to an amount unable to induce aggregation did not prevent the inhibition of the rise of Ca^{++} or aggregation, induced by the second dose of ADP (Fig. 3a, curves 1, 2, 4, 5). With an increase in the second dose of ADP, both the rise of Ca^{++} and aggregation of the platelets increased (Fig. 3a, curves 3, 6). Platelets suspended in medium not containing Ca^{++} were unable to aggregate under the influence of ADP; however, an increase in the Ca^{++} concentration in the cytoplasm of the cells took place and depended on the concentration of inducer. In this case also preliminary incubation of platelets with low doses of ADP caused a reduction of the increase in Ca^{++} concentration in the cytoplasm, induced by the second dose of ADP (Fig. 3b).

We showed that incubation of platelets with low doses of ADP did not cause any marked increase in the total cAMP concentration in the platelets by the time of addition of the second dose of ADP.

Inhibition of the rise of Ca·f under the influence of ADP found in the refractory state may actually be the cause of the decrease in platelet aggregation in the refractory state. Similar inhibition in the presence and absence of extracellular Ca^{++} evidently indicates a common mechanism of inhibition of the rise of Ca·f in these cases.

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CARDIAC OUTPUT OF CONSCIOUS RATS MEASURED BY AN ULTRASONIC DOPPLER TECHNIQUE

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UDC 612.13.088.53

KEY WORDS: cardiac output; ultrasonic Doppler technique; labeled microspheres.

To measure cardiac output in small laboratory animals the methods most widely used are the isotope-labeled microspheres method [3] and the thermodilution method [5], but a defect common to both of them is the discreteness of measurement of cardiac output and the fact that they can be correctly used only under steady-state conditions of function of the cardiovascular system.

In the present investigation the cardiac output of conscious rats was determined by an ultrasonic Doppler technique, the working principle of which is measurement of changes in the frequency of ultrasound reflected from the moving blood cells. The main advantage of the Doppler method is absence of zero line drift, a matter of particular importance when measurements are to be made under chronic experimental conditions.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 674-676, December, 1988. Original article submitted January 4, 1988.

0007-4888/88/0012-1702$12.50

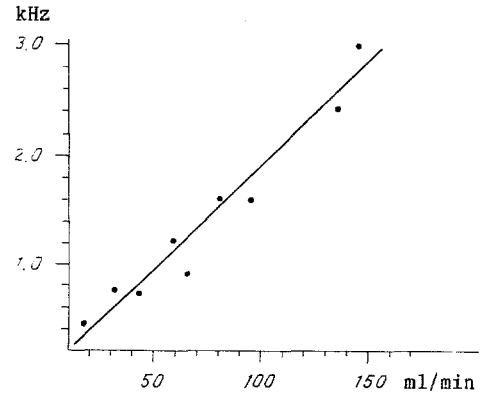

Fig. 1. Comparison of results of simultaneous measurement of cardiac output in rats by the isotope-labeled microspheres method (abscissa; in ml/min) and with an ultrasonic transducer (ordinate; in kHz).

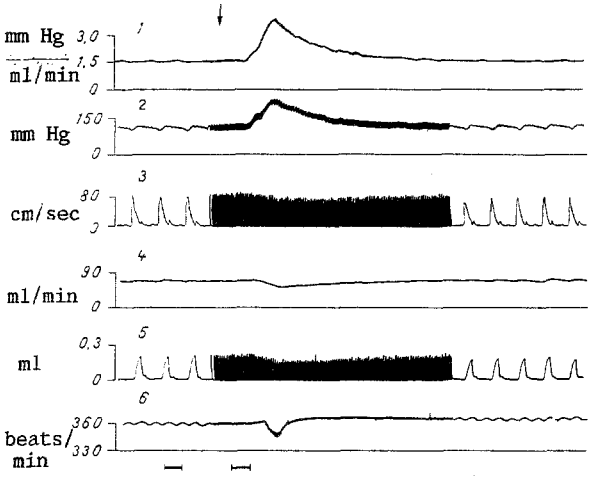

Fig. 2. Response of cardiovascular system of conscious rat to intravenous injection of adrenalin (in a dose of 0.004 mg/kg). Time marker: 0.1 sec (left) and 10 sec (right). Arrow indicates time of injection. 1) Total peripheral resistance (in mm Hg/ml/min); 2) blood pressure in abdominal aorta (in mm Hg); 3) linear velocity of blood flow in ascending aorta (in cm/sec); 4) cardiac output (in ml/min); 5) stroke volume (in ml); 6) heart rate (in beats/min).

EXPERIMENTAL METHOD

To measure the cardiac output of rats bandage transducers 2.5-3 mm in diameter, with a narrow longitudinal slit, with superior characteristics to the types used previously [4], were developed. The length of the transducer is 2.5-2.7 mm, so that it can be easily applied to the ascending part of the arch of the aorta. A piezoceramic with resonance frequency of 8 MHz was used as the ultrasound generator and receiver. The signal to noise ratio is 40 dB or more, providing the basis for reliable recording of the shape of the blood flow curve in the ascending aorta. The electronic circuit enables connection to be made with the implanted transducer by an unscreened lead (an insulated highly elastic multistrand steel wire with an external diameter of 0.3 mm). The use of a bandage type transducer leads to stabilization of the area of cross section of the aorta, so that it is possible, in principle, to determine the volume velocity of the blood flow from data for the mean velocity of the blood flow. It is difficult to calculate absolute values of mean velocity for each concrete transducer, for it depends on the velocity profile, on the width of the ultrasonic beam, and on various other factors [1, 2]. Assuming that the area of cross section of the aorta is assigned by the rigid transducer and that none of the characteristics of individual transducers change in the course of 10-14 days after implantation, experiments were carried out to calibrate the ultrasonic transducers, by means of the isotope-labeled microspheres method.

TABLE 1. Parameters of Blood Flow in Ascending Aorta of Rats

Body weight of animal, g	Parameters of blood flow	Day of implantation of transducer							
		1-st	2-nd	3-d	4-th	7-th	9-th	11th	14-th
250	CO, ml/min	72	76	81	80	84	82	79	83
	SV, ml	0,2	0,24	0,27	0,3	0,3	0,28	0,28	0,29
	LVBF, cm/sec	80	90	90	95	95	100	90	95
300	CO, ml/min	81	92	98	102	109	118	108	110
	SV, ml	0,26	0,29	0,32	0,34	0,38	0,42	0,42	0,4
	LVBF, cm/sec	90	100	100	105	110	110	015	110
270	CO, ml/min	68	73	82	85	92	90	95	97
	SV, ml	0,18	0,2	0,22	0,26	0,3	0,29	0,32	0,33
	LVBF, cm/sec	85	90	95	95	100	95	100	100
350	CO, ml/min	88	96	100	104	115	123	128	118
	SV, ml	0,27	0,3	0,3	0,32	0,38	0,41	0,43	0,42
	LVBF, cm/sec	70	85	90	90	100	95	105	100

Male Wistar rats were anesthetized with pentobarbital (40 mg/kg body weight), intubated, and artificially ventilated. The chest was opened in the fourth intercostal space and a polyethylene catheter with a length of silicone rubber at its end was inserted into the auricle of the left atrium for subsequent injection of a suspension of microspheres. The ultrasonic transducer was placed on the ascending part of the arch of the aorta. A polyethylene catheter was passed through the femoral artery into the abdominal aorta to measure blood pressure and for taking blood samples. When the blood pressure and cardiac output had stabilized 20-30 min after the operation (as shown by readings of the ultrasonic blood flowmeter) the first injection of microspheres was given, in accordance with the method described previously [3]. Later the rat was given an intravenous injection of physiological saline or blood was withdrawn in order to increase or reduce the cardiac output artificially. Measurements were made with the microspheres and ultrasonic apparatus at raised or lowered levels of cardiac output.

EXPERIMENTAL RESULTS

Data obtained in experiments with simultaneous determination of cardiac output by means of labeled microspheres and relative to the Doppler frequency shift indicate sufficiently good linearity of the ultrasonic blood flowmeter (Fig. 1). The calibration coefficient for one of the transducers used was 0.02, and for the other it was 0.0166 kHz/ml/min. The differences in the calibration coefficients depended on differences in the internal diameters of the bandage-type transducers. There was virtually no difference in the values of the calibration coefficients based on the results of several experiments provided that the transducer was firmly applied to the arch of the aorta, thereby preventing any change in the lumen of the vessel inside the transducer. An example of an original trace of cardiac output and of several of its derivatives, obtained in conscious rats by the use of the technique described, is given in Fig. 2. Experiments on conscious animals showed that the microsphere and ultrasonic methods of measurement of cardiac output can be combined on the same animal, and they also confirm that identical values of the calibration coefficient are obtained in animals with an open and closed chest.

It follows from the data in Table 1 that the linear velocity of the blood flow (LVBF) the stroke volume (SV), and the cardiac output (CO) all increase appreciably during the first 2-3 days after implantation of the transducers, and later become stabilized.

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