J. Physiol. (1953) 121, 374-389

THE EFFECT OF INHIBITORY NERVE IMPULSES ON A CRUSTACEAN MUSCLE FIBRE

By P. FATT and B. KATZ

From the Biophysics Department, University College, London

(*Received* 28 *January* 1953)

Synaptic inhibition has recently been studied by Brock, Coombs & Eccles (1952), who found that an inhibitory nerve impulse increases the resting potential of a motor nerve cell, thereby raising its threshold to excitatory impulses. The authors concluded that this was the sole mechanism by which direct inhibition of spinal motoneurones is produced, and it is of interest to inquire to what extent this conclusion may be generalized. A very different preparation for a study of direct synaptic inhibition is the crustacean nerve-muscle system (Biedermann, 1887; Hoffmann, 1914; Marmont & Wiersma, 1938; Kuffler & Katz, 1946) which enables one to stimulate single inhibitor and motor nerve axons and record their effects on individual muscle fibres (see Fatt & Katz, 1953*a*). Previous work indicated that the inhibitory process interferes with the crustacean muscle response at two stages: (*a*) in blocking transmission from the nerve to the muscle membrane; and (*b*) in uncoupling excitation and contraction processes within the muscle fibre. Only the first of these two actions is considered in this paper, which deals particularly with the electrical membrane changes set up in a crustacean muscle fibre by inhibitory nerve impulses.

It will be shown that the main effect of inhibitory impulses is to *attenuate the 'end-plate potentials'*, i.e. to diminish the local depolarization produced by motor impulses. Inhibitory impulses do not by themselves change the resting potential of the muscle fibres, unless this has previously been displaced from its normal level. But even though no potential change may be recorded, the inhibitory impulse was found to have a peculiar effect on the electrical properties of the muscle membrane: it always produces a transient increase of membrane conductance (or 'ion permeability') whose nature will be discussed below.

METHODS

The two nerve-muscle preparations used for most experiments were: the opener of the claw of the hermit crab (*Eupagurus bernhardus*) and the flexor of the dactylopodite of the shore crab (*Carcinus maenas*). The former muscle is supplied by two axons, one motor and one inhibitor, which run in

separate nerve bundles (see Wiersma & Harreveld, 1935) and thus can be stimulated separately without requiring isolation. In *Carcinus*, the flexor muscle is supplied by two motor axons which provide a double innervation for the muscle fibres (cf. Fatt & Katz, 1953*b*) and one inhibitor axon. The motor axons are easily found because they are the two outstanding fibres in the thicker nerve bundle and can be isolated without difficulty. Muscle fibres were exposed by carefully removing the part of the shell which covered them.

The preparations were mounted on glass slides under rubber bands; a rubber tube was slipped over the opened tip of the dactylopodite (*Carcinus*) or propodite (*Eupagurus*) and connected to a perfusion bottle. The preparation was then immersed in crab Ringer (Fatt & Katz, 1953*a*) in a large Petri dish which also contained the 'indifferent' stimulating and recording electrodes.

The motor and inhibitor axons were stimulated separately, without lifting them from the saline bath, by applying capillary electrodes close to their surface (cf. Fatt & Katz, 1953*b*). This was a convenient procedure, but sometimes gave rise to rather large shock artifacts (e.g. Figs. 1 and 2). Two pulse stimulators were used to provide excitor (*E*) and inhibitor (*I*) shocks of adjustable intervals and independently variable intensities.

The electric responses of crustacean muscle increase in amplitude with the frequency of nerve impulses (Katz, 1936; Wiersma, 1941; Katz & Kuffler, 1946), and the response produced by a single impulse is generally very small. It was necessary, therefore, to use repetitive stimulation: the frequency was kept at about 30 per sec, adjusted so as to give moderately large potential changes without vigorous mechanical responses. The records shown below were all due to multiple cathode-ray sweeps whose frequency was the same as, or one half of (Fig. 6), that of the stimuli.

Two intracellular electrodes were used, one to record membrane potentials of individual muscle fibres and the other, when required, to pass current through the fibre membrane. The procedures which were followed in recording membrane potentials, displacing the resting potential and measuring the fibre constants have been described in detail in previous papers (Fatt & Katz, 1951, 1953*a*).

RESULTS

The effect of inhibitory impulses on the 'end-plate potential'

When the motor nerve to a crustacean muscle is stimulated at a frequency of about 30 per sec, non-propagated action potentials are set up in the muscle fibres accompanied usually by a weak contraction (cf. Katz & Kuffler, 1946). The action potentials are in many ways analogous to the end-plate potentials (e.p.p.) of vertebrate muscle (Wiersma, 1941; Katz, 1949), and this term has been used to describe them in spite of morphological differences between crustacean and vertebrate nerve-muscle junctions. In Fig. 1, *E*, e.p.p.'s are shown obtained with an intracellular electrode from the opener of the claw of a hermit crab. The record is due to repetitive responses during stimulation at 33 per sec. The size of the e.p.p. varies in different muscles and different fibres, and depends upon the frequency and number of nerve impulses (see Katz & Kuffler, 1946). The time course of the e.p.p. was also found to vary widely: in some fibres the e.p.p. decayed with a time constant of 2–3 msec, in others 10 or 20 times more slowly. This large difference might be due either to a variable persistence of the nerve-muscle transmitter, or to a variable time constant of the muscle membrane. We observed that the longest e.p.p. (decay constant approximately 65 msec) occurred in a fibre of exceptionally large

membrane time constant (53 msec) determined by the rectangular pulse technique (Hodgkin & Rushton, 1946; Fatt & Katz, 1951). In a few other experiments in which both time constants were measured, a similarly good agreement was obtained, and it appears therefore that in these experiments the rate of fall of the e.p.p. is controlled by the resistance and capacity of the muscle fibre, rather like that of the e.p.p. in curarized vertebrate muscle.

There are, however, certain exceptions: for example, in some fibres of the flexor muscle of the dactylopodite (*Carcinus maenas*) e.p.p.'s of different rates of decay were produced by stimulating different motor axons (Fatt & Katz, 1953*b*) an effect which cannot be explained on the present simple hypothesis.

Usually, the decline of the e.p.p. in a crustacean muscle fibre, though not strictly exponential, approaches the exponential time course much more closely than does that of the 'focal' e.p.p. in a frog muscle fibre (Fatt & Katz, 1951). The analysis is thus simplified considerably; the reason for the observed exponential decay is almost certainly to be found in the much more uniform spatial spread, along the fibres, of the crustacean e.p.p. (Fatt & Katz, 1953*b*).

Another peculiarity was the large amplitude fluctuation of the response with successive impulses (e.g. Fig. 1, *E*). It could not be attributed to mechanical movement artifacts, for it was observed even when no muscle contraction was seen. It might be due to an intermittent failure of nerve impulses to conduct into all the terminal axon branches, or alternatively to some random event affecting each nerve terminal individually.

When both inhibitor (*I*) and motor (*E*) axons were stimulated, the e.p.p. was reduced in size as previously reported by Marmont & Wiersma (1938) and Kuffler & Katz (1946). The attenuation of the e.p.p. depended upon the time interval between *I* and *E* shocks; it was greatest when *I* slightly preceded *E*. The maximum reduction of the e.p.p. varied considerably; in some fibres it amounted to as much as 90%. Typical results are shown in Figs. 1, 2 and 4. When measuring the e.p.p. size, the 'phasic' amplitude of the responses was used (see Fig. 3), not the total deflexion which is complicated by summation of successive potentials. An 'inhibited' e.p.p. differed from the normal not only in amplitude, but also in time course, its rate of decay being faster. Thus, in the example of Figs. 1 and 2 the decay constant (fall to $1/e$) of the normal e.p.p. was 27 msec, that of the maximally 'inhibited' e.p.p. was 10 msec. When an inhibitory impulse arrived during the falling phase of an e.p.p. it caused the rate of decline of the e.p.p. to increase abruptly, by at least 50% (e.g. Fig. 4, record 1*a*).

Inhibited e.p.p.'s showed even more pronounced amplitude fluctuations than normal e.p.p.'s (cf. Fig. 5). It seems that fluctuations occurring simultaneously at excitatory and inhibitory nerve endings have additive effects; moreover, fluctuations in the time of arrival of *I* and *E* impulses would also accentuate the phenomenon.

In Fig. 5 'inhibition curves' are plotted, showing the attenuation of the e.p.p. at various $I-E$ intervals. The results are similar to those already

described by Marmont & Wiersma (1938) and Kuffler & Katz (1946). The curve is of interest because it provides an indication of the time course of inhibitory activity. When the inhibitor impulse arrives at a nerve terminal, it presumably releases some agent which has the power of locally suppressing the subsequent e.p.p.'s. This power gradually disappears so that less and less attenuation is produced as the excitor impulse arrives progressively later. The gradual return of the 'inhibitor curve' from maximum to no attenuation

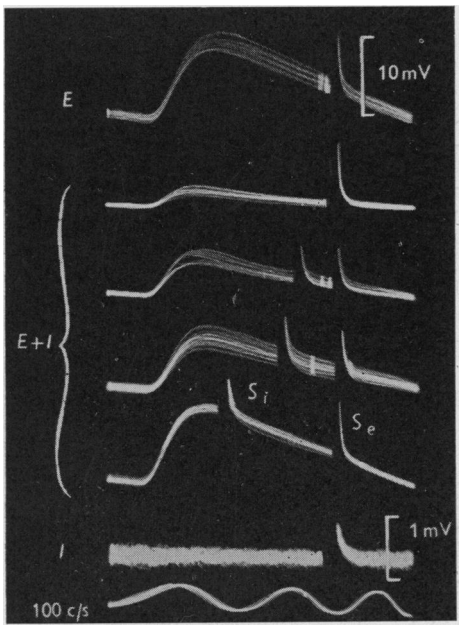

Fig. 1.

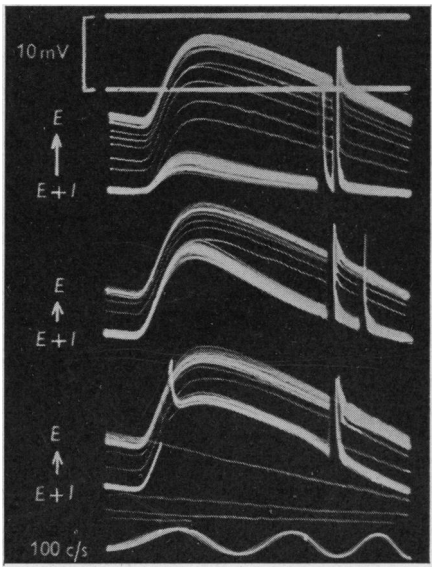

Fig 2.

Fig. 1. Effect of inhibitory impulse on crustacean 'end-plate potential'. Intracellular recording. Muscle fibre of opener of claw of hermit crab. Stimulation at 33/sec. Each record is a photograph of multiple sweeps, the stimulus artifacts (S_e and S_i) appearing on the falling phases of preceding responses. E (low amplification): stimulation of excitor axon, producing e.p.p. I (at higher amplification): stimulation of inhibitor axon alone, producing no potential change. $E+I$ (low amplification): combined stimulation of both axons, producing attenuated e.p.p. E shock was fixed (right hand artifact), the I shock preceding by varying intervals (successively from above: 1·2, 4·2, 6·1 and 11·3 msec).

Fig. 2. Effect of inhibitory impulse on crustacean 'e.p.p.' Repetitive time-base sweeps and stimulation, at 33 per sec, as in Fig. 1. Three recordings at different $I-E$ intervals, showing the response to combined ($E+I$) stimulation and the transition to a pure E response, when inhibitory impulses are stopped. Note the difference between the 'inhibited' and the first 'uninhibited' e.p.p. in each recording. Time interval between I and E shocks: top records, I precedes E by 1·4 msec; middle, I follows E after 3·8 msec; bottom, I half-way between E shocks (15 msec interval). The decline of the last e.p.p. towards the base-line is also shown in this recording.

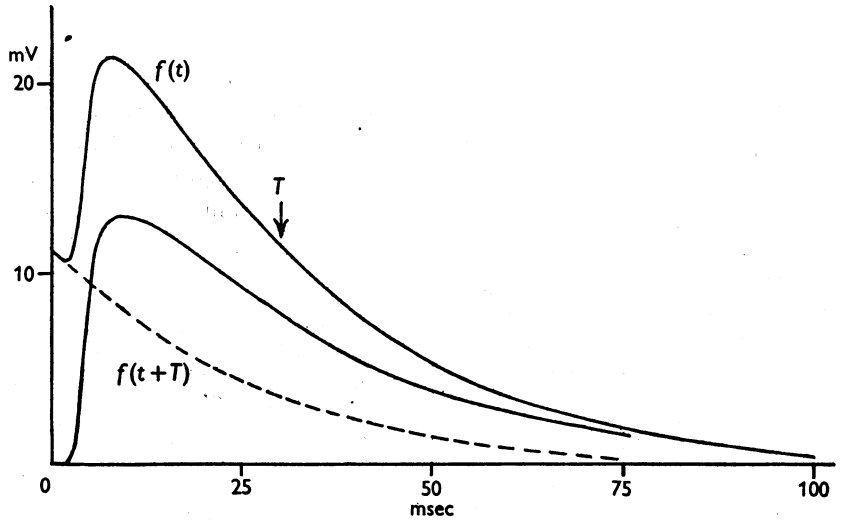

Fig. 3. Procedure used in measuring 'phasic' amplitude of e.p.p. during repetitive stimulation. The top record $f(t)$ shows the last of a series of e.p.p.'s obtained during steady stimulation at 33 per sec. The broken curve $f(t+T)$ shows the remainder of the potential after time T, T being the interval between stimuli. Assuming that the recorded potential is due to algebraic summation of successive e.p.p.'s, the phasic component is given by the difference between $f(t)$ and $f(t+T)$.

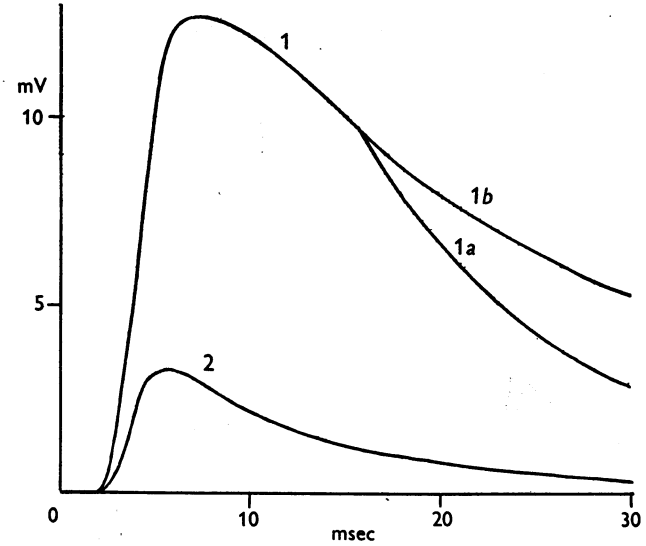

Fig. 4. Superimposed drawings of 'phasic' e.p.p.'s, from the experiment illustrated in Figs. 1 and 2. (These curves are single examples; to appreciate the random fluctuations of successive e.p.p.'s, Figs. 1 and 2 should be consulted.) 1: I shock follows 15 msec after E and accelerates the decay of the e.p.p.; 1a, during steady $E+I$ stimulation; 1b, first response after cessation of inhibitor impulses. 2: I precedes E by 1·6 msec, attenuating the amplitude of the e.p.p.

indicates, therefore, the time course of subsidence of the inhibitory transmitter potency. The initial rise of the inhibitor action cannot be deduced from Fig. 5; it may well be faster than the initial falling phase of the 'inhibition

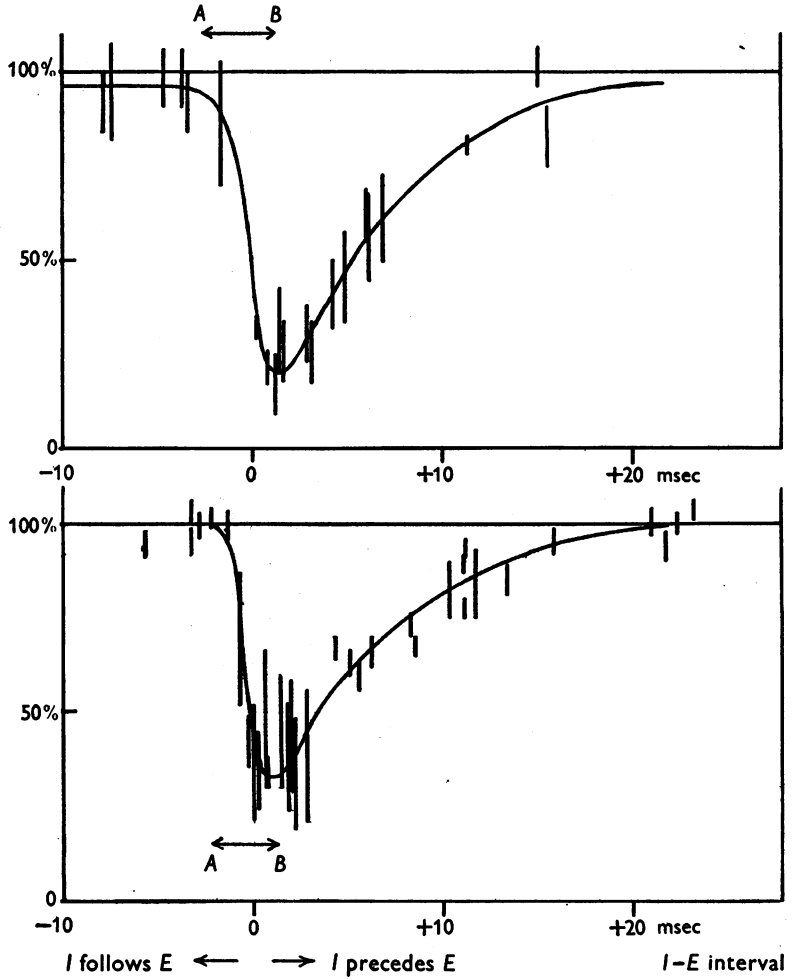

Fig. 5. 'Inhibition curves.' Results of two experiments showing dependence of e.p.p. amplitude on time interval between *I* and *E* impulses. Abscissa: interval between inhibitor and excitor shocks, in msec. Ordinate: 'phasic' amplitude of e.p.p. as a percentage of the normal. The vertical bars indicate the range of scatter observed in the recordings (cf. Figs. 1 and 2). Both experiments from opener of claw of hermit crab.

curve' $(A-B)$. When the *I* impulse arrives *after the beginning of the e.p.p.* it can only stop its further ascent; hence, the initial portion $(A-B)$ of the 'inhibition curve' has no special significance, except that it cannot be shorter than the rising phase of the e.p.p.

In considering the interaction of excitor and inhibitor impulses we have encountered, so far, three events of different time course, (1) the excitatory transmitter process ($E-$action) which gives rise to the e.p.p.: it is probably a brief event little longer than the rising phase of the e.p.p. (in the example of Fig. 3, about 5–10 msec); (2) the e.p.p. itself whose falling phase appears to be governed by the membrane time constant; (3) the I action which is the counterpart to the excitatory transmitter action, but has a longer duration (in the experiments of Fig. 5, judging from the length of the 'inhibition curves', about 20 msec).

The action of inhibitory impulses on the resting muscle membrane

The attenuation of the e.p.p. by an inhibitor nerve impulse could be explained by a competition between two antagonistic transmitter substances, reacting with a single receptor substance. The effect has previously been compared (see Kuffler & Katz, 1946) with the 'competitive' block which curare is believed to produce at the vertebrate motor end-plate, attenuating

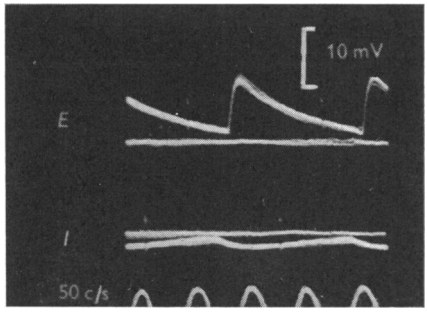

Fig. 6. Example of an increase of resting potential produced by inhibitor impulses. Flexor of dactylopodite, *Carcinus maenas*. E: e.p.p. due to stimulation of motor axons. I: electric response due to stimulation of inhibitor axon.

the depolarization due to acetylcholine without by itself changing the membrane potential. However, the evidence that the inhibitory impulse produces by itself no electrical change in a crustacean muscle fibre is not unequivocal. Biedermann (1887) claimed that inhibitory nerve impulses increase the resting potential of the muscle, while other authors (Wiersma & Harreveld, 1935; Kuffler & Katz, 1946) found no such effect.

In our own experiments, we did observe at times that stimulation of the inhibitory axon produced an increase of the resting membrane potential, but the effect was small and irregular, being only seen in relatively few fibres. An example is shown in Fig. 6 where a small *inverted* e.p.p. (henceforth called 'I-potential') can be seen which is of slower time course than the 'motor e.p.p.'.

The presence or absence of this *I*-potential had no relation to the reduction of the e.p.p. which was always observed. In the experiment of Fig. 1, for instance, there was no trace of an *I*-potential, but e.p.p.'s were attenuated by 80%.

What is the explanation of this irregular potential change produced by the inhibitor impulse? It might be thought that in some, possibly damaged, preparations a steady leakage of transmitter substances occurs from the nerve endings. The muscle membrane being thus exposed to a 'steady background concentration' of *E* substance might be kept slightly depolarized. Under these conditions an inhibitory nerve impulse would indeed produce a transient repolarization (just as an application of curare repolarizes a vertebrate motor end-plate previously exposed to acetylcholine). This suggestion then implies that the *I*-potential is simply a reduction of a steady 'background e.p.p.', and that the inhibitor impulse affects the membrane potential of the muscle fibre only indirectly, by stopping the *E* transmitter from depolarizing it. This explanation, however, became untenable in the course of further experiments described below. In these experiments *I*-potentials were provoked, abolished, or *reversed* by passing conditioning currents directly through the muscle membrane, without involving excitatory nerve endings at all.

The effect of inhibitor impulses on electrically applied potential changes in the muscle fibre. When the membrane potential of the muscle fibre was displaced by an applied current, an interesting and unexpected result was obtained. There was always a unique level of the membrane potential at which inhibitory impulses produced no potential change at all. Often this level was identical with the resting potential, but occasionally several millivolts higher. When the membrane potentials had been reduced below this equilibrium level, *I*-potentials like that in Fig. 6 were observed, giving a transient *increase* of membrane potential, the increase being approximately proportional to the initial depolarization. Conversely, when the membrane potential had been raised above the equilibrium level, the *I*-potentials became reversed (e.g. Fig. 7, record *Ic*), the inhibitory nerve impulse now producing a transient *depolarization*.

Thus, in fibres in which no *I*-potential was normally seen, it could be provoked by a preliminary depolarization and reversed by a preliminary hyperpolarization of the membrane. In fibres in which a small *I*-potential was present 'normally', it could be increased in size by an applied outward current, or abolished and reversed by an applied inward current.

Hence, the inhibitory nerve impulse diminishes any displacement of the membrane potential, in either direction, from a certain equilibrium level; the presence, sign and magnitude of the *I*-potential is merely a manifestation of this effect and depends upon the sign and magnitude of the initial displacement. The situation is illustrated by the circuit diagram of Fig. 8, in which four membrane components are shown, a fixed capacity C_m, a fixed battery V_0 representing the 'equilibrium potential', and two variable conductances G_1

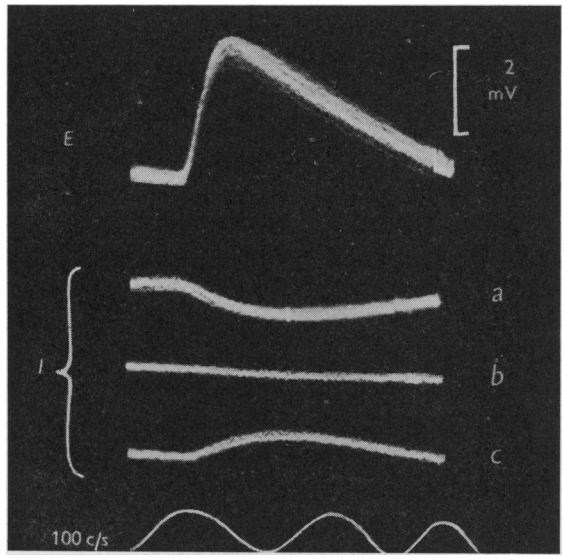

Fig. 7. *I*-potentials in a crustacean muscle fibre. Opener of claw, hermit crab. *E*: e.p.p. due to stimulation of motor axon. *I*: electric potential changes due to inhibitor impulses. The level of the resting membrane potential was lowered to 48 mV (cathodic) in *a*, and raised to 95 mV (anodic) in *c*. The resting potential in the other records was 73 mV. Note the dependence of the *I*-response on the level of the membrane potential: its absence at the normal level (*b*), and its reversal during hyperpolarization (*c*).

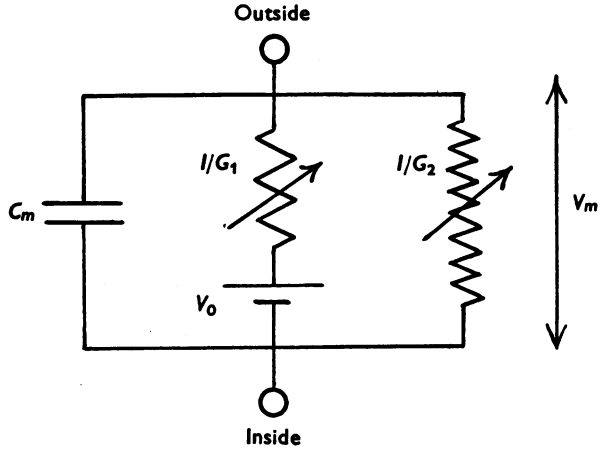

Fig. 8. Circuit model of crustacean muscle membrane. For details see text.

and G_2 which represent the permeability of the membrane to two groups of ions: (1) those whose movement serves to restore the resting 'equilibrium potential' (e.g. K and Cl, represented by the conductance G_1 in series with V_0); (2) those ions whose movements tend to depolarize (e.g. Na, represented by the leakage conductance G_2).

Suppose the inhibitory nerve impulse releases an agent which causes the 'permeability' G_1 to increase. As a result, the membrane potential V_m will tend to approach the equilibrium level V_0. If initially the 'leakage conductance' G_2 was nil, and no net current flowed through the membrane, then the membrane potential V_m was already at the equilibrium level and the inhibitory impulse, though always increasing G_1, produces no potential change.

However, if the value of V_m was initially lowered either by passing outward current through the membrane, or by introducing a leakage through G_2, then the inhibitory impulse would cause the membrane potential to increase towards V_0.

Thus, the *I*-potential could be attributed to an increase of membrane permeability to those ions (e.g. K) whose movement normally serves to maintain and restore the resting potential.

Size and time course of the I-potential. The 'phasic' amplitude of the *I*-potential amounted to only about 5% of the initial displacement of membrane potential; i.e. if the resting potential had been reduced by 20 mV below the 'equilibrium level', the *I*-deflexion after each inhibitory impulse was about 1 mV. This percentage value varied in different preparations between 3 and 10% and, as with the e.p.p., the amplitude depended upon the frequency of impulses. The total deflexion was much greater than the 'phasic', because at frequencies of 20–30 per sec successive *I*-potentials summed to at least twice their individual height.

The time course of the *I*-potential was generally slower than that of the e.p.p. observed in the same fibre. An example is shown in Fig. 9 where the time of rise of the e.p.p. is 5 msec and that of the *I*-potential about 15 msec.

Now, as with the e.p.p., the *I*-potential must be slower than the process which produces it. Suppose, for instance, that the *I*-potential is due to a transient increase of the series conductance G_1. Then even when G_1 has returned to normal, a residual *I*-potential will be left which gradually subsides with the normal time constant of the membrane. Qualitatively, it can be said that the fundamental change must be briefer than the potential which it produces, but at least as long as the period of rise of the *I*-potential.

In Fig. 9 three events, measured on the same fibre, are shown on the same time scale: the e.p.p., the *I*-potential and the 'inhibition curve' (cf. Fig. 5) which, as discussed earlier, indicates the time course of the inhibitory transmitter action. It is noteworthy that the time relations between *I*-potential and 'inhibition curve' are not unlike those to be expected between a mem-

brane potential change and its causative agent. It thus seems possible that a *single* process, or a single reaction between inhibitor substance and membrane substrate, may simultaneously produce two effects: (*a*) attenuation of the e.p.p., and (*b*) an increase of membrane conductance G_1, which then gives rise to the *I*-potential.

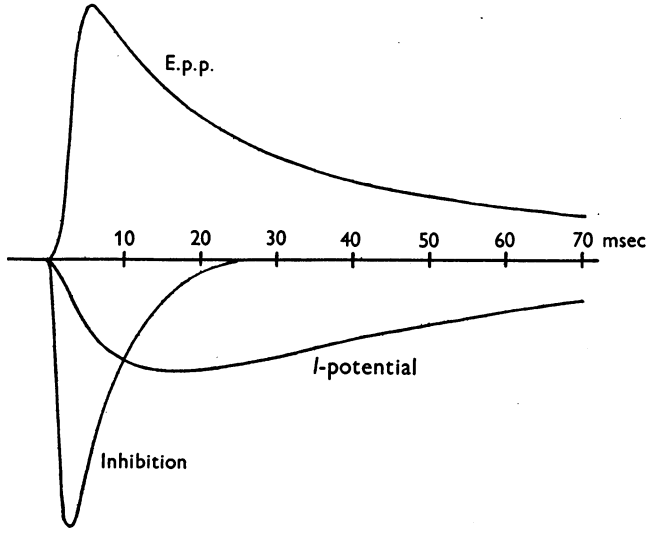

Fig. 9. Time relations of e.p.p., 'inhibition curve' (cf. Fig. 5) and *I*-potential taken from an experiment on a fibre of the opener of the claw (hermit crab). The amplitudes of the three curves have been chosen arbitrarily.

A further point of interest is the *magnitude* of the conductance change. A fairly simple estimate can be obtained in two ways. (*a*) We can make use of the sudden increase in the rate of decay of the e.p.p. which occurs when an inhibitory impulse impinges during its falling phase (e.g. Fig. 4, record 1*a*). Measurements of these rates indicate a transient increase of membrane conductance of about 50%. (*b*) It can be shown that the initial steep rate of rise of the *I*-potential ($\mathrm{d}p/\mathrm{d}t$) is related to the conductance change, in the following way:

$$r_m/r_m' = 1 - (\tau_m/p) \times \mathrm{d}p/\mathrm{d}t,$$

where τ_m is resting time constant of membrane, p displacement of membrane potential ($V_m - V_0$), r_m and r_m' membrane resistances, at rest and immediately following inhibitory impulse, respectively. Measurements of this kind were made in four experiments indicating a 20–50% increase of membrane conductance. Thus, while the peak of the *I*-potential amounts to only 5% reduction of the imposed p.d., the underlying conductance change appears to be several times greater.

The relation between I-potential and electric inhibition. It need hardly be emphasized that the *I*-potential, as such, has no significance as an inhibitory *agent*: the real inhibitory effect is the reduction of the e.p.p., which was always observed, regardless of presence or absence of the *I*-potential or of its electric sign.

When the membrane potential was raised by anodic current, the inhibitor impulse still caused a reduction of the e.p.p., but the effect tended to become obscured by the simultaneous appearance of the *I*-potential which was itself a depolarization summing with the e.p.p. This is illustrated in Fig. 10, where the electric responses to *E*, *I* and *E* + *I* are shown at two levels of the membrane

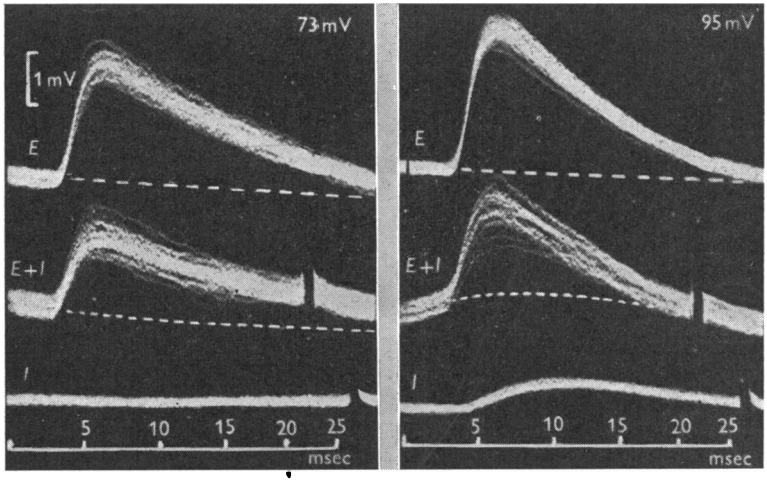

Fig. 10. E.p.p. and inhibitory effects at different levels of membrane potential. Opener of claw, hermit crab. The responses in the left-hand part were obtained at the normal resting potential (73 mV), those on the right at an anodically increased level (95 mV). *E*: e.p.p. due to stimulation of motor axon. Note increased size of e.p.p. at higher level of membrane potential. *I*: potential change due to inhibitor impulses. (The gaps are due to inhibitor shock artifacts.) *E* + *I*: combined stimulation of both axons. The broken lines have been drawn to indicate approximately the 'base-lines' of the phasic e.p.p.'s.

potential, (*a*) at the resting level, 73 mV, and (*b*) 22 mV higher. The 'phasic' amplitude and rate of rise of the e.p.p. were reduced by the inhibitory impulse in both cases; but in the 'hyperpolarized' state, the contribution of the *I*-potential was so large that the combined deflexion was only slightly less than the uninhibited e.p.p.

It would clearly be difficult to resolve the two components of the (*E* + *I*) potential with any accuracy, because mutual interaction must be expected, and it is probable that the *I*-potential becomes reduced by the action of the excitor impulse, just as the e.p.p. is diminished by the action of the inhibitor.

Although the I-potential appears to be merely a by-product of the inhibitory process, the question arises whether the mechanism which produces it, namely an increase of the membrane conductance G_1, might not directly account for the attenuation of the e.p.p.; for the e.p.p. itself is a displacement of the membrane potential from the equilibrium level V_0. Returning to the scheme of Fig. 8 one might suggest that the excitatory transmitter substance E reacts with the membrane so as to increase the shunt conductance G_2 (e.g. sodium permeability) and thereby produces the observed depolarization. The inhibitory substance I then counteracts this effect by increasing the series conductance G_1. This simple explanation, however, fails on quantitative grounds. The increase of G_1 accounts for only a 5% reduction of an applied p.d.; it is therefore difficult to see how this mechanism could account for 80–90% reduction of an e.p.p. The attenuation of the e.p.p. is clearly a much more powerful effect and probably depends on a more specific antagonism between transmitters E and I.

DISCUSSION

The electrical effects of an inhibitor nerve impulse are twofold: there is attenuation of a subsequent e.p.p. and, *in addition*, a lowering of the membrane resistance (more specifically, of the resistance in series with the resting e.m.f. of the membrane). These two actions appear to be synchronous, within the limited accuracy of our analysis. Yet, they must be regarded as two distinct mechanisms. To explain the dual nature of the inhibitory effect, the following hypothesis may be considered. In Fig. 8, the ion permeability of the membrane is represented by two variable conductances, G_1 and G_2, one in series and the other in parallel with the resting e.m.f. (By analogy with other tissues, G_1 might be thought to signify potassium and G_2 sodium permeability (Hodgkin, 1951), but in crustacean muscle the evidence is not sufficient for such specific conclusions.) We assume that motor and inhibitor nerve impulses affect conductances G_1 and G_2 by way of a competitive reaction with a single receptor substance.

The reactions may be described as $E+R \rightleftharpoons ER$, and $I+R \rightleftharpoons IR$ (hence $ER+I \rightleftharpoons IR+E$), where R is the receptor substance in the muscle fibre, and E and I are transmitter substances released, respectively, at excitor and inhibitor nerve endings. Suppose that the value of G_2 increases with the concentration, or surface density, of ER, and that G_1 increases with IR. A motor nerve impulse will then depolarize the fibre by increasing the shunt conductance G_2; an inhibitor impulse produces two effects: (*a*) by its competitive reaction with the receptor it reduces ER and thereby attenuates the e.p.p., (*b*) its reaction-product increases G_1 and thus can give rise to an I-potential.

Thus, in spite of their apparent complexities, the observed phenomena

might all be due simply to interaction of three basic entities: one receptor and two antagonistic transmitter substances.

In connexion with this hypothesis, two observations may be mentioned. (1) When the membrane potential was varied by an applied current, the size of the e.p.p. was found to vary in the same sense (e.g. Fig. 10), as it should if the effect of the motor impulse is to shunt the membrane. The effect was similar to that obtained at the amphibian nerve-muscle junction (Fatt & Katz, 1951), but the results were less accurate and can only be stated qualitatively. (2) The effect of the inhibitory impulse was studied when the muscle was soaked in a potassium-free medium. If the *I*-potential and the underlying change of G_1 were due exclusively to an increase of potassium permeability, then it should be impossible to reverse the *I*-potential in a potassium-free medium, because the theoretical equilibrium potential for potassium has been raised to infinity. However, while the resting potential increased in the potassium-free medium by some 10 mV (cf. Fatt & Katz, 1953*a*) the *I*-potential which was observed could still be reversed by 'anodizing' the membrane and raising its potential by a further 5–10 mV. It would appear, therefore, that potassium is not exclusively or specifically involved in the presumed change of G_1.

The mechanism of synaptic inhibition described here differs substantially from that found by Brock *et al.* (1952) in the cat's spinal cord. A 'hyperpolarization' of the post-synaptic membrane, superficially resembling that described by Brock *et al.*, is occasionally observed in crustacean muscle, but only when the fibres are partly depolarized, and in any case the effect is small and must be regarded as a by-product, rather than an agent, of the inhibitory process.

On the other hand, the attenuation of the e.p.p. is a very powerful effect, reaching in some cases as much as 80–90%. Brock *et al.* (1952) found no such effect in the spinal motoneurone of the cat, and perhaps we are dealing here with a mechanism which is peculiar to the crustacean nerve-muscle system. But it is also possible that a number of different synaptic mechanisms are at play at the different points in the central nervous system where inhibitory effects are produced.

The question of a direct mechanism by which the inhibitory nerve impulse can inactivate muscular contraction (Marmont & Wiersma, 1938; Kuffler & Katz, 1946) has not been investigated in the present experiments, but our results have some bearing on it. In the earlier studies, inhibitory impulses were found to stop contraction even though they arrived too late to reduce the amplitude of the e.p.p. It was suggested, therefore, that the inhibitory impulse can interfere with the contractile process directly, without operating on the fibre membrane. Our present results throw considerable doubt on this interpretation.

In the first place, it has now been shown that the inhibitor impulse does alter the membrane properties even when no potential changes are observed. Secondly, while it is true that the 'phasic' amplitude of successive e.p.p.'s may remain unchanged, if the *I* impulses arrive after their peaks, the decay of the e.p.p.'s becomes accelerated and their summation less effective. In this way, the mean level of the membrane potential may rise and the 'total' depolarization diminish, possibly below the level needed for contraction. The effect is illustrated in Fig. 2 where the level of the membrane potential is seen to shift by several millivolts even though the 'phasic' amplitude of the e.p.p. remains unaltered. Although this may not explain all cases of mechanical inhibition which have been observed, we feel that this matter needs re-examination.

SUMMARY

1. Experiments were made to study the effect of single excitor and inhibitor nerve fibres on the membrane of single crustacean muscle fibres.

2. An inhibitor impulse reduces the amplitude of the 'end-plate potential' (e.p.p.) due to a subsequent motor nerve impulse. This effect is observed when the interval between the antagonistic impulses is less than about 20 msec.

3. When the inhibitor impulse arrives during the falling phase of the e.p.p., it accelerates the decay of the e.p.p.

4. A 'direct' effect of the inhibitor impulse on the resting membrane potential of the muscle fibre is not usually seen. However, when the membrane potential has been displaced in either direction, by means of an applied current, the inhibitor impulse produces a potential change which is directed towards the normal resting level.

5. This action, and the acceleration of decay of the e.p.p. by the inhibitor impulse, can be explained by a lowering of the membrane resistance of the muscle fibre (more specifically of the resistance *in series* with the resting e.m.f.).

6. All the electrical effects of the inhibitor impulse are compatible with the concept of a single inhibitor/receptor reaction ($I + R \rightleftharpoons IR$), which changes the ion permeability of the fibre membrane and, at the same time, competes with the action of the excitatory transmitter ($E + R \rightleftharpoons ER$) on the common receptor molecule.

We are greatly indebted to Mr J. L. Parkinson for his invaluable help. This work was carried out with the aid of a grant for scientific assistance made by the Medical Research Council.

REFERENCES

BIEDERMANN, W. (1887). Beiträge zur allgemeinen Nerven und Muskelphysiologie. XX. Über die Innervation der Krebsschere. *S.B. Akad. Wiss. Wien*, Abt. 3, **95**, 7–40.

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1952). The recording of potentials from motoneurones with an intracellular electrode. *J. Physiol.* **117**, 431–460.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intracellular electrode. *J. Physiol.* **115**, 320–370.

FATT, P. & KATZ, B. (1953*a*). The electrical properties of crustacean muscle fibres. *J. Physiol.* **120**, 171–204.

FATT, P. & KATZ, B. (1953*b*). Distributed 'end-plate potentials' of crustacean muscle fibres. *J. exp. Biol.* (in the Press.)

HODGKIN, A. L. (1951). The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26**, 339–409.

HODGKIN, A. L. & RUSHTON, W. A. H. (1946). The electrical constants of a crustacean nerve fibre. *Proc. Roy. Soc.* B, **133**, 444–479.

HOFFMANN, P. (1914). Über die doppelte Innervation der Krebsmuskeln. Zugleich ein Beitrag zur Kenntnis nervöser Hemmungen. *Z. Biol.* **63**, 411–442.

KATZ, B. (1936). Neuro-muscular transmission in crabs. *J. Physiol.* **87**, 199–221.

KATZ, B. (1949). Neuro-muscular transmission in invertebrates. *Biol. Rev.* **24**, 1–20.

KATZ, B. & KUFFLER, S. W. (1946). Excitation of the nerve-muscle system in crustacea. *Proc. Roy. Soc.* B, **133**, 374–389.

KUFFLER, S. W. & KATZ, B. (1946). Inhibition at the nerve-muscle junction in crustacea. *J. Neurophysiol.* **9**, 337–346.

MARMONT, G. & WIERSMA, C. A. G. (1938). On the mechanism of inhibition and excitation of crayfish muscle. *J. Physiol.* **93**, 173–193.

WIERSMA, C. A. G. (1941). The efferent innervation of muscle. *Biol. Symp.* **3**, 259–289.

WIERSMA, C. A. G. & VAN HARREVELD, A. (1935). On the nerve-muscle system of the hermit crab (*Eupagurus bernhardus*). III. The action currents of the muscles of the claw in contraction and inhibition. *Arch. néerl. Physiol.* **20**, 89–102.