CONTRACTIONS OF THE FROG SUBMANDIBULAR MUSCLE AS AN OBJECT FOR STUDYING THE MICROCIRCULATION

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Amplitudinal and temporal characteristics of contractions of the submandibular muscle of the frog were studied. The nerves were stimulated with pulses 0.15 msec in duration and with a frequency of 20 Hz. The threshold amplitude of the pulses varied from 0.6 to 1.3 V. Maximal contraction of the muscle occurred at 4 V. The optimal frequency which the muscle could reproduce during supramaximal stimulation was between 30 and 40 Hz. The variable component of the tetanic contraction of the muscle fell off sharply at frequencies of between 12 and 20 Hz. Judging from the character of the myograms, the submandibular muscle belongs to the "fast" type.

During the study of responses of the cardiovascular system to various factors the need frequently arises for intravital microscopic studies to be made of the blood vessels of skeletal muscles. A convenient object to use for this purpose is the flat and relatively thin submandibular muscle of the frog, the reactions of whose vessels as a result of direct contraction were observed by Gaskell [4]. Access to the muscle and its nerve is easy, and their exposure is bloodless, an important feature when the normal functional state of the vessels must be assured. In addition, the forces developed by both halves of the muscle in response to stimulation of the symmetrical nerves can be expected to be equal, and the displacement of the muscle is slight, a very important factor for microscopic examination of the vessels in a chosen field of vision actually in the course of contraction.

The object of the present investigation was to study the amplitudinal and temporal characteristics of contractions of the frog submandibular muscle as functions of stimulus frequency and amplitude in the motor fibers.

EXPERIMENTAL METHOD

The submandibular muscle was exposed in frogs (Rana temporaria) anesthetized with urethane (3-3.5 ml of an 8% solution, injected into the lymph sac). The two mandibular nerves were dissected and divided, and then stimulated by monopolar silver electrodes. Contractions of the muscle were measured by a contactless method based on the use of Hall's transducer [2]. A permanent magnet (weight 60 mg) was fixed to the muscle with Cyacrin glue. Hall's transducer, fixed in a micrometer holder, was moved through a distance of 1-2 mm away from the magnet. The micrometer holder was so arranged that the direction of movement of the transducer when the micrometer screw was turned coincided with the direction of movement of the magnet during contraction of the muscle. This allowed graduation of the instrument used to measure the Hall emf, directly in units of length (the amount of displacement of the magnet during contraction of the muscle). The initial position of the transducer relative to the magnet was chosen so that for the greatest displacement of the magnet produced by contraction of the muscle the working point still remained on the linear segment of the calibration characteristic curve. The transducer was

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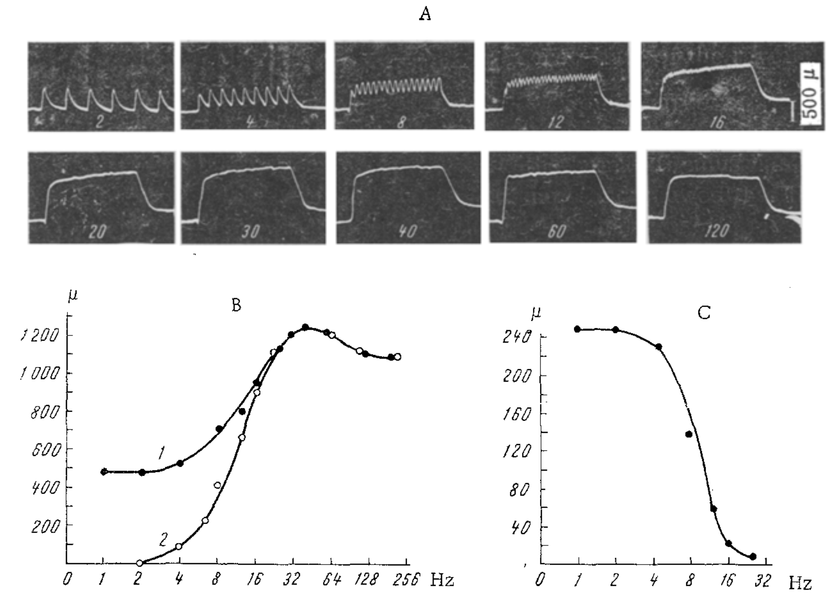

Fig. 1. Amplitude of isotonic contraction of frog submandibular muscle as a function of frequency of stimulation: A) myograms: numbers show frequency of stimulation (in Hz); B) total amplitude of shortening of muscle (1) and steady component of tetanic contraction of muscle (2) as functions of frequency of stimulation; C) amplitude of variable component of tetanic contraction of muscle as a function of frequency of stimulation. In B and C: abscissa, frequency of stimulation (in Hz), ordinate, contraction of muscle (in μ).

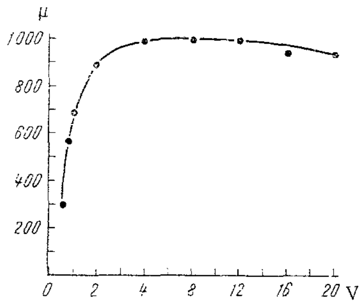

Fig. 2. Degree of contraction of frog submandibular muscle as a function of amplitude of stimulating pulses. Abscissa, amplitude of stimulation (in V); ordinate, contraction of muscle (in μ).

supplied with an alternating current (5 kHz) so that an ordinary UBP-1-0.2 biopotential amplifier could be used to amplify the signal. The choice of frequency was determined by the frequency of the process to be investigated (the frequency of the power supply must be at least 2 orders of magnitude higher than the highest frequency of the process investigated to permit qualitative detection) and by the noise level of the amplifier giving a transmission band of adequate width. The signals were recorded from the screen of a C1-4 CRO (dc amplification).

EXPERIMENTAL RESULTS

During unilateral supramaximal stimulation of the nerve, the muscle contracted by 1 ±0.2 mm toward the border of the mandible, while in response to bilateral stimulation of the symmetrical nerves, it moved through only 50-100 μ.

As Fig. 1A shows, the muscle responded to supramaximal stimulation of the nerve by single contractions up to a frequency of 4 Hz. With a further increase in stimulus frequency, the steady component of the tetanic contraction continued to increase, virtually reaching its upper limit at 30-40 Hz (Fig. 1B). The variable component of the contractions was correspondingly reduced to a value (Fig. 1C) whose determination was limited by the sensitivity of the method.

During stimulation of the nerve at a frequency of 20 Hz, the complete amplitude of contraction was close to the possible maximum. The threshold amplitude of the pulses at this frequency of stimulation varied from 0.6 to 1.3 V. Maximum shortening of the muscle in 7 of the 10 experiments occurred at 4 V.

The intensity of the working hyperemia in "fast" and "slow" muscles is unequal both in mammals and in birds [3, 5, 6]. The possibility is not ruled out that the same differences may exist also in frog muscles with different fiber compositions.

Judging by the character of the myograms (Fig. 1A), the submandibular muscle can be classed as "fast." However, the fact that relaxation of the muscle was incomplete during stimulation of the nerve at frequencies over 20 Hz suggested that it also contained tonic fibers. To test this hypothesis, Zhukov's experiment was reproduced [1]. Stimulating the nerve at 100 Hz for 0.3 sec showed that the threshold amplitude of stimulation for the tonic fibers was 3-4 V (Fig. 2). The nerve was then stimulated at the same frequency and below the threshold strength for tonic fibers for 2-2.5 min. During this time the muscle relaxed slightly because of disconnection of the "fast" fibers. With an increase in the amplitude of stimulation to supramaximal the muscle again contracted. This fact confirmed the hypothetical existence of a certain number of tonic fibers in the muscle. Since the amplitude of the tonic "tail," which appeared after stimulation of the nerve at high frequency, was small (Fig. 1A), the number of these fibers in the submandibular muscle likewise is small.

The optimal frequency of stimulation which the frog submandibular muscle can reproduce is 30-40 Hz. The variable component of the tetanic contraction of the muscle falls off sharply at frequencies between 12 and 20 Hz (Fig. 1C). It can therefore be considered that the rigidity of the muscle fibers during stimulation at about 20 Hz may be sufficient to cause compression of the blood vessels.

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