CLINICAL RESEARCH

Intact individual heart cells isolated from human ventricular tissue

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Abstract

The intricate architecture of heart muscle, comprising irregularly shaped cells which interdigitate in a complex three-dimensional array, has often compromised clear interpretation of experimental data obtained from the whole organ. One approach to minimise some of the difficulties is to use individual muscle cells in suspension, and data have already been reported using myocytes isolated from mammalian ventricles. It is difficult, however, to extrapolate results obtained from animal tissues to situations of medical relevance in man. Intact isolated muscle cells were obtained from human ventricular tissue by modifications of methods used for isolating smooth muscle, atrial, and ventricular tissue from animals. Electrical studies showed that these myocytes had functional characteristics similar to those observed in the whole heart.

Such cells will prove a useful preparation for studies on both the mechanisms underlying myocardial performance in normal and diseased states and the response of heart tissue at the cellular level to conditions found during cardiac surgery.

Introduction

Studies on the mechanisms underlying myocardial performance in both normal and diseased states have often been hampered by the complex morphology and syncytial function of the whole heart. One promising experimental approach to minimise some of these complexities has been the enzymic dispersion of adult

cardiac tissue into a suspension of intact cells. Work in our laboratory has shown that such a technique can provide individual myocytes from the ventricles of rats,¹⁻³ and other workers have obtained cells from the hearts of rabbits⁴ and dogs.⁵ Our studies have shown that isolated cells retain the morphological features characteristic of myocytes studied in the whole organ,^{1-3, 6-7} and have acceptable electrical properties^{3, 8} and responses to the administration of catecholamines.⁹⁻¹¹ Nevertheless, it is difficult and often misleading to extrapolate experimental results in animal tissues to man. We describe here, for the first time, a technique for obtaining isolated intact myocardial cells from human ventricular tissue and show that these individual myocytes have morphological and electrical characteristics consistent with those observed in the whole heart.

Methods

Tissue, excised from patients undergoing corrective open-heart surgery, was obtained in theatre immediately on removal. Samples were from either the right ventricle of children undergoing correction of Fallot's tetralogy or papillary muscle of patients undergoing mitral valve replacement. These samples, which varied in size and shape but generally had a maximum length of 3-8 mm, were placed in a sterilised container holding a Krebs-Ringer solution of the following composition: NaCl 118.5 mmol/l, KCl 4.2 mmol/l, NaHCO₃ 14.5 mmol/l, KH₂PO₄ 1.18 mmol/l, MgSO₄ 1.18 mmol/l, glucose 11.1 mmol/l; this solution was gassed with 95% O₂/5% CO₂ to maintain pH at 7.40 ± 0.05.

The tissue was transported to the laboratory within 10-15 minutes of removal and then cut into cubes of varying dimensions by a mechanical tissue chopper³ with a blade interval setting of 1 mm. The chopped tissue was placed in a polytetrafluoroethylene beaker containing 10 ml of Krebs-Ringer solution, which had been pre-incubated at 37°C and to which had been added 1 mg/ml lyophilised trypsin (236 U/mg), 1.5 mg/ml crude bacterial collagenase (type 1, *Clostridium histolyticum*, 175 U/mg), 10 mg/ml bovine serum albumin, and 100 U/ml of benzylpenicillin and streptomycin. This mixture was incubated at 37°C for 40 minutes, stirred continuously at 2 Hz by a stainless steel paddle, and gassed from above with humidified oxygen-carbon dioxide mixture.

At the end of this period the stirring was stopped and the tissue allowed to settle. The supernatant was removed carefully and replaced by 10 ml of preincubated buffer containing an additional 1 mg/ml of bovine serum albumin free of fatty acids. This incubation was carried out for five minutes, the solution was then removed from the

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settled tissue fragments, and 10 ml of Krebs-Ringer solution containing 0.5 mg/ml of crude collagenase was added. Incubation was continued, with stirring, for up to 75 minutes, with frequent microscopic examination of the supernatant (every 10-15 minutes) to assess the quality and quantity of cell yield.

When cell numbers had stabilised the incubation medium was filtered through 250- μ m nylon gauze and the cells washed by one of two methods. Either the filtered suspension was centrifuged at 22 g for one minute or 2.5 ml aliquots were placed in 10-ml siliconised tubes, diluted to 5 ml with albumin-free Krebs-Ringer solution, and the cells allowed to sediment under gravity for 15 minutes at room temperature. After centrifugation cell pellets were suspended in 5 ml Krebs-Ringer solution containing 10 mg/ml bovine serum albumin. For sedimented cells the bottom 2 ml of solution in each sedimentation tube was diluted with an equal volume of buffer and the fractions pooled. Cell suspensions were stored at room temperature in siliconised flasks and gassed at regular intervals. These isolation procedures were modifications of methods used for dispersing smooth muscle,^{12 13} atrial,¹⁴ and ventricular^{3 15 16} tissue from animals. We used conventional techniques³ for recording cellular electrical activity, using intracellular glass microelectrodes containing 3 M KCl and having resistances in the range 35-80 M Ω . A system incorporating a bridge circuit^{3 11} was used in most of the experiments.

Results

Attempts to dissociate cells from tissue obtained from adults were unsuccessful, but six successful experiments were carried out on samples taken from the infundibulum of the right ventricles of children (aged 4 to 10 years, both boys and girls) undergoing correction of Fallot's tetralogy. Maximum yields of cells were obtained after 40-50 minutes of the final incubation with collagenase (see Methods); fig 1 shows the appearance of two isolated myocytes as typical examples.

FIG 1—Light micrographs of two isolated ventricular muscle cells. Note regular cross striations and also irregular cell contours, particularly at the ends; both of these characteristics are similar to those observed in cells in vivo. Cell a is 118 μ m long and cell b 121 μ m long.

Cells were irregular, 60-140 μ m long and 10-30 μ m wide, with clear cytoplasm and distinct cross-striations. These features were consistent with those observed for myocytes isolated from other mammalian species,^{1 2 4-6 16} the non-uniformity of cell shapes reflecting the complex geometrical arrangement of ventricular muscle.

Although cells were quiescent, they could be stimulated electrically to give action potentials (fig 2) which evolved from membrane potentials more negative than -80 mV. The action potential in fig 2(a) was from a cell with a resting potential of -90 mV, with an overshoot of 31 mV and a duration (to 90% of repolarisation) of 75 ms. In fig 2(b) the initial membrane potential was -82 mV, overshoot 26 mV, and duration of action potential 520 ms. These values indicate the range of measurements obtained from individual cells and are comparable with recordings which have been reported for human multicellular preparations.¹⁷ Furthermore, cells could be paced at varying rates by either an intracellular microelectrode or field stimulation with plate electrodes. Results such as those shown in fig 2 could be obtained for at least six hours after the cell was isolated.

FIG 2—Action potentials recorded from two individual myocytes with a glass microelectrode. Both cells were paced at 1 Hz and bathed in Krebs-Ringer solution containing 1 mmol/l calcium chloride, temperature 25.5°C.

Discussion

Individual cells isolated from mammalian ventricles of animal origin have already provided interesting information on the electrical characteristics of myocytes uncoupled physically from their usual syncytial state,³ which is relevant to cells in vivo uncoupled electrically in regions of ischaemia or local mechanical damage. Experimental results have been reported on the ionic currents important for both cardiac impulse conduction¹⁸⁻²² and development of the force of contraction.^{11 23 24} Data have also been published on the biochemical characteristics of isolated cells,²⁵⁻³⁰ including their responses to hormones^{25 26} and the onset of anoxia.^{26 30} Our demonstration that it is now possible to obtain isolated cells from human tissue should not only prompt experiments designed to provide comparable evidence of direct clinical relevance in man but will also be an impetus to investigate both the response and preservation of myocardial tissue at cellular level in circumstances commonly encountered during cardiac surgery.

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Diaphragm pacing in ventilatory failure

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Abstract

Diaphragm pacing, which entails electrical stimulation to the phrenic nerve, is an effective means of managing patients with ventilatory insufficiency and intact lower-motor-neurone innervation of the diaphragm. The pacing apparatus is used to pace the right and left hemidiaphragms alternately to avoid fatigue, which may damage the muscle irreversibly.

Among the important benefits of pacing in quadriplegics with paralysis of respiratory muscles are the social and psychological advantages of not being dependent on a mechanical ventilator.

Introduction

Electrical stimulation of the phrenic nerve to effect ventilation for prolonged periods was first reported by Judson and Glenn in 1968. Since then the technique of diaphragm pacing has been used in over 180 patients in America, and we describe here what we believe to be the first case of its use in Britain.

Case report

A 42-year-old man was admitted to hospital in January 1980 having fallen headlong downstairs. On examination he was quadriplegic, with all respiratory movements being achieved by the sternomastoid and scalene accessory muscles. X-ray examination of the cervical spine showed an odontoid anomaly that was stable. He was observed initially and by the following day was becoming exhausted, so after endotracheal intubation positive-pressure ventilation was started and a tracheostomy performed. When the ventilator was stopped he could maintain a tidal volume of about 100 ml for up to six minutes. Operative treatment was not indicated, and as the neurological lesion was incomplete there was a slight chance of recovery of phrenic activity. X-ray screening of

the diaphragm in March, however, showed no voluntary movement on either side, and his suitability for implanted diaphragm pacemakers was further investigated. Percutaneous stimulation of each phrenic nerve produced adequate tidal volumes and visible excursion of the diaphragm on screening.

Receivers and electrodes of the pacemaker system were implanted by one of us (JJM) on the right side in September and on the left side six weeks later. The diaphragm was paced for short periods initially, but the duration of stimulation was gradually increased and the time spent on the ventilator became less. The pacing apparatus was adjusted to produce 12 respirations a minute with a tidal volume of about 440 ml during stimulation of the right and 460 ml during stimulation of the left hemidiaphragm with the patient supine. Arterial tensions of oxygen and carbon dioxide were in the normal range after four hours' continuous pacing on either side. The tracheostomy was maintained with a 24 French gauge plain Portex tube, which was occluded during the day, except when required for tracheal suction, to facilitate normal speech. At present each hemidiaphragm is paced alternately for eight-hour periods, so removing altogether the need for positive-pressure ventilation.

Discussion

The diaphragm pacer uses integrated solid-state electronic circuitry to permit delivery of an adjustable electrical stimulus to the phrenic nerve. The apparatus has been described in detail by Glenn *et al*^{2,3} (Avery Laboratories, Farmingdale, New York 11735). An external battery-powered transmitter develops a radiofrequency signal, which is radiated from a loop antenna. This antenna is placed over a subcutaneous radioreceiver, which is usually implanted just above the costal margin in the mid or anterior axillary line. The receiver is encapsulated in an epoxy disc and covered in silicone rubber. It demodulates the signal, and the resulting direct-current pulses pass to an electrode placed on the phrenic nerve usually in the neck (figure). The delivery of a train of about 25 electrical pulses to the phrenic nerve over approximately 1.3 seconds produces contraction of the diaphragm. By using the controls on the external transmitter it is possible to alter the respiration rate, which represents the time interval between adjacent pulse trains, and the inspiration time, which is determined by the duration of each pulse train. The amplitude of the stimulating current determines the depth of inspiration and is also adjustable.

Glenn has had the most extensive experience with diaphragm pacing. He has reported its use in 36 patients with central alveolar hypoventilation, in one patient with chronic obstructive lung disease, and in 20 quadriplegics with paralysis of the

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