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THERMOANALYTICAL STUDY OF ORGANS OF SPONTANEOUS HYPERTENSION RATS

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The thermal behavior of the kidney, heart and liver of spontaneous hypertension rats (SHR) were studied by thermogravimetry (TG) and differential thermal analysis (DTA). SHR are, by far, the most widely used rat model in the study of heart diseases in mammals, because they develop chronic hypertension, which leads to heart failure during their lives. TG curves showed two steps for all samples: at 800°C less residues remained for an SHR kidney (4.0±1.8%) than for an SHR heart (6.9±0.4) and liver samples (7.5±1.9%). It probably happened due to the presence of inorganic substances such as iron, calcium, magnesium, potassium and sodium. DTA curves depicted three endothermic events for kidney (70, 120 and 310°C), heart (85, 250 and 300°C) and liver samples (70, 250 and 310°C), indicating protein denaturation, as well as protein degradation and fat degradation, respectively. TG/DTG/DTA profiles of organs samples showed peculiarities that permit correlation between them. These methods might serve as a simple alternative to investigations over these vital organs.

Keywords: *heart, kidney, liver, thermogravimetry*

Introduction

Kidneys are large, reddish, bean-shaped organs. They function in excretion as well as in the regulation of body fluid composition and volume. The kidney is embedded in perirenal fat and invested by a thin, loosely adhering capsule, consisting mainly of a dense irregular collagenous connective tissue, with occasional elastic fibers and smooth muscle cells. The functional unit of the kidney is the uriferous tubule that consists of two parts: nephron and collecting tubule. The nephrons are composites of renal corpuscule (located in the cortex) and tubular parts (located in the medulla). The renal corpuscule is composed of a tuft of capillaries, the glomerulus (connective tissue component), which is invaginated into Bowman's capsule, composed of modified epithelial cells, squamous epithelial cells and pedicels (with podocalyxin). The glomerulus is invested with a basal lamina, consisting of type IV collagen, laminin, fibronectin and a polyanionic proteoglycan rich in heparin sulfate. Fibronectin and laminin help pedicels and endothelial cells to maintain their attachment. The tubules are composed of cuboidal epithelium and squamous epithelium cells [1–3].

The heart is the pump for the cardiovascular system. Its muscular wall (myocardium) is composed of cardiac muscle cells arranged in complex spirals around the chamber of orifices [1, 2]. The cardiac muscle is classified as a striated muscle, which is composed of two types of myofilaments (thin and thick filaments) responsible for muscle cell contraction [4]. Thin filaments are composed of some proteins (actin, tropomyosin and troponin) associated with α-actinin; and the thick ones are composed of the protein myosin, among others. Almost half of the volume of the cardiac muscle cell is occupied by mitochondria, attesting to its great energy consumption [1]. The sarcoplasm is relatively abundant and rich in glycogen. In the regions of sarcoplasm, there are often a few droplets of lipid and, in older animals, deposits of lipofuscin pigment [2]. Both the glycogen and the lipid may be used as sources for the contractile activity of the myocardium [1, 2]. Because the oxygen requirement of cardiac muscle cells is high, they contain an abundant supply of myoglobin. Calcium ions play an important role in the forces that bind cells together, but the majority of Ca^{2+} is transported into the cardiac muscle cell from extracellular fluid compartments [1].

Finally, the liver is the largest gland in the body. It functions both as an exocrine gland (secreting bile) and as an endocrine gland (synthesizing a variety of substances that are released directly into the blood stream). The liver is composed of epithelial cells arranged in plates or laminae that are connected to form a continuous tridimensional lattice. The bulk of the liver is composed of uniform parenchymal cells: the hepatocytes. Much of nutritive materials delivered to the liver are converted by the hepatocytes into storage products, such as glycogen. The cell membrane that

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forms the walls of bile canaliculi (intercellular spaces in lateral domains of hepatocyte membrane) display high levels of Na^+–K^+ATPase and adenylate cyclase. Hepatocytes contain varying amounts of inclusions in the form of lipid droplets (very-low-density lipoprotein) and glycogen, among others [1–4].

Spontaneously hypertensive rats (SHR) are by far the most widely used rat model in the study of heart diseases in mammals. SHR develop chronic hypertension leading to heart failure during their last six months of life span, which is of about 2 years. As hypertrophy in humans is usually associated with chronic hypertension, SHR are real models of human heart behavior [5].

Little is known about the thermal behavior of organs, tissues and/or cells, and regarding the potential of thermal analyses tools in many other fields, this work aims to study the thermal behavior of the organs (kidney, heart and liver) of SHR.

Experimental

Animals

SHR used came from colonies of Paulista School of Medicine (CEDEME), São Paulo, Brazil. During the period when the animals were from 3 to 7 months old, they were maintained in the State University of Rio de Janeiro. This investigation conforms to the 'Guide for the Care Use of Laboratory Animals' published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985).

Five male SHR rats were kept in a polypropylene cage, in a temperature-controlled (21±1°C) and humidity-controlled (60±10%) room. They were submitted to a 12 h light and dark cycle (artificial lights, 7 a.m.–7 p.m.) and to an air exhaustion cycle (15 min h^{-1}). They received a balanced diet (Nuvilab) and water ad libidum. At the end of the experiment, animals were deeply anaesthetized (intraperitoneal sodium pentobarbital) and their vascular system was perfused with a constant pressure (90 mmHg) through the left ventricle. A large amount of a tap physiologic solution was used, followed by a fixative solution (freshly prepared – 4% *w*/*v* formaldehyde in a 0.1 M phosphate buffer pH 7.2) until the rigidity of the animal body was obtained. Parts of the rats' kidney, heart and liver were removed and analyzed.

Coupled thermogravimetry-differential thermal analysis (TG-DTA experiments)

The analyses – TG and DTA – have been performed on a TA 2960 simultaneous TG-DTA model (TA Instruments, USA). SHR organ samples (10–11 mg) were heated from 30 to 800°C, at a 10°C min^{-1} heating and nitrogen atmosphere. Two parallel thermal runs for each rat organ sample (total of ten samples per organ) and an average were obtained for each organ.

Results and discussion

We carried out a total of 30 samples of TG/DTG and DTA analyses: 10 analyses for kidney, 10 analyses for heart and 10 analyses for liver. One of them was picked out to be shown here. Figure 1 shows TG/DTG and DTA curves for a single SHR kidney sample. TG curve shows two decomposition stages: the first stage occurs 60.0±1.0% of mass loss from 50 to 140°C, suggesting water and formalin evaporation, some residual blood loss [6] and water decomposition, while the second occurred from 295 to 395°C with 11.2±1.7%, a stage which is likely related to the decomposition of substances like proteins, lipids and others. The residue at 800°C was 4.0±1.8%, probably due to the presence of inorganic substances such as potassium and sodium [6, 7].

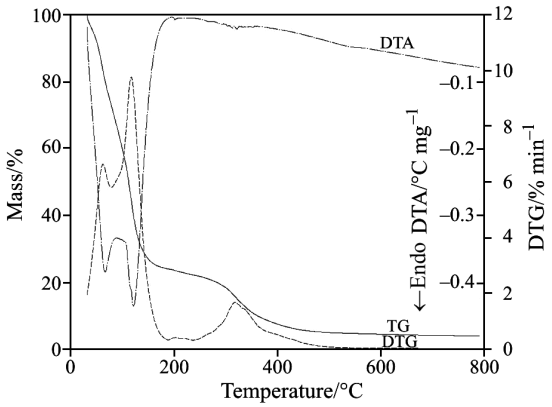

Fig. 1 TG/DTG and DTA curves for SHR kidney samples

DTG curves (Fig. 1) showed two mass degradation stages around 75, 160, 200°C and multiple stages from 280 to 370°C. DTA curve depicts three endothermic events at 70, 120 and 310°C.

TG, DTG and DTA curves of an SHR heart sample are presented in Fig. 2. TG curve of SHR heart sample shows two mass loss stages. The first and main degradation stage starts at 45°C and is completed at around 105°C causing 54.8±1.6% of mass loss. The second one occurred from 280 to 370°C with 11.2±1.4%. The residue at 800°C was 6.9±0.4%, likely associated with the presence of metals such as iron, calcium, magnesium, potassium and sodium [7]. DTG curves also showed two mass degradation stages around 80 and 250°C and multiple stages from 280 to 380°C [7]. The DTA curve indicates endothermic processes all along in the investigated temperature range (T_{peak1}: 85°C; T_{peak2}: 250°C;

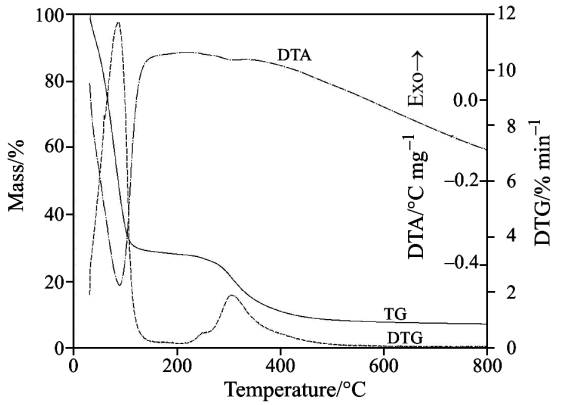

Fig. 2 TG/DTG and DTA curves for SHR heart samples

T_{peak3}: 300°C). These peaks are likely to indicate protein denaturation [6, 7], protein degradation and fat degradation [8], respectively.

TG curves of SHR liver samples also showed only two mass loss stages (Fig. 3). The first stage occurred from 50 to 110°C with 54.0±8.2% of mass loss. In the second stage multiple stages occurred, from 270 to 390°C. At 800°C the samples of SHR liver presented the biggest amount of residues (7.5±1.9%). DTG peaks showed two mass loss stages around 90 and 250°C for samples of SHR liver. The DTA curve indicates endothermic events all along in the investigated temperature range (T_{peak1}: 70°C; T_{peak2}: 250°C; T_{peak3}: 310°C).

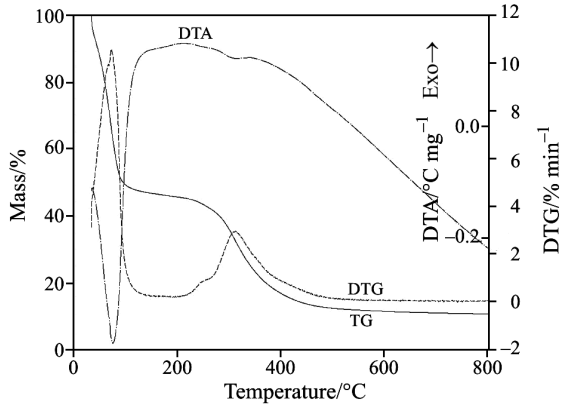

Fig. 3 TG/DTG and DTA curves for SHR liver samples

Conclusions

Thermal analysis seems to be an interesting tool in the thermal study of organ samples. TG curves showed two steps for all samples. Less residues remained in the TG analysis of SHR kidney samples (4.0±1.8%), heart samples (6.9±0.4) and liver samples (7.5±1.9%). DTA curves depict three endothermic events for kidney (70, 170 and 300°C) and heart samples (85, 250, 300°C); and only two events for liver samples (70, 310°C).

TG/DTG and DTA profiles of organs samples showed some peculiarities that permit correlation between them. However, even more extended research must be done to obtain sufficient evidence on this matter. These methods might serve as a simple alternative to investigations over these vital organs.

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