A DEVICE FOR RECORDING LEFT VENTRICULAR CONTRACTION AND ELECTROCARDIOGRAM IN NONWORKING ISOLATED PERFUSED RAT HEART

Kazushige SAKAI and Yasuyuki SHIRAKI
Department of Pharmacology, Research Laboratories, Chugai Pharmaceutical Co., Ltd., Toshima-ku, Tokyo 171, Japan

Accepted November 7, 1977

Abstract—Using the isolated Langendorff rat heart preparation perfused at a constant flow rate (about 6 ml·min⁻¹ pro heart) with a modified Krebs-Henseleit solution, methods for recording left ventricular cavity pressure and electrocardiogram (ECG) changes were devised. Stable levels of mechanical activities were reached 60-90 min after start of perfusion and were maintained for at least 4 hr. When either norepinephrine (0.05-0.15 μg) or quinidine (50-200 μg) was injected into the aortic bulb of the heart, sensitive changes in the measurable cardiac parameters were observed and changes in ECG patterns were particularly characteristic. Our results indicate that this experimental model may be useful for evaluating numerous functions of the isolated heart.

Since Langendorff (1) originally developed an isolated perfused heart preparation in 1895, such has been employed in a variety of ways to study the hemodynamic and biochemical changes. Many workers (2-6) have demonstrated the stability and versatility of the preparation. Although this Langendorff heart preparation offered many advantages in the evaluation of cardiac function, some modifications are required for diverse purposes of experiments.

The aim of our investigation was to devise a method for recording left ventricular cavity pressure and electrocardiogram in the isolated rat heart perfused according to the Langendorff technique. Also described is the stability of the preparation and the sensitive changes in the measurable cardiac parameters after the administration of norepinephrine and quinidine.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing about 300 g with free access to water and standard laboratory diet were used. The rats were injected with heparin sodium (5 mg) i.p. 30 min before sacrifice by a blow on the head. The heart was quickly excised and washed in ice-cold, O₂-saturated Krebs-Henseleit (K-H) solution. After a glass cannula filled with the solution had been inserted into the aorta, the heart was mounted in a water-jacketed chamber (37-38 °C) (Fig. 1). According to the Langendorff technique, the heart was perfused at a constant flow (about 6 ml·min⁻¹ pro heart) by means of a peristaltic pump consisting of six parallel channels of tygon tubing (Desaga, Heidelberg, West Germany). The K-H solution as the perfusate contained the following substances (in mM/l): Na⁺, 143; K⁺, 5.9; Ca²⁺, 1.7; Mg²⁺, 1.2; Cl⁻, 124; HCO₃⁻, 25.0; H₂PO₄⁻, 1.2; SO₄²⁻, 1.2; and glucose, 6;
A total concentration of about 310 mOs/1) and was aerated thoroughly with a gas phase mixture containing 95% \( \text{O}_2 \): 5% \( \text{CO}_2 \). The oxygen tension of the perfusate (pH, 7.4) was always in excess of 600 mmHg. The temperature was maintained between 37 and 38°C as it entered the heart. From the reservoir the perfusate traversed a Millipore filter (Toyo Roshi Co., KS-13), the pump and bubble trap and then entered the perfusion cannula. The perfusion pump was precalibrated and re-checked at the end of the experiments. Unless the heart had been damaged during the operation, it began to beat immediately after start of the perfusion.

For the measurement of left ventricular cavity pressure (LVP), a glass cannula (outer diameter, 2 mm; inner diameter, 1.5 mm) was inserted via the left atrium into the left ventricle. The glass cannula, the tip of which consisted of a small ball (outer diameter, 3 mm; inner diameter, 2.5 mm) with three holes (diameter, 1–2 mm), was filled with water and connected by a water-filled polyethylene tubing to a pressure transducer (Nihon Kohden, RP-5). The first derivative of left ventricular pressure \((\frac{dP}{dt})_{\text{max}}\) was obtained by using a resistance-capacitance differentiating circuit (Nihon Kohden, RPD-5). Electrocardiogram (ECG) was taken according to a bipolar lead from two (different and indifferent) silver-wire electrodes touching the origin of the aorta and from one touching the left intraventricular wall (Fig. 1C). The bioelectric amplifier (Nihon Kohden, RB-5) was set with a lower cutoff frequency of 0.3 Hz. Heart rate (HR) was measured with a cardiotachometer (Nihon Kohden, RT-5) or calculated from the R-R interval of ECG. Perfusion pressure (PP) was measured with a pressure transducer (Nihon Kohden, RP-5) from a side arm near the aortic cannula. Pressure and ECG signals were recorded on an ink-writing multipurpose polygraph.
(Nihon Kohden, RM-85-M). Measurements of $P_{O_2}$ and $pH$ in the perfusate were carried out using the blood gas analysing system (Radiometer, BMS3-MK2, Copenhagen, Denmark). Osmolarity was determined using a microosmometer (Knauer, Berlin, West Germany).

At the conclusion of the experiments, the heart was cut away from the position of the aortic cannula, then weighed, and dried for 24 hr at 100°C to constant weight. The weight of wet heart prior to perfusion can be calculated by multiplying the dry weights after perfusion with the factor 5 \( (7) \).

The drugs used were (+)-norepinephrine hydrochloride (Sankyo) and quinidine sulfate (Wako Junyaku). Doses are given in terms of their salts. Drugs were dissolved in or diluted with 0.9% saline solution and injected in a volume of 0.1 ml in 10 sec into the aortic bulb of the heart.

All results were expressed as means \( \pm \) standard error (SE) of the mean. Student's $t$-test was used to statistically analyze the data. A $P$ value of 0.05 or less was considered statistically significant.

**RESULTS**

*Control observations:* Eight isolated hearts were perfused at a constant flow rate of initially 6 ml-min$^{-1}$ pro heart. Soon after the perfusion the values of the measurable cardiac

---

![Fig. 2](image-url)  
**Fig. 2.** A) Representative recordings of cardiac parameters at 60 min intervals. Soon after the preparation became stable (zero time), observations over 4 hr were started. B) Summarized data. Vertical bars represent means $\pm$ SE (N=8). See Table 1 for control values of ECG patterns.
parameters were extremely low. In the time course of perfusion, however, these parameters rose progressively and reached stable levels 60 to 90 min after the perfusion. The flow rate

![Graph](image-url)

**Fig. 3.** Changes in cardiac parameters 30–60 sec after the administration of either norepinephrine or quinidine. A) Typical recordings. B) Dose response curves for changes in cardiac parameters after the administration of norepinephrine (0.05–0.15 μg). Values are expressed in the percent changes to the corresponding preadministered values, and vertical bars represent mean ± SE (N=8). C) Dose response curves for changes in cardiac parameters after the administration of quinidine (50–200 μg). Explanation is similar to that in Fig. 3B.
was then adjusted to maintain the perfusion pressure of about 100 mmHg, and, thereafter was not changed throughout the experimental time course. The flow rate and dry weights of the perfused hearts averaged 6.69 ± 0.09 ml·min⁻¹ pro heart and 0.202 ± 0.006 g, respectively. So, the calculated flow rate was 6.67 ± 0.17 ml·min⁻¹·g⁻¹ wet wt.

As shown in Fig. 2A and B, left ventricular cavity pressure (LVP) and perfusion pressure (PP) tended to increase progressively, whereas heart rate (HR) tended to decrease. However, the changes in these parameters were slight. Maximum dP/dt remained unchanged over 4 hr. When each value of ECG changes at zero time was compared with that measured at 60 min intervals over 4 hr, there were statistically no significant differences (Table 1). Thus, the present preparations remained in good condition over 4 hr or more.

**Drug responses:** When the preparation became stable, norepinephrine (0.05–0.15 µg) and quinidine (50–200 µg) were injected into the aortic bulb. Typical results are shown in Fig. 3A. The peak

| Duration (sec) | 0      | 0.042±0.002 | 0.043±0.002 | 0.043±0.002 | 0.045±0.002 | 0.045±0.002 |
|---------------|--------|-------------|-------------|-------------|-------------|-------------|
| Perfusion time (min) | 60     | 0.019±0.000 | 0.018±0.000 | 0.019±0.001 | 0.019±0.000 | 0.019±0.000 |
|               | 120    | 0.120±0.004 | 0.123±0.006 | 0.116±0.006 | 0.118±0.004 | 0.121±0.005 |
|               | 180    | 0.226±0.005 | 0.234±0.004 | 0.235±0.005 | 0.245±0.006 | 0.250±0.006 |
|               | 240    |             |             |             |             |             |

Soon after the preparation stabilized (zero time), observations were made. Values shown are means±SE (N = 8). Compared with initial (zero time) values, the corresponding values at each time were not significantly changed. Body weights of animals, 296±12 g; dry heart weights, 0.202±0.006 g; and perfusion flow rate, 6.67 ± 0.17 ml·min⁻¹·g⁻¹ wet wt.

**TABLE 1. Control durations of the basic components of ECG**

![Typical ECG patterns before (control) and after the administration of either norepinephrine (0.1 µg) or quinidine (100 µg).](image-url)
responses of the cardiac parameters, which appeared 30-60 sec after the injection of each drug, were expressed as the percent changes from the corresponding preadministered levels, respectively (Fig. 3B and C).

As shown in Fig. 3A and B, norepinephrine decreased PP, while it increased LVP and maximum dP/dt as well as HR. All of the P-Q, Q-T and R-R intervals were shortened, even if the changes in the QRS complex were not distinct.

As shown in Fig. 3A and C, the large doses (150-200 µg) of quinidine decreased LVP and maximum dP/dt, while the smaller doses caused a decrease preceded by an initial increase in the parameters. PP and HR were always decreased by the administration of quinidine in the dose range of 50 to 200 µg. Changes in ECG patterns were characteristic. All of the P-Q, Q-T and R-R intervals were prolonged definitely in a dose-dependent manner. Also, an increased duration of the QRS complex was prominent. Typical ECG patterns for control, norepinephrine (0.1 µg) and quinidine (100 µg), respectively, are illustrated in Fig. 4.

DISCUSSION

In this study, a constant volume perfusion technique was employed to perfuse simultaneously several isolated rat hearts, under uniform conditions. Previously, one of the present authors (7-8) perfused the isolated guinea pig hearts at a constant flow rate of 4 ml · min⁻¹ · g⁻¹ wet wt. This was based on the finding that the different flow rates ranging from 4 to 8 ml · min⁻¹ · g⁻¹ wet wt. produced no significant differences on the rates of lactate output determined at various time intervals over one hour after start of perfusion. At this flow rate, however, the observed perfusion pressure and heart rate were considerably low, although stable situations of the preparations were maintained over 5 hr.

Shipp et al. (9), using isolated working rat hearts, claimed that perfusion rates of 5 ml · min⁻¹ · g⁻¹ wet wt. or higher were needed to avoid metabolic changes suggestive of oxygen deficiency. Schreiber et al. (10) also presented a similar statement in the guinea pig heart preparations. Despite the affirmative report by Fisher and Williamson (11), the flow rate of 4 ml · min⁻¹ · g⁻¹ wet wt. appears to be critical for maintaining the preparation. In the present study, a constant flow rate of 6.13 ± 0.10 ml · min⁻¹ · g⁻¹ wet wt. (N = 24) was selected for maintaining the perfusion pressure (about 100 mmHg) near mean blood pressure levels of intact rats. At the flow rate, all of the measurable parameters remained relatively stable over at least 4 hr.

In Langendorff perfused hearts of small animals, there have been some difficulties in recording left ventricular cavity pressure (LVP) and ECG. Taylor and Cerny (12) using rat hearts measured the LVP by piercing the ventricle with a needle attached to a pressure transducer. However, with this technique, the ventricular muscle is injured. According to the method of Kadas and Opie (13), the LVP can be successfully measured by inserting a fluid-filled latex balloon via the left atrium into the cavity of the left ventricle of rats. This technique, however, involves complexities in attaching the balloon to the cannula and filling the balloon with water.
On the other hand, in most previous investigations ECG electrodes were attached to the surface of the heart either by pinching the muscle or through moistened cotton. It should be pointed out, however, that in the former procedure there is tissue injury and in the latter instability of recording ECG is inevitable during a long perfusion period. Kadas and Opie (13) obtained the ECG from two platinum plates touching the ventricles and from the aortic metal cannula. With this technique it is possible to continuously record ECG without injuring the ventricular surface. Our method described herein is somewhat similar.

As described in the results, the present attempts were characterized by the stability of the preparation and by the sensitive and well-known changes in the measurable cardiac parameters after drug administration. Therefore, this isolated perfused heart preparation appears to be a convenient and simple experimental model for evaluating cardiac function. Also, as the devised perfusion apparatus permits several hearts to be perfused simultaneously under relatively the same conditions, uniformity of data and efficiency of experiments are easily facilitated.

Acknowledgements: We thank M. Hiruta and M. Akima for their skill in building of the perfusion apparatus, and T. Yamazaki for technical assistance.

REFERENCES

1) LANGENDORFF, O.: Untersuchungen am überlebenden Säugethierversuchen. Pflügers Arch. 61, 291-332 (1895)
2) MORGAN, H.E., HENDERSON, M.J., REGAN, D.M. AND PARK, C.R.: Regulation of glucose uptake in muscle: I. The effects of insulin and anoxia on glucose transport and phosphorylation in the isolated, perfused heart of normal rats. J. biol. Chem. 236, 253-261 (1961)
3) OPIE, L.H.: Coronary flow rate and perfusion pressure as determinants of mechanical function and oxidative metabolism of isolated rat heart. J. Physiol. 180, 529-541 (1965)
4) WEISFELDT, M.L. AND SHOCK, N.W.: Effect of perfusion pressure on coronary flow and oxygen usage of nonworking heart. Am. J. Physiol. 218, 95-101 (1970)
5) DAVIDSON, S., MAROKO, P.R. AND BRAUNWALD, E.: Effects of isoproterenol on contractile function of the ischemic and anoxic heart. Am. J. Physiol. 227, 439-443 (1974)
6) ARONSON, C.E. AND SEDER, B.R.: Effects of prolonged perfusion time on the isolated perfused rat heart. Toxicol. appl. Pharmacol. 38, 479-488 (1976)
7) SAKAI, K., GEBHARD, M.M., SPEICKERMANN, P.G. AND BRIESCHNEIDER, H.J.: Enzyme release resulting from total ischemia and reperfusion in the isolated, perfused guinea pig heart. J. mol. cell. Cardiol. 7, 827-840 (1975)
8) SAKAI, K. AND SPEICKERMANN, P.G.: Effects of reserpine and propranolol on anoxia-induced enzyme release from the isolated perfused guinea pig heart. Arch. Pharmacol. 291, 123-130 (1975)
9) SHIPP, J.C., MATOS, O.E., KNIZLEY, H. AND CREVASSO, E.E.: CO2 formed from endogenous and exogenous substrates in perfused rat heart. Am. J. Physiol. 207, 1231-1236 (1964)
10) SCHREIBER, S.S., ROTHSCHILD, M.A., EVANS, C., RUFT, F. AND ORATZ, M.: The effect of pressure or flow stress on right ventricular protein synthesis in the face of constant and restricted coronary perfusion. J. clin. Invest. 55, 1-11 (1975)
11) FISHER, R.B. AND WILLIAMSON, J.R.: The oxygen uptake of the perfused rat heart. J. Physiol. 158, 86-101 (1961)
12) TAYLOR, F.B. AND CERNY, F.J.: Evaluation of the isolated paced rat heart. J. appl. Physiol. 41, 328-331 (1976)
13) KADAS, T. AND OPIE, L.H.: Isolated perfused rat heart adapted for simultaneous measurement of left ventricular contraction, electrocardiogram and metabolism of 14C-labelled substrates. J. Physiol. 167, 6p-7p (1963)