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PRESYNAPTIC CHANGES ASSOCIATED WITH POST-TETANIC POTENTIATION IN THE SPINAL CORD

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Lloyd (1949) has put forward the following hypothesis of the mechanism of post-tetanic potentiation (PTP) of the monosynaptic reflex in the spinal cord of cats. After a tetanus, the presynaptic nerve fibre undergoes a phase of hyperpolarization, associated with larger spike potentials which initiate synaptic transmission more effectively. How much experimental evidence is available in support of this hypothesis?

Gasser & Grundfest (1936) have analysed the post-tetanic hyperpolarization of mammalian peripheral nerves in some detail; they described a prolonged second phase (P_2) which could last 1 min or even longer. Woolsey & Larrabee (1940) observed a similar phase of hyperpolarization in the dorsal spinal roots of cats, whose occurrence was later confirmed by Lloyd (1952). Koketsu (1956) and Wall & Johnson (1958) also found in cats evidence of reduced excitability of primary afferent fibres within the cord after a tetanus. In a preceding paper (Eccles & Krnjević, 1959*b*) we have demonstrated in the cat's spinal cord a prolonged phase of membrane hyperpolarization in primary afferent fibres.

There is much less evidence that the spike potential is enhanced after a tetanus. Woronzow (1924) suggested that spikes are larger in frog nerves and, in a recent paper, Gasser (1958) mentions that augmented spikes were often seen in the cat phrenic nerve, but the increases were very small, usually only of 1% or less. Lloyd (1949) himself recorded the intramedullary spike potential evoked by afferent volleys. This triphasic spike was appreciably greater after a tetanus, and it gradually declined to its original size with a time course similar to that of the concurrent PTP of the monosynaptic reflex. Comparable changes in the presynaptic spike during PTP were seen by Liley & North (1953) at the neuromuscular junction in rats. Lloyd's observation was only

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partly confirmed by Eccles & Rall (1951) who found, in particular, no correlation between the intramedullary presynaptic spike and the phase of early PTP which reaches its peak within 200 msec of the end of a tetanus.

Changes in composite spike potentials recorded within volume conductors are notoriously difficult to interpret, especially when they are associated with a reduction in conduction velocity, as is always the case after a tetanus. In the present series of experiments, it was found possible to study with intracellular micro-electrodes changes in the membrane potential of afferent fibres inside the spinal cord over substantial periods of time (cf. Woodbury & Patton, 1952; Frank & Fuortes, 1955). This presented an opportunity to test Lloyd's hypothesis directly by recording the resting and spike potentials of fibres before and after a suitable tetanus.

As a further test, it was of interest to know whether hyperpolarization was in itself sufficient to enhance spike potentials, irrespective of the manner in which the hyperpolarization came about. It is well known that peripheral nerve spikes can be much increased by artificial anodic polarization (e.g. Bernstein, 1866; Gotch & Burch, 1898; Bishop & Erlanger, 1926; Graham, 1942), but this had not been shown under the conditions of our experiments, and not while recording the full membrane potential of mammalian fibres. Finally, an attempt was made to imitate PTP by polarizing afferent fibres artificially, in the hope that the enhanced spikes produced in this way might be equally effective in bettering synaptic transmission (cf. del Castillo & Katz, 1954; Hagiwara & Tasaki, 1958).

A brief report of the present results has already appeared (Eccles & Krnjević, 1959*a*).

METHODS

Experiments were performed on fifteen adult cats anaesthetized with pentobarbitone. The various techniques employed for intracellular recording, for stimulating afferent fibres and for polarizing spinal roots were similar to those already described in the previous paper (Eccles & Krnjević, 1959*b*).

In most cases post-tetanic changes in membrane resting and action potentials were studied as follows: when the micro-electrode entered an afferent fibre with a good resting and action potential, both of which remained reasonably stable, regular stimulation of the corresponding peripheral nerve was begun at a frequency of about 1/sec. After some 30 sec, the nerve was subjected to a standard tetanus at a frequency of 400/sec lasting 10 sec. Precautions were always taken to keep the strength of stimulation well above the threshold for the fibre, to ensure effective tetanization at the point of recording. The recorded potentials were displayed on an oscilloscope screen after d.c. amplification, and photographic records were obtained throughout the series at intervals of 2 sec. Each frame therefore shows usually two superimposed traces. After the tetanus, stimulation was continued at 1/sec for 1 or 2 min.

In a number of experiments a capacity neutralizing input stage was used to improve the frequency response of the recording system (cf. Eccles & Krnjević, 1959*b*). This made it possible to record spikes with a very much faster time course than usual.

RESULTS

Post-tetanic changes in resting and spike potentials of primary afferent fibres

In contrast to motoneurones, it is difficult to record resting and spike potentials from nerve fibres over an extended period, even for the 2–3 min required for a study of PTP. In the majority of cases resting potentials tended to diminish more or less rapidly after inserting the micro-electrode; as a rule the spike potential followed the resting potential fairly closely in its downward course.

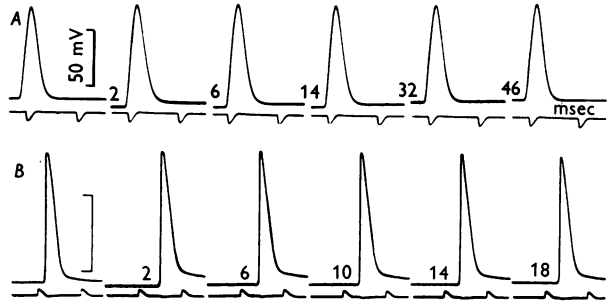

Fig. 1. Post-tetanic changes in resting and spike potentials recorded in low-threshold primary afferent fibres with micro-electrodes inserted into spinal cord of cat. In each series first record at extreme left is control obtained at 1/sec, 2–5 sec before stimulating fibre at 400/sec for 10 sec; other traces, also at 1/sec, followed end of tetanus at times indicated in seconds. Each record shows two traces superimposed; changes in resting potential can be seen with reference to fixed d.c. level of time trace. *A*: muscle afferent from flexor digitorum longus with conduction velocity of 90 m/sec, at a depth of 1·3 mm; initial resting potential −70 mV. *B*: cutaneous afferent from sural nerve with conduction velocity of 67 m/sec, at depth of 0·8 mm; initial resting potential −60 mV. Second series was recorded with improved frequency response provided by neutralized capacity input: some of the spikes have been retouched.

When the rate of fall was steady and not too rapid, post-tetanic changes could be seen superimposed on a sloping base line; such results gave useful information, although they are clearly not as satisfactory as those obtained under stable conditions. In many cases the fibre deteriorated so rapidly as to obscure any superimposed changes; these observations were rejected. It necessarily follows that our results are biased in favour of observations made on stable fibres; this need not be a disadvantage, since we were primarily interested in larger afferents from the skin and muscles, which are likely to remain more stable.

The kind of change which was seen is illustrated in Fig. 1. Two sets of traces are shown, *A* from a muscle afferent fibre (flexor digitorum longus) and *B* from a cutaneous afferent fibre (sural). The first trace in each sequence is a control recorded before the standard tetanus (400/sec for 10 sec). The other traces

were recorded at the various times indicated in seconds after the end of the tetanus. Since d.c. amplification was employed, changes of the base line in relation to the fixed time reference trace represent alterations in resting potential. It can be seen that a tetanus was followed by a phase of membrane hyperpolarization during which there was a pronounced increase in spike potential. In the best series the change in spike height was somewhat greater than the concurrent hyperpolarization, so that the summit of the spike was at an appreciably higher voltage than the normal (as in Fig. 1).

Certain other changes could also be detected: the spike evidently travelled more slowly and it had a slightly greater duration. All these changes were usually maximal within 10 sec of the end of a 10 sec tetanus, and then progressively diminished, so that, as a rule, the spike returned to normal within 1 min. The full time courses of the series of which samples are shown in Fig. 1*A* and *B* can be seen in Figs. 2 and 3, respectively. Other examples of typical observations are in Fig. 4, which shows changes in a fibre from a mixed nerve (posterior tibial (4*A*)) and in another cutaneous (superficial peroneal) afferent fibre (4*B*).

This type of definite change affecting both the resting and spike potential was observed on twenty-two occasions. The amount of change was highly variable, but it never exceeded 12% of the initial level: in most cases, the change was of the order of 5%. In a further number of experiments smaller changes in resting or action potential, or both, were seen, which were much less definite, often because of an unstable base line. Altogether, post-tetanic hyperpolarization was observed in thirty-one cases and an increase in spike height in twenty-three.

Results were considered to be negative only in those experiments in which no changes could be detected, even though recording conditions were sufficiently stable throughout the control and post-tetanic periods. By this criterion, there were only eight series with negative results.

A record was kept of the depth (measured from the dorsal surface of the cord) at which observations were made. Positive results were obtained at all depths at which we were able to record from afferent fibres (down to 1·5 mm); there was no obvious correlation between depth and magnitude of post-tetanic changes.

Repeated tetanus. In several cases conditions were so stable that it was possible to repeat the tetanus whilst recording from the same fibre. An example of such an experiment is shown in Fig. 5. About 90 sec after the first standard tetanus, a second tetanus was given to the nerve, again at a frequency of 400/sec, but lasting 30 sec instead of the usual 10 sec. There was a second, perfectly clear phase of PTP, although the amount of change was rather less than during the first trial, as was commonly the case. The rate of decay after a 30 sec tetanus was slower than after a 10 sec tetanus, in

agreement with the time course of PTP observed on monosynaptic reflexes (Lloyd, 1949).

Other post-tetanic changes. Certain slow potential changes which are associated with activity can be recorded from afferent fibres with intracellular electrodes. An after-depolarization follows the spike in all fibres, and a very slow depolarization is conducted passively in cutaneous fibres from the region

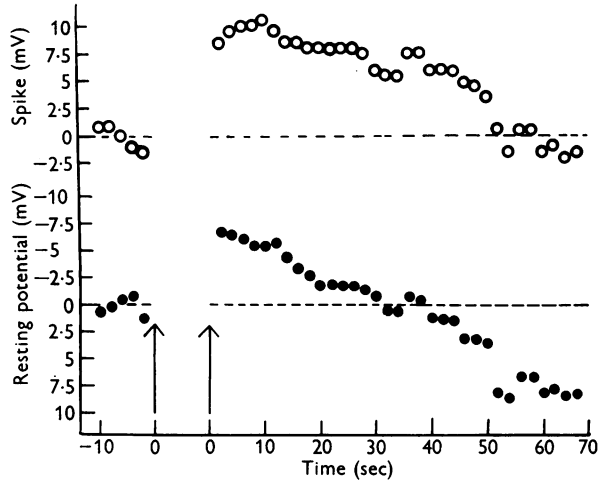

Fig. 2. Complete sequence of post-tetanic changes in resting and spike potentials in muscle afferent fibre, already partly illustrated in Fig. 1*A*; two arrows indicate 10 sec tetanus at 400/sec.

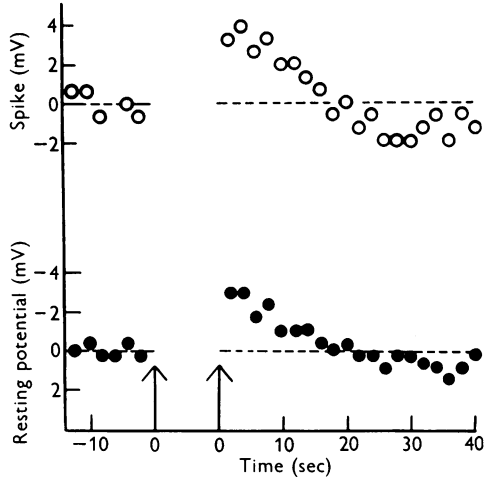

Fig. 3. Complete sequence of post-tetanic changes in resting and spike potentials in cutaneous afferent fibre already partly illustrated in Fig. 1*B*; two arrows indicate 10 sec tetanus at 400/sec.

of their terminals. These slow potential changes, but especially the after-depolarization, are substantially enhanced after tetanus, and gradually return to normal with a time course comparable with that of the PTP of the spike. These changes have already been fully described (Eccles & Krnjević, 1959*b*).

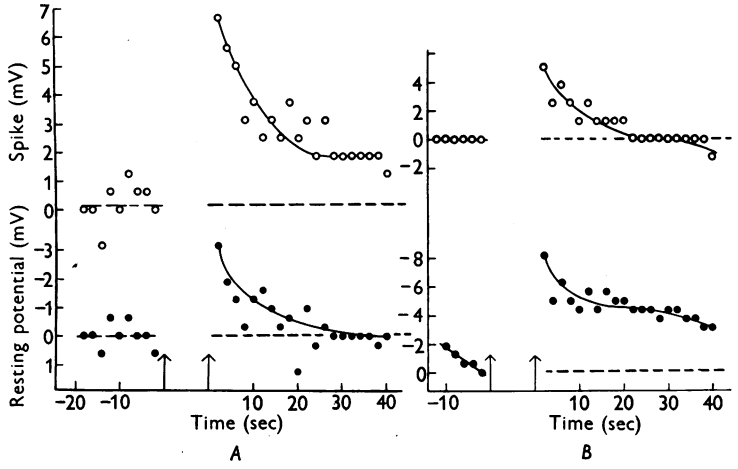

Fig. 4. Typical post-tetanic changes recorded in two other primary afferent fibres with micro-electrodes inserted into spinal cord; arrows indicate 10 sec tetanus at 400/sec. *A*: afferent fibre from mixed nerve (posterior tibial) with conduction velocity of 66 m/sec, at depth of 1·2 mm; initial resting potential −55 mV and spike height 65 mV. *B*: cutaneous afferent fibre (from superficial peroneal nerve) with conduction velocity of 71 m/sec at depth of 1·3 mm; initial resting potential −60 mV and spike height 50 mV.

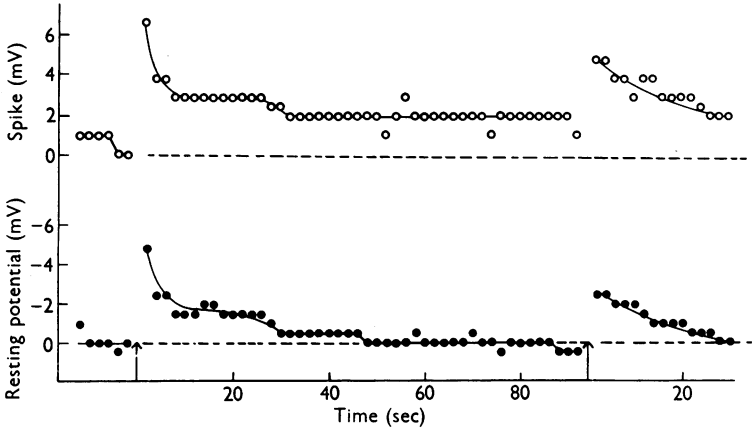

Fig. 5. Repeated post-tetanic changes recorded in same afferent fibre from hamstring nerve with conduction velocity of 100 m/sec, at depth of 1·2 mm inside spinal cord; initial resting potential −70 mV and spike height 75 mV. Note that first arrow indicates 10 sec tetanus at 400/sec and second arrow 30 sec tetanus at 400/sec.

Dorsal root reflex

A dorsal root antidromic reflex discharge was not infrequently observed in fibres from cutaneous or mixed nerves (cf. Toennies, 1938; Frank, 1953; Koketsu, 1956; Wall, 1959). As a rule there was only a single reflex spike, several milliseconds after the orthodromic spike (Fig. 6), even when very strong stimuli were used. In some cases, however, the reflex response became repetitive as the stimulus intensity was raised, but the number of reflex spikes was never greater than 3, as found by Frank (1953), but in disagreement with Koketsu (1956) and Wall (1959). On several occasions a repetitive discharge with a train of five or more impulses was recorded from interneurones during

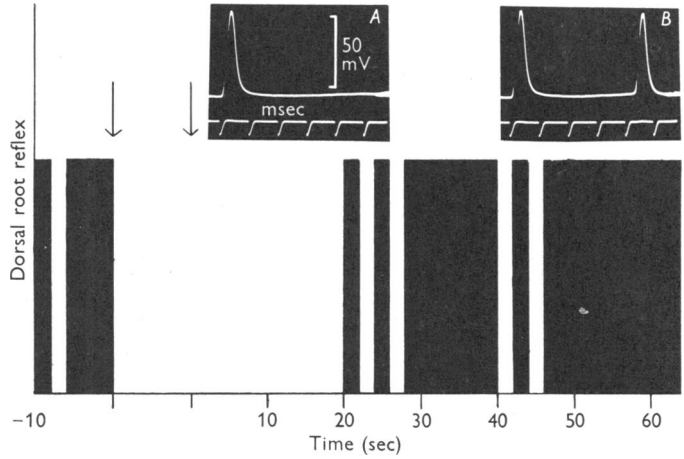

Fig. 6. Antidromic dorsal root reflex recorded in afferent fibre of a mixed nerve (posterior tibial) at depth of 0·6 mm, before and after 10 sec tetanus at 400/sec (shown by arrows). Fibre conduction velocity was 67 m/sec and initial resting potential − 65 mV. Rate of stimulation was 1/2 sec, and records showing no reflex response are indicated by gaps (except during tetanus). Samples of records *A* without, and *B* with, reflex response are included; note that this consisted of only one additional spike.

stimulation at a high intensity; such a discharge is in other respects similar to a dorsal root reflex (cf. Wall, 1959), but it can be distinguished if, for example, two stimuli are given in close succession, revealing the prolonged blocking effect of the antidromic dorsal root reflex. We confirmed previous observations that a reflex can be evoked with a stimulus which is subthreshold for that particular fibre (e.g. Frank, 1953; Wall, 1959).

A reflex discharge was only observed in cutaneous or mixed nerve afferent fibres, never in a fibre from a pure muscle nerve: but on some occasions it could be elicited in a cutaneous afferent fibre by volleys in a muscle nerve. According to Koketsu (1956) the reflex response is depressed temporarily after a tetanus. In some of our experiments we examined the effect of a

tetanus on the incidence of reflex spikes. In nine out of ten trials the reflex discharge either vanished completely or became substantially less frequent for a period of some 30 sec (Fig. 6); only in one case did it become apparently more frequent. After this period of depression there was some suggestion of a rebound, so that the reflex discharge seemed to occur more regularly than before the tetanus.

Effects of artificial polarization on membrane potentials

Very large alterations in resting and action potentials of fibres were very easily produced by passing currents between two non-polarizable electrodes, one on the cut dorsal root distally, and the other either on the proximal part of the root, or touching the surface of the cord close to the point of attachment of the root (cf. Eccles & Krnjević, 1959*b*). The intensity of current was varied so that a sequence of changes was observed, up to the point where excessive current caused a block of conduction (cf. Fig. 7). Observations were made on fibres at depths down to about 1·5 mm.

A characteristic sequence of changes recorded at a depth of 1·2 mm is shown in Fig. 7. (When describing the effect of the polarizing current, the sign of the impressed polarization refers of course to that at the proximal electrode.) Increasing anodic polarization (Fig. 7*A*) caused a progressive increase in resting potential, indicated by lowering of the base line in relation to the fixed d.c. level of the time reference trace. There was a corresponding increase in spike amplitude, which did more than match the change in resting potential, so that the peak of the spike tended to a higher absolute potential than before; the conduction velocity was at the same time much reduced. The spike height reached its maximum with a polarizing current of 15 μA; further increase to 20 μA raised the resting potential, and particularly the conduction time, even further, but the traces now showed intermittent block of conduction.

Changes in the opposite direction were induced by cathodic polarization (Fig. 7*B*). The membrane was progressively depolarized and spikes diminished, but the spike latency did not decrease appreciably, in fact, the latency became substantially longer with stronger currents. The reduction in spike height was exaggerated by the onset of partial nodal block, which became complete with a current of 25 μA. Post-cathodic or post-anodic overshoot was usually observed immediately after stopping the polarizing current, as can be seen in the last records of Fig. 7*A* and *B*.

Such pronounced effects of polarizing currents were observed in thirty-one trials in four cats. The amount of change in spike height was usually between 10 and 20 % of the initial height, but greater changes, up to 40 % of the initial height, were observed on several occasions, even at a depth of 1·5 mm. For a given intensity of current anodic effects were consistently greater than catho-

dic. These observations are in close agreement with the results of previous experiments described in the literature (e.g. Graham, 1942; Schoepfle, 1959).

Effects of artificial polarization on monosynaptic transmission. An attempt was made to change excitatory post-synaptic potentials (EPSPs) recorded in motoneurones during stimulation of dorsal roots, by polarizing the relevant

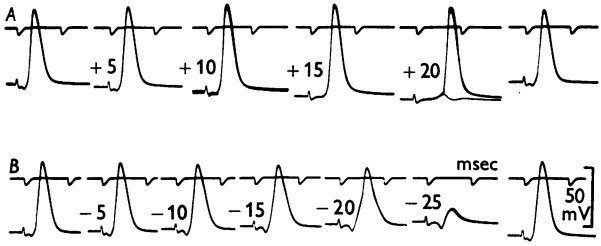

Fig. 7

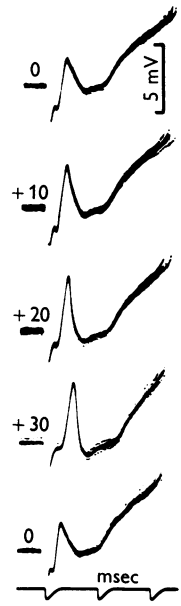

Fig. 8

Fig. 7. Effect of artificial polarization of dorsal root on resting potential and spike of primary afferent fibre at depth of 1·2 mm inside spinal cord. In *A*, proximal polarizing electrode was anode; in *B*, cathode. Strength of current is indicated in μA and the first and last trace in each series is a control recorded immediately before and after polarizing. Initial resting potential was −70 mV; each record consists of several traces superimposed.

Fig. 8. Effect of anodic polarization of dorsal root on monosynaptic EPSPs recorded inside motoneurone. Each record shows from left to right, stimulus artifact, spike indicating arrival of afferent volley, then rising phase of EPSPs leading to beginning of spikes at top right-hand corner; about ten traces are superimposed in each record. Top and bottom records are controls, others show EPSPs during polarization with currents of increasing strength (indicated in μA); note progressive change in afferent spike, and greater latency and steeper slope of EPSP.

afferent fibres at or close to the point of entry into the spinal cord. Although we were primarily interested in the monosynaptic EPSPs it was necessary to use quite strong afferent stimuli in order to mask changes in threshold produced by the polarizing current at the site of stimulation on the cut dorsal root. The monosynaptic component of the EPSPs was therefore best studied by examining the slope of the initial rising phase. In most cases, the maximal

effects of polarizing currents were compared with the changes seen during PTP, after a standard 10 sec afferent tetanus at 400/sec.

Experiments were performed on twenty-nine motoneurones in four cats. On the whole, it was not possible to produce consistent changes in EPSPs, except a depression as a result of a block of conduction in the dorsal roots caused by excessive polarization, either anodic or cathodic. Only in seven cases was there any reason to believe that anodic polarization of the dorsal roots might enhance the EPSPs, and the changes in every case but one were so small as to be no more than suggestive. In the one case, anodic polarization had a quite definite and reversible potentiating effect, which could be repeated. This effect is illustrated in Fig. 8, where the slope of the rising phase of the EPSPs (at the right of each trace) becomes steeper, and a spike is initiated earlier, as the strength of the anodic polarizing current is raised to 30 μA. The potentiation is seen most clearly when the EPSPs are superimposed (allowing for the change in latency), but it is quite obvious if the third trace is compared with the first and the last, which are control records.

DISCUSSION

Post-tetanic changes in resting and action potentials were not seen in every trial but this was usually because conditions for recording were not sufficiently stable. Positive results were obtained so often as to suggest that here was a regular phenomenon which might always be observed under ideal conditions. It was seen equally clearly in cutaneous and in muscular afferent fibres, and could be elicited more than once in the same fibre.

An association between post-tetanic hyperpolarization and increase in spike height has thus been confirmed. This kind of change had been searched for in peripheral nerves but with only meagre results (Gasser, 1958). The greater increase in spike height observed in our experiments may represent a difference in the properties of nerve fibres near their terminals. Previous evidence (Woolsey & Larrabee, 1940; Lloyd, 1952; Rudin & Eisenman, 1954; Wall, 1958) suggests that post-tetanic hyperpolarization is more pronounced in the terminal region of the afferent fibres, inside the spinal cord, than elsewhere along their course. The presence of this gradient in the properties of the fibres is significant, as it might be objected that our observations were probably not made in the immediate vicinity of the fibre terminals and were therefore irrelevant from the point of view of synaptic transmission. All the evidence suggests that even greater effects would have been observed at the terminals. It should be added that even though intracellular recording from the actual terminals is very unlikely to have occurred, many of our observations were made well within 1 mm of the terminals of cutaneous afferents in the dorsal horn, and within 2–3 mm of the terminals of muscle afferents in the ventro-

lateral horn. Other observations confirm our belief that post-tetanic hyperpolarization also occurred at or very near the terminals; e.g. the clear PTP of the dorsal root potential conducted passively from the terminal region (Eccles & Krnjević, 1959*b*) and the post-tetanic depression of the dorsal root reflex discharge (cf. Koketsu, 1956), which also probably originates at a point close to the terminals.

Relation between hyperpolarization and spike height

The similarity between the time courses of the changes in spike height and resting potential indicate a simple relation between them. In support of this there was also usually good agreement with respect to the amount of change, although in the best series the increase in spike height was slightly more than was needed to compensate for the higher resting potential. Further support is given by the ease with which the spike height could be raised when fibres were hyperpolarized artificially. These various observations suggest that the post-tetanic hyperpolarization is the basic phenomenon and that most other changes are mainly of a secondary nature: e.g. the reduced excitability (Wall & Johnson, 1958); the PTP of the after-depolarization and of the dorsal root potential (Eccles & Krnjević, 1959*b*); the depression of the antidromic dorsal root reflex discharge, and, of course, the augmented spikes.

Connexion between presynaptic potential changes and PTP

There seems little doubt that a prolonged tetanus is followed by a period during which afferent spikes are augmented, as was suggested by Lloyd (1949), and that the rate at which spikes return to their normal amplitude is often comparable with the time course of the PTP of the post-synaptic potential observed in motoneurones (cf. Eccles, Krnjević & Miledi, 1959). Post-synaptic PTP actually lasts somewhat longer than the observed phase of augmented presynaptic spikes; this may be a real difference, but may perhaps be an experimental artifact caused by the very much greater difficulty of recording stable potentials from the afferent fibres for any length of time.

One can postulate several ways in which post-tetanic increase in presynaptic spike amplitude might improve synaptic transmission:

1. On an electrical hypothesis, the effectiveness of transmission is clearly dependent upon the magnitude of the presynaptic current.

2. All the numerous terminal filaments of a single afferent fibre may not normally be invaded by an impulse. After a tetanus even a small change in spike height may improve the safety factor for conduction at a significant number of bifurcations to cause invasion of many more terminals (cf. Adrian & Lucas, 1912; Brown & Harvey, 1938; Frank, 1950).

3. A larger spike may release the chemical synaptic transmitter in greater amounts. Del Castillo & Katz (1954) and Liley (1956*b*) found that the spontaneous release of transmitter at the neuromuscular junction can be altered by polarizing the nerve. According to Liley (1956*b*) there is a simple but very steep logarithmic relation between the potential of the nerve terminals and the rate of release: if such a mechanism obtained in the spinal cord, quite a small change in spike height might well be enough to produce the observed increase in EPSP (about 70%, Eccles *et al.* 1959). However, the magnitude of this effect will depend very much upon whether it is determined by the change in absolute voltage at the summit of the spike, as suggested by Liley, or by the change in spike amplitude as measured from the hyperpolarized base line.

Existing evidence tends to support the last hypothesis. Hagiwara & Tasaki (1958) have been able to study directly the relation between the size of the presynaptic spike and post-synaptic potentials at the squid giant synapse, which has no presynaptic branching. They found that small presynaptic changes had a very large post-synaptic effect, and concluded that their observations were most consistent with a chemical mechanism of transmission. It seems that, in so far as PTP can be ascribed to a temporary increase in the presynaptic spike, the process is probably of the type described in the third hypothesis. Our observations do not allow us to decide what part, if any, is played by various other factors held to be responsible for PTP, such as an alteration in ionic balance, swelling of the synaptic boutons, or greater mobilization of the transmitter stored in the terminals (cf. Liley & North, 1953; Liley, 1956*a*).

The inconclusive results of our attempts to change monosynaptic EPSPs in motoneurones by polarizing afferent fibres are in keeping with the negative results previously obtained in comparable experiments on the spinal cord (Frank, 1950). They are probably a reflexion of the difficulty of producing adequate electrotonic changes in membrane potential at the presynaptic nerve endings on the motoneurones (cf. Wall, 1958), caused by the relatively great distance between the polarizing electrodes and the endings, the profuse branching of the afferent fibres, and a high rate of spatial decrement along the fine terminal branches. At the neuromuscular junction the end-plate potential can be altered only if the motor nerve is polarized close to the nerve endings, and even then only in a certain proportion of cases (del Castillo & Katz, 1954).

SUMMARY

1. Intracellular recording from low-threshold primary afferent fibres inside the spinal cord of cats has shown that a tetanus (400/sec for 10 sec) is usually followed by a rise in membrane resting potential and larger spikes.

2. The magnitude of the changes in membrane potentials is commonly of

the order of 5–10%; when recording conditions are optimal, the increase in spike height clearly exceeds the amount of hyperpolarization.

3. Such effects of a tetanus persist for periods of up to 1 min; they are repeatable, and they can be seen as often in afferent fibres from muscle as from the skin.

4. Similar but even more pronounced changes can be produced in afferent fibres by anodic polarization of the dorsal roots; however, attempts to alter post-synaptic potentials, recorded in motoneurones, by polarizing presynaptic fibres in the dorsal roots have not given very conclusive results, probably because the effects of dorsal root polarization do not extend as far as the presynaptic terminals.

5. These observations are consistent with the hypothesis that larger presynaptic spikes are at least partly responsible for post-tetanic potentiation of synaptic transmission.

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