E X P E R I M E N T   S I X

6 Guinea Pig Atria

The spontaneously beating atria from the heart of a small animal can be used to show a variety of responses to drugs that affect the frequency and strength of muscle contraction.

The atria from the heart of a small laboratory animal survive well in vitro, and continue to beat spontaneously for hours provided that the SA node is not damaged during dissection. Inotropic and chronotropic responses to a range of adrenomimetic and cholinomimetic drugs are easily demonstrated. Tissue responses to these drugs show little fade during prolonged exposure; thus cumulative dose–response analysis is possible.

Dissection

Remove the heart from a rat or guinea pig and transfer it to a dish of well-oxygenated physiological saline. Gently press the heart to help empty any blood remaining. Carefully dissect away most of the ventricular tissue. Tie the preparation to a tissue holder and attach another thread for connection to the force transducer. Allow the tissue to equilibrate in the organ bath for at least 20 minutes at a temperature of 32–33 °C.

Plate 8 shows guinea pig atria attached to a tissue holder, ready for insertion into an organ bath. As shown in Plate 9, the tissue holder slides into the organ bath, where the spring-mounting clip holds it firmly in place, allowing reasonable tension to be exerted on the tissue.

Experiment

Chart Settings

This is a three-channel arrangement with a PowerLab, Bridge Amp, and force transducer (connected to Channel 1). In Chart, the range for Channel 1 (Force) 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 0 to 2 g wt. The sampling rate should be at least 200/s (the high rate ensures reasonably accurate recording of the twitch contractions). The view compression should be 20:1. A low-pass filter of 10 or 20 Hz should be chosen in the Bridge Amplifier dialog box. Channel 2 should be set up to show the rate calculated from Channel 1 using the Ratemeter function from its Computed Input dialog box (computed range 500 BPM, fastest tracking, average 1, set up to trigger correctly from the force trace).

Channel 3 should be set up to show the rate calculated from Channel 1 using the Ratemeter function from the 'Cycle Variables' Chart extension, also adjusted (Figure 6–1) to give correct triggering from the force trace.

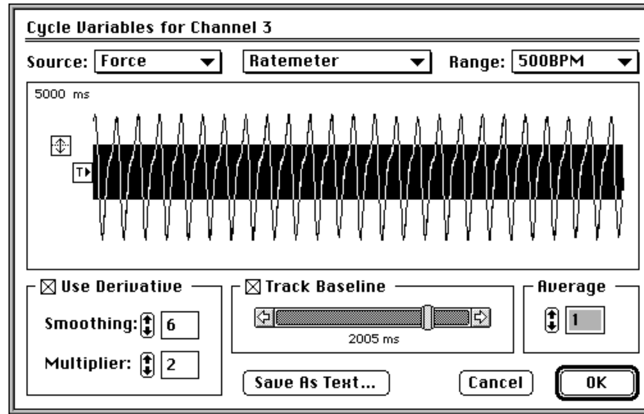

Figure 6–1
The Cycle Variables dialog box, with suggested settings to measure the beating rate of isolated atria.

When set up correctly, both ratemeters work equally well to calculate and display heart rate. The Computed Input Ratemeter has the advantage that it shows the rate on-line during recording. Changes in the amplitude or waveform of the force trace, however, may prevent correct triggering of the ratemeter (for example after drug application). The advantage of the ratemeter from Cycle Variables is that you can adjust its settings after recording has finished, although you cannot see it while recording.

Protocol

Perform a cumulative dose–response analysis for isoprenaline, using half-log concentrations from 10^{-10} M to 10^{-5} M or until a maximum is reached. Wash thoroughly. Choose a dose of isoprenaline that produced approximately 50% of maximal effect and test it for reproducibility. Add propranolol to bath (5×10^{-6} M) and incubate for 10 minutes. Retest the dose of isoprenaline. Wash thoroughly and allow rate to recover to a steady baseline level (10–15 minutes).

Figure 6–2 shows the response of spontaneously beating guinea pig atria to increasing doses of isoprenaline (isoproterenol) from 10^{-9} to 3×10^{-8} M. Note the absence of fade; responses are well maintained and allow cumulative dose–response studies.

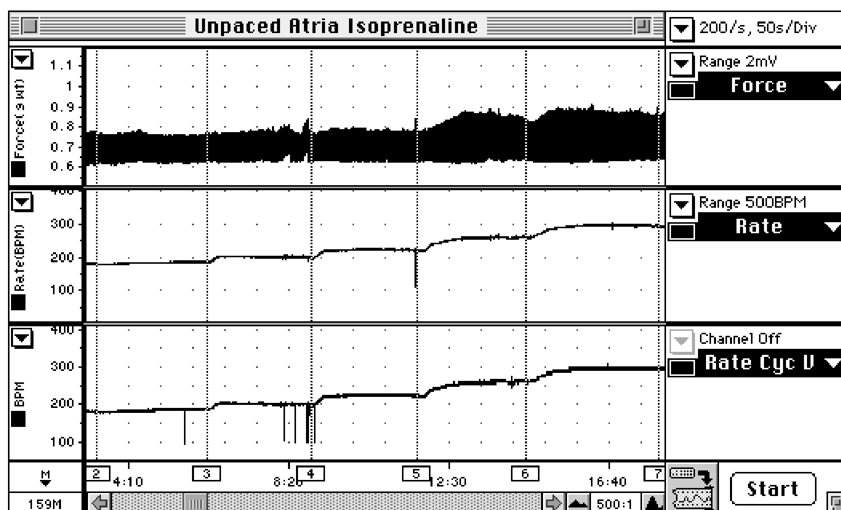

Figure 6–2
Response of spontaneously beating guinea pig atria to increasing concentrations of isoprenaline from 10^{-9} to 3×10^{-8} M, showing response maintenance.

Perform a cumulative dose–response analysis of acetylcholine (half-log, 10^{-8} M to 10^{-5} M or until a maximum is reached). Wash thoroughly. Again, choose a dose that produced 50% of maximal effect, and test it for reproducibility. Add atropine to the bath (10^{-6} M), incubate for 10 minutes, and retest the dose of acetylcholine in presence of atropine.

Figure 6–3 shows the response of spontaneously beating guinea pig atria to increasing doses of acetylcholine from 10^{-7} to 10^{-5} M. The disturbances in the traces seen around the comments are artefacts from adding the drug to the organ bath.

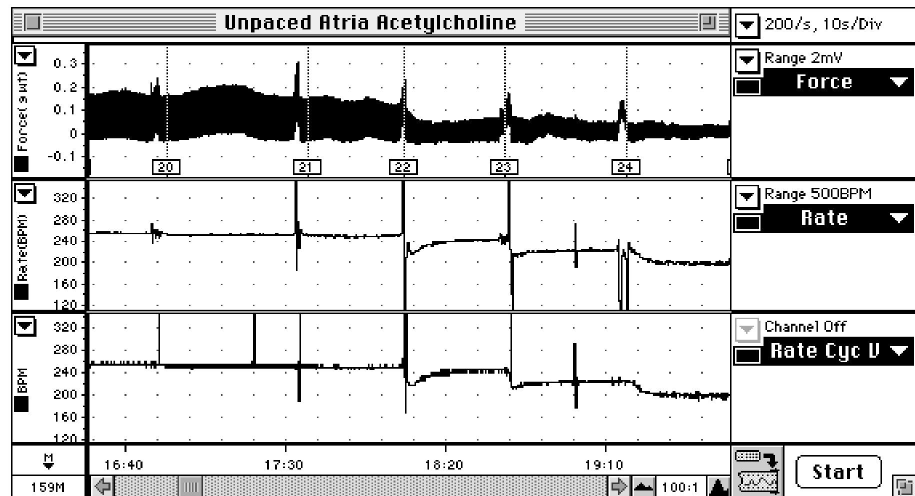

Figure 6–3
Response of spontaneously beating guinea pig atria to increasing concentrations of acetylcholine (from 10^{-7} to 10^{-5} M).

Further Work

Marked potentiation of response to acetylcholine (10–50 times) can be shown by perfusing with the cholinesterase inhibitor physostigmine (5×10^{-6} M). Allow several minutes before testing the effects of acetylcholine.

If a pair of platinum stimulating electrodes is placed around the atria, stimulus pulses (0.5 ms) can excite vagal and sympathetic postganglionic fibres to produce good chronotropic responses. Although the stimuli tend to excite the heart directly, this unwanted effect does not outlast the stimulus train, and the responses of interest are easily distinguished. Short trains (1–3 pulses at 10 Hz) give mainly vagal effects: the heart rate is reduced for a few seconds. With longer trains, the vagal slowing is followed by a much longer-lasting increase in heart rate. These two effects are inhibited by atropine (10^{-6} M) and propranolol (5×10^{-6} M) respectively.