Fictive Rhythmic Motor Patterns Induced by NMDA in an In Vitro Brain Stem–Spinal Cord Preparation From an Adult Urodele

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Delvolvé, Isabelle, Pascal Branchereau, Réjean Dubuc, and Jean-Marie Cabelguen. Fictive rhythmic motor patterns induced by NMDA in an in vitro brain stem–spinal cord preparation from an adult urodele. J. Neurophysiol. 82: 1074–1077, 1999. An in vitro brain stem–spinal cord preparation from an adult urodele (Pleurodeles waltl) was developed in which two fictive rhythmic motor patterns were evoked by bath application of N-methyl-D-aspartate (NMDA; 2.5–10 μM) with d-serine (10 μM). Both motor patterns displayed left-right alternation. The first pattern was characterized by cycle periods ranging between 2.4 and 9.0 s (4.9 ± 1.2 s, mean ± SD) and a rostrocaudal propagation of the activity in consecutive ventral roots. The second pattern displayed longer cycle periods (8.1–28.3 s; 14.2 ± 3.6 s) with a caudorostral propagation. The two patterns were inducible after a spinal transection at the first segment. Preliminary experiments on small pieces of spinal cord further suggested that the ability for rhythm generation is distributed along the spinal cord of this preparation. This study shows that the in vitro brain stem–spinal cord preparation from Pleurodeles waltl may be a useful model to study the mechanisms underlying the different axial motor patterns and the flexibility of the neural networks involved.

INTRODUCTION

The neural networks generating axial muscle activations during locomotion have been investigated extensively in the lamprey (Grillner et al. 1995) and Xenopus embryo (Roberts et al. 1997). In vitro preparations have been developed more recently in higher vertebrates and provided information on the overall structure of the spinal locomotor networks. The complexity of the mammalian spinal cord has precluded, however, a detailed understanding of the circuitry responsible for the generation of stepping (Kiehn et al. 1997). In this context, the adult urodele appears as a useful model because it exhibits spontaneous swimming and overground stepping. In a previous study we have characterized the patterns of activation of epaxial myomers (intersegmental coordination patterns) during swimming and stepping in an intact adult urodele (Pleurodeles waltl) (Delvolvé et al. 1997). To study the neural networks responsible, an in vitro brain stem–spinal cord preparation from the adult urodele, P. waltl was used (see also Wheatley et al. 1992). The present paper reports on results obtained with this in vitro preparation.

METHODS

Experiments were performed on 21 adult urodele amphibians (Pleurodeles waltl) previously kept at 15°C for >1 wk. The animals were deeply anesthetized by immersion in a 0.1% aqueous solution of tricaine methanesulfonate (Sigma; MS 222). They were perfused transcardially with cold oxygenated Ringer solution (in mM: 130 NaCl, 2.6 CaCl2, 2.1 KCl, 0.2 MgCl2, 1 NaHCO3, 5 glucose, and 4 HEPES, pH 7.5) and transected at lumbar level. The rostral part was dissected in continuously perfused cold Ringer (3 ml/min). The dorsal part of the skull and vertebrae was removed, and the preparation was decerebrated by removing the brain tissue rostral to the mesencephalon. The more caudal spinal cord (5th to 16th segments) was completely isolated from the vertebrae, and special care was taken to dissect the ventral roots (VRs) over a length of 1–2 mm (Fig. 1A). The dura mater and the choroid plexus were removed to facilitate oxygen diffusion into the preparation. The preparation was pinned down and stored overnight at ∼5°C in oxygenated Ringer solution containing a neuromuscular blocking agent (α-bungarotoxin 1 μM, Sigma) to prevent the remaining muscle fibers from contracting. All preparations remained viable for >48 h.

After a recovery of 12 h, motor activity was induced by bath application of a mixture of 2.5–10 μM N-methyl-D-aspartate (NMDA; Sigma) and 10 μM d-serine (Sigma). Ventral root activity was recorded by means of tight-fitting glass suction electrodes. The neurograms were amplified, filtered (70 Hz to 3 kHz), and stored on a computer for subsequent analyses using the Cambridge Electronic Design 1401 system. In each of five animals, one piece of two or three spinal segments was cut with small scissors, and ventral root activity was recorded after 1 h of recovery.

RESULTS

One of the 21 preparations displayed spontaneous rhythmic activity alternating between the 2 sides in normal Ringer. This activity never lasted >1 min. After adding NMDA (2.5–10 μM) with d-serine (10 μM) to the bath, sustained rhythmic bursts of activity occurred within ∼20 min in 20 of the 21 preparations. This activity persisted for up to 2 h and disappeared after wash out of the drugs. The threshold concentration of NMDA for evoking rhythmic activity was 2.5–5.0 μM. At concentrations above 15 μM, the activity in all VRs became tonic.

Two distinct patterns of rhythmic activity were observed. The first one was characterized by a rostrocaudal propagation of the rhythmic activity along the spinal cord (Fig. 1B1). The second one consisted of a slower rhythm with a caudorostral propagation (Fig. 1B2). Increasing the concentration of NMDA...
from 5.0 to 7.5 μM increased progressively the slow rhythm frequency in five experiments (Fig. 1C; t-test: \( P < 0.0001 \)) while it elicited a switch to a fast rhythm in two experiments.

The cycle periods, defined as the time intervals between the onset of two successive bursts in a given VR, were distributed in two distinct ranges (measured in 4 animals): 2.4–9.0 s (4.9 ±1.2 s, mean ± SD; \( n = 86 \); Fig. 2A; 

The delay between motor burst onsets in the 9th and 11th VRs was measured during both rhythmic motor activities in three animals. The phase lag (delay/cycle duration in the 9th VR) had a positive value (0.13 ± 0.10) during the fast rhythmic activity (Fig. 2B; empty bars) and a negative value (−0.16 ± 0.09) during the slow rhythmic activity (Fig. 2B; filled bars).

The phase lag values for the two rhythmic patterns were significantly different from zero (t-test: \( P < 0.001 \)) and each other (t-test: \( P < 0.0001 \)).

At 5 μM NMDA, 17 preparations displayed a slow rhythmic motor pattern, whereas one showed the fast rhythmic pattern. Interestingly in two preparations, both patterns of rhythmic activity were observed as the rhythmic activity spontaneously switched from one pattern to the other without any changes in the concentration of NMDA. This is exemplified in Fig. 3A, where the rhythmic activity first switched from a slow rhythm to a fast one, and then again to a slow rhythm, with a short recurrence of the slow rhythm in the middle of the fast activity (arrow). The transition from one pattern to the other occurred within 1–2 cycles. In preparations displaying both patterns, the slow rhythm predominated with longer and more numerous episodes. In seven experiments it was also possible to selectively induce a switch from slow to fast activity by increasing the NMDA concentration from 5 to 10 μM (Fig. 3B). A spinal transection was performed at the first spinal segment in five experiments; three preparations displayed a slow rhythm, one a fast rhythm, and one both rhythms, indicating that both patterns were present in the isolated spinal cord.

Preliminary experiments were carried out to determine how distributed the neural network-generating rhythmic axial motor activity was. Figure 3C shows a bilaterally alternating rhythmic motor activity (slow rhythm) in a piece of three spinal segments (11th to 13th) during coapplication of NMDA (5 μM) and D-serine (10 μM) in the bath. Ventral root discharges on one side were nearly synchronous in this short piece of spinal cord. A similar rhythmic activity was observed in the other pieces of the three spinal segments (2nd to 4th; 8th to 10th; 14th to 16th) and in one piece of two segments (14th to 15th).

**DISCUSSION**

Our results show that the in vitro brain stem–spinal cord preparation from the adult *P. waltl* superfused with a mixture of NMDA and D-serine displays a sustained rhythm motor activity in the absence of rhythmic sensory inputs (fictive activity). Two distinct patterns were observed with different intersegmental coordination.

The fast rhythm displayed a rostrocaudal propagation as seen during swimming in intact urodeles (Delvolve et al. 1997; Frolich and Biewener 1992). It remains to be determined whether this pattern of coordination reverses at two specific sites of the trunk, as reported for myomere activations during forward swimming in intact animals (Delvolve et al. 1997).
During the slow pattern, the intersegmental coordination was reversed with a caudorostral propagation. This feature is similar to that reported for the axial motor pattern recorded during real or fictive struggling in Xenopus embryos (Green and Soffe 1996; Kahn and Roberts 1982). However, further experiments are needed to establish whether the adult Pleurodeles waltl can exhibit a behavior similar to struggling seen in Xenopus tadpoles.

The NMDA-evoked rhythms observed in the present study were slower than the locomotor rhythms in intact animals (Devolve et al. 1997). The activation of non-NMDA receptors might speed up the fictive rhythm as reported in lamprey (Brodin et al. 1985).

Our preliminary experiments further suggest that the rhythm-generating capability is distributed along the spinal cord. Hence the axial locomotor network in P. waltl might have a similar longitudinal organization (chain of coupled segmental oscillators) to the lamprey or Xenopus embryo locomotor networks (Cohen and Wallén 1980; Roberts and Alford 1986). Although in lampreys, every isolated spinal segment shows equal rhythm-generating capability (Grillner et al. 1983), in the Xenopus embryos, caudal spinal segments show progressively less ability to sustain a rhythm (Roberts and Alford 1986). Further experiments are in progress to investigate whether the frequency and pattern of motor activity are similar in every piece of the spinal cord of P. waltl.

We thank Dr. T. Bem for valuable comments on the manuscript. This study was supported by grants from Université Bordeaux I, the Conseil Régional d’Aquitaine, a France-Québec exchange program, and a Group grant from the Canadian Medical Research Council to R. Dubuc.

Received 24 December 1998; accepted in final form 22 April 1999.

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