ELSEVIER

Comparative Biochemistry and Physiology Part C 126 (2000) 203–208

CBP

www.elsevier.com/locate/cbpc

Circaannual changes in antioxidants and oxidative stress in the heart and liver in rats

A. Belló-Klein [a], M.I. Morgan-Martins [a], J. Barp [a], S. Llesuy [b], A.A. Belló [a], P.K. Singal [c,*]

[a] *Laboratório de Fisiologia Cardiovascular, Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

[b] *Catedra de Fisicoquimica, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Buenos Aires, Argentina*

[c] *Institute of Cardiovascular Sciences, St Boniface General Hospital Research Centre, Room R3022, 351 Tache Avenue, Winnipeg, Man. Canada MB R2H 2A6*

Received 26 November 1999; received in revised form 24 January 2000; accepted 14 April 2000

Abstract

Reactive oxygen species are formed in physiological and pathological conditions in mammalian tissues. Because of their high reactivity, they may interact with biomolecules, inducing oxidative injury. Increases in lipid peroxidation can result in oxidative damage to cellular membranes. Protection against oxidative damage is provided by enzymatic and non-enzymatic antioxidant defenses. Antioxidant enzyme activities and lipid peroxidation, as an index of oxidative stress injury, were evaluated in different seasons over one year in the heart and liver of rats, maintained on a 12 h light and dark cycle. Glutathione peroxidase and catalase activities, in both tissues, were maximal in the summer season. Lipid peroxidation in the heart was maximal in the spring as compared to the other seasons and it did not vary in the liver during the year. These findings suggest that any study of antioxidants or oxidative stress must take into account such seasonal variations for a more precise analysis of changes due to any pathological condition.

Keywords: Catalase; Glutathione peroxidase (GSHPx); Lipid peroxidation; Seasonal variations

1. Introduction

When the production of reactive oxygen species (ROS) exceeds the capacity of cells to neutralize them, it creates a condition of oxidative stress (Sies, 1991). It has been reported that oxidative stress plays a role in the pathogenesis of heart failure (Khaper and Singal, 1997; Singal et al., 1998). Oxidative stress has also been suggested to be involved in a variety of conditions such as cataract, Parkinson's disease, diabetes, carcinogenesis, aging and ischemia-reperfusion injury (Halliwell, 1987). The generation of ROS, and the associated oxidative stress, may also correlate with the basal metabolic rate. A high correlation between the basal oxygen consumption and the body content of cytochrome oxidase, a key enzyme of the oxidative metabolism, has been reported (Jansky, 1961). Therefore, the higher the metabolic rate or oxygen consumption, the higher the production of harmful free radicals. Since metabolism in different body tissues is known to

* Corresponding author. Tel.: +1-204-2353416; fax: +1-204-2336723.

E-mail address: psingal@sbrc.umanitoba.ca (P.K. Singal)

0742-8413/00/$ - see front matter
PII: S0742-8413(00)00112-2

vary with seasons (Jansky, 1961; Buzadzic et al., 1990), there may also be changes in antioxidants and oxidative stress.

Even the incidence of many diseases, including cardiovascular diseases, tends also to occur in a circadian pattern. For example, blood pressure is characterized by a nocturnal fall and a diurnal rise. This oscillation seems to be mediated mainly by the circadian oscillations of the sympathetic tone linked to changes in physical and mental activities (Imai et al., 1990). Reports of many other circadian changes can be found in the literature, such as circadian rhythm of body temperature, trace metals (such as iron and zinc) and several other blood variables (Scales et al., 1988). Although literature is replete with studies on circadian changes, not much is known about seasonal changes in oxidative stress, especially in rat heart and liver.

There is only one report of seasonal or circaannual oscillations of oxidative stress in rats in which lipid peroxidation in the bone marrow was found to be maximal during the summer (Sólar et al., 1995). Therefore, the aim of this work was to examine changes in the antioxidant enzyme profile and oxidative stress in heart and liver of rats in the four seasons of the year in Brazil. These two tissues were chosen because they have markedly different antioxidant status.

2. Material and methods

2.1. Animals

Male Wistar rats (250 ± 12 g) were purchased from the Animal Quarter House (ICBS — Federal University of Rio Grande do Sul, Brazil) at different times of the year, i.e. 1 week prior to the start of spring (September–November), summer (December–February), fall (March–May) and winter (June–August). For each season, six animals were housed in plastic cages lined with sawdust, in air-conditioned quarters (20–22°C) and had free access to tap water and pelleted food. The animals were maintained under 12 h light/dark cycle.

2.2. Tissue homogenates

Rats were sacrificed with a blow to the head followed by cervical dislocation and their heart and liver were rapidly removed. In order to avoid any possible daily cyclic variations in the measurements, animals were always sacrificed between 08:00 and 10:00 h. Tissue samples were homogenized in an Ultraturrax homogenizer at 0–4°C in KCl (1.15%) and PMSF (100 mmol/l) and centrifuged at $1000 \times g$ for 10 min (Llesuy et al., 1985). The supernatant was used for the determination of antioxidant enzyme activities and lipid peroxidation.

2.3. Antioxidant enzyme activities

Glutathione peroxidase (GSHPx) activity was determined as previously described (Flohé and Gunzler, 1984). It was assayed in a 3-ml cuvette containing 140 mmol/l phosphate buffer (pH 7.4). The following solutions were then added to obtain final concentrations indicated in parentheses: reduced glutathione (GSH) (5 mmol/l), glutathione reductase (GR) (0.25 U/ml), sodium azide (1 mmol/l, NADPH (0.24 mmol/l) and EDTA (1 mmol/l). The reaction was started by the addition of *tert*-butyl hydroperoxide (*t*BOOH) (0.5 mmol/l final concentration) and the conversion of NADPH to NADP was monitored (in a spectrophotometer Varian, model Cary) by a continuous recording of the change in absorbance at 340 nm for 5 min. GSHPx activity was expressed in nanomoles of NADPH oxidized/min/mg protein, with a molar extinction coefficient for NADPH of 6.22×10^6 mol/l per cm.

Catalase (CAT) activity was determined according to a procedure previously described (Boveris and Chance, 1973). Supernatant was added to a 3-ml cuvette that contained 2.9 ml of 50 mmol/l potassium phosphate buffer and 100 μl of 0.3 mol/l hydrogen peroxide. Changes in absorbance at 240 nm were followed for 5 min. CAT activity was expressed as pmol/min/mg protein.

2.4. Lipid peroxidation assay

The lipid peroxidation was assessed by studying thiobarbituric acid reactive substances (TBARS) by the method previously described (Buege and Aust, 1978). Aliquots of the supernatant were added to a pyrex tube that contained trichloroacetic acid (10%) and thiobarbituric acid (0.67%) and incubated at 100°C for 15 min. The mixture was allowed to cool on ice for 5 min. This was followed by the addition of 1.5 ml of *n*-butyl-

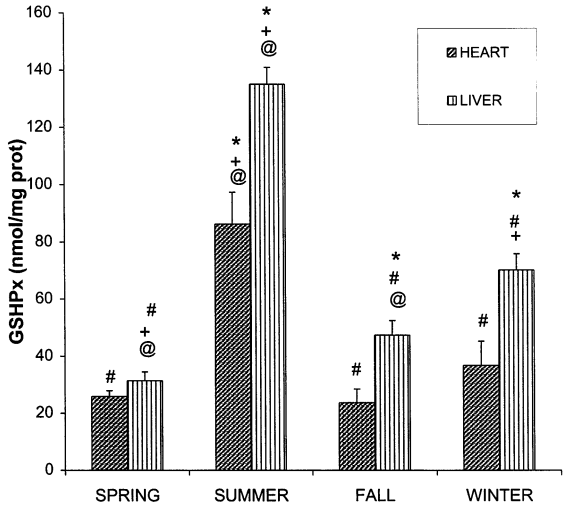

Fig. 1. GSHPx activity in the heart and the liver of rats in different seasons of the year. Data are mean ± S.E.M. of six animals. *, significantly different from the respective value in the spring group ($P < 0.05$); #, significantly different from the respective value in the summer group ($P < 0.05$); +, significantly different from the respective value in the fall ($P < 0.05$); @, significantly different from the respective value in the winter group ($P < 0.05$).

alcohol; the mixture was vigorously agited for 40 s and centrifuged at 1000 × *g* for 15 min, in order to extract the resulting chromogen. The absorbance of the organic phase was determined at 535 nm in a spectrophotometer Varian (model Cary). TBARS formation was quantified and expressed as nmol of malonaldehyde/mg protein. Commercially available malonaldehyde was used as a standard.

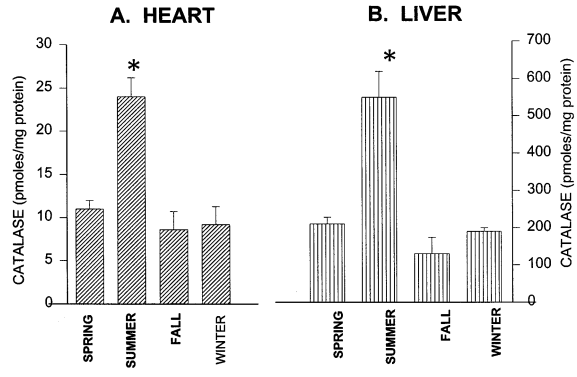

Fig. 2. CAT activity in the heart (panel A) and the liver (panel B) of rats in different seasons of the year. Data are mean ± S.E.M. of six animals. *, Significantly different from all other groups ($P < 0.05$).

2.5. Proteins and statistical analysis

The protein content of homogenates was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard. Results are reported as mean ± SEM. For a statistical analysis of the data, group means were compared by one-way ANOVA followed by Student–Newmann–Keuls test. The differences were considered significant at $P < 0.05$.

3. Results

GSHPx and CAT activities were measured in the heart and liver of six rats in each of the four different seasons of the year. GSHPx activity was found to be significantly increased in the summer as compared to the other seasons. This increase was about 57% in the heart and 48% in the liver when compared to the winter group (Fig. 1). In the heart, GSHPx values in spring, fall and winter were not significantly different from each other. GSHPx activity in the liver was relatively higher than heart in the summer, fall and winter. GSHPx activity in the liver was found significantly different from one season to another (Fig. 1). It was increased from spring to summer by 77%, decreased by 185% from summer to fall, increased by 32% from fall to winter and decreased from winter to spring by 124%.

CAT activity in the liver was about 20 times more than heart in all four seasons. CAT activity was also found to be maximal in the summer as compared to the other seasons in both heart and liver (Fig. 2). In the spring, fall and winter groups in the heart and liver, CAT activity did not differ significantly.

Lipid peroxidation was studied by assessing TBARS. The levels of TBARS in the heart and liver were not different from each other in any of the seasons. Cardiac values of TBARS were found to be increased in the spring as compared to the other seasons (Fig. 3). From the winter to the spring group TBARS content was increased by 59% and it was decreased by 34% from spring to summer in the heart (Fig. 3). This value was decreased (142%) from the summer to the fall. No significant differences in cardiac TBARS were observed between fall and winter and between summer and winter. TBARS concentration did not change significantly in the liver in the four seasons of the year.

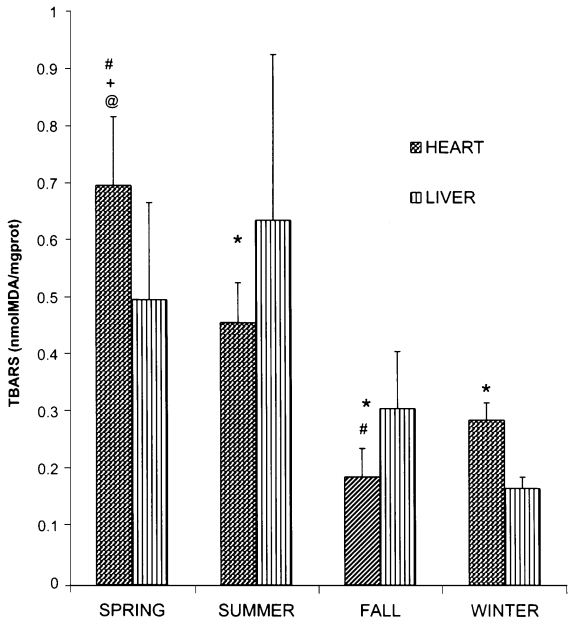

Fig. 3. Lipid peroxidation as indicated by TBARS in the heart and the liver of rats in different seasons of the year. Data are mean ± S.E.M. of six animals. Abbreviations are the same as in Fig. 1.

4. Discussion

Although circadian fluctuations due to the light and dark cycles in reduced GSH, lipid peroxidation, GSHPx and GR have been reported in various rat organs (Farooqui and Ahmed, 1984; Díaz-Muñoz et al., 1985; Lapenna et al., 1992), the present study is the first to examine seasonal changes in GSHPx, CAT and lipid peroxidation in the rat heart and liver. It should be noted that circadian changes depend mostly on light/dark cycle, while seasonal alterations depend more on weather conditions such as temperature, humidity and atmospheric pressure. Thus the underlying mechanisms for diurnal and circaannual changes may differ significantly. Since the light and dark cycle as well as temperature were controlled in the present study, the observed changes cannot be attributed to these factors.

Significantly higher CAT activity in the liver as compared to the heart has been reported before (Doroshow et al., 1980). However, our study showed that this difference is seen in all seasons. Antioxidant enzymes (GSHPx and CAT) were found increased in the summer time as compared to the other seasons in heart and liver, whereas lipid peroxidation in the heart was increased in the spring season. These increased oxidative stress observed in the spring could determine these increased antioxidant enzyme activities, constituting, at least in the heart, an adaptive response. There are now several examples of oxidative stress induced up-regulation of antioxidant enzymes (Halliwell and Gutteridge, 1999). Similarly an adaptive response is observed in the ischemic preconditioning, where the myocardium becomes more resistant to severe oxidative stress applied subsequently (Sun et al., 1996). Clearly, seasons influence some enzymatic activities in mammals. Blagojevic et al. (1998) have observed an increase in antioxidant enzyme activities during the spring in ground squirrels. They also found a decrease in the antioxidant enzyme activities during the winter with a concomitant increase in the low molecular weight antioxidants such as ascorbic acid.

The oscillations of shorter periodicity such as circadian rhythms have been more extensively studied. A decrease in GSHPx and an increase in glutathione transferase activities was found during the dark phase of the day in rat hearts (Lapenna et al., 1992). In cerebral cortex, circadian changes in GSHPx, GR and superoxide dismutase (SOD) activities have been reported. The reduction in the activity of the enzymes that remove hydroperoxides (GSHPx and GR) during the night was found to be related with increased oxidative stress (Díaz-Muñoz et al., 1985). Biological rhythms are regulated by internal time clocks. Melatonin, the chief secretory product of pineal gland, may be the main synchronizer of the light-dependent periodicity (Bubenik et al., 1998). This substance can also be considered as an antioxidant as it is suggested to neutralize partially reduced forms of oxygen such as hydroxyl radical, superoxide anion, singlet oxygen and peroxynitrite anion (Reiter et al., 1998).

Some information on human material is also available. Kosugi et al. (1994) have observed diurnal variation in urinary TBARS in male and female patients, with afternoon levels higher than morning levels. The highest values of lipid hydroperoxides in women were found from October to December in the northern hemisphere (Gladen et al., 1999). In terms of circadian oscillations, Farooqui and Ahmed (1984) found an increase of TBARS concentrations in many tissues in the daylight hours and a decrease in the dark hours. These oscillations were inversely proportional to reduced GSH content changes, e.g. when TBARS

increased it was found lesser amounts of GSH in the tissues. These authors have associated this enhanced production of GSH in the dark period to the feeding time of the animals.

In our experiments, we have observed circaannual oscillations in lipid peroxidation only in the cardiac tissue and not in the liver. Maximal lipid peroxidation was observed during the spring in rat hearts. Sólar et al. (1995) have obtained similar result in the bone marrow of rats. One possible explanation for this observation could be the enhanced food intake reported in these animals in this season (Rietfeld et al., 1980). More pronounced changes in the thyroid hormones, that can modulate the metabolic rate of the rats, have also been reported for the spring season (Àhlersová et al., 1991). Increased lipid peroxidation in the spring, in our study, may correlate with increased feeding and physical activity.

In conclusion, we observed evident changes in the oxidative stress as well as antioxidant enzyme activities during different seasons of the year in rats where light and dark cycle and ambient temperature were carefully controlled. Since the environment in these conditions was controlled, any change in the enzyme activity can be attributed to some type of genomic memory responsible for the adaptation to different seasons. In the last few years, a great amount of information has been published on oxidative stress, not only in animals but also in humans. Data in this study suggest the necessity to consider seasonal variations in any evaluation of lipid peroxidation as well as antioxidant enzyme activities.

Acknowledgements

PKS is a Career Awardee of Medical Research Council of Canada. Student fellowships (MIMM and JB) from CNPq, visiting scientist grant (SL) from FAPERGS, research productivity grant (AAB) from CNPq and post-doc grant (ABK) from CAPES are gratefully acknowledged. CNPq, FAPERGS and CAPES are agencies from Brazilian government. We would like also to thanks Tânia Regina Gattelli Fernandes for her technical assistance

References

Àhlersová, E., Ahlers, J., Smajda, B., 1991. Influence of light regimen and time of year on circadian oscillations of thyroid hormones in rats. Physiol. Res. 40, 305–315.

Blagojevic, D., Buzadzic, B., Korac, B., et al., 1998. Seasonal changes in the antioxidative defense in ground squirrels (Citellus citellus): possible role of GSHPx. J. Environ. Pathol. Toxicol. Oncol. 17 (3-4), 241–250.

Boveris, A., Chance, B., 1973. The mitochondrial generation of hydrogen peroxide. Biochem. J. 134, 707–716.

Bubenik, G.A., Blask, D.E., Brown, G.M., et al., 1998. Prospects of the clinical utilization of melatonin. Biol. Signals Recept. 7 (4), 195–219.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Meth. Enzymol. 52, 302–309.

Buzadzic, B., Sasic, M.B., Saicic, Z.S., Radojicic, R., Petrovic, V.M., Halliwell, B., 1990. Antioxidant defence enzymes in the ground squirrel Citellus citellus 2: the effect of hibernation. Free Rad. Biol. Med. 9, 407–413.

Díaz-Muñoz, M., Suarez, J., Hernandez-Muñoz, R., Chagoya de Sanchez, V., 1985. Day- night cycle of lipid peroxidation in rat cerebral cortex and their relationship to the glutathione cycle and superoxide dismutase activity. Neuroscience 16, 859–863.

Doroshow, J.H., Locker, G.Y., Myers, C.E., 1980. Enzymatic defenses of the mouse heart against reactive oxygen metabolites: alterations produced by doxorubicin. J. Clin. Invest. 65, 128–135.

Farooqui, M., Ahmed, A.E., 1984. Circadian periodicity of tissue glutathione and its relationship with lipid peroxidation in rats. Life Sci. 34, 2413–2418.

Flohé, L., Gunzler, W.A., 1984. Assays of glutathione peroxidase. Meth. Enzymol. 105, 114–121.

Gladen, B.C., Tabacova, S., Baird, D.D., Little, R.E., Balabaeva, L., 1999. Variability of lipid hydroperoxides in pregnant and nonpregnant women. Reprod. Toxicol. 13, 41–44.

Halliwell, B., 1987. Oxidants and human disease: some new concepts. FASEB J. 1, 358–364.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine, third ed. Oxford University Press, London, pp. 247–249.

Imai, Y., Abe, K., Munakata, M., et al., 1990. Circadian blood pressure variations under different pathophysiological conditions. J. Hypertens. Suppl. 8 (7), S125–S132.

Jansky, L., 1961. Total cytochrome oxidase activity and its relation to basal and maximal metabolism. Nature 189, 921–922.

Khaper, N., Singal, P.K., 1997. Effects of afterload-reducing drugs on pathogenesis of antioxidant

changes and congestive heart failure in rats. J. Am. Coll. Cardiol. 29, 856–861.

Kosugi, H., Enomoto, H., Ishizuka, Y., Kikugawa, K., 1994. Variations in the level of urinary thiobarbituric acid reactant in healthy humans under different physiological conditions. Biol. Pharm. Bull. 17, 1645–1650.

Lapenna, D., De Gioia, S., Mezzetti, A., et al., 1992. Circadian variations in antioxidant defences and lipid peroxidation in the rat heart. Free Rad. Res. Commun. 17 (3), 187–194.

Llesuy, S., Milei, J., Molina, H., Milei, S., Boveris, A., 1985. Comparison of lipid peroxidation and myocardial damage induced by adriamycin and 4'-epiadriamycin in mice. Tumori 71, 241–249.

Lowry, O.H., Rosebrough, A.L., Farr, A.L., Randall, R., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Reiter, R., Guerrero, J.M., Garcia, J.J., Acuna-Castroviejo, D., 1998. Reactive oxygen intermediates, molecular damage, and aging: relation to melatonin. Ann. NY Acad. Sci. 854, 410–424.

Rietfeld, W.J., Flory, W., Kooij, M., Ten Hoor, F., 1980. Observations on total food intake and 24 h food intake patterns during long-term registration of food intake behaviour in rats. Z. Versuchstierk. 22, 156–160.

Scales, W.E., Vander, A.J., Brown, M.B., Kluger, M.J., 1988. Human circadian rhythms in temperature, trace metals, and blood variables. J. Appl. Physiol. 65 (4), 1840–1846.

Sies, H., 1991. Oxidative Stress, Oxidants and Antioxidants. Academic Press, New York.

Singal, P.K., Khaper, N., Palace, V., Kumar, D., 1998. The role of oxidative stress in the genesis of heart disease. Cardiovasc. Res. 40, 426–432.

Sólar, P., Toth, G., Smajda, B., Ahlers, J., Ahlersová, E., 1995. Circadian and circaannual oscillations of tissue lipoperoxides in rats. Physiol. Res. 44 (4), 249–256.

Sun, J.Z., Tang, X.L., Park, S.W., Qiu, Y., Turrens, J.F., Bolli, R., 1996. Evidence for an essential role of reactive oxygen species in the genesis of late preconditioning against myocardial stunning in conscious pigs. J. Clin. Invest. 97, 562–576.