HYPERPOLARIZING ACTION OF GLYCINE ON MOTONEURONS BLOCKED BY TETANUS TOXIN

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Microelectrophoretic injection of glycine, the probable mediator of postsynaptic inhibition in the spinal cord, close to the outer surface of the membrane of motoneurons in the cat spinal cord, in which generation of IPSPs is blocked by tetanus toxin, causes hyperpolarization of the postsynaptic membrane of these motoneurons. The inhibitory action of glycine on motoneurons blocked by tetanus toxin, consisting of hyperpolarization of the membrane, blocking of antidromic excitation of the motoneurons, a decrease in the input resistance of the membrane, and a decrease in amplitude of the EPSPs, is analogous to that on motoneurons of healthy cats. It is concluded that inhibition of IPSPs by tetanus toxin is evidently due to blocking of liberation of inhibitory mediator from presynaptic endings of inhibitory neurons.

The writers have previously shown [1] that spinal cord motoneurons of cats, in which IPSP generation is blocked by tetanus toxin, remain sensitive to the inhibitory action of the probable mediator of postsynaptic inhibition, namely glycine. If glycine is injected electrophoretically close to the outer surface of membranes blocked by tetanus toxin, it prevents their antidromic excitation, reduces the input resistance of the membrane, and reduces the amplitude of EPSPs.

However, for technical reasons it was impossible to obtain convincing proof that glycine causes hyperpolarization of the postsynaptic membrane of motoneurons blocked by tetanus toxin. This proof was obtained in the course of further investigations, the results of which are described below.

EXPERIMENTAL METHOD

Cats weighing 2-4 kg received an injection of 1500-2000 mouse LD_{50} of tetanus toxin into the posterior group of muscles of the left leg. When rigidity of the muscles of the injected limb had developed, 40 h after the injection, the lumbosacral enlargement of the spinal cord was exposed under nembutal anesthesia (25-30 mg/kg, intravenously). The ventral roots of segments S_6-S_2 were divided. The spinal cord was transsected at the level of the last rib. Cutaneous and muscular nerves in the left hind limb were dissected for stimulation. Coaxial microelectrodes were used. The inner core of the electrode, filled with 0.6 M K_2SO_4 solution and with a tip about 1 μ in diameter, was used for intracellular recording of potentials. The outer core, with a tip 3-5 μ in diameter, was separated by a distance of 30-70 μ from the protruding inner core, and was filled with 1 M glycine solution (pH 3.0), which could be injected electrophoretically close to the outer surface of the punctured motoneuron. Details of the method were described previously [1].

EXPERIMENTAL RESULTS AND DISCUSSION

Intracellular recordings of potentials of a motoneuron in segment S7 of a cat receiving tetanus toxin are shown in Fig. 1A. A monosynaptic EPSP and an accompanying spike discharge were generated in res-

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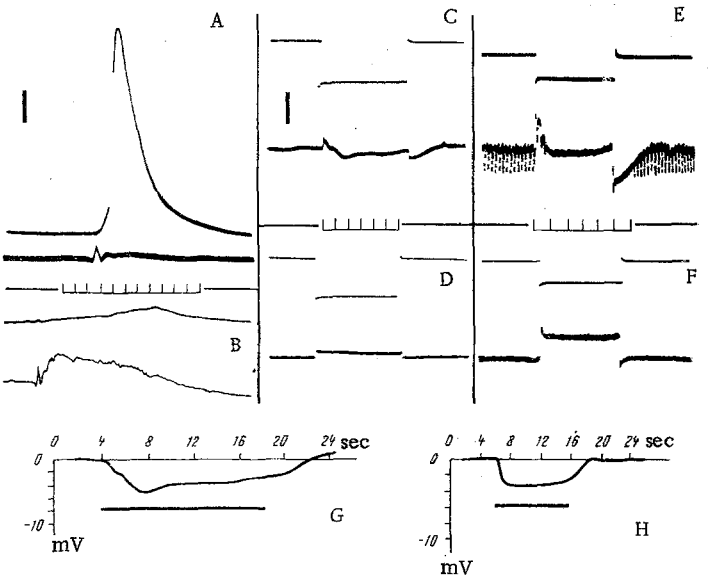

Fig. 1. Action of glycine on membrane potential of motoneurons blocked by tetanus toxin. A, B: Top beam shows intracellular recording of potentials of motoneuron in segment S7 in response to stimulation of nerves G (in A) and PP in (B) by single pulses above the threshold for group 1 afferent fibers; bottom beam shows dorsal surface potential of segment L6 in zone of entry of afferent fibers into spinal cord; C, D: bottom beam shows changes in potential of inner core of coaxial microelectrode in intracellular (C) and extracellular (D) position, evoked by injection of glycine; top beam used to record intensity of injecting current (downward deflection of beam corresponds to beginning of injection); G) curve showing true changes in membrane potential evoked by glycine. Abscissa: time from an arbitrary moment to beginning of injection of glycine; ordinate: changes in potential recorded by intracellular microelectrode during injection of glycine, disregarding jump of potential due to presence of resistance in connection between inner and outer cores (see text). Potential of inner core at moment of time corresponding to zero on abscissa taken as zero. Horizontal line indicates time of injection of glycine; E (bottom beam) shows action of glycine on antidromic potentials of FDHL motoneuron; F) change in potential of inner electrode (bottom beam) in extracellular position during injection of glycine; H) curve showing changes in membrane potential of FDHL motoneuron, plotted in a similar manner to curve G for recordings E and F (see text). Vertical line in C represents 10 mV for bottom tracings in C and D; 4 mV for bottom tracings in E and F; 200 nA for top tracings in C and D, and 500 nA for top tracings in E and F. Time for A and B 1 msec, for C and F 2 sec.

ponse to a volley of afferent impulses evoked by stimulation of the nerve to the gastrocnemius muscle (G). According to the criteria of Eccles et al. [4], this motoneuron must belong to the motor nucleus of the extensor G muscle.

Stimulation of the nerve to the antagonistic muscles – the deep peroneal nerve (PP) by stimuli above the threshold strength for group 1 afferent fibers did not evoke IPSPs in this motoneuron (Fig. 1 B), just as in the motoneurons of the G nucleus in normal cats. It is also clear from the curve in Fig. 1 B that afferent volleys in PP produce depolarization of the G motoneuron, characteristic of the action of impulses in afferents of the flexor reflex (AFR) on motoneurons of extensor muscles in tetanus [2].

Changes of potential recorded by the microelectrode when inside that same motoneuron, during electrophoretic injection of glycine close to the outer surface of the motoneuron, are shown in Fig. 1 C. The

tracing in Fig. 1 D was made after withdrawal of the inner core of the microelectrode from the motoneuron. Injection of glycine in this case by a current of the same strength as in recording 1 C changed the potential of the inner electrode. The steady shift of potential was due to the presence of a definite resistance in the connection between the inner and outer cores of the coaxial microelectrode.

The curve in Fig. 1 G is the result obtained by subtracting the change in potential of the inner core during injection of glycine (Fig. 1 D) with the electrode in the extracellular position from the change in potential of the inner core during injection of glycine (Fig. 1 C) with the electrode in the intracellular position. It evidently shows the true changes in membrane potential evoked by the action of glycine. Definite hyperpolarization of the membrane (an increase in negativity of the intracellular electrode) can be seen, reaching a maximum (about 5 mV) 4 sec after the beginning of injection. The membrane potential returned to its initial level 4 sec after the end of injection of glycine.

An action of glycine analogous to that shown on these recordings was also observed on motoneurons of other extensor and flexor muscles of the hind limb, in which IPSPs were blocked by tetanus toxin. In Fig. 1 E, for example, the action of glycine is demonstrated on antidromic discharges of a motoneuron in the motor nucleus of the flexor muscles of the ankle joint (flexor digitorum, flexor hallucis longus – FDHL), in which development of IPSPs in response to stimulation of type 1a afferent fibers of nerves to the antagonistic muscles was absent. Antidromic discharges were evoked by stimulation of the ventral root of L_7 by repetitive stimuli (3/sec) and were recorded at slow scanning speed. Fast waves, corresponding to spike potentials, were not seen on the recording, but slow deflections of the beam, corresponding to after-hyperpolarization, were clearly visible. Injection of glycine was accompanied by total blocking of antidromic discharges from the motoneuron, recovering soon after the end of the injection.

The change in potential of the inner electrode, when in the extracellular position, at the time of injection of glycine by a current of the same strength as in curve 1 e, is shown in Fig. 1 F.

Curve 1 h was plotted by the same method as curve 1 g, without allowing for fluctuations due to antidromic excitation of the motoneuron. In this case also, hyperpolarization of the motoneuron membrane was clearly apparent, reaching a maximum 2 sec after the beginning of glycine injection.

Glycine, therefore, produces hyperpolarization of the postsynaptic membrane of spinal motoneurons in which the generation of IPSPs is blocked by tetanus toxin. A particularly important feature is that glycine hyperpolarization can be recorded in motoneurons of extensor muscles, blocked by tetanus toxin, in which volleys of impulses in the AFR not only do not evoke IPSPs, but produce depolarization of the postsynaptic membrane. This fact clearly shows that substitution (during the action of tetanus toxin) of the inhibitory action of impulses in the AFR on motoneurons of the extensor muscles by an excitatory action is not the result of the conversion of inhibition into excitation, but is due the demasking of the excitatory action of some fibers of the AFR system on these motoneurons after inhibition of the IPSP.

In conjunction with other data [1, 3], the results described above show that motoneurons, when blocked by tetanus toxin, respond to the action of an inhibitory mediator in precisely the same way as motoneurons of normal cats. Inhibition of IPSPs by tetanus toxin is therefore due, probably, to blocking of the liberation of inhibitory mediator from the presynaptic endings of inhibitory neurons.

LITERATURE CITED

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