Research Journal of Pharmacology 1 (1): 1-4, 2007

New Variant of the Isolated Perfused "Working" Rat Heart

[1]Bozana Alexander, [1]Malini Rao, [2]Sanja Milutinovic and [1]Rajko Igic
[1]Department of Anesthesiology and Pain Management,
J.H. Stroger Hospital of Cook County Chicago, IL 60612, USA
[2]Department of Clinical Pharmacology, Medical Faculty,
University of Belgrade 11000 Belgrade, Serbia and Montenegro

Abstract: The aim of this study was to explore whether it is possible to perfuse the isolated rat heart through a needle inserted into the left ventricle through the apex of the heart and determine the cardiac rhythm of such preparation. The heart was excised and the perfusion started via the aorta. Then a needle was inserted into the left ventricle via the apex of the heart through which the perfusion solution is pumped. If the aortic column is closed, all the perfusion solution passes the coronary vascular bed. If the aortic column is open, the afterload could be set by adjusting the height of the fluid in the aortic column above the heart. Drugs were given via an in-line injection port placed at the perfusion line. ECG and aortic pressure in the aortic cannula were recorded. Heart rhythm and rate remained stable when the heart was perfused for 2 h at the afterload of 55 mm Hg, at the pump rate of 40 mm min^{-1}. The initial heart rate was 250+/-23 beats min^{-1} and after 2 h it was 238+/-27 (n=7). Epinephrine induced transitory tachycardia. Oxotremorine (50 ng) caused transient cardiac arrest, while 250 ng of this agent caused ventricular fibrillation. Glycopyrrolate prevented the effect of oxotremorine. The insertion of a needle did not cause significant damage of the heart with this technique. The preparation is stable for more than 2 h. The preparation is suitable for study of drug effects on the heart, especially heart rate and dysrhythmias.

Key words: Epinephrine, glycopyrrolate, isolated "working" heart, langendorff, oxotremorine, rat

INTRODUCTION

The isolated heart is a research tool that has been used in biomedical investigations for more than 150 years (Zimmer, 1998). It helped to establish the role of calcium on muscle contraction (Ringer, 1883) and the existence of chemical transmission in the vagal nerve (Loewi, 1921). The isolated mammalian heart perfused retrogradly through the aorta was introduced by Oscar Langendorff more than a century ago (Langendorff, 1985) and has been widely used since. In this preparation, the coronary vascular bed is perfused; the empty left ventricle contracts, but it does not pump fluid. Such a heart is beating but not "working." To make the preparation work, it needs an infusion of solution or the insertion of a small balloon filled with fluid into the left ventricle. The preparations with infusion of solution into the left ventricle in the isolated rat heart were developed (Neely *et al*., 1967; Igic, 1996). The "balloon method" helps to make the heart work and allows the measurement of the exact contractile ventricular force by a small balloon attached to the catheter tip and filled with a liquid. Such a balloon, preferably prepared of non-stretchable plastic foil (Curtis *et al*., 1986) is inserted into the left ventricle through the left atrium and the mitral valve.

The isolated mammalian heart perfused with a blood-free medium, whole blood, or blood components has provided reliable information on the condition of the working musculature, the conduction system and the coronary vessels in various conditions, including cardiac effectiveness of different biologically active substances (Doring and Dehnert, 1988) and adenovirus-mediated gene transfer (Abunasra *et al*., 2006). Recent advances in the fields of genomics, molecular and cell biology and chemistry often require a simple, reliable and efficient test to estimate global function at the organ level and various modifications of the isolated mammalian heart are used for this purpose (Igic, 2004).

The aim of the present study was to explore whether it is possible to perfuse the isolated rat heart through a needle inserted into the left ventricle through the apex of the heart and to determine the cardiac rhythm of such preparation.

Corresponding Author: Bozana Alexander, Department of Anesthesiology and Pain Management,
J.H. Stroger Hospital of Cook County Chicago, IL 60612, USA

MATERIALS AND METHODS

Female Sprague-Dawley (retired breeder) rats, 280-310 g, were anesthetized with sevoflurane. The abdomen was opened up to the diaphragm and heparin (500 USP units 100 g^{-1} body weight) was injected in the inferior vena cava. Ventral part of the diaphragm was removed from the ribs and cold (+4°C) Krebs-Henseleit solution was then poured into the thoracic cavity. The heart was excised by making a single cut with the scissors and placed in a cold Krebs-Henseleit solution. A cannula was positioned into the ascendant portion of the aorta approximately 2 mm above the coronary ostia and tied. The perfusion was started according to the Langendorff method, the pulmonary artery was identified and a small incision made into it so that the right ventricle would not be overstretched during the heart perfusion. The time from removal of the heart to establishment of the perfusion was less than 60 s. The perfusion was done with Krebs-Henseleit solution that was gassed with carbogen (5 vol% CO_2 and 95 vol% O_2) at 37°C using a Radnoti perfusion system (Radnoti Glass Technology, Inc., Monrovia, CA). The pH of the gassed solution was adjusted to 7.4. The flow rate of the perfusate, delivered from the oxygenated perfusion fluid to the heart, was controlled by a peristaltic pump and silicone tubing.

A side arm was mounted above the aortic cannula line (Fig. 1) in order to record aortic pressure and to set the afterload by adjusting the height of the fluid column above the heart (aortic outflow line). Above this side arm, a 3-way valve was mounted at the aortic cannula so that the perfusion solution could be diverted to propylene tubing that has a needle at the end which may be inserted into the left ventricle via the apex of the heart. The needle, 15 mm long cut from the 18 g hypodermic needle was bended and a small metal thickening (1 mm thick) was soldered close to the tip of the needle (Fig. 1). After the needle was inserted, the perfusion solution was directed to the needle and then the heart was immersed approximately 1.5 cm deep into the thermo-controlled organ bath at 37°C, filled with its own perfusate. If the aortic column was closed, all perfusion solution passed the coronary vascular bed. If the aortic column was open and the flow rate of the perfusion fluid was higher than the flow rate of the coronary flow, then the afterload could be set by adjusting the height of the fluid in the column above the heart and the outflow fluid may be collected and measured.

Aortic pressure and Electrocardiogram (ECG) were recorded with two sets of Hewlett-Packard (models 78534B and 78574A) instruments using chlorinated silver wire electrodes that were placed in the fluid present in the bath and a pressure transducer. To test durability of this preparation, we separately injected via the in-line injection port two compounds: Epinephrine and oxotremorine. It is well known that the later compound induces atrial fibrillation (Chiba *et al.*, 1972). Drug solutions were injected as a timed bolus via an in-line injection port placed at the perfusion line by a 100 μL microsyringe. The results are expressed as mean " standard deviation. Prior approval for animal use to conduct this educational project was obtained from our Institutional Animal Care and Use Committee.

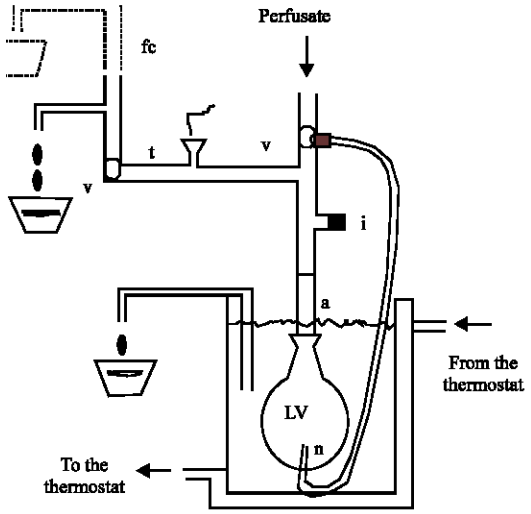

Fig. 1: Schematic diagram of the perfusion system. A needle is inserted into the left ventricle via the apex through which perfusion fluid is pumped. The needle, 15 mm long cut from a hypodermic needle (18 g) was bended and a small metal thickening (1 mm thick) was soldered close to the tip of needle. The other end of the needle was connected to the polyethylene tubing that delivers perfusion fluid when the fluid was diverted from the aortic cannula to the left ventricle. The perfusate coming from the coronaries via a siphon tube was collected in a measuring cylinder. Legend: n--needle, lv--left ventricle, a--aortic cannula, i--injection port, t--pressure transducer, v--three-way stopcock, fc--fluid column (aortic outflow line)

Drugs and solutions: Epinephrine hydrochloride and oxotremorine were purchased from Sigma Chemical Co., St. Louis, MO. Epinephrine HCl was dissolved in isotonic saline that contained 10 mg mL^{-1} of ascorbic acid. Glycopyrrolate Injection, USP, was a product of Gensia Pharmaceuticals, Inc., San Diego, CA. Pentobarbital

sodium (Nembutal) was purchased from Abbott Laboratories, North Chicago, IL. Heparin sodium was from Elkins-Sinn, Cherry Hill, NJ. Krebs-Henseleit solution contained the following compounds (in mmol/L): NaCl 118, KCL 4.70, $CaCl_2$ 2.52, $MgSO_4$ 1.66, $NaHCO_3$ 24.88, KH_2PO_4 1.18, glucose 5.55, Na-pyruvate 2.00. The solution was filtered through a 0.45 μm membrane to remove particles.

RESULTS AND DISCUSSION

Heart rhythm and rate remained stable when the heart was perfused for 2 h with an afterload of 55 mm Hg, open aortic cannula and a pump flow rate of 40 mL min^{-1}. The initial basal heart rate was 250+/-23 beats min^{-1} and after 2 h it was 238+/-27 (n=7). Coronary perfusion flow of the Langendorff and "working" mode were 13.0 +/- 0.8 and 12.6 +/- 0.7 mL min^{-1} (n = 4), respectively. Injection of 1microgram of epinephrine increased the heart rate from 240 +/- 18 to 345 +/-21 (n = 3); the beats were rhythmic (Fig. 2) and no one dysrhythmic beat was recorded during transitory tachycardia that lasted about 2 min. Twenty ng of oxotremorine caused transient bradycardia, 50 ng caused transient cardiac arrest and 250 ng caused permanent ventricular fibrillation (Fig. 3). Glycopyrrolate, 10 micrograms given also as a bolus 1 min before 50 ng of oxotremorine, prevented the effect of oxotremorine.

The heart perfusion via a needle inserted into the left ventricle permits the isolated rat heart to pump perfusion solution to the aorta and thus supply the coronary vessels. Such working heart preparation is stable for more than two hours and it seems that the insertion of a needle at the apical part of the heart does not cause significant damage of the conducting system or an escape route for coronary flow, that may alter normal heart function. The durability test, using epinephrine and oxotremorine, showed robustness of the preparation.

In this preparation, the coronary flow can be measured by collecting the effluent in a graduated cylinder (Fig. 1) or by a drop counter placed below a siphon tube. The fluid pumped through open aortic outflow line may also be measured. This working heart preparation can easily be transformed to a non-working (Langendorff mode) and vice versa.

Certain improvements of this method could be explored. For example, one may insert an ultra-miniature catheter for continuous measurement of pressure in the left ventricle. Also, instead immersing the heart in a thermo-constant organ bath filled with the perfusate to reduce tissue edema (Doring and Dehnert, 1988), it is possible to use a bovine serum albumin to prevent interstitial edema of the tissue

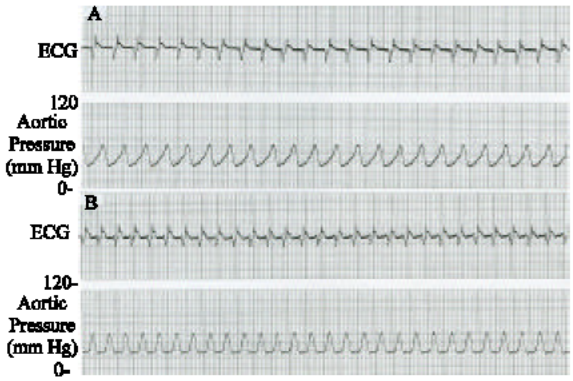

Fig. 2: ECG and aortic pressure recordings during the rat heart perfusion via a needle inserted into the left ventricle. Aortic outflow line was open and the afterload set at 55 mm Hg. The flow rate was 40 mL min^{-1}. A. Control recordings. B. Recording done 10 sec after 1 microgram of epinephrine was injected via an in-line port. Chart speed: 25 mm min^{-1}

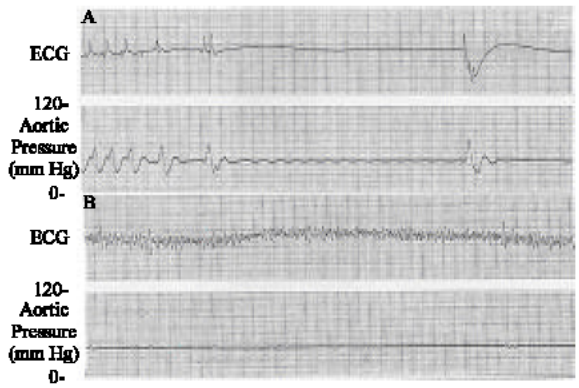

Fig. 3: Effect of oxotremorine on the isolated "working" rat heart perfused via a needle inserted into the left ventricle. Aortic outflow line was open, and the afterload was set at 55 mm Hg. The flow rate was 40 mL min^{-1}. ECG and aortic pressure recordings were done 10 sec after injection of 50 (panel A) and 250 (panel B) micrograms of oxotremorine, respectively, via an in-line injection port. Chart speed: 25 mm min^{-1}

(Clements-Jewery *et al*., 2002). Thus, the preparation may be kept working much longer at optimal contractile force without immersion in its own perfusate, so that the coronary flow could be measured by drops of the effluent that directly trickle from the isolated heart.

Recent advances in molecular and cell biology often need simple, reliable and efficient tests for estimating the global function at an organ level. Isolated perfused mouse

(Larsen *et al*., 1999; MacGown *et al*., 2004) and neonatal rat (Wiechert *et al*., 2003) hearts are important research tools for such estimation. However, we doubt that it is possible to apply our method to such small hearts.

This variant of the isolated perfused rat heart can be prepared faster than other working heart preparations. Hence, it is a useful as a teaching model for the isolated perfused heart as students may prepare-Langendorff/ working mode-independently. This model is also suitable for study of drug effects on the heart, especially heart rate and dysrhytmias. It remains to be established if it could be used to study the ischemia-reperfusion dysrhythmias, as well.

ACKNOWLEDGMENT

The authors thank Dr. Sara F. Rabito for reading the manuscript and Dr. Milutinovic started cooperation on this project when she was an exchange visitor at the Department of Physiology, UIC, Chicago, Illinois. This investigation was supported in part by a grant-in-aid from the American Heart Association (9708074A) awarded to R. I.

REFERENCES

Abunasra, H., R.T. Smolenski, J. Yap, J. Jayakumar, M. Sheppard and M.H. Yacoub, 2006. Comparison of two gene transfer models for the attenuation of myocardial ischemia-reperfusion injury following preservation for cardiac transplantation. Eur. J. Cardiothorac. Surg., 29: 772-8.

Chiba, S., R. Igic and T. Nakajima, 1972. Effect of tremorine and oxotremorine on the S-A node of the dog heart *in vivo*. Tohoku J. Exp. Med., 107: 381-385.

Clements-Jewery, H., D.J. Hearse and M.J. Curtis, 2002. The isolated-perfused rat heart: An inappropriate model for the study of ischemia-and infarction-related ventricular fibrillation. Br. J. Pharmacol., 37: 1089-1099.

Curtis, M.J., R. MacLeod, R. Tabrizchi and M.J.A. Walker, 1986. An improved apparatus for small animal hearts. J. Pharmacol. Methods, 15: 87-94.

Doring, H.J. and H. Dehnert, 1988. The isolated perfused heart according to Langendorff. March, Germany, Biomesstechnik.

Igic, R., 1996. The isolated perfused "working" rat heart: A new method. J. Pharmacol. Toxicol. Methods, 35: 63-67.

Igic, R., 2004. Isolated-perfused heart: The preparation that has been used as a research tool for more than a century. Drug Design Reviews-Online, 1: 175-184.

Langendorff, O., 1895. Untersuchungen um hberlebenden S@ugetierherzen. Pflhgers Arch. ges Physiol., 61: 291-332.

Larsen, T.S., D.D. Belke, R. Sas, W.R. Giles, D.L. Severson, G.D. Lopaschuk and J.V. Tyberg, 1999. The isolated working heart: methodological considerations. Pflugers Arch., 437: 979-985.

Loewi, O., 1921. Uber humorale gbetagbarkeit der Herznervenwirkung. I. Mitteilung. Arch. Physiol., 189: 239-242.

MacGowan, G.A., C. Evans, T.C. Hu, D. Debrah, S. Mullet, H.H. Chen, C.F. McTiernan, A.F. Stewart, A.P. Koretsky and S.G. Shroff, 2004. Troponin I protein kinase C phosphorilation sites and ventricular function. Cardivasc. Res., 63: 245-245.

Neely, J.R., H. Liebermeister, E.J. Battersby and H.E. Morgan, 1967. Inhibition of pressure development on oxygen consumption by isolated rat heart. Am. J. Physiol., 212: 804-814.

Ringer, S., 1883. A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol. (Lond.), 4: 29-42.

Wiechert, S., A. El-Armouche, T. Rau, W.H. Zimmermann and T. Eschenhagen, 2003. Twenty four h Langendorff-perfused neonatal rat heart used to study the impact of adenoviral gene transfer. Am. J. Physiol. Heart Circ. Physiol., 285: H907-14.

Zimmer, H.G., 1998. The isolated perfused heart and its pioneers. News Physiol. Sci., 13: 203-210.