

Research Article

Cerebral Epiphyseal Proteins and Melatonin Modulate the Hepatic and Renal Antioxidant Defense of Rats

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The cerebral epiphysis (pineal gland) secretes melatonin and number of other proteins and peptides. It was thus hypothesized that antioxidant properties of epiphyseal proteins and melatonin could potentially benefit from exogenous therapies. In view of the therapeutic potential of these proteins, the present experiment was conducted to investigate the effect of buffalo epiphyseal proteins (BEP, at 100 µg/kg BW, i.p.) and melatonin (MEL, at 10 mg/kg BW, i.p) on changes in hepatic and renal antioxidant enzymes of adult female Wistar rats. Buffalo epiphyseal proteins significantly ($P < .05$) increased hepatic lipid peroxidation (LPO), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), reduced glutathione (GSH), and renal LPO, catalase (CAT), GR, GSH, GPx levels as compared to control animals. Similarly, MEL treatment significantly ($P < .05$) up-regulated hepatic SOD and GPx activity, whereas CAT, GR, GPx, and GSH levels in renal tissues were increased while SOD and LPO remained unaffected. Buffalo epiphyseal protein treatment produced greater effects on hepatic GPx and renal CAT and GSH levels than did MEL. These findings support the conclusion that buffalo epiphyseal proteins and melatonin activate a number of antioxidant mechanisms in hepatic and renal tissues.

1. Introduction

The cerebral epiphysis (pineal gland) is broadly involved in the synchronization of bodily functions(s) with the environment and serves as a “regulator of regulators”. It is known that, in addition to its synthesis of melatonin (MEL) from serotonin, this organ secretes various proteins and peptides [1, 2]. The epiphysis additionally has a rich supply of adrenergic nerve fibers that greatly influence its secretory activity. The cerebral epiphysis, through its production of MEL and its effect on serotonin, affects many neuroendocrine functions [1]. However, many researchers

regard MEL as the sole mediator of epiphyseal functions. Recently, a number of studies reported that epiphyseal proteins have the ability to regulate various physiological functions in numerous animals and thus hypothesized that these proteins effectively acted as epiphyseal hormones [3–7]. In spite of these studies, the functional role of epiphyseal hormones and proteins in antioxidant defense system of vital organs remains poorly understood. Oxidative damage to vital organs, particularly to the liver and kidney, becomes very important in humans and animals when the antioxidant defense system is either absent or functioning inefficiently [8]. Inasmuch as the liver and kidney are metabolically highly

active in xenobiotic metabolism and excretion, they have, compared to other organs, a greater load of free radical activity and thus are more prone to oxidative damage [8, 9]. As a consequence, reactive oxygen species (ROS) tissue injuries are found more commonly in these organs, as are their sequelae of toxic damage, disease, and the ultimate death of the biological systems in which they occur [10, 11]. In healthy animals, there is a balance between the production of various ROS and antioxidant defenses [8]. It has been noted that the antioxidant defense systems of the organs of many species are inadequately equipped to take up an excessive load of free radicals and ROS, and that when this does take place it is associated with increased oxidative damage in the affected organs [10–12]. Having to contend with oxidative stress brought about by uncontrolled oxidation of important molecules in foods and body tissues is thus a significant biological challenge faced by most living organisms [13, 14]. Antioxidant therapies, which are based on upregulation of body antioxidant defense system, are now a commonly employed strategy for combating molecular damage in various tissues [12]. Variations of this approach, which make use of exogenously administered antioxidant agents, could potentially provide an important and inexpensive alternative treatment for diseases related to oxidative stress. Hence, the present study was based on the perceived value of investigating several molecules for their potential benefit in bolstering the antioxidant defense systems of the liver and kidney. Melatonin (*N*-acetyl-5-methoxytryptamine), which has been long known as the pineal's major secretory product, has potent antioxidant properties [15–17]. Earlier, we reported on the antioxidant action of buffalo (*Bubalus bubalis*) epiphyseal proteins (BEPs) under fluoride and arsenic-induced oxidative stress in blood, brain, and kidney [2–6, 18–20]. However, the effect of epiphyseal hormone and proteins on liver and kidney antioxidant defense system has not previously been studied. Thus in view of findings reported by ourselves and others, we hypothesized that pineal BEP and MEL might enhance levels of antioxidant defense activity (enzymatic and nonenzymatic) in the liver and kidney and could therefore be of benefit for animals undergoing oxidative stress.

2. Methods

All the procedures, conducted on the experimental animals were duly approved by the Institutional Animal Ethics Committee (IAEC) of Indian Veterinary Research Institute (IVRI) for the purpose of control and supervision of experiments on animals.

2.1. Chemicals. All chemicals used in the study were of analytical grade from HiMedia, Loba Chemie (Mumbai, India), SRL Chemicals, India. Melatonin was procured from Sigma Chemical Co. (St. Louis, USA). Buffalo (*Bubalus bubalis*) epiphyseal proteins were supplied by the Neurophysiology Laboratory, Division of Physiology and Climatology, IVRI (Izatnagar, India).

2.2. Experimental Animals. The present study was carried out on eighteen sexually mature and healthy female Wistar rats of 130–142 g body weights, procured from the Laboratory Animal Resource (LAR) Section of the IVRI. Rats were examined on arrival for any abnormality or overt ill health. Rats were housed in polypropylene cages in a light/dark (LD) cycle of 12 h, in a pathogen-free, temperature- and humidity-controlled environment (set at $21 \pm 2^\circ\text{C}$ and relative humidity at $50 \pm 10\%$, resp.). After an acclimatization period of 1 week, they were weighed and randomly assigned to various groups with approximately equal initial group mean body weights. Following allocation, the animals were marked with picric acid solution for individual identification. All the animals had free access to the standard laboratory animal diet and water, which were replenished on daily basis. The animals were also checked daily for the health and husbandry conditions.

2.3. Experimental Design. The experimental design for the present study, including various groups, doses, route of administration, and duration of treatment is presented in Table 1. BEPs were used as the experimental agent in as much as its safety and utility were confirmed in a study that we have previously published. Appropriate dosages of BEP and MEL were optimized from experience in our previous work and thereafter they were dissolved in a suitable vehicle before administration at exactly 16.00 hrs [6].

2.4. Sample Collection. Daily observations were taken for the behavioral changes and mortality, if any, throughout the experimental period. The samples were collected at the end of the experiment (day 28). The rats were euthanized using ether at the end of the experiments. The liver and kidney were collected, cleaned, rinsed in chilled saline, blotted, weighed, and stored at -20°C . Frozen liver and kidney tissues samples were partially thawed, and 200 mg of sample was weighed and taken in 2 mL of ice-cold saline. Another 200 mg of the samples were weighed separately and taken in 2 mL of 0.02 M EDTA for GSH estimation. Organ homogenates were prepared using an IKA homogenizer (Germany), under ice-cold conditions and collected, and then centrifuged for 10 min at 3000 rpm. Thereafter, cell-free supernatant was collected and transferred to precooled microfuge tubes in duplicate and stored at below -20°C . These supernatants were used for estimation of total proteins (organs), lipid peroxidation (LPO), and enzyme activity namely, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) as well as non-enzymatic namely, reduced glutathione (GSH) antioxidant defense level.

2.5. Analytical Procedures. Estimations of different antioxidant defense-related biochemical parameters in hepatic and renal tissues were carried out using a Double Beam UV-VIS Spectrophotometer (UV 5704 SS, ECIL, India).

TABLE 1: Distribution of experimental rats to different treatments.

Groups (N/group = 6)	Treatments	Dose	Route of administration
Control	Drinking water + Normal saline	<i>Ad libitum</i>	Oral Intraperitoneal
BEP	Buffalo epiphyseal proteins (BEPs)	100 μ g/kg BW	Intraperitoneal
MEL	Melatonin (MEL)	10 mg/kg BW	Intraperitoneal

Lipid Peroxidation (LPO). Renal and hepatic tissues LPO was determined in terms of malondialdehyde (MDA) production by the method of Rehman [21].

Reduced Glutathione (GSH). The concentration of GSH in renal and hepatic tissues was estimated by evaluating free-SH groups, using the 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) method as described by Sedlak and Lindsay [22].

Catalase (CAT). Activities of catalase enzymes were estimated as described by Bergmayer [23] and were expressed as nM H₂O₂ utilized per minute per milligram protein.

Superoxide Dismutase (SOD). Superoxide dismutase activities were estimated using the method described by Madesh and Balasubramanian [24] and are expressed as SOD units [one unit of SOD is the amount (μ g) of protein required to inhibit the MTT reduction by 50%].

Glutathione Peroxidase (GPx). Glutathione peroxidase activities were determined by the method of Paglia and Valantine [25]. The enzyme activity is expressed as U/mg of protein, and one unit of enzyme activity is defined as 1 nM of substrate (NADPH) utilized/min/mg protein at 25°C.

Glutathione Reductase (GR). The enzyme activities were assayed by the method of Goldberg and Spooner [26], and activity is expressed as nM NADPH oxidized to NADP/min/mg protein.

Protein Assay. Protein contents in liver and kidneys homogenates were determined and calculated by the method of Lowry et al [27].

Statistical Analysis. Differences between groups were statistically analyzed by one-way ANOVA, and the differences between the means of groups were separated by the least significant difference (LSD) test. All data were presented as mean \pm standard error. Values differing by $P < .05$ are regarded as significant. A computer program (SPSS 10.01, SPSS Inc. Chicago, IL, USA) was used for statistical analysis.

3. Results

All the animals were healthy, and no mortality was observed during the entire period of the experiment. The activities of hepatic and renal glutathione peroxidase (GPx), superoxide dismutase (SOD), and the levels of lipid peroxidation (LPO) and glutathione (GSH) were measured to assess the level

of antioxidant defense in female rats. BEP significantly increased ($P < .05$) hepatic LPO over the control and melatonin-treated animals (Table 2). However, no effects were observed on CAT activity in hepatic of BEP- and MEL-treated groups. Interestingly, GPx and SOD activity in hepatic tissues was significantly increased ($P < .05$) in both BEP- and MEL-administered groups. No effect of MEL was recorded on hepatic GSH and GR level, however, their levels were significantly higher ($P < .05$) in BEP-treated animals.

Similarly, the renal LPO level was significantly ($P < .05$) greater in BEP administered animals (Table 3). On the other hand, renal CAT, GSH, GR, and GPx levels were markedly enhanced in MEL and BEP-supplemented groups as compared to control animals (Table 3). However, effects on CAT and GSH were greater in BEP-treated animals than in those which were given MEL (Table 3). Superoxide dismutase activity in kidney tissues was unaffected in all groups.

4. Discussion

Markers of oxidative stress damage can be found in many disease states including renal damage, chronic heart disease, and liver disease [28, 29]. Thus, excess free radical formation is associated with many disease states. Many diseases and increased toxicity are often associated with oxidative stress in different vital organs and are characterized by the reductions in the activity of specific enzymes activity, including catalase, SOD, GPx, and GR [15].

We observed marked increases in renal CAT, GSH, GR, and GPx in MEL- and BEP-supplemented animals. Interestingly, GPx and SOD activities in hepatic tissues were also significantly increased ($P < .05$) in BEP- and MEL-administered groups. However, only hepatic GSH and GR levels were significantly higher ($P < .05$) in BEP-treated animals. These pharmacological effects of BEP and MEL may be relevant to their potentiation of antioxidant defense activity in renal and hepatic tissues [17–19]. Present study findings do not support the hypothesis of increased endogenous antioxidant activity in response to the deleterious/toxic effect of BEP in the rat's liver and kidney. Since, liver and kidney protective responses to any deleterious agents are immediate and transient, and so these endogenous protective/beneficial effects cannot be for long periods (beyond 2-weeks), as in our study. Therefore, findings of the present study support the antioxidant properties of BEP. Also, BEP increased not only the catalase (kidney), SOD (liver), GPx, and GR enzymes but also GSH level. GSH levels should have decreased if BEPs had a deleterious effect.

GPx removes H₂O₂ by coupling its reduction to H₂O₂ with oxidation of reduced glutathione, GSH. It is the most important enzyme for extraperoxisomal inactivation of

TABLE 2: Effects of different treatments on lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) in liver of female rats.

Group	Parameters					
	LPO (nM MDA/mL)	CAT (nM/min/mg protein)	SOD (U)	GR (nM/min/mg protein)	GPx (nM/min/mg protein)	GSH (μ M/g tissue)
Control	4.61 ^a \pm 0.11	291.03 ^a \pm 14.93	6.15 ^a \pm 0.37	145.85 ^a \pm 7.33	25.67 ^a \pm 1.87	3.30 ^a \pm 0.06
BEP	5.78 ^b \pm 0.21	293.45 ^a \pm 9.20	7.94 ^b \pm 0.24	189.17 ^b \pm 5.79	37.44 ^c \pm 1.70	4.49 ^b \pm 0.08
MEL	4.89 ^a \pm 0.13	300.40 ^a \pm 11.68	7.60 ^b \pm 0.33	139.32 ^a \pm 13.12	30.28 ^b \pm 1.03	3.50 ^a \pm 0.15

Values ($n = 6$; Means \pm S.E) in the same column bearing no superscript (^{a,b,c}) common vary significantly ($P < .05$).

TABLE 3: Effects of different treatments on lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) in kidney of female rats.

Group	Parameters					
	LPO (nM MDA/mL)	CAT (nM/min/mg protein)	SOD (U)	GR (nM/min/mg protein)	GPx (nM/min/mg protein)	GSH (μ M/g tissue)
Control	4.99 ^a \pm 0.13	251.70 ^a \pm 4.58	5.98 ^a \pm 0.17	126.64 ^a \pm 8.47	20.13 ^a \pm 1.11	3.50 ^a \pm 0.06
BEP	5.80 ^b \pm 0.11	327.49 ^c \pm 18.14	5.46 ^a \pm 0.31	168.14 ^b \pm 6.20	36.99 ^b \pm 3.69	5.91 ^c \pm 0.15
MEL	4.95 ^a \pm 0.12	284.10 ^b \pm 2.79	6.12 ^a \pm 0.34	152.82 ^b \pm 10.54	32.48 ^b \pm 1.92	4.19 ^b \pm 0.113

Values ($n = 6$; Means \pm S.E) in the same column bearing no superscript (^{a,b,c}) common vary significantly ($P < .05$).

H₂O₂, especially in the liver and kidney. Since the liver is a major source of GSH, metabolism of xenobiotics in the liver, which can drastically deplete liver GSH, may also result in GSH depletion in other tissues [30]. In numerous reactions, GPx, GR, and GSH act as free radical scavenging molecules and therefore the finding in our study that these enzymatic and nonenzymatic antioxidant defense systems are upregulated in liver and kidney of animals that exhibit oxidative stress underscore the critical importance of BEP and MEL as potentiators of antioxidant activity. Melatonin, the chief secretory product of the cerebral epiphysis, is a direct free radical scavenger and indirect antioxidant [31]. In addition to its direct free radical scavenging activity, MEL also enhances the synthesis of SOD, GSH, CAT, GR, and GPx [32].

In the present study, it was shown that BEP from the cerebral epiphysis possesses antioxidant properties exceeding in some cases the effects of the MEL. The superior effects of BEPs might be due to their direct antioxidant effects, but also because BEPs have been implicated in the stimulation of MEL production [33]. In recent studies, the role of BEP in the stimulation of MEL production was demonstrated [3]. Of relevance here is that MEL has a very short half-life and is metabolized and excreted within a few minutes. However, the presence of BEP may represent a constant source of stimulation for MEL synthesis. This might also be the reason that certain enzymes have greater antioxidative effects when compared to MEL action alone. Besides increasing the activity of the antioxidative enzymes, it is known that melatonin also increases their expression. This observation suggests that the indoleamine may have a physiological role in promoting endogenous antioxidative defense activity. These findings support the hypothesis that BEP and MEL modulate antioxidant defense of liver and kidney, and also demonstrate that these agents are generally equivalent in the potency of their of antioxidant activities.

5. Conclusions

These experimental findings establish that buffalo epiphyseal proteins and melatonin are effective antioxidants and that they may play a protective role against hepatic and renal damage induced by oxidative stress. The findings suggest that epiphyseal proteins and melatonin may have therapeutic potential as antioxidants drugs for the management of oxidative stress.

Conflict of Interests and Disclosure Statement

S. R. Pandi-Perumal is a stockholder and the President and Chief Executive Officer of Somnogen Inc., a New York Corporation. He declared no competing interests that might be perceived to influence the content of this paper. All remaining authors declare that they have no proprietary, financial, professional, nor any other personal interest of any kind in any product or services and/or company that could be construed or considered to be a potential conflict of interest that might have influenced the views expressed in this paper.

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