TECHNICAL NOTES

Gated Blood-Pool Studies of Cardiac Function in the Rat and Marmoset

Graeme H. McIntosh, Leighton R. Barnden, Ian H. Buttfield, and John S. Charnock

C.S.I.R.O., Glenthorne Laboratories, Adelaide, South Australia, and Queen Elizabeth Hospital, Woodville, South Australia

To develop a sensitive, relatively noninvasive method for monitoring cardiac function in small laboratory animals, equilibrium gated blood-pool imaging (with Tc-99m RBC) was adapted for use in an inbred strain of laboratory rats of 400–470 g body weight and in marmosets of 320–400 g body weight. A 2-mm pinhole collimator was fitted to the gamma camera to produce a distinct image of the left ventricle following intravenous injection of 5 mCi of pertechnetate. Reproducible and consistent data for heart rate, left-ventricular ejection fraction, and peak ejection rate were obtained from studies on 13 male rats and five marmosets maintained on constant diets. An intravenous injection of 0.4 μg of isoprenaline led to predictable increases in heart rate, left-ventricular ejection fraction, and peak ejection rate, and provided evidence of the sensitivity of the method in monitoring heart function in small laboratory animals.

J Nucl Med 24: 728–731, 1983

Equilibrium gated blood-pool imaging after in vivo labeling of erythrocytes with Tc-99m has proved to be a very effective tool for the noninvasive assessment of cardiac function in man (*1*,*2*). To study cardiac function in the rat and marmoset, highly invasive approaches are commonly used, requiring death of the animal at the end of the study (*3*). The application of equilibrium gated blood-pool imaging to the small laboratory animal enables heart function to be assessed with minimal disturbances and without killing the animal. This has the potential for long-term monitoring of the influence of diet on heart function in experimental animals, in our case the rat and common marmoset (*Callithrix jacchus*). The animals can then be preserved for biochemical analysis at the conclusion of the experiment. In terms of the reproducibility of the results and their applicability to man, this approach to the monitoring of cardiac function has many advantages over the various preparations of an isolated perfused heart commonly used in cardiac research using small animals.

METHODS

Male inbred wooded Wistar rats, 8–12 mo of age and 400–470 g body weight, and mature marmosets 12–18 mo of age and 320–400 g body weight, were anesthetised by intraperitoneal injection of sodium pentobarbitol. The dose was 60 mg/kg body wt. for the rat and 30 mg/kg for the marmoset. After anesthesia each animal was laid on its back and the right external jugular vein exposed and cannulated using a polyethylene cannula (0.5 mm i.d., 0.9 mm o.d.), the tip being placed as close as possible to the heart. All subsequent injections were given intravenously via a three-way tap connected to the cannula, which was kept filled with sterile physiological saline between injections. A bolus of stannous pyrophosphate containing 20 μg of stannous ions was administered and was followed 10 to 20 min later by 5 mCi of sodium [Tc-99m]pertechnetate. This labeling procedure was used in preliminary studies in which the rats were killed and the erythrocytes separated, washed, and counted; the efficiency turned out to be 90% or better. In the early marmoset studies, however, satisfactory in vivo labeling was not obtained. This problem was overcome by withdrawing 2 ml of blood after the pyrophosphate injection, adding the pertechnetate, then reinjecting via the cannula 10 min later (*4*). A temperature-controlled table was used to maintain the anesthetised animal at 38 ± 0.5°C.

To accommodate to the very small hearts in rats and marmosets, a collimator with a 2-mm pinhole was used in place of the parallel-hole clinical collimator. This improved the system's resolution and sensitivity* from 9.2 mm FWHM at 10 cm from the collimator face, yielding 300 cpm/μCi, to 1.2 mm FWHM and 450 cpm/μCi at a distance of 2.5 cm from the pinhole aperture. At this distance, which was typical for our experiments, the pinhole also produced a sevenfold image magnification.

Received Nov. 23, 1982; revision accepted Mar. 1, 1983.

For reprints contact: Dr. Graeme H. McIntosh, CSIRO, Div. of Human Nutrition, Glenthorne Laboratories, Majors Rd., O'Halloran Hill, Adelaide, South Australia 5158.

TABLE 1. THE INFLUENCE OF CORRECTION FACTORS ON DATA OF MARMOSETS AT REST

	Left-ventricular ejection fraction (%)			Peak ejection rate (EDV/sec)		
	Mean	s.d.	n	Mean	s.d.	n
1. Uncorrected data	48.5	10.7	9	8.1	3.3	9
2. Flood-corrected data	50.9	10.8	9	7.9	2.7	9
3. Geometry-corrected data	51.1	10.4	9	8.7	3.4	9

The animal was positioned supine under a 37-photomultiplier (PMT) gamma camera. The image of the left ventricle was centered in the field of view and its dimension restricted to less than 20% of the field diameter by adjusting the distance between the pinhole and the heart (typically 20-25 mm). Angling of the camera to isolate the image of the left-ventricle blood pool from the rest of the heart was facilitated by a computer program that provided a real-time display of images of end-diastole and end-systole during set-up.

ECG electrodes were attached to both forelimbs and one hind limb. Heart-beat synchronization pulses were generated by an ECG R-wave trigger circuit. Together with 2-msec time marks, these pulses were inserted into the sequence of photon origins during computer list-mode acquisition. Twenty to thirty images were formatted from the list-mode data. In later studies, 24 images were gated in real time into the computer memory. Each study accumulated 2-6 million gamma counts, which corresponded to 3000-4000 heart beats acquired over 7-10 min.

From these data, heart rate, left-ventricular ejection fraction (LVEF), and peak ejection rate (PER) were determined (*7*). The ejection fraction was derived from ventricular regions of interest selected independently on the end-diastolic image (sum of two raw images) and the end-systolic image (sum of three raw images), using a trough-detection algorithm (*5*). The background region of interest was defined on the end-systolic image on or within the end-diastolic ventricular edge. Peak ejection rate (PER) represents the maximum ejection rate of the left ventricle, in end-diastolic volumes/sec (EDV/sec), and was derived from the slope of the time-volume curve.

To investigate the effects of the sensitivity variations associated with a pinhole system (*6*), LVEF and PER were computed without uniformity correction, after pinhole flood-field correction, and after correction with a pinhole "geometry flood" from which the camera's PMT intensity modulation was removed. The results for seven studies are presented in Table 1. There is no significant difference between either the means or the standard deviation for the three sets of data, for either LVEF or PER. We chose to apply the pinhole flood-field correction to all studies reported here.

Cardiac function was monitored at rest and then under the influence of a pharmacological stress, following administration of the beta adrenergic receptor agonist isoprenaline hydrochloride (IPA) (*8,9*). A total dose of 0.4 μg IPA was injected, 0.2 μg initially followed by a further 0.1 μg after 4 and 7 min, during which period a second accumulation of data was undertaken.

Results were analysed using the Student's t-test.

RESULTS

Well-defined images of the left ventricle of the rat and marmoset were obtained, as shown in Fig. 1. In plates (A) and (B) respectively, the left ventricle of the resting rat heart is clearly visible, at end-diastole and end-systole. The left ventricle of the marmoset is also shown at end-diastole (C) and end-systole (D) for comparison. All of these images have been 'zoomed' by a factor of 2.

In the rat both ventricles appeared more elongated than in the marmoset, and the right ventricle appeared comparable in volume with the left and quite well separated from the right atrium. This latter feature was readily seen on the cine replay. The marmoset's left ventricle, on the other hand, appeared larger in volume than the right ventricle, which overlapped the right atrium to such an extent that visualization of right ventricular contraction was not possible. The ejection fraction in the rat was greater than in the marmoset, as is shown in Fig. 1 and Table 2. The anterior oblique angle (AO), which yielded best separation of the ventricular blood pools, was 5° RAO with 20° caudal tilt in the rat, and 15° LAO with 15° caudal tilt in the marmoset.

The quantitative data derived by computer-assisted assessment of these images are given in Table 2. The standard deviations give a clear indication of the consistency of the data, heart rate being most variable. Administration of 0.4 μg isoprenaline to the rat produced a 28% increase in heart rate accompanied by a similar increase (23%) in LVEF. In the marmoset, heart rate showed a greater increase (49%) whereas LVEF was increased by a fraction (29%) similar to that seen in the rat. Changes of this magnitude are well within the method's sensitivity of detection. Heart rate of the resting rat reported here was similar to that found earlier in many other studies.

It is of particular interest in the application of this technique to nutritional studies (unpublished data) that the isoprenaline-induced change in the left-ventricular PER of the rat heart showed the greatest change of any of the parameters derived by gated blood-pool imaging. Here the PER increased 65% above the mean level of the animals at rest, which was significant at the 0.1% level.

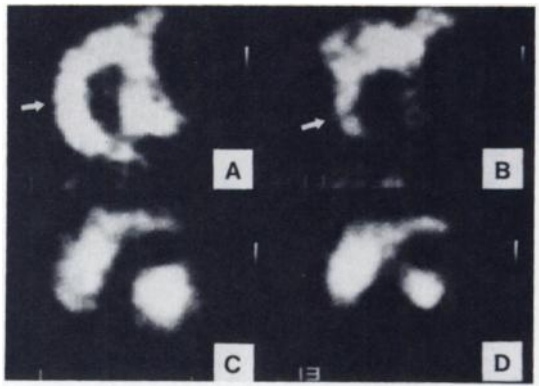

FIG. 1. Anterior images of blood pools in rat and marmoset hearts. Images A and B show rat heart at end-diastole and end-systole, respectively. Corresponding images of marmoset heart are shown in (C) and (D). Arrows indicate position of valve plane between right atrium and right ventricle in rat. In marmoset these two blood pools overlap.

TABLE 2. THE LEFT-VENTRICULAR CARDIAC DATA FROM NORMAL MALE RATS AND MARMOSETS AT REST AND UNDER ISOPRENALINE STRESS

Animal	Experimental conditions	n	Heart rate (beats/min)	Left-ventricular ejection fraction (%)	Peak ejection rate (EDV/sec)
Rat	At rest	13	329 ± 95	70.1 ± 8.0	13.0 ± 2.4
	Isoprenaline (0.4 μg)	12	421 ± 68	86.5 ± 6.5	21.4 ± 6.0
	%Δ		+28	+23	+65
	Sig. (t-test)		p <0.01	p < 0.001	p < 0.001
Marmoset	At rest	5	260 ± 21	50 ± 8.3	7.2 ± 1.7
	Isoprenaline (0.4 μg)	5	387 ± 27	64.6 ± 7.4	14.8 ± 2.9
	%Δ		+49	+29	+106
	Sig. (t-test)		p <0.001	p <0.05	p <0.05

All values are given as mean ± s.d.

Under isoprenaline stress, the marmoset showed an even greater increase in PER (106%), which was significant at the 5% level.

DISCUSSION

Gated blood-pool imaging using in vivo Tc-99m-labeled erythrocytes has been successfully adapted for use in the laboratory rat and marmoset by replacement of the normal parallel-hole collimator with a pinhole on the gamma camera, and by an increase in the amount of radiotracer per unit body weight. Because the pinhole was so close to the subject, the system's sensitivity was actually better than with a parallel-hole collimator. Scaling the human radiation dose by body weight, we estimate that the blood dose to these experimental animals is 35 rem. The shape of the left ventricle and the angle of the camera used to effect maximal septal separation, which indicates the degree of rotation of the heart in the thorax, demonstrated clear differences in the heart and thorax anatomy of the marmoset and rat (*10*). This was also the case with ejection fraction, which was very high in the rat relative to the marmoset.

Our result, that a pinhole system can yield the same LVEF and PER whether or not regional sensitivity correction is applied, was perhaps unexpected. We attribute this to the concentric nature of the ventricular blood-pool contraction, and to the precautions taken to limit the ventricle size on the camera field and to select background on the end-systolic image close to the ventricular edge.

Results were highly reproducible between animals, as was shown by the size of standard deviations. This verifies both the homogeneity of animals studied and the reproducibility of the method. Heart rate was the most variable component, although it was improved by the introduction of firm body-temperature control in these studies. PER showed increased variability under the influence of isoprenaline, whereas both heart rate and LVEF showed reduced variability about the increased means.

The results are consistent with the known effects of isoprenaline in producing a significant increase in activity of the rat heart (*8,9*). A similar and even more dramatic response was observed in the primate, indicating its greater sensitivity to the drug. The LVEF gives an indication of cardiac efficiency, and is of prime importance to the clinician as a measure of cardiac function. PER, on the other hand, may be used to compare isotonic contraction force between animals, if it can be assumed that end-diastolic left-ventricular volume is constant in a group of inbred experimental animals matched for age, weight, and sex. This uniformity certainly applies to the rats used in this study, and to a lesser degree to the marmosets. Left-ventricular volumes measured directly at necropsy and from preparations of the isolated working heart enabled us to obtain a calculation of cardiac output of 65 ml/min from the data for the rat, which is consistent with figures for the rat derived by other means (*10*).

Barbiturate anesthesia is required for adequate restraint in laboratory animals like the rat and marmoset, and represents an interference with normal conscious cardiac function. The dosage we used, although relatively high in the rat, was effective in obtaining complete anesthesia throughout the procedure (60–90 min) and was followed by uneventful recovery within 2–3 hr. It is essential, however, to control body temperature by external means, as barbiturates interfere with normal thermoregulatory control in the anesthetised animal, which may lead to spurious heart rates.

In conclusion, then, we have established a gated blood-pool procedure yielding parameters of cardiac function that are reproducible and sensitive to pharmacologic stress. The method should be of value in the repeated monitoring of cardiac function in long-term nutritional studies spanning months or years.

FOOTNOTES

* NEMA-US National Electrical Manufacturers Association—definitions.

ACKNOWLEDGMENTS

We acknowledge the excellent assistance given by Professor W. F. Dryden, Ms. Felicia Bulman, Ms. Ruth Ryan and Mr. Peter McCabe with these studies.

REFERENCES

1. BUROW RD, STRAUSS HW, SINGLETON R, et al: Analysis of left ventricular function with multiple gated acquisition cardiac blood pool imaging. *Circulation* 56:1024-1028, 1977
2. BODENHEIMER MM, BANKA VS, HELFANT RH: Nuclear

cardiology 1. Radionuclide angiographic assessment of left ventricular contraction: Uses, limitations and future directions. *Am J Cardiol* 45:661-673, 1980

3. YEAGER JC, IAMS SG: Isoproterenol-induced cardiac failure in the spontaneously hypertensive rat. *Proc Soc Exp Biol Med* 168:137-142, 1981
4. CALLAGHAN RJ, FROELICH JW, MCKUSICK KA, et al: A modified method for in vivo labelling of red blood cells with Tc-99m: Concise communication. *J Nucl Med* 23:315-318, 1982
5. HUTTON BF, CORMACK J, FULTON RR: Software package for the analysis of gated cardiac bloodpool studies. *Aust Phys Eng Sci* in Medicine 5:128-134, 1982
6. HINE GJ: Instrumentation in Nuclear Medicine, Vol. 1. Academic Press, New York, 1967, p 517
7. BACHARACH SL, GREEN MV, BORER JS, et al: Left-ventricular peak ejection rate, filling rate, and ejection fraction—Frame rate requirements at rest and exercise: Concise communication. *J Nucl Med* 20:189-193, 1979
8. MUIR AL, HANNAN WJ, SAPRU RP, et al: The effects of isoprenaline atropine and dobutamine on ventricular volume curves obtained by radionuclide ventriculography. *Clin Sci* 58:357-364, 1980
9. GILMAN AG, GOODMAN LS, GILMAN A: The Pharmacological Basis of Therapeutics, Sixth edition. McMillan Publishing Co, New York, 1980, p 152
10. BIVINS WS, CRAWFORD MP, BREWER NR: Morphophysiology. In *The Laboratory Rat—Biology and Diseases.* Baker HJ, Lindsey JR, Weisbroth SH, Eds. New York, Academic Press, 1979, Chap. 4