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Melatonin modulates drug-induced acute porphyria

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ABSTRACT

This work investigated the modulation by melatonin (Mel) of the effects of the porphyrinogenic drugs 2-allyl-2-isopropylacetamide (AIA) and 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-collidine (DDC) on oxidative environment, glucose biosynthesis and heme pathway parameters. Administration of Mel before rat intoxication with AIA/DDC showed a clear beneficial effect in all cases. Mel induced decreases of 42% and 35% in the excretion of the hemeprecursors 5-aminolevulinic acid (ALA) and porphobilinogen (PBG), respectively, and a 33% decrease in the induction of the heme regulatory enzyme 5-aminolevulinic acid-synthase (ALA-S). The activity of the glucose metabolism enzyme phosphoenolpyruvate carboxykinase (PEPCK), which had been diminished by the porphyrinogenic treatment, was restored by 45% when animals were pre-treated with Mel. Mel abolished the modest decrease in glucose 6-phospatase (G6Pase) activity caused by AIA/DDC treatment. The oxidative status of lipids was attenuated by Mel treatment in homogenates by 47%, whereas no statistically significant AIA/DDC-induced increase in thiobarbituric acid reactive substances (TBARS) was observed in microsomes after Mel pre-treatment. We hypothesize that Mel may be scavenging reactive species of oxygen (ROS) that could be damaging lipids, PEPCK, G6Pase and ferrochelatase (FQ). Additionally, Mel administration resulted in the repression of the key enzyme ALA-S, and this could be due to an increase in glucose levels, which is known to inhibit ALA-S induction. The consequent decrease in levels of the heme precursors ALA and PBG had a beneficial effect on the drug-induced porphyria. The results obtained open the possibility of further research on the use of melatonin as a co-treatment option in acute porphyria.

1. Introduction

Melatonin (Mel), which derives from the serotoninergic pathway of tryptophan, is a neurohormone with antioxidant properties [28]. It is mainly produced in the pineal gland but also in other organs. Mel has a number of physiological effects including the regulation of circadian rhythms, oncostasis, stimulation of the immune system and the regeneration of sexual activity, as well as being anti-inflammatory [8]. Mel has been identified as a powerful direct free radical scavenger and an antioxidant of broad spectrum even more potent than glutation (GSH). It can cross the hemato-encephalic and placental barriers [32,29]. Numerous investigations have shown its ability to reduce the molecular damage caused by reactive species of oxygen (ROS) and reactive nitrogen species (RNS) [8]).

Porphyrias are hereditary disorders caused by a de-regulation of the heme pathway due to deficiencies in the activity of some of its enzymes, which lead to the accumulation of heme precursors and diminished heme formation. This deficiency triggers the induction of the regulatory enzyme 5-aminolevulinic acid-synthase (ALA-S) [3]. Life-threatening acute porphyrias are biochemically characterized by the accumulation of heme precursors such as 5-aminolevulinic acid (ALA), which promotes oxidative stress by generating ROS [3,6].

The porphyrinogenic drug 2-allyl-2-isopropylacetamide (AIA) enhances the destruction of liver heme, particularly cytochrome P-450, whereas the porphyrinogenic drug 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-collidine (DDC) strongly depletes heme by combination of heme destruction and inhibition of heme synthesis [18]. AIA/DDC treatment results in acute heme deficiency, an impairment of ferrochelatase (FQ) activity, a marked de-repression of ALA-S and, as a consequence, an exacerbated production of ALA and other heme precursors in the liver [14]. This combined treatment has been reported to induce a rat experimental porphyria

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resembling quite accurately acute variegate porphyria [14]. Accumulated ALA has been associated with iron-mediated oxidative damage to biomolecules and cell structures through ROS generation [6]. ROS are able to oxidize nucleic acids, proteins, lipids, and carbohydrates, affecting key cellular functions [6]. It has been demonstrated that AIA/DDC treatment promotes the establishment of an oxidative environment with increases in ROS [14].

Glucose administration is known to have beneficial effects on acute porphyria patients, significatively improving biochemical parameters and clinical conditions [3]. The prevention of acute experimental porphyria through a carbohydrate-rich diet in animal models illustrates the effect of glucose, which prevents the induction of the heme pathway regulatory enzyme ALA-S [33]. Furthermore, it has been reported that treatment with AIA/DDC hinders gluconeogenesis and glycogenolysis leading to a reduction in glucose availability in hepatocytes. In particular, hepatic phosphoenolpyruvate carboxykinase (PEPCK) and glycogen phosphorylase (GP) activities have been found impaired in AIA/DDC-induced rat porphyria [14,20].

The relationship between carbohydrates and oxidative status with porphyria, plus the known anti-oxidant and radical-scavenging properties of Mel, prompted the present investigation about Mel as a possible modulator of AIA/DC-induced experimental porphyria. Effects were studied at the level of oxidative environment, glucose biosynthesis and heme pathway by measuring key enzymes of the heme and glucose pathways as well as porphyria and lipid peroxidation markers.

2. Materials and methods

2.1. Materials

AIA was a gift from Roche Co. (Germany). DDC was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI). Melatonin, bovine serum albumin, deoxyguanosine 5′-diphosphate, glucose-1-phosphate, malate dehydrogenase, NADH, Dowex 1, Dowex 50 W and thiobarbituric acid were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade.

2.2. Animal treatments

Female Wistar rats (180–200 g) were purchased from the National Committee of Atomic Energy (CONEA, Argentina). They were maintained on food and water ad libitum and housed under conditions of controlled temperature (25 °C) and light (12 h light–dark cycle, light from 6am to 6pm).

Animals were treated according to International Guidelines (Guide for Care and Use of Laboratory Animals, National Research Council, USA, 1996, the Council of the European Communities Directive, 86/609/ECC) and to guidelines from the Animal Care and Use Committee of the Argentine Association of Specialists in Laboratory Animals (AADEALC). AIA was dissolved in saline solution (0.9% NaCl, w/v) and DDC was dissolved in corn oil [5] just before administration. Melatonin was dissolved in pure ethanol. The solution was protected from light and kept at 4 °C until injection. Ethanolic stocks were diluted with 0.9% NaCl (w/v) to achieve a ratio 0.9% NaCl/ethanol of 20/1 (v/v, melatonin vehicle). Mel was dissolved in saline just before being ip administered.

A total of 3 experiments were performed, each involving 8 randomly selected animals divided in 4 groups of 2 animals. The four groups were as follows: "−Mel,−AIA/DDC", not pretreated with Mel nor treated with AIA/DDC; "−Mel,+AIA/DDC", not pretreated with Mel and treated with AIA/DDC; "+Mel,−AIA/DDC", pretreated with Mel but not treated with AIA/DDC; and "+Mel,+AIA/DDC", pretreated with Mel and then treated with AIA/DDC. Mel (10 mg/kg) body weight (bw) or Mel vehicle were intraperitoneally (ip) injected 3 times a day at 8am, 12pm and 4pm during 2 days. At the end of this treatment, intoxication with AIA (300 mg/kg), bw, subcutaneously (sc) and DDC (50 mg/kg bw, ip) was performed. In the case of controls, Mel, AIA and DDC vehicles were administered. Mel dosage was based on studies about its drug toxicity-reduction and drug protective ability [27,11,13]. Rats were fasted 8 h before and 16 h after intoxication. Access to drinking water was free. Parameters were measured in duplicate at 16 h post-intoxication, in all cases. Urine was collected during the whole period of 16 h after intoxication by using individual metabolic cages for each animal.

2.3. Liver extracts

Rats were euthanized by carbon dioxide inhalation followed by decapitation, 16 h after AIA/DDC administration. Animals had been fasted for a total of 24 h before death, as this has been previously shown to result in reliable measurements of PEPCK and glucose 6-phospatase (G6Pase) activities [22]. Livers previously perfused with ice-cold saline solution, were removed and portions from them were homogenized in a Potter-Elvehjem homogenizer using different solutions according to the assays to be performed, in all cases at 0–4 °C. Liver portions of approximately 1 g were excised and immediately homogenized in 3 ml of a solution containing 0.9% NaCl, 0.1 mM Tris–HCl pH 7.4 and 0.5 mM EDTA. These extracts were employed to determine ALA-S activity [19]. For PEPCK activity, liver was homogenized (1:3 w/v) in 0.25 M sucrose. Homogenates were centrifuged for 1 h at 100000*xg* and the resulting supernatants were used for PEPCK activity measurements while the pellets, properly resuspended, were used for microsomal G6Pase enzymatic determination. All procedures were carried out at 4 °C. For hepatic FQ determination, homogenates were prepared with 0.154 M KCl (1:5 w/v). Homogenates were centrifuged for 25 mim at 11000x*g* and the resulting pellets were frozen until hepatic FQ activities were determined. Lipid peroxidation was determined in homogenates (those used also for PEPCK activity tissue preparations) and microsomal fractions (those used also for G6Pase enzymatic determination)

2.4. Metabolite levels and enzyme assays

2.4.1. 5-Aminolevulinic acid and porphobilinogen in urine

0.3–1.0 ml samples from urine collected during 16 h after intoxication adjusted to pH 4.5–6.0, was placed on the top of two column, anion–cation exchange assembly as described by Piper et al. [25]. Then the column assembly was washed three time with 8 ml of distilled water and then the columns were separated. PBG, bound to the anion exchange Dowex 1 column, was eluted by 2 ml of 1 M acetic acid followed by 3 ml of 0.2 M acetic acid, whereas ALA bound to the cation exchange Dowex 50W column was eluted with 7 ml of 1 M sodium acetate. ALA pyrrole and PBG were determined colorimetrically as described by Mauzerall and Granick [21].

2.4.2. 5-Aminolevulinic acid synthase activity

ALA-S activity was assayed in whole liver homogenates by the method of Marver et al. [19]. Incubation mixtures containing 0.1 M glycine, 0.01 M EDTA, 0.08 M Tris–HCl buffer pH 7.2 and 0.5 ml of homogenate in a final volume of 2 ml were incubated at 37 °C for 60 min. The reaction products were determined spectrophotometrically at 553 nm [21].

2.4.3. Ferrochelatase activity

FQ activities were measured in liver mitochondrial fractions. Pellets containing mitochondria were used after being frozen at −20 °C for 24–48 h. Enzymatic activities were determined in