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Normal Hemodynamic Parameters in Conscious Wistar Rats*

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The evolution of different hemodynamic parameters with ponderal growth has been studied in conscious Wistar rats. The thermodilution method has been used to determine cardiac output and related variables. The results suggest that, between animal weight and the different hemodynamic parameters, there is a direct proportional relationship to blood volume, mean arterial pressure, cardiac output, stroke volume and total peripheral resistance, and an indirect proportional relationship to heart rate, cardiac index and stroke volume index. Body weight, therefore, plays a major role in hemodynamic determination, this having to be kept in mind when designing the experiment.

Key words: Cardiac output, Thermodilution, Wistar rats.

Hemodynamic studies in small animals are common nowadays in many research laboratories (4, 8, 11, 13). Wistar rats have been frequently used as an adequate animal for this purpose; however, its fast growth and the influence of some experimental handlings on weight, especially in chronic studies can produce important differences between experimental and control animals (1, 13, 15). Some authors have tried to avoid this problem by giving their results as index per unit of weight (1, 2, 13, 15). Nevertheless, the normal values of these hemodynamic indices could change with the ponderal growth in such a way that the contrast of these variables between animals of different weight can induce error.

The aim of the present work has been to study the changes in different hemodynamic parameters with the evolution of animal weight in the conscious, unrestrained Wistar rat. The thermodilution method has been used to obtain cardiac output and related variables.

Materials and Methods

Male Wistar rats (177-463 g weight) (n = 79) were used in this study.

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Blood volume determination. For blood volume determination 45 rats (177-456 g weight) were used. On the day of study, each rat was anesthetized with ethylic ether and a catheter (PE-50 polyethylene tubing) was placed in the left ventricle via the right common carotid artery. The catheter was brought out through the skin at the dorsal side of the neck. The wound was closed with suture and the rat was allowed to recover from anesthesia for 3-5 h.

Plasma volume was measured by dilution of radioiodinated (^{125}I) human serum albumin (RISA) (CIS-Sorin). RISA (0.75 μCi) in a volume of 0.15 ml was injected through the left ventricular catheter and back-flushed twice with 0.2 ml of blood to insure complete infusion. For determination of plasma radioactivity concentration arterial catheter blood samples (0.2 ml) were obtained at 10, 20, 30 and 40 minutes after injection. Plasma samples were obtained by centrifugation. A 0.05 ml aliquot from each plasma sample was placed in 1.0 ml of water and counted on a gamma radiation detector (Kontron MR480). Plasma samples and RISA stock solution (also used for injection) were counted for 10 min; all total counts were greater than 10,000. Monoexponential regression analysis of gamma radioactivity, contained in sequentially collected samples, yielded the theoretical instantaneously distributed concentration of RISA (RISA-To) in counts/min/ml of sample (cpm/ml). The bound fraction of ^{125}I was determined for each batch of RISA by precipitation of the protein with 10 % trichloroacetic acid (3), ranging from 0.96 to 0.99. Plasma volume (Pv) was calculated by the formula:

$$Pv\ (ml) = ml\ injected \times cpm \times ml^{-1}\ RISA\ stock\ solution \times bound\ fraction / cpm \times ml^{-1}\ plasma\ sample\ extrapolated\ at\ zero\ time$$

The blood volume was calculated using Pv and hematocrit.

Other hemodynamic parameter determinations. Male Wistar rats (n = 34) (180-463 g weight) were used for mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), cardiac output per 100 g (CI), stroke volume (SV), stroke volume per 100 g (SVI) and total peripheral resistance (TPR) determination. On the day of the experiment, the rats were weighed and anesthetized with ethylic ether and then cannulated with indwelling arterial (left femoral) and venous (cava superior via right jugular) catheters (PE-50 polyethylene tubing). The right carotid artery was isolated and a thermistor (Columbus Instruments) inserted into the aortic root. The three catheters were brought out through the skin at the dorsal side of the neck. The wound was closed with suture and the rats were allowed to recover from anesthesia (for 3-5 h) in a small, but unconfining, box. After this recovery period, the jugular cannula was connected to a system allowing consecutive injections and the femoral catheter was connected to a pressure transducer (Bell and Howell, 4-327-I). The thermistor and the pressure signals were processed in a microcomputer system for cardiac output determination (Cardiomax IIR, Columbus Instruments). After 30 min of stabilization, 0.2 ml of isotonic saline (20-21°C) was injected as a bolus in the jugular catheter and the thermodilution curve recorded (680M Hewlett Packard); SV and CO were digitally obtained by the microcomputer, as well as MAP and HR. SV and Co indices (SVI and CI respectively) were calculated by dividing SV and CO by animal weight/100 g TPR was obtained using the formula TRP=MAP/CI. This manoeuvre was repeated at least three times for each animal.

The formula used for CO calculation was:

$$CO = \frac{(BT - IT) \times \text{Injection volume (ml)}}{\int_{\cdot}^{\infty} T\ dt}$$

Where: BT = Blood animal temperature before injection (°C).

IT = Injection solution temperature (°C).

$\int_{0}^{\infty} T\ dt$ = Integral on time of change in the blood temperature due to injection (°C × min).

The difference between both animal and saline temperatures was always greater than 15°C. When the experiment had finished location of catheters was checked by autopsy.

Statistical treatment of data (three for each animal), consisted of regression analysis to study and measure the dependence or association between variables.

Results and Discussion

In figures 1 and 2 the representation of different relationships between the hemodynamic parameters and animal body weight, with their corresponding equations, confidence intervals (95 %), correlation coefficients and significance levels can be observed.

The major finding of the present work is the proportional relationship between the different hemodynamic variables in respect to body weight, since blood volume (fig. 1a), MAP (fig. 1c), CO (fig. 2a), SV (fig. 2c) and TPR (fig. 1b) are in direct relation to body weight; HR (fig. 1d), SVI (fig. 2d) and CI (fig. 2b) have an indirect relationship.

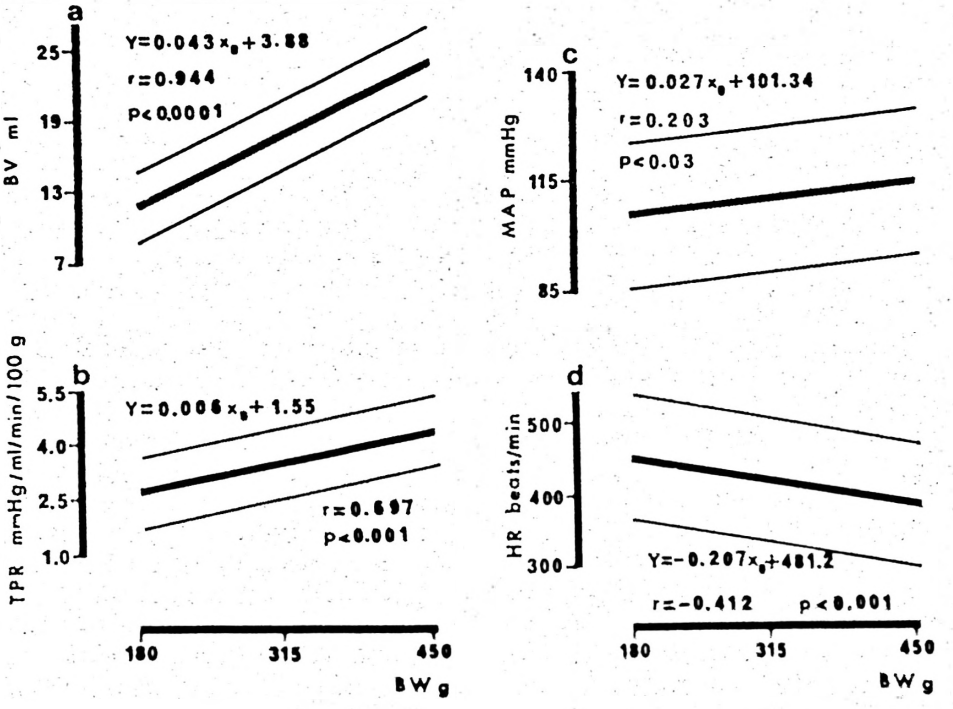

Fig. 1. *Evolution of the different hemodynamic parameters with rat body weight (BW), showing corresponding equations, correlation coefficients, significance level and confidence limits to 95 %.*

1a = Blood volume (BV); 1b = Total peripheral resistance (TPR); 1c = Mean arterial pressure (MAP); 1d = Heart rate (HR).

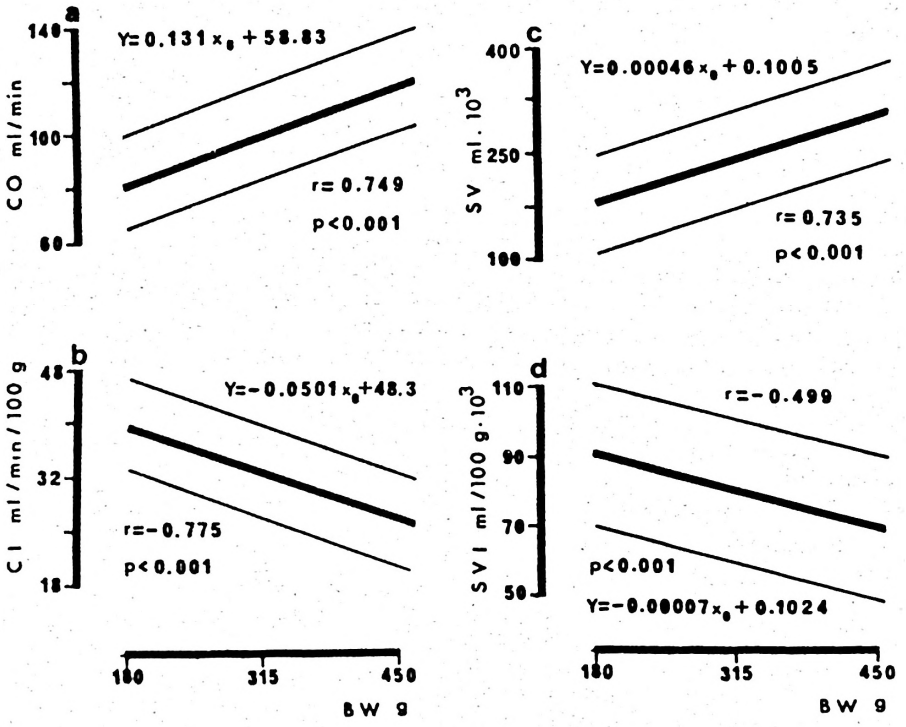

Fig. 2. *Evolution of the different hemodynamic parameters with rat body weight (BW), showing corresponding equations, correlation coefficients, significance level and confidence limits to 95 %.*
2a = Cardiac output (CO); 2b = Cardiac index (CI); 2c = Stroke volume (SV); 2d = Stroke volume index (SVI).

The determination of CO by the thermodilution method, as described by FEGLER (6), based on the Stewart-Hamilton principle, has many advantages over other methods: by dilution (1, 5), O_2 arterio-venous difference (20), radiactive microspheres (11, 12), electromagnetic (4, 10) and ultrasonic (17) flowmeter, thus being simple, accurate and non contaminating, as well as allowing good reproducibility to be obtained (8, 9). On the other hand, the thermodilution method enables hemodynamic parameters to be measured in the conscious animal, thereby avoiding the use of anesthetics which can alter cardiovascular homeostasis, as has been reported with ether, pentobarbital sodium and chloraloseurethan mixture (19).

Recently, HAYES *et. al.* (8) criticized the used of thermodilution in rats, on the basis of heat loss from their vascular system. However, Hayes' method differed from that used in the present study, since they used urethane as anesthetic, and injected saline at 13.7°C, whereas the present rats were conscious and the difference between injected saline (20-21°C) and animal temperature was less, so that the possibility of cold loss through the vascular wall was inconsistent. On the other hand, HAYES *et al.* (8) placed the three thermistors in the right ventricle, pulmonary artery and mid-thoracic

aorta, although it is well established that the correct place is the aortic root (9). Finally, the present results agree with previous authors, even when other methods have been used (16, 17, 21). It is widely known that alterations in body weight, related to ponderal growth, change the hemodynamic parameters in mammals (14, 18); however, we are not aware of any published work, to date, where such a variation in conscious Wistar rats has been measured.

The direct relationships between SV and CO with the body weight, found in the experiments described here, could be related to the increase of blood volume which occurs throughout ponderal growth. On the other hand, since the body weight is increasing, the decrease showed by the SVI and CI could be due to the smaller metabolic needs per 100 g of tissue in larger animals. This could also explain the direct relationship found between body weight and TPR, since changes due to aging in the vascular distensibility did not take place, the larger rats in this study being adults but not old. The relationship found between body weight and MAP and HR respectively show the smallest correlation coefficient. In the former, the slight increase of MAP when body weight is increasing could be a consequence of the TPR increase and secondary to the CO elevation. In the latter, the higher HR in young animals could be related to their greater metabolic activity.

These findings make it advisable, when comparing hemodynamic data, to introduce a weight-matched group if there are weight differences between the experimental and control animals, thus avoiding the differences observed in the present study.

Resumen

Se estudia en rata Wistar consciente la evolución de diferentes parámetros hemodinámicos con el crecimiento ponderal, utilizando la técnica de termodilución. Los resultados indican que entre el peso de los animales y los parámetros hemodinámicos existe una relación proporcional, siendo ésta directa entre crecimiento ponderal y volumen sanguíneo, presión arterial media, gasto cardíaco, volumen sistólico y resistencias periféricas totales e inversa entre peso y frecuencia cardíaca, gasto cardíaco por 100 gramos y volumen sistólico por 100 gramos. Se concluye que, en las determinaciones hemodinámicas, el peso corporal se comporta como un factor determinante que debe ser tenido en cuenta en el momento del diseño experimental.

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