ACTION OF THE HYPAXIAL MUSCLES DURING WALKING AND SWIMMING IN THE SALAMANDER *DICAMPTODON ENSATUS*

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Summary

Activity of the hypaxial muscles during walking and swimming was measured in the salamander *Dicamptodon ensatus.* During walking, there was activity on both sides of the trunk simultaneously. The obliquus externus superficialis and profundus muscles were active on the side towards which the trunk was bending (flexing), while the obliquus internus and transversalis muscles were active on the opposite side. In contrast, during swimming, all four of these muscles were active on the side of the trunk that was bending to become concave. These observations are consistent with the hypothesis that during terrestrial ambulation the obliquely oriented hypaxial muscles do not contribute to lateral bending of the trunk or to horizontal propulsion, but to stabilization of the trunk against long-axis torsion.

Introduction

The precise coupling of breath and locomotor cycles in running mammals (Attenburrow, 1982; Bramble and Carrier, 1983; Baudinette *et al.* 1987; Young *et al.* 1992) and the inability of lizards to breathe when they run (Carrier, 1991) illustrate a tight mechanical linkage between the locomotor and ventilatory systems of amniotic vertebrates. This linkage results from both functions being produced, at least in part, by some of the same elements of the hypaxial musculo-skeletal system. The various mechanisms by which the hypaxial musculo-skeletal system elicits breathing have received considerable attention and have been well characterized in tetrapods. In contrast, the role that this system plays in terrestrial locomotion is poorly understood.

Observations in walking lizards suggest that the hypaxial muscles function to stabilize the trunk against the horizontal and vertical components of the propulsive force that the limbs exert on the ground (Carrier, 1990). Hypaxial muscles that have a predominantly longitudinal orientation appear to stabilize the trunk against the horizontal component. Those muscles with oblique orientations appear to resist long-axis torsion of the trunk resulting from the vertical component of the propulsive force. Specifically, muscles with anterodorsal oblique orientation, such as the external oblique, are active contralateral to

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hindlimb support while muscles with an anteroventral orientation are active ipsilateral to hindlimb support. This pattern of simultaneous activity on the two sides of the trunk seems to be inappropriate for the production of lateral bending of the trunk, but would be effective in resisting long-axis torsion which occurs during diagonal limb support.

This investigation tests the hypothesis that obliquely oriented hypaxial muscles stabilize the trunk against the vertical component of the propulsive force by comparing the action of these muscles during swimming to that during walking in the Pacific giant salamander, *Dicamptodon ensatus*. Salamanders provide a natural test of this hypothesis. Lateral bending of the trunk occurs in both swimming and terrestrial ambulation, but neutral buoyancy eliminates the force of gravity during swimming. Consequently, if the obliquely oriented hypaxial muscles act to stabilize the trunk against the vertical component of the propulsive force, one would expect very different patterns of activity during lateral undulation in swimming compared with lateral bending in terrestrial walking.

Materials and methods

Experiments were performed on six adult Pacific giant salamanders (*Dicamptodon ensatus* (Eschscholtz)) collected in the Santa Rosa Mountains of northern California. Specimens had a mean snout–vent length of 12.7±0.4cm. They were housed in a cold room maintained at 15˚C, with a 12h:12h light:dark photoperiod, and were fed a diet of crickets. Two additional specimens were preserved for dissection of the hypaxial muscles.

Activity of the m. obliquus externus, m. obliquus internus and m. transversalis was monitored during swimming and terrestrial walking. Patch electrodes were used because they provide an electrically non-conductive barrier in one direction. If applied with care, patch electrodes greatly reduce the risk of cross-talk from adjacent muscles (Loeb and Gans, 1986). This is particularly helpful in situations where the muscles of interest are thin and packed closely together, as is the case in vertebrate hypaxial muscles. Electrodes were constructed from Dow Corning 501-1 Silastic reinforced sheeting and Teflon-insulated 75 μm diameter stainless-steel wire (Carrier, 1989). The non-insulated portions of the two electrode wires were approximately 1mm long and separated by roughly 1mm. Prior to surgery, the animals were anesthetized by immersion in a 2% solution of ethyl *p*-aminobenzoate. Electrodes were implanted through small incisions (approximately 2cm) in the skin overlying the site of interest. Electrode wires were passed subcutaneously to a single exit point on the mid-line of the back. Electrodes on the m. obliquus externus superficialis were implanted on the medial surface of the muscle and faced laterally. Those on the m. obliquus externus profundus, m. obliquus internus and m. transversalis were implanted on the lateral surfaces of the muscles and faced medially. Electrical signals from the muscles were passed to Tektronix FM 122 preamplifiers, amplified 1000 times and filtered below 80Hz and above 10kHz. Signals were then amplified an additional 10 times by home-built A/C amplifiers, and simultaneously stored on a Crown International 700 tape recorder and printed out on a Beckman 611 chart recorder.

Following recovery from the anesthesia, the salamanders swam and walked in a trough

(200cm×20cm×20cm), while activity of the hypaxial muscles was recorded. The trough was filled with 10cm of water for swimming. For walking, the trough was dry and the bottom was lined with wet paper towels. The animals were easily induced to walk or swim the length of the trough by touching or gently pinching the base of the tail. Each walking and swimming sequence was recorded on video tape (60images s^{-1}) with a camera suspended directly over the trough. To monitor lateral bending of the trunk, three white plastic dots were sutured along the midline of the back; one each above the pectoral and pelvic girdles and one at mid-trunk. To determine the relationship between timing of muscle activity and timing of lateral bending of the trunk and of limb support, an electrical circuit was used that simultaneously turned on a light in the view of the camera and sent a small pulse to the recorders. Video recordings were analyzed with a PEAK motion measurement system.

Results

Anatomy

The hypaxial musculature of *Dicamptodon ensatus* consists of five muscles: obliquus externus superficialis, obliquus externus profundus, obliquus internus, transversalis and rectus abdominis (Fig. 1). Four of these have oblique orientations and were investigated in this study.

M. obliquus externus superficialis

The superficial layer of the external oblique originates on an aponeurosis that ties into the lateral margin of the epaxial muscles. Fibers course ventrally and caudally, at an angle of approximately 60˚ to the horizontal, to insert on the lateral edge of the rectus abdominis muscle and a very thin aponeurosis that extends to the ventral midline. Fibers run from the origin aponeurosis to the insertion aponeurosis. Thus, although this muscle is arranged into semi-discrete bands, it is not segmented into myomeres.

M. obliquus externus profundus

The deep layer of the external oblique also has a ventrocaudal orientation, but its fibers are oriented at a more longitudinal angle of approximately 30˚ to the horizontal. This muscle originates on the neural arches of the vertebrae. Fibers extend ventrally to the epaxial muscles and dorsally to the ribs to form a sheet which largely encloses the body cavity. Ventrally, the fibers run above (dorsal) the rectus abdominus muscle and insert on the linea alba. Posteriorly, the fibers also insert on the pubic bones. Anteriorly, at the level of the glenoid, the fibers rotate to assume a more longitudinal orientation and course anteriorly to form the rectus cervicus muscle with the internal oblique, which inserts on the hyoid apparatus and tongue. The entire muscle is segmented into myomeres.

M. obliquus internus

The internal oblique is oriented ventrocranially at an angle of approximately 35˚ to the horizontal. It originates on the anterior surface of the lateral portion of the very short ribs. Fibers run cranially and ventrally to insert on the linea alba in the middle and posterior
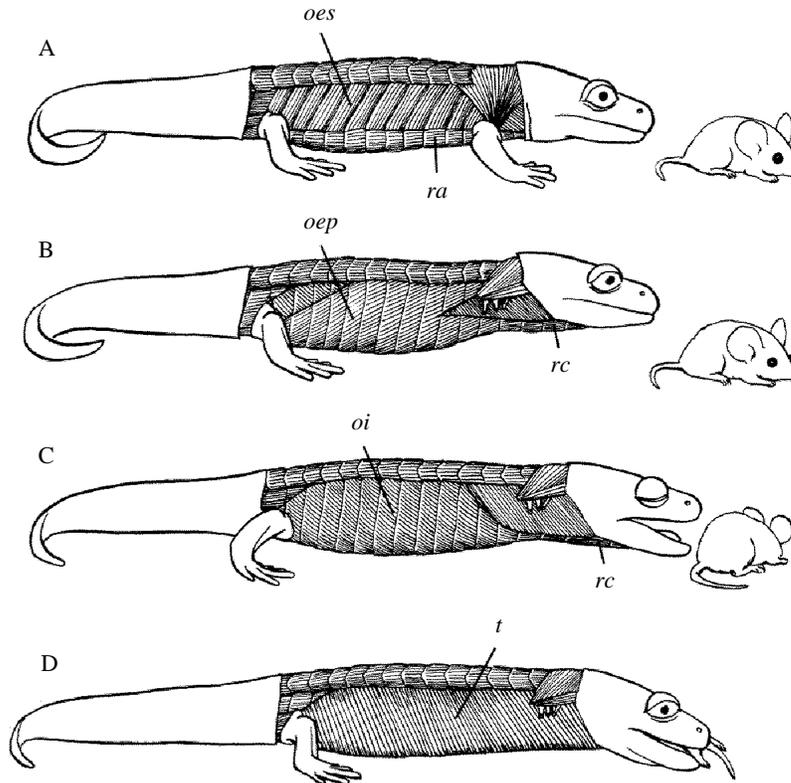
Fig. 1. Hypaxial musculature of *Dicamptodon ensatus*. (A) Lateral view of the superficial muscles. (B–D) Lateral views showing successively deeper muscles. *oep*, m. obliquus externus profundus; *oes*, m. obliquus externus superficialis; *oi*, m. obliquus internus; *ra*, m. rectus abdominis; *rc*, m. rectus cervicus; *t*, m. transversalis.

portions of the trunk. Anteriorly, the fibers take a more longitudinal orientation. They join with the anterior fibers of the deep external oblique to course forward to form the rectus cervicus muscle, which inserts on the hyoid apparatus and body of the tongue. Like the deep external oblique, this muscle is segmented into myomeres.

M. transversalis

The transversalis muscle originates on an aponeurosis that lies ventral to the epaxial muscles and attaches to the neural arches of the vertebrae. Fibers run ventrally and slightly cranially at an angle of approximately 65° to the horizontal to insert on a short aponeurosis which extends to the ventral midline. Fibers run from origin to insertion and the muscle is not segmented.

These four obliquely oriented hypaxial muscles form two sets of opposing helical sheaths which wrap around the trunk. The first set consists of the superficial external oblique and the transversalis on the opposite sides of the body. Together, these two muscles form two helical layers with fibers conversely oriented at approximately 60° to the horizontal. The other set of helical sheaths consists of the deep external oblique and,
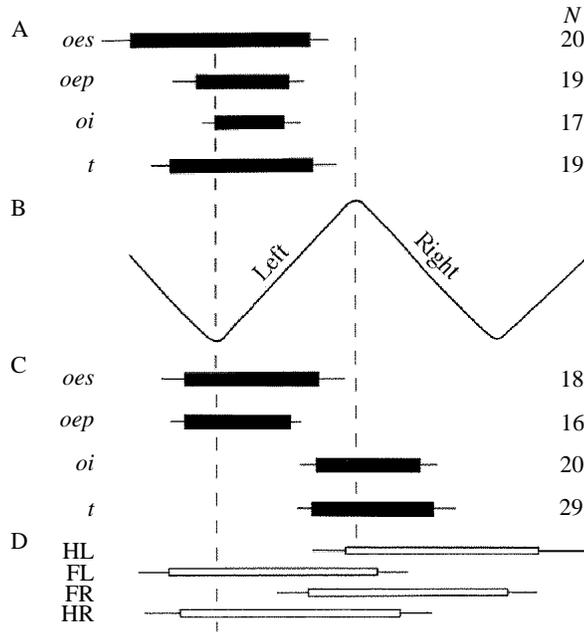
Fig. 2. Onset and offset times of the hypaxial muscles on the left side of the body during walking and swimming in *Dicamptodon ensatus*. (A) Means and standard deviations of activity of hypaxial muscles relative to trunk bending during swimming. Muscle names are abbreviated as in Fig. 1. (B) Trace of lateral bending of the trunk, indicating the timing of flexion to the left and right. The peak represents the point at which the trunk is maximally flexed (bent concavely) to the left. The valleys represent the points when the trunk is maximally flexed to the right. (C) Means and standard deviations of the activity of hypaxial muscles relative to foot support and trunk bending during walking. Data for each muscle represents equal sampling from at least two individuals. (D) Summary of foot support during walking relative to trunk bending. FL, left forefoot; FR, right forefoot; HL, left hindfoot; HR, right hindfoot.

on the opposite side, the internal oblique. Together, these two muscles form two layers with fibers conversely oriented at approximately 30° to the horizontal.

Muscle activity during swimming

During swimming, all four of the lateral hypaxial muscles were active on the side of the body to which the trunk was being flexed (Fig. 2). Hence, the four muscles on the left side of the body were active as the trunk bent to assume a concave curve on the left side. Activity ceased slightly before the trunk became maximally flexed on the left side. Within a given myotome, the four muscles became active at slightly different times. On the left side, the deep layer of the external oblique and the internal oblique were activated at approximately maximum flexion to the right, the point at which the trunk began to bend back to the left. In contrast, the superficial layer of the external oblique and the transversalis became active earlier in the cycle, as the trunk was approaching maximum flexion to the right, and remained active longer than the deep layer of the external oblique and internal oblique. The superficial external oblique and transversalis were active for
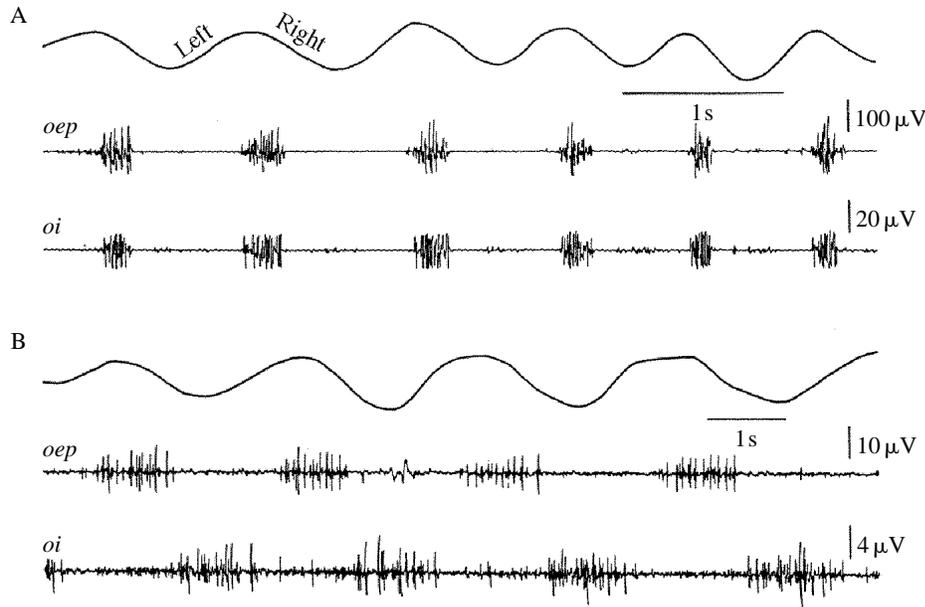
Fig. 3. Sample recordings comparing muscle activity recorded during swimming (A) with that recorded during walking (B) from the right m. obliquus externus profundus (*oep*) and right m. obliquus internus (*oi*) in *Dicamptodon ensatus*. Swimming and walking signals are recorded from the same two electrodes.

approximately 64% and 51% of the cycle, respectively, while the deep external oblique and internal oblique were active for 32% and 25% of the cycle, respectively.

Muscle activity during walking

The pattern of hypaxial muscle recruitment during walking differed from that observed during swimming (Figs 2, 3). During walking, only the superficial and deep external oblique muscles were active on the flexing side of the trunk. Simultaneously with the activity in these two muscles, the internal oblique and transversalis muscles were active on the extending (convex bending) side of the trunk.

Muscle activity during walking can also be correlated with the phase of limb support (Fig. 2). The two external oblique muscles were active on the opposite side of the body from hindlimb support, and the internal oblique and transversalis muscles were active on the same side of the body as hindlimb support. Thus, the two muscles that are obliquely oriented anterodorsally were active contralateral to hindlimb support, while the two muscles that are obliquely oriented anteroventrally were active ipsilateral to hindlimb support.

Discussion

Hypaxial muscles which have oblique orientations serve different functions in swimming and walking. During swimming in the Pacific giant salamander, all four hypaxial muscles are active on the side towards which the trunk is flexing. This pattern of
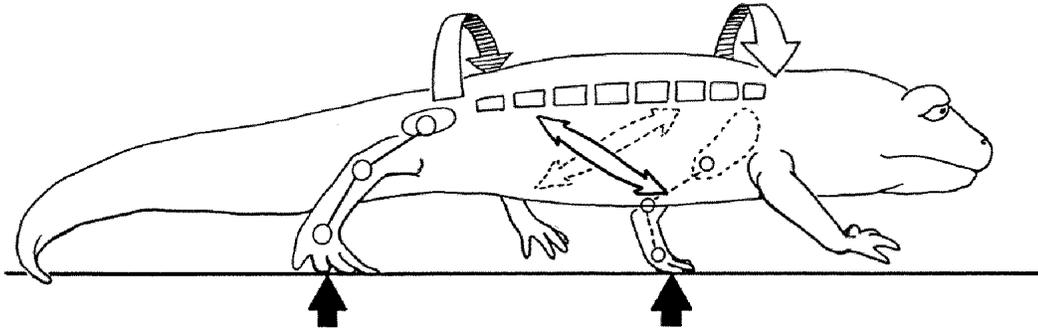
Fig. 4. Schematic representation of the loading of the trunk due to the vertical component of the propulsive ground force. The salamander is supported by diagonal limbs (filled arrows), which impose long-axis torsion (curved arrows) on the axial musculo-skeletal system. To stabilize the trunk against this torsion, hypaxial muscles with oblique orientations are necessary. The filled white arrow indicates the orientation of muscles that must be active on the side of the body ipsilateral to hindlimb support. The dashed white arrow indicates the orientation of muscles that must be active on the side of the body contralateral to hindlimb support.

unilateral firing is what one would expect for the production of lateral bending of the trunk and is similar to the activity of the caudal hypaxial musculature in fishes (Bone, 1966) and the epaxial muscles of salamanders (Frolich and Biewener, 1992) during swimming. In contrast, during walking, activity occurs on both sides of the trunk simultaneously. At the same time that the two external oblique muscles are active on the side towards which the trunk is flexing, the internal oblique and transversalis muscles are active on the other side. Any tendency that the external oblique muscles have to bend the trunk laterally appears to be countered by the action of the internal oblique and transversalis muscles of the opposing side. Therefore, during walking, these muscles are unlikely to contribute in a significant way to the production of lateral bending.

The observed pattern could, however, act to stabilize the trunk against the vertical component of the propulsive force (Fig. 4). When salamanders walk they are largely supported by diagonal limbs; the left hindlimb and right forelimb are on the ground while the right hindlimb and left forelimb are in swing phase. As a consequence of diagonal support, there is a tendency for the pectoral and pelvic girdles to collapse to the sides of the unloaded limb, which produces long-axis torsion in the axial musculo-skeletal system. During each locomotor cycle, the axial system experiences a torsional load with a right-handed orientation during right hindlimb and left forelimb support and then a torsional load with a left-handed orientation during left hindlimb and right forelimb support (Carrier, 1990). The oblique orientations of the hypaxial muscles provide a way to resist this long-axis torsion. The left external oblique muscles combined with the right internal oblique and transversalis muscles form two helical sheaths, which are oriented in a way that can resist right-handed torsion, whereas the right external oblique muscles combined with the left internal oblique and transversalis muscles make two helical sheaths that can resist left-handed torsion.

Two observations suggest that the hypaxial muscles stabilize the trunk against long-

axis torsion during walking. First, the timing of limb support and muscle activity shows the hypaxial muscles to be active at the appropriate time for torsional stabilization. Furthermore, this same pattern of activity characterizes the hypaxial muscles of walking lizards (Carrier, 1990). Second, during swimming, when salamanders are neutrally buoyant, the hypaxial muscles are active in a manner that is consistent with lateral bending of the trunk, rather than torsional stabilization. Indeed, the results presented here suggest that the oblique orientations of the hypaxials are not required for swimming; a single longitudinally oriented muscle would be more effective in the production of lateral trunk bending than four separate muscles which are oriented obliquely. Thus, the evidence suggests that during terrestrial ambulation the obliquely oriented hypaxial muscles stabilize the trunk against long-axis torsion that is induced by the vertical component of the propulsive force.

If we assume that the forces produced during walking by the two external oblique muscles are roughly equivalent to the forces produced on the opposite side of the body by the internal oblique and transversalis muscles, then the pattern of hypaxial muscle activity is not compatible with the production of lateral trunk bending or with the need to stabilize the trunk against forward acceleration and deceleration. Both of these functions could be accomplished, most efficiently, by unilateral activity of axial muscles. The muscles with the most appropriate orientations for this are the epaxial muscles or the longitudinally oriented rectus abdominus muscle of the ventral body wall. Timing of activity in the epaxial muscles during terrestrial locomotion in the salamander *Ambystoma tigrinum* is correlated with lateral trunk bending (Frolich and Biewener, 1992) and does appear to be appropriate for stabilization against forward acceleration and deceleration.

Although this study provides additional support for the role that the hypaxial muscles play in terrestrial locomotion, the findings raise an important question. If the oblique orientations of hypaxial muscles are necessary for walking but not swimming in salamanders, why are the hypaxial muscles of fishes obliquely oriented? Elasmobranch, actinopterygian and sarcopterygian fishes all have a hypaxial musculature that is differentiated into external and internal oblique layers in the trunk region (Maurer, 1913). The orientation of these muscles suggests that fish experience axial torsion during swimming. There is some evidence that this occurs. Lauder (1989) measured the strain patterns induced in the caudal skeleton of sunfishes during steady swimming and fast-starts. He found that the mean principal strains were aligned parallel to the body axis during fast-starts, but were inclined anterioventrally at a mean angle of $-34°$ to the body axis during steady locomotion. When the propulsive force is aligned parallel to the body, axial torsion will not occur. (This may be the situation during straight-line swimming in salamanders.) However, if thrust is oriented at an angle to the body axis, axial torsion can be expected.

Early fishes are likely to have experienced long-axis torsion during swimming as well. Many of the early fishes had caudal fins which were angled dorsally (Romer, 1966). The functional significance of such 'heterocercal tails' has received considerable attention and is generally thought to have provided a lifting component to the generated thrust (Affleck, 1950; Alexander, 1965). This upward component of the thrust is suggested to have helped to counter the tendency of these heavily armored animals to sink in the water column

(Thomson, 1971). If this were true, thrust would have been oriented at an anterodorsal angle to the body axis and would have induced axial torsion. These fishes were equipped with helically arranged ganoid scales, and these might have provided a means for stabilizing the trunk against long-axis torsion. However, passive stabilization (i.e. that produced by bone or collagen) is not always advantageous because it places strict limits on mobility. Stabilization of a moveable joint, in the direction of movement, can only be accomplished with muscle. Thus, if early fishes had any capacity to twist along their body axis, obliquely oriented hypaxial muscles may have provided a mechanism for resisting axial torsion that was inherent in a locomotor system which generated forward thrust with an asymmetrically shaped caudal fin.

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