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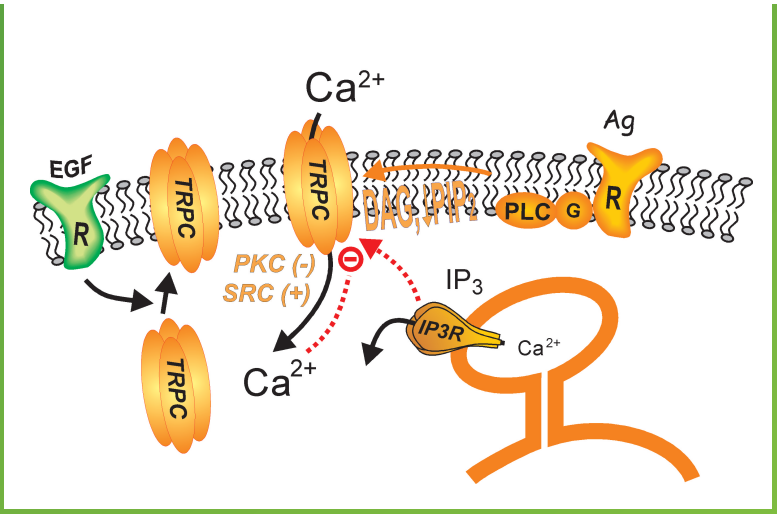

Melatonin regulation of some important metabolic enzymes during intestinal ischemia/reperfusion injury in rats

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ABSTRACT

Intestinal ischemia/reperfusion (I/R) injury is known to result from both tissue hypoxia and the consequences of reperfusion of ischemic tissues. In addition, ATP stores are depleted during ischemia because of the relatively inefficient production of ATP by glycolysis. In this study, the effect of melatonin on some important metabolic enzymes hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) responsible for generation of energy was investigated. Melatonin was determined to cause a distinct regulation of these enzymes. While the activity of the enzyme HK of the first step of glycolysis was significantly higher in pre-melatonin treated I/R group than group I/R animals, the activities of LDH and G6PD were decreased markedly with melatonin application. Although, the MDH levels of pre-melatonin treated I/R group were higher than the sham control group, this might not be a result of melatonin application, since both I/R and I/(Mel/R) groups also showed even higher MDH activity than the control group.

Key words: ischemia-reperfusion, melatonin, hexokinase, glucose-6-phosphate dehydrogenase, malate dehydrogenase, lactate dehydrogenase

INTRODUCTION

The consequences of mesenteric ischemia are devastating to the patient and usually result in diarrhea, malabsorption, short gut syndrome, and even death (Koltuksuz et al., 1999). The mechanism of injury in intestinal ischemia involves both hypoxia and reperfusion (Schoenberg and Berger, 1993). Under both conditions tissues or cells experience stress. Hypoxic conditions (as the result of ischemia) causes cells to remain in reduced state, while hyperoxic conditions (as the result of reperfusion) causes a burst in oxygenation of tissues thus a more oxidized state is achived in the cells. The tissues under both ischemia and reperfusion are far from normal and thus severe clinical problems arise.

As a result of ATP depletion during ischemia, the energy balance of tissues is disturbed and cells are forced to compensate these unfovorable conditions by re-regulating their energytic pathways (Zhong et al., 1998). The generation of ATP and the production of reduced cofactors of energytic type (NADH, FADH2) and biosynthetic type (NADPH) are through intermediary pathways. Given that those pathways are affected by the presence/absence of oxygen, ischemia and reperfusion would cause some shifts in the flow of these schemes.

Melatonin plays several important physiological functions in mammals, such as reproductive regulation, immune enhancement, and regulation of dark-light signal transductions (Reiter, 1991). Also, it as well as its metabolites (6-hydroxymelatonin, 2-hydroxymelatonin, cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramin, N-acetyl-5-methoxykynuramine, 1-nitromelatonin and nitrosomelatonin) are a ubiquitously acting direct free radical scavenger and an indirect antioxidant (Tan et al., 2003; Allegra et al., 2003; Tan et al., 2007; Peyrot and Ducrocq, 2008; Ozen et al., 2008; Reiter et al., 2008). In addition to melatonin stimulates antioxidant enzyme such as glucose-6-phosphate dehydrogenase (G6PD) activity (Reiter, 1998). The aim of this study was to investigate the effect of pre-ischemic and post-ischemic melatonin treatment on activities of some metabolic enzymes important for the energetic status of cells: hexokinase □HK□, G6PD, lactate dehydrogenase (LDH) and malate dehydrogenase (MDH).

MATERIALS AND METHODS

Treatment of animals

The study was performed using 32 male Sprague-Dawley rats (weighing 200-250 g) randomly divided into 4 groups (n = 8); sham, I/R, melatonin (10 mg kg-1, obtained from Sigma Chemical Co., St Luis, MO, USA) followed I/R, and I plus melatonin (10 mg kg-1) (Ateş et al., 2004) followed R. All animals were kept in individual cages in a controlled room The rats were fed ad libitum with standard rat food and drinking water. Rats were deprived of food for 12 h before experimentation but allowed free access to drinking water throughout. Experimentation on animals was carried out following a protocol approved by the Animal Research Committee of Inonu University. The melatonin solution was prepared freshly by dissolving the molecule in 1 volume absolute ethanol and 5 volumes 0.9 □ NaCl to a final concentration of 0.25 mg.ml-1. Animals in sham group were given vehicle

solution in equal volumes as the melatonin solution via the intraperitoneal route.

Experimental design

The rats were anaesthetized by intramuscular injection of ketamine/xylazine hydrochloride (20/2 mg kg-1 b.w.). A midline laparotomy was performed after shaving and local cleansing with an antiseptic solution. Intestines were exteriorized, and the superior mesenteric artery was dissected carefully and occluded using atraumatic microvascular clip (ischemia) for 45 min. Then, the clamp was removed and reperfusion occured for 120 min. Melatonin (10 mg kg-1) was given intraperitoneally to Mel-I/R group before ischemia while for I-Mel-R group melatonin is was give just prior to the clamp being removed before reperfusion. After reperfusion, all rats were sacrificed and their intestines, beginning from duodenum to the middle of the colon, were removed. They were rinsed with 0.9□ NaCl. Tissues for enzyme activities were homogenized (Kinematica Homogenizator; Kinematica GmbH, Littau Lazern, Switzerland) in phosphate buffered saline (pH 7.4). The homogenate was sonicated with an ultrasonifier (Bronson Ultrasonic Co., Bronson Sonifier 450; Danbury, CT, USA) using six cycles (20-s sonications and 40-s pause, on ice). The extracts were centrifuged (15,000 xg, 10 min, 4 oC) and cell-free supernatants were assayed for enzyme activities.

Enzyme assays

Activities of all enzymes were measured in NAD(P)/NAD(P)H-dependent reactions at 340 nm using a molar extinction coefficient of 6,220 M-1 cm-1. The activities of enzymes HK, G6PD, LDH, and MDH were determined according to manufacturer's handbook (Boehringer 1973). MDH and LDH activities were measured from the oxidation of NADH, and HK and G6PD activities were measured from the reduction of NADP+ at A340 nm at 25 °C for a 5-minute period. Tissue protein levels were determined by the Lowry procedure, with bovine serum albumin as a standard (Lowry et al., 1951). Activities were given in milliunits (mU) per milligram protein. All samples were assayed in duplicate.

Statistical Analysis

All statistical analyses were carried out using SPSS statistical software (SPSS for windows; Chicago, IL, USA). The one-way ANOVA analysis of variance and post hoc multiple comparison tests (LSD) were performed on the data of biochemical variables to examine the differences among groups. All data were given as mean ± S.E.M and $p<0.05$ was considered as statistically significant.

RESULTS

The activity results of the metabolic enzymes HK, G6PD, LDH, and MDH level are shown Figure 1. There was a significant ($p<0.04$) increase in the activity of HK in the Mel/(I/R) group compared to I/R group. There was no significant difference in the activity of HK among the sham, I/R, and I-Mel-R groups. There was no significant difference in the activities of G6PD and LDH among the all groups. There was a significant ($p<0.02$) increase in the activity of MDH in the I/R group compared to sham group. There was a significant ($p<0.003$) increase in the activity of MDH in the I-Mel-R group compared to sham group. There was no significant difference in the activity of MDH between the sham and Mel-I/R groups.

DISCUSSION

The HK activity of I/R group animals were determined to be the lowest among any other group, including the sham control group. There was a significant ($p<0.04$) increase in the activity of HK in Mel/(I/R) group compared to I/R group animals. The application of melatonin after I but before R, however, showed no marked effect on HK activity, as those animals showed similar enzyme activity to that of sham (control) group animals. The low HK activity in I/R group might result from higher oxygenation of tissues during 2 h of R after a 45 min I which would lead to anaerobiosis. It is known that, under hypoxic conditions the degradation of glucose would increase to compensate the energetic need of cells. The R, however, would cause a diminish in HK activity as cells experience an immediate oxygen uptake. The application of melatonin prior to I/R seems to reverse this process; causing significantly higher HK activity. This indicates that melatonin act as an negative regulator for oxygen uptake/utilization which would otherwise cause a lower HK activity. Furthermore, the low LDH activity supports this assumption as R would cause a burst in oxygenation of tissues and direct the NADH formed during the glycolysis to oxidative phosphorylation of ATP generation instead of its use in reduction of pyruvate to lactate through LDH. Cellular oxidant-antioxidant status is in balance under physiological conditions. But, this stability is disturbed by I/R injury. A continuing loss of oxygen results in high anaerobic metabolism for energy production and high lactic acid concentration in the tissue (Grace, 1994). High ATP need due to ischemia (anaerobiosis) induces glycolysis, and thus a higher activity of HK, an enzyme with a central role on glycolysis (Magnani et al., 1984). The effect of R is evident from higher MDH activity in Mel/(I/R) group compared to that in sham group animals, as a high oxygen uptake would result a higher activity of this citric acid cycle enzyme. The higher activity of MDH, however, might not be solely a result of melatonin application, since I/R group animals showed even a

significantly ($p<0.02$) higher MDH activity than sham control group animals. The highest MDH activity was determined in group I/(Mel/R).

Antioxidant enzyme G6PDH is the first rate-limiting enzyme in the hexose monophosphate shunt pathway, playing an important role in the regeneration of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH maintains glutathione in its reduced form, which is essential for the detoxification of reactive free radicals, lipid hydroperoxides and toxic compounds of endogenous and exogenous origin (Agrawal et al., 2004). The melatonin application on the activity of G6PD was opposite to that of HK. In animals treated with melatonin before I/R, there was a marked decrease in G6PD activity. The melatonin treratment after I, however, caused similar enzyme activity to I/R group.

In conclusion, in the presence of melatonin the activity of metabolic enzymes studied here might be restored in cells experiencing hypoxic and hyperoxic conditions. Although there have been some suggestions regarding how melatonin might improve cellular bioenergetics (Acuna-Castroviejo et al., 2001; Ateş et al., 2004), further experimental settings are needed to determine the exact role of melatonin in cells under metabolic stress conditions.

Table 1. Changes after pre- and post ischemia melatonin treatment on HK, G6PD, LDH, and MDH activities in intestinal tissue of rats subjected to ischemia/reperfusion (I/R) injury.

	HK	G6PD	LDH	MDH
Groups	(mU/mg protein)			
Sham (n=8)	5.83 ± 0.62	1.80 ± 0.19	60.9 ± 5.5	779 ± 107
I/R (n=8)	5.05 ± 0.38	1.56 ± 0.25	57.6 ± 8.4	1692 ± 235[b]
Mel-I/R (n=8)	6.81 ± 0.42[a]	1.09 ± 0.24	45.5 ± 2.8	1333 ± 259
I-Mel-R (n=8)	5.62 ± 0.74	1.59 ± 0.28	56.3 ± 6.0	2004 ± 372[c]

Each data point represents the mean ± SEM. a Statistically significant increase ($p<0.04$) compared to I/R group. bStatistically significant increase ($p<0.02$) compared to sham group. c Statistically significant increase ($p<0.003$) compared to sham group.

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