A New Method of Measuring of Contractility of the Isolated Perfused Neonatal Rat Heart

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For studying the functional performance of the adult heart in vitro, the perfusion technique already introduced by Langendorff (1895) is frequently used. This method consists of perfusion of the coronary bed of the isolated heart via retrograde perfusion of the aorta. The intraventricular pressure curve is measured isovolumically by means of a fluid-filled balloon introduced into the ventricle through the left atrium and connected to a pressure transducer (Fallen et al. 1967, Bergnam et al. 1979). The applicability of this method is, however, limited by the dimension of the ventricular cavity. It is, therefore, suitable for adolescent and adult rat hearts or larger laboratory animals only. For the measurement of cardiac performance during the neonatal period, the rabbit heart has repeatedly been used (e.g. Seguchi et al. 1986, Otani *et al.* 1987). The only study using the isolated perfused neonatal rat heart (Hoerter and Opie 1978) deals exclusively with the metabolic changes, but its contractility was not measured. We, therefore, tried to develop a method for measuring the contractility of the perfused heart muscle of neonatal rats.

The small weight of the neonatal heart together with its weak contraction force does not allow the classical Langendorff arrangement to be used, because of the inertia of the transfer pulley system and the necessity of using a flexible fibre for transmission of the force. The inertia of the pulley lowers the maximal frequency response of the system to unacceptable values and a long light fibre can distort the isometricity of the system due to its elasticity.



Fig. 1

Arrangement of the measurement. **1a**. 1) perfusing canula, 2) heart muscle, 3) cardiac appex, 4) two-arm lever, 5) jewel bearing, 6) glass fibre, 7) stimulating electrodes. **1b**. Detail of the bearing.

Our arrangement of the measuring procedure is shown in Fig. 1a. The neonatal rat heart (2) is attached by a fine ligature around the aorta to the perfusing cannula (1). A thread of surgical polyester sutured in the cardial apex (3) forming a short loop transmits the contraction force by means of a two-arm lever (4) and a glass fibre (6) to the isometric force transducer. To reduce the weight and so the moment of inertia of the lever a thin titan plate was used. The shaft made of stainless steel is fastened in the centre of gravity of the lever. To minimize the friction of the shaft, a jewel bearing (5) was used. For details see Fig. 1b. The other end of the lever is connected to the force transducer by means of a glass fibre (6). The perfused isolated heart together with the lever and its holder is placed in a double-wall cup with flowing water which is temperature stabilized to prevent desiccation and to ensure a stable temperature.

The heart is electrically stimulated with two elastic metal electrodes (7) connected to the stimulator. The suitable position of the perfusing cannula is fixed at the beginning of the experiment and the transducer can be shifted by means of a screw for setting and finely adjusting the preload force of the perfused myocardium.

Care was taken to prevent the lever from influencing the parameters of the system. The lever has to be rigid enough to ensure the isometricity and its moment of inertia should be as small as possible. A lever of titan sheet of 0.3 mm thickness of the shape shown in Fig. 1b, with a moment of inertia of 3.8×10^{-9} kgm² (which is smaller by at least one order of magnitude than that of the equivalent pulley) fulfils these requirements. The sagging of the lever to a force

of 1 g is <0.5 μ m, so that the isometricity is not affected. The influence of the moment of inertia of the lever on the maximal frequency depends on the parameters of the transducer in the system. For example, for the most often used Cambridge 403 transducer the maximal frequency of the system is reduced to 510 Hz (15%). A 0.2 mm titan sheet (which we did not have at our disposal) should have been quite sufficient from the point of rigidness. In this case the maximal frequency of the transducer would even be less affected.

Fig. 2 shows a record of the contraction curve of a heart isolated from a two-day-old rat.

Further experiments have shown that the method described also allows measurements on the perinatal rat heart even weighing 17 mg.





Record of the contraction curve of the heart isolated from a 2-day-old rat. (Perfusion: oxygenated Krebs-Henseleit solution, pressure 0.98 kPa, temperature $30 \,^{\circ}$ C; stimulation: polarity alternating pulses 1 ms, $12 \,\text{V}$, 2.5 Hz).

References

- BERGNAM S.R., CLARK R.E., SOBEL B.E.: An improved isolated heart preparation for external assessment of myocardial metabolism. Am. J. Physiol. 236: H644, 1979.
- FALLEN E.L., ELLIOT W.C., GORLIN R.: Apparatus for study of ventricular function and metabolism in the isolated perfused rat heart. J. Appl. Physiol. 22: 836, 1967.
- HOERTER J.A., OPIE L.H.: Perinatal changes in glycolytic function in response to hypoxia in the incubated and perfused rat heart. *Biol. Neonate* 33: 144-1261, 1978.

LANGENDORFF O.: Untersuchungen an überlebenden Säugetierherzen. Pflügers Arch. 61: 291, 1895.

- OTANI, H. ENGELMAN R.M., ROUSOU J.A., BREUER R.H., LEMESHOW S., DAS D.K.: The mechanism of myocardial reperfusion injury in neonates. *Circulation* 76 (Suppl. V): V161-V167, 1987.
- SEGUCHI M., HARDING J.A., JARMAKANI J.M.: Developmental change in the function of sarcoplasmatic reticulum. J. Mol. Cell Cardiol. 18: 189-195, 1986.

Reprint Requests

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