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How porphyrinogenic drugs modeling acute porphyria impair the hormonal status that regulates glucose metabolism. Their relevance in the onset of this disease

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ABSTRACT

This work deals with the study of how porphyrinogenic drugs modeling acute porphyrias interfere with the status of carbohydrate-regulating hormones in relation to key glucose enzymes and to porphyria, considering that glucose modulates the development of the disease. Female Wistar rats were treated with 2-allyl-2-isopropylacetamide (AIA) and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) using different doses of AIA (100, 250 and 500 mg/kg body weight) and a single dose of DDC (50 mg DDC/kg body weight). Rats were sacrificed 16 h after AIA/DDC administration. In the group treated with the highest dose of AIA (group H), hepatic 5-aminolevulinic acid synthase (ALA-S) increased more than 300%, phosphoenolpyruvate carboxykinase (PEPCK) and glycogen phosphorylase (GP) activities were 43% and 46% lower than the controls, respectively, plasmatic insulin levels exceeded normal values by 617%, and plasmatic glucocorticoids (GC) decreased 20%. GC results are related to a decrease in corticosterone (CORT) adrenal production (33%) and a significant reduction in its metabolization by UDP-glucuronosyltransferase (UGT) (62%). Adrenocorticotropic hormone (ACTH) stimulated adrenal production 3-fold and drugs did not alter this process. Thus, porphyria-inducing drugs AIA and DDC dramatically altered the status of hormones that regulate carbohydrate metabolism increasing insulin levels and reducing GC production, metabolization and plasmatic levels. In this acute porphyria model, gluconeogenic and glycogenolytic blockages caused by PEPCK and GP depressed activities, respectively, would be mainly a consequence of the negative regulatory action of insulin on these enzymes. GC could also contribute to PEPCK blockage both because they were depressed by the treatment and because they are positive effectors on PEPCK. These disturbances in carbohydrates and their regulation, through ALA-S de-repression, would enhance the porphyria state promoted by the drugs on heme synthesis and destruction. This might be the mechanism underlying the "glucose effect" observed in hepatic porphyrias. The statistical correlation study performed showed association between all the variables studied and reinforce these conclusions.

1. Introduction

The hepatic heme pathway is closely regulated by its end product. Heme exerts a negative feedback control on 5-aminolevulinic acid (ALA)-synthase (ALA-S), which is the rate-limiting step of the pathway (Fig. 1).

Porphyrias are hereditary disorders in the heme metabolic pathway. They are caused by the de-regulation of its synthesis route due to a deficiency in some of the enzymes of the pathway, leading to lower heme formation. This deficiency triggers the accumulation and excretion of porphyrins and/or their ALA and porphobilinogen (PBG) precursors, and the induction of regulatory enzyme ALA-S (Kappas et al., 1995).

Acute porphyrias are the most dangerous type since they can be fatal. They are also the most susceptible to be triggered and exacerbated by drugs and metabolic factors. Acute porphyrias are hepatic diseases characterized by the accumulation of ALA and PBG precursors and by a neuropsychiatric syndrome (Kappas et al., 1995), which can be promptly relieved by intravenous heme infusion (Kappas et al., 1995; Litman and Correia, 1983).

2-Allyl-2-isopropylacetamide (AIA) increases the destruction of liver heme, particularly that of cytochrome P-450 (Smith and De

Abbreviations: ACTH, adrenocorticotropic hormone; AIA, 2-allyl-2-isopropylacetamide; ALA, 5-aminolevulinic acid; ALA-S, ALA-synthase; CORT, corticosterone; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; GC, glucocorticoids; GP, glycogen phosphorylase; HCB, hexachlorobenzene; PBG, porphobilinogen; PCT, porphyria cutanea tarda; PEPCK, phosphoenolpyruvate carboxykinase; UGT, UDP-glucuronosyltransferase.

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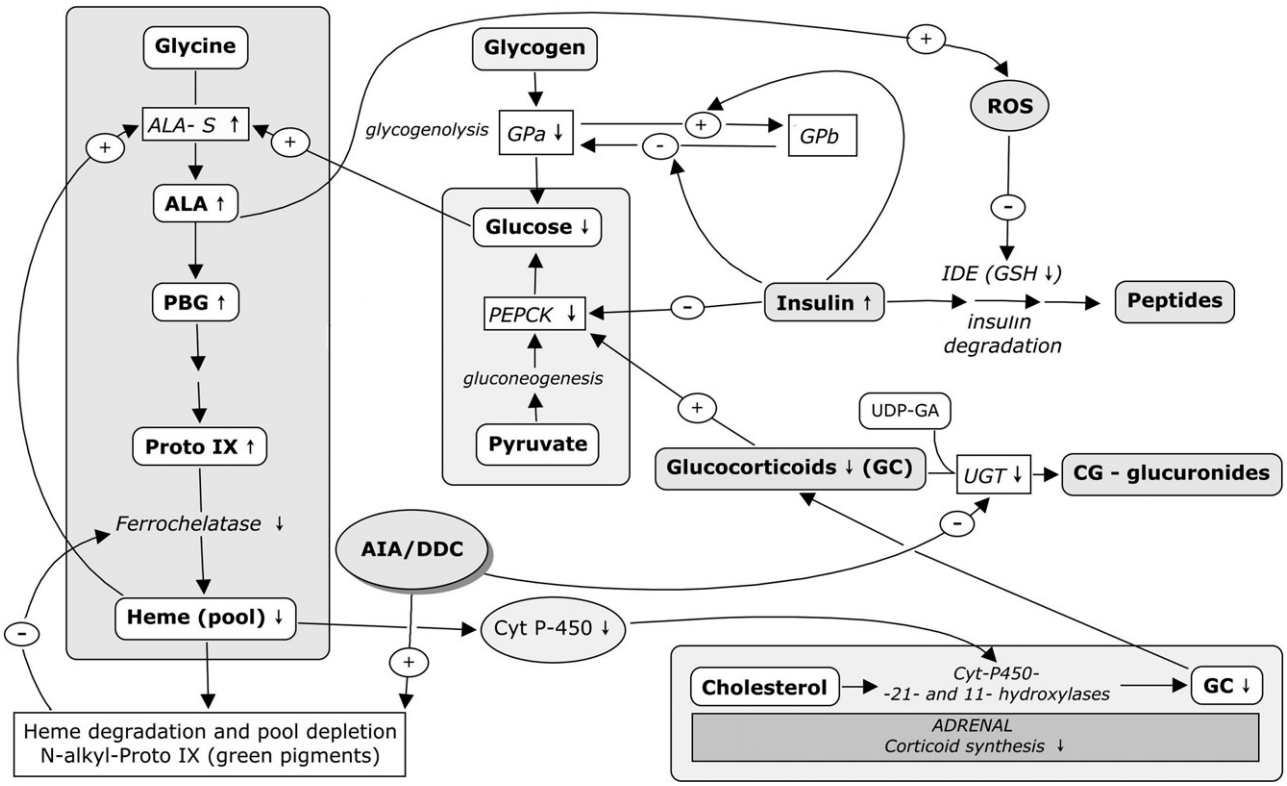

Fig. 1. Scheme on the effect of AIA/DDC treatment on different metabolic routes (carbohydrates, glucocorticoids and heme pathway) and impact on the gluconeogenic and glycogenolytic blockages of PEPCK and GP levels leading to glucose decrease. ALA, 5-aminolevulinic acid; ALA-S, ALA-synthase; PBG, porphobilinogen; Proto IX, protoporphyrin IX; Cyt P-450, cytochrome P-450; GPa and GPb, active and inactive forms of glycogen phosphorylase; GSH, glutathione; UDP-GA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase; IDE, insulin-degrading enzyme; ROS, reactive oxygen species.

Matteis, 1980). 3,5-Diethoxycarbonyl-1,4-dihydrocollidine (DDC) is a potent depletor of hepatic heme since it is able to both degrade heme and inhibit its synthesis (Marks et al., 1988). The combined treatment of AIA and DDC resulted in acute heme deficiency, marked ALA-S de-repression and, consequently, exacerbated production of ALA and other heme precursors in the liver (Ortiz de Montellano et al., 1981; Lelli et al., 2005) (Fig. 1). This combined treatment has been reported to induce an experimental porphyria resembling quite accurately acute variegate porphyria in rats (Lelli et al., 2005). Moreover, it has been demonstrated that ALA promotes the generation of reactive oxygen species (Monteiro et al., 1989) (Fig. 1).

Glucose administration is known to have beneficial effects on acute porphyria patients, by significantly improving their clinical and biochemical condition (Bonkovsky, 1990; Doss et al., 1985; Kappas et al., 1995). The prevention of acute experimental porphyria through high carbohydrate and/or protein intake is an example of the effect of glucose on ALA-S, with carbohydrates preventing its induction (Tschudy et al., 1964).

Phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme of gluconeogenesis, plays a key role in glucose synthesis both in the liver and the kidney (Hanson and Reshef, 1997). PEPCK gene expression can be increased by several factors such as cyclic AMP, thyroid hormones and glucocorticoids (GC). Conversely, it has been found that it can be inhibited by insulin (Hanson and Reshef, 1997) (Fig. 1). Recently, it has been reported that combined AIA and DDC treatment not only alters the heme pathway but also impairs carbohydrate metabolism by producing, among other disturbances, the gluconeogenic blockage of PEPCK (Lelli et al., 2005).

Glycogen phosphorylase (GP) catalyzes the rate-limiting step in the degradation of glycogen in animals. Glucagon and epinephrine activate GP, whereas insulin decreases its activity. A marked reduction in the glycogenolytic activity of GP has been reported in a rat AIA/DDC acute porphyria model (Lelli et al., 2005).

GC are cholesterol-synthesized steroid hormones that stimulate the expression of gluconeogenesis enzymes, particularly in the liver.

As regards steroid hormone disturbances caused by porphyria-inducing drugs, Lelli et al. (2007) have shown that hexachlorobenzene (HCB) promotes a significant decrease in plasmatic corticosterone (CORT) adrenal synthesis, as well as in their hepatic receptors. It has also been reported that the porphyrinogenic agent AIA alters the hydroxylation of testosterone in rat liver microsomal fraction (Hodgins et al., 1973).

Microsomal UDP-glucuronosyltransferases (UGTs) are a family of isoenzymes which transfer glucuronic acid from UDP-glucuronic acid to endogenous and exogenous compounds and/or their metabolites (Fig. 1), rendering these substances more polar and facilitating their excretion through bile and urine (Burchell and Coughtrie, 1989; Tephly and Burchell, 1990). Two families of UGT (designated UGT1 and UGT2) have been found both in humans and rats. UGT1 isoenzymes are encoded by a single gene (Owens and Ritter, 1995) and are known to conjugate bilirubin and phenols. Protein-encoded UGT2 are steroid-metabolizing enzymes with distinct but overlapping substrate specificities (Mackenzie et al., 1996; Turgeon et al., 2001).

Taking into account that AIA/DDC treatment blocks PEPCK and GP, that PEPCK is positively regulated by GC and negatively regulated by insulin, and that AIA alters the level and synthesis of cholesterol (the biosynthetic precursor of GC) and also impairs the metabolization of testosterone (another steroid hormone), it seemed interesting to study the effect of porphyrinogenic drugs AIA and DDC on the synthesis, metabolization and release of GC, as well as on plasmatic insulin levels, in order to find the potential