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CENTRAL INHIBITORY ACTION ATTRIBUTABLE TO PRESYNAPTIC DEPOLARIZATION PRODUCED BY MUSCLE AFFERENT VOLLEYS

BY J. C. ECCLES, ROSAMOND M. ECCLES AND F. MAGNI*

From the Department of Physiology, Australian National University, Canberra, Australia

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Frank & Fuortes (1957) and Frank (1959 and personal communication) have shown that muscle afferent volleys produce inhibition by diminishing the size of the monosynaptic excitatory post-synaptic potential (EPSP) of motoneurones without having any other demonstrable action on those motoneurones. Thus there was no change in the ionic permeability of the post-synaptic membrane, such as occurs with other types of inhibition (Coombs, Eccles & Fatt, 1955*a*), for there was no associated membrane potential change either at the normal resting potential, or when the membrane potential was altered by a background depolarizing or hyperpolarizing current. Furthermore, there was no associated change in motoneuronal excitability as tested either by the intracellular application of current pulses or by the response to invasion by an antidromic impulse in the motor axon. Evidently it should be concluded that the diminution of the EPSPs is due to a diminished excitatory action of the Group Ia presynaptic impulses. However, Frank (1959) in addition proposed an alternative explanation, which attributed the EPSP depression to an action exerted so far out on the dendrites of the motoneurone that no trace of the inhibitory influence itself could be detected by a micro-electrode in the motoneuronal soma. Since both these alternative explanations would locate inhibitory action at a site remote from the motoneurone soma, Frank (1959) employed the term 'remote inhibition' for the phenomenon. It is proposed here to use the non-committal term 'EPSP depression' instead, until a more appropriate term can be developed later in this paper.

There are many suggestions in the literature that central inhibitory action occurs in the presynaptic pathway, there being block or depression of presynaptic excitatory impulses (Barron & Matthews, 1935, 1938; Renshaw, 1946; Brooks, Eccles & Malcolm, 1948; Howland, Lettvin, McCulloch, Pitts & Wall, 1955). These various suggestions will be examined

* Present address: Istituto di Fisiologia, Della Universita de Pisa, Italy.

after an account has been given of the present investigation, which confirms and extends the pioneer studies of Frank & Fuortes (1957) and Frank (1959). A preliminary report has already been published (Eccles, Eccles & Magni, 1960*a*), and reference to this work has also been made in lectures (Eccles, 1961*a*, *b*) already published.

METHODS

The experimental procedures conformed to the conventional pattern as reported in previous publications from this laboratory and in the preceding paper (Eccles, Kozak & Magni, 1961), which also may be referred to for the symbols corresponding to the various nerves (see this volume, p. 129). In some experiments the temperature of the animal was kept as low as 34° C, because larger EPSP depressions were observed under these conditions. The temperatures given in this paper were recorded by a thermometer inserted under the scapula and just outside the thoracic cage. Usually the temperature of the oil pool over the cord was 2 or 3° C cooler.

RESULTS

Time course of EPSP depression

The time course of onset of EPSP depression is illustrated in Fig. 1B–D, where a maximum Group I volley in the nerve to posterior biceps and semitendinosus (PBST) preceded at progressively increasing intervals the testing volley in gastrocnemius soleus (GS) nerve that by itself set up the monosynaptic EPSP shown in A. The EPSP depression is seen to increase from a negligible action at a brief interval (2·5 msec in B), to a large effect at 10·2 msec in D. The diminution of the EPSP was not associated with any change in its time course, nor did the conditioning volley produce any appreciable change in the membrane potential of the impaled gastrocnemius motoneurone, as may also be seen in Fig. 2C–E. Measurements from the series partly illustrated in Fig. 1A–D are plotted in E to show all except the final stages of the time course of the EPSP depression.

The depression of the EPSP in Fig. 1 was much larger than that usually observed for a single afferent volley, which often was only 10% or less, as illustrated in Fig. 2A. Much larger depressions are produced by repetitive afferent volleys. For example, 2 volleys at 3·7 msec interval doubled the depression (records of Fig. 2B–E, plotted in F), while with 7 volleys at 270/sec the depression was increased nearly threefold (Fig. 2G), and had a slower time course of decay.

As is shown in Figs. 1E and 2F, G, the onset of the EPSP depression has been gradual, and no precise latency can be given; but usually a significant depression occurred with testing intervals as brief as 5 msec. The maximum depression occurred with test intervals of 15–20 msec. The time course of Fig. 1E would be prolonged on account of the abnormally

low temperature (34·5° C), but even at 37° C (Fig. 2G) the depression persisted beyond 200 msec.

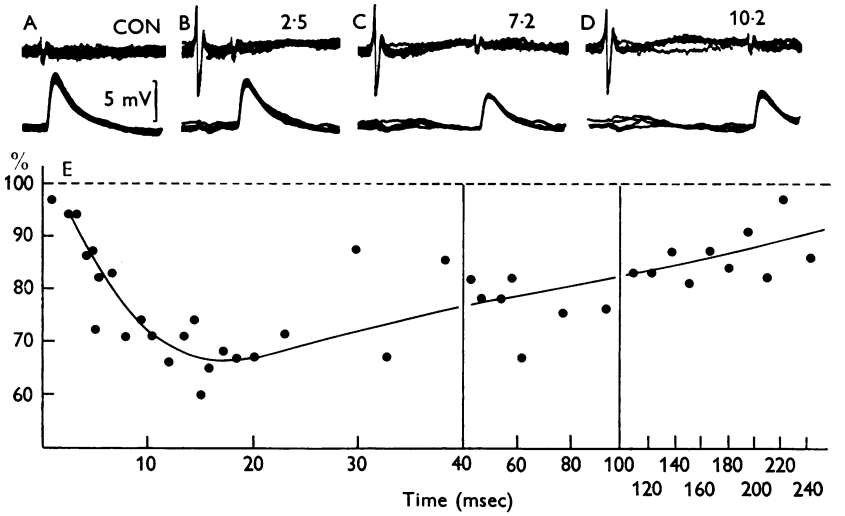

Fig. 1. Time course of EPSP depression. In A–D the lower records give monosynaptic EPSPs intracellularly recorded from a gastrocnemius motoneurone, and evoked by maximal Group Ia volleys in the GS nerve. In the upper records are the potentials recorded from the L7 DR as it enters the cord, negativity being recorded downwards. All records are formed by superposition of about 5 traces recorded at a frequency of repetition of about 2/sec. A shows the control EPSP, and in B–D the GS volley was preceded by a maximum Group I PBST volley at the intervals marked in msec. The depression of the EPSP was small in B and large at the longer intervals, C, D. The time course of the depression is displayed in E, where the ordinates are the sizes of the EPSPs plotted as percentages of the control EPSPs, and the abscissae the intervals after the conditioning PBST volley. The perpendicular lines denote changes in the abscissal scaling. Body temperature 34·5° C.

Types of afferent impulses giving EPSP depression

In agreement with Frank & Fuortes (personal communication), such investigations as those of Figs. 1 and 2 establish that EPSP depression is due to Group I volleys. It is therefore of particular interest to discover if EPSP depression is due to a specific property of the Ia or of the Ib impulses. For this purpose the PBST nerve was especially suitable, because often an almost complete threshold separation is possible (Bradley & Eccles, 1953; Eccles, Eccles & Lundberg, 1957*a*; Laporte & Bessou, 1957). The experimental investigation is illustrated in Fig. 3, where conditioning volleys in the PBST nerve were evoked by a wide range of stimulus strengths as illustrated by A–D, E–H, I–L and M–O, and their actions tested against the monosynaptic EPSPs produced in a gastrocnemius motoneurone by a maximum Ia GS volley 20·5 msec later (Fig. 3P),

the control EPSPs being shown in B, F, J and N. Immediately after this series the conventional tests were applied (cf. Fig. 3D, H, L) in order to determine the Ia–Ib composition of the conditioning afferent volleys evoked by the various strengths of stimuli. The curves for percentage Ia–Ib composition of the afferent volleys set up by the stimuli of the

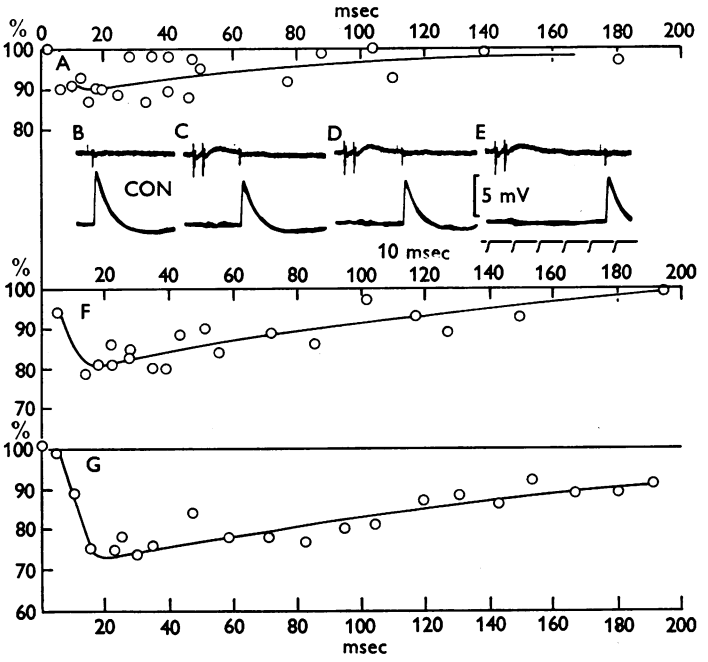

Fig. 2. Time courses of EPSP depression produced by one or more conditioning volleys. Monosynaptic EPSPs produced in a plantaris motoneurone, membrane potential −75 mV, by a maximum Ia plantaris volley and depressed by 1, 2, or 7 maximum Group I PBST volleys. A, plotting of depression by a single PBST volley as in Fig. 1E, but illustrating the much smaller depression usually observed. B–E show records as in Fig. 1A–D, but for depression by two PBST volleys 3·7 msec apart, F showing the plotted points for the whole series. With G there is a similar plotting after conditioning by 7 PBST volleys at a frequency of 270/sec. With all records the frequency of repetition was 0·5/sec. Body temperature was 37° C. With F and G zero time corresponds to first PBST volley.

various strengths are plotted in Fig. 4B, as has been done previously (Eccles *et al.* 1957*a*). When the EPSP depression is plotted on the same abscissal scaling (Fig. 4A), it is seen that almost all of this depression came in at a range of stimulus strengths which corresponded closely to the Ib range, there being only a negligible effect over the Ia range.

However, this almost exclusive relationship of Ib to EPSP depression was unusual, and Fig. 5E shows clearly two steps in the size of the

depression as the conditioning stimulus was increased. The component attributable to the Ib volley was only a little larger than that due to the Ia volley (Fig. 5E). Most of our series have conformed to Fig. 5 in showing that a substantial component of the EPSP depression was due to Ia impulses, and with some the Ia component was even a little larger than the Ib. So far as has been determined, the EPSP depressions produced by Ia volleys (cf. Fig. 5C) were similar in every respect to the depressions produced by the combined Ia–Ib volleys (Fig. 3E–H).

In Figs. 3 and 4A there was no further increase in the EPSP depression when the stimulus was increased to 4 or 8 times threshold, so as to add Group II and III impulses to the conditioning volley. In Fig. 5E it appeared that somewhat larger depression was produced when the

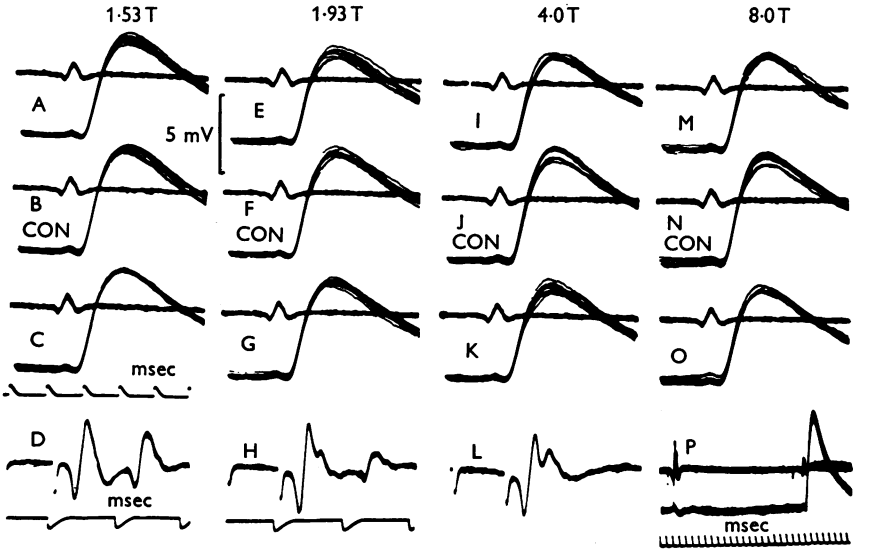

Fig. 3. Type of muscle afferent fibre responsible for EPSP depression. As is shown in P, the conditioning PBST volley preceded the monosynaptic EPSP of the gastrocnemius motoneurone (membrane potential, −80 mV) at a fixed interval of 20·5 msec. The conditioning volley was set up by stimuli of varying strengths up to 8 times threshold (8 T) and specimen records of the conditioned EPSPs (set up by maximum Ia GS volleys) are shown in 4 columns at the indicated strengths: A–C being for 1·53T, E–G for 1·93T, etc. In each series a control EPSP (CON) is shown between two conditioned EPSPs. Note the expanded time scale of these EPSPs relative to that of P. Each record is formed by the superposition of about 10 faint traces at 2/sec. The Ia composition of the PBST conditioning volley used in each vertical column was tested by the double-volley technique (see Eccles *et al.* 1957*a*, and preceding paper, Eccles *et al.* 1961) and the result illustrated in D, H and L. Thus a testing stimulus 0·75 msec after the conditioning found that virtually all Ia and no Ib fibres were refractory in D, while in H about half Ib fibres were refractory and in L all Ia and Ib were refractory.

conditioning stimulus was increased from maximal for Group I (2·2T) to 3·2 and 4·0T, but it may be doubted if this effect is significant. So far there has been no convincing demonstration that Group II and III afferent impulses are effective in producing EPSP depression.

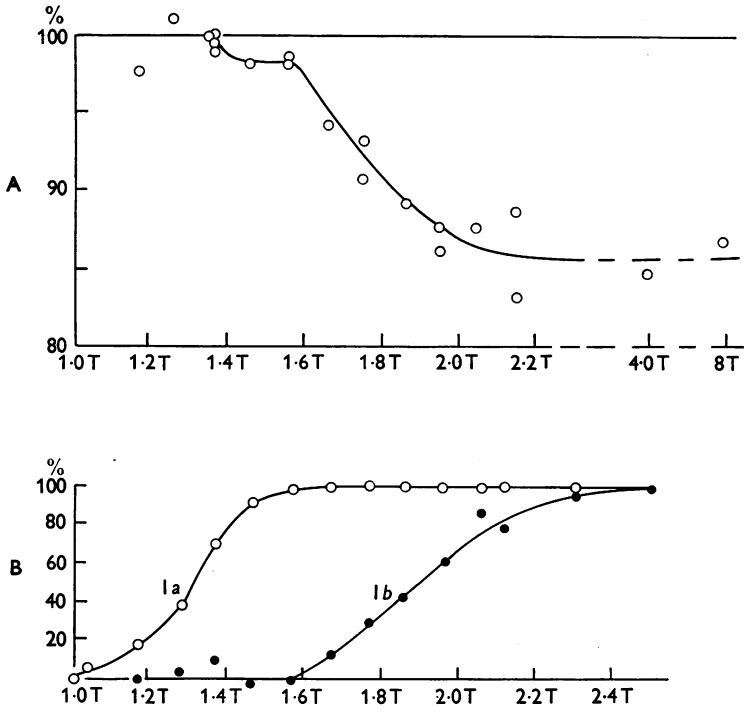

Fig. 4. Types of muscle afferent fibre responsible for EPSP depression. A. The sizes of the conditioned EPSPs partly illustrated in Fig. 3 are expressed as percentages of the control EPSPs and plotted as ordinates against the stimulus strengths relative to threshold as abscissae. In B the sizes of the Ia and Ib components of the conditioning afferent volleys (PBST) were measured from records such as Fig. 3D, H, L, and expressed as percentages of the maximal size, exactly as was done by Eccles *et al.* (1957*a*). Note that the abscissal scalings of A and B are identical.

EPSP depression by repetitive nerve volleys

When the EPSP depression was tested during repetitive stimulation of the conditioning afferent nerve, there was a rapid increase in the depression, as indicated in Fig. 6A, a maximum value being reached with a very few impulses. After cessation of the train of conditioning volleys there was a fairly rapid decline of the EPSP depression, as indicated in the records of Fig. 6B–E, but full recovery could take more than 1 sec (Fig. 6F).

When repetitive stimulation was continued after the maximum EPSP depression had been attained, there was usually a slow decline over several seconds, but eventually the depression tended to stabilize at some intermediate level. For example, Fig. 7B, C, D illustrates the EPSP depression

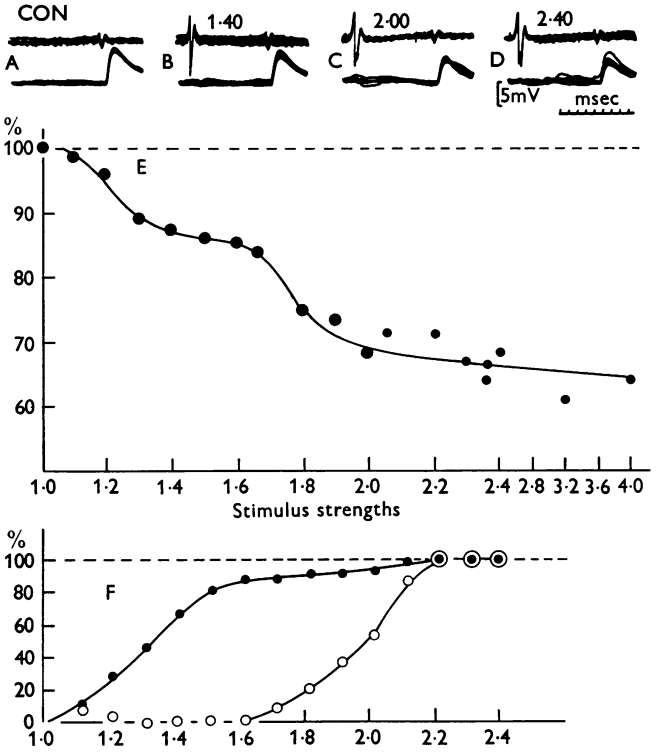

Fig. 5. Types of muscle afferent fibre responsible for EPSP depression. In B–D a conditioning PBST volley was evoked by stimuli of the indicated strengths relative to threshold and followed at a fixed interval (9·0 msec) by a monosynaptic EPSP produced by a maximum Group Ia gastrocnemius volley in the same gastrocnemius motoneurone as in Fig. 1. The points in E and F are plotted as in Fig. 4A, B. Note that in contrast to Fig. 4, a considerable EPSP depression was produced by a Group Ia afferent volley, i.e. with stimulus strengths up to 1·6T. Body temperature 34·5° C. In E the larger plotted points up to 2·0T are means of two or three records.

at 5, 10 and 20 sec after the onset of repetitive stimulation of the PBST nerve at 300/sec, while Fig. 7G, H, I gives a similar series for repetitive PDP stimulation. Measurements from these and other similar tests on this same EPSP are plotted in Fig. 7E and J, and show that the weaker depression produced by the PDP volleys is less well maintained than the

PBST depression. The very large depression in another experiment (Fig. 7K) was well maintained even during 60 sec tetanization.

Summation of the depressant actions of repetitive volleys has been illustrated in Figs. 2 and 6 for high-frequency stimulation at 270 and 210/sec respectively. In Fig. 8A–E the frequency of the conditioning tetanus was varied. As is shown in the plotted curve (F), more than half of the

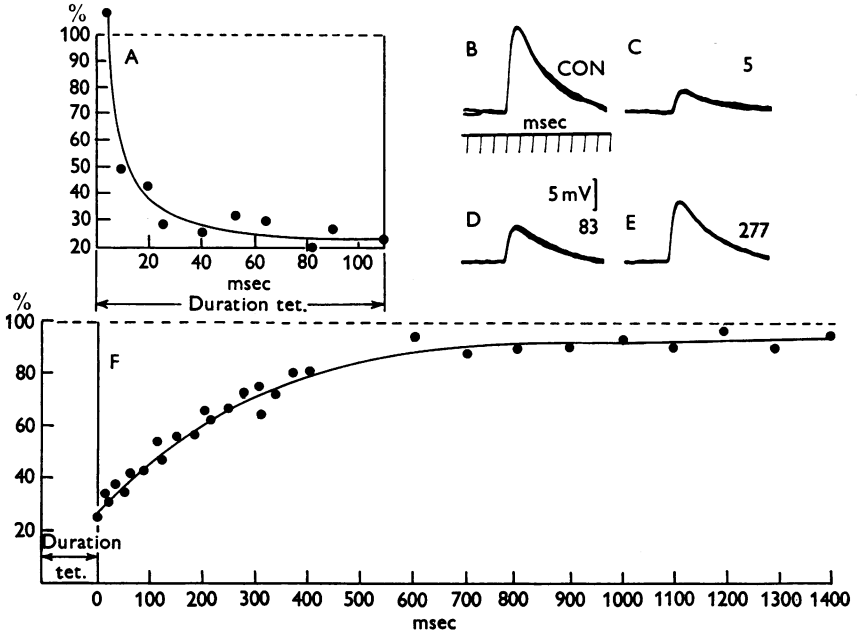

Fig. 6. Time course of EPSP depression during and after repetitive conditioning volleys. The PBST nerve was stimulated for 109 msec at a just-supramaximal strength for Group Ia and at a frequency of 210/sec (22 volleys). The EPSP depression was measured by the size of the maximum monosynaptic EPSP set up by a gastrocnemius volley in a gastrocnemius motoneurone. A shows the rapid increase of the depression during the stimulation. B–E show specimen records (two superimposed traces) of the control EPSP (B) and at the intervals (indicated in msec) after the end of the conditioning tetanus. The time course of recovery from the depression is shown by the plotted points of F for the whole series that was partly illustrated in B–E. In order to allow time for full recovery there was an interval of 2 sec between each conditioning tetanus, and there was, of course, only one testing EPSP after each tetanus. Body temperature 34·5° C.

maximum depression was produced by a frequency of 65/sec, and there was little further increase above 200/sec. Figure 8G–K shows much the same frequency dependence for the very large depression illustrated in Fig. 6.

Besides thus producing a deep and long-lasting EPSP depression, a prolonged tetanization of the conditioning nerve also resulted in a typical

post-tetanic potentiation of the EPSP depression produced by a single conditioning volley. For example, in Fig. 9, following the control records of the depressed EPSP (A) and of the EPSP alone (B), there was a long tetanus of the conditioning nerve. Two seconds later there was still a small residual depression of the EPSP alone in one control response of D (cf. Fig. 6F). The conditioning volley exerted a greatly increased depression (C), as may also be seen at 12 sec (E, F), and even to a small extent at 90 sec (G, H). The full time course of the post-tetanic potentiation of

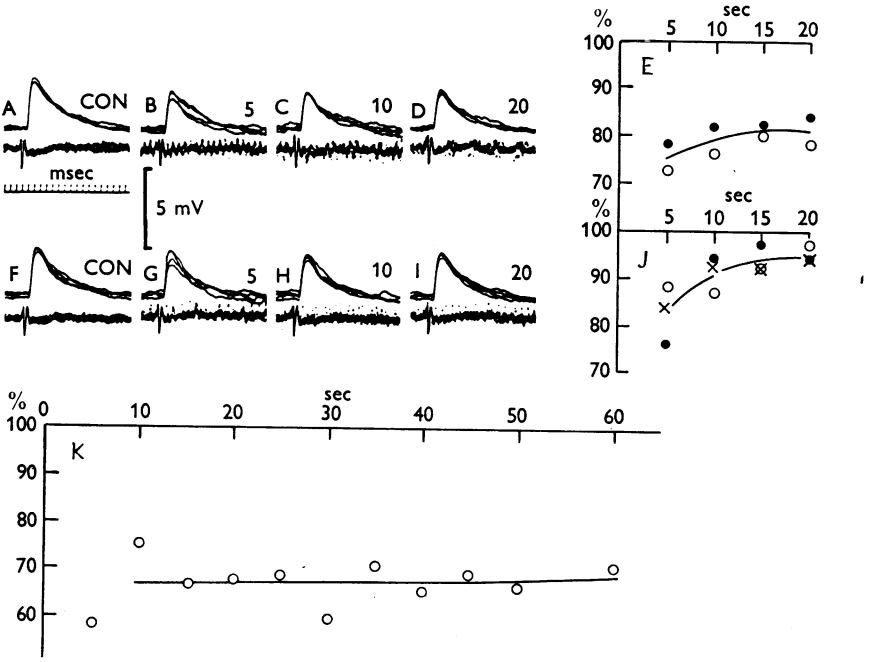

Fig. 7. EPSP depression during prolonged tetanization of the conditioning afferent nerve. The GS nerve was stimulated continuously at 5/sec at a strength supramaximal for Group Ia and the EPSPs were recorded intracellularly from a gastrocnemius motoneurone (membrane potential, −70 mV), there being four superimposed traces in each record of A–D and F–I, A and F being the control EPSPs. After record A the PBST nerve was stimulated at 300/sec at a strength sufficient to maintain it supramaximal for Group I during a prolonged tetanus. After 5 sec conditioning tetanus the EPSPs were still depressed (B), as also at 10 and 20 sec in C and D during this same conditioning tetanus. E gives the plotted points for these records and for one other series taken just afterwards. A similar series of traces for depression of the same EPSP during a PDP tetanus at 300/sec is shown in G–I and plotted in J along with two other series on the same EPSP. It is seen that the depression is less well maintained than during the PBST tetanus. Body temperature 37° C. In K the EPSP depression is seen to be even better maintained than in E. This is also for a PBST tetanus at 220/sec on to an EPSP of a gastrocnemius motoneurone.

the EPSP depressant action (Fig. 9I) exhibited a relative potentiation and a duration comparable with that observed for monosynaptic actions after a similar conditioning tetanus (Eccles, Krnjević & Miledi, 1959; Curtis & Eccles, 1960).

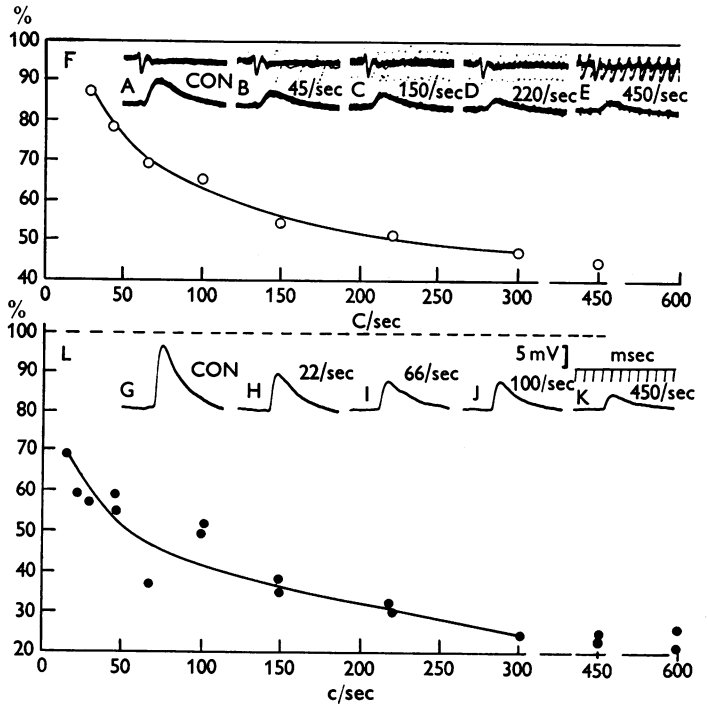

Fig. 8. Effect of frequency of conditioning stimulation on intensity of EPSP depression. A–F, specimen records and plotted curve of EPSP depression produced on the monosynaptic EPSP of a gastrocnemius motoneurone (membrane potential, −35 mV) by maximum Group I PBST volleys at various frequencies. A gives control EPSP and in B–E the EPSP was photographed after a brief duration (about 1 sec) of the conditioning tetanus at the indicated frequencies. Thus the depression was recorded before there had been the decline shown in Fig. 7. The EPSP were recorded at a repetition frequency of about 20/sec, and each record of A–E was formed by the superposition of about 10 faint traces. The traces above the EPSPs show that there was no appreciable change in the sizes of the gastrocnemius afferent volleys as recorded from the L7 dorsal root at cord entry. The plotted points of F are for the series partly illustrated in A–E. Body temperature 37° C. G–K give specimen records of control EPSP and depressed EPSPs at the stated frequencies of condititioning tetani for the same motoneurone as Fig. 6, while L gives the curve for EPSP depression–frequency relationship. Body temperature 34·5° C. Same time scale for A–E and G–K.

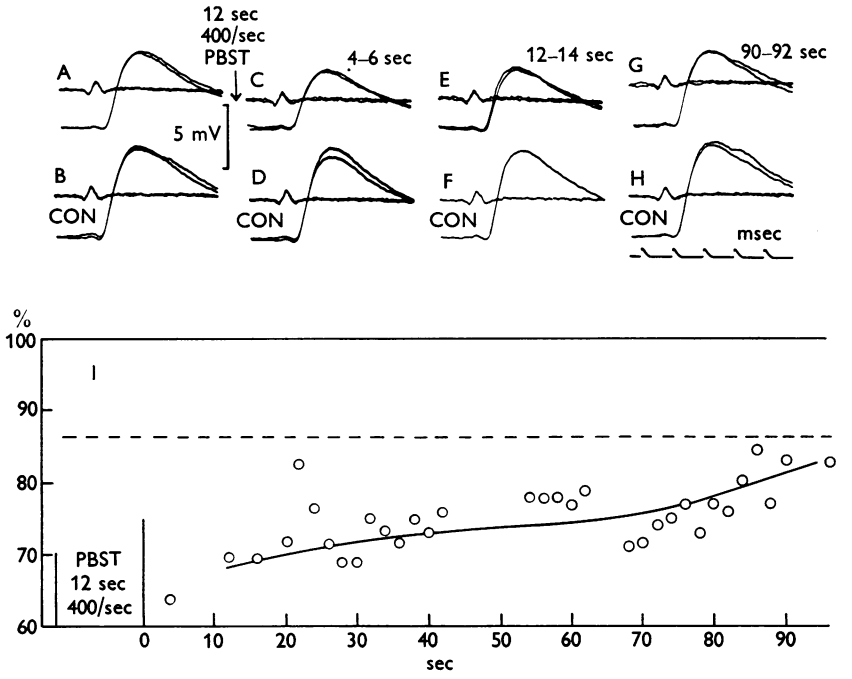

Fig. 9. Post-tetanic potentiation of EPSP depression. A and B illustrate the control level of EPSP depression (A/B) produced by a maximum Group I PBST volley on the monosynaptic EPSP of a gastrocnemius motoneurone (membrane potential, −75 mV). After a conditioning PBST tetanus (of 400/sec for 12 sec) a much larger depression, C/D, was produced by the PBST volley, as also at 12 sec post-tetanically, E/F; but at 90 sec there was recovery (G/H) almost to the pre-tetanic ratio. Testing EPSPs were evoked every 2 sec, there being two superimposed in each trace, and the control EPSPs (D, F and H) were usually taken in alternating sequence. These controls were remarkably steady except for the depression of the first one after the conditioning tetanus (one record of D). The series partly illustrated in A–H is fully plotted in I to show the time course of the post-tetanic potentiation of the EPSP depression.

EPSP depression in relation to the various species of muscle

Hitherto EPSP depression has been illustrated and discussed in relation to the actions of PBST volleys on monosynaptic EPSPs of gastrocnemius and plantaris (Fig. 2) motoneurones. With this arrangement there is the advantage of having large depressions, and in addition the tests are generally free from complications by the various actions of Group I afferent volleys that occur by the conventional synaptic pathways. PBST Group Ia volleys usually exert no excitatory or inhibitory action on GS and PL motoneurones (Eccles, Eccles & Magni, 1960*b*), and in only about one quarter of gastrocnemius motoneurones have Group Ib PBST volleys

any excitatory or inhibitory action (Eccles, Eccles & Lundberg, 1957*b*). By taking advantage of these favourable conditions, it has been possible to establish many of the distinguishing features of EPSP depression; as a consequence it can be recognized even when the conditioning volleys exert post-synaptic inhibitory or excitatory actions on the motoneurone under observation. The very long duration of the EPSP depression is of particular value in this respect. It is justifiable to assume that no other Group I actions continue beyond 25 msec in the isolated spinal cord under sodium pentobarbital anaesthesia. Furthermore, during rapid repetitive stimulation the polysynaptic actions of Group Ib volleys rapidly diminish and even disappear.

The simplest method of surveying the relative potencies of afferent volleys from different muscles in depressing the monosynaptic EPSPs of motoneurones is illustrated in Fig. 10. The EPSPs are generated by stimulating the appropriate afferent nerve at 5/sec and are recorded by superimposition of about 3 or 4 faint traces. The conditioning nerve is stimulated at 300/sec and the EPSPs are again recorded for about 1·9 sec, beginning a fraction of a second after the onset of the conditioning tetanus, which is itself discontinued immediately after the EPSPs have been photographed. In this way full advantage is taken of the optimum conditions for detecting any EPSP depression that may be present. There is of course a risk that post-synaptic inhibitory actions might depress the EPSP, as described by Coombs, Eccles & Fatt (1955*b*) and by Curtis & Eccles (1959), but this effect rapidly declines during repetitive stimulation at 300/sec, though some Group Ia inhibition may not be thus eliminated.

Table 1 summarizes the effects thus observed on monosynaptic EPSPs in seven motoneurones in one experiment. Invariably the PBST volley was the most effective, but the P and DP volleys were also surprisingly potent. When their relatively small sizes are taken into account, they must be at least as effective as the PBST volleys. With all the other nerve volleys the EPSP depression was either absent or so small as to be negligible. In other experiments GS and Q (quadriceps) volleys have produced significant depressions. The most significant features of Table 1 are the EPSP depressions produced by PBST, P and DP volleys in all motoneurones. This has been the general rule; but much more systematic examination is needed before the full pattern of action is disclosed.

Correlation of EPSP depression with dorsal root reflexes

It will be sufficiently obvious that there is a very close parallelism between the investigations on the dorsal root reflexes (DRR) in muscle afferent fibres as reported in the preceding paper (Eccles *et al.* 1961) and

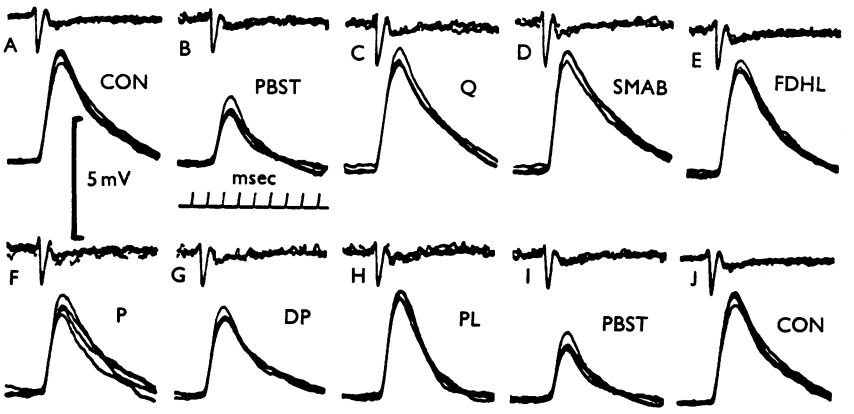

Fig. 10. Monosynaptic EPSPs during conditioning by repetitive stimulation of many different muscle afferents. The EPSPs were generated in a gastrocnemius motoneurone (membrane potential, −66 mV) by maximum Group Ia GS volleys at a repetition rate of 5/sec and each record is formed by the superposition of 3 or 4 traces. This repetitive GS stimulation was continued throughout the whole series. In B–I various muscle nerves, as indicated by the symbols, were stimulated at 300/sec and just-supramaximally for Group I. About 1 sec after the onset of this tetanization several EPSPs were recorded superimposed, the conditioning tetanus being then changed to some other nerve and the EPSPs again recorded. Thus B–I show the sequence of observations with the various conditioning tetani and A and J the control EPSPs before and after the series. Note that, as is shown by the records above the EPSPs, there was no sign of depression of the GS afferent volley as recorded from L7 dorsal root at its cord entry, negativity being downwards. Body temperature 37° C.

TABLE 1

	RP (mV)	Conditioning nerves: PBST	P	DP	SMAB	PL	FDHL	GS	Q
GS	−58	72	85	91	100	—	—	—	100
	−66	60	79	79	—	104	96	—	102
	−30	50	66	82	100	100	—	—	—
	−35	48	66	80	98	91	—	—	—
PL	−75	68	86	84	99	—	100	99	98
P	−59	42	—	68	96·	100	—	96	—
PBST	−77	—	83	84	98	101	—	98	—
Means		57	78	81	98·5	99	98	98	100

The numbers give the sizes of the monosynaptic EPSPs of the various motoneurones listed to the left when subjected to brief conditioning tetanization at 300/sec of the nerves shown above each column. The sizes are measured relative to the mean values of the control EPSPs recorded before and after each series of conditioning tetanizations. Many of the percentages are means of several determinations. Specimen records are shown in Fig. 10 for the EPSPs of the second GS motoneurone. Column labelled RP shows membrane potential.

the results reported here for EPSP depressions. For example, Group Ia and Ib afferent impulses conjointly contribute to both phenomena, and the fields of influence are parallel, e.g. PBST afferent volleys are very effective generators of DRRs into the afferent fibres of all muscles of that limb and also are very effective depressants of the EPSPs of their motoneurones. This parallelism is well illustrated when two effects of the testing gastrocnemius stimulus are recorded simultaneously (Fig. 11): the gastrocnemius afferent volley as it enters the spinal cord in S1 and L7 dorsal

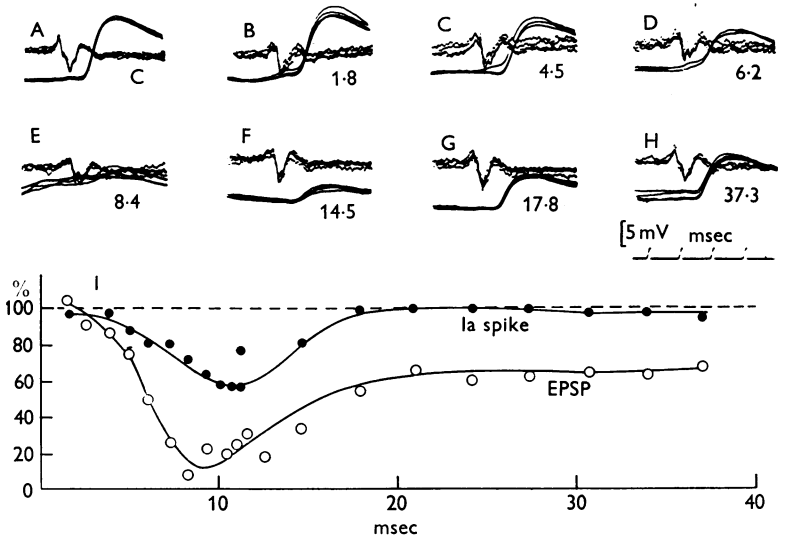

Fig. 11. Relation of EPSP depression to depression of testing afferent volley. In A–H monosynaptic EPSPs are recorded intracellularly in a gastrocnemius motoneurone and the gastrocnemius afferent volley is also recorded from L7 dorsal root at cord entry, negativity being recorded downwards. A is the control EPSP and in B–H it is preceded by a maximum Group I PBST volley at the intervals indicated in msec. Each record is formed by superposition of 3 or 4 faint traces at a repetitive frequency of 0·5/sec. The sizes of the EPSPs as percentages of the control are plotted as open circles in I. The sizes of the spike potentials produced by the gastrocnemius afferent volleys are measured between the initial positive and negative spike summits, are calculated as a percentage of the control and are plotted as filled circles in I. Body temperature 34·5° C.

roots; and the monosynaptic EPSP which it produces in a gastrocnemius motoneurone. Both were diminished by the conditioning PBST volley in Fig. 11D, E, but, as is shown in the plotted points of Fig. 11I, the afferent spike potential had fully recovered at 18 msec test interval, whereas the EPSP was depressed almost to 60% at the longest test interval. Possibly the very large EPSP depression in Fig. 1E at testing intervals up to 20 msec is in part due to diminution of the testing pre-

synaptic volley by the DRR, just as in Fig. 11I. When the stimulus to the GS nerve was weakened so that it virtually excited only Group Ia afferent fibres (Fig. 12A–E), the proportional depression of the gastrocnemius afferent volley was greater, as reported in the previous paper (Eccles *et al.* 1961), but it was still less than the EPSP depression. Evidently in Fig. 11I two factors were concerned in the depression of the EPSP at intervals up to 18 msec: diminution of the Ia afferent volley on account of block of incoming impulses by collision (or later refractoriness) with impulses discharged out along the Ia fibres, i.e. by the DDR; and depression of the

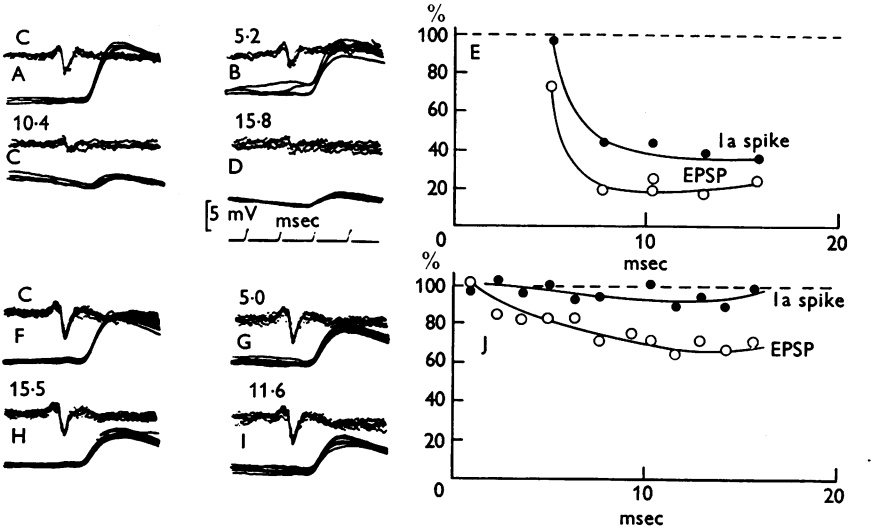

Fig. 12. Relation of EPSP depression to depression of the afferent volley. Continuation of the series of Fig. 11. The stimulus to the gastrocnemius nerve was diminished until it was submaximal for Group Ia, A being the control as in Fig. 11A, while B–D resemble Fig. 11B–H, being recorded at the indicated intervals after the same conditioning PBST volley, at a repetition rate of 0·5/sec. Measurements from the series partly illustrated in A–D are plotted in E with the same symbols as in Fig. 11I. F–I resemble Fig. 11A–H, F being the control and G–I at the test intervals indicated in msec, except that the frequency of repetition was raised to 5/sec, so as virtually to eliminate complications due to dorsal root reflexes (Eccles *et al.* 1961). Measurements from the series partly illustrated in F–I are plotted in J with the same conventions as in E. Further explanation in text.

EPSP produced by the remaining fraction of the Ia volley. This inference is supported by observing the effect of a tenfold increase in repetition rate, from 0·5/sec in Fig. 12A–E to 5/sec in Fig. 12F–J. At such frequencies the DRRs in muscle afferent fibres are virtually eliminated (Eccles *el al.* 1961), and correspondingly there was no more than a 5 % depression of the Ia spike potential of the testing afferent volley (Fig. 12G), yet the EPSP

was still depressed to below 70 %. The large EPSP depressions produced by prolonged high-frequency (200/sec or more) stimulation of the conditioning muscle afferent nerve (Figs. 6, 7, 8) certainly establish that the EPSP depression occurs independently of a peripheral block of the incoming testing afferent volley. Under such conditions there would be no DRRs after the initial discharge of about 20 msec duration (Eccles *el al.* 1961), so there would be no diminution of the testing afferent volley by collision with DRR discharges; yet the largest EPSP depressions occurred under such conditions.

DISCUSSION

There has been no systematic attempt to confirm the report by Frank & Fuortes (1957) and by Frank (1959 and personal communication) that the EPSP depression is not associated with any change in the post-synaptic membrane that is exhibiting the EPSP. However, it can be stated that depression of the monosynaptic EPSP of a motoneurone occurs in the absence of any change in the membrane potential, or in the excitability, of the motoneurone which has been tested both by antidromic invasion and by the threshold depolarization for generation of an impulse; furthermore, even when the EPSP was depressed to less than 25 % of its control size (Figs. 6, 8), there was no detectable change in its time course, and this also indicates the absence of any change in the post-synaptic membrane. On the other hand the EPSP depression is precisely correlated with depolarization of the presynaptic fibres. When sufficiently large this depolarization manifests itself by the generation of impulses in the presynaptic fibres, i.e. by the dorsal root reflex (DRR) described in the preceding paper (Eccles *et al.* 1961). However, it has been more directly displayed by intracellular recording from the primary afferent fibres and by their enhanced excitability as tested by brief current pulses (Eccles, Magni & Willis, unpublished observations). In every experimental test that has been applied there is excellent correlation between this presynaptic depolarization and the EPSP depression; hence it can be concluded that EPSP depression is a manifestation of a presynaptic mechanism of inhibition. It is appropriate therefore to designate this inhibitory phenomenon as 'presynaptic inhibition', which was the term originally used by Frank & Fuortes (1957).

It is postulated that presynaptic depolarization results in EPSP depression because it depresses the size of the presynaptic impulse and hence decreases the liberation of excitatory transmitter substance. A very steep relationship between size of presynaptic impulse and the EPSP was demonstrated by Hagiwara & Tasaki (1958) with the giant synapse of the squid stellate ganglion, where there was virtually complete suppression of the EPSP when a depolarizing current depressed the presynaptic

impulse to 80% of its normal size, while an increased presynaptic spike gave a great increase in the EPSP. More indirect evidence led Liley (1956) to postulate that at the rat neuromuscular junction the rate of quantal liberation of transmitter was increased tenfold for every 15 mV of presynaptic depolarization. On such a relationship a 5 mV diminution of the presynaptic spike would reduce the liberation of transmitter to 45%, which would account for all but the largest EPSP depressions reported above. Evidently it is of the greatest importance to investigate the effect of presynaptic depolarization on the size of the presynaptic spike and on the output of transmitter by that spike. There is much more evidence for the opposite action of presynaptic hyperpolarization in greatly increasing the output of transmitter: at the giant synapse of the squid stellate ganglion (Hagiwara & Tasaki, 1958); at the frog neuromuscular junction (del Castillo & Katz, 1954); and with monosynaptic EPSPs in the cat cord, where it provides at least part of the explanation of post-tetanic potentiation of monosynaptic reflexes and EPSPs (Lloyd, 1949; Eccles & Rall, 1951; Eccles & Krnjević, 1959).

It may be provisionally concluded that the EPSP depression is fully explained by the presynaptic depolarization together with the blockage that occurs during the outward passage of the impulses of the DRR. Of course such impulses in Group Ia fibres of the testing muscle nerve will themselves produce monosynaptic EPSPs that precede the EPSP produced by the remaining impulses of the testing afferent volley. Such an EPSP is seen in Fig. 11 D–F, where the testing EPSP was superimposed on an earlier EPSP in D–E and occurred during its decline in F, as also may be seen in Fig. 12C, D. Other examples are shown in the preceding paper (Eccles *et al.* 1961, Fig. 12B, C).

A full discussion of the production of presynaptic depolarization cannot be attempted until the more direct investigations have been reported. There have been many previous suggestions that inhibition is brought about by some presynaptic mechanism. In almost all cases it has been attributed to blockage of the presynaptic impulses by the flow of currents that exert either a hyperpolarizing or depolarizing action on the branching presynaptic fibres (Barron & Matthews, 1935, 1938; Renshaw, 1946; Brooks *et al.* 1948; Howland *et al.* 1955). In part this earlier experimental evidence for presynaptic blockage is explained by the block produced by DRRs as illustrated here (Figs. 11, 12; Eccles *et al.* 1961, Fig. 11) and previously suggested (Brooks *et al.* 1948). This latter investigation is of particular interest in relation to the present findings. Focal recording of presynaptic spikes and monosynaptic EPSPs showed that at a sufficiently long interval after the conditioning volley there was no block by DRR discharge, but there was a reduction in size of the presynaptic spike in

the motoneuronal nucleus and a corresponding reduction in the EPSP. This effect was attributed to presynaptic depolarization blocking the presynaptic impulses close to their synaptic terminals. It now seems likely that this was an example of a true presynaptic inhibition and that the presynaptic depolarization was displayed both by the diminished negative phase of the presynaptic spike and by the diminished EPSP. Other observations in that paper may now be interpreted as examples of EPSP depression that were due to presynaptic inhibition. The monosynaptic EPSPs exhibiting this depression were recorded either focally by an extracellular micro-electrode, or after electrotonic spread to the ventral root.

When produced by repetitive volleys presynaptic inhibition causes a remarkable depression in the sizes of testing EPSPs, as is impressively shown in Figs. 6 and 8. It would be expected that under such conditions there would be a deep prolonged inhibition of reflexes, and this has been observed. The long duration of the presynaptic inhibition produced by a single volley gives opportunity for summation of the asynchronous discharges from muscle receptor organs; hence it would be expected that through their presynaptic inhibitory action such discharges would be very effective in depressing reflexes.

SUMMARY

1. There has been detailed investigation of the finding of Frank and Fuortes that a muscle afferent volley depresses the size of the monosynaptic EPSP produced by another muscle afferent volley in a motoneurone that otherwise is unchanged in its responses.

2. EPSP depression occurs with a testing interval as brief as 5 msec; it reaches a maximum in 10–20 msec and slowly decays, having a total duration of 200 msec or more.

3. EPSP depression is produced by both Group Ia and Ib afferent impulses, and little if at all by Group II and III afferent impulses.

4. During prolonged repetitive stimulation of the conditioning nerve, the EPSP depression reaches a maximum after the first few impulses and there is subsequently a partial decline, though it may be well maintained for 60 sec. With increases in frequency up to 100/sec there is a commensurate increase in EPSP depression, but there is little further increase as the frequency rises above 200/sec. Following prolonged tetanization there is a post-tetanic increase in the EPSP depression produced by a test volley; it persists for as long as 60 sec.

5. Afferent volleys from knee and ankle flexors give potent depression of the monosynaptic EPSPs of all motoneurones, whereas volleys in extensor afferents are always much less effective.

6. There is good correlation between the conditions producing dorsal root reflexes (DRRs) in muscle afferent fibres and those giving the EPSP depression, but the latter is not due to blockage of the testing volley by centrifugal impulses of the former.

7. It is concluded that both the DRR and the EPSP depression occur on account of depolarization of presynaptic fibres in the spinal cord; hence EPSP depression and the resultant depression of reflexes is designated 'presynaptic inhibition'.

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