ACTION OF STRYCHNINE ON INHIBITORY NEURONAL SYNAPSES OF THE ISOLATED CRUSTACEAN STRETCH RECEPTOR

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Strychnine is one of the drugs most commonly used for blocking inhibitory transmission in the central nervous system. The depressant action of strychnine on postsynaptic inhibition has repeatedly been demonstrated in experiments using intravenous injection, local application, and microphoretic injection techniques [3, 4]. It is not certain, however, whether it acts on the subsynaptic membrane of the chemoreceptor or on the presynaptic endings of the inhibitory synapses. The fact that some types of inhibitory influences are not blocked by strychnine has been attributed to their presynaptic character [5]. Nevertheless, work has recently been published in which the existence of strychnine-resistant postsynaptic inhibition of neurons has been demonstrated in various parts of the central nervous system (spinal cord motoneurons, Purkinje cells, neurons of the hippocampus and thalamus, Betz cells) [1, 2, 7, 8]. Washizu and co-workers [13], who studied the action of strychnine on the neuron of the crustacean stretch receptor, observed that the inhibitory effect was preserved during stimulation of the inhibitory fiber.

These results, together with the comparative simplicity of synaptic organization and the high level of study of its physiology [6, 9], have provided the most favorable conditions for studying certain aspects of the action of strychnine on inhibitory synapses of crustacean stretch receptor neurons. The results of these investigations are described in the present paper.

EXPERIMENTAL METHOD

The preparation was immersed in Harreveld's solution by means of a micromanipulator, and the nerve trunk, held on two silver electrodes, was lifted into a layer of mineral oil. One of the two neurons was of the fast-adapting, the other of the slow-adapting type, so that with constant tension of the muscle bundles rhythmic activity of only the slow-adapting neuron was observed. Intracellular recordings were made by means of microelectrodes with a resistance of 20-80 m Ω, filled with 3 M KCl solution. Intracellular polarization was carried out through the recording microelectrode by means of a bridge circuit. Strychnine nitrate (0.5%) was injected by a syringe into the solution close to the preparation.

EXPERIMENTAL RESULTS

Rhythmic action potentials (AP) of a slowly adapting neuron are shown in Fig. 1, 1. The resting potential of the soma was 75 mV. During moderate stretching of the muscle bundles a generator potential was produced in the distal parts of the dendrites, spreading electrotonically to cause depolarization of the soma. When the membrane potential was lowered by 12 mV, an AP developed, terminating with a positive deflection. In some cases of preparations taken from crustaceans which had recently completed molting, after injection of strychnine,AP of the fast-adapting neuron were recorded from the nerve trunk, usually of higher amplitude than the AP of the slow-adapting neuron. These AP of the fast-adapting neuron could be observed throughout the time of action of strychnine, which thus converted the fast-adapting neuron into a slow-adapting type. In addition, much smaller AP were recorded from the electrodes of the nerve trunk, reflecting the spread of excitation along the thinner inhibitory fiber (Fig. 1, 2). When intracellular recordings were taken these corresponded to hyperpolarization inhibitory postsynaptic potentials (IPSP), which effectively suppressed the activity of both the fast and slow adapting neurons. The AP of the inhibitory fiber, was negative-positive in direction (Fig. 1, 3), and together with its temporal relationship to the IPSP, this indicates that its point of origin was evidently the nonmedullated presynaptic ending.

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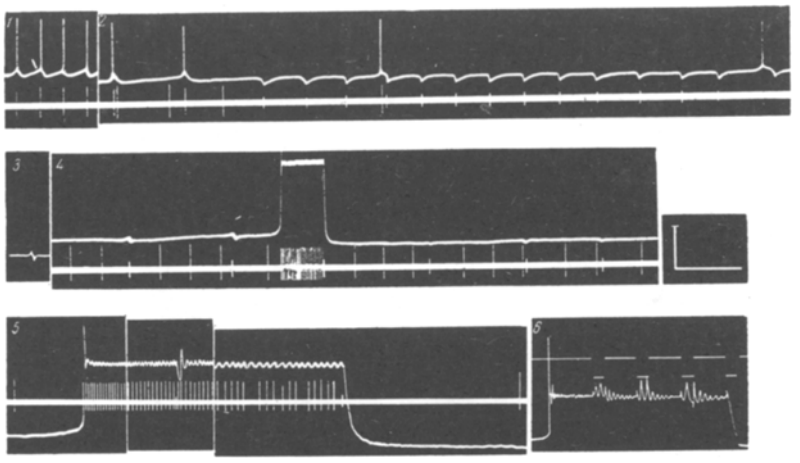

Fig. 1. Origin and effect of IPSP after injection of strychnine. 1) Normal; recording from axon (below) and soma; 2) beginning of action of strychnine; 3) AP of inhibitory fiber; 4) inhibition of AP and steep decline of prolonged action potentials of neurons; 5) effect of IPSP on plateau (to save space two portions of the plateaus have been cut out); 6) direct hyperpolarizing impulses during plateau (strength $5 \cdot 10^{-10}$ A); 1, 2, 4, 5) the same preparation. Amplification 44 mV (1, 2) and 22 mV (4, 5). Time calibration 40 msec (3), 80 msec (5), and 400 msec (1-2, 4-6).

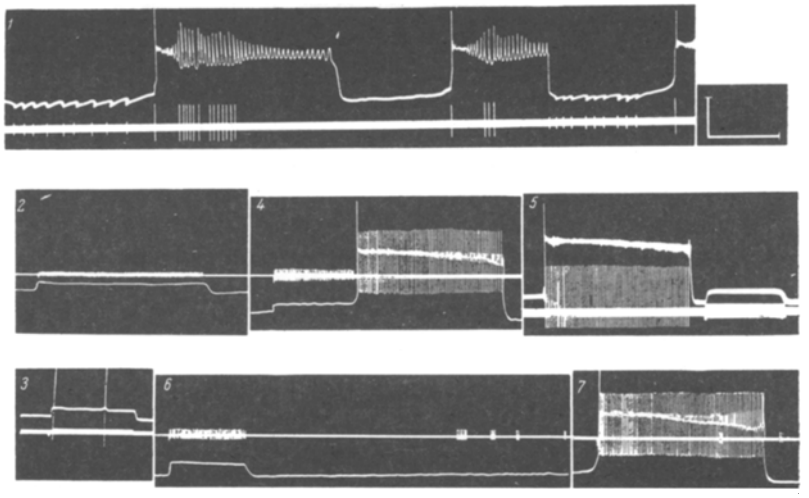

Fig. 2. Transformation of hyperpolarization IPSP into depolarization. 1) Beginning of action of strychnine; 2) multiple discharge of inhibitory fiber (above) and depolarization IPSP; 3) activation of neuron by means of IPSP; 4, 5) IPSP for different levels of membrane potential; 6, 7) two types of multiple discharges of inhibitory fiber. The top part (3) is cut off. Amplification 22 mV, time calibration 400 msec (1, 2) and 1100 msec (3-7).

According to some observations [11, 14], certain pharmacological substances increase the activity of motor presynaptic endings in warm-blooded animals, leading to the appearance of rhythmic activity or to an increase in such activity during stimulation. When the action of strychnine was prolonged, the repolarization phase of the AP of the slow-adapting neuron became drawn out into a depolarization plateau, accompanied by oscillations of membrane potential. The depolarization gradually diminished while the amplitude of the oscillations on the plateau increased, so that when a critical level was reached the plateau terminated abruptly and changed into a positive deflection. The oscillations on the plateau were accom-

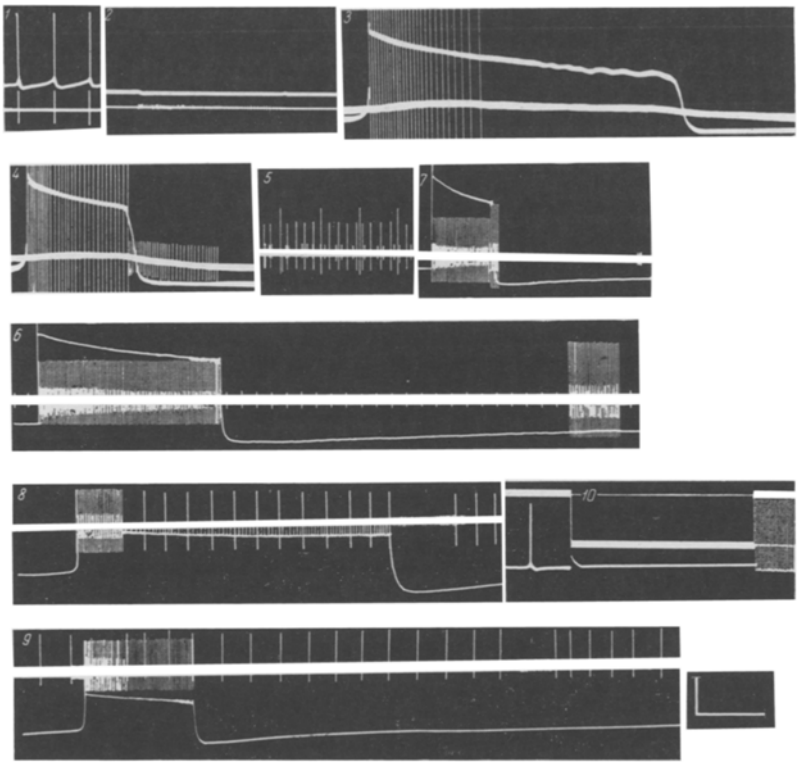

Fig. 3. Origin and action of multiple discharges of an inhibitory fiber. 1) Normal; 2) multiple discharge and its reflection in the soma; 3-4) two successive PAP, one of which was formed by a discharge of IPSP; 5) AP of axons of receptor neurons and inhibitory fiber; 6-7) AP and multiple discharges of inhibitory fiber during grouped discharges of neurons; 8, 9) inhibition of fast-adapting neuron; 10) action of direct hyperpolarizing current (strength $5 \cdot 10^{-9}$ A). 1-4) One preparation, and 5-9) another preparation. Amplification 44 mV (1-2, 8-10) and 22 mV (3-4, 6-7). 3-4) AP from axons amplified four times compared with AP in 1 and 2. Time calibration 400 msec (1-5, 10), 1100 msec (6, 8),and 1700 msec (7, 9).

panied by a multiple discharge of AP spreading to the axon, corresponding either to each oscillation on the plateau or only to the largest oscillations. A similar transformation, but with considerable delay, took place in the fast-adapting neuron. Prolonged action potentials (PAP) could be traced for several hours. The effect was completely reversible after rinsing the preparation free from strychnine. The spontaneous activity of the inhibitory fiber accompanying the IPSP also remained at these stages. It is clear from Figs. 1, 4 and 2, 1 that a single hyperpolarization IPSP caused disappearance of the SP from the rhythmic activity of the fast-adapting neuron and, if it coincided in time, a steep decline of the PAP plateau of the slow-adapting neuron. The action in the last case must be particularly effective, for the equilibrium potential of the inhibitory synapses of this particular preparation is 70 mV [9].

For comparison, two PAP are shown in Fig. 2, 1, one of which terminates "naturally," while the other is terminated abruptly by an IPSP. The relationship between amplitude of the IPSP and level of the membrane potential can also be seen from Figs. 1, 4 and 2, 1. An intracellular hyperpolarization pulse of current applied during the PAP plateau increased the membrane potential and amplitude of the oscillations on the plateau. As the level of depolarization of duration gave an increasing effect, and one pulse increased the membrane potential to the critical for abrupt termination of the plateau (Fig. 1, 6). The action of an intracellular pulse of hyperpolarizing current was similar to the effect of the IPSP arising during the plateau (Fig. 1, 5): the first IPSP arising during the PAP plateau caused a hyperpolarization deflection of 11 mV, followed by a large wave, while the second IPSP, appearing in the later part of the plateau, caused it to terminate abruptly.

A characteristic feature of the inhibitory fiber during its spontaneous activity was its ability to discharge with multiple AP when the action of strychnine was intensified. In these circumstances the IPSP become superposed and form a steady decrease in depolarization of the membrane, lasting throughout the period of discharge of the inhibitory fiber (Fig. 3, 2). Since the equilibrium potential is reached with the first IPSP, no summation of the IPSP was observed. Such prolonged hyperpolarization was able to produce effective inhibition and rapid decline of the plateau at a high level of depolarization (Fig. 3, 4). In some cases rhythmic activity of the inhibitory fiber was not accompanied by inhibition of the slow and fast-adapting neurons (Fig. 3, 5). Inhibition likewise did not take place when the PAP of these neurons appeared, and in this case intracellular recording from the slow-adapting neuron showed absence of IPSP in it (Fig. 3, 6) at various levels of depolarization of the membranes. When the action of strychnine was continued, rhythmic AP of the inhibitory fiber were grouped together and formed multiple discharges (Fig. 3, 7). Such a multiple discharge could inhibit the activity of the fast-adapted neuron, which again discharged as single AP as a result of a decrease in the strychnine concentration. In these circumstances the effective inhibitory action disappeared at the end of the multiple discharge, when its frequency was reduced (Fig. 3, 8). A similar "escape" could also be observed during inhibition by a weak intracellular current. At the same time, both IPSP and inhibition of the slow-adapting neuron were absent as before (Fig. 3, 9); the phenomenon of post-inhibitory facilitation was observed – a temporary increase in frequency of AP of the neuron after the end of a multiple discharge of the inhibitory fiber. This phenomenon was evidently analogous to "anode-closing excitation" caused by an intracellular pulse of hyperpolarizing current against the background of membrane depolarization (Fig. 3, 10).

In some cases the hyperpolarization IPSP were converted into depolarization. In Fig. 2, 2, for example, a multiple discharge of an inhibitory fiber can be seen, accompanied by a depolarization plateau, which developed from ordinary single hyperpolarization IPSP (Fig. 2, 1). The initial frequency of the multiple discharge was 220 per second. After the discharge had ceased the membrane potential was gradually restored, giving rise in the case of hyperpolarization to postinhibitory depression, preceding postinhibitory facilitation, as was also observed during high-frequency stimulation of an inhibitory fiber [9]. The multiple discharge accompanied by depolarization could evoke an AP in the neuron (Fig. 2, 3). The degree of depolarization produced by the IPSP depended on the level of the soma membrane potential (Fig. 2, 4 and 5). Since this depolarization was higher than the critical level of discharge of the neuron, and consequently, higher than the equilibrium potential of the IPSP, it could not be explained by an increase in polarization of the membrane. It was probably due to diffusion of Cl ions from the microelectrode, although special proof of this suggestion is required, for results have recently been obtained [10, 12] suggesting that changes in the equilibrium potential in the direction of greater depolarization may take place under the influence of strychnine. Besides prolonged multiple discharges of the inhibitory fiber, shorter discharges unaccompanied by changes in the soma membrane potential or by any influence on the PAP of the slow-adpating neuron could also be recorded in it (Fig. 2, 6 and 7). These potentials evidently reflected excitation of the presynaptic ending on the dendrite of the fast-adpating neuron, not crossing by axon-reflex to the branch terminating in synapses on dendrites of the slow-adapting neuron. In this case, absence of inhibition in the slow-adapting neuron during discharge of the inhibitory fiber, inhibiting the fast-adapting neuron (Fig. 3, 8 and 9) was also possibly of the same nature. It may be postulated that the multiple discharge of nonmedullated presynaptic endings of the inhibitory fiber is based on the same changes in their membrane as are found in the soma of both neurons, and that they lead to formation of a PAP and, as a result of this, to emergence of discharges on their axons.

The results obtained thus show that under the influence of strychnine a spontaneous activity arises in the inhibitory fiber, evidently in its presynaptic endings, and this is subsequently converted into multiple discharges. Even at the stage of PAP of the neurons, in these circumstances no block to inhibitory synaptic transmission is present.

LITERATURE CITED

1. P. Andersen, J. C. Eccles and P. E. Voorhoeve, Nature, 200, 843 (1963).
2. J. M. Crawford, D. R. Curtis, P. E. Voorhoeve et al., Ibid, 845.
3. D. R. Curtis, Int. J. Neuropharmacol, 1, 239 (1962).
4. J. C. Eccles, P. Fatt, and K. Koketsu, J. Physiol. (Lond.), 126, 524 (1954).
5. J. C. Eccles, R. F. Schmidt, and W. D. Willis, Ibid, 168, 500 (1963).

6. S. Hagiwara, K. Kusano, and S. Saito, J. Neurophysiol., 23, 505 (1960).
7. J. O. Kellerth, Acta Physiol. Scand., 63, 469 (1965).
8. K. Krnjevic, M. Randic, and D. W. Straugham., Nature, 201, 1294 (1964).
9. S. W. Kuffler and C. Eyzaguirre, J. Gen. Physiol, 39, 155 (1955).
10. D. A. Pollen and C. Marsan Ajmone, J. Neurophysiol., 28, 342 (1965).
11. W. F. Riker, Jr., G. Werner, J. Roberts et al., J. Pharmacol. Exp. Ther., 125, 150 (1959).
12. C. Stefanis and H. Jasper, Int. J. Neuropharmacol., 4, 125 (1965).
13. Y. Washizu, G. W. Bonewell, and C. A. Terzuolo, Science, 133, 333 (1961).
14. G. Werner, J. Neurophysiol., 23, 171 (1960).