Synaptic organization of dorsal root projections to lumbar motoneurons in the clawed toad (Xenopus laevis)

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Summary. Synaptic connexions between dorsal root primary afferents and lumbar motoneurons have been investigated in the isolated spinal cord of the clawed toad. The study of monosynaptic actions evoked in motoneurons by 9th or 10th dorsal root stimulation or by impulses in single primary afferents provided evidence for both electrical and chemical junctional transmission at the sensory-motor synapses. The anterograde filling of the 9th and 10th dorsal roots with horseradish peroxidase (HRP) shows that afferents do project to the motoneuron field of the segments IX and X. Some of the fibres not only reach the dorsally located motoneurons, but also cross the lateral motor column (LMC) and terminate in the marginal zone of ventral horn gray matter. The projections of the 9th and 10th dorsal root fibres are most numerous in the caudal part of segment X. Simultaneous HRP labeling of single motoneurons and the whole 10th dorsal root has revealed that afferent fibres make contacts not only on the distal dendrites of the motor cells, but also on the proximal ones. This latter finding is in a good agreement with the electrophysiological data.

Key words: Clawed toad - Dorsal root fibers - Lumbar motoneurons - Single-fiber EPSPs - Electrical-chemical transmission - HRP labeling

Introduction

The existence of a two-neuron reflex arc in Xenopus laevis lumbar spinal cord was postulated by Holemans et al. (1966), who were able to elicit short-latency strong synchronized activity in the motor-neurons by afferent impulses in the 10th dorsal root. The study of monosynaptic excitatory postsynaptic potentials (EPSPs) evoked in motoneurons by stimulation of the sensory nerves of the isolated spinal cord in the clawed toad (Shiriaev 1983) revealed the existence of electrical coupling between the primary afferents and motoneurons. All these data suggested that the organization of sensory-motor connexions in the lumbar spinal cord in this species had much in common with that of the other anuran amphibians.

However, the filling of the afferent axons with HRP (Nikundiwe et al. 1982; Katzenstain and Bohn 1984) showed that dorsal root projections in the lumbar spinal cord were very restricted and no fibers reached the LMC. Nikundiwe et al. (1982) claimed that the monosynaptic connexions in Xenopus could occur only between the primary afferent terminals and distal dendrites of motoneurons. It was supposed (Nikundiwe et al. 1982) that the well-developed lateral line system reduced the significance and prominence of the dorsal column pathway in this species.

On the basis of these morphological investigations Liuzzi et al. (1984) began to consider the organization of dorsal root projections in Xenopus as an exception among living anurans.

In this paper we compare the physiological and morphological data obtained in one series of experiments in order to resolve the contradictions existing with respect to the primary afferent input to lumbar motoneurons in the clawed toad.

Methods

The experiments were performed on the isolated spinal cord of clawed toads (Xenopus laevis) 5–10 cm in length. The results described in this paper are derived from 21 experiments. The technique of preparing and mounting the anuran spinal cord together with the ventral and dorsal roots has been described

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earlier (Shapovalov et al. 1978). The preparation was kept in a solution with the following composition (mM): NaCl 98.0; KCl 2.0; NaH2PO4 1.2; Na2HPO4 2.0; NaHCO3 9.0; MgCl2 0.5; CaCl2 1.5; glucose 5.5; gassed with 98% O2 and 2% CO2 to achieve a pH 7.4–7.6. The temperature of the bath was maintained between 15–18°C. After different periods in this normal solution, the test solution containing 0 mM – Ca2+, and 2 mM – Mn2+ was applied to eliminate chemically mediated transmission.

Cut ends of the 9th and 10th ventral and dorsal roots were placed on stimulating electrodes.

Intracellular recordings were made from lumbar motoneurones using bevelled micropipettes filled with 3 M – KCl or 2 M K citrate. Motoneurons were identified by antidromic activation.

In a few cases intracellular recordings were obtained with HRP-filled (10% HRP in 0.2 M KCl solution) microelectrodes. After these recordings HRP was iontophoresed into the motoneuron.

The microelectrode signals were amplified conventionally and monitored on an oscilloscope. The voltage response of the cell was frequently digitized using a DIDAC – 4000 (Intertechnique) computer. An extracellular average of the voltage response to dorsal root stimulation was also recorded. This average was later subtracted by computer from the intracellular average, to correct distortion of the intracellular response by the extracellular field.

When the intracellular recordings were made simultaneously from a lumbar motoneuron and a dorsal root fibre (using micropipettes filled with 3 M KCI), the fiber microelectrode was used for passing currents through a bridge circuit and for recording the presynaptic potential changes evoked by direct stimulation. Action potentials in fibres were evoked by intracellular injection of brief intense depolarising current pulses (2–4 nA, 0.2–0.5 ms).

The central projection from the primary afferent fibres was determined by placing HRP (5% HRP in 0.2 M KCl solution) on the cut end of the 9th or 10th dorsal root of the isolated spinal cord according to the technique described earlier (Shupliakov 1985). After 24 h the lumbar segment containing the HRP-filled elements was dissected out. It was immersed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h, and then in a mixture of phosphate buffer and 30% sucrose for 24 h. Transverse sections (60 μm) were cut from this segment on a freezing microtome. They were incubated according to the Graham and Karnovsky (1966) procedure with benzidine as chromogen. All sections were counterstained with cresyl-violet.

The criteria adopted for identification of presumed contacts follow closely those of Brown, Fyffy (1981) and Grantyn et al. (1982). HRP-filled boutons in close opposition to the labeled motoneuron were regarded as synaptic contacts if close examination under oil immersion (x2000) failed to detect any gaps between the pre- and postsynaptic elements.

Results

Synaptic actions produced by dorsal root and individual primary afferent stimulation

Monosynaptic and polysynaptic EPSPs were evoked in lumbar motoneurones by the 9th or 10th dorsal root stimulation. Figure 1 illustrates these two types of responses. It may be seen that the intracellular responses to the dorsal root volley (Fig. 1A1, B1) are distorted by the extracellular field as recorded after withdrawing the microelectrode to a position just outside the cell (Fig. 1A2, B2). The subtraction of extracellularly recorded voltage transient from the intracellular record of Fig. 1A reveals a long-latency response which is incompatible with monosynaptic linkage (Fig. 1A3). The latency of polysynaptic EPSPs recorded in lumbar motoneurons ranged from 3.6 to 4.8 ms, mean 3.98 ± 0.28 (standard deviation) ms (n = 7).