SEX DIFFERENCES IN RAT HEART. DIFFERENT PATTERNS OF CATALYTICALLY ACTIVE CREATINE KINASE ISOENZYMES

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RESUMEN

DIMORFISMO SEXUAL EN EL CORAZÓN DE RATA: PATRONES DIFERENCIALES DE LAS ISOENZIMAS DE LA CREATINA CINASA CATALÍTICAMENTE ACTIVAS

Objetivos. *Proponemos que en la rata, la síntesis anaeróbica de ATP mediada por el sistema creatina cinasa/fosfocreatina (CK/PCr) es sexualmente dimórfica durante la maduración y el envejecimiento del corazón.* **Antecedentes.** *En función de género, las diferencias morfológicas y funcionales durante el envejecimiento cardiovascular parecen explicar la mayor longevidad de las hembras de los mamíferos y de la mujer.* **Material y Métodos.** *Se estudiaron 46 ratas Wistar de ambos sexos, por parejas de peso semejante de 200, 250 y 300 g de peso corporal.* **Resultados.** *No se observaron diferencias sexuales en cuanto al peso del corazón y a su contenido de proteínas del sobrenadante post 27 000 xg, en ninguno de los pesos corporales estudiados en la rata. Los cocientes peso cardíaco/peso corporal no mostraron diferencias significativas de género durante todo el estudio. Se encontraron diferencias de la actividad específica de la CK cardíaca solamente a los 257 ± 6 g de peso corporal, debido al decremento de tal actividad en el macho. El corazón de la hembra mostró una mayor variedad de isoenzimas de la CK citosólica en todos los pesos corporales estudiados. En los corazones de rata de ambos sexos se encontraron consistentemente isoenzimas del tipo cerebral BB-CK citosólicas fuertemente teñidas catalíticamente, durante todo el estudio. Este hallazgo no está de acuerdo con la aceptada especificidad tisular de la CK cardíaca.* **Conclusiones.** *En este trabajo se encontraron diferencias significativas de género, principalmente en cuanto a los patrones y al número de isoformas catalíticas de la CK citosólica del corazón de rata. En relación con los mecanismos anaeróbicos de la producción de ATP, estas diferencias podrían explicar, en parte, la susceptibilidad diferencial sexual al compromiso hemodinámico, en respuesta al estrés cardiovascular, en favor de las hembras.*

SUMMARY

Objectives. *We hypothesized that the anaerobic ATP synthesis mediated by the creatine kinase/phosphocreatine (CK/PCr) system is sexually dimorphic during maturation and aging of the rat heart.* **Background.** *Gender-related morphological and functional differences in cardiovascular aging seem to explain the greater longevity of mammalian females, including women.* **Material and Methods.** *By means of heart CK specific activity and cytosolic CK isoenzyme analyses we studied 46 male and female Wistar rats of similar weight divided in groups of 200, 250, and 300 g of body weight.* **Results.** *No sex differences were observed in heart weight and post 27 000 ´ g heart protein content at any studied weight. Heart/body weight ratios did not show any significant gender difference along the study. Differences of heart CK specific activity were found only at 257 ± 6 g of rat body weight due to a decrease of the male enzyme activity. The female heart showed a larger variety of cytosolic CK isoenzymes at any studied weight. Heavily catalytically stained BB-CK type cytosolic isoenzymes were consistently found in the heart of rats of either sex at the studied weights, contrarily to the accepted view of CK tissue specificity.* **Conclusions.** *In this work, significant gender differences were mainly found in the patterns and number of catalytical cytosolic CK cardiac isoforms. Regarding the alternate anaerobic mechanism of ATP production, these differences may explain in part the sex differential susceptibility to hemodynamic compromise in response to cardiovascular stress, in favor of females.*

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RESUME

DIMORPHISME SEXUEL DANS LE COEUR DU RAT: PATRONS DIFFERENTIELS DES ISOENZIMES DE LA CREATINE CINASE CATALYTIQUEMENT ACTIVES

Objectifs. *Nous proposons que la synthèse anaérobique de l'ATP, faite au moyen du système créatine cinase/phosphocréatine (CK/PCr), est sexuellement dimorphique pendant la maturation et le vieillissement du coeur du rat.* **Antécédents.** *Les différences morphologiques et fonctionelles pendant le vieillissement cardiovasculaire semblent expliquer la longevité accrue des mammifères femelles et des femmes.* **Méthodes.** *Nous avons étudié 46 Wistar rats, mâles et femelles, de poids semblable de 200, 250 et 300 g, au moyen d'analyses d'activité spécifique de la CK du coeur et des isoenzimes de la CK cytosolique.* **Résultats.** *On n'a trouvé aucune différence sexuelle en ce qui concerne le poids et le contenu protéique du surnageant de 27 000 X g du coeur pour tous les poids des rats compris dans cette étude. Les rapports de poids coeur/corps n'ont montré aucune différence significative du genre pendant l'étude. Une différence dans l'activité spécifique de la CK du coeur a été trouvée seulement pour le poids du rat de 257 ± 6 g, due à la diminution de l'activité enzymatique des mâles. Les coeurs des femelles ont montré, pour tous les poids étudiés, une plus grande varieté d'isoenzymes de la CK cytosolique. Des enzymes du type BB-CK, teintes catalytiquement, ont été trouvées systématiquement dans les coeurs des rats des deux genres pour tous les poids étudiés, en contradiction avec l'idée acceptée couramment de la specificité tissulaire de la CK.* **Conclusions.** *Des différences significatives du genre ont été trouvées dans les patrons et dans le nombre d'isoformes catalytiques cytosoliques de la CK surtout en faveur des femelles.*

Palabras clave: Creatina cinasa. Isoenzimas. Corazón. Dimorfismo sexual.
Key words: Creatine kinase. Isoenzymes. Heart. Sex dimorphism.

INTRODUCTION

Previous studies have investigated age-related differences in cardiovascular performance,[6] but the impact of gender on such age-associated cardiovascular changes remains incompletely covered. Other studies have investigated sex-related differences in cardiovascular morphology[7,8] and function[9-14] in young adults.

The importance of gender-related differences during cardiovascular aging may help to explain partially the greater longevity of women and females of most mammalian species.[15]

ATP is the universal energy currency for the energy-requiring processes in biological systems.[16] Excitable cells and tissues, like skeletal and cardiac muscle, neural cells and electrocytes, depend on the immediate availability of vast amounts of energy that may be used in a pulsed or fluctuating manner. In the heart, that process begins since the very first embryonic myocardial beat until the end of life.

Simply increasing intracellular concentration of ATP for energy storage would represent a bad choice to meet the energy requirements of the above mentioned type of cells and tissues, since local concentrations of ATP, ADP, and adenylate monophosphate (AMP), as well as the ATP/ADP ratio, are key regulators that influence fundamental metabolic processes.[17,18] However, excitable cells contain only 2-5 mM concentration of ATP, which would be enough for a muscle contraction of only a few seconds.[19] Instead, large quantities of metabolically "inert" phosphagens are accumulated in these cells or tissues.[20] Phosphocreatine (PCr) is the sole phosphagen in vertebrates and some invertebrate species. In skeletal muscle, PCr concentrations are about 30 mM or more,[21] whereas in brain, electrical organ, and smooth muscle, PCr is in the range of 5-10 mM.[22] Although the cell pools of ATP are rather small, no significant changes in overall ATP levels are detected during activation of excitable cells,[23] because ATP is efficiently replenished from the large PCr pools.

In most muscles, the ATP regeneration capacity of creatine kinase (CK) by transphosphorylation is very high and considerably exceeds both ATP utilization and ATP replenishment by oxidative phosphorylation and glycolysis.[24] For example, the maximal rate of ATP synthesis by the CK reaction

$$MgADP^{-} + PCr^{2-} + H^{+} \rightleftharpoons MgATP^{2-} + Cr$$

in rat cardiac muscle (30 μmol s^{-1} g^{-1}) is much higher than the maximal rate of ATP synthesis by oxidative phosphorylation (2.5 μmol s^{-1} g^{-1}) or by ***de novo*** pathways (0.39 μmol s^{-1} g^{-1}).[25-27] In addition to regeneration of hydrolyzed ATP, the CK/PCr system, with CK as a low-threshold ADP sensor (Km of skeletal muscle-CK [MM-CK] for ADP is 10-35 μM or 10^{-5}M),[28,29] plays a critical role in preventing an elevation of ADP concentration during transient periods in which energy utilization exceeds energy production. By maintaining the ATP/ADP ratio high in the vicinity of an ATPase, CK increases the thermodynamic efficiency of ATP hydrolisis

$$\Delta G = \Delta G_{obs} - RT \cdot \ln [ATP]/[ADP] \cdot [Pi]$$[30]

The dimeric cytosolic CK isoenzymes are thought to be tissue-specific:

MM-CK for mature skeletal muscle, MB-CK for myocardial cells, and BB-CK for brain tissues.[31,32] CK isoenzymes are in part concentrated and specifically localized at sites of energy consumption as well as those of energy production.[33] In heart cells, generally depending on oxidative metabolism, energy derived from glycolysis can contribute to some extent to the maintenance of high energy phosphate levels and contractility if oxidative phosphorylation of myocardial cells is inhibited.[34] Nevertheless, the glycolytic flux and the functional coupling of CK, seen at low PCr/creatine ratios, is suppressed when respiration is normal and the PCr/creatine ratio is high. As a low threshold sensor of ADP, CK in heart is also exerting some control over the glycolytic flux.[35]

In the present study we hypothesized that the transphosphorylation mechanisms mediated by the CK/PCr system are sexually dimorphic during the physiological maturation and aging of the rat heart. Weight and sex-dependent differential cCK isoenzyme transition patterns were expected to occur accordingly during the studied period of life, contrarily to the idea of the post-natal "mature cytosolic CK tissue-specificity". Previously, but not as a function of sex, physiological complete isoenzyme transition of brain CK was demonstrated in the Wistar rat beginning after three months of age.[36]

MATERIAL AND METHODS

The changes in serum concentration of thyroid hormones (T_4, T_3 and reverse T_3) and in hemoglobin and hematocrit levels are not related to chronological age, but more adequately interpreted in terms of body height, weight, and surface area in human adolescents of either sex. They may represent adaptive changes directed toward energy production and conservation.[37,38] The CK/PCr system is directly involved in both processes.

Animals studied

Based on the above mentioned premise, to compare ponderal and biochemical parameters, male and female Wistar rats (CINVESTAV-IPN), with weights ranging from 200 to 303 g, were studied. To prevent circadian rhythm problems, pairs of opposite sex and similar body weight were systematically killed between 8:30 and 9:00 h. A careful dissection and removal of heart was performed before this organ was transferred through a series of Ringer and 0.145 M NaCl solutions at 4°C, until blood or extraneous loose tissue had disappeared. The heart was rapidly minced with scissors and homogenized at an approximate ratio of 1:6 (w/v) with 0.1M KCl at 4°C in a "45" Virtis apparatus (45 000 rpm). Thirty per cent of this speed was employed for 2 minutes, with a 5 seconds rest interval between minutes.

Biochemical procedures

The homogenate was centrifuged for 30 min at 4°C at 27 000 x g, in either a Beckman J2-21 or a Sorvall DC2B models, to eliminate nuclei, cell debris, and heavy and light mitochondria.[39,40] Aliquots of this supernatant (SN_1) were used as the source for the CK assay and protein determination.[36] The SN_1 was ultracentrifuged for 60 minutes at 4°C at 125 000 x g in an OTD-65B Sorvall model. Samples were taken from this high speed supernatant (SN_2), immediately, to separate the cytosolic CK isoenzymes by zone electrophoresis using cellulose polyacetate strips, since preparations cold stored for more than 24 h can produce spurious cytosolic CK dimeric hybrids.[36] The presence of contaminating mitochondrial CK in post 116 000 x g supernatants has been excluded by protein immunoblot analysis.[41] After electropho-

resis for 120 min, the strips were catalytically stained for CK using overlay reaction gels containing hexokinase and glucose-6-phosphate dehydrogenase as the coupling enzymes in the forward reaction for CK at pH 7.2,[42] and AMP to inhibit adenylate kinase activity.[39] To exclude interference by co-migration of other enzymes or nonspecific staining, appropriate blanks were prepared from each sample. Treatment of these controls was identical to the problems, except that PCr was omitted from the incubation mixture.[36]

Data analysis and statistics

The results (mean ± SD) were analyzed for gender differences. Statistical methods were completed using one way ANOVA. A value $p < 0.05$ was considered to be statistically significant.

RESULTS

The heart wet weight was similar in male and female rats at about 200 and 303 g of body weight. At about 257 g, the male heart weight seemed to be larger but it resulted non significant *(Table I)*. When heart weight was normalized for SN_1 protein content non significant differences were found between males and females at any of the above selected body weights *(Table II)*. When heart masses were normalized dividing by body weights, the ratios did not show significant differences between males and females *(Table III)*.

The weight-dependent changes in heart CK specific activity are ilustrated in *Table IV*. Sex differences of CK activity were found only at about 257 g of rat body weight (about 180 days for the male and 400 days for the female). The female heart CK specific activity was 2.3-fold the amount found in the male heart. Furthermore at the same rat weight, it must be emphasized that a decrease in CK activity occurred in the male heart, as compared to its respective CK specific activity value at about 200 g of body weight.

Table I.
Heart Weight of Wistar Rat.

Body Weight (g)	Male (g)	Female (g)	p Value
200 ± 8 (23)	0.80 ± 0.10 (10)	0.77 ± 0.02 (13)	NS
257 ± 6 (10)	0.90 ± 0.01 (5)	0.82 ± 0.06 (5)	NS
303 ± 8 (13)	0.99 ± 0.08 (5)	0.90 ± 0.07 (8)	NS

Body and heart weight data are mean values ± SD. The number of animals is given in parenthesis.

Table II.
SN_1 Protein per Heart of Wistar Rat.

Body Weight (g)	Male (mg protein)	Female (mg protein)	p Value
200 ± 8 (23)	30.75 ± 6.45 (10)	31.40 ± 4.55 (13)	NS
257 ± 6 (10)	20.30 ± 4.05 (5)	23.70 ± 2.45 (5)	NS
303 ± 8 (13)	37.15 ± 6.40 (5)	30.30 ± 4.80 (8)	NS

Body weight and total SN_1 protein data are mean values ± SD. The number of animals is given in parenthesis. SN_1 = post 27 000 x g supernatant of each homogenized heart.

Table III.
Heart/Body Weight Ratios (10^{-3}) of Wistar rat.

Body Weight (g)	Male	Female	p Value
200 ± 8 (23)	3.85 ± 0.59 (10)	3.98 ± 0.14 (13)	NS
257 ± 6 (10)	3.54 ± 0.12 (5)	3.16 ± 0.27 (5)	NS
303 ± 8 (13)	3.24 ± 0.26 (5)	2.94 ± 0.19 (8)	NS

Body Weight and Heart/Body weight data are mean values ± SD. The number of animals is given in parenthesis.

Table IV.
Rat Cardiac CK Specific Activity.

Body Weight (g)	Male	Female	p Value
200 ± 8 (23)	0.145 ± 0.017 (10)	0.139 ± 0.030 (13)	NS
257 ± 6 (10)	0.083 ± 0.016 (5)	0.192 ± 0.030 (5)	< 0.0001
303 ± 8 (13)	0.113 ± 0.040 (5)	0.137 ± 0.022 (8)	NS

CK specific activity (D μmol PCr × min^{-1} × mg^{-1}) was determined as described in Methods. Data are presented as mean values ± SD. Differences in CK specific activity were found only at 257 ± 6 g of rat body weight. The number of animals is given in parenthesis.

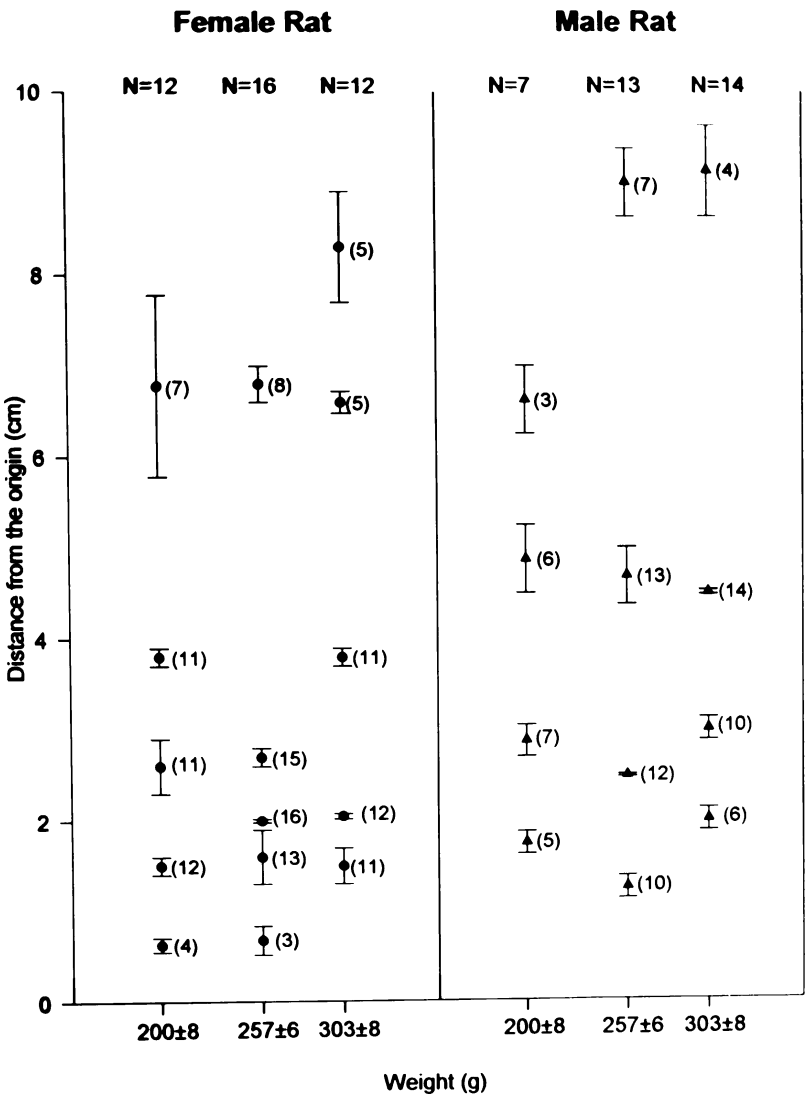

FIG. 1: Female hearts showed a larger variety of cytosolic CK isoenzymes at any studied weight. Samples were taken from the post 125 000 x g supernatant to separate the cytosolic CK isoenzymes according to the protocol described in Methods. Circles = female rats; triangles = male rats. The number of animals is given in parenthesis. Vertical bars=SD.

Regarding the catalytical cytosolic CK electrophoretic patterns, migrating from the origin toward the anode, more than two heavily stained bands (the expected "mature tissue-specific" skeletal and cardiac muscle cytosolic CK types) were consistently found in the hearts of male and female rats at all the above mentioned studied weights. Worthy of mention is the fact that female hearts showed a larger variety of cytosolic CK isoenzymes at all studied rat body weights.

The number of catalytical cytosolic CK isoforms was always higher in female hearts (5 bands), as compared to males (4 bands). Also, worth noticing is that along the study, brain type cytosolic CK bands, migrating a long distance from the origin toward the anode (more than 6 cm on the cellulose polyacetate strips), were found in both sexes confirming previous results obtained in the brain of rats of similar body weights,[36] as well as atypical migrating ones, as shown in *Figure 1*.

Representative cytosolic CK isoenzyme patterns of each sex are shown in *Figures 2 and 3*. In no case did spurious staining of NADPH appear in the blanks.

DISCUSSION

In an earlier study,[1] the polyacrylamide gel electrophoretograms of heart cytosolic proteins from rats of opposite sex, with similar body weights (no more than 15 g of difference, between 200 and 320

g), differed in the number and position of the protein bands, possibly reflecting differential epigenetic cardiac control of protein synthesis at the selected rat weights. In the present work, it is shown that male and female rat hearts of similar body weights (comparable to the above mentioned ones) did not differ significantly in relation to heart wet weight, total SN_1 protein per heart, and heart weight/body weight ratios along the study.

The relevance of the rat sexually dimorphic cardiac protein polyacrilamide gel electrophoresis patterns above mentioned notwithstanding, these ponderal findings are in agreement with changes in serum concentration of thyroid hormones, hemoglobin and hematocrit levels not related to chronological age, but to body weight, height, and surface area of human adolescents of either sex.[37,38]

In regard to the epigenetic control of catalytically functional protein synthesis in heart

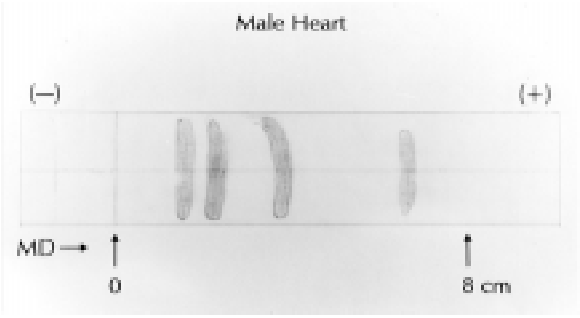

Fig. 2: A pattern of male rat heart electrophoretogram was selected to show a representative example of cytosolic CK isoenzymes. MD = Migration Distance; O = Origin.

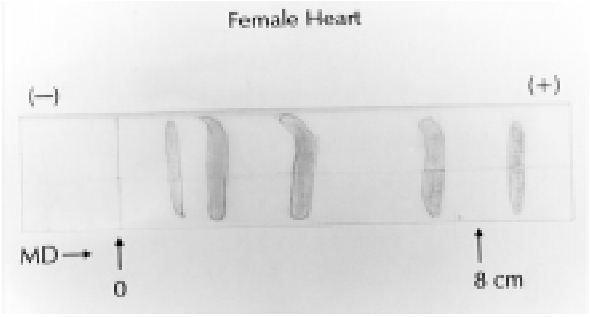

Fig. 3: A complementary pattern of female rat heart electrophoretogram was selected as a representative example of cytosolic CK isoenzymes. MD = Migration Distance, O = Origin.

tissues, one is tempted to speculate that, at about 257 g of body weight, the more than 2-fold higher CK specific activity found in the female rat heart, as compared to the male, is due to cardiac overload. The increase of ventricular mass and of CK activity has been observed in a number of cases in both spontaneously hypertensive rats[43] and in artificially induced high blood pressure after constriction of the rat aorta.[44,45] It is thought that muscle cell mass accounts for about 80% of myocardial mass,[46] and from data obtained in tissue culture, CK in muscle cells is more than 16-fold higher than in fibroblasts.[47] Nevertheless, in this study, the heart mass of females at about 257 g of body weight remained in its normal range regarding the parameters shown in *Tables I, II, and III*. Even more, at the same body weight, the male heart with an equivalent weight and protein content exhibited a decrease of CK specific activity of about 43%, as compared to the one exhibited at about 200 g of rat body weight.

Considering the rat sexual differences of cytosolic proteins from previous heart polyacrilamide results[1] and the above mentioned ones (regarding increase and/or decrease of heart CK specific activities in healthy normal animals of either sex), one should conclude that, at any time, the epigenetic control of the synthesis of catalytically active heart CK seems to depend on very specific regional ATP replenishment demands in equilibrium with the anaerobic glycolytic pathway and the mitochondrial oxidative phosphorylation capacity. It should be emphasized, however, that an increase in heart CK activity does not necessarily mean that a concomitant pathological ventricular hypertrophy has occurred.[43,44] The opposite consideration is also valid; a decrease in CK specific activity in rat heart is not always directly related to myocardial failure, in spite of the hypothesis that impaired ATP synthesis rates produced by the CK/PCr system is an important mechanism underlying cardiac failure.[27] The fine control of ATP replenishment seems to require also the participation of creatine transporter(s). Northern blot analysis has revealed the presence of significant amounts of mRNAs of CHOT1 creatine transporter in muscle-rich tissues of the rat, the heart included.[48] The Na^+-Cl^--dependent plasma membrane creatine transporter with a Michaelis constant of about 35 μM is also expressed promi-

nently in rabbit heart and in tissues known to possess high creatine uptake capacity.[49]

In order to discuss adequately the electrophoretic results, one must consider the physiological significance of the findings after the analysis of the employed techniques. The presence in the normal rat heart of more than two heavily stained catalytically active cytosolic CK isoforms, at all selected rat weights, seems to confirm previous evidence regarding post-natal rat brain CK isoenzyme transitions.[36] In both studies the following parameters were similar: a) animal and strain source, b) age-body weight conditions, c) composition of media for CK extraction, d) isolation and purification of subcellular fractions, e) electrophoretic procedures, and f) isoenzyme visualization techniques.

Age/Body Weight Data and Reference Tables of the Wistar Rat have shown that the heavier animals are older. Specifically, this seems to be the case for Wistar female rats (at about 300 g, female Wistar rats are 16 months old, whereas males are about 11 months old).[50]

To compare protein isoforms from young, adult, and senescent animals with a reasonable degree of confidence, it is necessary to review the literature regarding immunological detection of isoenzymes from aging animals, in contrast to catalytical procedures of the same isoforms. It is well known that the Krebs cycle and anaerobic glycolysis lose capacity with increasing age and with chronic decrease in physical activity.[51] Furthermore, it has been postulated that the error frequency in protein biosynthesis rises with the age of the animal to the point that the resulting protein molecules may be rendered less active or even inactive. This fact has been confirmed specifically with one glycolytic enzyme. Although the total amount of immunologically detectable aldolase remained unchanged in the muscles of aged animals, the isolated aldolase was only one third as active catalytically as aldolase isolated from younger animals.[52] Glyceraldehyde 3-phosphate dehydrogenase and pyruvate kinase, other two glycolytic enzymes, have also been described as presenting catalytically inactive isoforms during aging.[53,54] The presence of "antizyme" in the inactivation of ornithine decarboxylase in the mouse brain, which gives rise to active and inactive isoforms,[55] as well as maltase[56] and glucose 6-phosphate dehydrogenase[57] are other examples of inactive isoenzymes that are not necessarily participating in a metabolic pathway, although they could be functional parts of cellular structures.

In the case of CK specifically, previous work has also shown that large quantities of antigenically measured brain CK isoforms are catalytically inactive and can be separated from the catalytical CK species by DEAE-cellulose chromatography.[58]

The rat heart cytosolic CK isoenzyme data of the present work represent catalytically active isoforms that can participate physiologically in the CK/PCr system along the studied period. The differential affinities for ADP and PCr of MM, MB, and BB-CK are well documented as shown by their respective Michaelis constants. The brain type (BB-CK), the most anodic one, can produce ATP more actively than the other CK isoenzymes.[59] Affinities can change in the atypical cCK isoforms, thus affecting the speed of creatine and ATP formation. Atypical catalytical CK isoforms may arise by protein post translational modification(s) of the enzyme,[60,61] as well as by differential splicing of a unique CK gene,[62] or by differences in the transcription starting point of the CK promoter,[63,64] and from alternative ribosomal initiation.[65]

The simultaneous presence of more than one catalytical isoform of cytosolic CK found in the rat heart of both sexes along the study could be associated with a particular local energy demand not completely covered by anaerobic glycolysis and mitochondrial oxidative phosphorylation. In the adult human heart the presence of distinct cytoplasmic CK isoforms, besides the expected ones, has been described depending on the regionalization of the heart. The increase of BB-CK activity has been ascribed to differences in myocardial perfusion during the cardiac cycle in the normal left ventricle, and to a stressed intracellular energy transfer in patients suffering of mitral regurgitation.[66]

In the old male rats, hearts are larger, thinner, more fibrotic, and with high prevalence of mitral regurgitation with indexes of diminished performance, as compared to female rats.[15] In regard to blood flow distribution during normothermia and hyperthermia, mature laboratory rabbits showed sex differences in their blood flow regulation in

both peripheral organs which are active in heat dissipation (toe, ear skin, and nasal turbinates), and inner body organs.[67]

Irrespective of the migration distance of catalytically active heart cytosolic CK isoforms shown in *Figure 1*, the larger number and variety of cytosolic CK bands exhibited by the female heart at every studied rat body weight, in comparison to the male heart, seems to indicate that ATP replenishment by means of the CK/PCr system, the female rat heart is better endowed to respond to immediate and fluctuating vast energy requirements to cope with the differential susceptibilities of hemodynamic compromises along life. This finding in regard to energy requiring processes seems to confirm the gender-related anatomical,[7,8] and functional mechanisms,[9-14] and may explain in part the greater longevity of females of most mammalian species including women.

CONCLUSIONS

The aim of this biochemical study was to understand whether the anaerobic heart ATP synthesis, mediated by the CK/PCr system, is sexually dimorphic during maturation and aging of the rat. In this work, significant gender differences were found mainly in the patterns and number of catalytical cytosolic CK isoforms. Regarding the alternate anaerobic mechanism of ATP production, these differences may explain in part the sex differential susceptibility to hemodynamic compromise in response to cardiovascular stress, in favor of females.

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