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PHYSIOLOGICAL FEATURES OF THE OPERCULARIS MUSCLE AND THEIR EFFECTS ON VIBRATION SENSITIVITY IN THE BULLFROG *RANA CATESBEIANA*

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SUMMARY

The amphibian opercularis muscle connects a movable otic element (the operculum) to the pectoral girdle and can act in reception of ground vibrations. Various physiological parameters of the opercularis muscle of the bullfrog *Rana catesbeiana* were measured and compared with similar measurements on the iliofibularis muscle of the hindlimb. The opercularis muscle is a very slowly contracting muscle, with a V_{max} of 1·81 muscle lengths s^{-1} compared to a V_{max} of 6·24 muscle lengths s^{-1} for the iliofibularis muscle. The opercularis muscle develops tension slowly, taking about 10 s to attain maximum isometric tension when stimulated at 100 Hz. The muscle can retain high levels of tension for several minutes, and following stimulation has a time to half-relaxation of about 4–6 s. The slow velocity of contraction, slow rate of tension development, fatigue-resistance and slow rate of relaxation of the opercularis muscle support morphological evidence that it consists mostly of tonic muscle fibres. Experiments were also made to examine the effects of muscle tension on reception of ground vibrations as measured by inner ear microphonics. Severing the nerve supplying the opercularis muscle produced slight decreases of no more than 2 dB in responses to vibrations from 25 to 200 Hz. Artificial stimulation of the opercularis muscle after severing the nerve supplying the muscle increased responses to vibration across the entire frequency range. Higher tension levels produced greater increases in responses; at the highest tensions used (about 120 kN m^{-2}) responses were increased by as much as 4·5 dB. The opercularis muscle is therefore specialized for slow but prolonged contractions, and tension is important in its sensory function. A tensed opercularis muscle appears to transmit faithfully motion of the forelimb, produced by vibrations, to the operculum such that the latter moves relative to the inner ear fluids.

INTRODUCTION

The opercularis system of amphibians consists of an opercularis muscle that connects an operculum, a typically cartilaginous element lying next to the inner ear, to the suprascapula of the pectoral girdle. The system was originally considered to function in reception of ground vibrations (Kingsbury & Reed, 1909), and physiological evidence demonstrates that the system can increase inner ear responses

Key words: amphibian, opercularis muscle, vibratory sensitivity.

to vibration in the bullfrog *Rana catesbeiana* (Hetherington, 1985). The operc ularis muscle appears to act as a structural link between the forelimb and operculum such that the latter will move in response to substrate motion acting on the forelimbs. Movement of the operculum presumably produces fluid waves within the inner ear that stimulate appropriate end organs. However, there are additional hypotheses concerning the function of the opercularis system. Lombard & Straughan (1974) found that severing the opercularis muscle reduced midbrain responses to low-frequency sound (below about 1 kHz) in several anuran species. They speculated that the opercularis muscle can interlock the operculum and the stapedial footplate, thereby adding mass to the tympanum–stapes complex and increasing its responsiveness to low-frequency sound. However, these results could also be explained by a direct role of the opercularis system in reception of low-frequency sound independently of the tympanic ear. Wilczynski, Resler & Capranica (1981) demonstrated that an alternative pathway exists in *Rana pipiens* for reception of sound below about 500 Hz. The opercularis system could possibly act as such a pathway (Hetherington, 1985). Another major hypothesis for the function of the opercularis system is that of a protective mechanism. Wever (1985) suggests that the opercularis muscle can interlock the operculum with the stapes in such a way as to limit motion of the latter, thereby preventing overstimulation of the inner ear in the event of loud sounds.

Investigation of the morphological and physiological features of the opercularis muscle has provided important evidence about the functional capabilities of the opercularis system. Analysis of ultrastructural characteristics of the opercularis muscle of several different amphibians has established that it is a primarily tonic muscle (Becker & Lombard, 1977). Electromyographic analysis of the muscle has shown that in the bullfrog *Rana catesbeiana* it has a rhythmic pattern of activity linked with lung ventilation (Hetherington & Lombard, 1983). Given the evidence for the tonic nature of the muscle, the rhythmic pattern of stimulation suggests that the muscle is kept tensed in terrestrial situations. This corresponds well with the hypothesis that the opercularis muscle fundamentally acts as a connecting element to transmit motion of the pectoral girdle to the operculum. Such data make functional hypotheses linking the system with modulation of stapedial responsiveness less likely, as the opercularis muscle may be incapable of rapid contraction, a property that would be important for rapid modification of middle ear sensitivity.

Although physiological characteristics of the amphibian opercularis muscle have been inferred from the morphological and electromyographic studies noted above, there has been no direct analysis of such properties of the muscle. This paper describes experiments on opercularis muscle physiology that directly measure contractile features that should be functionally important. For purposes of comparison, these properties are measured for the iliofibularis muscle of the hindlimb, a muscle that in anurans is composed mainly of twitch fibres of the fast glycolytic category (Putnam & Bennett, 1983) and that therefore should contrast sharply with the opercularis muscle in many physiological characteristics. Additional experiments investigate how physiological characteristics of the opercularis muscle affect its role

in vibration sensitivity. By direct stimulation of the muscle, the influence of muscle tension on the responses of the inner ear to vibration is investigated.

MATERIALS AND METHODS

Muscle physiology

Studies were made on the opercularis muscle of the bullfrog *Rana catesbeiana*. All experimental techniques in this and following sections complied with the National Institutes of Health (USA) Guidelines on the use of vertebrate animals. Adult bullfrogs (12–16 cm in snout–vent length; 300–400 g body mass) were killed by pithing and the skin and superficial muscles overlying the shoulder and otic region were removed. If the suprascapular cartilage is lifted, both the opercularis and levator scapulae superior muscles are easily observed passing from the suprascapula towards the otic region. The opercularis muscle appears as a richly vascularized muscle (reddish in colour) lying on the anterior edge of the less-vascularized, paler levator scapulae superior. The distinct difference in colour is useful in dissecting the opercularis muscle from the levator scapulae superior, as the connective tissue separating the two muscles is thin and not easy to detect. The opercularis muscle has a fleshy origin on the inner surface of the anterior part of the suprascapular cartilage, and a tendinous insertion on the operculum. To remove the opercularis muscle, the tendon was cut close to the operculum and the cartilage from which the muscle originates was cut from the suprascapula. The dissected muscle therefore had a short tendon at one end (approximately 1·0–1·5 mm in length) and a small piece of cartilage attached to the other end. The original length of the muscle *in situ* was measured so that in isometric experiments the muscle could be tested at its approximate normal length. The iliofibularis muscle of the hindlimb was also dissected from the frogs. This muscle has tendons at both ends, and is well suited for physiological study (Putnam & Bennett, 1983). During surgery all muscles were kept constantly moist with amphibian Ringer solution (Gibbs & Chapman, 1974).

Following removal, the muscles were placed in Ringer solution at room temperature (about 22 °C). Nylon thread was tied to the tendinous end of the opercularis muscle and the cartilaginous end was secured in a small clamp. Nylon thread was tied to both tendinous ends of the iliofibularis muscles. During experiments, muscles were kept constantly moist with oxygenated Ringer solution. In tests on the opercularis muscle, the clamp holding the suprascapula fragment was tied by nylon thread to a Grass FT10.C force transducer, and the thread on the tendinous end was tied to one side of the muscle lever of a Harvard Bioscience isotonic transducer. In tests on the iliofibularis muscle, one tendon was tied to the force transducer, the other to the isotonic transducer. By altering the position of the muscle lever of the isotonic transducer, the muscles could be held at the resting lengths measured during dissection. Stimulation of the muscles was accomplished with two pairs of wire electrodes placed on either side of the muscles and connected to a Grass SD-9 stimulator.

Rate of tension development, maximum tension levels and rate of relaxation were measured during isometric contractions of opercularis muscles with the muscle lever of the isotonic transducer held in position. These parameters were measured for three muscles, each from a different frog. Muscles were stimulated by 10 V pulses lasting 2 ms at three different frequencies (2, 20 and 100 Hz) for up to 60 s. The 2 Hz pattern of stimulation was used to approximate the 2 Hz rhythm of muscle stimulation observed during electromyographic analysis of opercularis muscle activity in *Rana catesbeiana* (Hetherington & Lombard, 1983). Tension produced by muscle stimulation was measured by the force transducer, amplified, and plotted on a Grass Model 7 Polygraph. Rate of tension loss was determined by measuring changes in tension following the end of stimulation. Force production relative to the cross-sectional area of the muscle was calculated in $kN\,m^{-2}$. Muscle cross-section was approximated by dividing the mass of the muscles by its length times its specific gravity. As both the opercularis muscle (Hetherington, Jaslow & Lombard, 1986) and iliofibularis muscle (Putnam & Bennett, 1983) are parallel-fibred, this method provided an accurate approximation. Applied loads were converted to kN.

Velocity of contraction and force–velocity curves were determined for both opercularis and iliofibularis muscles using isotonic contractions. These data were obtained for five muscles of each type, (each muscle from a different frog). Muscles were stimulated by 10 V pulses lasting 2 ms at a frequency of 100 Hz. Loads on the muscles were produced by attaching weights to the muscle lever to which the muscle was attached. Muscle contraction was plotted on a Harvard Apparatus chart mover and velocity of shortening determined from the slope of the length *vs* time trace. Maximum velocity of contraction can be calculated from Hill's equation (1938)

$$(P + a)(v + b) = (P_0 + a)b\,, \tag{1}$$

which is an expression of the variation of velocity of contraction (v) with load (P). P_0 is the maximum isometric tension that the muscle can produce at the stimulation rate used in the isotonic tests, and a and b are constants. These constants were determined from a linearized regression of the above equation. Maximum velocity of contraction was then calculated from the following formula

$$V_{max} = P_0 b/a \tag{2}$$

Vibration sensitivity experiments

Experiments were designed to analyse the effects of muscle tension on responses to vibration in adult *Rana catesbeiana*. Vibration responses were measured as microphonic signals that emanate primarily from the saccular macula (Hetherington, 1985). Experiments first involved determining the effect of normal muscle tension on vibration responses in animals with the nerve to the opercularis muscle intact. After measurement of vibration responses with an intact nerve, the nerve was severed and responses to vibration were measured again and compared with the control values. Following this test, responses to vibration were measured as tension in the opercularis muscle was manipulated by direct stimulation with electrodes.

Adult *Rana catesbeiana* were anaesthestized with ethyl carbamate (1 ml of 20% solution per 50 g body mass), and a slit was made in the skin and connective tissue overlying the shoulder and otic regions. The operculari muscle was dissected away from the underlying levator scapulae superior, and a piece of Parafilm was slipped between the two muscles. This allowed for isolated electrical stimulation of the operculari muscle in later experiments. Care was taken not to damage the nerve innervating the operculari muscle on its medial side or the larger blood vessels supplying the muscle. Placement of electrodes into the inner ear required drilling a small hole through the dorsolateral otic capsule and into the periotic cistern region of the inner ear. Tungsten electrodes with tapered coatings of epoxy resin (thinnest towards the exposed tip) were then placed in the hole. The tapered insulation allowed the electrode to be firmly wedged into the hole and prevented leakage of inner ear fluids or displacement of the electrode.

All tests were conducted inside a dark, anechoic sound chamber. Frogs were tied by the waist and hindlimbs to a piece of metal screen that was suspended 5 mm above (but was not in contact with) an aluminium platform mounted on a Bruel & Kjaer 4820 vibration exciter. A hole was cut through the screen underneath the forelimb on the side of the body from which recordings were made, and that forelimb was placed on the aluminium platform attached to the vibration exciter. Therefore, only the forelimb of the appropriate side of the body was vibrated directly. Removal of the operculari muscle in animals tested in this manner produced more pronounced decreases in vibration responses than that observed in animals in which the entire body was in contact with the platform. Presumably, when the entire body is in contact with the platform, alternative routes for vibration to the inner ear may exist and, although removal of the operculari muscle still tends to lower responses to vibration (Hetherington, 1985), the effect is less pronounced.

Frogs were allowed to recover from anaesthesia before experimentation. This allowed the animals to be tested in a normal, sitting posture that would have minimal interference with normal operculari muscle function. The frogs displayed little restlessness during experiments and usually sat immobile in the dark sound chamber. Microphonic signals recorded by the electrodes in the inner ear were amplified and measured at twice the frequency of the vibratory stimulus (Hetherington, 1985) on a Hewlett-Packard 3600 wave analyser. Microphonic signals in amphibians are typically double the frequency of the stimulus because of a mixture of hair cell orientations in the sensory end organs (Paton, 1971). This is convenient because the microphonic responses are clearly distinct from any motion artefact signals that may occur at the stimulus frequency. The microphonic signals were also recorded on a Honeywell 7600 tape recorder. Animals were exposed to vibrations at several frequencies (25, 50, 75, 100, 125, 150, 175 and 200 Hz), although all of the vibratory stimuli had the same peak acceleration ($1{\cdot}0\,cm\,s^{-2}$). Sound pressure levels were monitored during all tests with a Bruel & Kjaer Type 2230 sound level meter and Type 1625 octave filter set, and control tests were performed on two animals to ensure that the microphonic signals were responses to vibrations and not responses to aerial sounds produced by the vibrating platform. It was established that to attain

responses measured during vibration tests using sound stimuli produced by an overhead speaker, sound pressure levels had to be 30–60 dB (depending on the frequency) above those measured during vibration experiments. Thus, the microphonic signals measured in these vibration experiments were indeed responses to vibration and not to sound (for further discussion see Hetherington, 1985).

Vibration responses were first recorded with the nerve to the opercularis muscle intact. Two series of these control measurements were made for each frog to establish consistency in responsiveness. The frogs were then cold-anaesthetized by immersion in iced water, and the nerve innervating the opercularis muscle was severed. As the muscle had already been exposed, this was a simple procedure that involved little disturbance of the muscle. In one animal, a 'mock' surgery, including manipulation of the muscle but no severing of the nerve, was performed and had no effect on responses recorded subsequently. After the temperature of the animals with severed nerves had returned to room level, as checked by cloacal temperatures, another two series of vibration responses were measured. Following these measurements, two metal wire electrodes were placed on the opercularis muscle. The muscle was stimulated by 2 ms square-wave signals at 10 V at a frequency of either 2 or 100 Hz. Previous physiological experiments (see above) had determined that the two different frequencies of stimulation produced different tension levels. Stimulation at 2 Hz lasted for 20 s, whereas stimulation at 100 Hz lasted for 10 s, the respective durations having been determined to be appropriate for tension development in the muscle. Transient potentials interfered with microphonic recording during muscle stimulation, but microphonic signals were recorded on tape before and immediately following stimulation. Following the end of stimulation, muscle tension decreased at a previously determined rate, and changes in vibration responses during this period of tension loss could be monitored. As in other vibration experiments, two series of measurements were made to control for any fatigue or change in responsiveness in the animal. Comparisons of microphonic responses before and following muscle stimulation acted as controls for any permanent changes in responsiveness caused by the electrical stimulation. Response levels were typically the same (±1 dB) before and after stimulation, and were not consistently elevated or depressed by the stimulation technique.

RESULTS

Muscle physiology

The opercularis muscle of *Rana catesbeiana* had a distinctly lower maximum velocity of contraction than the iliofibularis muscle (Table 1). The mean V_{max} for the opercularis muscle at 22°C was 1·81 $L\,s^{-1}$ (where L is muscle length), whereas the mean V_{max} for the iliofibularis muscle was 6·24 $L\,s^{-1}$. Representative force–velocity curves for an opercularis muscle and an iliofibularis muscle from the same specimen of *Rana catesbeiana* showed distinct differences, with a steeper decrease in velocity of contraction at light loads in the opercularis muscle than in the iliofibularis muscle (Fig. 1A). This difference in the shape of the curves is reflected by the different

values for a/P_0 (Table 1), which is a measure of curve shape. The steeper the decrease of V_{max} with increasing load, the lower the a/P_0 value. This value for the opercularis muscles is only about one-third that for the iliofibularis muscle. *In vivo,* the muscles are always loaded, so the velocity of contraction of the opercularis muscle will be even slower than is suggested by comparison of the V_{max} measurements. For example, at a load of about 50% of maximum isometric tension (P_0), the mean velocity of the opercularis muscle was approximately $0{\cdot}10\,L\,s^{-1}$, whereas the velocity for the iliofibularis at 50% of P_0 was approximately $1{\cdot}12\,L\,s^{-1}$. Therefore, whereas the V_{max} of the opercularis muscle is about 29% of the V_{max} of the iliofibularis muscle, velocity of the opercularis muscle at $50\%P_0$ is only about 9% of the velocity of the iliofibularis muscle at $50\%P_0$.

Table 1. *Force–velocity values for the opercularis and iliofibularis muscles of the bullfrog* Rana catesbeiana

	P_0 ($kN\,m^{-2}$)	V_{max} ($L\,s^{-1}$)	a/P_0	$V_{50\%P_0}$ ($L\,s^{-1}$)
Opercularis	121 ± 14	1·81 ± 0·22	0·11 ± 0·03	0·10 ± 0·02
Iliofibularis	292 ± 19	6·24 ± 0·47	0·33 ± 0·06	1·12 ± 0·13

P_0, maximum tetanic tension; V_{max}, maximum velocity of contraction; a/P_0, force–velocity curve constant (see text); $V_{50\%P_0}$, velocity of contraction at 50% of maximum tetanic tension; L, muscle length.

Values are means ± S.E. for a sample size of five animals.

The differences between all mean values for the two muscles are statistically significant ($P < 0{\cdot}05$).

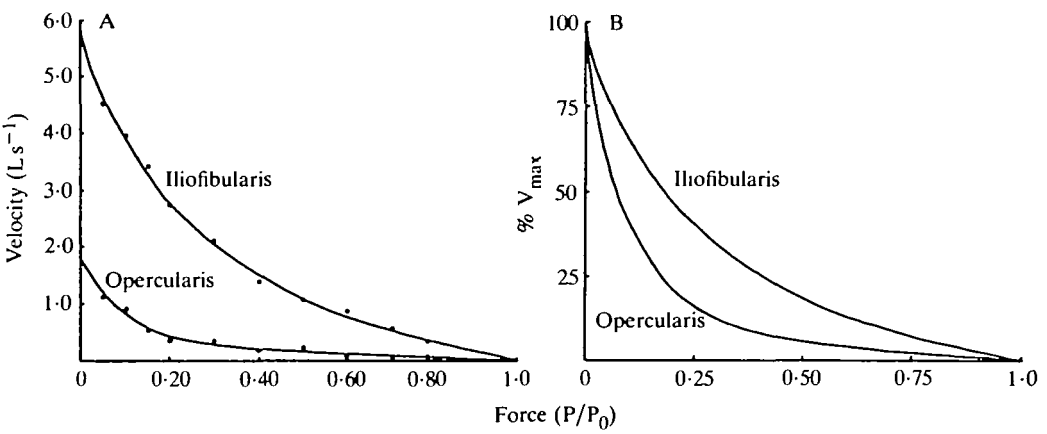

Fig. 1. (A) Force–velocity curves for an opercularis muscle and an iliofibularis muscle from an adult bullfrog (*Rana catesbeiana*). Velocity of contraction is measured as muscle lengths s^{-1} ($L\,s^{-1}$), and force (load) is plotted as the ratio of applied load to maximum isometric tension (P_0). (B) A comparison of the shapes of the two muscle curves above obtained by plotting percentage of V_{max} against the ratio of applied load to maximum isometric tension (P_0). Note that the velocity of contraction of the opercularis muscle is more readily decreased by the application of a load.

The opercularis muscle generated less isometric tension than the iliofibularis muscle and developed tension at a slower rate. At a stimulation of 100 Hz (the frequency of stimulation used in determining the force–velocity curves above) the opercularis muscle generated less force (121 kN m^{-2}) than the iliofibularis muscle (292 kN m^{-2}). The maximum tetanic tension developed by the opercularis muscle, and the rate of its development, varied with frequency of stimulation (Fig. 2). At 100 Hz, maximum tension (about 110 kN m^{-2} in this specimen) was reached after about 10 s. At 20 Hz, maximum tension (about 64 kN m^{-2}) was reached after about 20 s, and at 2 Hz maximum tension (about 26 kN m^{-2}) was reached after about 50–60 s. The iliofibularis muscle reached maximum tetanic tension more quickly than the opercularis muscle (0·1–0·2 s) at 100 Hz stimulation. At 20 Hz stimulation, the iliofibularis reached maximum tension levels (about 160–170 kN m^{-2}) after about 0·3–0·4 s, and at 2 Hz stimulation the iliofibularis displayed only a series of twitches with no fused tetanus. The opercularis muscle, therefore, could develop and maintain tension at a very low rate of stimulation (2 Hz) compared to the iliofibularis muscle.

Relaxation times for the opercularis and iliofibularis muscles following tetanic isometric contraction also differed dramatically. Following the end of stimulation at 100 or 20 Hz, tension in the iliofibularis muscle fell to 50 % levels after about 0·1–0·2 s and fell to the baseline in well under 1 s. Tension in the opercularis muscle fell at a much slower rate, reaching 50 % levels after 4–6 s (Fig. 2). Residual tension in the opercularis muscle remained for about 50–70 s (Fig. 2). Rate of tension loss in the opercularis muscle was approximately the same at all three frequencies of stimulation (2, 20 and 100 Hz).

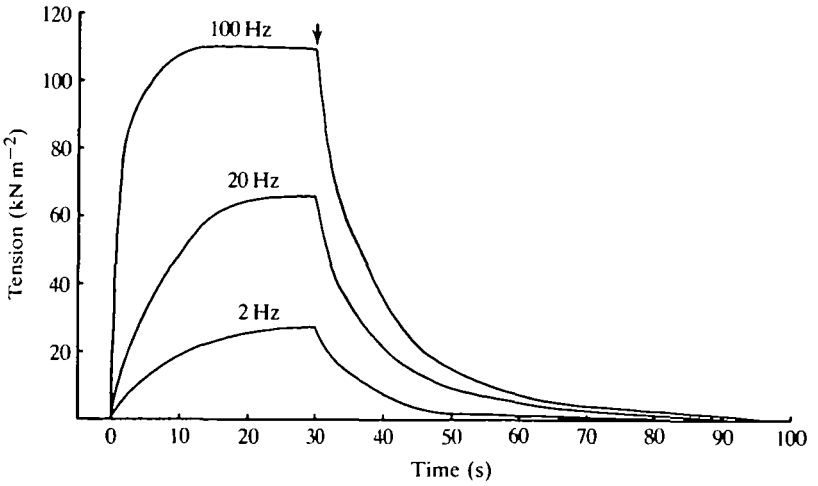

Fig. 2. Isometric tension development and loss in a bullfrog opercularis muscle stimulated for 20 s at three different frequencies; 2, 20 and 100 Hz. Note the rate of tension loss following the termination of stimulation (arrow) and the long retention of residual tension.

The opercularis muscle also displayed fatigue resistant characteristics compared to the iliofibularis muscle. In tests involving isometric contractions of fresh opercularis muscles, the muscles lost about 10 % of their maximum isometric tension after about 4–5 min at a stimulation rate of 100 Hz, after 6–7 min at 20 Hz stimulation, and after 10–12 min at 2 Hz stimulation. In contrast, fresh iliofibularis muscles lost 10 % of their isometric tension after about 20–25 s at 100 Hz stimulation and after 40–50 s at 20 Hz stimulation. At a stimulation rate of 2 Hz the iliofibularis did not develop a tetanic contraction, but isometric tension developed by individual twitches decreased by 10 % after about 40 s. Compared to the iliofibularis muscle, therefore, the opercularis muscle was more fatigue-resistant at all frequencies of stimulation.

Muscle effects on vibration responses

Severing the nerve to the opercularis muscle depressed vibratory responses at all frequencies tested (25–200 Hz), although the effect was not pronounced and slight increases were occasionally observed (Fig. 3). The greatest decreases measured were about 3 dB; these decreases were observed throughout the frequency range examined and were not restricted to any particular vibration frequencies. The mean decrease in

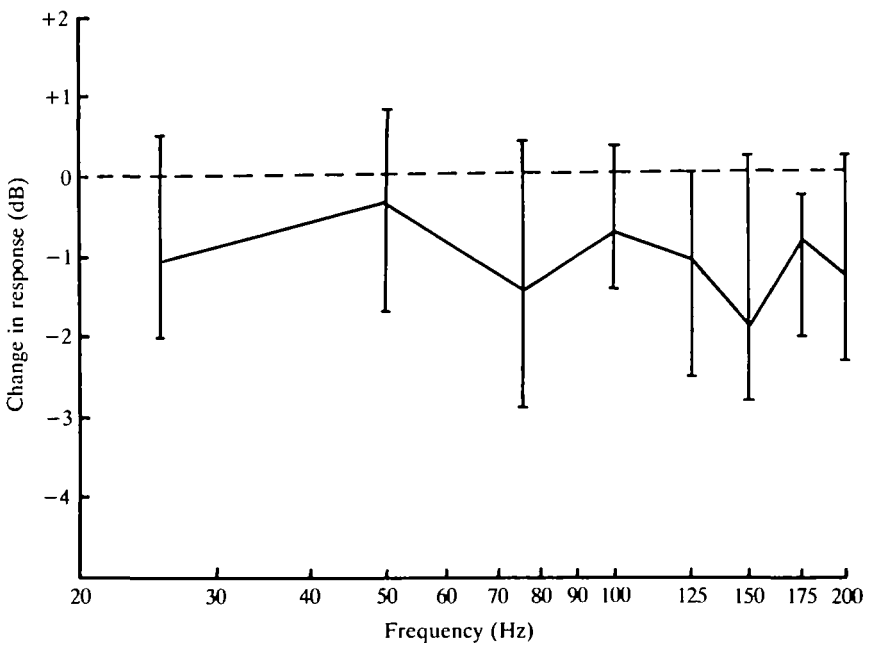

Fig. 3. Graph demonstrating the effect of severing the nerve to the opercularis muscle on vibration responses in four adult bullfrogs. The dashed line represents no change in response following severing of the nerve. Values above or below this line (expressed in dB) represent increases or decreases, respectively, in responses following severing of the nerve. The solid line represents the mean value and the vertical lines represent the range about that mean for the four specimens tested. Vibratory stimuli at all frequencies had an equal acceleration (1 cm s^{-2}).

the sample of four animals for any one frequency, however, was always less than 2 dB and as little as about 0·4 dB.

Artificial stimulation of the denervated opercularis muscle produced clear increases in vibratory responses, especially at the stimulation frequency of 100 Hz. Immediately following the end of muscle stimulation, the microphonic responses were elevated and then began to fall to levels approximating those measured before stimulation (Fig. 4). The data presented above show that tension decreases following stimulation, reaching about 50 % 4–6 s after the end of stimulation (see Fig. 2). The elevated microphonic responses fall at about the same rate, returning to the levels preceding muscle stimulation after about 10 s. Fig. 5 summarizes data from four animals during muscle stimulation at two frequencies, 2 and 100 Hz. Stimulation at 100 Hz produced clear increases in microphonic responses. Depending upon the vibration frequency, increases of as much as 7 dB were observed, although the mean increases at any one frequency ranged from about 2 to 4·5 dB and responses were sometimes depressed. Greater increases tended to occur at higher vibration frequencies, although there was no consistent relationship between the increases and vibration frequency. Stimulation of the opercularis muscle at a frequency of 2 Hz also produced increases in vibratory responses, although the increases were of smaller amplitude than those when the muscle was stimulated at 100 Hz (Fig. 5). Occasionally increases of up to 2 dB were observed, but the mean increase for the four animals at any given vibration frequency was only 0·5–1·5 dB, and occasionally a decrease in response was measured. The effect of muscle stimulation at 2 Hz showed a closer

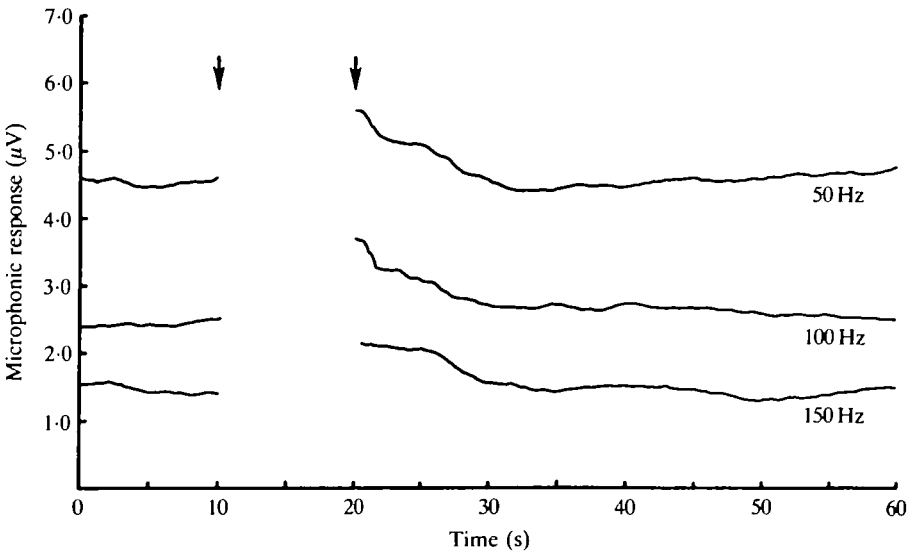

Fig. 4. Recorder traces demonstrating the effect of direct stimulation of the opercularis muscle on vibratory responses in one bullfrog. The traces represent microphonic recordings (at three different vibration frequencies; 50, 100 and 150 Hz) preceding and immediately following 10 s of muscle stimulation at 100 Hz. The arrows denote the onset and termination of stimulation. Note the increase in response at the end of muscle stimulation followed by a gradual decrease in response amplitude.

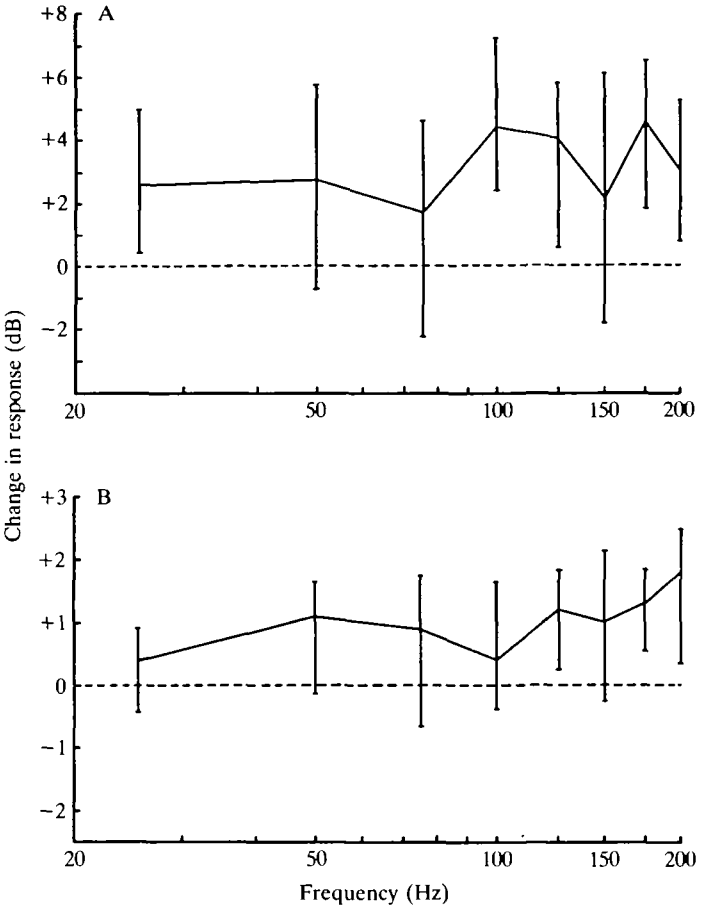

Fig. 5. Graphs demonstrating the effect of 10 s of 100 Hz stimulation (A) and 20 s of 2 Hz stimulation (B) of the opercularis muscle on vibration responses in four bullfrogs. The nerve to the opercularis muscle had previously been severed. The graph compares responses measured immediately after the end of stimulation to control responses preceding the stimulation. The dashed line represents no change in response following stimulation; values above or below the line (expressed in dB) represent increases or decreases, respectively, in responses at the end of stimulation. The solid line represents the mean value and the vertical lines the range around the mean value for the four specimens combined. Vibratory stimuli at all frequencies had an acceleration of 1 cm s^{-2}.

relationship with frequency of vibration, as greater increases were observed at higher frequencies.

DISCUSSION

Velocity of contraction

The opercularis muscle of *Rana catesbeiana* has physiological features consistent with the morphological evidence that it is a primarily tonic muscle (Hetherington & Lombard, 1983; Becker & Lombard, 1977). The maximum velocity of contraction of the opercularis muscle is quite slow (1·81 L s^{-1}) compared to that of the iliofibularis

muscle of the hindlimb ($6{\cdot}24\,L\,s^{-1}$). In anurans the iliofibularis muscle is predominantly composed of twitch fibres of the fast glycolytic category (Putnam & Bennett, 1983). Tonic muscle fibres respond to stimulation with graded contractions rather than twitches and are much slower than twitch muscle fibres (Kuffler & Vaughan Williams, 1953; Hess, 1970). Therefore, the differences in velocity of contraction between the opercularis and iliofibularis muscles would be expected if indeed the opercularis muscle were composed predominantly of tonic muscle fibres.

Velocity of contraction of the opercularis muscle is also more susceptible to loading, as demonstrated by the shape of the force–velocity curve and the relatively low a/P_0 value. This effect of loading has been observed in other studies of amphibian tonic muscle fibres (Lännergren, 1978) and appears to be characteristic of tonic muscles. In the animal, muscles always experience some degree of loading, so in natural conditions the opercularis muscle is slower still compared to the iliofibularis and other body muscles. At loads representing 50% of maximum isometric tension the opercularis muscle contracted only 9% as fast as the iliofibularis muscle. The susceptibility of the velocity of contraction of the opercularis muscle to loading may indicate that it is an energetically economical muscle. Woledge (1968) considers low a/P_0 values indicative of highly efficient muscle fibres that expend minimal energy during contraction.

The V_{max} determined in these tests for the opercularis muscle is not as low as that determined for slow or tonic fibres in other frog muscles. Floyd & Smith (1971) measured a V_{max} of $0{\cdot}11\,L\,s^{-1}$ for slow fibres in the iliofibularis muscle of *Rana*, and Lännergren (1978) measured a V_{max} of $1{\cdot}10\,L\,s^{-1}$ for single tonic fibres of *Xenopus laevis*. The opercularis muscle of amphibians typically contains some twitch fibres (Becker & Lombard, 1977), so the greater value of V_{max} measured here is not surprising. The V_{max} of the opercularis muscle is, however, similar to the V_{max} ($1{\cdot}8$–$2{\cdot}0\,L\,s^{-1}$) of tonic portions of the anterior latissimus dorsi muscle of the chicken measured at approximately the same temperature (Rall & Schotellius, 1973). The velocity of contraction of the opercularis muscle is therefore comparable to that of other vertebrate slow or tonic muscles, and in comparison with most skeletal muscles that consist primarily of twitch fibres, the opercularis muscle of *Rana catesbeiana* is a very slowly contracting muscle.

Tension development and maintenance

The opercularis muscle is also slow in developing isometric tension compared to the iliofibularis muscle, but is more fatigue-resistant and can retain tension for a longer time following stimulation. Residual tension was observed in the opercularis muscle for as long as 1 min after the end of stimulation. Electromyographic studies have found that the opercularis muscle of *Rana catesbeiana* shows a constant 2 Hz rhythm of activity when the animal is out of the water (Hetherington & Lombard, 1983). The muscle shows increased activity during lung ventilation, which occurs at a variable rate, but often at a frequency of about $1\,s^{-1}$. In experiments described here, stimulation of the isolated muscle at 2 Hz produces a tetanic contraction that reaches maximum tension after about 1 min and can maintain that tension level with

no significant reduction for 10–12 min. The maximum level of tension was somewhat less than that measured at higher frequencies of stimulation (20 and 100 Hz). The ability of the operculari muscle to develop and maintain tension at a low frequency of stimulation suggests that, at the natural stimulation rate of 2 Hz *in vivo*, the muscle may be maintained at a low level of tension. Additional stimulation during lung ventilation could act to produce and maintain a higher level of tension.

Functional implications of physiological features

The physiological properties of the operculari muscle, including the slow V_{max}, slow rate of tension development, fatigue-resistance and slow rate of relaxation, when combined with electromyographic information, suggest that the operculari muscle is normally maintained in tension when the frog is in terrestrial situations. Two hypotheses of operculari muscle function are supported by these physiological data. Lombard & Straughan (1974) proposed that the muscle is tensed in terrestrial situations, thereby acting to interlock the operculum and stapedial footplate so as to increase responsiveness of the middle ear to low-frequency sound. Hetherington (1985) also proposed that the muscle is tensed in terrestrial situations, but that it functions as a structural link between the operculum and shoulder girdle. Substrate motion affecting the forelimb and shoulder would, in turn, produce motion of the operculum relative to the inner ear fluids. Neither hypothesis would predict that a rapid speed of contraction would be an important characteristic of the operculari muscle, and indeed the slow V_{max} of the muscle corroborates these interpretations. The functional hypothesis of Wever (1985), however, that the operculari muscle acts to modify stapedial movement and protect the inner ear from loud sounds, appears unlikely in the light of the physiological characteristics of the operculari muscle. A fast velocity of contraction should be an important feature of such a muscle, as this would allow rapid modulation of middle ear responsiveness in the event of loud sounds. Also, the slow rate of relaxation observed in the operculari muscle would make it poorly suited for quickly reversible modifications of middle ear responsiveness. Middle ear muscles of other vertebrates that clearly have some sort of protective function, such as the stapedius and tensor tympani muscles of mammals, consist of fast twitch muscle fibres that are capable of rapid contraction (Densert & Wersall, 1974). Given the physiological differences between the operculari muscle and these middle ear muscles, any analogous function of the operculari muscle in protection of inner ear receptors seems unlikely.

Tension effects on vibration responses

The operculari muscle can enhance reception of substrate vibrations (Hetherington, 1985), and the effects of artificial stimulation of the operculari muscle on vibration microphonic responses provide evidence that tension contributes to the sensory function of this muscle. Although severing the nerve to the operculari muscle (and thereby removing natural levels of tension) produced only slight decreases in responses, direct stimulation of the muscle at a high frequency (100 Hz) clearly increased responsiveness to vibrations moving the forelimb of the animal.

Admittedly, the increase in vibratory sensitivity produced by elevated muscle tension was not pronounced (up to 4·5 dB). Muscle tension, therefore, may not be especially significant in determining sensitivity to natural vibratory signals in these animals, especially as the natural levels of tension seem to have the least effect. Perhaps the effects of tension may be more significant at vibratory accelerations higher or lower than the 1 cm s^{-2} level used in this study. In any case, as the increases in responses produced by stimulation at a lower frequency (2 Hz) were considerably less than the increases at 100 Hz, it would appear that the higher the tension in the muscle the more effective the operecularis system is in reception of motions affecting the forelimbs. In physiological tests, stimulation at 2 Hz produced tension levels 20–25 % of that produced by stimulation at 100 Hz. If the operecularis muscle functions as a taut structural connection between operculum and shoulder girdle, a high level of tension may allow more effective transmission of motion of the forelimb to the operculum. Higher levels of muscle tension would probably minimize any stretching of the muscle as a result of substrate forces acting on the forelimb and shoulder girdle. Forces moving the shoulder girdle would therefore produce motion of the operculum rather than be expended in stretching the muscle.

Interestingly, the increase in response produced by 2 Hz stimulation was similar in magnitude to the decrease in response caused by severing the nerve to the operecularis muscle. This may provide evidence that in the normal, resting animal, the muscle is maintained at a tension level similar to that observed at 2 Hz stimulation. It is not clear, given the increase in inner ear responses related to an increase in tension, why tension in the operecularis muscle may be maintained at relatively low levels. Perhaps at such tensions the muscle provides adequate vibration sensitivity while experiencing virtually no fatigue and utilizing a minimum of energy. The animals, however, may increase the rate of muscle stimulation, thereby increasing tension and vibration sensitivity, at particular times when it is especially beneficial. Perhaps when the frogs first detect a vibratory signal, they may increase muscle tension to enhance reception of that signal. Electromyographic analysis of the operecularis muscle gave no evidence that bullfrogs spontaneously alter the pattern of muscle stimulation (Hetherington & Lombard, 1983), but the animals were physically constrained in these tests and were not exposed to vibrations. Electromyographic monitoring of the pattern of muscle stimulation in unconstrained frogs exposed to substrate vibrations may answer the question of whether operecularis muscle tension is actively manipulated by these animals. Artificial stimulation of the muscle did not demonstrate any frequency-specific effects of muscle tension on vibration reception. At 2 Hz stimulation, responses to higher frequencies tended to show the greatest increases, but this effect was not pronounced. This study, therefore, provides no evidence that the animals could modulate frequency responsiveness of the operecularis system by manipulation of muscle tension.

Why is the operecularis muscle not a ligament, which would probably be an effective structural connection between the operculum and the shoulder girdle? The answer may lie in the amphibious nature of most amphibians, such as the bullfrog *Rana catesbeiana*. The operecularis system is typically absent in completely aquatic

amphibians (Hetherington *et al.* 1986), and the operecularis muscle of the bullfrog is active only when the animal is out of the water (Hetherington & Lombard, 1983). While the mechanics of underwater hearing in amphibians are not well understood (Hetherington & Lombard, 1982), the opercularis system clearly has no function in this regard, and perhaps an 'activated' opercularis system (with a tensed muscle) may interfere with underwater hearing. In species that spend time both in water and on land, such as the bullfrog, the presence of a muscle that can be contracted for terrestrial function and relaxed (turned off) in water, may be more advantageous than a constantly taut ligament.

Two previous studies have examined the effects of opercularis muscle tension on inner ear responses to aerial sound. In work on the bullfrog *Rana catesbeiana*, Paton (1971) found that increased tension (as a result of loading the muscle) produced an increase in microphonic responses to very low frequency sounds below about 150 Hz. No change in microphonic responses, however, was observed at higher sound frequencies. Wever (1985), working on species of *Rana*, found that increasing the tension of the opercularis muscle by applying loads increased microphonic responses to sound, but he did not specify the amplitude of the increase or describe different frequency effects. The role of the opercularis system in the reception of aerial sound needs to explored further. Lombard & Straughan (1974) observed significant decreases in auditory responses to sound below about 1 kHz in several species of hylid and leptodactylid frogs following removal of the opercularis muscle. These authors, as described above, hypothesized that the opercularis muscle acted to interlock the operculum and stapes and thereby increase the responsiveness of the middle ear to low-frequency sounds. While their functional hypothesis appears to be plausible in the light of the physiological characteristics of the opercularis muscle described in this study, their results could also be explained by a direct role of the opercularis system in the reception of low-frequency sounds. The general hypothesis of how the opercularis muscle functions in reception of substrate motions, in which the muscle acts only as a taut structural connection between the operculum and shoulder girdle, could also be applied to sound reception. It is possible that any mechanical stimulus, including aerial sound, could produce differential motion of the head and shoulder region of an amphibian. As the opercularis muscle links the operculum to the shoulder girdle, the operculum would move relative to the inner ear fluids, setting up waves within the inner ear that could stimulate appropriate end organs. Additional work is needed to explore the possible role of the opercularis system in sound reception. The physiological features of the opercularis muscle clearly enhance the ability of the muscle to function in reception of ground vibrations, and these features could allow the muscle to act in sound reception as well.

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