Extrasomatic spikes recorded from cat motoneurons
Possible dendritic spikes

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Abstract

Extrasomatic (ES-) spikes were recorded in motoneurons in cats anesthetized with pentobarbital sodium and immobilized with d-tubocurarine. ES-spikes, 40-79 mV in amplitude, were distinguished from intrasomatic spikes by their differences in responses to a train of 8 pulses at 500 Hz given to the L 7 or S 1 ventral root. In 23 motoneurons 3-6 different spikes could be recorded from the same site in the same cell in response to the ventral root stimulation. It was not possible to explain these multiple spikes as axon spikes or IS-(initial segment) spikes because too many spikes were observed at the same site. Accordingly, it was suggested that they were dendritic in origin. (J Nippon Med Sch 1997; 64: 323-328)

Key words: ventral root stimulation, extrasomatic spike, axon spike, dendritic spike, interaction between motoneurons

Introduction

It is reported that motoneuron spikes could be recorded not only intrasomatically, but also extrasomatically in response to ventral root stimulation1. The extrasomatic (ES-) spike could be distinguished by differences in positions of inflections from the intrasomatic spike which consists of the IS-spike originating in the initial segment and therewith dtriggered SD-spike originating in the some-dendritic membrane2-3. However, the easiest and surest way to distinguish both spikes may be the differences in responses to high frequency (e.g., 500 Hz) stimulation. Namely, the ES-spike could follow a high frequency stimulation with fairly constant waveform1, whereas in the case of the intrasomatic spike usually only the first pulse of a high frequency pulse train produces a full IS-SD-spike and all of thereto following pulses produce only IS-spikes which could follow the pulse train with fairly constant waveform2. These results appear to indicate that the ES-spike is the IS-spike or axon spike recorded at its site of origin. However, we obtained lines of evidence suggesting that at least some of the ES-spikes may be dendritic in origin, as will be described subsequently. For example, a single ES-spike could consist of 3-6 components. This is hard to explain if it were an axon spike. Conversely, if it does originate in the axon it would become an important subject how the axon could exhibit such a composite spike. In this case the most likely site in the axon would be the bifurcating point. Inasmuch as the ES-spike was encountered most frequently although to keep it in a stable state was of extreme difficulty, we thought it significant to describe its electrophysiological properties and made an attempt to identify its site of origin in the present paper.

Materials and Methods

The trachea and a vein in the forelimb of cats (2.6-3.9 kg) were cannulated under ether anesthesia. They were then anesthetized with pentobarbital sodium (Nembutal: 25-33 mg/kg, i.v.) and immobilized with d-tubocurarine (Amerizol: 1-2 mg/kg). The depth of the anesthesia was so controlled by
adding the anesthetic if necessary that the EEGs recorded monopolarly and bipolarly from the anterior sigmoid gyrus exhibited irregular slow waves with occasional spindle bursts throughout the experiments.

The L7 and S1 roots were exposed and cut to isolate the ventral root. It was stimulated with a train of 8 pulses of 0.1 ms in duration at 500 Hz because of the reason stated above by employing a pair of silver wire electrodes and an electronic stimulator (Nihonkoden SEN 7103). The rectal temperature and the mineral oil covering the exposed spinal cord were maintained at 36-38°C and 37-39°C, respectively.

Capillary microelectrodes filled with 4 M potassium acetate solution (15-40 MΩ) were employed. For observing intracellular potentials a cathode ray oscillograph (Nihonkoden VC 11) and a preamplifier (WPI M 707 A) were employed. EEGs and intracellular d.c. potentials were monitored with a pen-writing oscillograph (Nihonkoden RM 80).

Results

In 818 motoneurons ES-(extrasomatic) spikes were recorded. Their amplitudes were 40-79 mV and durations at half-amplitude 0.4-0.6 ms with resting membrane potentials of −36 to −69 mV. Except in 44 motoneurons each of the 8 pulses delivered to a ventral root produced a single ES-spike with a latency of less than 0.4 ms. The ES-spike usually did not show the inflection on the rising phase, in this respect differing from the intrasomatic spike (cf., ref. 1). In the 44 motoneurons one of the 8 pulses given to the ventral root could produce two Es-spikes or potentials were superimposed on the ES-spike. For example, in Fig. 1 this motoneuron was characterized by the existence of second spikes which were 0.7-1.2 ms in latency. There were three different second spikes as distinguished by differences in amplitude. The largest of them, spike 1 (Fig. 1, arrow 1) had an inflection on the rising phase (Fig. 1A-C, arrow head) and could arise from a smaller spike (Fig. 1C, arrow 3). These results suggest that spike 1 consists of 3 spikes.

Of utmost interest is that the first (shortest latency) Es-spike could exhibit two amplitudes in all-or-none fashion (Fig. 1B, lateral arrow) indicating that the ES-spike consists of two spikes. Of further interest is that the amplitude of the smaller ES-spike is approximately the same as that of the second spike which consists of 3 spikes. These results suggest that the first ES-spike consists of 4 spikes. In addition, this motoneuron exhibits a fifth spike (Fig. 1, arrow 2).

Fig. 2 illustrates another example in which there are second spikes. From Fig. 2A to B the strength of L7 ventral root stimulation was reduced. The second spikes were seen more frequently with weaker stimulation and arrows a and b indicate spikes larger and smaller, respectively, than the half amplitude of the first spike (Fig. 2A, B and E). The smaller second spikes, i.e., second spikes b, were
ES-spikes produced by 8-pulse stimulation of the L7 ventral root. Stimulus strength: 1.4T; B, D-G: 1.1T. C: the L7 dorsal root was stimulated with a single shock at 4.0T. A-C and D-G are of the same calibrations, respectively. E: responses produced by the first 3 pulses: F and G: those by the last 3 and 2 pulses, respectively. In A and B there are two kinds of second spikes, large (arrow a) and small (b). In D, F and G small second spikes (spikes b) were further classified according to differences in amplitude (arrows 1-4). In F and G note that some of small spikes b summate to form a composite spike. In H the number of second spikes produced by each pulse was related to the pulse number: Closed box: spike a; open box: spike b (spikes 1-4).

There were 4 different smaller second spikes (Fig. 2D, F and G, arrows 1-4) which could summate to form a composite spike (e.g., Fig. 2G, arrows 2-4). The second, seventh and eight pulses produced the second spikes more frequently than any other pulses (Fig. 2H). L7 dorsal root stimulation produced a slow depolarizing potential which presumably was an EPSP (excitatory postsynaptic potential) (Fig. 2C). The EPSP had no effects on production of spikes by the ventral root stimulation. The second spike was observed in 11 motoneurons and in 6 cells it was a composite spike.

In 21 motoneurons a spike was superimposed near the peak of the ES-spike and in 12 cells the superimposed spike was a composite spike by itself. For example, in Fig. 3 it is noted that near the peak of the ES-spike a composite spike was superimposed (Fig. 3B, arrow). This occurred always with the ES-spike elicited by the first pulse. However, the composite spike was made to be superimposed on the ES-spike elicited by the second pulse through simultaneously stimulating the corresponding dorsal root (Fig. 3D-F). The composite spike consisted of 2 spikes, b and c.

Fig. 3H-J were recorded from another motoneuron. In this cell the 8 pulses given to the L7 ventral root elicited only ES-spikes of the same waveform (Fig. 3H). L7 dorsal root stimulation consistently produced an EPSP which triggered an ES-spike (Fig. 3I). When both dorsal and ventral roots were stimulated together a potential was superimposed on an ES-spike (Fig. 3J, arrow). In 16 motoneurons an EPSP was produced by dorsal root stimulation and in 8 cells both EPSPs and multiple spikes were observed.

In 11 motoneurons the ES-spike was triggered by a spike of 2-15 mV in amplitude and in 5 cells it was a composite spike. For example, in Fig. 4A, 8 pulses delivered to the L7 ventral root produced ES-spikes of similar waveform. It is noted that there is an inflection on the rising phase of the ES-spike (Fig. 4A, arrow). By decreasing the stimulus strength the inflection turned out to be the peak of a composite spike which triggered the ES-spike (Fig. 4B, arrow head and arrow). By further decreasing the stimulus strength a small potential was revealed (Fig. 4C, triangle). This potential may be a spike because its time to peak is shorter than that of an electrical synaptic potential (cf., 4).

From Fig. 4D to I the strength of ventral root stimulation was reduced and at least 5 different spikes other than the ES-spike were shown to exist (arrows 1-5). All the potentials indicated with
Fig. 3  ES-spikes produced by 8-pulse stimulation of the L7 ventral root. A-G were obtained from a motoneuron and H-J from another. Stimulus strength: A, D, E, F: 1.3T; B: 1.1T; C: slightly above 1.0T (threshold). In D, E, G and I arrow head indicates a single shock stimulation of the L7 dorsal root. In D-F both ventral and dorsal stimulation were carried out together. In E responses produced by stimulating both were superimposed on the dorsal root elicited EPSP such as shown in G (4.0T). In F the responses to the first 2 pulses are illustrated, the first pulse being delivered at the beginning of the sweep. In B and D-F note that a composite spike consisting of 2 spikes (F, b, c) is sometimes superimposed on the ES-spike (arrow). H: ES-spikes of similar waveforms were produced by all the 8 pulses given to the L7 ventral root. I: a single shock stimulation of the L7 dorsal root consistently produced the ES-spike via an EPSP. In J both ventral and dorsal root stimulation as in H and I, respectively, were carried out simultaneously.

Fig. 4  ES-spikes triggered by small composite spikes. The L7 ventral root was stimulated with 8 pulses. A-C and D-I are of the same calibrations, respectively. From A (1.5T) to C (1.1T) the stimulus strength was reduced. Note that a composite spike triggers the ES-spike (B, arrow heads and arrow). From D (1.2T) to I (1.4T) the stimulus strength was increased. In D and E-I spikes produced by the last 4 and first 4 pulses, respectively, are shown. Note in D-H the existence of different small spikes (arrows 1-5). Also note that the firing level of the ES-spike could vary.

arrow 1 are probably the same spikes because all of them exhibited essentially a similar waveform despite changes in stimulus strength. If this is so, potential superimposed on them are of necessity spikes. By differences in waveform at least 4 different superimposed spikes could be distinguished (Fig. 4D and E, arrows 2-5). In addition, spikes indicated with arrow heads in Fig. 4E, F and G might be different spikes. The spikes indicated with arrow 1 + 2 in Fig. 4E, F and H could be interpreted as having been formed by superimposing spike 2 on spike 1 in Fig. 4D. The spike indicated with arrow
6 in Fig. 4f is the largest spike next to the ES-spike. It could have been formed either by superimposing another spike on the spikes produced by weaker stimulation in Fig. 4D-H or simply by synchronizing these spikes with stronger stimulation. A similar interpretation could be applied also to the potential indicated with triangle in Fig. 4E and F.

The most interesting phenomenon in this motoneuron was the fact that the firing level of the ES-spike could differ from one ES-spike to another. The firing level became lower and the latency longer as the pulse number increased in the case of strong stimulation (Fig. 4A, I). However, with a weaker stimulation such a rule did not exist although the ES-spike produced by the first pulse was always of the highest firing level and shortest latency (Fig. 4E-II). The ES-spike could arise even directly from the baseline (Fig. 4I, triangle).

Discussion

All the ES-spike occurred near the site where typical intrasomatic spikes\(^1\)-\(^3\) were observed. The site was shown to be the motoneuron pool by identifying the α-motoneuron through injecting neurobiotin into the cell (900 ms negative pulses, 3.5-5.2 nA, 6-12 min) in 8 cases. These results suggest that the ES-spike was recorded close to the soma of the α-motoneuron. If this is so the ES-spike must have originated in either the node of Ranvier nearest to the some\(^1\), the initial segment\(^1\)-\(^3\) or the first order dendrite. In 23 motoneurons 3-6 different spikes or components some of which summated to build up a composite spike could be produced in the same cell by ventral root stimulation. A single and/or composite spike could be superimposed on the falling phase (Figs. 1 and 2) or near the peak (Fig. 3) of the ES-spike, or the ES-spike was triggered by a composite spike (Fig. 4). These results could not possibly be explained as the ES-spike originating in the node of Ranvier (the M-spike) or in the initial segment (the IS-spike) (cf., ref. 1) because all the ES-spikes observed in the present experiments might have been dendritic in origin because all of them were common in the following three points: (1) All of them were similar in wave form exhibiting in most cases an inflection only in the falling phase (Fig. 1A, B, double arrow heads, Fig. 3A, H, F, arrow head). (2) In no cases did the site generating the ES-spike exhibit a high frequency injury discharge albeit there did exist such sites in the spinal cord. On the other hand, it is not easy to interpret the short latency of the ES-spike unless the axon arises from a dendrite or there are electrical synapses near the some between motoneurons (cf., ref. 4). Nevertheless, it seems to be of importance to explore the possibility suggested above because it is known that motoneurons do form the dendrite bundle which appears to be of functional significance\(^14\)-\(^23\) and was described earlier in cellule

Alternatively, multiple spikes could occur in motoneuron dendrites\(^4\) (cf., ref. 7, 8). That the firing level could differ from one ES-spike to another (n=3; Fig. 4) could be explained if the components of the composite spike triggering it originated in different dendritic regions. There are reports that not only chromatolyzed\(^4\), but also normal motoneurons\(^4\)-\(^11\),\(^12\) could generate dendritic spikes (cf., 7).

Of interest is that the second spike could be produced 0.8-1.4 ms after the first spike (n=5; Figs. 1, 2). This could be explained not as antidromic impulse transmission (cf., 1), but as excitatory interactions between motoneurons by impulses arising in axons other than its own because of the long latency differences between the first and second spikes (e.g., ref. 4, 12, 13, cf., 1). The existence of excitatory interactions could be suppoed by the facts that the multiple spikes were shown to exist in extrasomatic sites by changing the strength of the ventral root stimulation (Figs. 2, 4). In addition, the probability of producing second spikes was pulse-number-related in such a manner that it was not possible to explain in terms of the after-effects of preceding spikes (Fig. 2H).

Fig. 1 suggests that the first (shortest latency) ES-spike itself is a composite spike consisting of 4 components. This could possibly mean that the ES-spike is of dendritic origin. Then there seems to loom a possibility, however, slight it may be, that all the ES-spikes observed in the present experiments might have been dendritic in origin because all of them were common in the following three points: (1) All of them were similar in wave form exhibiting in most cases an inflection only in the falling phase (Fig. 1A, B, double arrow heads, Fig. 3A, H, F, arrow head). (2) In no cases did the site generating the ES-spike exhibit a high frequency injury discharge albeit there did exist such sites in the spinal cord. On the other hand, it is not easy to interpret the short latency of the ES-spike unless the axon arises from a dendrite or there are electrical synapses near the some between motoneurons (cf., ref. 4). Nevertheless, it seems to be of importance to explore the possibility suggested above because it is known that motoneurons do form the dendrite bundle which appears to be of functional significance\(^14\)-\(^23\) and was described earlier in Identified cellule
psychique (pyramidal cell) by Ramón y Cajal.

References

3. Eccles JC: The Physiology of Nerve Cells. 1957; The Johns Hopkins Press, Baltimore, MA, USA.

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