SYNAPTIC EFFECTS FROM THE VENTRAL COLUMNS ON LUMBAR MOTONEURONS IN THE FROG

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Stimulation of the ventral columns leads to the appearance of monosynaptic EPSPs in the spinal motoneurons of frog under normal conditions and in the late stages after division of the spinal cord leading to degeneration of descending fibers of supraspinal origin. Monosynaptic EPSPs recorded before and after transection of the spinal cord were similar in their temporal course and exhibited slight facilitation to paired stimulation. It is postulated that monosynaptic excitatory influences evoked by activation of fibers of the ventral columns are due chiefly to involvement of the descending propriospinal pathways.

Experiments on cats have shown that stimulation of the ventrolateral columns causes EPSPs in lumbar spinal motoneurons [4]. These effects may depend on the activity both of fibers of the cerebrospinal tracts and also of descending propriospinal fibers [1]. In amphibians descending projections with monosynaptic effects on lumbar motoneurons have been found in the lateral columns of the spinal cord [3].

The object of the present investigation was to study descending synaptic influences from fibers of the ventral columns on the lumbar motoneurons of the frog and to determine their supraspinal or propriospinal origin.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated spinal cord of frogs (Rana ridibunda). The spinal cord was placed in a transparent plastic chamber with a continuous flow of Ringer's solution. Motoneuron potentials were recorded intracellularly by glass microelectrodes filled with 3 M KCl solution with an impedance of 10-40 MΩ. The motoneurons were identified by their antidromic responses to stimulation of the 9th ventral root. The anterior and lateral columns were stimulated by bipolar nichrome electrodes with interpolar distance 100-200 μ, applied at the levels of the 6th-7th or 3rd segments. The strength of the stimulating current varied from 25 to 200 μA and the duration of the stimulus was 0.1 msec. Frogs used in some experiments had their spinal cord divided at the level of the 3rd segment 4-5 weeks before the experiment. The degeneration of the fibers of the cerebrospinal tracts arising as a result made it possible to distinguish effects due entirely to activation of the descending propriospinal fibers.

EXPERIMENTAL RESULTS

Altogether 162 motoneurons were tested in the experiments on intact frogs. Application of a single stimulus to the ventral columns at the level of the 6th segment evoked EPSPs with a short latent period of 1.2-2.2 msec (mean 1.74 ± 0.21 msec). A histogram of distribution of the latent periods is given in Fig. 1. In specimens kept in normal Ringer's solution the duration of the EPSPs was measured in tens of milliseconds (Fig. 1A). These EPSPs were unstable and irregular in shape, undoubtedly on account of the participation of polysynaptic components. After the addition of pentobarbital to the Ringer's solution in a concentration of 1:5000, the polysynaptic components of the response were inhibited, leaving only a short-latency,

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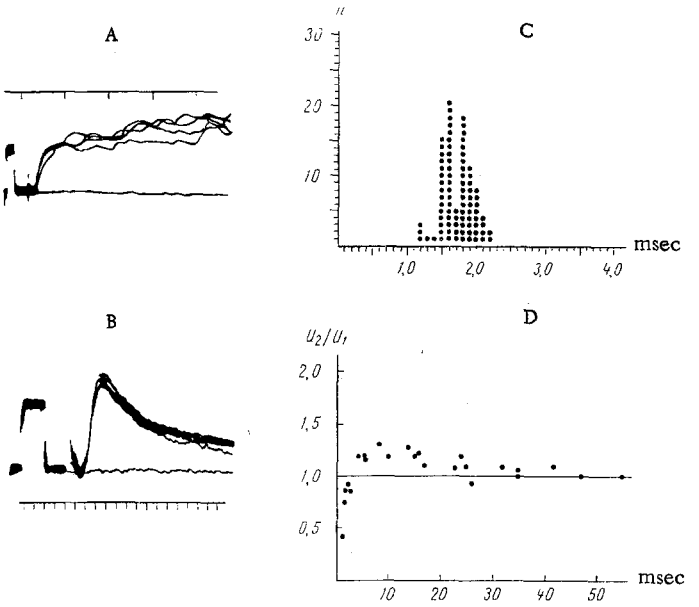

Fig. 1. EPSPs evoked from ventral column at level of 6th segment: A) response of motoneuron to single stimuli (before inhibition of polysynaptic activity by pentobarbital); B) monosynaptic EPSP evoked after inhibition of polysynaptic activity by pentobarbital; C) histogram of latent periods of EPSPs; abscissa–latent period (in msec), ordinate–number of cells; D) graph showing amplitude of test monosynaptic EPSP as a function of interval between paired stimuli; abscissa – interval between stimuli (in msec), ordinate – ratio between amplitude of test EPSP and amplitude of initial EPSP. Time marker for A and B, 10 and 1 msec, respectively. Calibration pulse for A and B, 5 and 2 mV, respectively.

stable EPSP of simple shape (Fig. 1B), and having regard to the latent period and segmental delay, which in frogs is 1.1-2 msec [2], this EPSP can be regarded as monosynaptic. The duration of the ascending phase of the monosynaptic EPSPs evoked from the ventral columns at the level of the 6th segment varied from 1.2 to 2.6 msec, with a mean value of 1.66 ± 0.33 msec, and the time constant of the descending phase was 7.8-12.6 msec (mean 9.5 ± 0.5 msec). By these parameters the monosynaptic EPSPs from the ventral columns were very similar to the monosynaptic EPSPs evoked from the lateral columns and described previously by Fadiga and Brookhart [3]. These responses were not due to activation of the same descending pathways, for if stimulation of the ventral and lateral columns was combined, summation was almost linear. On repeating the stimulation slight facilitation of the monosynaptic EPSPs was observed if the interval between stimuli was between 3 and 40 msec (Fig. 1D). The maximal degree of facilitation was 110-150%. With shorter intervals, inhibition of the second response was usually observed. The maximal amplitude of the monosynaptic EPSPs evoked from the ventral columns at the level of the 6th segment was 1.5-4.7 mV. With an increase in the strength of stimulation the amplitude increased either gradually (Fig. 2A-G) or stepwise (Fig. 2J). In some cases monosynaptic EPSPs of "all or nothing" type appeared (Fig. 2L). In the latter case involvement of a limited number of descending axons can reasonably be assumed.

A distinguishing feature of the monosynaptic EPSPs evoked from the ventral columns was a short wave of potential observed frequently (in 30% of cases) before the beginning of the EPSP (shown by an arrow in Fig. 2G). This prepotential, which was particularly marked during strong stimulation, disappeared as soon as the electrode was withdrawn from the cell. This prepotential can evidently be regarded as an electrotonic effect connected with the influence of presynaptic fibers on the motoneuron tested. In the case of stimulation of the later columns, electrotonic prepotentials were never observed.

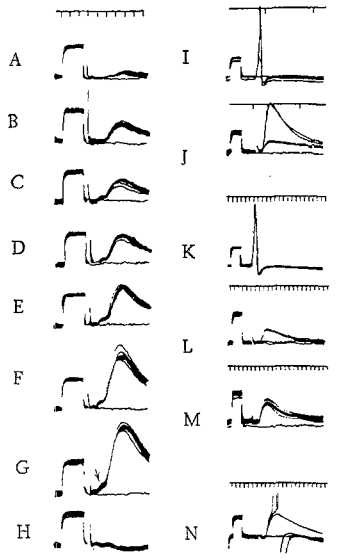

Fig. 2

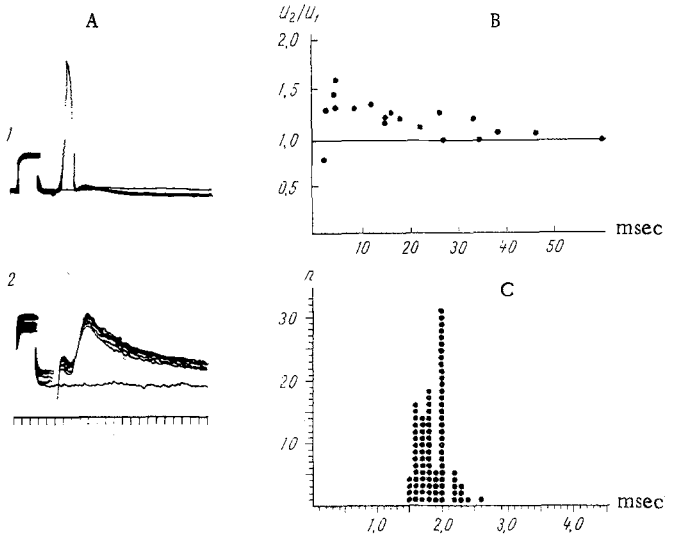

Fig. 3

Fig. 2. Monosynaptic EPSPs evoked from ventral column at level of 6th segment. Recording from 4 different motoneurons: A–H, I–J, K–M, N. A–G) response to single stimuli of gradually increasing strength (20, 25, 30, 40, 45, 70, and 100 μA, respectively). Arrow on record G denotes component preceding monosynaptic EPSP; H) extracellular recording immediately after withdrawal from cell; I, K) antidromic action potentials; J) monosynaptic EPSPs in response to single stimuli with a strength of 50, 70, 80, and 100 μA; response increases stepwise; L) monosynaptic EPSP of "all or nothing" type; strength of stimulus 70 μA; M) same response to stronger stimulus (120 μA); N) appearance of action potential at peak of monosynaptic EPSP; strength of stimulus 300 μA. Time marker 1 msec for A–H, K–N and 10 msec for I, J. Calibration pulse 2 mV for A–H, J, L, M, 5 mV for N, and 20 mV for I, K.

Fig. 3. Monosynaptic EPSPs evoked in preparations in the late period after transection of the spinal cord: A, 2) response of motoneurons to single stimulation of ventral columns; A, 1) antidromic action potential of the same motoneurons; B) graph showing amplitude of test EPSP as a function of interval between paired stimuli; abscissa – interval between stimuli (in msec), ordinate – ratio between amplitude of test EPSP and amplitude of original EPSP; C) histogram of latent periods of EPSPs; abscissa – latent period (in msec), ordinate – number of cells. Time marker 1 msec, calibration pulse for A, 1 and 2) 20 and 2 mV, respectively.

Short-latency EPSPs could also be evoked by stimulating the ventral columns at the level of the 3rd segment. The latent periods of these responses, recorded in 71 motoneurons, varied from 1.6 to 3.4 msec with a mean value of 2.32 ± 0.31 msec, indicating that the latent period was increased slightly on account of an increase in the length of the descending pathway. The conduction velocity of this pathway, measured from the difference between the latent periods and the distance between the points stimulated, was 10-20 m/sec. The amplitude of the short-latency EPSPs of this group varied from 1.4 to 3 mV, with a mean value of 2.22 ± 0.7 mV. The duration of the ascending phase of the EPSP was 1-2.2 msec (mean 1.74 ± 0.42 msec), and the time constant of the descending phase was 7-14 msec (mean 10.6 ± 2.92 msec). The EPSPs arising in response to stimulation of the more rostral regions of the ventral column thus did not differ significantly from responses evoked from the level of the 6th segment.

Monosynaptic EPSPs evoked from the ventral column continued to appear even after preliminary transection of the spinal cord. In this whole series of experiments activity of 97 motoneurons was recorded. An example of a monosynaptic EPSP evoked from the ventral column at the level of the 6th segment and a histogram of distribution of the latent periods of these EPSPs in experiments on animals after preliminary transection of the spinal cord are shown in Fig. 3A, 2. Clearly transection did not lead to any significant change in the distribution of the latent periods of the EPSPs. The change in their parameters showed that

the duration of the ascending phase was 1.3-2.2 msec (mean 1.7 ± 0.26 msec), and the time constant of the descending phase was 7.5-12 msec (mean 10.12 ± 0.61 msec). The amplitude of the monosynaptic EPSPs of this group evoked by stimuli 2-3 times stronger than the threshold varied from 1 to 3.3 mV, with a mean value of 2.14 ± 0.48 mV. Statistical analysis (by Student's method) showed that the differences between the parameters of the monosynaptic EPSPs before and after transection of the spinal cord were not statistically significant ($P > 0.05$). The dynamic characteristics of the monosynaptic EPSPs (the degree of partial potentiation) likewise was unchanged in the animals undergoing the operation (Fig. 3B).

It can thus be concluded from these results that monosynaptic excitatory influences evoked by activation of fibers of the ventral columns are due mainly to involvement of descending propriospinal pathways.

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