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**INTRACELLULAR AUTOGENETIC EFFECTS OF MUSCULAR
CONTRACTION ON EXTENSOR MOTONEURONES.
THE SILENT PERIOD**

BY R. GRANIT, J.-O. KELLERTH AND A. J. SZUMSKI*

*From the Nobel Institute for Neurophysiology, Karolinska
Institutet, Stockholm 60, Sweden*

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SUMMARY

1. Intracellular records have been taken from cat motoneurones belonging to gastrocnemius and soleus or to popliteal synergists during contractions of gastrocnemius and soleus, acting separately or jointly. Such contractions were elicited by brief tetani or single shocks to the peripheral end of the cut ventral roots L7 or S1.

2. Hyperpolarization of the motoneurone accompanies rise of tension in contraction. The amount of it increases when at constant extension the contraction of the muscle is increased by increasing stimulus strength, as well as when it is increased by augmenting extension at constant stimulus strength. It is therefore tension-sensitive.

3. The duration of the hyperpolarization induced in this manner reflects the duration of the contraction itself, being considerably longer in the slow soleus than in the faster gastrocnemius. It is often preceded by a brief wavelet of depolarization ascribed to the so-called back-response.

4. Early in relaxation there occurs a transient 'hump' of membrane depolarization. This corresponds to the moment characterized by phasic bursts from the spindle primaries. The 'hump' terminates hyperpolarization.

5. When the cell is stimulated by injected current to maintained repetitive firing, the 'silent period' in contraction begins with the phase of hyperpolarization and ends with the hump of depolarization as described above.

6. Later during relaxation, delayed inhibition, may or may not follow often accompanied by hyperpolarizing activation noise and sometimes also visible as an extension of the silent period of a firing cell. There is, however, no marked hyperpolarization of the motoneurone in delayed inhibition.

* Vocational Rehabilitation Administration Research Fellow on leave of absence from the Department of Physiology, Medical College of Virginia, Richmond, Va., U.S.A.

7. In the Discussion the events described above are related to previous studies employing monosynaptic testing or electromyography for the analysis of the variations of excitability caused in extensor motoneurons by autogenetic contractions as well as to known properties of stretch receptors.

INTRODUCTION

A missing link in our knowledge of the autogenetic effects of contraction on extensor motoneurons is filled in by the results reported below. All previous work has been extracellular and carried out by electromyography, myography or by monosynaptic testing. It is reviewed by Granit (1955), Hufschmidt (1960, 1961) and by Jansen & Rudjord (1964). The experiments making use of monosynaptic testing have recently been repeated and extended by Bianconi, Granit & Reis (1964*a*, with full references). The latter authors devoted particular attention to a late inhibition which extended far into the relaxation period of an extensor contraction. In flexor contractions this phase was excitatory on the flexor motoneurons (autogenetic excitation). This being in accordance with what is known about the reflex pattern of the spindle secondaries, it seemed reasonable to ascribe the late autogenetic inhibition of extensors in relaxation to the activity of the secondaries.

Monosynaptic testing when the muscle is conditioned by stretch or contraction appears to be a perfectly reliable indicator of excitation. Employed in this context it means that motoneurons are added to the test response from the subliminal fringe. The method may, however, be deceptive in stretch if a reduction of the monosynaptic test response is interpreted as an unequivocal consequence of (pre- or post-synaptic) inhibition. This was pointed out long ago by Granit & Job (1952) who found that a decrease of the monosynaptic response ran parallel with an increase of the stretch reflex in active motoneurons. These findings were ultimately explained by the intracellular studies of Granit, Kellerth & Williams (1964*b*). The nature of the autogenetic inhibitions induced by contraction will be explored below.

The best known index of inhibition after a muscle contraction (cf. Denny-Brown, 1928) is the silent period of Hoffmann (1922) which is so prominent whenever a jerk or an electrically induced twitch is superimposed upon a muscle in reflex or volitional activity. An electrical silence is then visible in the electromyogram. The results to be reported, using intracellular recording, not only throw further light on the silent period, but explain it in terms of membrane potential changes of the motoneurone. They also contribute to the understanding of the late inhibition mentioned above.

The simple plan of this work has been to study the effect of extensor muscle contractions, elicited from the peripheral stump of the cut ventral root, on the muscle's own motoneurons. Many of these motoneurons have been discharged by transmembrane stimulation from the electrode tip. Observed silent periods are thereby localized to the cell membrane. The actual membrane events were mostly studied in silent motoneurons at high d.c. sensitivity. The salient point is whether and to what extent such events can be shown to be dependent on the amount of tension developed.

METHODS

The general methods were those described by Granit, Kellerth & Williams (1964*a*) in their study with intracellular recording of the effects of muscle stretch on motoneurons. In the present work the strain gauge myograph was connected to a sliding metal rod provided with a catch making it possible to extend the muscle millimetre by millimetre. All animals were anaesthetized with an initial minimum dose of pentobarbitone of 35 mg/kg. Small additional doses were given from time to time to prevent movement. Artificial respiration was usually employed.

Stimulating electrodes were placed on the central ends of the cut left hamstring, peroneal and popliteal as well as on the intact left gastrocnemius-soleus nerves. Otherwise both legs were denervated. The gastrocnemius-soleus (GS) muscles were mostly used together, but sometimes they were separated. The left femur was broken. The lumbar cord was exposed and the left ventral roots L7 and S1 were cut across in the middle. After fixation of the animal and its left leg, denervation was completed by stimulating the peripheral end of the ventral root to be used in the experiment and any contractions around the hip and spine were prevented by cutting nerves and muscles in these regions so that finally the gastrocnemius-soleus muscles alone responded to the stimulus.

A microcapillary (2 M-potassium citrate) was inserted into a ventral horn cell of (mostly) L7. Often the L6 dorsal roots were cut across to give easier access to these ventral cells. Stimulation of the central stump of the cut ventral root was used for locating the fields leading to the motoneurons. The peripheral end of ventral L7 was usually used for stimulation of the muscle, but sometimes S1 was used. Inevitably the contraction from one ventral root stimulation was a good deal below the maximum obtainable from the muscle nerves but by this technique it mostly proved possible to avoid firing the cell that the microcapillary had penetrated.

Zero extension was defined by pulling slowly on the muscle and noting when the strain gauge picked up the slack. This was done at a myograph sensitivity of 300 g per 2.5 nA on a scale readable to 0.5 nA. An electrically heated blanket was folded around the leg.

Controls. Our fixation techniques for use during muscle stretch (Granit *et al.* 1964*a*) proved adequate during contraction. Mechanical artifacts checked themselves in that of two perfectly good adjacent penetrations the one cell would display effects of contraction on the membrane potential which were missing or of different character in the next one. Occasionally stimulus spread from the cut ventral root was noted but this effect was rare and easily recognized by its dependence on stimulus strength alone in the absence of an influence of variations due to size of contraction and amount of initial tension. The latter was regularly varied from 'slack', i.e. muscle unhooked from myograph, to at least 10 mm extension. Flaxedil (gallamine triethiodide) was often used and then, with contraction gone, the effect of muscle stimulation on the membrane potential of motoneurons also disappeared. This drug was chiefly employed in connexion with long-lasting supramaximal tetani, not to be discussed in the present paper. In all, seventy cells could be held long enough for analysis, thirty-one belonging to gastrocnemius-soleus (GS) motoneurons, eighteen to popliteal synergists. Other combinations will not be included in this paper.

RESULTS

The silent period. The GS (gastrocnemius-soleus) motoneurone of Fig. 1 was maintained firing by current injected from the tip of the micro-electrode, the muscle being at two extensions. In both cases maintained transmembrane stimulation gradually led to a diminution of the rate of repetition. This is the slow process studied by Granit, Kernell & Shortess (1936*a, b*) in motoneurones and by Fuortes & Mantegazzini (1962) in the excentric cell of *Limulus*. At slower rates of discharge there was an increase in the duration of the silent period, perhaps less striking than it appears to be because the GS contractions are independent of the running discharge and so, when the frequency of the latter slows down, they stand a better chance of hitting an interval between two spikes in the middle.

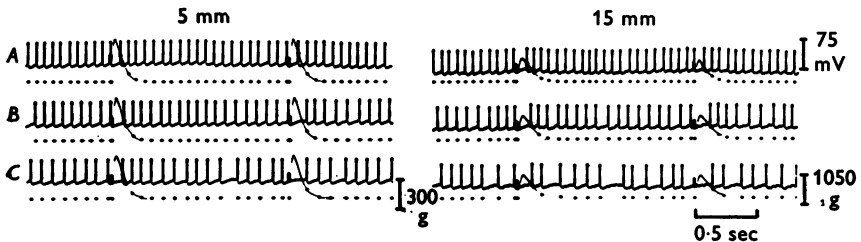


Fig. 1. Gastrocnemius-soleus motoneurone; spike height about 70 mV (without depolarizing current). The motoneurone discharge, seen in the upper trace, is maintained by current injected from the tip of an intracellular micro-electrode at around 10 nA; it gradually runs down in frequency from *A* to *C*. The GS muscle is kept extended at 5 or 15 mm and contractile tension is recorded in the lower trace. Note two different myograph sensitivities. Double shocks, delivered to the peripheral portion of the cut ventral root L7, cause muscle twitches and the afferent impulses from the muscle pass by the intact dorsal roots to bring about silent periods in the motoneurone discharge.

This postpones the next discharge. With respect to phase of contraction there is far less variation in the location of the spike by which the silent period is terminated. The effect of initial tension on the length of the silent period seems small.

Figures 2 and 3 extend the analysis of the same experiment. Records *A* of Fig. 2 serve to identify the cell as a GS motoneurone, and records *B* demonstrate at higher sensitivity the hyperpolarization caused by contraction, in this particular case initiated by a spike and its after-hyperpolarization. (While the observations illustrated in Fig. 1 were made, the cell did not fire to contraction which here, as nearly always, was elicited from the ventral root.) In Fig. 2 there is a 'trough' of hyperpolarization whose depth is tension-sensitive and whose duration exceeds that of pure afterhyperpolarization. At 10 and 15 mm extension, the

double shock gives more hyperpolarization than the single shock. It can only have done this by producing more tension from the muscle; for silence in de-efferented spindles is absolute and not graded and a single maximal shock would have silenced all the spindles. Highly significant is the slight hump of depolarization replacing hyperpolarization early in relaxation.

It proved possible to inactivate the cell by maintained transmembrane stimulation. While this went on, the records of Fig. 3A were taken (note the diminished sensitivity) and now there was no initial, but only a terminal spike. In the five contractions reproduced the location of this spike early in relaxation varied but little. When slightly delayed in some records, it was preceded by a visible hump of depolarization. It is well

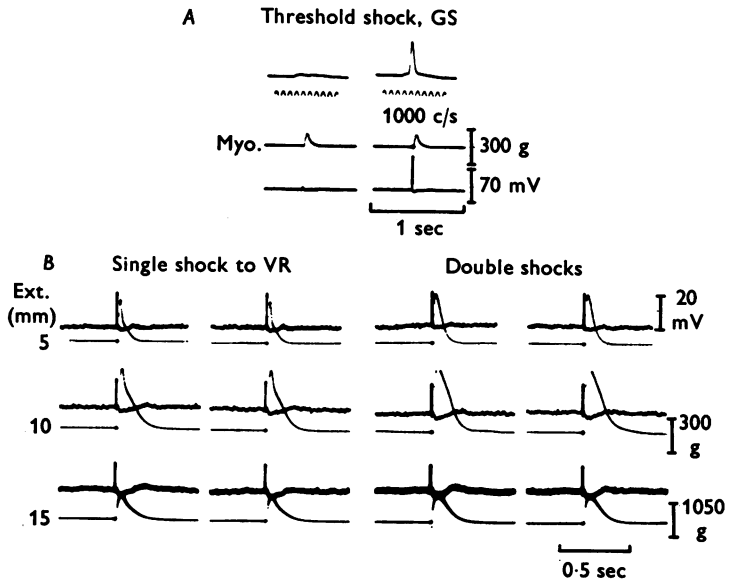


Fig. 2. Same motoneurone as in Fig. 1. The upper trace in *A*, with fast sweep, shows motoneurone just giving a monosynaptic spike in response to a threshold shock to the GS nerve. The small myographic responses caused by the same two threshold shocks are reproduced in the middle trace at slow speed. In the lowermost trace the effects of the same shocks on the motoneurone are shown at slow speed. These records identify the motoneurone as a GS cell. In *B*, series of records of motoneurone discharge above and GS muscle tension below, when the muscle is stimulated from the peripheral stump of the cut ventral root L7. Muscle at three extensions. The motoneurone is now slightly more depolarized than before (in records of Fig. 1) and the GS contraction elicits a spike followed by afterhyperpolarization. In addition there is tension-sensitive hyperpolarization because the larger contractions by double shocks at greater extensions produce larger hyperpolarizations than those obtained with single shocks. Note the subsequent hump of transient depolarization.

known that an inactivated cell may become generative after a transient hyperpolarization (e.g. Coombs, Eccles & Fatt, 1955; Kuffler & Eyzaguirre, 1955; Ito, 1957 and others). In the present case we have used this finding as a contrivance by means of which the initial spike has been removed while the 'rebound' effect due to the transient hump of depolarization has been retained. The records of Fig. 3*B*, at higher sensitivity,

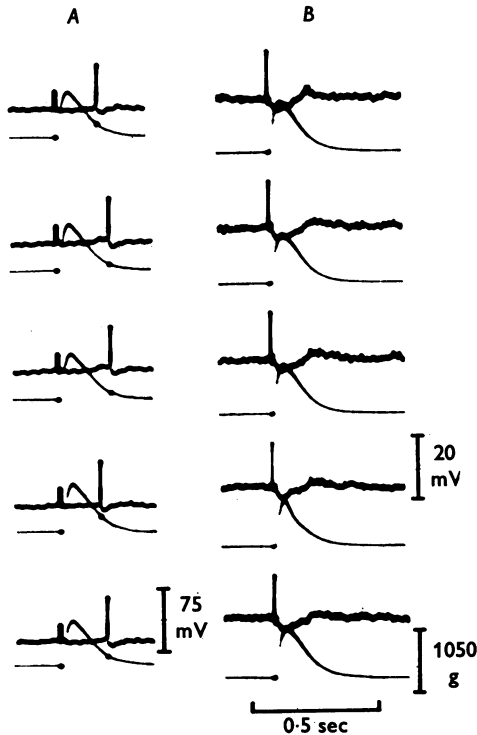


Fig. 3. Same GS motoneurone as in Figs. 1 and 2. Motoneurone discharge above, muscle tension below. Muscle extension 10 mm. *A*, during inactivation of the motoneurone by maintained transmembrane stimulation (low amplification). The twitch of the GS muscle in response to peripheral root stimulation now brings about a delayed spike occurring in much the same place relative to relaxation. In the second and third records the spike is slightly further delayed and there is a transient hump of depolarization visible. *B*, later, after recovery of motoneurone from inactivation. There is now the initial spike and hyperpolarization succeeded by a transient hump of depolarization as in Fig. 2. Note increased sensitivity to show these events clearly.

were taken after recovery of the cell. They have been added in order, once more, to demonstrate the perfectly normal sequence of hyperpolarization-depolarization caused by autogenetic contractions. The hump of depolarization serves as a 'timing device' switching off hyperpolarization. Clearly it also (Figs. 1-3) terminates the silent period.

When stimulating GS motoneurons in this manner by transmembrane currents the events described above are perfectly regular in good preparations. Occasionally the transient hump of depolarization early in relaxation may be missing (cf. below, section on separation of soleus and gastrocnemius). It can then mostly be produced by adding initial tension. The same events are seen in a large number of popliteal synergists.

It should be recalled now that there are three types of stretch receptors in the muscle; spindle primaries, spindle secondaries and Golgi tendon organs. Obviously they must project in different proportions to different motoneurons. The two latter types have polysynaptic projections on the motoneurons and hence their effects are facultative and not inevitable

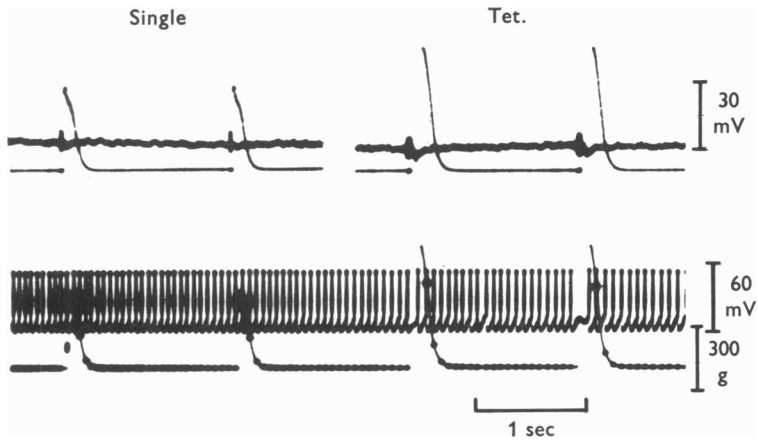


Fig. 4. Popliteal motoneurone influenced by GS contraction. Spike height 75 mV. Upper trace, motoneurone discharge, lower trace, tension record of GS contraction. Muscle at 5 mm extension. Stimulation of peripheral portion of cut ventral root L7 with single shocks (left) and 3 shocks at 330/sec (right). The silent motoneurone (above) responded by hyperpolarization which deepened with the larger contraction. The cell was then fired by transmembrane stimulation (below) to show that the larger hyperpolarizations caused by larger contractions produced more definite silent periods. Strength of stimulus was just above threshold for full contraction.

as are those of the spindle primaries. A perfectly physiological variation of the contribution of the three types of afferents to the changes of potential recordable during autogenetic contraction is therefore to be expected. In addition there is the early discharge, to be discussed below. Some of the more important variations will now be described. (As to interference between gastrocnemius and soleus, see last section, p. 500.)

In popliteal synergists of which Fig. 4 provides an example, a relatively large contraction may sometimes be needed for the production of a silent period. It is an old observation (Denny-Brown, 1928) that the silent

period may be reflected in muscles other than the one contracting. In these records the silent period is longer for the second of the two large contractions. This is so because there the rising phase of contractile tension happened to fall well inside the interval between two spikes, actually at the very moment when the motoneurons was about to fire, as seen from the increasing depolarization. Statistical variations of this character would hardly be visible by the mass-indicator of electromyography. This would rather tend to emphasize the constant location of the spike terminating the silent period as predictable from earlier electromyographic work (Merton, 1951; Jansen & Rudjord, 1964). In the case of Fig. 4 there was no spike elicited from the motoneurone by contraction

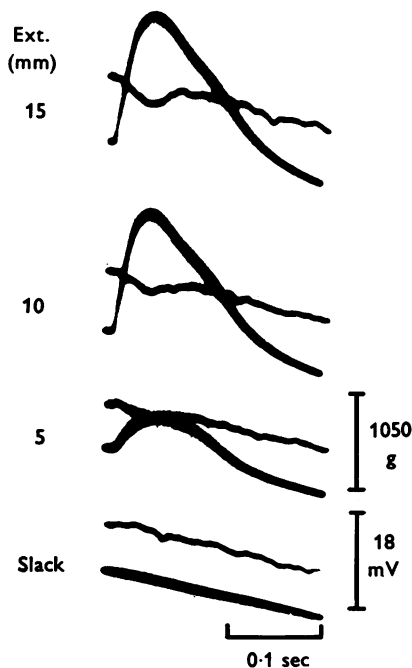


Fig. 5. GS motoneurone of spike height 71 mV. GS muscle contracted at different extensions by triple shocks to peripheral portion of cut ventral root L7. Stimulus strength just above threshold for full contraction.

(see upper row of records). In subsequent illustrations GS motoneurons will be used which have not been fired by the contraction elicited from the ventral root. This was the large majority of them.

Tension and extension as variables. Close inspection of many records from motoneurons influenced by autogenetic contractions has made it possible from time to time to distinguish two phases of hyperpolarizing potential. (i) The first is a gently sloping increase of membrane potential

coinciding precisely with the rising contractile tension and absent in slack muscle. This is shown in Fig. 5. It is always present in GS motoneurons and in many synergists and is most characteristic. (ii) The second phase of post-synaptic inhibition is the trough of hyperpolarization strikingly in evidence in Fig. 6. In some motoneurons the second phase was also visible in slack muscle (Fig. 6). The second phase has been seen in only four extensor motoneurons.

The first phase is reasonably pure in the experiment of Fig. 5. Something of the second phase may turn up at greater extensions. The relevant point, however, is not that the distinction between the two phases need be hard and fast but that it occasionally should be possible to make it. In Fig. 5 should be noted the hump of depolarization at 15 mm extension early in relaxation.

The GS motoneuron of Fig. 6 was selected for reproduction because it clearly demonstrated that while the first phase again turned up early in contraction and greatly depended on contractile tension, the second phase was present in slack muscle in the absence of the first phase. Nevertheless, the second phase also increased when the muscle was extended (three first vertical rows) as well as when stimulus strength was increased to give greater contractions at slight (zero) extension (fourth vertical row, right). The first two vertical rows are repetitions to illustrate range of variation, the third shows the same events as recorded by the standing spot on slowly running film. At 10 mm extension (in the first two rows) the two phases of hyperpolarization can still be differentiated, at 15 mm they merge.

Finally, there is shown in Fig. 6 the slowly developing effect of an injection of gallamine triethiodide (horizontal, below). Time after injection is inserted (note the change of myograph sensitivity). The first phase soon went and the second disappeared before contraction was abolished. The records on the extreme right in this row show the effect of pressure on the wire joining muscle and myograph. There is clearly an effect of stretch left in the paralysed muscle. In other similar experiments the hyperpolarization by contraction, though diminished, has been visible as long as it has been possible to detect a contraction.

In Fig. 6 there is often a wavelet of depolarization at the foot of contraction, illustrated also in Fig. 10. Such wavelets were quite common in many motoneurons and, when large, caused the discharge of an initial spike, as in Figs. 2 and 10. Occasionally the wavelet was hyperpolarizing in direction. An instance is the cell of Fig. 7. Now, such very early wavelets must be caused by the 'back-response' (Lloyd, 1942; Leksell, 1945). Both muscle spindles and tendon organs contribute to it (Hunt & Kuffler, 1951; Granit, Pompeiano & Waltman, 1959) and it has been split

into two components, one ephaptic, the other due to the (intra- or extra-fusal) muscle itself (Granit *et al.* 1959). Hunt & Kuffler called it the 'early discharge'. Apparently the spindle primaries dominate in this effect, as early in extensor contraction the average effect by the monosynaptic indicator is facilitation (Granit, 1950; Bianconi *et al.* 1964*a*).

In Fig. 6 the hump of transient depolarization can be seen in several records. Especially interesting are the events later in relaxation, best visible at slow speed of recording (in the third vertical row). There is intense, hyperpolarizing activation noise suggesting delayed or late inhibition.

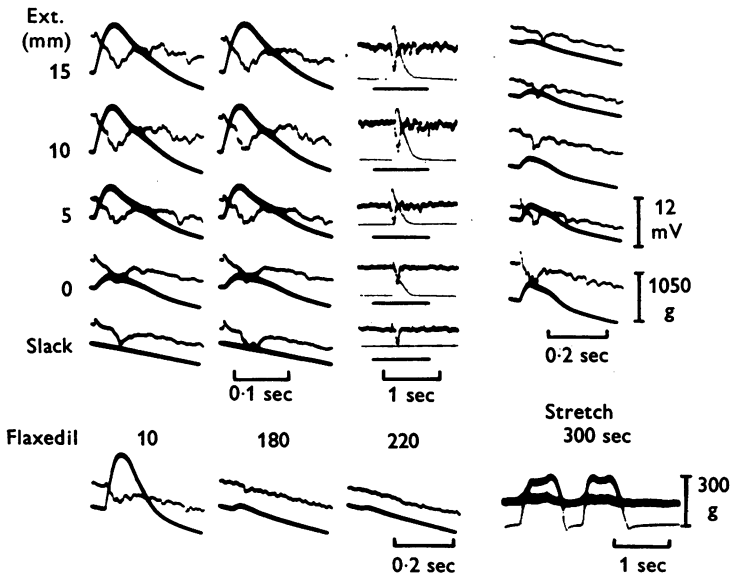


Fig. 6. GS motoneurone of 68 mV spike height stimulated by triple shock (except vertical right row) at 330/sec to peripheral portion of cut ventral root L7. The two vertical rows on the left (at threshold strength for full contraction) refer to different extensions of GS muscle, the third, same at slow speed. The fourth vertical row on the right illustrates effect of stimulus strength, for single shock and zero extension. Flaxedil (bottom, horizontal) 2 mg/kg given at zero time (not shown) and its effect on contraction and the hyperpolarization caused by it illustrated from 10 sec onwards. After 300 sec (extreme right) twice pressure on myograph string.

The late inhibition. Since the work of Bianconi *et al.* (1964*a*) had shown that autogenetic inhibitions in relaxation are quite common with monosynaptic testing of excitability after extensor contractions, we expected to see them more regularly than they were actually found by the intracellular approach. The late inhibition is prominent in Fig. 7, with a.c.

recording at high sensitivity (above) and d.c. recording at lower sensitivity (below). Arrows point to the very late activation noise of hyperpolarization. This motoneurone was one that responded by an initial wavelet of hyperpolarization at the foot of contraction, again too early for anything but the 'back-response' to have been its causative agent.

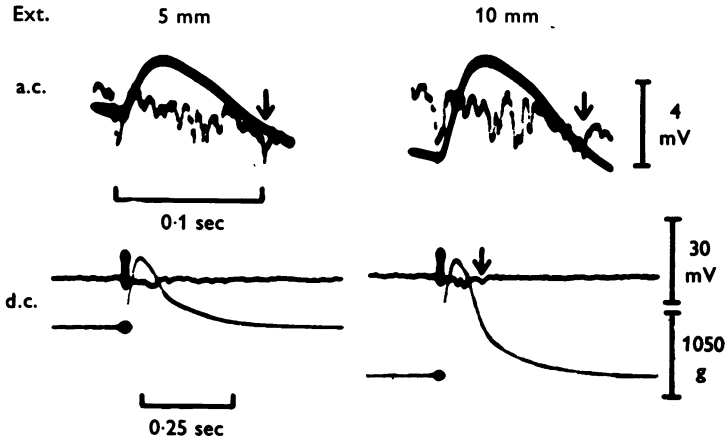


Fig. 7. Synergist popliteal motoneurone of 85 mV spike height. Most data inserted alongside records. Effect of GS contraction by brief tetanus to ventral root L7 as in previous Figures. Note the delayed inhibition in relaxation marked by arrow. Inhibitory wavelet preceding contraction.

The motoneurone of Fig. 8 is of particular interest because the muscle contraction was large. Stimulus strength to the ventral root was varied and it appears that with large contractions there was a delayed inhibition of the spontaneous discharge but that only the silent period proper was regularly present with small contractions (below). It is seen in the two uppermost records that the silent period again coincided with a large hyperpolarization, while the delayed inhibition did not show up as a definite shift of membrane potential. It is further notable that as soon as the contraction became small enough for the late inhibition to disappear, the spike terminating the silent period, as usual, arose in a definite position relative to relaxation.

Some motoneurons could be activated by both the GS nerve and the cut popliteal and then it became possible to use the disappearance of a threshold popliteal spike as a test of inhibition caused by a conditioning contraction from the ventral root. An instance is the motoneurone used for the graph of Fig. 9. As a rule there was good overlap in such experiments between amount and site of hyperpolarization in contraction on the one hand and the probability of disappearance of the spike on the other. Also, if hyperpolarization was unusually small or diminished much during

the course of an experiment, inhibition of the monosynaptic spike was unobtainable from the beginning or became so in the end. The method of testing probability of suppression or appearance of a response was developed for single spikes in ventral root filaments by Granit & Ström (1951). Lloyd & McIntyre (1955) made it the basis of their 'firing index' in excitation and it has since become better known by that term.

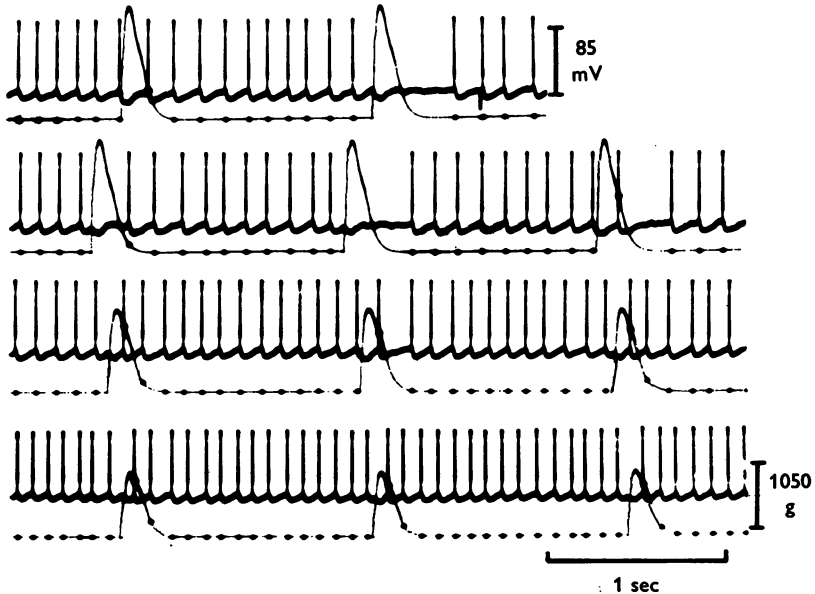


Fig. 8. Popliteal motoneurone firing spontaneously. Some initial tension in GS was necessary in order to get silent periods when the peripheral portion of the cut ventral L7 was stimulated. Records taken at 11 mm extension. Variations of stimulus strength reflected in variations of size of contraction. Note that the large contractions tend to produce silent period *plus* delayed inhibition during relaxation while the small contractions produce silent periods alone. Inhibitions during silent period accompanied by hyperpolarization, characteristically deeper with the larger contractions.

Figure 9A is of interest because here the maximum of the probability of inhibition at an interval of 40–70 msec smoothly tailed off into a delayed inhibition without any sign whatever of an intervening transient hump of excitation (as in the records of Fig. 8). Figure 9B refers to the same cell and is added merely to show the difference between the hyperpolarizations caused by a contraction preceded by a spike and one without it. The latter is repeated at slow film speed (below the others).

Separation of gastrocnemius and soleus. With three cats the two ankle extensors were separated in order to investigate the possibility of having been misled by interference effects due to their different contraction times.

The results are shown in Fig. 10. With these animals it turned out that it was easier to find effects on motoneurons from gastrocnemius than from soleus contractions. This is to be expected. The two gastrocnemii are much larger muscles than soleus. In addition their motoneurons are also likely to be larger than those of soleus (Eccles, Eccles & Lundberg, 1958) and hence easier to find and hold. The characteristic difference between the effects of the two muscles in contraction is presented in Fig. 10, records 1. This is the most common case. Soleus produces a small amount of hyperpolarization by comparison with gastrocnemius. Both possess a hump of

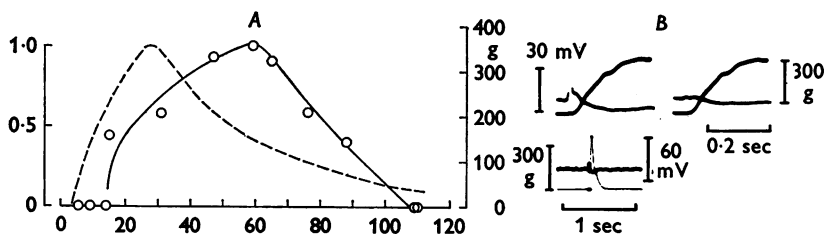


Fig. 9. GS motoneurone of 85 mV spike height. This cell also gave a spike to stimulation of the cut popliteal nerve. *A*, ordinates (left) the probability (P) of failure (in 95 tests) of the popliteal spike to occur when conditioned by the GS contraction due to stimulation of the peripheral stump of the cut ventral root with 4 shocks at 450/sec. This probability is plotted against the interval (abscissae) between shocks to the root and threshold popliteal test spikes. The interrupted line shows tension of muscle contraction (ordinates right) on the same time course. Note a late inhibition merging with that caused by the rising contraction. *B*, same motoneurone. The upper records show the beginning of the contraction, due to ventral root stimulation, with and without the popliteal spike inserted. The spike adds after-hyperpolarization to the hyperpolarization caused by the contraction. The lower record shows a full contraction at slow speed of film and the ensuing hyperpolarization.

depolarization, much larger with the latter muscle. It is not unlikely that in records from the undivided triceps (less plantaris) the hump of depolarization early in relaxation occasionally may have been smothered by interference. The records 2 and 3 are from two different motoneurons in which stretch of soleus had caused far more activation noise than pull on the gastrocnemius (stretch was used fairly regularly as a test in our experiments). The cell of Fig. 10, record 2, had the long duration of after-hyperpolarization (about 150 msec) found in soleus motoneurons (Eccles *et al.* 1958). The two lower records in both cases (2 and 3) are ones in which the early discharge at the foot of contraction produced large enough wavelets of depolarization to fire the cell. Clearly afterhyperpolarization in them emphasizes the trough of inhibitory hyperpolarization caused by contraction alone. The lowermost record (4) is again from the undivided

GS muscle. Possibly the 'hump' in gastrocnemius relaxation is cut short in this experiment by maintained soleus hyperpolarization.

The main conclusion from the experiments with the two muscles separated is that the post-synaptic shifts of membrane potential caused in motoneurons by autogenetic contractions depend on the duration of contraction in the expected manner.

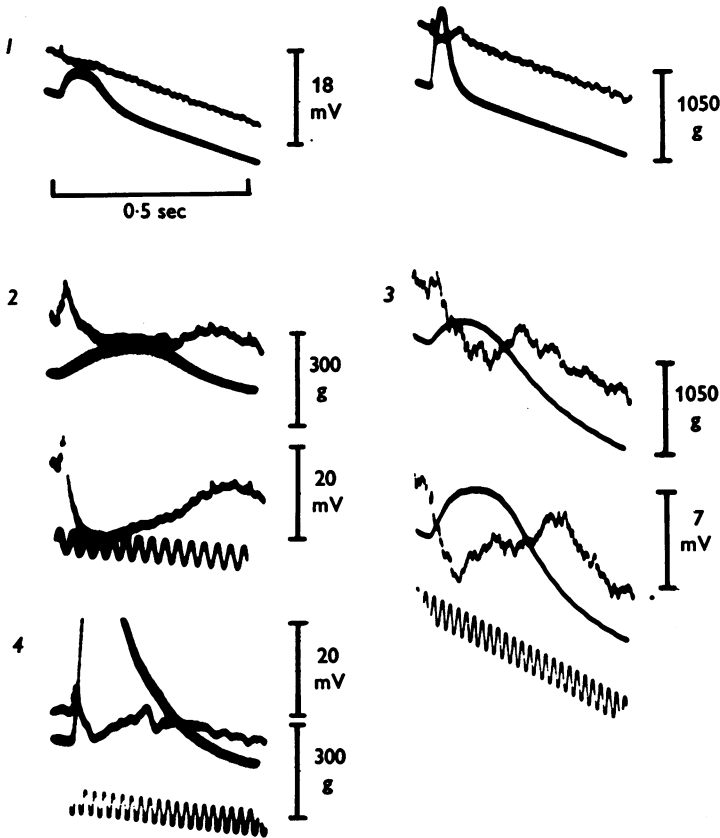


Fig. 10. Various motoneurons subjected to the effect of autogenetic contraction from cut ventral root. 1, GS cell of 70 mV; soleus at 12 mm extension (left), gastrocnemius at 14 mm (right). 2, GS-cell of 64 mV spike height. Soleus at 12 mm extension. Upper contraction only eliciting initial wavelet of depolarization, while lower contraction also caused spike and after-hyperpolarization. Recording beam for contraction switched to time marker, 100 c/s. 3, GS cell of 66 mV spike height. Soleus at zero extension, upper record without, lower with initial spike (as in 2). 4, synergist popliteal spike (not photographed, because soon lost). Soleus and gastrocnemius at 6 mm extension unseparated. Note, dominance of gastrocnemius (cf. 1, on the right above).

DISCUSSION

Comparison with monosynaptic testing. Monosynaptic testing has the advantage over electromyography that changes in motoneurone excitability can be demonstrated in contraction without a background discharge. The events which take place are well known from previous work (Granit, 1950; Bianconi *et al.* 1964*a*) and can now be compared with the intracellular results obtained above. The first visible event in contraction, occurring a little before a rise of tension can be myographically recorded, is a short-lasting facilitation of the monosynaptic response, ascribed above to the early discharge or 'back-response'. Intracellularly it corresponds to a brief wavelet of depolarization, seen in a fair number of cells. During the next phase, that of the rising contraction, there is inhibition which is deeper the greater the amount of tension (Granit, 1950; Hagbarth & Naess, 1950; Hufshmidt, 1960). Intracellularly this corresponds to the hyperpolarizing responses described above. Early in relaxation the next phase is a transient facilitation of the monosynaptic response. This has its exact intracellular equivalent in the hump of depolarization and is therefore a post-synaptic excitation. Ultimately this event is succeeded by a depression of the average monosynaptic response often lasting far into the relaxation period. With the membrane potential as indicator, this delayed or late inhibition tends to be 'remote' in the sense of Granit *et al.* (1964*b*). But since it is often accompanied by hyperpolarizing activation noise and likewise may emerge as a depression of firing rate in transmembrane stimulation, it, too, is a genuine post-synaptic event.

It is concluded from this comparison that the monosynaptic index has given an essentially correct picture of what happens in extensor motoneurons and in many of their synergists during contractions of the muscles they innervate. If there is presynaptic inhibition, it is hardly necessary to invoke it in order to explain the effects of autogenetic contraction on extensor motoneurons.

Source of events recorded. The early facilitation at the foot of contraction has already been discussed in connexion with Fig. 6. The subsequent inhibition is obviously in the main of muscular origin because such inhibitions (i) do not require activation of a cellular spike delivering after-hyperpolarization; (ii) they are favoured by an increase of tension be it by extension or by increase of stimulus strength at constant extension; (iii) their duration reflects the duration of contraction, different for gastrocnemius and soleus; they occur in all GS motoneurons as long as the preparation is in good condition and hence cannot all be explained by recurrent inhibition from adjacent cells firing to the back-response because a considerable number of gastrocnemius motoneurons do not possess

recurrent inhibition (Granit, Pascoe & Steg, 1957). It has been demonstrated above (Figs. 9 and 10) that when afterhyperpolarization does occur it adds emphasis to the inhibition caused by contraction. Similarly, it is to be expected that recurrent inhibition will contribute to it whenever present. This inhibition will obviously be of much greater importance in experiments with intact ventral roots into which volleys from the nerve are backfired (cf. Holmgren & Merton, 1954). It is then the earliest genuine inhibition recordable.

The regularly noticeable inhibition, described as phase (i), possessing a slope of rising hyperpolarization mimicking the rising tension, is likely to arise in the Golgi tendon organs. The duration of the 'pause' of the primaries in contraction varies very little with tension and extension (Granit & Van Der Meulen, 1962). The tendon organs have recently been studied in detail by Jansen & Rudjord (1964) who have demonstrated that the large majority of these receptors in the cat soleus are far more sensitive to contraction than to a passive rise of tension. A typical response is shown in Fig. 11*B*. Quite small twitches stimulate them, the average threshold being of the order of 44 g. Of forty-two organs studied twelve failed to give a maintained discharge to full physiological extension and six could be fired only by active contraction. Similarly, autogenetic inhibition was found to be dependent on active contraction (Granit, 1950; Hagbarth & Naess, 1950). Clearly this is wholly in accordance with the results presented above for the hyperpolarization caused by contraction.

We have proved beyond any reasonable doubt, as is illustrated in Figs. 2, 4, 6 and 8 that an increase of contractile tension at constant extension augments the hyperpolarization thereby excluding disfacilitation by the pause in the discharge of the large spindle afferents as the cause of this augmentation. Actually hyperpolarizations by contraction tend to be wholly removed by injecting chloride through the micro-electrode tip (criterion of Coombs *et al.* 1955), as we have reported at a recent Symposium (Granit, 1965). Disfacilitations would not react in this manner. Here is therefore additional evidence that the hyperpolarizations produced by contraction in the present work are largely if not wholly due to genuine post-synaptic inhibitions, as is also shown by the presence of wavelets of hyperpolarizing activation noise (cf. Fig. 7) in cases where the noise has not fused to form a trough of hyperpolarization. In the course of work on intracellular effects of contraction (from the root) from other points of view Granit & Kernell (personal communication) have also had opportunities of testing by hyperpolarizing currents motoneurons responding to such contractions by hyperpolarization (as above): the effect could generally be changed into a depolarization. Our de-efferented and anaesthetized preparation is not a favourable one for demonstrating disfacili-

tation from tonic spindle activity. There is therefore no escape from the conclusion that active post-synaptic inhibition is an important factor in determining excitability during the silent period of extensor muscles in contraction.

The second phase of hyperpolarization in contraction, extending a little into relaxation, has been seen in a smaller number of cases. It is too late to be a special contribution of the spindle pause because this begins as soon as the muscle starts to produce contractile tension (see Fig. 11). The number of cells giving the characteristic 'trough' has been too small to enable us to reach definite conclusions as to its nature. The most likely

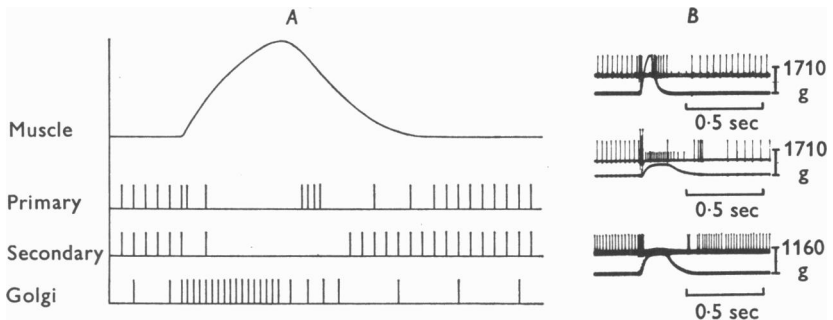


Fig. 11. *A*, diagram to illustrate characteristic features of discharge of spindle and tendon organs in contraction of their common muscle of origin assumed to be extended 10 mm. *B*, some original records from the laboratory collection (Granit & Van Der Meulen). Recording from dorsal root filaments. Contraction is from a brief tetanus to GS nerves in cats under Nembutal. *Uppermost* record is a de-efferented gastrocnemius spindle-primary showing early burst and slow pick-up of discharge afterwards. Gastrocnemius extended 12 mm. The *middle* record contains both de-efferented soleus spindle-primary and soleus tendon organ in the same thin dorsal root filament. Very characteristic response of both. Muscle extended 4.5 mm. *Lowermost* record, soleus spindle primary with intact motor innervation. Muscle extended 13 mm.

explanation is that the 'trough' is due to a large contribution from the slower soleus imposing itself upon a diminishing inhibition from gastrocnemius. The relatively late hump of depolarization in Fig. 6 also suggests a strong influence from soleus (cf. Fig. 10). Before this simple and attractive hypothesis has been tested by experiment, there is no need to consider alternatives. The existence of some hyperpolarization in slack muscle during contraction need not cause much concern because Jansen (personal communication) reports that tendon organs have been observed to respond in flexors under such circumstances and so it is perfectly possible that they could do the same in a preparation consisting of gastrocnemius and soleus together. The highly plausible explanation, given by Jansen & Rudjord (1964) of the necessity for contractile tension (instead of passive

stretch) to stimulate tendon organs effectively, is based on the distribution of mechanical vectors of pull which can be very complex. Besides, there has been no systematic study of tendon organs in the gastrocnemii.

The diagram given as Fig. 11*A* and the original records in Fig. 11*B* account for the hump of transient depolarization by the bursts from a large number of phasic spindle primaries. For a more detailed comparison we refer to the special study of the pause of these organs by Granit & Van Der Meulen (1962). The diagram and the records emphasize only highly characteristic features. Some primaries belong to the category of 'long-pause spindles', not included in the diagram.

During relaxation both phasic and long-pause spindle primaries may require some time for picking up the steady rate of discharge set by a given degree of extension. This gives the tonic secondaries a chance of dominating for a while and it was assumed by Bianconi *et al.* (1964*a*) that this is the explanation of the delayed inhibition lasting far into relaxation. Spindle secondaries have been shown to be inhibitory on extensor motoneurons (Hunt, 1954; Laporte & Bessou, 1959). They tend to resume their original firing rate as soon as contraction is over (Harvey & Matthews, 1961; Bessou & Laporte, 1962). We therefore subscribe to the explanation of Bianconi, Granit & Reis (1964*b*) of delayed inhibition.

The silent period. Ever since the work of Matthews (1933) which so decisively confirmed the explanation of the silent period by spindle silence, as adumbrated by Fulton & Pi-Suñer (1927-28), the role of the 'pause' in the discharge of the spindle primaries during contraction has hardly been in doubt, nor do we doubt it now, in spite of providing fresh and unequivocal evidence for the existence of an inhibition induced by contractile tension and ascribed to the tendon organs. It is merely being denied that the relatively constant duration of the silent period can be used as evidence against the existence of a preceding inhibitory process (Merton, 1951; Jansen & Rudjord, 1964). The duration of the silent period is determined by a 'clock' of its own, the transient hump of depolarization that was ascribed to the spindle bursts early in relaxation. For this reason duration alone can give no information whatever as to whether or not true inhibition was present during the period of observed silence. Actually, the contracting extensor is putting on double 'brakes', post-synaptic inhibition and the spindle pause. How much the latter contributes to hyperpolarization caused by contraction will depend upon the original contribution of the spindle primaries to the pre-existent level of depolarization. This is, for instance, likely to be high in decerebrate animals and low in those anaesthetized with pentobarbitone and—as in our case—lacking their efferent γ -supply. We have actually succeeded in firing silent motoneurons by tetani to the ventral root across the γ -loop

thus demonstrating that the spindle primaries *can* depolarize motoneurons.

The transient hump of depolarization also provides an explanation of old findings beginning with myographical observations on the knee jerk by Sherrington (1915) and Ballif, Fulton & Liddell (1925). In decerebrate animals there is often a prominent hump in relaxation, called by the latter authors the myotatic 'appendage' or 'hump'. There may be in it an element of post-hyperpolarization overswing or 'rebound' of the membrane towards depolarization but since at times the 'hump' can be large in cells with but little hyperpolarization, the most important factor must be the spindle bursts, as pointed out above.

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