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THE INHIBITORY SUPPRESSION OF REFLEX DISCHARGES FROM MOTONEURONES

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In a preceding paper (Coombs, Eccles & Fatt, 1955*b*) an account was given of the ionic events (increases in membrane permeability and the consequent ionic fluxes) that account for the potential changes which are produced in motoneurones by inhibitory impulses, i.e. the inhibitory post-synaptic potential (i.p.s.p.). The present paper provides evidence on two further questions that arise in an inquiry into central inhibitory action: What is the time course of the postulated change in membrane permeability? How does this membrane change together with the consequent ionic fluxes cause the inhibitory suppression of reflex discharges from motoneurones? The intensity-time course of this inhibitory suppression has been thoroughly studied with direct inhibitory action on monosynaptic reflex discharges (Lloyd, 1946; Laporte & Lloyd, 1952; Bradley, Easton & Eccles, 1953). Actually the intensity of inhibition is indirectly measured by the depression of the size of a testing monosynaptic reflex discharge which is applied at various times during the direct inhibitory action of a single afferent volley. The time course of the intensity so measured may be called an inhibitory curve. It will be shown below that it is possible to explain in detail the inhibitory curve that is produced with direct inhibitory action on a population of motoneurones.

METHODS

The techniques used for intracellular recording and for investigations on the inhibitory and excitatory post-synaptic potentials have been described in recent papers (Brock, Coombs & Eccles, 1952*a*; Eccles, Fatt, Landgren & Winsbury, 1954; Coombs *et al.* 1955*a*, *b*). The technique of superposition of many faint traces has been employed throughout in order to minimize disturbances by background noise (cf. Figs. 1, 2, 4 and 7). When determining the time course of the inhibitory post-synaptic potential, it has been important to reject all experiments in which it was appreciably modified by changes in the ionic composition of the motoneurone, e.g. by chloride dosage from the microelectrode (cf. Coombs *et al.* 1955*b*).

RESULTS

A. *The time course of the inhibitory post-synaptic potential*

The present investigations have confirmed earlier reports on the time course of the i.p.s.p. (Brock *et al.* 1952*a*, *b*). The i.p.s.p. produced in a motoneurone by the inhibitory action of a single group I*a* afferent volley rises steeply to attain a summit at about 1·5 msec after its onset and declines therefrom along a time course which becomes exponential at about 2 msec after the onset (cf. Fig. 1). The half-time of this exponential decay has varied from 1·5 to 3·5 msec in our more reliable experiments, the time constant (1·44 times the half-time) being on the average about 3·5 msec. As shown in Fig. 1, with increase in the size of the group I*a* afferent volley there was a corresponding increase in the size of the i.p.s.p. with but little alteration in its time course. Presumably, therefore, the observed time course obtains for the i.p.s.p. set up by each inhibitory synapse.

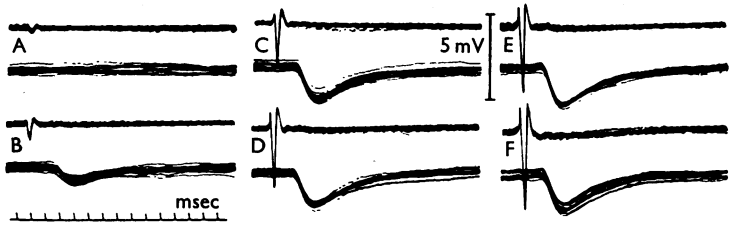

Fig. 1. Lower records give intracellular responses of a biceps-semitendinosus motoneurone to a quadriceps volley of progressively increasing size, as is shown by the upper records which are recorded from the L6 dorsal root by a surface electrode (downward deflections signalling negativity). Note three gradations in the size of the i.p.s.p.; from A to B, from B to C and from D to E. Voltage scale gives 5 mV for intracellular records, downward deflexions indicating membrane hyperpolarization.

When other nervous pathways are used for eliciting an i.p.s.p., it has a more prolonged time course, which is probably formed by the summation of a succession of elementary inhibitory responses due to the repetitive arrival of impulses in the inhibitory presynaptic terminals on the motoneurone. Each such elementary response is presumed to have an active phase, i.e. a phase during which the transmitter is actively changing the properties of the membrane, that has the same time course as is revealed for the direct inhibitory response. For example, in the case of the i.p.s.p. generated by a volley in motor axons and operating via Renshaw cells, the response is prolonged over 50 msec, but under suitable conditions it may be seen to be composed, at least in its early part, of a succession of waves, each having a rising phase of about the same duration as that of the i.p.s.p. of direct inhibition (Eccles, Fatt & Koketsu, 1954). In other cases of prolonged inhibitory response, asynchronism

in the arrival of impulses in different inhibitory endings on the motoneurone may prevent the appearance of such repetitive components. Because it is generated by single virtually synchronous synaptic bombardments, the i.p.s.p. of direct inhibition provides the best conditions for investigating the relationship of the i.p.s.p. to the suppression of the discharge of motoneurones.

It has been assumed that the i.p.s.p. generated by group I*a* afferent impulses is the cause of direct inhibitory action. Hyperpolarization of the surface membrane would be expected to depress excitability, and the observed hyperpolarization (i.p.s.p.) had a time course bearing some resemblance to the inhibitory curve for direct inhibition. The only serious discrepancy would appear to concern the respective latent periods. In the next section an analysis of the latent period of direct inhibitory action will reveal that it is precisely in accord with the observed latency and rising phase of the direct i.p.s.p.

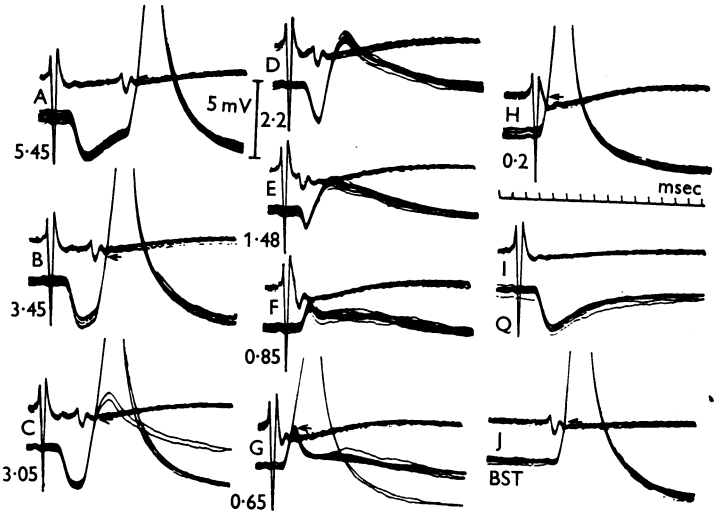

Fig. 2. Lower records give intracellular responses evoked in a biceps-semitendinosus motoneurone by group I*a* volleys in quadriceps and biceps-semitendinosus nerves at the indicated intervals apart, I and J being the respective control responses. Potential scale gives 5 mV for intracellular records, upward deflexions indicating membrane depolarization. Upper records are recorded from the L 6 dorsal root as in Fig. 1. The horizontal arrows mark the approximate potentials at which the spikes are initiated. The low values for these threshold voltages are presumably attributable to depolarization of the motoneurone (cf. Coombs *et al.* 1955*a*), the resting potential having fallen to − 66 mV during this series from an initial value of − 72 mV.

B. *Latent period of direct inhibitory action*

In Fig. 2 the inhibitory volley in quadriceps I*a* afferent fibres entered the spinal cord at various intervals before the testing excitatory volley in biceps-semitendinosus afferent fibres. When alone, this monosynaptic excitatory volley always evoked the discharge of an impulse by the motoneurone (Fig. 2 J).

Suppression of this discharge was invariably observed with volley intervals lying within the range 3·05 to 0·65 msec (Fig. 2D–F), while at the extreme values of this range there was sometimes a failure to inhibit (Fig. 2C, G). A volley interval of 0·65 msec thus provided the situation which gave a measure of the shortest latency for inhibitory action on this particular motoneurone.

Fig. 3A allows an analysis to be made of the factors concerned in this inhibition at minimum latency. The i.p.s.p. and e.p.s.p. were observed in Fig. 2 to begin 1·5 and 0·5 msec respectively after the quadriceps and biceps-semitendinosus afferent volleys entered the spinal cord. In the control observations the e.p.s.p. took 0·55 msec from its onset until it generated an impulse in the motoneurone. Thus at the testing interval of 0·65 msec (interval between first two arrows) the generation of the impulse (indicated by the third arrow) would be at 1·7 msec after the entry of the quadriceps volley into the spinal cord (first arrow), which would be 0·2 msec after the onset of the i.p.s.p. as signalled by the vertical broken line: i.e. the latest time at which the inhibitory volley may arrive at the spinal cord and still inhibit was observed in Fig. 2G when 0·2 msec was available for effective inhibitory action by the i.p.s.p. Evidently no inhibitory action could have been expected with the inhibitory volley 0·45 msec later, as was the case with the briefest test interval (0·2 msec) in Fig. 2H, for the impulse would have been discharged 0·25 msec before the onset of the i.p.s.p. However, it is possible that at this interval inhibition would perhaps have occurred in other motoneurones, or even in this motoneurone when less strongly excited by the testing volley. For example, in the uninhibited response at the 3·05 msec interval (Fig. 2C), the impulse was generated as long as 0·9 msec after the onset of the e.p.s.p., which was 1·4 msec after the entry into the cord of the excitatory test volley. If occurring normally, such a delayed initiation of an impulse would be 1·6 msec after entry of the inhibitory volley at a test interval of 0·2 msec. Thus the onset of the i.p.s.p. would be 0·1 msec before the anticipated initiation of the impulse by the e.p.s.p. and hence inhibition would be just possible.

Similar calculations have been made with direct inhibitory action on other motoneurones (cf. Eccles, 1953, p. 158), and it has always been found that the i.p.s.p. is set up early enough to cause inhibition. It may be concluded that, despite its relatively long latency, the i.p.s.p. satisfactorily explains the short latency of the direct inhibitory action. The temporal analysis of Fig. 3A differs in only one feature from that of Renshaw (1942). He employed an excitatory latent period derived from the motoneurones with briefest latent periods. As shown above, the motoneurones responding with the longest latencies are those which remain susceptible to inhibitory action at the briefest test intervals. Hence Renshaw should have used the longest and not the briefest latency for the testing monosynaptic reflex discharge, which would have added about 0·5 msec to the value which he derived for the central latency of direct

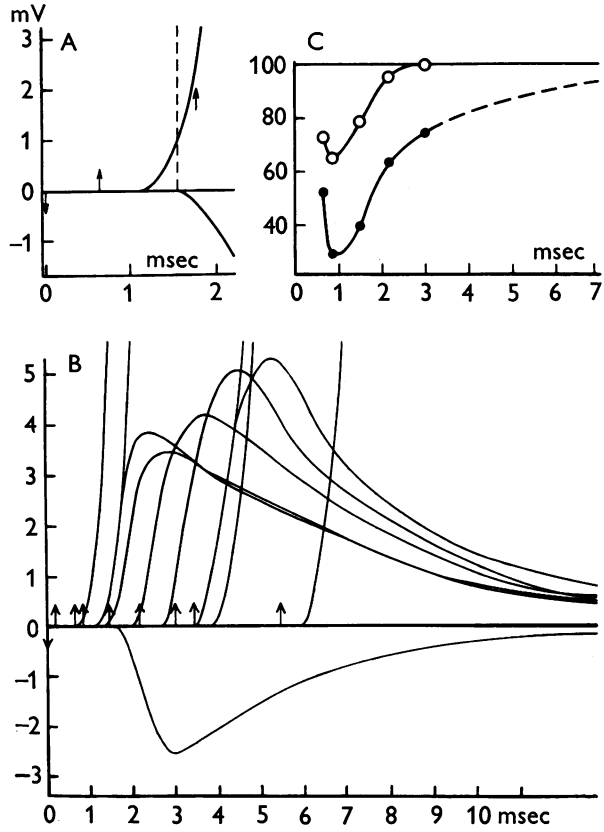

Fig. 3. (A) Plot of time course of events during the 0·65 msec test interval of Fig. 2. The left lower and upper arrows show respectively the times of arrival of the inhibitory and excitatory volleys at the spinal cord. Plotted upwards is the e.p.s.p. beginning 0·5 msec after the excitatory volley and rising up to generate a spike at 0·55 msec after its onset as indicated by the arrow. Plotted downwards is the i.p.s.p. beginning 1·5 msec after the inhibitory volley, the vertical broken line at this time giving the earliest possible onset of inhibition. (B) At each test interval there is plotted upwards the potential which the excitatory volley added to the assumed background i.p.s.p. for the various records of Fig. 2. The i.p.s.p. is plotted downwards at the fixed position that obtains for all records. Test intervals are measured between the times of entry of the inhibitory and excitatory volleys into the spinal cord, as indicated by the downward arrow at zero time and the respective upward arrows. (C) On the assumption that the e.p.s.p. was fully recovered at 3·05 msec test interval (cf. Fig. 5A), the percentage sizes of the e.p.s.p.'s when they are superimposed earlier on the i.p.s.p. (measured from Fig. 3B) are shown by ○ points, the abscissae giving volley intervals. ● points give for the various volley intervals the actual heights of the e.p.s.p.'s which are measured in Fig. 2 relative to the resting potential, and are calculated as percentages of the same control e.p.s.p. as for the ○ points. Thus it is again assumed that complete recovery of the e.p.s.p. has occurred at 3·05 msec volley interval, so beyond that interval the depression would be attributable to superposition on the background i.p.s.p., its course being shown by the broken line.

inhibition. The reassessed value of 1·5–1·7 msec for his calculation of the latency of direct inhibition reveals that the onset of the i.p.s.p. is just early enough for it to be the causal agent.

C. *Relationship of inhibitory action to hyperpolarization*

In Fig. 2 arrows indicate the inflexion that was caused as the impulse arose from the e.p.s.p., so approximate estimates may be made of the amount of depolarization (relative to the resting level) at which an impulse was initiated. It will be seen that the critical depolarization was approximately 2·5 mV (range 2·0–2·7) both for the control and for the various test intervals at which impulses were initiated despite the inhibitory action. However, at the test interval of 2·2 msec the summit of the e.p.s.p. (3·3 mV) was considerably above this cricital level and yet no impulse was generated. This discrepancy may be attributed to the ineffectiveness of the later slowly rising part of the e.p.s.p., which may also be seen in the two records where no impulse was generated at 3·05 msec test interval, even though the depolarization rose to 3·7 mV. Almost invariably it has been found that the later part of the rising phase of the e.p.s.p. has been ineffective in generating impulses (Coombs, Eccles & Fatt, 1955*c*, fig. 4D).

Thus Fig. 2 indicates that an impulse is initiated when the depolarization attains a critical level (relative to the resting potential) within a critical time. The hyperpolarization of the i.p.s.p. would therefore be directly effective in suppressing the initiation of impulses by the e.p.s.p. However, it is not neeessary to assume that the inhibitory action is entirely attributable to hyperpolarization. If there is a critical depolarization for generation of an impulse, effective inhibitory action would also occur if there were a direct interaction between the excitatory and inhibitory actions on the motoneurone.

D. *Interaction between inhibitory and excitatory post-synaptic potentials*

In the experimental series illustrated in Fig. 2 the potential which the excitatory volley added to the background hyperpolarization may be determined by simple subtraction of the control i.p.s.p. from any combined response. This is justified as an initial procedure, though it will later emerge that the excitatory and inhibitory synapses do not act independently in changing the membrane potential of the motoneurone. Usually there is significant interaction. The subtraction procedure must be regarded merely as a device for revealing the characteristics of this interaction. It would be more justifiable to adopt the reverse subtraction procedure, i.e. to assume constancy of the e.p.s.p., and determine the changes in the i.p.s.p. by subtracting the control e.p.s.p. from the combined responses. However, this method has not been adopted because the results so obtained are less readily relatable to the time course of the inhibitory suppression of reflex discharges.

The subtracted curves plotted as in Fig. 3B reveal that there was an apparent depression of the e.p.s.p. when it was set up during the incrementing phase of the i.p.s.p. Since, in the control responses to the excitatory volley alone, the e.p.s.p. generated a spike, a comparison is only possible during the brief rising phase of the e.p.s.p.'s. In the most depressed e.p.s.p. (0·85 msec test interval) the slope of the rising phase was depressed by about 20%. When the e.p.s.p. was set up later relative to the i.p.s.p., the rising phase was indistinguishable from that of the control. The most depressed e.p.s.p. (at 0·85 test interval) had a summit height that was only about 65% of the e.p.s.p. observed at a test interval of 3·05 msec.

Assessment of the apparent depressant effect of inhibitory action on the e.p.s.p. is more conveniently done when the e.p.s.p. alone was not generating the discharge of an impulse (Figs. 4, 5). As shown in Fig. 5A and B (○ points) the e.p.s.p. was depressed considerably (about 15%) below the control level when its rising phase occurred during the early part of the i.p.s.p. At longer testing intervals the e.p.s.p. was virtually unchanged, the depression being observed for a range of volley intervals covering less than 3 msec, which is in good agreement with Fig. 3C. Fig. 3C is constructed on the assumption that the control size of the e.p.s.p. is given by the e.p.s.p. observed in Fig. 3B at 3·05 msec test interval.

The other plotted curves of Figs. 3C and 5B (● points) are of great interest in relationship to the standard curves which are derived from direct inhibition of monosynaptic reflex discharges and which give the time course of direct inhibitory action (Lloyd, 1946; Laporte & Lloyd, 1952; Bradley *et al.* 1953). Against the various volley intervals there are plotted the actual membrane potentials at the summits of the e.p.s.p.'s as directly measured from Figs. 2 and 4. It will be seen that these curves through the ● points represent approximately the sum of two depressant effects on the level of depolarization attained by the e.p.s.p.'s: depression of the actual sizes of the e.p.s.p.'s as determined by subtraction (curves through the ○ points); and depression due to their superposition upon the hyperpolarization of the i.p.s.p. Presumably, by depressing the magnitude of the membrane depolarization produced by the testing excitatory volley, both these effects would contribute to the observed time course of direct inhibition, as will be further considered in the Discussion.

Altogether, the interaction between the direct i.p.s.p. and the monosynaptic e.p.s.p. has been investigated with seventeen motoneurones. In ten motoneurones there was the apparent depressant action on the e.p.s.p. as in Figs. 3C and 5B, whose magnitude varies from about 8 to 35%. A comparable depression has been reported for inhibitory action on synaptic potentials recorded either focally or from the ventral root (Brooks, Eccles & Malcolm, 1948). Thus, while the inhibition of motoneurones is effected through the inhibitory

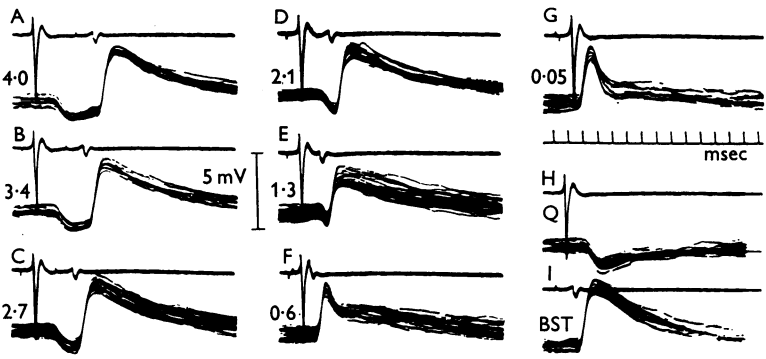

Fig. 4. Lower records give as in Fig. 2 intracellular responses evoked in a biceps-semitendinosus motoneurone by quadriceps and biceps-semitendinosus volleys which are recorded in the L 6 dorsal root (upper records). The volley intervals are marked on each record, being progressively shortened from A to G. H and I give control responses to quadriceps and to biceps-semitendinosus volleys alone. Voltage scale gives 5 mV for intracellular recording.

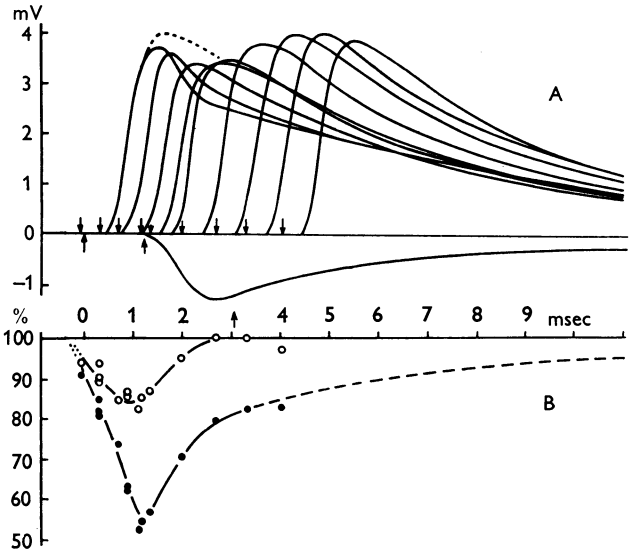

Fig. 5. (A) Excitatory responses of Fig. 4 have been subtracted and plotted as in Fig. 3B, the i.p.s.p. being again shown below the base line at the fixed position. The downward-pointing arrows give the times of the respective excitatory volleys, while the left upward-pointing arrow gives the time of the inhibitory volley. (B) Plotting as in Fig. 3C for the heights of the e.p.s.p.'s in the subtracted records of A (○ points) and the actual e.p.s.p. heights directly measured in Fig. 4 relative to the initial base-line (● points), both series being expressed as percentages of the control e.p.s.p. The curve through the ● points has been extended on the assumption that at the longer intervals unchanged e.p.s.p.'s are superimposed on the background i.p.s.p. (cf. Fig. 3C).

volley preventing the e.p.s.p. from reaching the critical degree of depolarization at which an impulse is generated, the action of the inhibitory volley is not simply to generate an i.p.s.p. which sums algebraically with the e.p.s.p. There is, in addition, a brief initial interaction giving an apparent depression in the size of the e.p.s.p. as determined by the subtraction procedure. An explanation of this action will be given in the Discussion.

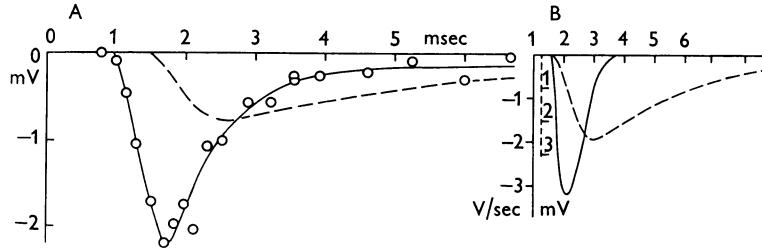

Fig. 6. (A) ○ points plot the diminution produced in the summit of the antidromic spike-potential of a motoneurone when it is set up at various times relative to an i.p.s.p. (shown by the broken line). Abscissae give the times of onset of the antidromic spikes relative to the arrival of the inhibiting quadriceps volley on the cord. (B) Plot of i.p.s.p. from the record in Fig. 2I (broken line) and the time course of the active phase of ionic permeability (continuous line), which is calculated on the assumption that the exponentially decaying phase of the i.p.s.p. is attributable to a passive process governed by the electric time constant of the neuronal membrane, which in this case was 3·4 msec (see text).

The curves through the open circles in Figs. 3C and 5B give some indication of the time course of this brief phase of interaction. However, there would be a considerable distortion because the test response (the rising phase of the e.p.s.p.) was itself about 1·5 msec in duration. A briefer test response is provided by the neuronal spike-potential which is set up by an antidromic impulse (cf. Brock *et al.* 1952*a*). As shown in Fig. 6A the summit of the antidromic spike-potential was apparently depressed by the i.p.s.p. in much the same manner as the e.p.s.p. The maximum depression occurred when the spike summit was late on the rising phase of the i.p.s.p., and significant depression was observed for a range of volley intervals extending over little more than 2 msec. Similar brief depressant actions on the neuronal spike were observed with the three other neurones so tested.

E. *Effects of alterations of the i.p.s.p.*

When the concentration of chloride or of other small anions, such as nitrate or bromide, in the motoneurone is sufficiently increased by injection from the microelectrode, the i.p.s.p. is converted from a hyperpolarizing to a depolarizing response (Coombs *et al.* 1955*b*). If the depolarization is sufficient, the i.p.s.p. will generate a spike discharge, the threshold level for this excitatory action being identical with that for the e.p.s.p. (cf. Coombs *et al.* 1955*b*, figs. 3, 9). Not only has the increased anion concentration displaced the equili-

brium potential for inhibition below the resting potential, but an increase in the ionic fluxes occurring across the active inhibitory areas would also be expected; hence conditions should be more favourable for interaction between the inhibitory and excitatory responses. For example, in Fig. 7D at 0·6 msec interval, when the peaks of the i.p.s.p. and e.p.s.p. would be virtually synchronous, the combined response was very little larger than the control e.p.s.p. Actually slightly larger combined responses were observed with both longer

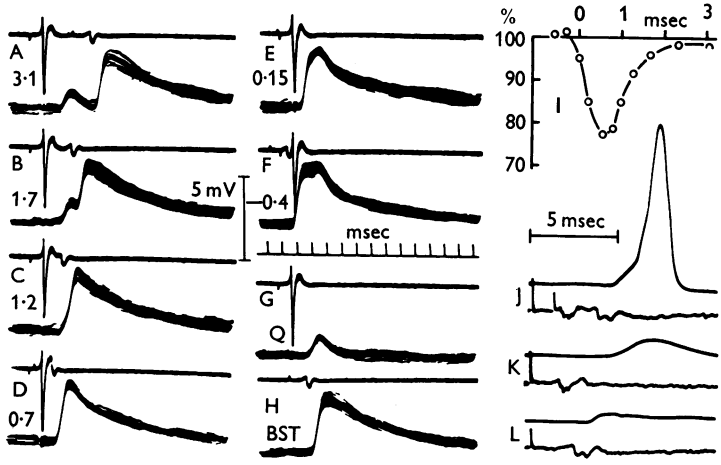

Fig. 7. (A–H) Series of records as in Fig. 4 and for the same motoneurone, but after reversal of i.p.s.p. due to dosage with chloride. The volley intervals are marked on each record. G and H give control resposes to quadriceps and to biceps-semitendinosus volleys alone. Voltage scale gives 5 mV for intracellular recording. (I) Plot as in Fig. 5B (○ points) for the heights of the e.p.s.p.'s in subtracted records for series partly illustrated in Fig. 7A–H. (J–L) Intracellular potentials produced by simultaneous i.p.s.p. and e.p.s.p. in a motoneurone heavily dosed with chloride. In J summation of the two depolarizing responses generates a spike, whereas either alone fails in K and L respectively.

and shorter intervals (Fig. 7C, E). If the potential added by the e.p.s.p. is calculated by subtraction as in Fig. 5A, it is found that the apparent depression of the e.p.s.p. runs practically the same time course as in Fig. 5B, but is almost twice as large (Fig. 7I). The failure of the i.p.s.p. to add any appreciable depolarization on to the summit of the e.p.s.p. (Fig. 7D) indicates that the equilibrium potential for the i.p.s.p. is at a membrane potential very little more depolarized than the summit of the e.p.s.p. When, by increasing the intracellular chloride concentration, the equilibrium potential for the i.p.s.p. is displaced further in the depolarizing direction, significant summation with the e.p.s.p. occurs, and can cause the generation of a reflex discharge as in Fig. 7J.

Strychnine acts as a specific depressant of inhibitory action on reflexes, a sub-convulsant dose of 0·1 mg/kg depressing the inhibition of a test reflex to about one-third of its initial magnitude (Bradley, *et al.* 1953). This depressant

action is closely paralleled by the depression produced in the i.p.s.p. For example, in Fig. 8 the injection of 0·1 mg/kg reduced the i.p.s.p. by more than one-half (from A to D), while having no effect on the monosynaptic e.p.s.p. (from B to E). It is further seen in C that the hyperpolarizing effect of the i.p.s.p. is greatly increased when it is superimposed on the depolarization produced by the e.p.s.p. (cf. Fig. 4G), while after the injection of strychnine this effect is correspondingly smaller (Fig. 8F). Finally when, for a series of records

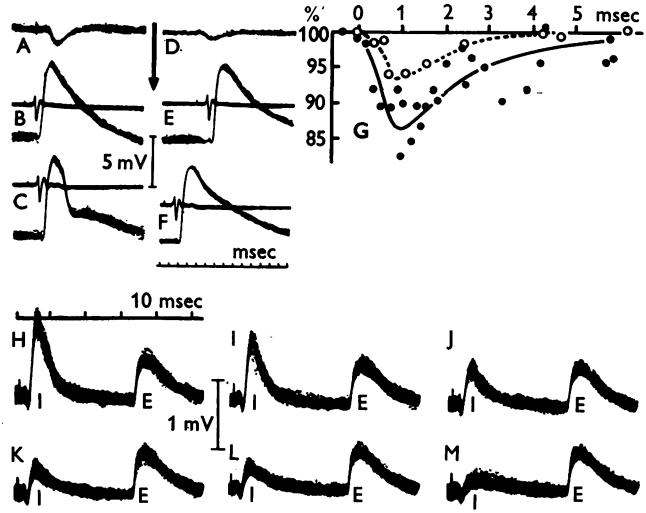

Fig. 8. (A–F) Intracellular records from a biceps-semitendinosus motoneurone evoked by a quadriceps afferent volley (A, D), by a biceps-semitendinosus afferent volley (B, E) and by both volleys, the quadriceps being later by 0·6 msec interval (C, F). The vertical arrow indicates the intravenous injection of 0·1 mg/kg of strychnine salicylate, A, B, C being before injection and D, E, F after. (G) Shows plot of apparent depression of e.p.s.p. by the inhibitory volley just as in Figs. 3C, 5B, the ● points being before and the ○ points after 0·1 mg/kg of strychnine salicylate. (H–M) Intracellular recording of biceps-semitendinosus motoneurone whose Cl^- ion concentration was greatly increased by diffusion out of the microelectrode. In all records the initial response (I) is an i.p.s.p. of depolarizing type evoked by a quadriceps I*a* volley and the later response (E) a monosynaptic e.p.s.p. 0·1 mg/kg of strychnine salicylate was intravenously injected after H and the records I–L were recorded at successive intervals of 10 sec thereafter. Record M was recorded after full development of the inhibitory depression produced by a second injection of 0·1 mg/kg of strychnine salicylate.

as in Fig. 4, the potential added by the e.p.s.p. to the i.p.s.p. is calculated by subtraction, as in Fig. 5A, the depression is greatly diminished by strychnine (Fig. 8G). It has already been reported that strychnine in a dose of 0·1 mg/kg also reduces the antidromically evoked i.p.s.p. to less than one-half (Eccles *et al.*, 1954, fig. 4D–G). Strychnine similarly diminishes the i.p.s.p. when it has been converted to a depolarizing response by increase in the intracellular chloride concentration. For example, Fig. 8H–M shows the progressive diminution of the i.p.s.p., while the e.p.s.p. remains unaffected. Following the

intravenous injection of strychnine the depression of the i.p.s.p. commences at about 10 sec and reaches a steady level in about 30 sec (cf. Fig. 8H–L). A similar time course of onset is observed for the depressant action of strychnine on the inhibition of reflex discharge.

DISCUSSION

The active phase of the inhibitory transmitter action

Apart from its longer latent period, the i.p.s.p. of direct inhibition is virtually a mirror image of the excitatory post-synaptic potential, e.p.s.p. (cf. Fig. 4H, I) produced by a group I*a* volley (cf. Brock *et al.* 1952*a*, *b*). Interaction between an antidromic impulse and such an e.p.s.p. has indicated that its later exponentially decaying phase is attributable to the passive recharging of the motoneuronal surface membrane, whose electric time constant is thus shown to be about 4 msec (Brock *et al.* 1952*b*; Coombs *et al.* 1955*c*). Similarly, it may be postulated that the time course of the direct i.p.s.p. set up by a single afferent volley is attributable to an initial active hyperpolarizing process and a passive decay which, as expected, follows an exponential time course with a time constant approximately identical with the electric time constant of the membrane (4 msec) as determined from the e.p.s.p. Fig. 6B gives an analysis of the i.p.s.p. of Fig. 2I on the basis of this postulate. Both the postulate and the analysis are identical with those already used for other junctional potentials such as the e.p.p. of muscle (Eccles, Katz, & Kuffler, 1941; Fatt & Katz, 1951) and the excitatory synaptic potential of a motoneurone (Eccles, 1952). In Fig. 6B the time course of the active hyperpolarizing process is seen to rise steeply to a summit in about 0·5 msec from which there is a rapid decline so that its total duration is less than 2 msec. A similar rapid time course would also be given by analysis of the i.p.s.p. of Fig. 4H. With the motoneurone, conditions appear to be particularly favourable for an analysis of this type. Histological examination reveals that the synapses are dispersed widely over the membrane whose potential is being recorded; consequently there would be no distortion of the passively decaying process by a spatial redistribution of charge, which with the amphibian muscle causes a considerable departure from the expected exponential decay (cf. Eccles *et al.* 1941; Fatt & Katz, 1951). It has previously been suggested (Fatt & Katz, 1953) that the wide dispersal of excitatory endings is likewise responsible for the closer approximation to an exponential decay which is exhibited by the crustacean e.p.p.

Interaction of excitatory and inhibitory responses

When allowance is made for distortion by the temporal duration of the test response, i.e. the e.p.s.p. (Figs. 3C, 5B) or the spike-potential (Fig. 6A), it may be estimated that with direct inhibition by a single afferent volley the apparent depressant action on the e.p.s.p. is exerted by the i.p.s.p. elements for no more

than 2 msec. For example, in Fig. 5B the e.p.s.p. as determined by subtraction was no longer depressed when it was set up by an excitatory volley entering the spinal cord 2·7 msec after the inhibitory volley. It will be seen in Fig. 5A that at this interval the e.p.s.p. did not begin until the point marked by the arrow on the i.p.s.p. curve, which was less than 2 msec from the onset of the i.p.s.p. The absence of any interaction with the e.p.s.p. may be correlated with the circumstance that at its onset the exponential decay of the i.p.s.p. had already begun. At the other end of the series there is some depression of the e.p.s.p., even when it is set up by an excitatory volley that precedes the inhibitory by about 0·1 msec. In Fig. 5A it is seen that only the terminal part of the rising phase of the e.p.s.p. was then affected by the depression, the broken line showing the anticipated time course of the e.p.s.p. as given in control responses. It is further seen in Fig. 5A that the prolonged rising phase of the e.p.s.p. (1·2 msec) provided the opportunity for the inhibitory volley to exert this depressant action. As would be expected, the i.p.s.p. and the interaction with the e.p.s.p. begin simultaneously. Thus in Fig. 5A the actual duration of the depressant action lies between the two arrows pointing upwards towards the i.p.s.p. curve, one at its origin and the other 1·9 msec later. This is in good agreement with the estimated duration of the active phase of inhibitory transmission, as determined more simply and accurately by the method of analysis used for Fig. 6B. It may be concluded that both actions of the inhibitory volley, the generation of the i.p.s.p. and the interaction with the e.p.s.p., are satisfactorily explained by a single process which may be identified as the increased ionic permeability which the inhibitory transmitter produces in the motoneurone membrane. Further evidence identifying the inhibitory action as due to the i.p.s.p. has been given in 'Results', § E, particularly the close parallelism observed in the respective depressant actions produced by strychnine.

As shown by Coombs *et al.* (1955*b*), the inhibitory transmitter momentarily renders the post-synaptic membrane highly permeable to K^+ and Cl^- ions. As a consequence there will be an intense ionic flux across the post-synaptic membrane tending to increase the potential of the whole neuronal membrane from its resting value of about -70 mV to an equilibrium potential of about -80 mV, which represents a compromise value between the equilibrium potentials of the K^+ and Cl^- ions, which are about -90 and -70 mV respectively. The contrary effect is produced by the ionic flux giving the e.p.s.p., which tends to displace the neuronal membrane to an equilibrium potential near to complete depolarization (Coombs *et al.* 1955*c*). Since the equilibrium potential for the i.p.s.p. is only about 10 mV more than the resting potential, even relatively small e.p.s.p.'s, e.g. those above 3 mV, will significantly increase this potential difference, and hence increase the net ionic flux through any inhibitory patches that are activated.

The electrical interactions may be represented in a formal electrical circuit diagram (Fig. 9) in which the i.p.s.p. and e.p.s.p. generating elements are shown in parallel with each other and with the resting areas of the motoneuronal membrane. The situation is simplified by neglecting the rectifying characteristics which are present in a conductance involving ions distributed unequally across a membrane.

A similar electrical interaction of excitatory and inhibitory effects has been demonstrated in the crustacean nerve-muscle preparation (Fatt & Katz, 1953). With the motoneurone, however, there is no evidence that requires the additional postulate of a chemical interaction between junctional transmitters—a mechanism which provides a very effective inhibition in the crustacean preparation.

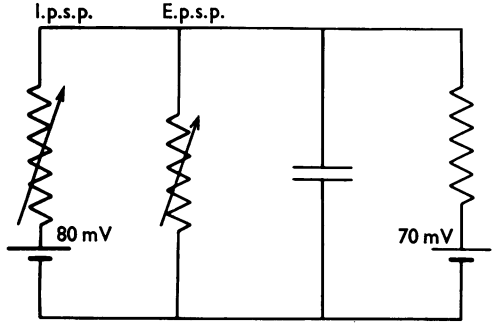

Fig. 9. Electric circuit diagram, illustrating impedance elements and voltage sources in the motoneuronal membrane. The right half of the diagram represents the condition of the general (i.e. non-synaptic) membrane with a resting potential of −70 mV. The variable resistances represent the alterations produced during the i.p.s.p. and e.p.s.p. as indicated.

Suppression of reflex discharge and the direct inhibitory curve

The direct inhibitory curve has been derived by plotting the size of a testing monosynaptic reflex response at various intervals after a conditioning inhibitory volley (Lloyd, 1946; Laporte & Lloyd, 1952; Bradley *et al.* 1953). When the testing reflex response occurs in a considerable fraction of the motoneurones, it has been assumed (cf. Bradley *et al.* 1953) that the number of inhibited motoneurones is approximately in direct proportion to the intensity of the inhibition, i.e. that the inhibitory curve gives the time course of inhibitory action on any one motoneurone. It has been shown above that by means of two separate actions an inhibitory volley depresses the amount of depolarization produced by an excitatory volley (● points of Figs. 3C, 5B). A brief action due to the increased ionic currents through the i.p.s.p. elements is superimposed on a more prolonged action due to the hyperpolarization of the motoneuronal membrane (the i.p.s.p.). It is of interest, therefore, that inhibitory curves have been observed to have a configuration indicative of a double

composition. For example, the two curves reproduced in Fig. 10 have an initial rapid decline merging at about 2–3 msec into a later, approximately exponential decay. The broken line continues this exponential decay further to the left along the time course of an assumed i.p.s.p. Thus the hatched area would give that component of the inhibitory curve attributable to the hyperpolarization of the i.p.s.p. The remaining component has a duration little longer than 2 msec having much the same time course as the direct interaction between i.p.s.p. and e.p.s.p. (cf. Figs. 3C, 5B). Thus the observed time course of the direct inhibitory curve may be satisfactorily explained without recourse to consideration of the spatial relationships of excitatory and inhibitory synaptic endings on the motoneurone (cf. Eccles, 1953; Bradley *et al.* 1953).

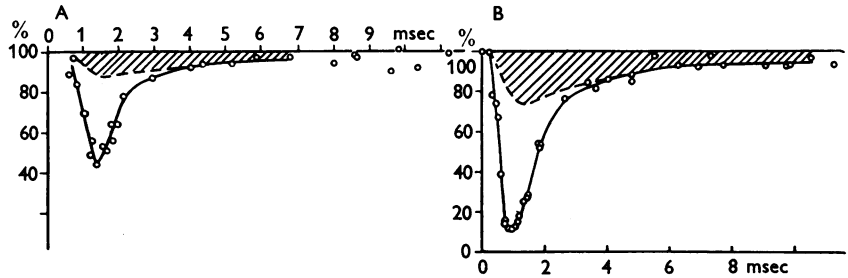

Fig. 10. Reproductions of direct inhibitory curves with plotted points as already illustrated (Fig. 2A, C, Bradley *et al.* 1953). Ordinates show, for the various test intervals, the mean reflex spikes expressed as percentages of the control value. The broken lines delimit the depression which can be attributed to the hyperpolarization of the i.p.s.p., and which is distinguished by hatching. There is a large additional inhibitory action at shorter test intervals, which is attributed to the direct action which the ionic flux through the inhibitory patches exerts on the e.p.s.p. mechanism.

As originally described (Lloyd, 1946), the direct inhibitory curve simply decayed exponentially from its maximum with a time constant of about 4 msec. More recently, Laporte & Lloyd (1952) have reported that this simple exponential decay for the 'direct' inhibitory curve was only occasionally observed. Usually there was a rapid decay from the maximum, much as shown in Fig. 10. They attributed this deviation to the superposition of a disynaptic excitatory action by group I*b* impulses. However, curves such as those of Fig. 10 have been obtained with an afferent volley which could be shown, by its time course, where it entered the spinal cord, to be restricted to group I*a* fibres (cf. Bradley *et al.* 1953). Further investigation is necessary in order to determine the actual contribution made by the disynaptic excitatory action of group I*b* impulses.

It will be observed in Fig. 5B that an inhibitory volley appears to exert a considerable depressant action of the e.p.s.p. when it is synchronous with the excitatory volley, or even a little later. A direct inhibitory action might

therefore be expected at such intervals, whereas none is observed until the inhibitory volley precedes the excitatory (Laporte & Lloyd, 1952; Bradley *et al.* 1953). An explanation of this apparent discrepancy is provided by the observation that at such intervals there is depression only of the last part of the rising phase of the e.p.s.p. (cf. Fig. 5B), and this part of the e.p.s.p. is normally ineffective in generating an impulse (Coombs *et al.* 1955*c*).

The simple exponential decay that is sometimes observed for the direct inhibitory curve (Lloyd, 1946; Laporte & Lloyd, 1952) is an unexpected finding in view of the double composition that has been demonstrated for inhibitory action. In part an explanation may be provided by the relatively small depressant action on the e.p.s.p. that is sometimes observed (cf. 'Results', § D). The time course of the hyperpolarization then plays a dominant role in determining the inhibitory curve. A further explanation is based on the inadequacy of the assumption that, within the population of motoneurones discharged by the control excitatory volley, the degree of excitatory action is uniformly distributed over the whole range in which inhibition can be effected. Under conditions in which nearly all the motoneurones can be inhibited (cf. Fig. 10B), the expected deviation from this uniform distribution would cause a delayed decline of the early stages of the inhibitory curve and hence a closer approximation to a simple exponential decay. In addition, brief repetitive discharges of the intermediate neurones in the group I*a* inhibitory pathway would provide a continuing inhibitory action which would serve to retard the initial rapid decay of the inhibitory curve from its maximum. A single group I*a* volley was found to evoke double or even triple discharges from some I*a* intermediate neurones (cf. Eccles, Fatt & Langdren, 1954, fig. 2). Such repetitive discharges are likely to be more common and more prolonged in Lloyd's unanaesthetized preparations.

CONCLUSIONS

It has been experimentally demonstrated (Eccles, Fatt, Landgren & Winsbury, 1954; Eccles, Fatt & Landgren, 1955) that in the direct inhibitory pathway the primary afferent fibres (the group I*a* fibres from muscle) synaptically relay on to intermediate neurones which in turn provide the inhibitory synapses for motoneurones. It is postulated that impulses in these interneurones liberate a specific inhibitory transmitter substance from their synapses, which acts on the inhibitory patches of the motoneuronal post-synaptic membrane greatly increasing their permeability to small ions, particularly K^+ and Cl^- ions (Coombs *et al.* 1955*b*). As a consequence of this brief increase of ionic permeability (about 2 msec duration), there is an outward current through these inhibitory areas which hyperpolarizes the whole neuronal membrane, so producing the inhibitory post-synaptic potential. It is shown above that this increased ionic permeability has a double depressant action on the effectiveness with which excitatory synaptic action evokes the discharge of an impulse. It is

further shown that the latent period and the time course of direct inhibitory action (the inhibitory curve) are satisfactorily accounted for. Finally it must be assumed that the transmitter substance liberated from an inhibitory synapse is removed within 2 msec from the region of its action on the post-synaptic membrane.

With other types of inhibitory action the chain of experimental evidence is less complete. In every case the post-synaptic inhibitory membranes are shown to have the same permeabilities to ions (Coombs *et al.* 1955*b*). However, with these other types of inhibition there has been no investigation of the way in which depression of excitatory synaptic action is accomplished. The more irregular and prolonged time courses of these inhibitions made it difficult to carry out experiments of the type described in this paper. However, since the ionic mechanisms have been shown to be similar, it seems likely that any individual inhibitory synapse is effective in suppressing the reflex discharges from motoneurones by the same complex mechanism that has here been proposed for direct inhibition. Thus it may be postulated that all inhibitory synapses are similarly effective on motoneurones, the differences between the various types of inhibitory action arising on account of differences in their pathways.

SUMMARY

1. This investigation is concerned with the manner in which the excitatory responses of a motoneurone are depressed by the events which are produced by inhibitory impulses. It is restricted to direct inhibitory action by a single afferent volley in group I*a* fibres in the anaesthetized cat.

2. It is shown that the time course of the inhibitory post-synaptic potential can be accounted for on the basis of a brief (about 2 msec) generating process, which is attributed to an increased ionic permeability (to K^+ and Cl^- ions) of the inhibitory areas of the motoneuronal membrane. The consequent hyperpolarization of the motoneuronal membrane passively decays along an exponential curve with the time constant of about 4 msec, which is characteristic of the membrane. There is evidence that the ionic permeability of the inhibitory areas may be several times larger than for the whole of the remaining motoneurone membrane.

3. By means of intracellular recording there has been a detailed study of the changes which the inhibitory process produces in the excitatory post-synaptic potential of a motoneurone. During the brief phase of high ionic permeability there is a direct antagonism, which is attributable to an increased hyperpolarizing current through the inhibitory areas. Thereafter there is a simple algebraic summation of the respective hyperpolarizations and depolarizations.

4. The very brief latency of the direct inhibitory suppression of motoneuronal discharges is accounted for by an analysis of the direct inhibitory and the

monosynaptic excitatory responses of a motoneurone. A qualitatively satisfactory explanation is given for the observed time course of the direct inhibitory curve with its two-phase decay. The initial peak of inhibitory action is due to the direct interaction between the conductance changes in the inhibitory and excitatory post-synaptic areas, which can occur only if the excitatory action operates during the initial two or three milliseconds of the inhibition. Thereafter the inhibitory action is attributable to the residual hyperpolarization of the inhibitory post-synaptic potential and exhibits the characteristic slow decay. Explanations are offered for the simple exponential decay sometimes observed for the direct inhibitory curve.

5. The relationship of these observations and explanations to other types of inhibitory action on motoneurones is discussed briefly.

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