INFLUENCE OF CEREBELLAR STIMULATION ON THE ACTIVITY OF THE INTERNUNCIAL AND MOTOR NEURONES OF THE SPINAL CORD AS RECORDED BY INTRACELLULAR ELECTRODES

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Many investigators have already established that the cerebellum may exert either a facilitatory or an inhbitory influence on spinal reflexes [7, 11, 18, 19]. By recording potentials intracellularly from extensor motor-neurones stimulated monosynaptically, antidromically, or directly, Terzuolo [21] showed that inhibitory effects which were facilitated by tetanic stimulation of the cerebellum were associtated with hyperpolarization of the membrane of an inhibitory cell. In this even the size of the monosynaptic excitatory post-synaptic potentials showed no change. The effects observed on stimulation of the cerebellum differ from segmental motoneurone reactions. Further, there have been reports of the characteristic action of certain substances, for example strychnine [7, 8, 15, 17] on the activity of the spinal cord during stimulation of the cerebellum or other suprasegmental structures.

It will be interesting to carry out further study of the physiological mechanisms of cerebellar inhibition and facilitation, and to determine the influence on these processes of various drugs. We have investigated the inhibitory and facilitatory effects developed in response to single or tetanic stimuli applied to the anterior cerebellar lobe as shown by the response of motor neurones and internuncial cells of the spinal cord, which were either firing spontaneously or were activated polysynaptically or antidromically; we have also studied the influence on these effects of various drugs having an excitatory or depressor action. The pharmacological results will be presented in separate articles.

Experimental Method

The experiments were carried out on cats. The operative procedures were performed under nembutal anesthesia (35-45 mg/kg intraperitoneally). The operation consisted of laminectomy, division of the posterior and anterior roots, and exposure of the cerebellum; after the operation and before the experiment 5-8 hours were allowed to elapse, which was sufficient for recovery of reflex activity. At the start of the experiment the animals were immobilized with muscular relaxants which exerted no appreciable influence on synaptic transmission in the cord [2]. Stimulation of the anterior cerebellar lobe was made with unipolar silver electrodes and supramaximal pulse stimuli. The frequency was 0.5-300 stimuli per second, and the duration 0.5-1 msecond. Potentials from the motor and internuncial neurones of the seventh lumbar and first sacral segments were led off intracellularly by capillary microelectrodes having a tip diameter less than 1 μ. Detailed descriptions [3-6] of the microelectrode technique and means of identification of the cellular elements have been given elsewhere.

We recorded spontaneous rhythmic activity of neurones in response to synaptic and antidromic stimulation. Stimuli from an electronic stimulator were applied orthodromically to the central ends of the cut posterior roots. Division of the roots between the 6th lumbar and 1st sacral segments was carried out in order to exclude activation of the gamma-system during cerebellar stimulation [10, 13].

Experimental Results

Altogether 228 cells were investigated (190 motor and 38 internuncial neurones); the effects observed included facilitatory and inhibitory responses to cerebellar stimulation. The large number of neurones whose activity was not affected by cerebellar stimulation have not been considered in this article.

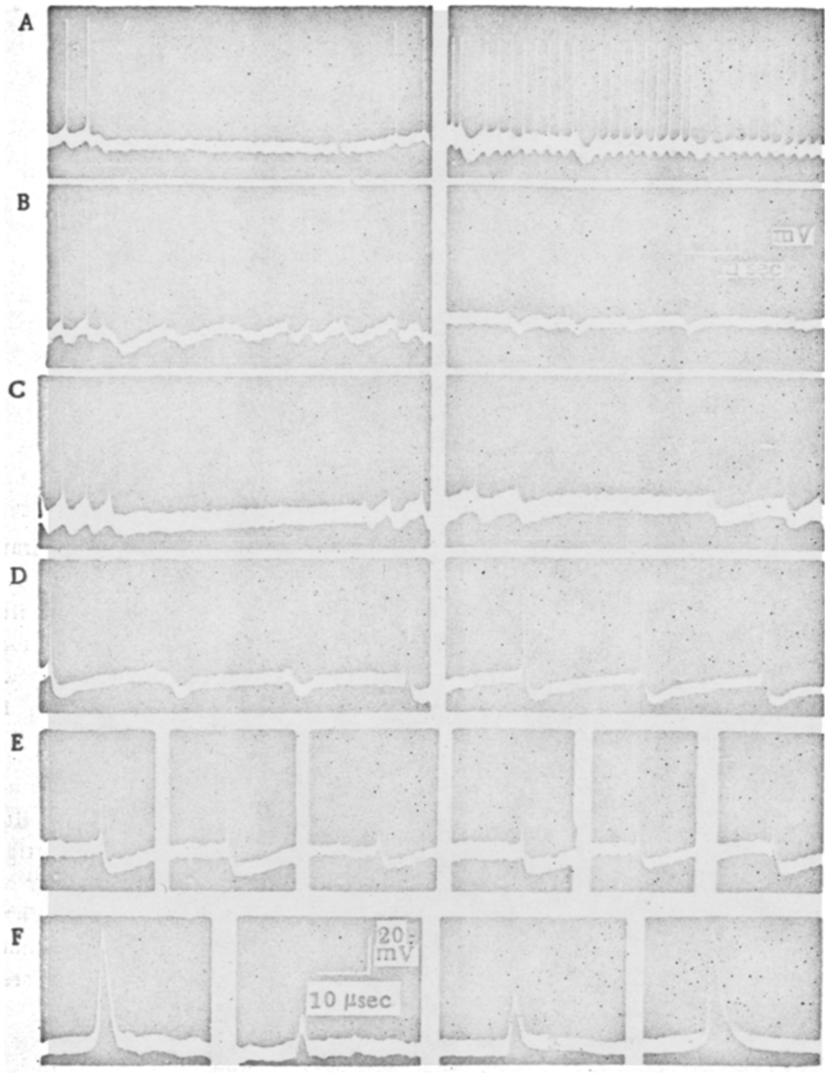

Fig. 1. Examples of inhibition of motoneurones caused by cerebellar stimulation. A, B, and C) each square represents the response of different cells; D, E, F) all squares represent the responses of a single cell. Tetanic stimulation of the cerebellum is shown by a horizontal line, solitary stimuli by points.

Inhibition of Motoneurones was observed in 119 cells. In 92 cells inhibition of activity occurred in association with hyperpolarization of the membrane, in 19 inhibition was not accompanied by any appreciable change of polarization of the cell, and in 8 cells there was depolarization.

Inhibitory cerebellar influence was most easily demonstrated when connection was made with cells in a condition of sustained rhythmic activity. A considerable proportion of the spontaneously active neurones (47 cells) were inhibited as a result of the development of hyperpolarization which greatly exceeded the repolarization following spontaneous peaks. The hyperpolarization developed both with high-frequency tetanic stimulation and in response to isolated stimuli (Fig. 1 A). For a given intensity of cerebellar stimulation and a particlar rate of discharge of different motoneurones the extent of the suppressive influence varied from a very slight reduction in frequency to complete arrest of the spontaneous discharge. Nevertheless the greater inhibitory effect on spontaneous as contrasted with evoked activity was always easily seen. Therefore, quite frequently when the spontaneous rhythm was completely suppressed the evoked potentials were in no way inhibited, even when they had been elicited by rhythmic stimulation of a dorsal root. Most frequently the intensity of hyperpolarization and the inhibitory effect associated with it was greatest at the onset of stimulation. Subsequently, despite the continued tetanization of the cerebellum the inhibitory effect waned, and we observed that the rhythmic activity of the cell escaped completely from cerebellar influence.

By use of occasional stimuli applied to the cerebellum the magnitude of the subsequent inhibitory post-synaptic potentials was also greatly reduced during the course of stimulation (see Fig. 1, A). The effects observed resembled inhibition of background activity by rhythmical stimulation of the afferent pathways of the spinal cord [4]. With sufficiently prolonged cerebellar tetanization (for hundreds of milliseconds) the hyperpolarization developing in a cell was frequently not a continuous process but was, as it were, broken up into a separate inhibitory postsynaptic potential whose frequency was much lower (5-10 per sec). than the rhythm of cerebellar stimulation (300 per sec). This effect was observed both in the spontaneously active and in the "silent" neurones (Fig. 1, B). The "rebound" phenomenon quite often developed after cessation of cerebellar stimulation.

Inhibition of background discharge by cerebellar stimulation was not always associated with hyperpolarization at a level above that of the repolarization observed after each spontaneous peak. This kind of inhibition was found in 30 spontaneously active neurones (Fig. 1, C). In a portion of the cells of this group, during development of the inhibitory influence the membrane potential became stable at a level corresponding approximately to the maximum value before cerebellar stimulation. In other neurones the rhythmic activity ceased despite the fact that the membrane potential remained at a level close to the critical level for generation of an action potential. The characteristic feature of this kind of inhibition is the marked reduction in the rate of growth of the prepotentials preceding the action potentials. This moment is known to be extremely important in relation to the course of the spontaneous rhythm in nerve and muscle cells [1,5, 22]. Apparently in this case the inhibition is brought about by IPSP whose amplitude does not exceed the level of the after-hyperpolarization but which effectively reduces the rate of depolarization of the neurone.

Inhibition of the evoked potentials of the motoneurones may develop in association with hyperpolarization of the cell, or it may occur without noticeable change of the membrane potential, or together with depolarization. In many cases inhibition was associated with a reduction in amplitude of the EPSP. This shows that the inhibitory cerebellar influence may depend upon internuncial neurones taking part in the activation of motor cells. Depolarization of the membrane sometimes observed during inhibition of evoked potentials may be related to a combined inhibitory-facilitatory effect [12]: one and the same stimulus causes depolarization of a motoneurone and inhibits its discharge through suppression of polysynaptic pathways through which it is activated as a result of segmental stimulation. With different types of inhibition of evoked motoneurone reactions the phenomenon of escape from the inhibitory cerebellar influence was clearly observed (Fig. 1, D).

As in the study of spontaneously active cells or of cells showing no background activity, inhibitory effects were most easily achieved by tetanic cerebellar stimulation at a frequency of about 300 per sec. These results agree with others that have been published [12]. However, certain neurones could be inhibited even with single stimuli applied to the cerebellum. An example of the inhibition of motoneurone responses to polysynaptic stimulation under the influence of isolated inhibitory stimuli is shown in Fig. 1, E. The cause of the inhibitory effect was in this case IPSPs evoked by cerebellar stimulation. The extent of the inhibition depended upon the interval between the inhibitory stimulus and the evoked response, which was related to the duration of the cerebellar IPSP and to its latent period.

In some motoneurones a feature of the development of the inhibitory action was the gradual reduction of the amplitude of the evoked action potential, an effect which could be explained on the assumption that the inhibitory influence spreads chiefly to the dendrites of the motoneurone, and in this way impedes the spread of the action potentials from the cell body on to the neighboring parts of the dendrites.

Hyperpolarization of a membrane caused by tetanization of the cerebellum sometimes attains a level sufficient to block the spread of action potentials on to the cell body. This effect is most easily observed during rhythmic antidromic stimulation. However, in some cases it is shown also during activation of a motoneurone by isolated antidromic stimuli (Fig. 1, F). The inhibition which develops during cerebellar tetanization may be maintained for some tens of milliseconds after cessation of the stimulus. In many cases during the recovery period there is a gradual increase of the amplitude of the first component of the action potential (the A component, see [14]). This indicates an inequality in the suppression of the different parts of the cell body. Probably with cerebellar inhibition the spread of the action potential is most effectively inhibited in the region of the dendrites and in the neighboring parts of the

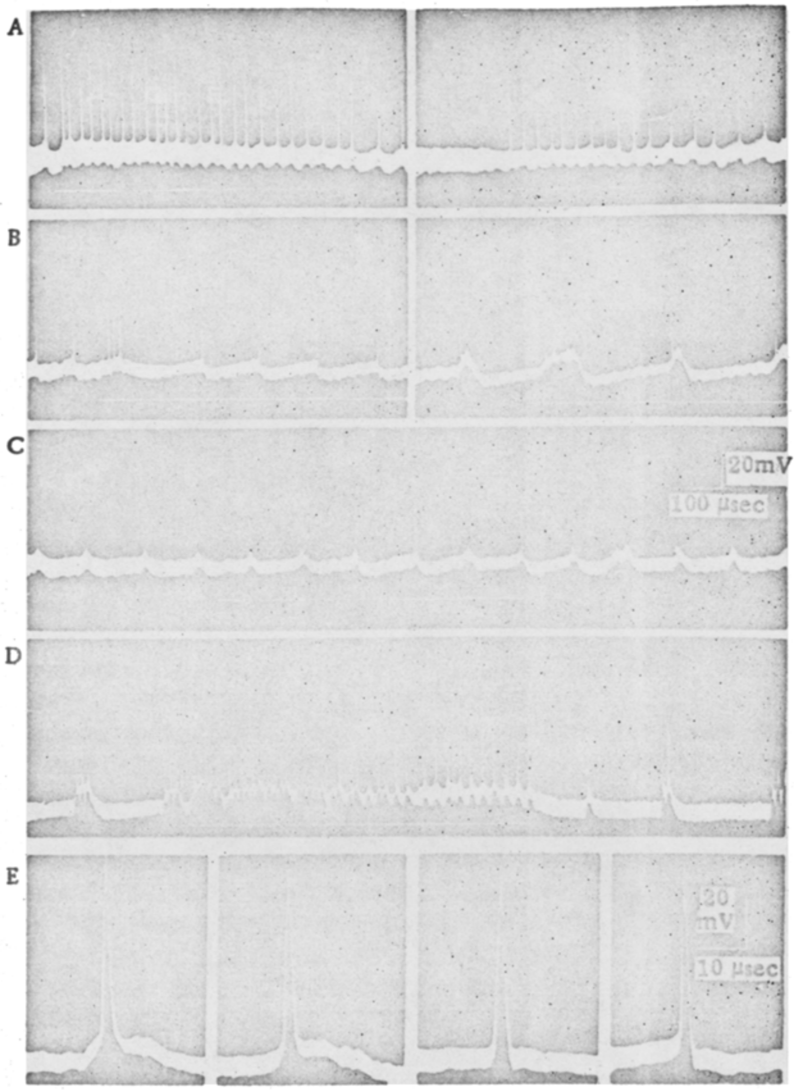

Fig. 2. Examples of excitation of motoneurones on cerebellar stimulation. A, B, C, D) each square shows the responses of different cells; E) all the squares show the responses of a single cell. Tetanic stimulation of the cerebellum is shown by a horizontal line.

cell body. Near to the axon hillock the suppressive influence appears to be less marked. This view is in line with the fact that changes of the membrane potential evoked by cerebellar stimulation have a characteristically slow rise, a more gradual decline, and a long duration. According to Brooks et al. [9] suprasegmental influences on motoneurones are also mediated primarily through the dendrites.

Facilitation was found in 71 of the motoneurones. Just like inhibitory influences, facilitatory effects of the cerebellum were always most easily manifested in relation to spontaneously active cells. The facilitation was shown by an increased frequency of discharge and was associated with a marked depolarization of the neurone. With prolonged tetanization of the cerebellum the intensity of the depolarization and the associated increased frequency of discharge was gradually reduced (Fig. 2, A, 1). Quite frequently the facilitatory effect observed in a spontaneously active or in a synaptically activated cell occurred only at the very start of cerebellar stimulation (Fig. 2, B). In certain "silent" cells cerebellar stimulation induced the development of a rhythm which very much resembled a spontaneous rhythm (Fig. 2, A, 2). The frequency of this rhythmic response did not correspond to the stimulus frequency; before each action potential there was a prepotential. In many cells showing signs of facilitation cerebellar tetanization caused a continuous plateau of depolarization, and on it there was either no action potential or one which arose only at the very start of stimulation. One of the manifestations of the facilitatory effect of the cerebellum was the development of action potentials in cells where the EPSP had not reached the critical limit for generation of the former (Fig. 2, C). During antidromic stimulation of a motoneurone, depolarization which produced a facilitatory effect increased the number of action potentials spreading on the cell body (Fig. 2, D).

Some of the motoneurones are able to respond to EPSPs by attaining the threshold for generation of action potentials in response to a solitary or occasional (5 per sec) cerebellar stimuli. As a rule the ability of these cells to reproduce a given frequency of rhythmic stimulation was not great. A very small increase of stimulus frequency led to the drop in the subsequent responses. Substances with an excitatory action (strychnine, corasole) appreciably increased the ability of the cells to respond by producing action potentials at higher rates (up to 30-60 per sec). We may compare the extent to which one particular cell responded to cerebellar stimulation, stimulation of the afferent pathways, or to antidromic stimuli capable of generating spontaneous discharges (Fig. 2, E); it can be seen that the amplitude and duration of the action potentials with different types of activation was approximately the same. The distinctive features of the responses produced by cerebellar stimulation were their long latent period (18-25 msec) and the time course of the EPSP. The latter show a characteristically slow rise and have a considerable duration (up to 20-100 sec). Because of the shallow slope of the ascending phase of the EPSP, the discharge which it evokes develops several milliseconds after the onset of the postsynaptic response, while the critical value of the depolarization is considerably greater (15-25 mv) than it is with monosynaptic activation. There thus develops an additional delay between the onset of the excitatory postsynaptic potential and the action potential, a delay which is considerably longer than with segmental polysynaptic activation, and far greater than with monosynaptic activation. In some cases it is as long as 3-5 msec. In this connection the latent period of the action potentials was even longer than the latent period of the postsynaptic potential. Because of the duration of the latter it was only very slightly suppressed by the action potential which developed. This circumstance, the features of the time course of the EPSP, and the results which we have quoted allow us to conclude that activation of cells by cerebellar stimulation is brought about chiefly by changes in the region of the dendrites.

When considering facilitatory influences exerted by the cerebellum on motoneurones it is important to note that the facilitation of segmental reactions was very frequently additive, i.e., responses from the cerebellum were shown only when superimposed on stimulation of segmental pathways.

The Influence of the Cerebellum on the Activity of the Internuncial Neurones. With intracellular recording from internuncial neurones, in most cases (30 cells) there was facilitation, and only in 6 cases was the activity of the internuncial cells inhibited by cerebellar stimulation. Characteristically these findings agree with those obtained in connection with an investigation of special influences on internuncial elements of the spinal cord by the reticular nuclei of the brain stem [16].

Stimulation of the cerebellum activated both spontaneously active and "silent" internuncial neurones. Cells showing no spontaneous activity responded to simuli applied to the cerebellum either by solitary or multiple discharges. In most cases the internuncial neurones excited by the cerebellum also responded to simulation of the afferent pathways, and the response to the latter was more intense. This was shown by the greater duration of the usual response and by the far greater facility to follow the tetanic stimulus rate. The effectiveness of cerebellar influences increased sharply under the influence of the stimulants strychnine, corasole, and caffeine.

Internuncial cells capable of maintaining a spontaneous discharge reacted to cerebellar stimulation in various ways: there could be an increase of the spontaneous rate, a change from multiple discharges to discharges which were single but maintained at a higher rate, an increased repetition frequency of the multiple discharges, or multiple discharges might replace the single action potentials (Fig. 3, A). A change in the spontaneous firing rate of the internuncial neurones depended upon the duration and frequency of cerebellar stimulation. The spontaneous condition was one in which the multiple discharges consisted of groups of a fixed number of action potentials,each group separated from the next by an approximately constant interval; at the onset of cerebellar stimulation there was no appreciable change despite the occurrence of occasional responses in between the spontaneous groups (Fig. 3,B). As stimulation continued the nature of the neuronal activity gradually altered. The spontaneous bursts were completely replaced by responses corresponding to the frequency of cerebellar stimulation. At the end of stimulation there was a return to the initial type of activity.

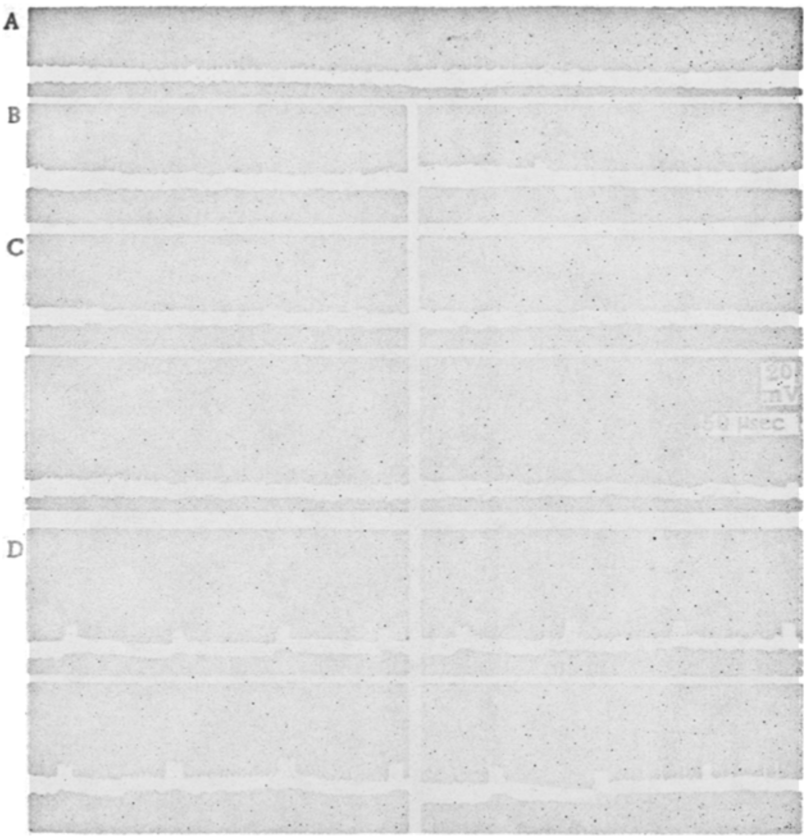

Fig. 3. Effect of cerebellar stimulation on the activity of [illegible] neurones. A and B) Each square shows the responses of different cells; C, D) all squares represent the responses of a single cell. Tetanic stimulation of the cerebellum is shown by a horizontal line.

The frequency of cerebellar stimulation is of great importance. If we take as example one of the neurones (Fig. 3, D) it can be seen that low-frequency stimulation leads to the generation of action potentials in the cell without any alteration in the nature of the spontaneous discharges, whereas higher frequency stimulation disturbs the spontaneous rhythm also.

Thus, the activity of motor and internuncial neurones of the spinal cord is highly sensitive to cerebellar influences. Facilitatory and inhibitory effects [illegible] of activity, whether spontaneous or evoked; in addition, [illegible] about as a result of hyper- [illegible] membrane potential, and even in [illegible] cell of [illegible] circuit [illegible] the [illegible] of [illegible] discharge.

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