EFFECT OF STRYCHNINE ON ACTIVITY OF MOTOR AND INTERNUNCIAL NEURONS OF THE SPINAL CORD DURING STIMULATION OF THE ANTERIOR LOBE OF THE CEREBELLUM

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According to reports in the literature [7,11], inhibition of the activity of the spinal cord under the influence of stimulation of the cerebellum is not abolished by strychnine, although this drug effectively weakens various forms of segmental inhibition [6,9,10,11]. The reason for this unique action of strychnine on the effects of stimulation of the suprasegmental structures is unexplained.

By means of the intracellular technique of recording the potentials of the motor and internuncial neurons of the spinal cord, information may be obtained concerning the mechanism of the effect of strychnine on the inhibitory and facilitating reactions arising as a result of stimulation of the cerebellum.

EXPERIMENTAL METHOD

Experiments were conducted on cats immobilized by muscle relaxants. Potentials were recorded intracellularly. The operative procedures, the methods of recording, and the technique of stimulating the cerebellum have been described previously [4,5]. Strychnine was injected intravenously in the form of a 0.01% solution of the nitrate in doses of 0.05-0.6 mg/kg. Altogether more than 100 neurons were investigated. In some experiments the preparation was injected after preliminary recording of the activity of the cell (24 neurons); in the remaining cases potentials were recorded after the development of established strychnine poisoning with marked strychnine activity [3].

EXPERIMENTAL RESULTS

Effect of strychnine on motor neuron activity. In those cases in which inhibitory postsynaptic potentials (IPP) were recorded in the motor neurons in response to stimulation of the cerebellum, strychnine in subconvulsant doses (0.05-0.1 mg/kg) lowered their amplitude (10 units). These results are in agreement with those obtained by Curtis [9]. The weakening of the IPP in some cells was accompanied by the appearance of depolarization, which was not observed before administration of strychnine (Fig. 1a, 1-3). Depolarization, when appearing after administration of strychnine, may be regarded as excitatory postsynaptic potential (EPP). As a result, the hyperpolarization response of the motor neuron was converted into a depolarization response. During conversion of the hyperpolarization evoked by the cerebellum, depolarization of the membrane sometimes reached a level adequate for generating action potentials.

Similar changes were observed during activation of the polysynaptic afferent pathways (Fig. 1a, 4-6). In the last case two components could usually be distinguished in the postsynaptic potential before the injection of strychnine: an initial depolarization, and a later hyperpolarization component, corresponding to the EPP and IPP. Under the influence of strychnine the first component was increased in amplitude and the second diminished. Hence, the conversion of the cerebellar IPP caused by strychnine was evidently brought about as a result of masking of the EPP appearing after administration of the drug by the more powerful IPP. The diminution of the postsynaptic hyperpolarization caused by stimulation of the cerebellum was accompanied by a diminution or disappearance of inhibition of the evoked responses of the motor neuron.

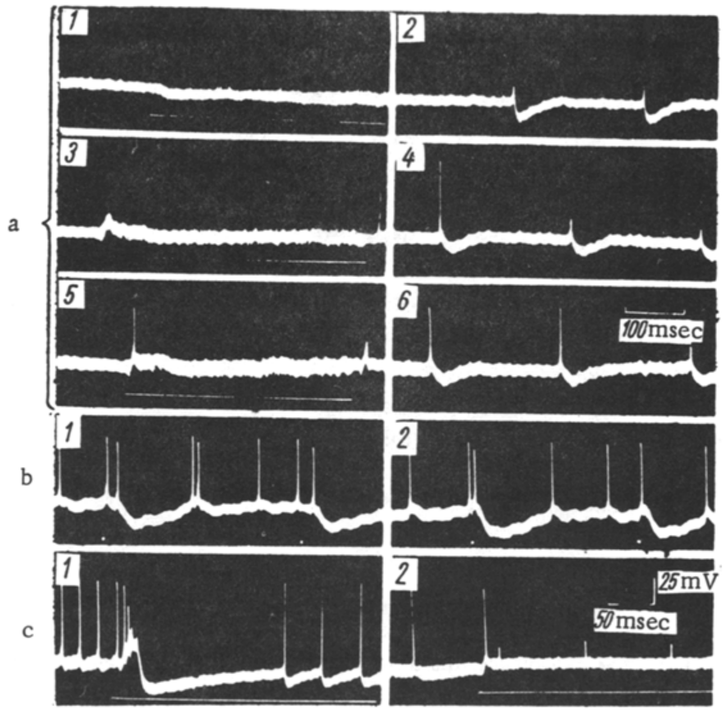

Fig. 1. Effect of strychnine on inhibition of motor neurons. a) Responses to stimulation of cerebellum (1) and posterior roots (2) before administration of strychnine and 30 sec (3,4) and 2 min (5,6) after administration of strychnine in a dose of 0.1 mg/kg; b) responses of a rhythmically active cell after injection of strychnine to cerebellar (1) and posterior root (2) stimuli; c) responses of a rhythmically active cell after administration of strychnine to stimulation of the cerebellum (1); inhibition of antidromic responses of the cell during stimulation of the cerebellum (2). Tetanic stimulation of the cerebellum is denoted by a horizontal line; single stimuli by dots.

Particularly marked changes were observed after injection of strychnine in the EPP arising in response to cerebellar stimulation. Their amplitude and duration increased sharply. The responses caused by single stimuli increased to such an extent that they resembled discharges provoked by stimulation of the posterior roots. As previously demonstrated [1,3,4,5], strychnine causes a sharp increase in the amplitude of the polysynaptic reflexes of the spinal cord during stimulation of the afferent tracts. However, the relative increase in the amplitude of the responses to cerebellar stimuli was still more marked. For this reason, after injection of strychnine, the discharges of the motor neurons obtainable from the cerebellum "caught up" with the discharges of these same cells, in amplitude and duration, in response to afferent stimulation. This process progressed as the dose of strychnine was increased, and after injection of large doses (0.2-0.6 mg/kg) in many cases the responses to segmental and suprasegmental stimuli became identical. For instance, whereas under the influence of strychnine the motor neuron began to respond to afferent stimulation with a powerful EPP, on which a discharge and several peaks were superimposed, a discharge and a similar number of action potentials developed in exactly the same way during stimulation of the cerebellum. Under the influence of strychnine the ability of the motor neurons to reproduce without transmformation the rhythm of cerebellar stimulation was appreciably increased (up to 30-60/sec). However, during afferent stimulation at up to 80-100/sec, this ability always remained higher.

A characteristic feature of many polysynaptic postsynaptic potentials arising in the motor neurons after injection of strychnine was the existence of two phases: a powerful EPP with a single or multiple discharge at its apex was followed by hyperpolarization, reflecting the onset of a polysynaptic IPP. The probable explanation is that, despite

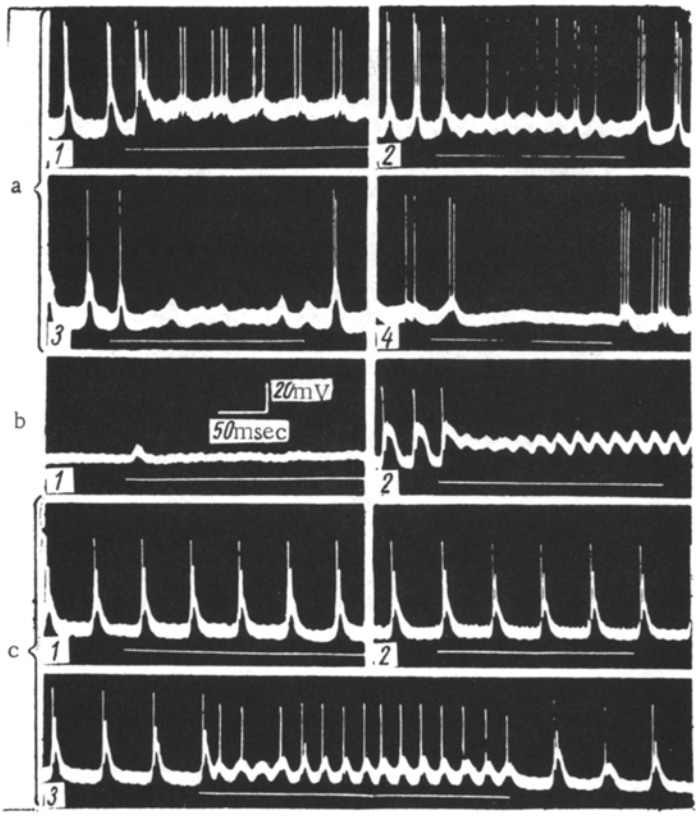

Fig. 2. Effect of the cerebellum on motor neuron activity after injection of strychnine. a) Examples of effect of tetanization of the cerebellum on strychnine tetanus (4) of different motor neurons; b) responses of motor neurons to tetanization of the cerebellum before (1) and 20 sec after (2) injection of strychnine; c) effect of tetanization of the cerebellum with a frequency of 40/sec (1), 100/sec (2), and 300/sec (3) on the strychnine tetanus of the same motor neuron.

the depression of the IPP by strychnine, facilitation of activity of the internuncial neurons could lead to an increase in the amplitude of the inhibitory potentials of the internuncial neurons of the long polysynaptic tracts. When such responses were present, especially when they were evoked against the background of rhythmic activity of the neuron, the effect was mixed, i.e., at first the frequency of the background rhythm was increased, and later it was diminished. This effect was also analogous to the changes arising in response to afferent stimulation (Fig. 1b). If tetanic stimulation was applied to the cerebellum, the hyperpolarization following the initial multiple discharge diminished in amplitude, despite the continuation of tetanization (Fig. 1c, 1).

The amplitude of the slow depolarization waves appearing in response to single cerebellar stimuli, in conditions of severe strychnine poisoning, reached 20-40 μV, so that the action potentials arising at their apex were reduced in size. The depolarization plateau produced in the motor neuron by tetanic stimulation of the cerebellum could even disturb the spread of antidromic impulses to the cell body. It is clear from Fig. 1c, 2 that depolarization of the cell, accompanying stimulation of the cerebellum, removes the second component of the antidromic action potential but leaves intact the first component, corresponding to activation of the initial segment of the axon. The changes observed in the action potential of the motor neuron demonstrate disturbances of the membrane mechanism responsible for the generation of this potential. They may be explained by partial inactivation of sodium transfer on account of excessive depolarization of the cell. Similar changes have previously been reported in the motor neurons of the spinal cord of the toad [13] and cat [3], activated by afferent stimuli.

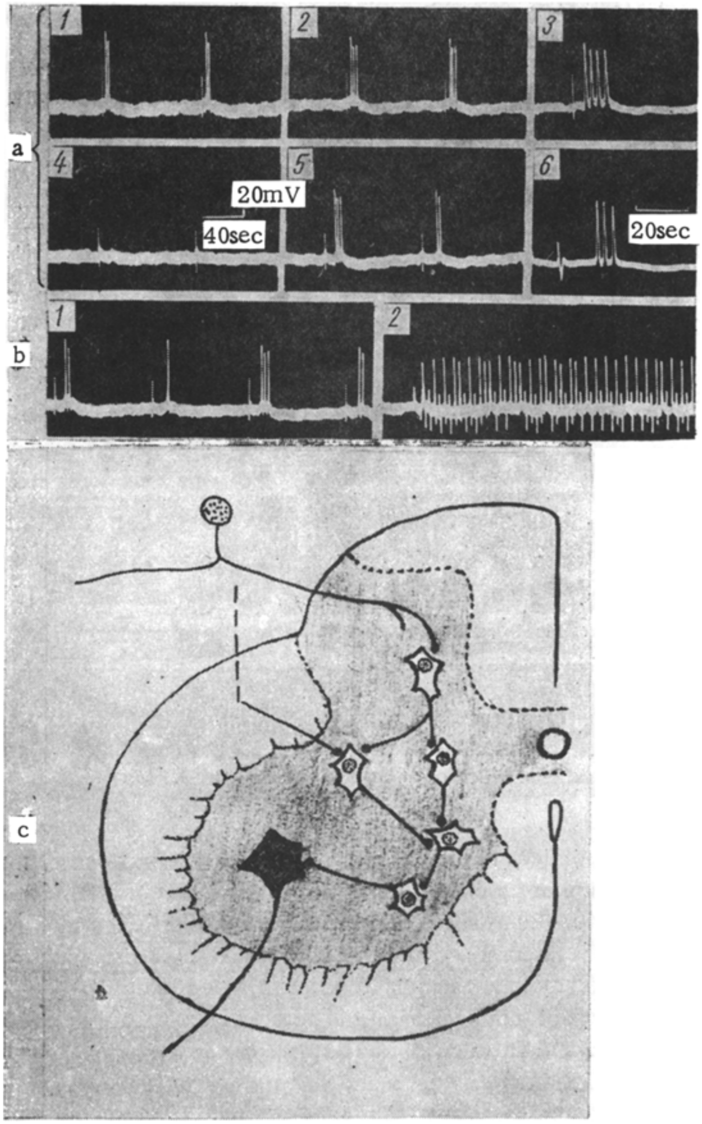

Fig. 3. Effect of strychnine on activity of internuncial neurons and schematic illustration of multineuronal pathways subjected to the action of strychnine. a) Responses of internuncial neuron to afferent stimulation (1) and to stimulation of the cerebellum (4) before (2,3) and 40 sec after (5,6) injection of strychnine in a dose of 0.08 mg/kg; b) responses of internuncial neuron of an animal receiving an injection of strychnine to cerebellar stimuli of different frequency; c) scheme of the afferent and suprasegmental pathways to motor cell of spinal cord.

The increased strength of the excitatory influences from the cerebellum on the motor neurons was also revealed by the fact that a single stimulus during strychnine poisoning could transform the state of the neuron into one of stabilized strychnine rhythmic activity. The activity thus developing was identical with the rhythm appearing in some of the neurons "spontaneously." It was characterized by slow depolarization waves and by multiple discharges. Very often the amplitude of individual slow waves, the interval between them, and the number of action potentials to each wave were remarkably constant for the particular neuron [3]. This fact, and also the long duration of the tetanus, created ideal conditions for studying the effect of cerebellar stimulation on strychnine tetanus. The changes in strychnine tetanic activity arising under the influence of cerebellar stimulation could be subdivided into four main variants.

1. During stimulation of the cerebellum, a continuous plateau of depolarization developed in a rhythmically active neuron, very close in amplitude to that of the individual depolarization waves, or actually greater in amplitude. Against the background of this developing constant depolarization the frequency of the action potentials of the motor neuron increased, although now they did not appear as groups of potentials, as they did before stimulation, but in a different, and often irregular rhythm (Fig. 2a, 1). If the depolarization plateau reached a considerable amplitude (30-35 μV) and the duration of the stimulus was prolonged (hundreds of milliseconds or a few seconds), the action potentials arising in these conditions showed signs of cathodic depression or were actually completely blocked (Fig. 2b, 2).

2. Under the influence of stimulation of the cerebellum the frequency of the developing slow depolarization increased by 50-100%, but its amplitude fell at the same time. These changes were accompanied by a regrouping of the action potentials. They no longer appeared as groups of potentials separated by very short intervals within the group, but as single discharges (see Fig. 1a, 2).

3. Stimulation of the cerebellum led to a marked increase in the amplitude of the strychnine slow waves and to disturbance of the regularity of the rhythm of these waves. This was accompanied by complete cessation of generation (Fig. 2a, 3). Hence, complete or partial inhibition of the strychnine discharges was observed. A special feature of this particular type of inhibition was the absence of hyperpolarization or of excessive depolarization of the neuron.

4. Finally, during tetanization of the cerebellum, complete disappearance of the slow waves and of the discharges evoked by them was observed (Fig. 2a, 4). The polarization of the cell under these circumstances remained at a constant level, corresponding to the polarization in the intervals between the strychnine waves. This type of inhibition was seen least frequently.

It is noteworthy that all the types of changes in strychnine tetanus mentioned above are unconnected with the hyperpolarization IPP, but are due to the excitatory action of the cerebellum. It may be seen from the example of the cell shown in Fig. 2b that the neuron responding to stimulation of the cerebellum by a small initial EPP showed a characteristic modification of the course of its slow depolarization waves after injection of strychnine and the development of spontaneous strychnine activity in the cell, and this was accompanied by blocking of the spreading discharges.

It should be noted that these types of disturbance of strychnine tetanus were also observed in response to stimulation of the segmental afferent tracts, although in the latter case stimulation most frequently caused the appearance of a plateau of depolarization and prevented the development of action potentials, mainly as a result purely of the appearance of cathodic depression.

The effectiveness of the action of cerebellar stimulation on the spontaneous strychnine activity of the motor neurons was dependent on the frequency of stimulation. As an example of this relationship, tracings of the electrical activity of a neuron are shown in Fig. 2c. The regular spontaneous activity of this cell was completely unchanged when the cerebellum was stimulated with a frequency of 40 and 100 per second. Well-defined changes in activity appeared when the frequency of tetanization of the cerebellum was increased to 300 per second. These observations agree very well with our previously reported findings concerning the effect of the frequency of cerebellar stimulation on the activity of internuncial neurons [5].

Effect of strychnine on activity of internuncial neurons. Altogether 29 units were investigated. After injection of strychnine the facilitating effects from the cerebellum on the internuncial neurons of the spinal cord were considerably increased. In the cells capable of responding to cerebellar stimulation before injection of the preparation an increase in the frequency and number of action potentials of the individual discharge was observed, and the ability to reproduce the different frequencies of rhythmic stimulation was also enhanced. Changes were especially marked in the neurons which, before injection of strychnine, were not activated by cerebellar stimuli (Fig. 3a). Strychnine increased the amplitude of their responses to afferent stimuli, but stimuli applied to the cerebellum unexpectedly (15-60 millisec after injection of strychnine) began to give rise to discharges closely similar in their intensity to the responses to afferent stimulation. As when recordings were made from motor cells, the character of the discharges from the internuncial neurons was almost identical whether evoked from the cerebellum or from the afferent tracts. If single stimuli were used, the difference consisted mainly of inequality of the length of the latent periods (see Fig. 3a). Meanwhile, many internuncial neurons (11 units) gave discharges of much greater duration and frequency in response to afferent stimulation, despite their increased ability to respond to stimulation of the cerebellum.

When rhythmic stimuli were applied to the cerebellum, the discharges of the internuncial neuron evoked by each stimulus showed a tendency to decrease rapidly or to appear in a transformed rhythm. Similar results were obtained during rhythmic afferent stimulation [2], although in this case the ability to reproduce the driving rhythm, while preserving the multiple character of each individual response, was always much greater in amplitude. Responses consisting of single action potentials were also well reproduced at high rhythms of stimulation after injection of strychnine (Fig. 3b).

These observations show that under the influence of strychnine a marked increase is observed in the facilitating effects of the cerebellum on the motor and internuncial neurons, together with weakening of the primary inhibitory effects accompanied by hyperpolarization (IPP). Meanwhile the spontaneous strychnine activity may be very effectively inhibited by stimulation of the cerebellum, despite the absence of hyperpolarization of the cell membrane.

The ability of the cerebellum and other suprasegmental formations to inhibit the motor neuron activity after injection of strychnine has been reported by several workers [7,8,12,14,16]. These observations attracted attention originally several years ago, when Eccles and his collaborators [6,9,10,11] showed that strychnine depresses segmental inhibition as a result of depression of the IPP. It was accordingly suggested that suprasegmental inhibition is effected by means of special mediators, different from the inhibitory mediator of the segmental pathways [10]. Investigations of the effect of strychnine on the suprasegmental and segmental inhibition have been conducted with the use of different methods. In the first case the activity of the spinal cord was assessed by recording potentials from the surface of the cord or roots, and in the second the electrical reactions of the motor neurons were recorded intracellularly. Only Terzuolo and Gernandt [16] and Curtis [9] used microelectrodes to study the effect of strychnine on the results of cerebellar stimulation, yet the first of these cited authors used intracellular electrodes, so that they could record only the action potentials of the spinal cord neurons, while Curtis studied the effect of small doses of strychnine on the IPP (and, moreover, in a limited number of experiments) and made no attempt to investigate the effect of the cerebellum on strychnine tetanus.

The results of our experiments showed that the IPP evoked by cerebellar and afferent stimuli are depressed by strychnine. Consequently, there is no reason to suppose that they are due to the action of different mediators. The ability of the cerebellum to depress strychnine tetanus is explained by the individual features of the mechanisms of cerebellar inhibition. As we showed previously [5], hyperpolarization of the membrane by IPP is not the only form of inhibition brought about by the cerebellum. For this reason, an increase in the effectiveness of the excitatory influences may itself create conditions leading in certain circumstances to inhibition of the activity of the spinal neurons. The character of the changes observed following injection of strychnine against the background of cerebellar stimulation may be explained by the scheme given in Fig. 3c. In this scheme the principal part is played by the internuncial neurons on which both cerebellar and afferent influences converge. If it is assumed that these internuncial neurons of segmental polysynaptic pathways, then this suggested scheme may help to explain all the effects observed after administration of strychnine.

1. The increase in the facilitating effects is due to more intensive activation of the internuncial neurons by the cerebellum and to a strengthening of their influence on the neurons of the polysynaptic pathway, which, in turn, are activated by strychnine. Hence, strychnine may bring the effectiveness of the cerebellar stimulus up to the characteristic standard of the afferent stimulus, and this may actually lead to cathodic depression of the motor neuron.

2. During simultaneous excitation of the segmental polysynaptic pathways (the activity of which undoubtedly is mainly responsible for strychnine tetanus) and stimulation of the cerebellum, interference of the different excitatory influences may take place in the common internuncial neurons, as a result of which the activation of the motor neuron may be disturbed.

Increased activation of the internuncial neurons exciting the motor cell may be accompanied by stimulation of the activity of the synapses terminating on inhibitory internuncial neurons, giving rise to a hyperpolarization phase at the end of the enlarged EPP, as was sometimes observed during cerebellar and afferent stimulation.

SUMMARY

Experiments were done on cats. The effect of strychnine on the activity of the motor neurons and the interneurones of the spinal cord was studied with the aid of intracellular potential leads. Under the effect of strychnine there is a reduction of the inhibitory post-synaptic potentials and a marked rise in the excitatory post-synaptic potentials of the motor neurons, caused by the cerebellum stimulation. Depression of the strychnine tetanus of the motor

neurons under the effect of cerebellar stimulation develops as a result of disturbed process of appearance of slow depolarization waves as a result of the formation of a stable depolarization plateau. Under the effect of strychnine there is a marked increase of the interneural responses to which the cerebellar and afferent actions converge. There is an assumption that the effects observed after the administration of strychnine are caused mainly by its action on the activity of the interneurones.

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