SYNAPTIC EFFECTS ON FROG MOTONEURONS DURING STIMULATION OF THE DORSAL ROOTS AND POSTERIOR COLUMNS OF THE SPINAL CORD

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In response to stimulation of the dorsal roots, good agreement was obtained between the ventral root potentials (VRPs) and the excitatory postsynaptic potentials recorded intracellularly. Monosynaptic VRPs generated during stimulation of the dorsal roots and posterior columns had a similar temporal course and did not show facilitation in response to paired stimuli or tetanization. Simultaneous stimulation of the dorsal roots and posterior columns led to occlusion of the combined VRP on the average by 64%. Ascending collaterals of dorsal-root fibers are evidently activated by stimulation of the posterior columns.

There is morphological evidence [2, 3] that in amphibians the dorsal root fibers give off collaterals after entering the spinal cord, which can be followed in the posterior columns as far as the cerebellum.

Since only monosynaptic effects from the dorsal roots on motoneurons of the same segment have so far been fully investigated in frogs [1], and effects of stimulation of the posterior columns have not been studied, it was decided to examine synaptic effects of stimulation of the dorsal root and posterior column projections on motoneurons.

EXPERIMENTAL METHOD

The isolated spinal cord of the frog Rana ridibunda was used. After its isolation, the spinal cord of the frog was placed in a chamber with a constant flow of Ringer's solution oxygenated with 95% O_2 and 5% CO_2. The 9th and 10th pairs of ventral and dorsal roots, corresponding to the lumbar enlargement, were placed on silver stimulating electrodes for orthodromic and antidromic stimulation. The posterior columns were stimulated through bipolar nichrome electrodes, with an interpolar distance of 100-200 μ, placed at different levels rostrally from the recorded segments. Ventral root potentials (VRPs) were recorded by a unipolar technique between the point where the ventral root left the Ringer's solution to enter mineral oil at a distance of about 0.5 mm from the point of emergence of the ventral root from the spinal cord, and the electrode placed on the distal end of the ventral root. In this way synaptic potentials of motoneuron populations could be recorded. Intracellular recordings were made with glass microelectrodes filled with 3 M KCl solution, having a tip of 0.5-1 μ in diameter and an impedance of 15-30 MΩ. The microelectrode was inserted into the spinal cord from the lateral surface (before the ventral and dorsal roots), from which the pia mater had first been removed, in steps of 2 μ by means of a remote-controlled micromanipulator. Square pulses (10-100 μA, 0.1-0.3 msec) were used for stimulation. To inhibit polysynaptic responses, Nembutal was injected into the Ringer's solution in a concentration of 1:5000. The experiments were carried out at 14-18°C. VRPs were investigated in 47 preparations. Intracellular recordings were obtained successfully from 97 motoneurons. All cells were identified as motoneurons by the presence of an antidromic action potential (AP). The amplitude of the antidromic AP varied from 27 to 82 mV, and the amplitude of the membrane potential from 20 to 60 mV. The duration of the intracellular recording was usually 10-30 min.

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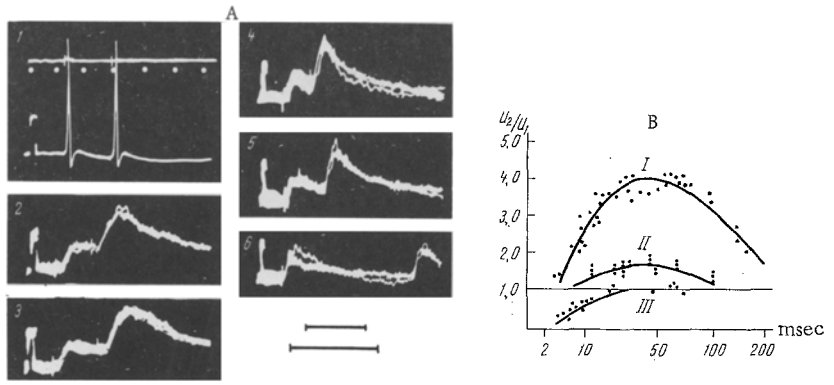

Fig. 1. Facilitation of synaptic responses to paired stimuli applied to dorsal roots (intracellular recording): A, 1) antidromic discharge. Calibration: 20 mV. Time marker 10 msec; 2-6) EPSP in response to paired stimuli applied to dorsal roots at different intervals. Calibration: 5 mV. Time marker: top, for curves 2 and 3, 20 msec; bottom, for curves 4-6, 60 msec. B) Relationship between amplitude of test EPSPs and interval between stimuli. Curve I plotted for polysynaptic EPSP with duration 150 msec, curve II for EPSP shown on records 2-6, and curve III for monosynaptic EPSP. Abscissa, interval between stimuli; ordinate, ratio between amplitude of test EPSPs and amplitude of initial EPSPs.

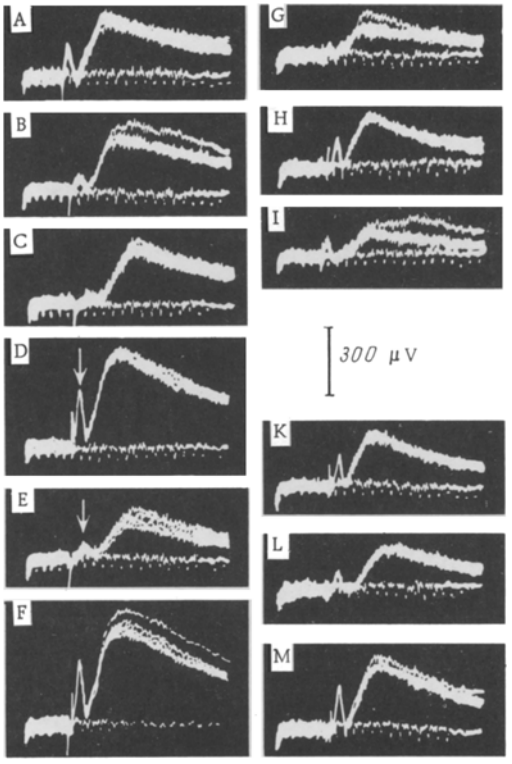

Fig. 2. Monosynaptic potentials of 9th ventral root in response to stimulation of posterior column and dorsal root: A) at level of 7th segment; B) at level of 5th segment; C, E, L) at level of 2nd segment; G) dorsal root of 8th segment; K, H) 9th segment; I) 10th segment; D) 8th, 9th, 10th segments; F) summation of response of D and E; M) summation of responses of K and L; 5 sweeps of beam on each record. Strength of stimulating current for A 30 μA, B 45 μA, C 100 μA, D 60 μA, E and L 70 μA, G, H, I, and K 40 μA. Time marker 1 sec.

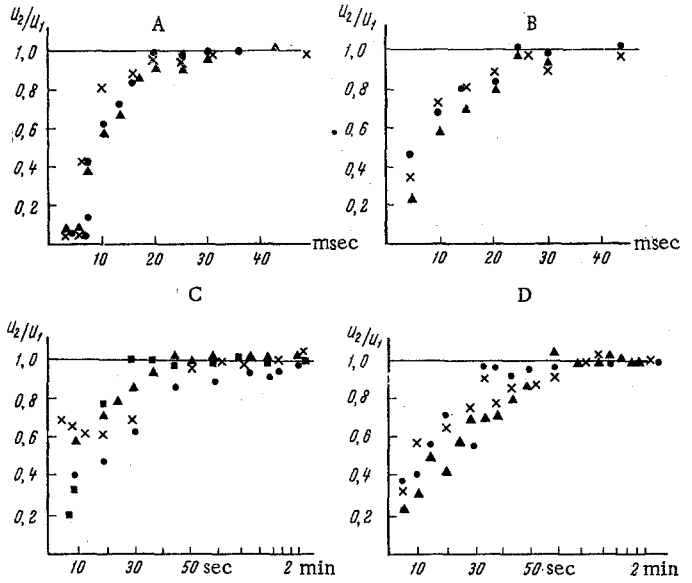

Fig. 3. Depression of testing monosynaptic VRPs in response to paired stimuli applied to dorsal roots (A) and posterior columns (B) and posttetanic depression of monosynaptic VRPs in response to stimulation of dorsal roots (C) and posterior columns (D). In A and B: abscissa, interval between stimuli (in msec); ordinate, ratio between amplitude of test VRP and amplitude of control VRP. In C and D: ordinate, ratio between amplitude of test VRPs and amplitude of initial VRPs, abscissa, time after end of tetanization.

EXPERIMENTAL RESULTS

In response to stimulation of the dorsal roots in a spinal cord not treated with Nembutal, VRPs with an amplitude of between 0.1 and 2 mV, depending on the strength of stimulation, were recorded. The thresholds of onset of these VRPs was usually 10-20 μA for stimuli 0.1 msec in duration. The latent period of the VRPs varied from 1.3 to 1.8 msec, with a mean value of 1.5 msec. The VRPs in response to stimulation of the dorsal roots consisted almost always of a mixture of mono-, di-, and polysynaptic components. The total duration of the responses was 50-200 msec. VRPs in response to stimulation of the dorsal roots easily attained the threshold level for discharge to take place. The latent period of discharge indicated that the APs were generated at the peak of the di- and polysynaptic components of the VRP. In response to paired stimuli, facilitation could be obtained if the 2nd stimulus was applied after an interval of 10-100 msec. Polysynaptic components of the responses exhibited facilitation. Tetanization (50 Hz, 10 sec) by stimuli 3 times stronger than the testing stimuli led to very slight potentiation (up to 20%) of the polysynaptic components of the responses for up to 1 min. Intracellular recordings revealed good agreement between the excitatory postsynaptic potential (EPSP) and the VRPs recorded during dorsal root stimulation. The latent period of the EPSP averaged 1.4 msec, while the threshold response varied from 10 to 30 μA, and the total duration of the response was 40-200 msec. The polysynaptic components of the EPSP easily attained the threshold level for AP generation, averaging 9 mV. EPSPs evoked by stimulation of the dorsal root showed facilitation of the polysynaptic components up to 400% in response to paired stimulation (Fig. 1). EPSPs of more complex composition showed greater facilitation to paired stimuli (Fig. 1, curve I) than those which rapidly reached their maximum and were shorter in duration (Fig. 1, curve II). Monosynaptic EPSPs, which were recorded after partial inhbition of activity by Nembutal, generally speaking did not exhibit facilitation (Fig. 1, curve III).

The VRP generated in response to stimulation of the posterior columns had a threshold of 10-100 μA for a stimulus 0.1 msec in duration, and like the VRP in response to stimulation of the dorsal roots in the spinal cord not treated with Nembutal, it consisted of a mixture of mono-, di-, and polysynaptic components. The threshold current was between 10 and 30 μA, regardless of the distance from the segment recorded.

Isolation of the ipsilateral and contralateral columns and placing them on the stimulating electrodes showed that responses arose from both sides. According to anatomical findings [4], dendrites of motoneurons can run for a distance of up to 1 mm into the contralateral half of the spinal cord. Activation of such dendrites was evidently responsible for the generation of responses from the contralateral side of the spinal cord. The threshold current for VRP generation was the same on both sides. The latent period was reduced as the stimulating electrode was moved from the second to the seventh segment from 2.6 to 1.4 msec (mean results of 9 measurements) (Fig. 2: A, B, C). The level of the 2nd segment was 10-15 mm away from the recorded segment, and the corresponding variations in latent period were between 2.6 and 1.8 msec. Measurement of the conduction velocity of the posterior column fibers gave a value of 5-10 m/sec. After administration of Nembutal, the dorsal-root and posterior-column VRPs gradually lost their di- and polysynaptic components and assumed the shape shown in Fig. 2: A-C, G-I). The duration of the ascending phase was 2 and 1.8 msec for the dorsal-root and posterior-column VRPs respectively, the duration of their descending phase was 17.5 and 18 msec respectively, and the amplitude of the responses 0.20 and 0.24 mV respectively for a stimulus with a strength of 50 μA (mean results). The latent periods of the dorsal-root and posterior-column VRPs were the same as before inhibition of Nembutal. These VRPs can accordingly be regarded as monosynaptic, for their parameters showed good agreement with the monosynaptic EPSPs recorded intracellularly by Fadiga and Brookhart [1]. The latent period of EPSP generation during dorsal root stimulation, according to their observations, was 1.8 msec, the duration of the ascending phase 2.7 msec, and the duration of the descending phase 15-20 msec. When paired stimuli were applied, both types of responses showed inhibition of the testing responses for intervals of between 3 and 25 msec between stimuli (Fig. 3: A, B). Tetanization (50 Hz, 10 sec) by stimuli 3 times above the threshold strength led to posttetanic depression both of the dorsal-root VRPs and of the VRPs generated in response to stimulation of the posterior columns, for up to 1 min (Fig. 3: C, D). The VRPs from the dorsal roots and from the posterior columns were thus similar both in their general characteristics and in their responses to paired stimuli and to tetanization. This suggests that collaterals of the dorsal-root fibers may be concerned with the evoking of responses from the posterior columns. To test this hypothesis, 6 experiments were carried out with summation of the responses evoked by stimulation of the dorsal roots and posterior columns in which marked occlusion was found. The stimuli were so applied that the presynaptic components (indicated by arrows in Fig. 2: D, E) and the beginning of the postsynaptic potentials obtained from both sources coincided. Stimulation of the 9th dorsal root with the dorsal column led to occlusion of the combined response on the average by 64% (Fig. 2: K, L, M).

According to the anatomical evidence [3], dorsal root fibers giving off collaterals into the posterior columns may terminate a few segments lower or higher than the point of entry into the spinal cord, as the present experiments confirmed. Monosynaptic VRPs were invariably recorded both during stimulation of the dorsal root corresponding to the recorded segment (Fig. 2: H) and during stimulation of the neighboring dorsal roots (Fig. 2: G, I). During stimulation of the posterior columns, the possibility of collaterals of neighboring dorsal roots being recruited into the response cannot be ruled out, and this would naturally lead to a decrease in the occlusion. It would therefore be expected that on activation of 3 dorsal roots (8th, 9th, and 10th) and the posterior columns simultaneously, more marked occlusion would take place. In fact, simultaneous stimulation of 3 dorsal roots and the posterior columns led to more marked occlusion (on the average 92%, see Fig. 2: D, E, F) than in the case of summation from only one dorsal root and one posterior column (Fig. 2: K, L, M). Stimulation of the posterior columns at different levels evidently led to activation of the same tract fibers, for combining these effects led to the almost total occlusion of the combined response (on the average 94%). These tract fibers, as was shown above, are most probably ascending collaterals of afferent fibers in the dorsal roots.

LITERATURE CITED

1. E. Fadiga and J. M. Brookhart, Am. J. Physiol., 198, 693 (1960).
2. B. S. Joseph and D. P. Whitlock, Brain Behav. Evolut., 1, 2 (1968).
3. C. N. Liu and W. W. Chambers, Anat. Rec., 127, 326 (1957).
4. M. S. Silver, J. Comp. Neurol., 77, 1 (1942).