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A NEW METHOD FOR THE VISUALIZATION OF SUBEPICARDIAL CORONARY ARTERIES IN SMALL ISOLATED MAMMALIAN HEARTS

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Abstract: A model is described which permits direct visualization of large coronary arteries in a supported modified perfused heart preparation, using a perfluorochemical (FC-43) as perfusate. Filling of a large coronary artery with Patent Blue Dye is recorded by gated photography (color arteriography). The technique is applicable to the study of reactivity (spasm) of coronary arteries in hearts of small and large animals (rats, rabbits, dogs). The technique has the following advantages: preservation of vascular endothelium, adequate oxygenation, avoidance of major surgical intervention to implant sensors for the detection of changes in coronary diameter, quantitative evaluation of time dependent changes in geometry of large coronary arteries and simultaneous measurement of large coronary vessel and total coronary vascular resistance.

Introduction: Visualization of the coronary arteries in man has hitherto been primarily accomplished by means of selective coronary arteriography (1). In larger animals, such as dogs, changes in diameter of larger coronary arteries have been ascertained by the use of piezoelectric crystals and ultrasonics (2,3). This method has the advantage of uninterrupted measurements but is technically difficult in smaller hearts because of the dimensions of the coronary arteries. To overcome these difficulties, a method has been devised which makes possible visualization of the larger coronary arteries in the isolated heart of rabbits and guinea pigs. The method consists of injection of Patent Blue Dye into the left atrium and visualization of the larger coronary arteries by means of gated pictures synchronized with cardiac systole. We have termed this procedure, "color arteriography." The main advantage of the procedure is the quantitation of the extent of changes in diameter of epicardial coronary arteries. Therefore, repetitive observations are feasible. The system is useful as a model for the study of coronary spasm in the perfused isolated rabbit heart.

Materials and Methods: A supported modified heart preparation was used as previously described (4). A perfluorochemical (FC-43) was employed as perfusate, providing an oxygen concentration of 6 vol% in the left atrial inflow. Unpublished results from this laboratory show that this perfusate resulted in physiological levels of coronary flow and myocardial oxygen consumption and, compared to electrolyte solutions, in considerable reduction of myocardial edema as well as in improved left ventricular power.

Patent Blue Dye for injection was prepared as 0.1% solution in Krebs-Henseleit solution. 0.5 ml of diluted dye were injected into the left atrium. This can be carried out every 2 minutes. During injection drainage from the aorta and right ventricle (coronary sinus) was discarded for a period of 30 seconds. Immediately following injection of the dye a Nikon F3 camera and two Vivitar 283 flashlights were activated, triggered by the peak of left ventricular pressure. Usually from 7 to 10 frames were taken over a period of 3 seconds, using a motordrive Nikon MD4. The objective used was a (1:4) Micro-Nikkor 105 mm. The

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distance between the heart and the film was 46 cm. Ektachrome films (100 ASA) were used (Eastman Kodak Co., Rochester, NY). The obtuse marginal coronary artery was best suited for color arteriography. After development of the film, the pictures were transferred to slides which were projected onto a screen at a magnification of x10. The internal coronary diameter (d_i) could then be measured at several points. From the inside diameter d_i, the index of large coronary vascular resistance could be calculated using Poiseuille's formula on a per-unit length basis (5):

$$\text{Large vessel resistance} = 8\,\mu/\pi r_i^4$$

Since 8, π and μ (the viscosity) are constant, the formula can be reduced to:

$$\text{Large vessel resistance (R)} = \left(\frac{1}{r_i}\right)^4$$

Using $r_i = d_i/2$, R can be calculated:

$$R = \left(\frac{2}{d_i}\right)^4 \ (\text{mm}^{-4})$$

Total coronary vascular resistance was calculated as follows:

$$R_{(\text{total})} \ (\text{mm Hg} \times \text{min} \times \text{ml}^{-1}) = \frac{\text{mean aortic pressure (mm Hg)}}{\text{coronary flow (ml/min)}}$$

Pressure and flows were recorded as previously described (4).

For intraatrial histamine injection 10 µmoles (= 1.84 mg) of histamine were dissolved in 1 ml Krebs-Henseleit solution and injected over a period of 30 seconds into the rubber tubing leading to the left atrium. This resulted in a final histamine concentration of 4×10^{-5} M in the perfusate. For local application of histamine onto the cardiac surface, 40 µmoles were dissolved in 2 ml Krebs-Henseleit solution and sprayed through a 27-gauge needle at an approximate pressure of 2 lbs/in^2 over a period of 30 seconds onto a visible large coronary artery (obtuse marginal artery). The outflow from the heart chamber was discharged during histamine application.

The values were analyzed for significant differences ($p < 0.05$) with Student's t-test for paired data.

Results: Fig. 1a and b shows that after intraatrial injection of 10 µmoles of histamine, dissolved in 1 ml Krebs-Henseleit solution, complete occlusion of the obtuse marginal coronary artery has occurred. Total coronary vascular resistance rose from 2.10 to 2.51 mm Hg/ml/min. In another experiment in which histamine (40 µmoles in 2 ml Krebs-Henseleit solution) was sprayed on the epicardium in close proximity to the obtuse marginal coronary artery, the diameter of that vessel narrowed considerably (from 0.71 mm to 0.10 mm) (Fig. 2a and b). Large vessel resistance increased from 63 mm^{-4} to 150,000/mm^{-4}. In contrast to intraatrial injection of histamine, the increase in total vascular resistance was less (from 2.08 to 2.56 mm Hg/ml/min). Table 1 illustrates the changes in total coronary resistance and large vessel resistance following epicardial and intraatrial administration of histamine. As in the individual experiments described above, changes in large vessel resistance could be calculated independently of total coronary vascular resistance. Control observations in individual experiments prior to histamine showed a high degree of reproducibility of d_i and vascular resistance.

Discussion: A method is presented which permits direct visualization of the large coronary arteries by means of color arteriography. Visualization is achieved by injection of Patent Blue Dye into the left atrium. Filling of large coronary arteries with the dye is then recorded by gated photography during the height of systole. By magnifying the pictures obtained on the screen (x10) resolutions of 0.1 mm can be obtained. Reproducibility of the method is demonstrated in serial color arteriograms taken during steady state conditions.

The method is particularly useful in hearts of smaller animals such as rabbits, guinea pigs and rats, in which the small diameter of the epicardial coronary arteries makes the implantation of piezoelectric crystals difficult (2,3). It is also applicable to the study of reactivity of coronary arteries in larger animals. The vascular endothelium is well preserved because the coronary arteries are perfused as they are in vivo. The usual disadvantage of employing crystalloid solution with low oxygen carrying capacity is overcome by perfusing the hearts with the perfluorochemical (FC-43) which guarantees adequate oxygenation of the heart (6 vol% O_2 in the perfusate) (4,6).

The value of color arteriography in the detection of changes in large vessel resistance is demonstrated in Figs. 1 and

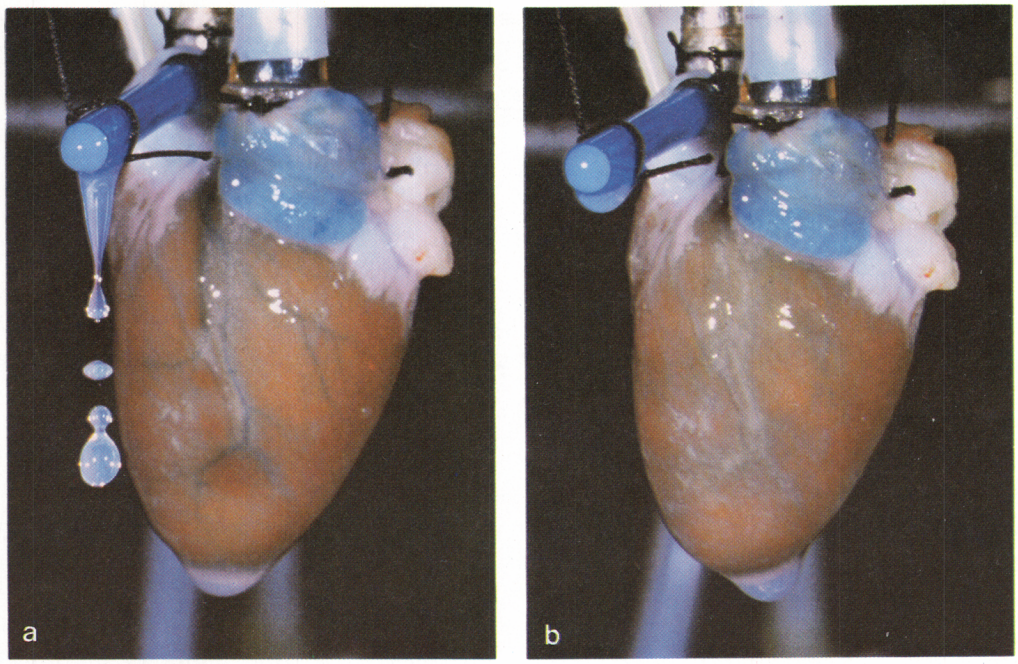

Figure 1a: Color arteriogram (left obtuse marginal artery; control)

Figure 1b: Following the intraatrial injection of histamine

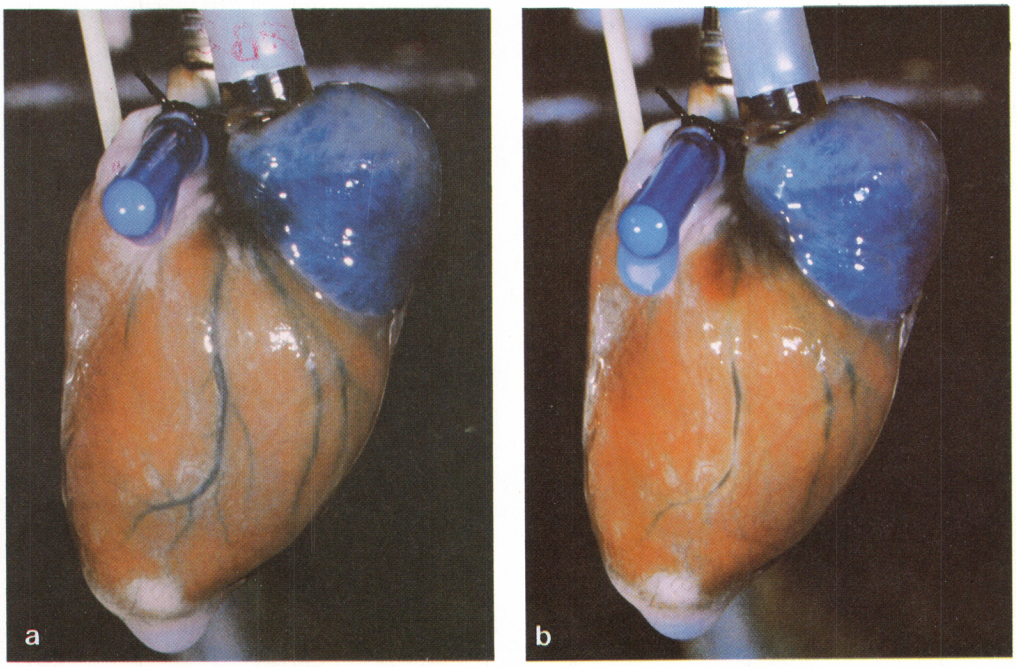

Figure 2a: Color arteriogram (left obtuse marginal artery; control)

Figure 2b: Following topical spray of histamine

TABLE I

	Large vessel resistance d_i [mm]	R [mm^{-4}]	Total vascular resistance R_{total} [mm Hg·min·ml^{-1}]
Control (n=20)	0.53 ± 0.04	453 ± 167	2.10 ± 0.20
Histamine epicardial (n=13)	0.23 ± 0.04*	5700*	2.51 ± 0.26*
Histamine intraatrial (n=7)	0.07 ± 0.03*	666000*	4.98 ± 0.62*

* $p < 0.05$ compared with control; Large vessel resistance was calculated using the d_i values.

2. The color arteriograms illustrate that coronary vasoconstriction after topical administration is strictly localized (Figs. 2a and b), in contrast to intraatrial injection where constriction extends to all large coronary arteries (Figs. 1a and b). The photographs demonstrate that pictures of sufficient clarity can be obtained to make possible quantitative assessment in changes of diameter of large coronary arteries. Furthermore, the time dependent effect of different experimental procedures on the geometry of large coronary arteries can be studied by repeated injections of the dye.

The method is of considerable value in determining whether changes in total vascular resistance are accompanied by those in large vessel resistance. Because of the clinical implications of coronary artery vasospasm (7), the function of large coronary arteries has become of considerable interest. The main purpose of the topical administration of histamine is to test if localized constriction of large coronary arteries, as observed in vivo, can be reproduced in our model. The concentration of vasoactive material released in vivo is not known and there is no way to quantitate accurately the amount of histamine reaching the large coronary arteries after surface application. Our method is useful in the study of vasoactive substances on large coronary arteries independent of humoral substances or innervation. Therefore it constitutes a valuable supplementation to studies carried out on the intact conscious animal (3,5).

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