curves in Fig. 1*C* represent the discharges of five single group IV units which responded in a regular manner to all the doses of bradykinin applied. It has to be pointed out that such a dose–response relation was not present in all the group IV units tested. Some responded with a prolonged, bursting type of discharge to bradykinin, especially when the dosage was high. Because of the long duration of such discharges and the possible occurrence of unspecific effects (e.g. tissue oedema) after administration of greater amounts of bradykinin, the doses were injected in increasing order.

According to the dose–response curves in Fig. 1*C*, the injection of 26 μg bradykinin was adopted as a distinctly suprathreshold stimulus for this study. The equieffective doses (in terms of activation of group IV afferent units) for the other substances used were taken from an earlier investigation (Fock & Mense, 1976) viz. 5-HT 67·5–135 μg, histamine 90–180 μg and potassium 3·8 mg. By intra-arterial injections of such doses of the algesic agents 46% of the muscular group IV afferent units could be activated.

Group III afferent units

Of sixty-three group III units that were tested with intra-arterial injections of the above doses of pain-producing substances, forty-five (71%) proved to be responsive. Thus, in comparison with group IV fibres, the

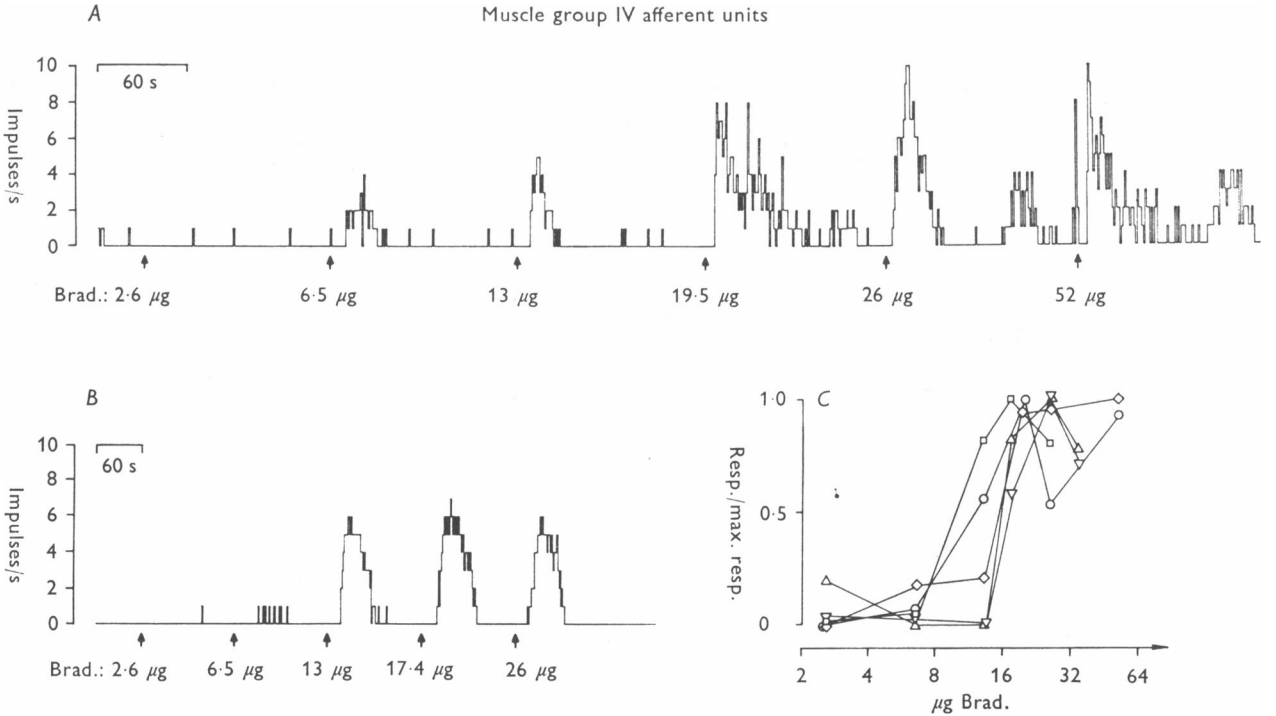

Fig. 1. Responses of single group IV muscle afferents to increasing doses of bradykinin. *A*, appearance of separate afterdischarges at a higher dosage (19·5–52 μg). Unit from lateral gastrocnemius-soleus, conduction velocity 0·76 m/s. Recording from sciatic nerve. Arrows indicate start of injection, which was completed in 10–15 s. Bin width of time histogram: 1 s. *B*, occurrence of saturation in the magnitude of response at a bradykinin dosage exceeding 13 μg. Unit from lateral gastrocnemius-soleus, conduction velocity 0·64 m/s. Recording from sciatic nerve. Bin width of time histogram: 1 s. *C*, log dose–response plots of five group IV afferent units from GS muscle, all recorded from sciatic nerve. Ordinate, relative magnitude of response in terms of impulses evoked per injection. Abscissa, doses of bradykinin applied as single injections at intervals of 2 min (logarithmic scale).

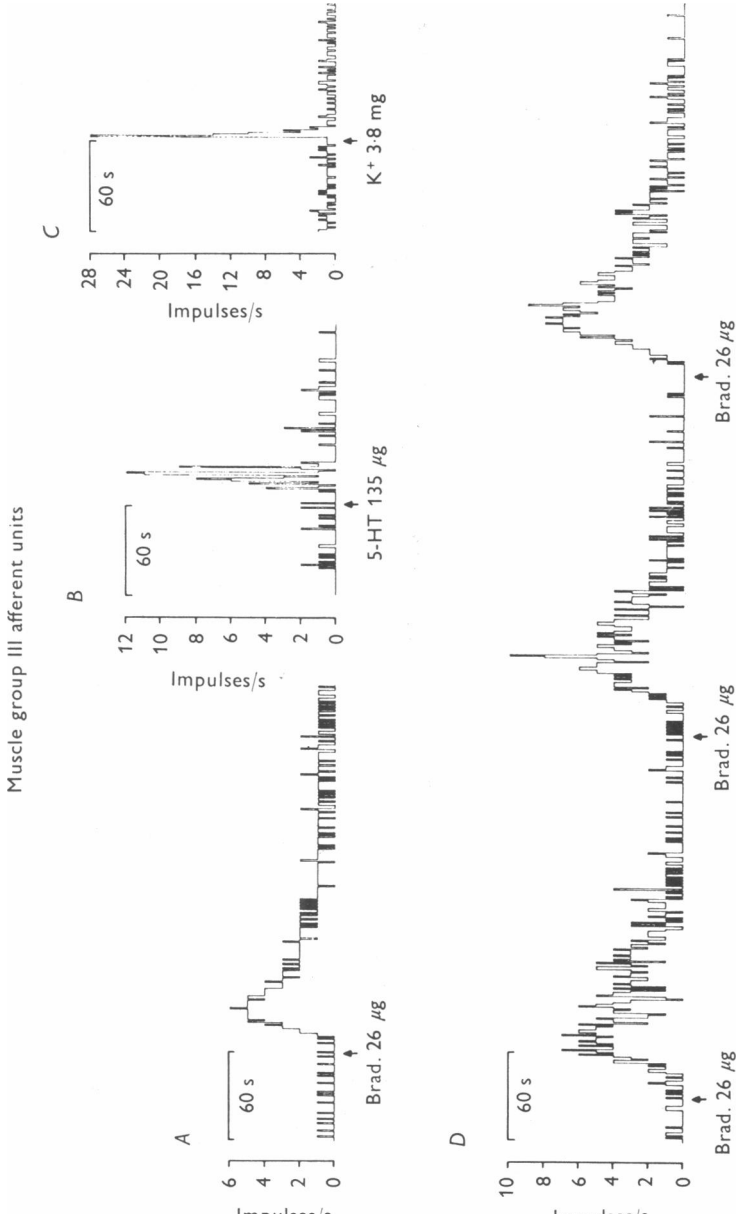

Fig. 2. Responses of group III muscle afferents from medial gastrocnemius to intra-arterial injection of pain-producing substances. *A*, activation induced by a dose of 26 μg bradykinin. The unit conducted at 8·33 m/s, recording from sciatic nerve. *B*, response to 5-HT. Conduction velocity of the unit 4·67 m/s, recording from dorsal root. *C*, response to K^+. Conduction velocity of afferent fibre 4·98 m/s, recording from dorsal root. *D*, effects of repeated administrations of bradykinin 26 μg at intervals of 4 min. The unit conducted at 9·38 m/s, recording from sciatic nerve. Bin width of all time histograms: 1 s.

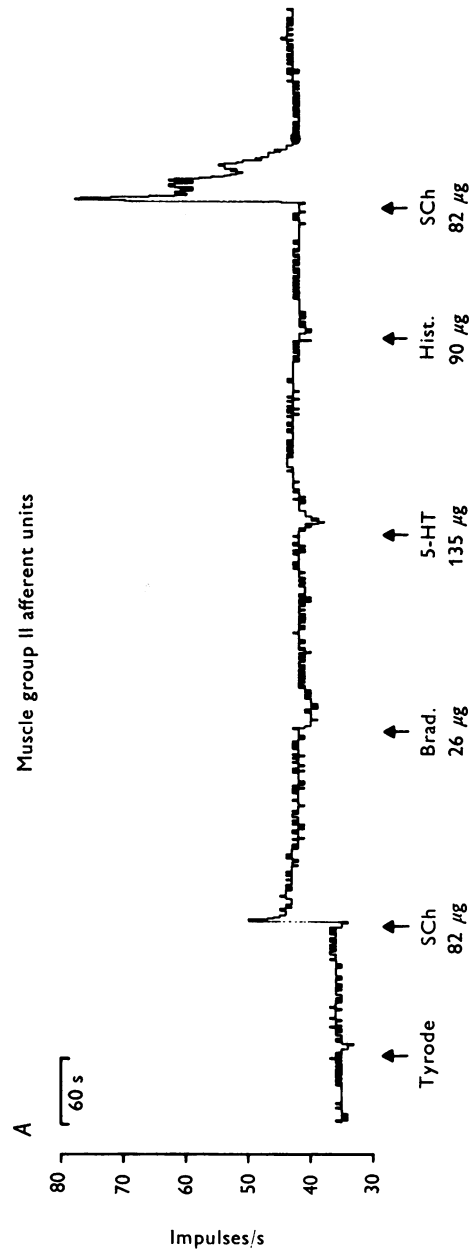

Fig. 3.4. For legend see opposite page.

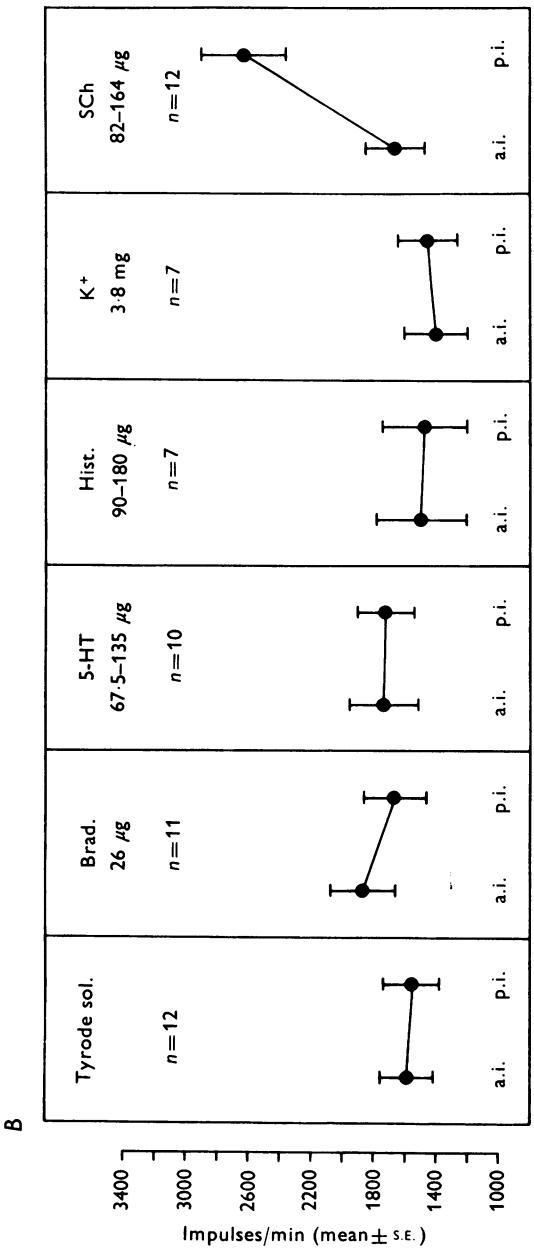

Fig. 3. Impulse activity of secondary muscle spindle afferents in response to intra-arterial injections of pain-producing substances and of succinylcholine. *A*, single secondary ending from muscle spindle with origin in the lateral gastrocnemius-soleus, conduction velocity 51·7 m/s. Recording from dorsal root. Bin width of time histogram 1 s. *B*, impulse activity (mean ± S.E.) of secondary endings of GS muscle spindles before and after administration of pain-producing substances and of succinylcholine. Tyrode solution was injected as a control. The pairs of values in each column represent the sum of impulses occurring during a period of 60 s before (a.i.) and 60 s after (p.i.) the respective injection.

portion of chemo-nociceptive afferents among group III units seems to be greater.

The qualitative features of the responses to the various algesic agents observed in group IV units (cf. Fock & Mense, 1976) were present also in group III afferents: (1) The long latency together with the slow rise and decay of the response to bradykinin (Fig. 2*A*, *D*), (2) the steeper onset and decay of the response to an about equieffective dose of 5-HT (Fig. 2*B*), and (3) the short-lasting high frequency discharge following application of potassium (Fig. 2*C*). As in group IV afferents, the responses of group III units to bradykinin were usually well reproducible (Fig. 2*D*).

The magnitude of the bradykinin-induced response was significantly greater in group III than in group IV units. The mean number of impulses elicited by injection of 26 μg bradykinin was 163·5 ($\pm$ 29·5 S.E., range 20–663, $n = 21$) in group III afferents, against 104·2 ($\pm$ 17·3 S.E., range 22–383, $n = 20$) in group IV units ($P < 0{\cdot}05$, U test of Wilcoxon, Mann & Whitney).

Group II afferent units

Of the thirty-three group II afferents studied, twenty-one could be classified as secondary muscle spindle afferents. In the results, only those units are included which were activated by intra-arterial administration of SCh 82–164 μg indicating that they were accessible via the vascular route.

By the injection of algesic agents in a dosage that was suprathreshold for chemosensitive group IV and group III afferents, none of the secondary spindle endings and of the other group II units was definitely activated. If an effect at all occurred, it consisted of a reduction of the fibre activity rather than of an activation. An example is shown in Fig. 3*A*. The secondary muscle spindle afferent was excited by injections of SCh 82 μg but showed a decrease in discharge frequency after administration of pain-producing substances. Such effects of the algesic agents occurred after a short latency of a few seconds and were present only in trials in which the injection of Tyrode solution likewise led to a decrease in the fibre activity. Therefore, these effects are considered to be unspecific ones, probably induced by the injection procedure itself.

A quantitative evaluation of the impulse activity of secondary muscle spindle afferents following administration of the various stimulants (Fig. 3*B*) yielded a significant change in discharge frequency for SCh only ($P < 0{\cdot}001$, according to the Wilcoxon matched pairs signed rank test).

Group I afferent units

Primary endings from muscle spindles (Ia fibres). Thirty-two group I afferents were isolated which had response characteristics of primary

endings from muscle spindles. Only Ia units being activated by SCh 82–164 μg are included in the quantitative evaluation of the results. The Ia afferent in Fig. 4*A* responded vigorously to SCh but showed no such activation after administration of algesic agents. Yet the discharge frequency was not totally unaffected by the pain-producing substances, for after administration of 5-HT, bradykinin and potassium effects with a long latency occurred. The injection of 5-HT led to a slow rise of the discharge frequency to a new level. Such 5-HT-induced effects were present in 33% of the Ia afferents, they occurred in three out of eleven group II afferents as well. In Fig. 4*A* the injection of bradykinin 26 μg and K^+ 3·8 mg induced a transient rise in discharge frequency followed by a phase of complete depression. Such effects were also observed after injection of histamine (and sometimes 5-HT), they were present in 63% of the Ia and in 50% of the group II spindle afferents. The transient increase in discharge frequency usually did not exceed the extent shown in Fig. 4*A*, whereas the duration of the silent period sometimes lasted for several minutes. The mean latency of the responses of primary muscle spindle afferents to SCh 82–164 μg (10·6 s $\pm$ 3·4 S.E., range 2–48, $n = 17$) was much shorter than that of the effects induced by algesic agents (30·3 s $\pm$ 3·0 S.E., range 8–64, $n = 29$).

The pain-producing substances did not cause a significant increase in the mean discharge frequency of Ia afferents (Fig. 4*B*); these units were excited by SCh only ($P < 0{\cdot}001$, according to the Wilcoxon matched pairs signed rank test). The above effects of algesic agents on group I (and group II) muscle afferents can probably not be attributed to a direct interaction with the spindle. Besides their long latency period, a further finding supporting this assumption was that the effects were present also in spindle afferents not responding to SCh, i.e. in units that were not accessible via the vascular route.

Afferents from Golgi tendon organs (Ib fibres). Of nineteen Ib afferents tested with injection of the various pain-producing substances, only two were activated, one responding to bradykinin and histamine, the other one being weakly excited by 5–HT, histamine and K^+. Long latency effects as occurring in group II and group I spindle afferents after application of algesic agents were not observed in Ib units.

DISCUSSION

The results demonstrate that the intra-arterial injection of algesic agents in doses which elicit pain in man and pain reactions in animals evokes an increased nervous outflow from skeletal muscle which is practically restricted to group IV and group III fibres.

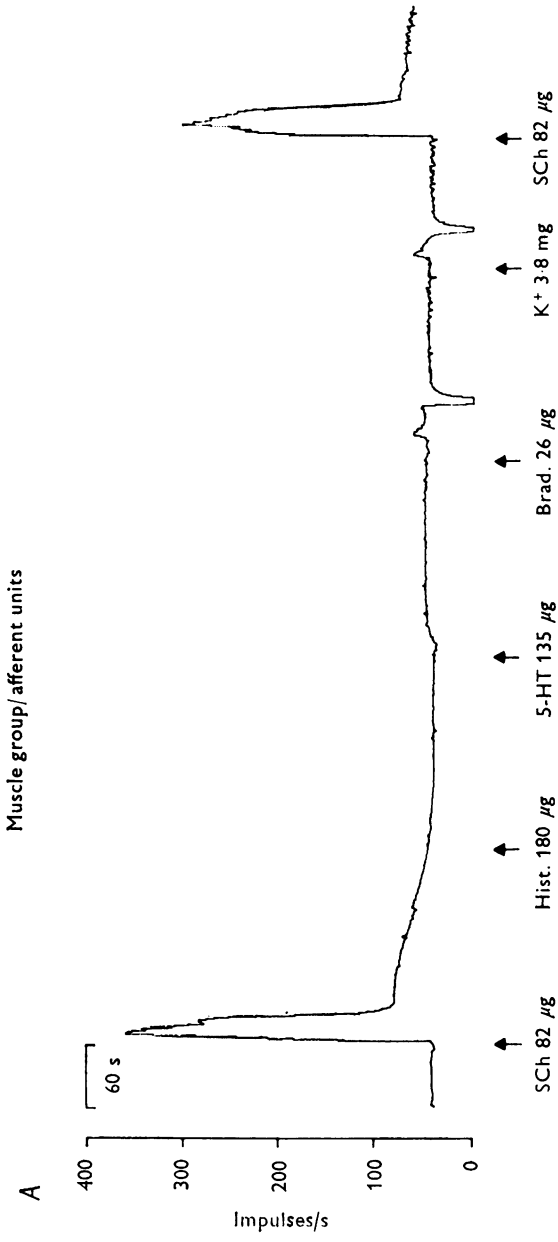

Fig. 4 *A*. For legend see opposite page.

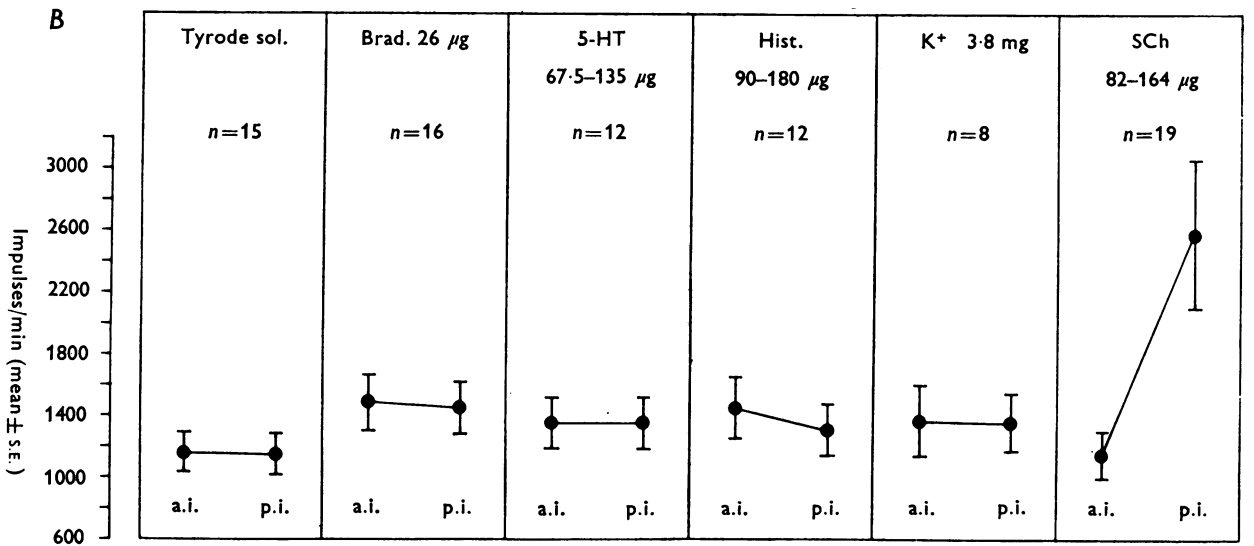

Fig. 4. Impulse activity of primary muscle spindle afferents in response to intra-arterial injections of pain-producing substances and of succinylcholine. *A*, single primary ending from medial gastrocnemius, conduction velocity 81·0 m/s. Recording from dorsal root. Bin width of time histogram: 1 s. *B*, impulse activity (mean ± S.E.) of primary endings of GS muscle spindles before and after injection of pain-producing substances and of succinylcholine. For further explanation see legend, Fig. 3*B*.

The bradykinin-induced excitation of part of the group IV units can be described by dose–response curves which are rather consistent in threshold and shape (cf. Fig. 1*C*). Some units, however, had a dose-response relation which was not sigmoid in shape but rather resembled an all-or-nothing phenomenon. The slopes of dose–response curves for nociceptive effects of bradykinin in animals are less steep but start at similar concentrations as used in our experiments (Collier & Lee, 1963).

The finding that the discharges evoked by pain-producing substances in group III muscle afferents resembled those of group IV units very closely may indicate that the chemically induced muscle pain is mediated by similar sets of afferent units within both groups. The thin myelinated afferents from skeletal muscle possibly participate also in other forms of muscular nociception, since part of them have been shown to have high thresholds on mechanical stimulation (Paintal, 1960). In addition, their central effects, e.g. on the flexion reflex (Bessou & Laporte, 1961; Paintal, 1961), suggest that they are involved in the induction of nocifensive reactions.

In contrast to group IV and group III units, the thick myelinated afferents from skeletal muscle were not appreciably excited by the administration of pain-producing substances. Thus, the situation in skeletal muscle differs from that in the skin, where in addition to group IV and group III units also a certain portion of the group II afferents is activated by these agents (Fjällbrant & Iggo, 1961; Beck & Handwerker, 1974).

If effects at all were induced in the thicker myelinated muscle afferents of the present study, they consisted predominantly of depressions of the fibre activity which occurred after a long latency period. The mechanisms underlying these effects are not clear. Besides an oedema of the muscle tissue possibly induced by the injection of bradykinin and histamine (Ferreira, 1972; Grega, Kline, Dobbins & Haddy, 1972), efferent influences mediated by α- and γ-fibres (Laporte & Boër, 1955; Voorhoeve, Laporte & Bessou, 1959) or by sympathetic efferents (Eldred, Schnitzlein & Buchwald, 1960; Hunt, 1960) have to be taken into account. Although in most of our experiments with recordings of spindle afferents the ventral roots L7 and S1 were cut, a reflex activation of adjacent α- and γ-motoneurones cannot be excluded.

It seems highly improbable that the changes in discharge frequency induced by pain-producing substances in group II and group I muscle afferents are related to the painful sensations evoked by these agents, since the time course of the afferent discharge lacks any resemblance with that of chemically induced pain in man (Burch & DePasquale, 1962; Coffman, 1966) and pseudo-affective responses in animals (Guzman *et al.* 1962).

Thus it appears that the injection of algesic agents into a muscle artery leads to an excitation of group IV and group III muscle afferents, whereas the activity in group II and group I afferent units is reduced rather than increased. The group III units seeem to be more sensitive to painful chemical stimulation, since the percentage of units being activated was higher among group III than among group IV afferents, and since in an individual group III fibre the magnitude of response to bradykinin was likewise greater.

As effective dosage and time course of activation for both group IV and group III units agree well with those of painful sensations in man and pain reactions in animals, it is likely that the peripheral afferent pathway for muscular nociception is formed by certain portions of the group IV and group III muscle afferents.

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