THE EFFECT OF STIMULANTS ON THE ELECTRICAL ACTIVITY OF SOLITARY NEURONS OF THE SPINAL CORD

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The intracellular method of recording of the potential of individual neurons enables very precise information to be obtained regarding the character of the changes arising in nerve cells under the influence of drugs. An important defect of this method, considerably limiting its application, is the difficulty of obtaining stable responses over a fairly long period of time. This is because of the traumatizing action of even the thinnest microelectrodes on the nerve cell; moreover, unless the animal is completely immobilized, the tip of the microelectrode may slip out of the cell at any given moment. In a series of cases we have succeeded in recording potentials with an intracellular microelectrode without definite signs of injury to the cell for periods of 60-70 min from motor neurons, and 20-30 min from intercalary neurons. In most cases, however, this period was much shorter. Nevertheless, in view of the rapid development of the effect of drugs when injected intravenously, intracellular recording for as little as 10-15 min can yield sufficiently clear information on the effect of the drug on the electrical activity of the neuron under test.

In the present study, concerned with the investigation of the action of strychnine, securinine, corazole (pentamethylenetetrazole), and caffeine on the electrical activity of the neurons of the spinal cord, we investigated about 100 individual motor and intercalary neurons. The results of the experiments described in this paper are in agreement with data relating to the action of these substances obtained by investigations using the extracellular method of recording the activity of individual neurons of the spinal cord [8].

EXPERIMENTAL METHOD

Experiments were conducted on decerebrate (section at the level of the posterior colliculi) and spinal cats, or on intact cats anesthetized with nembutal (40-50 mg/kg, intraperitoneally). In the last case the animals' natural respiration was maintained. The decerebrate and spinal animals were immobilized with diplacin or tubocurarine, which have no significant effect on the transmission of excitation in the spinal cord [6], and were maintained on artificial respiration. A push-pull apparatus was used, so that in most cases the respiratory movements did not constitute a mechanical obstacle to working with microelectrodes. We therefore did not use a pneumothorax to reduce the movements of the chest wall, as suggested by some writers [3]. The use of muscular relaxants enabled the action of analeptics to be investigated, even in convulsive doses.

The microelectrodes used in the experiments had a tip with a diameter of 0.5-1.0 μ. In most cases they were filled with a 3 M solution of potassium chloride. In some experiments in which the inhibitory postsynaptic potentials were recorded, the electrodes were filled with 0.6 M potassium sulfate [12]. Besides the usual single electrodes, we also used two-channel intracellular microelectrodes [7], permitting direct stimulation of the cell, simultaneously with the registration of its potentials, with pulses of depolarizing current, or enabling its polarization to be modified [5, 6]. The microelectrode was connected through a cathode repeater with a direct current amplifier. The microelectrodes were buried by means of a micromanipulator from the lateral or dorsal surface of the brain.

The neutrons were identified in the usual manner [14]. We used stimulation of the dorsal and ventral roots of the 7th lumbar and 1st sacral segments, and also of the nerves of the lower limbs. All drugs were injected into the external jugular vein.

EXPERIMENTAL RESULTS

The drug with the clearest action on the electrical activity of the spinal cord neurons was strychnine. Recordings made from the motor cells of the anterior horns revealed a considerable increase in the polysynaptic postsynaptic potentials, leading to the appearance of multiple motor neuron discharges [10]. In agreement with reports in the literature [13, 15], we observed depression of the inhibitory postsynaptic potentials during antidromic and polysynaptic activation. Even after large doses of strychnine (0.5-1.0 mg/kg), however, the depression of the inhibitory synaptic potentials was not complete. This was demonstrated particularly clearly during recording of the polysynaptic inhibitory potentials, which could attain an amplitude of 10-20 mv. Doses of strychnine causing a marked change in the electrical activity of the neuron diminished the amplitude of the polysynaptic inhibitory potentials by more than 50-60%. Strychnine had its most marked depressing action on the inhibitory hyperpolarization in cases in which polysynaptic stimulation had a mixed effect on the motor neuron: after application of stimulation a polysynaptic excitation potential developed first, as shown by depolarization of the membrane of the neuron; this then changed smoothly into an inhibitory postsynaptic potential, as found in hyperpolarization. Effects such as these may be obtained by stimulation of the cutaneous nerves. In such cases strychnine may cause depolarization to increase to such an extent that it leads to the generation of an action potential, and the subsequent hyperpolarization is diminished. Similar results have been obtained with the motor neurons of the toad [16]. Although the sensitivity of the individual motor neurons to strychnine varied, the changes described above were recorded in all the cells investigated.

Strychnine also caused a sharp increase in the rhythmic activity of the intercalary neurons, in which multiple discharges developed both after stimulation of the afferent pathways and in the neurons with spontaneous activity. If the cell showed a tendency towards the generation of group responses even before injection of strychnine, then after the latter had been given the number of action potentials rose sharply (Fig. 1, B). This was accompanied by an increase in the exciting postsynaptic potential. In large doses, strychnine sometimes caused the action potential to be separated into two components, presumably as a result of a considerable increase in the local depolarization [10].

The process of inhibition in the intercalary neutrons were much better defined and constant in character than in the motor neurons. Frequently, for instance, the inhibitory effect was not accompanied by perceptible hyperpolarization changes. The most obvious sign of inhibition in the intercalary neurons was the cessation of rhythmic activation under the influence of inhibiting stimuli [9]. After injection of strychnine, the inhibition in the intercalary neurons was weakened (Fig. 1, A). However, in contrast to the experiments on motor cells, the depressing action of strychnine on the inhibition of the intercalary neurons was less constant. Weakening of inhibition was observed in 4 of the 7 neurons investigated.

Securinine had a similar action to strychnine by causing an increase primarily in the polysynaptically exciting postsynaptic potentials of the motor neurons (see Fig. 1). A reduction of the strength of stimulation was accompanied by a decrease in the postsynaptic responses, increased by securinine. However, its action was less constant than that of strychnine: even in large doses (2-5 mg/kg), the drug did not produce such a sharp increase in the polysynaptic responses as did strychnine. Large doses of securinine very rarely caused spontaneous activity of the motor and intercalary neurons, so characteristic of strychnine poisoning. The action of securinine on the inhibitory postsynaptic potentials of the motor neurons was very weak and inconstant.

After injection of caffeine in doses of 0.5-3.0 mg/kg, spontaneous rhythmic activity developed in certain motor and intercalary neurons. In those cells in which rhythmic activity was recorded before injection of the drug, the frequency of the rhythm was increased. In contrast to the spontaneous rhythmic activity arising after injection of strychnine, that caused by caffeine usually consisted of single discharges rather than groups. It should be pointed out that the responses to afferent stimulation arising in cells with spontaneous rhythmic activity following administration of caffeine were not significantly modified. This applied not only to the responses to single stimuli but also to their ability to reproduce tetanic stimulation. The changes in the polysynaptic potentials were most marked in the motor cells, in which synaptic transmission was facilitated by caffeine: both their amplitude and duration were increased. In these cases the ability of the cell to give polysynaptic responses to rhythmic stimulation was also increased, and they could also generate multiple responses. If the dose of caffeine were increased to 10-20 mg/kg, in many neurons the spontaneous rhythm and the responses to afferent stimulation were depressed after a transient burst of activity.

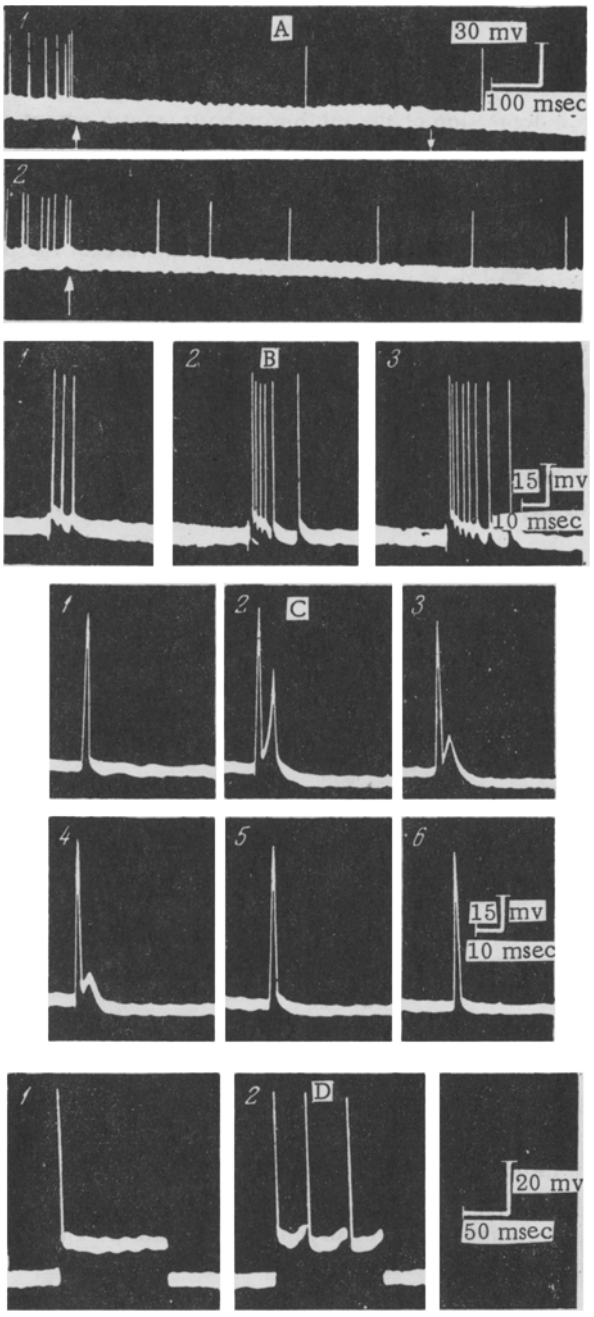

Fig. 1. Effect of strychnine on the electrical activity of single intercalary and motor neurons. A) Inhibition of the rhythmic activity of an intercalary neuron under the influence of afferent stimulation before (1) and after (2) injection of 0.2 mg/kg strychnine. The beginning and ending of stimulation are shown by arrows; B) responses of an intercalary neuron to single afferent stimuli before (1) and 2 min (2) and 5 min (3) after injection of 0.15 mg/kg strychnine; C) responses of a motor neuron to single stimuli applied to a posterior root before (1) and 6 min (2-6) after injection of 2 mg/kg strychnine. Reduction of the strength of stimulation from 2 to 6; D) responses of the motor neuron to direct stimulation by pulses of depolarizing current before (1) and 4 min after (2) injection of 2 mg/kg caffeine.

Even in large doses (10-20 mg/kg), corazole comparatively rarely increased the responses of the motor neurons of the spinal cord. An increase in the amplitude of the exciting postsynaptic potentials was observed in 4 of the 16 cells examined. More often (in 7 cells) corazole did not significantly affect either the monosynaptic activation or the polysynaptic responses of the motor neurons. An example of the responses of the motor neuron to single and rhythmic stimulation of the homolateral posterior root is given in Fig. 2, A. After injection of corazole, the responses of the cell to afferent stimuli were unchanged (Fig. 2, B). Fifteen minutes after the corazole, a small dose of strychnine (0.02 mg/kg) was injected, causing rhythmic activity to develop in the same cell. Against the background of this developing rhythmic activity, single stimuli began to produce multiple discharges, but rhythmic stimulation, in contrast to the recordings made before and after injection of corazole, led to the appearance of an action

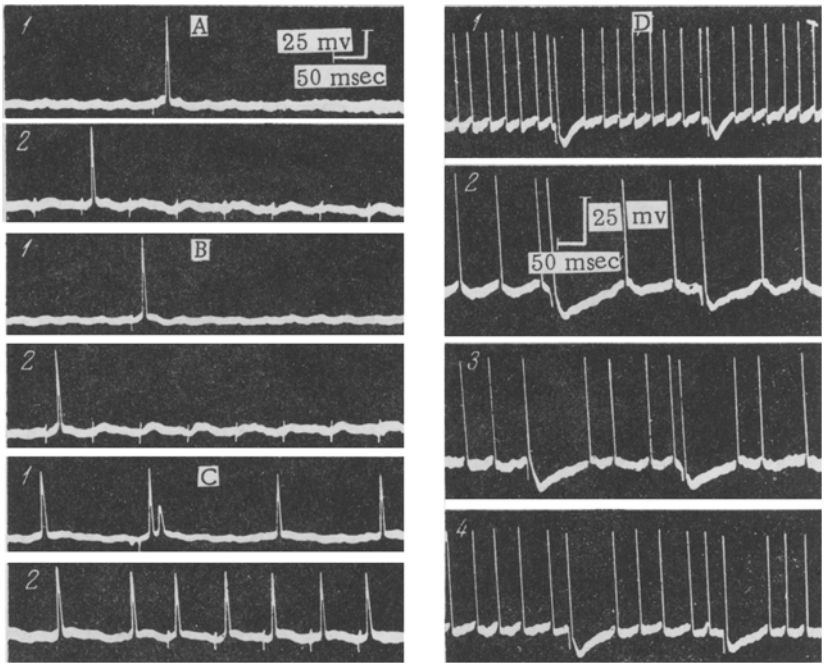

Fig. 2. Effect of corazole and strychnine on the electrical activity of motor neurons. A) Responses of a motor neuron to single (1) and rhythmic (2) stimulation; B) responses of the same cell 5 min after injection of 9 mg/kg corazole (1, 2); C) responses of the same cell 2 min after injection of 0.02 mg/kg strychnine (1, 2); D) spontaneous rhythmic activity of another motor neuron, on which are superimposed inhibitory postsynaptic potentials before (1) and 2 min (2), 5 min(3) and 10 min (4) after injection of 12 mg/kg corazole. Positive stimuli are denoted by loops of current directed downward.

potential in response to each positive stimulus (Fig. 2, C). Meanwhile, in some motor cells (2 units), the injection of corazole was accompanied by an appreciable increase in the amplitude and duration of the inhibitory postsynaptic potentials and a slowing of the background rhythmic activity of the neuron (Fig. 2, D).

Finally, motor neurons were found (3 units) in which the amplitude of the exciting postsynaptic potentials was clearly reduced by corazole (Fig. 3). When different frequencies of stimulation were used, the depressing effect of corazole was most apparent at the lowest frequencies. High-frequency stimulation potentiated the subsequent responses to low-frequency stimuli (Fig. 3, B). That the action of corazole and not injury to the cell was responsible for the observed depression was proved by the gradual restoration of the amplitude of the postsynaptic potentials to a magnitude sufficient to generate an action potential after the administration of strychnine (Fig. 3, C and D). Under the influence of corazole the frequency of the discharges of the intercalary neurons was increased, whether during spontaneous or induced activity.

Our findings suggest that the exciting action of analeptics on the spinal cord is mainly due to the increased activity of the intercalary neurons. It should be noted that after administration of strychnine and caffeine, the

ability of certain motor neurons (3 units) to respond to direct stimulation was increased. Motor neurons reacting to a rectangular pulse of depolarizing current (of a strength of 6-9 $\times 10^{-6}$ A) with a subthreshold response, generated an action potential after the administration of these drugs, while cells responding with a single action potential began to give a multiple discharge. I. A. Zemlyanoi [2] observed an increase in excitability during direct intracellular stimulation of the nuclei of the spinal cord under the influence of caffeine. In every case in which facilitation of the responses of the neuron to direct stimulation was observed, however, either a rhythmic activity developed in the cell or there was an increase in "synaptic noise" and slight depolarization of the membrane. Evidently the change in the responses of the motor neutrons to direct stimulation was not associated with the direct action of the drugs on their membrane, but was due to the background depolarization of the neuron as a result of an increase in presynaptic activity. This point, however, needs further clarification.

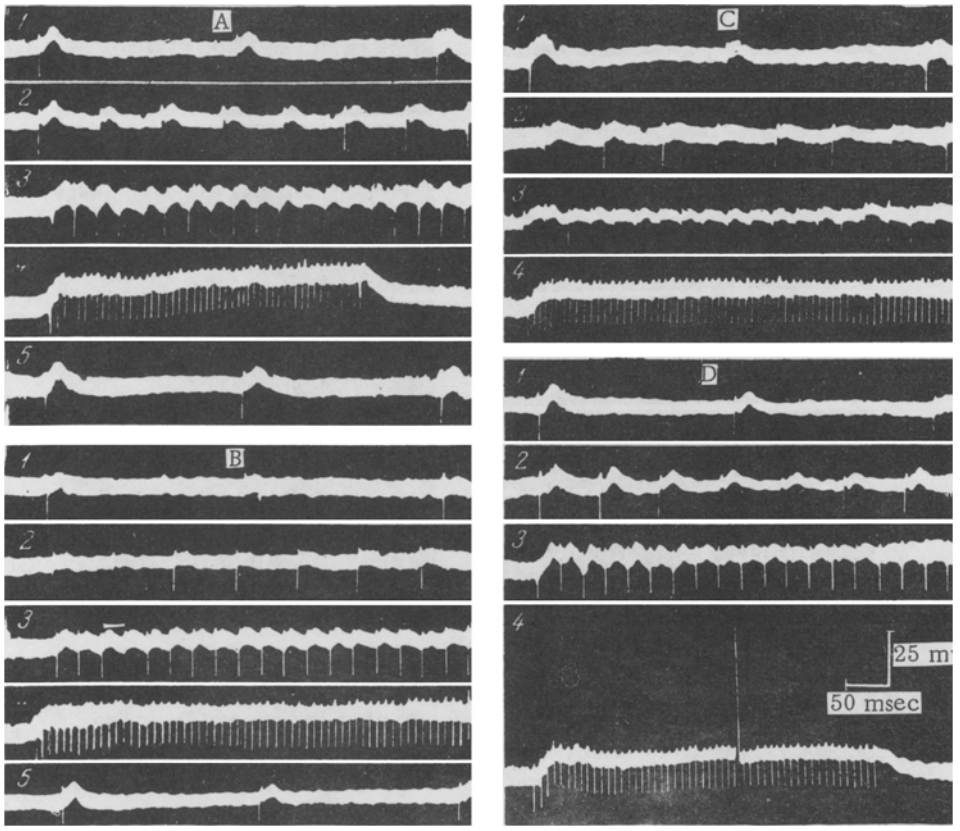

Fig. 3. Effect of corazole and strychnine on the electrical responses of a motor neuron. A) Responses to stimulation of a posterior root with different frequencies (1-5); B) responses of this same cell 3 min after injection of 10 mg/kg corazole (1-5); C) 7 min after injection of corazole (1-4); D) 2 min after injection of 1 mg/kg strychnine (1-4). Stimuli are denoted by loops of current.

The main differences in the action of the various investigated analeptics on the electrical activity of the spinal cord cells may be attributed, firstly, to their different effects on the inhibitory postsynaptic potentials of the motor neurons, and secondly, to their different effects on the intercalary neurons of the spinal cord.

The characteristic feature of strychnine is its ability to depress the inhibitory postsynaptic potentials of the motor neurons, which may be regarded as the result of a blocking of the chemoreceptive areas of the membrane, sensitive to the action of the inhibitory mediator [15]. This property is weak in securinine and is absent in caffeine and corazole. The last, in fact, may increase the inhibitory postsynaptic potentials. In our opinion, the action of strychnine cannot be explained with complete certainty by the removal of the postsynaptic inhibition in various neurons (the lower sensitivity of the polysynaptic inhibiting potentials of the motor neurons and of the inhibition of

the intercalary neurons to strychnine suggests that its effect on the inhibitory processes of these neurons is much weaker than on the motor neurons). Nevertheless there is no doubt that the depressing effect of strychnine on inhibition distinguishes it essentially from the other analeptics.

The special features of the action of drugs such as corazole may be explained by variations in the sensitivity of the different intercalary neurons to them. Under the influence of corazole, intercalary neurons possing an inhibiting action on other cells may beclme activated. This accounts for the slowing of the background activity and the increase in the amplitude of the inhibitory postsynaptic potentials observed in our experiments. It should be pointed out that other workers [17] have reported the possible activation of intercalary neurons of the inhibiting pathways by pentamethylenetetrazole. This may also explain the decrease in the amplitude of the exciting postsynaptic potentials observed in some of our experiments. There is also the possibility that corazole can activate not only the neurons through which the postsynaptic inhibition is effected, but also the intercalary cells, responsible for presynaptic inhibition [11], leading to a reduction in the efficacy of the exciting action of the presynaptic nerve endings.

The differences in the degree of the stimulating effect of the investigated analeptics on the electrical activity of the spinal cord neurons are in agreement with the reports in the literature [1] indicating that each of these substances acts preferentially on particular divisions of the central nervous system.

SUMMARY

The effect exerted by strychnine, securinine and corazole (pentamethylenetetrazole) on the electric activity of single motor and neurons of the spinal cord, was studied by intracellular recording of the potentials. The data obtained demonstrated that the stimulants studied had a different effect on inhibitory processes in the spinal cord. Due to this, with the action of some substances (corazole) there was not only a rise, but also a depression of the activity of the individual neurons. The excitatory action of the stimulants was mainly directed upon the neurons of the spinal cord.

LITERATURE CITED

1. V. V. Zakusov, The Pharmacology of the Nervous System [in Russian] (Leningrad, 1953).
2. I. A. Zemlyanoi, Byull. Éksper. Biol. 1, 63 (1961).
3. P. G. Kostyuk, Microelectrode Technique [in Russian] (Kiev, 1960).
4. P. G. Kostyuk and A. I. Shapovalov, Byull. Éksper. Biol. 9, 8 (1960).
5. P. G. Kostyuk and A. I. Shapovalov, Biofizika, 5, 586 (1960).
6. A. I. Shapovalov, Fiziol. Zh. SSSR, 8, 952 (1959).
7. A. I. Shapovalov, Biofizika 1, 79 (1960).
8. A. I. Shapovalov, Proceedings of the Ninth All-Union Pharmacological Conference [in Russian], p. 305 (Sverdlovsk, 1961).
9. A. I. Shapovalov, Doklady Akad. Nauk SSSR 141, 5, 1267 (1961).
10. A. I. Shapovalov, Doklady Akad. Nauk SSSR 145, 6, 1424 (1962).
11. J. Eccles, P. G. Kostyuk, and R. Shmidt, Fiziol. Zh. (Ukr.), 1, 21 (1962).
12. J. S. Coombs, J. C. Eccles, and P. Fatt, J. Physiol. (London, 1955), v. 130, p. 374.
13. D. R. Curtis, J. Physiol. (London, 1959), v. 145, p. 175.
14. J. C. Eccles, The Physiology of Nerve Cells (Baltimore, 1957).
15. J. C. Eccles, Biochem. Pharmacol. 1961, v. 8, p. 105.
16. M. Kuno, Jap. J. Physiol. 1957, v. 7, p. 42.
17. J. Lewin and D. W. Esplin, J. Pharmacol. exp. Ther. 1961, v. 132, p. 245.
