—Originals—

Reduction in amplitude of the ventral root elicited
small spike by dorsal root stimulation
in cat motoneurons

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Abstract

Ventral root stimulation is known to produce two spikes, large and small, in the α -motoneuron. Since it is the small spike that triggers the outgoing impulse from the cell it is regarded as the key potential in information processing of the cell and referred to either as the IS-spike or as the A-spike depending upon whether one considers it as a purely single spike or as a composite spike. The small spike was elicited by stimulating the L 7 or S 1 ventral root in cats anesthetized with pentobarbital sodium and immobilized with *d*-tubocurarine. During the later phase of the excitatory postsynaptic potential and thereto following inhibitory postsynaptic potential (IPSP) produced by stimulating the corresponding dorsal root the amplitude of the small spike was reduced by about 20%. This could be either explained as due to reduction in impedance of the membrane generating the small spike or interpreted as suggesting that the small spike is a composite spike and some of its components were inhibited by the IPSP. This interpretation was favored because the reduction occurred not when the safety factor for antidromic impulse transmission was high, but when it was low. (J Nippon Med Sch 1997; 64: 337–343)

Key words: A-spike, IS-spike, dendritic potential, motoneuron, dorsal root stimulation

Introduction

It is a well known fact that ventral root stimulation produces in the α -motoneuron two kinds of spikes, large (60–100 mV) and small (20–40 mV)^{1,2}. The small spike is regarded as the key potential in information processing of the motoneuron because it triggers not only the large spike, but also the outgoing impulse from the cell³. The small spike is referred to as either the IS-spike^{1–3} or A-spike⁴ depending upon whether one considers it as a purely single spike or as a composite spike. It is assumed in the former case that the IS-spike is the spike of the initial segment (IS) and in the latter case that the potentials originating in some sites other than the IS are superimposed on the spike of the IS. On the other hand, it seems to have unanimously been

agreed upon that at least the soma membrane is mainly responsible for generation of the large spike. This spike is called either the SD-spike^{1–3} (soma-dendritic spike) or B-spike⁴ according to one's view on the origin of the small spike as described above. Fluctuations in amplitude of the small spike have been noticed when the small spike is preceded by another spike ever since the small spike was described. If the small spike is a single spike the fluctuations must be due to the refractoriness developed in the wake of the preceding spike. On the other hand, if it is a composite spike the fluctuations could be produced through suppression of some of the components forming the small spike.

The present experiments were planned to obtain facts which could at least suggest which of the two views on the small spike is more relevant. We discovered that the amplitude of the small spike

could consistently be reduced during the Cl-non-sensitive IPSP produced by dorsal root stimulation. Inasmuch as the reduction did not occur when the safety factor for antidromic impulse transmission was high, the possibility was excluded that the impedance of the membrane supporting the small spike was reduced during the IPSP. Accordingly, the view that the small spike is a composite spike was favored and the reduction in its amplitude was attributed to suppression of some of the components of the composite spike.

Materials and Methods

The trachea and a vein in the forelimb of cats (weight: 2.6–3.9 kg) were cannulated under ether anesthesia. They were then anesthetized with pentobarbital sodium (Nembutal: 21–32 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 EEGs recorded monopolarly and bipolarly from the anterior sigmoid gyrus exhibited irregular slow waves with occasional spindle bursts throughout the experiments.

The lumbar enlargement was exposed and cov-

ered with mineral oil of 37–38°C (the rectal temperature: 36–37°C). The L7 and S1 roots were cut to isolate ventral and dorsal roots. Silver wire electrodes were used to stimulate or record from the root. Pulses of 0.1 ms duration were delivered every second by stimulators (Nihonkoden SEN 3201, SEN 3203).

Glass capillary microelectrodes were filled with either 4 M K-acetate or 3 M KCl solution (d.c. resistance: 5–20 MΩ). A cathode ray oscillograph (Nihonkoden VC 11), a preamplifier (WPI M 707 A) and a pen-writing oscillograph were employed for observing electrical activities.

Antidromic spikes elicited in 48 cells were in amplitude 69–100 mV (mean: $77.4 \pm \text{SD: } 4.16$ mV) with resting membrane potentials of -57 to -83 mV (-68.1 ± 7.81 mV).

Results

Reduction in amplitude of the ventral root elicited small spike during the later phase of the excitatory postsynaptic potential (EPSP)

Fig. 1A shows a full spike elicited by stimulating the L7 ventral root. Two inflections are recognizable on the rising phase of the spike. This spike

Fig. 1 Reduction of amplitude of the L7 ventral root elicited small spike (IS- or A-spike) by L7 dorsal root stimulation. Except in **Fig. 4** all records were taken with K-acetate solution filled microelectrodes. **A:** a ventral root elicited full spike. Upward arrows in **B-G** indicate the ventral root stimulation. Strength of dorsal root stimulin: **B:** slightly below 2.1T, **C:** 2.1T, **D:** slightly above 2.1T, **E-G:** 4.0T. From **E** to **G** the interval between ventral and dorsal root stimulation was decreased. The difference between the intervals in **F** and **G** is 0.1 ms. Note in **D-F** that the amplitude of the small spike is reduced as compared with that in **C** and **G** (arrow head). The graphs show that a slight increase in strength of dorsal root stimulation (**H**) or interval (**I**) between ventral and dorsal root stimulation results in a discontinuous reduction in amplitude of the small spike (single and double arrow heads).

serves as the control response. The upper (oblique arrow) and lower (lateral arrow) inflections are concave and convex upwards, respectively. It will be shown below that it is the portion of the spike between the two inflections that is subject to reduction by dorsal root stimulation. The upper inflection (oblique arrow) denotes the transition from the small to the large (full) spike. In **Fig. 1B-G** the responses produced by stimulating both ventral and dorsal roots were superimposed on postsynaptic potentials produced by stimulating the dorsal root alone. The postsynaptic potentials consist of a slow depolarization and thereto following hyperpolarization. They were interpreted as the EPSP and IPSP, respectively.

From **Fig. 1B** to **D** the strength of dorsal root stimulation was increased, whereas the interval between the dorsal and ventral root stimulation remained the same. In **Fig. 1B** the inflection denoting the onset of the full spike (oblique arrow) became clearer as compared with the control (**Fig. 1A**). In **Fig. 1C** the inflection had become even clearer and sometimes the full spike was not produced to expose the variations in amplitude of the small spike (double arrow heads). A slight increase in strength of the dorsal root stimulation resulted in abolishment of the full spike (**Fig. 1D**).

Further increase in the strength resulted only in a slight reduction in the amplitude (**Fig. 1E** and **H**). The strength of the dorsal root stimulation was the same in **Fig. 1E-G**. But the interval between the dorsal and ventral root stimulation in **Fig. 1G** is shorter by only 0.1 ms than that in **Fig. 1F**, yet the amplitude of the small spike in **Fig. 1G** sometimes attained a magnitude large enough to trigger a full spike (double arrow heads, see also **Fig. 1I**). These results suggest that the small spike consists of two component, i.e., one that is suppressed and the other that is unaffected by dorsal root stimulation.

Reduction in amplitude of the ventral root elicited small spike during the inhibitory postsynaptic potential (IPSP)

In **Fig. 2A** L7 ventral root stimulation with 4 pulses elicited a full spike followed by small spikes and there was an underlying depolarization which could possibly be summated after-depolarizations. From **Fig. 2B** to **G** the interval between dorsal and ventral root stimulation was increased. In **Fig. 2B** the inflection denoting the onset of the full spike (arrow) is less clear as compared with the control (**Fig. 2A**). This indicates that antidromic impulse transmission during the early phase of the EPSP is facilitated. In **Fig. 2C** the first pulse fell on the later

Fig. 2 Reduction in amplitude of the L7 ventral root-elicited small spike by L7 dorsal root stimulation (4.0T). In A-C the baseline is shown. A: spikes elicited by 4 pulses (500 Hz) given to the ventral root. In B-G the responses elicited by stimulating both ventral (4 pulses) and dorsal (a single pulse) roots stimulation were superimposed on postsynaptic potentials produced by single shock stimulation of the dorsal root. From B to G the interval between dorsal and ventral root stimulation was increased. Note in C-F that the first pulse given to the ventral root could not produce the full spike because it produced a small spike whose peak was lower than the inflection of the full spike in A, B and G (arrow). H shows schematically the magnitudes of the ventral root elicited small spike (a), underlying depolarization (b) and postsynaptic potentials (c) as measured from the baseline (see **Fig. 3**).

phase of the EPSP and the full spike was no longer produced evidently because the small spike was unable to attain a magnitude large enough to trigger the full spike. Since no spikes preceded the first small spike its small amplitude could be attributed not to "refractoriness", but to the effects of the dorsal root stimulation. It is noted in **Fig. 2C** that all the spikes were practically of the same amplitude and in **Fig. 2B** and **C** that the underlying depolarization had disappeared. As the stimulus interval was increased the EPSP was replaced by a slow hyperpolarization which presumably was an IPSP and the underlying depolarization reappeared (**Fig. 2D-G**, **Fig. 3**, curves b and c); however the first pulse continued to trigger only the small spike (**Fig. 2D-F**, **Fig. 3**, curve a) until it recovered its amplitude almost in all-or-none fashion to trigger a full spike at the stimulus interval of about 70 ms (**Fig. 2G**).

Two kinds of IPSPs are known to exist. One is Cl-sensitive² and the other Cl-non-sensitive⁵. In order to know which IPSP was that which reduced the amplitude of the small spike in **Fig. 2** the KCl filled microelectrode was employed in **Fig. 4**. In **Fig. 4** the dorsal root stimulation can still produce an EPSP-IPSP sequence and the ventral root stimulation can not produce the full spike (**Fig. 4D, E**) exactly as in **Fig. 2**. It should be noted that as the interval between dorsal and ventral root stimulation was increased the recovery of amplitude of the small spike was heralded by the sudden, occasional appearance of a larger small spike which sometimes

Fig. 3 Graphs showing the magnitudes of the ventral root elicited small spike (a), underlying depolarization (b) and postsynaptic potentials produced at various intervals between dorsal and ventral root stimulation when the full spike did not occur in the same motoneuron as in **Fig. 2**. Arrows 1 and 2 indicate the potential level from which a full spike develops (**Fig. 2A, B, G**, arrows) and the baseline, respectively.

became large enough to trigger a full spike (**Fig. 4F**, double arrow heads). A similar variation in amplitude heralded also the onset of the reduction in amplitude when we started to increase the stimulus interval (**Fig. 4C**, double arrow heads) similarly as in **Fig. 1G** and **I** (double arrow heads). Otherwise, the small spike triggered by the first pulse during the IPSP (**Fig. 4C-E**, arrow) was of similar magnitude to the other small spikes (e.g., **Fig. 4A-E, G**, arrow head).

Fig. 4 Reduction in amplitude of the L7 ventral root stimulation by the L7 dorsal root stimulation (4.0 T). The K-acetate solution filled microelectrode was used. In A and E-G the baseline is shown. In B and E-G responses produced by stimulating both dorsal and ventral roots at various intervals are superimposed on postsynaptic potentials produced by stimulating the dorsal root. Note in E-G that the EPSP (plus the reversed IPSP due to Cl ion diffusion) is followed by the IPSP. During the IPSP the first pulse given to the ventral root could not produce a full spike.

Fig. 5 High safety factor for antidromic impulse transmission and reduction in amplitude of the L7 elicited small spike by L7 dorsal root stimulation. A: spikes elicited by the ventral root stimulation. Note that the full spike hardly exhibits inflections on its rising phase (cf., Fig. 1). This indicates that the safety factor for antidromic impulse transmission is high. Also note that the small spike elicited by the fourth pulse exhibits variations in amplitude (double arrow heads). In B and C responses produced by stimulating both roots were superimposed on postsynaptic potentials produced by the dorsal root stimulation (downward arrow). Upward arrow indicates the first of 4 pulses given to the ventral root at 500 Hz. In B note that the amplitudes of the small spikes (arrow heads) were reduced as compared with the corresponding spikes in A. In C an orthodromic full spike (oblique arrow) was sometimes produced and the first pulse to the ventral root produced only a small spike (arrow head). Otherwise the antidromic full spike was unaffected by the dorsal root stimulation.

High safety factor for antidromic impulse transmission and amplitude reducing effects of dorsal root stimulation

In Fig. 5A 4 pulses were delivered to the L7 ventral root and the small spike elicited by the fourth pulse exhibited a large variation in amplitude, sometimes attaining a magnitude large enough to trigger a full spike (double arrow heads). The amplitudes of the small spikes elicited by the third and fourth pulses were reduced during the later phase of the EPSP in Fig. 5B (arrow head) as compared with the corresponding spikes in Fig. 5A (arrow head). In Fig. 5C the interval between the dorsal root stimulation and first pulse was exactly the same as that between the dorsal root stimulation and the third pulse in Fig. 5B. Yet the first pulse continued to elicit a full spike except when the dorsal root stimulation produced a full spike (Fig. 5C, oblique arrow) and the first pulse elicited the smallest small spike (arrow head) evidently due to the refractoriness. It is noted that the inflections on the full spike elicited by the first pulse are almost unrecognizable in contradistinction to the ventral root-elicited full spike shown in Figs. 1,2,4. This could be taken to indicate that the safety factor for antidromic impulse transmission is high in the case of Fig. 5 and in such a case the dorsal root stimulation could not reduce the amplitude of the small spike. In 6 cases the safety factor was high and dorsal root stimulation could not affect the antidromic impulse transmission. In 20 motoneurons the amplitude of the ventral root elicited small spike was reduced. In 7 of these cells the KCl filled

microelectrode was employed and the IPSP was confirmed to be Cl⁻-non-sensitive. In 28 motoneurons the orthodromic full spike occurred consistently at low threshold and it was not known if dorsal root stimulation could affect the antidromic impulse transmission.

Discussion

The central fact in the present experiments is that dorsal root stimulation can reduce the amplitude of the ventral root elicited small spike, thereby preventing the antidromic impulse transmission into the same (Figs. 1-4). Only the following two interpretations are possible. (1) The small spike is a purely single spike, i.e., the IS-spike¹⁻³, and reduction of its amplitude by dorsal root stimulation is due to a decrease in impedance of the IS (initial segment) membrane. (2) The small spike is a composite spike, i.e., the A-spike⁴ which is equivalent to the IS-spike and thereon superimposed potentials, and reduction of its amplitude is attributed to inhibition or suppression of the superimposed potentials. The present results are in favor of the latter interpretation for the following reasons. (a) The amplitude of the small spike is reduced in a discontinuous manner as the strength of dorsal root stimulation is reduced or the interval between the dorsal and ventral root stimulation is changed (Fig. 1-4). This suggests that the small spike consists of two distinct parts. (b) The amplitude of the small spike could be reduced during the later phase of the EPSP (Fig. 1), whereas the antidromic impulse transmis-

sion is facilitated during the early phase of the EPSP (**Fig. 2B**). On the other hand, the impedance change during the EPSP could possibly occur not during the later phase, but during the early phase². (c) The amplitude of the small spike was reduced during the Cl-non-sensitive IPSP, which could be dendritic in origin⁵. (d) When the safety factor for antidromic impulse transmission is high, dorsal root stimulation is unable to reduce the amplitude of the small spike supporting a full spike, although the amplitude of the same small spike in the same cell which was isolated from a full spike due to of the existence of a preceding spike can be reduced by the same dorsal root stimulation (**Fig. 5**). That the reduced part of the small spike, i.e., the potentials superimposed on the IS-spike (see above), consists of local responses of the SD (soma-dendritic) membrane could be gainsaid because of their shorter latencies as compared with the latency of the SD-spike (**Fig. 1C, G, Fig. 4F, Fig. 5A**).

If the interpretation that the small spike consists of the IS-spike and thereon superimposed potentials is accepted, the sites of origin of the potentials must be considered. As already suggested by Fuortes et al.⁴ the dendrites should be considered as the likely sites of origin not only because there are a number of reports indicating that motoneuron dendrites could generate spikes⁶⁻¹² (cf., ref. 13-15), but also because it has already been shown morphologically that nerve cell dendrites are functionally significant¹⁶⁻²⁴ and proliferate as the phylogenetic scale is ascended, as eloquently exemplified on the cellule psychique of Ramón y Cajal²⁰. Furthermore, dendrites could possibly carry out information processing of their own^{5,25} (cf., 26, 27). If this is so, they may be likened to the personal computer. Further studies are to be expected.

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