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EXPERIMENT ONE

Mammalian Diaphragm

Stimulation of the phrenic nerve causes twitch responses in rat diaphragm muscle. The effects of drugs on this fast response are examined, along with reversal of those effects.

The mammalian diaphragm is a focally-innervated skeletal muscle whose response to nerve stimulation is a fast twitch. Acetylcholine released from the motor nerve terminals acts very rapidly on postsynaptic nicotinic receptors, and is then destroyed by acetylcholinesterase in the synaptic cleft. Neuromuscular block by tubocurarine and suxamethonium is easily demonstrated. Reversal of non-depolarising block by a cholinesterase inhibitor such as edrophonium is slightly less reliable.

The phrenic-innervated diaphragm muscle consists of a thin sheet and remains viable for hours in an organ bath, so drug penetration is good, and the preparation is stable. It is thus well suited for experimentation.

Dissection

Hemi-diaphragms, each with its phrenic nerve intact, are dissected along with part of the ribcage and placed in a dish containing physiological saline. Each hemi-diaphragm can then be 'cleaned up' as follows. A triangular or wedge-shaped piece is prepared, with its base at the rib margin, its apex in the central tendon of the diaphragm, and with the phrenic nerve attaching in the interior of the triangle. Tie a thread to the free end of the phrenic nerve.

The diaphragm is attached to a special tissue holder along its origin at the rib margin, and transferred to an organ bath. The central tendinous part is attached by a thread to a tension transducer. The position of the transducer is adjusted so that the muscle is slightly stretched (tension 0.1 to 0.5 g wt) at rest. The phrenic nerve is pulled gently through a bipolar stimulating electrode (ring electrode). Alternatively, the end of the nerve can be taken into a suction electrode. Take care not to stretch the nerve. The phrenic nerve can be kept under slight tension through the ring electrode by attaching the thread from the nerve to the top of the organ bath with a blob of Blu Tack or a similar adhesive.

The preparation should be left to equilibrate for at least 30 minutes, and the bathing fluid changed several times during this period.

Experiment

Chart Settings

This is a single-channel arrangement with a PowerLab, Bridge Amp, and force transducer. In Chart, the range should be chosen to suit the maximum force to be exerted on the transducer. The final scale after units conversion and so on should be -1 to 6 g wt. The sampling rate should be at least 200 samples per second (200/s). The high rate ensures accurate recording of the twitch contractions. The view compression should be 50:1 or 100:1. A high-pass filter of 200 or 100 Hz should be chosen in the Bridge Amplifier dialog box. Data Pad miniwindows can be set up to show the comment number and text, and the twitch amplitude (using the Max–Min function).

Stimulation

Apply single pulses of 0.2 ms duration at 5 s intervals. Find the threshold stimulation voltage (usually 0.2–2 V) and increase the voltage until a maximum contraction is achieved. Increase the stimulus intensity by a further 1 V. Stimulate the nerve at 0.2 Hz for the remainder of the experiment.

Protocol

Test the effect of acetylcholine 10^{-6} M. Wash thoroughly. Then add suxamethonium 10^{-6} M. If the drug has no effect on the amplitude of contraction after 5–15 min, increase the concentration of suxamethonium to 2×10^{-6} M. When the response to 0.2 Hz stimulation has decreased to about 75% of the control level, retest the effect of acetylcholine 10^{-6} M. Wash out, then add edrophonium 10^{-5} M.

Wait 3 min and note whether the block by suxamethonium is affected. Wash out several times over 15 min. Test the effect of acetylcholine 10^{-6} M. Wash thoroughly.

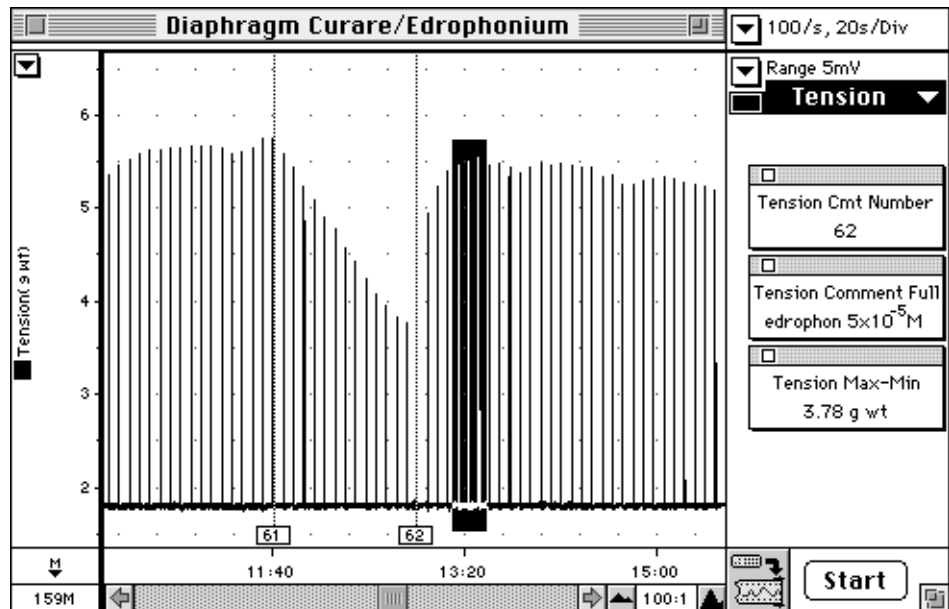
Add tubocurarine 10^{-6} M. If the drug has no effect on the amplitude of contraction, after 5–10 min, increase the concentration of tubocurarine to 2 or 5×10^{-6} M. When the response to 0.2 Hz stimulation has decreased to about 75% of the control level, retest the effect of acetylcholine 10^{-6} M. Wash out, then add edrophonium 10^{-5} M.

Wait 3 min and note whether the block by tubocurarine is reversed. If not, increase the dose of edrophonium to five times the starting concentration.

Figure 1–1 shows the effects of tubocurarine and the subsequent addition of edrophonium. The Data Pad function Max–Min has been used to measure the twitch contractions in a selection.

Figure 1–1

Twitch contractions of rat diaphragm muscle, showing effect of tubocurarine (2×10^{-6} M) at comment #61, and edrophonium (5×10^{-5} M) at comment #62.



Further Work

The corresponding preparation from the guinea pig or mouse has similar behaviour to the rat. Dissection of the mouse phrenic nerves is best done with the aid of a dissecting microscope.

A second stimulator (or second stimulator channel) can be set up with platinum wire electrodes on each side of the diaphragm. Strong stimuli (20–40 V, 0.5 ms) activate muscle fibres directly, and can be used to show that non-depolarising blockers abolish the nerve-mediated response without affecting the muscle itself (an observation made originally by Claude Bernard). A further refinement is to connect the two stimulator channels so that stimuli are delivered alternately to the nerve and the muscle.

The experiment described uses twitch responses to single-pulse stimulation. Tetanic responses (for example 0.5 s bursts at 40 Hz, every minute) show somewhat different behaviour. A tetanus during non-depolarising neuromuscular blockade rapidly declines, presumably because of run-down of transmitter release. After the tetanus, single twitches are often temporarily increased (post-tetanic potentiation). In contrast, during depolarising block, the tetanus, though depressed, is well sustained. After the tetanus, single twitches are neither increased nor further depressed.

With the settings described above, Chart records continuously at a moderately high rate, so that over the course of the experiment the data file can become inconveniently large. An alternative recording setup favoured by some workers is 'start-stop', in which recording is made only of the twitch contraction, and not of the flat baseline between twitches (Figure 1–2 and Figure 1–3). Even with a high sampling rate (500 or 1000 samples per second), files remain manageably small.

Figure 1-2

Twitch contractions recorded in 'start-stop' mode, with a sampling rate of 1000/s. Times shown along the horizontal axis are the time of day at the start of each data block.

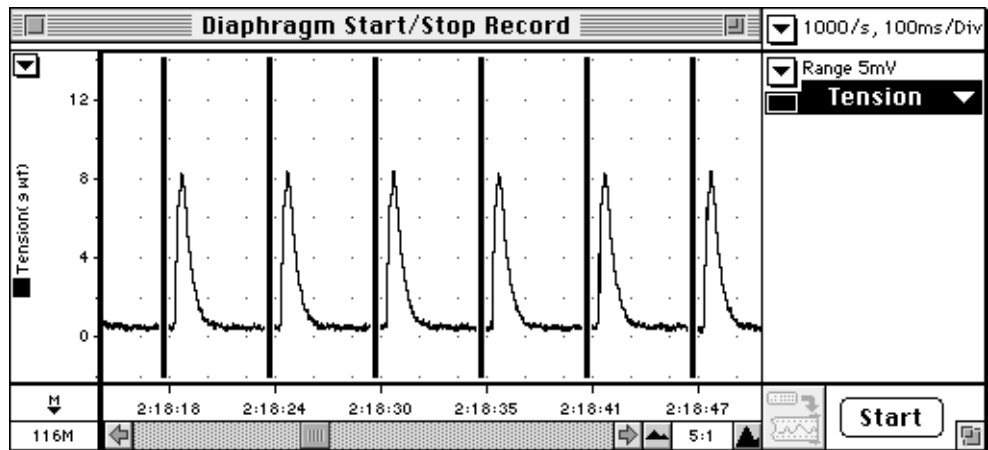


Figure 1-3

Chart's Trigger window, showing settings for 'start-stop' recording of data blocks. A trigger pulse from the external stimulator is used to start each block recording.

