MELATONIN STIMULATES BRAIN GLUTATHIONE PEROXIDASE ACTIVITY

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(Received 19 September 1994; accepted 7 November 1994)

Abstract—Exogenously administered melatonin causes a 2-fold rise in glutathione peroxidase activity within 30 min in the brain of the rat. Furthermore, brain glutathione peroxidase activity is higher at night than during the day and is correlated with high night-time tissue melatonin levels. Glutathione peroxidase is thought to be the principal enzyme eliminating peroxides in the brain. This antioxidative enzyme reduces the formation of hydroxyl radicals formed via iron-catalyzed Fenton-type reactions from hydrogen peroxide by reducing this oxidant to water. Since the hydroxyl radical is the most noxious oxygen radical known, induction of brain glutathione peroxidase might be an important mechanism by which melatonin exerts its potent neuroprotective effects.

Melatonin acts as a primary non-enzymatic antioxidative defense against the devastating actions of the extremely reactive hydroxyl radical (Reiter et al., 1994, 1995b). Since the highly reactive and toxic hydroxyl radical cannot be detoxified enzymatically, such as the superoxide anion radical and hydrogen peroxide, low molecular weight antioxidants such as melatonin are the only endogenous protective defense against oxidative stress and damage induced by this electrophilic intermediate (Poeggeler et al., 1993, 1994).

Oxidative stress in general, and hydroxyl radicals in particular, inactivate superoxide dismutase, catalase and glutathione peroxidase, the enzymes involved in detoxifying reactive oxygen intermediates such as superoxide anion radicals and hydrogen peroxide (Nistico et al., 1992; Pigeolet and Remacle, 1991; Raes et al., 1987; Tabatabaie and Floyd, 1994). Glutathione peroxidase (EC 1.11.1.9) plays a key role in the enzymatic defense system against oxygen free radicals generated in the presence of peroxides by reducing hydrogen peroxide and lipid peroxides with the concomitant oxidation of glutathione (Michiels et al., 1994; Raes et al., 1987). However, compared to superoxide dismutase and catalase, glutathione peroxidase is also much more vulnerable to peroxide-induced and hydroxyl radical-mediated inactivation and degradation (Pigeolet and Remacle, 1991).

In aged animals, superoxide dismutase activity is increased without a concomitant, compensatory increase in glutathione peroxidase (Haan et al., 1992). Hydrogen peroxide accumulates and is converted partially to the highly reactive hydroxyl radical in a Fenton-type reaction catalyzed by transition metals (Fig. 1). Therefore hydroxyl radical formation is enhanced in aged animals and hydroxyl radical mediated oxidative stress and damage finally lead to neuronal cell degeneration and death (Beal, 1993; Haan et al., 1992; Poeggeler et al., 1993). It has been proposed that neuronal damage and death is the pacemaker of the aging process (Poeggeler et al., 1993).

Glutathione peroxidase protects neurons from oxidative stress and damage (Mirault et al., 1994). An enhanced glutathione peroxidase activity could slow

$$\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GSH-Px} \rightarrow 2\text{H}_2\text{O} + \text{GSSG}$$

Fig. 1. Reduction of hydrogen peroxide to water catalyzed by glutathione peroxidase and reduction of hydrogen peroxide to the highly hydroxy radical in the presence of iron chelates.
the supernatant decanted and recentrifuged at 100,000 g for 60 min at 4°C, excess fat was removed, and the supernatant (3 μl) was taken for protein determination with 50 mmol/l potassium chloride, pH 7.6. This and all chemicals were purchased from Sigma Chemical Co., St Louis, MO, except potassium cyanide which was obtained from Fluka Chemical Corp., Ronkonkoma, N.Y. The homogenate was centrifuged with 50 mmol/l potassium chloride, pH 7.6. The samples were also extracted with the same volume of chloroform. The chloroform extract of the brain tissue and the serum samples were evaporated overnight in the darkness and the dried extract was redissolved in the same volume of radioimmunoassay buffer. Recovery of exogenous standard melatonin was 69% on average for brain tissue (rat brain frontal cortex) and 66% on average for the serum samples. Serial dilution and parallel inhibition of endogenous and exogenous melatonin was established for both brain and serum extracts. Melatonin content was determined as previously described (Fraser et al., 1983).

**Statistical analysis**

Data are expressed as mean ± SEM. Statistical analysis of the data was performed using a Student's t-test.

**RESULTS**

Melatonin, administered at a pharmacological dose of 500 μg/kg i.p. during the day, elicited an immediate and long lasting increase in brain glutathione peroxidase activity (Fig. 2). Glutathione peroxidase activity is increased 2-fold at 30 min after administration of melatonin (Fig. 2); this increase persists for at least 180 min (Fig. 2) after the i.p. injection of the indoleamine. Nocturnal brain glutathione peroxidase activity is 2-fold higher than diurnal glutathione peroxidase activity (Fig. 3).

Brain melatonin levels are increased at least 10-fold 30 min after melatonin administration (Fig. 2), while brain melatonin levels are only 2-fold higher after 180 min (Fig. 2) compared to non-injected controls. Night-time melatonin levels are higher than daytime melatonin levels (Fig. 3). Circulating melatonin levels are 1000 times higher 30 min after administration of the indoleamine (Table 1), but are reduced to supraphysiological levels 3 h later (Table 1). Circulating serum melatonin levels are much higher in the night than during the day (Table 1).

Melatonin tissue levels are 1000 times higher than the circulating levels in the serum (Figs 2, 3 and Table 1).
Melatonin increases glutathione peroxidase

**DISCUSSION**

Melatonin boosts the most important endogenous enzymatic antioxidative defense mechanism in the brain, glutathione peroxidase activity (Figs 2 and 3). The increase in glutathione peroxidase activity is substantial and long lasting (Fig. 2). As shown herein, glutathione peroxidase can be induced by the administration of pharmacological amounts of melatonin. However, the diurnal rhythm in brain glutathione peroxidase activity might be related to the endogenous circadian rhythm of physiological tissue concentrations of the indoleamine.

It is apparent that melatonin administration not only increases the detoxification and elimination rate of the highly reactive and toxic hydroxyl radical by direct hydroxyl radical scavenging, but it also reduces the generation and formation of hydroxyl radicals by reducing the accumulation of peroxides through induction of glutathione peroxidase (Figs 2 and 3). Thus, the potent antioxidative effects of melatonin are caused by at least two different mechanisms, which might, however, be interdependent (Beloqui and Cederraum, 1986; Hardeland et al., 1993; Poeggeler et al., 1993; Reiter et al., 1994, 1995). It has been demonstrated that the glutathione–glutathione peroxidase system suppresses hydroxyl radical generation and prevents oxidative damage and destruction due to this highly reactive radical (Beloqui and Cederbaum, 1986).

Other indolic compounds with glutathione peroxidase stimulating activity such as the ergot alkaloid dihydroergocryptine are also able to prevent age-dependent and glutamate-induced neuronal degeneration due to peroxide accumulation and hydroxyl radical generation (Favit et al., 1993). The potent enzyme inducing effects of melatonin and structurally related
Fig. 3. Diurnal rhythm of glutathione peroxidase activity and melatonin immunoreactivity in the brain. The tissue was collected at 1200 and 2400 h (n = 8).

Table 1. Effects of melatonin (500 μg/kg i.p., at 1200 h) on daytime serum melatonin levels and diurnal rhythm of circulating melatonin levels. The serum was collected 30 min and 180 min after the administration of the indoleamine, and at 2400 h (n = 8).

<table>
<thead>
<tr>
<th>Time</th>
<th>Serum melatonin (pg/ml)</th>
<th>Mean</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime</td>
<td></td>
<td>20.50</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>30 min after injection</td>
<td></td>
<td>15,800.00</td>
<td>3,600.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>180 min after injection</td>
<td></td>
<td>78.00</td>
<td>12.00</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>Nighttime</td>
<td></td>
<td>126.00</td>
<td>18.00</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Indoles might also contribute significantly to the neuroprotective activity of these compounds and to the potent anti-aging effects elicited by melatonin and its naphtalenic analogs (Hoffen et al., 1993; Pierpaoli and Regelson, 1994; Poeggeler et al., 1993; Reiter et al., 1995).

Hydrogen peroxide accumulates in the brain of aged animals because superoxide dismutase gene expression and activity are increased without a concomitant, compensatory increase in the activity of catalase or glutathione peroxidase to detoxify this reactive oxygen intermediate (Haan et al., 1992). Aged animals are melatonin deficient and more sensitive to oxidative stress (Poeggeler et al., 1993; Reiter et al., 1994, 1995).

Recently a comparison was made between the short-lived house mouse (Mus musculus) and the long-lived white-footed mouse (Peromyscus leucopus). The glutathione peroxidase activity in the brain of white-footed mice is much higher than the enzyme activity in house mice (Sohal et al., 1993). The long-lived rodents have a much higher rate of hydrogen peroxide detoxification and a lower rate of hydrogen peroxide generation than do short-lived animals (Sohal et al., 1993). Likewise, the level of hydroxyl radical-mediated oxidative damage is significantly lower in the long-lived mice than in their short-lived counterparts (Sohal et al., 1993).

The efficiency of hydrogen peroxide elimination is a major factor in determining longevity and life span potential in mammals (Haan et al., 1992; Sohal et al., 1993). Aged rodents lose the ability to detoxify hydrogen peroxide efficiently (Haan et al., 1992). Hydroxyl radical-mediated oxidative damage to neurons increases exponentially with age, possibly in part related to the fact that aged animals and humans lose their melatonin-mediated antioxidative protection (Beal, 1993; Poeggeler et al., 1993; Reiter et al., 1994, 1995).

Melatonin and structurally related aromatic compounds with high resonance stability and electroreactivity are a class of chemoprotectants that act by several mechanisms, including radical scavenging, protection against thiol depletion and calcium over-load as well as enzyme induction (Chen et al., 1993, 1994; Hardeland et al., 1993; Poeggeler et al., 1993, 1994; Reiter et al., 1993–1995; Tan et al., 1993a, 1994). To date the putative interactions between the specific melatonin receptors, their activation and antioxidative protection, as controlled by glutathione peroxidase gene expression have not been investigated (Reiter et al., 1995).

It is extremely difficult to distinguish between specific receptor mediated mechanisms, e.g. enzyme induction and non-receptor mediated mechanisms such as radical scavenging, e.g. enzyme protection (Beloqui and Cederbaum, 1986; Reiter et al., 1994). Neither is it known whether the radical scavenging and receptor-mediated actions are exerted independently or whether they are highly interdependent (Reiter et al., 1995). The use of specific receptor agonists and antagonists as well as melatonin analogs with similar molec-
cular structure will enable us to distinguish between the direct and indirect antioxidative actions of melatonin and other structurally related molecules.

Acknowledgements—Work by the authors was supported by NSF grant IBN 9121262 (to R. J. Reiter), a Fedor Lynen Stipendium of the Alexander von Humboldt Foundation (to B. Poggeler) and a grant from the Japan Foundation for Aging and Health (to M. Abe). Dedicated to the memory of A. Menendez-Pelaez, a true and selfless colleague without whom the world will miss.

REFERENCES


