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Review article

The neurotoxic effects of prenatal cardiac glycoside exposure: A hypothesis

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Abstract

Cardiac glycosides (CGs) are beneficial in treating cardiac conditions; depending on time and dosage, they can also be toxic as they regularly cross the blood brain barrier and the placenta and may affect the unborn baby. This paper therefore focuses on the effects of CGs administered to the mother on normal cellular physiology of the foetus with specific reference to neural tissue. CGs act by binding to the Na^+/K^+-ATPase and decrease or inhibit Na^+-K^+ pump activity. In the foetus, CGs may disrupt ion homeostasis. An over-dosage of CGs or when it is taken during pregnancy, can also affect the neuro-energy levels of brain tissue in particular. We conclude and hypothesize that CGs in this case will not only cause severe alterations in neuronal function due to disruption of membrane activity, but also in glutamate clearance, affecting neurotransmission in general. Furthermore, elevated cytosolic Ca^{2+} will lead to permeabilization of the mitochondrial membranes, resulting ultimately in mitochondrial dysfunction. This will result in neurotoxicity — ensuing in neural cell damage or death, and we propose the mechanism to be due to neuro-necrapoptosis.

Keywords: Cardiac glycosides; Na^+/K^+ pump; Pregnancy; Mitochondria; Neuro-necrapoptosis

Contents

1. Introduction

Pharmaceutical products with cardiovascular actions used for stimulating the heart muscle are classified as cardiac glycosides (CGs). These products act on conducting tissue by changing the functioning of the depolarisation of the membrane-linked Na^+/K^+ pump. The therapeutic actions of CGs are

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well-recognized. As early as 1775 Withering described the value of CGs in the treatment of cardiac failure [11]. One of the most well-known CGs comes from the foxglove family of plants of which the genus *Digitalis* is probably the most important, renowned for its CG action. The foxgloves contain many CGs with similar actions. Digoxin is probably also one of the most important CGs from a therapeutic point of view, and Ouabain is another CG, but more short-acting.

Cardiac glycosides are beneficial in treating cardiac conditions, but their effect is dependent on timing and dosage. For instance, an overdose can be toxic [74], affecting not only cardiac cells but also other conducting tissues such as the brain [40,120]. CGs readily cross the blood–brain barrier (BBB) [10,63], and the brain contains specific binding sites for CGs [73]. Encephalopathy is a common occurrence after overdose [10,63]. Cardiac glycosides are toxic to cardiac and neural cells because of a combination of direct effects on the myocardium and of neurally mediated increases in autonomic activity [43].

Furthermore, CGs are often prescribed in pregnancy for the treatment of maternal as well as foetal heart conditions [93], and these drugs may be life-saving for both the mother and baby. CGs are readily transferred across placenta [84,93,100]: after i.v. administration, they are detected within two minutes in the umbilical blood of the foetus, while foetal CG plasma concentrations approximate the maternal values within five hours after oral drug administration [104]. In addition, CGs administered to the mother may alter the maternal–placental transfer of other essential amino acids to the foetus [128]. This paper focuses on the effects of CGs on normal cellular physiology of the foetus with specific reference to neural tissue, when administered to the mother. We also relate possible cytotoxic effects to apoptosis and necrosis in a foetus prenatally exposed to CGs, since it affects the Na^+/K^+ pump system of conducting tissues, including neural cells.

2. Literature review

2.1. CGs and the NA^+/K^+ pump

CGs act by binding to the Na^+/K^+-ATPase and *decrease or inhibit* $Na^+–K^+$ pump activity [12,71]. During binding of CGs to these pumps, Na^+ enters the cell, resulting in the cell attempting to maintain the osmotic balance by pumping Na^+ out. Consequently, Ca^{2+} is being pumped into the cell. This increase of intracellular Ca^{2+} is considered to be a critical event as it may affect intracellular organelles, e.g., the ER and mitochondria; these two organelles are both involved in intracellular Ca^{2+} regulation and homeostasis. Increases in intracellular Ca^{2+} may, therefore, cause dysfunction there and, additionally, enhance free radical generation [80], resulting in elevated peroxidation of cellular and subcellular membranes. Furthermore, increased intracellular Ca^{2+} may also lead to increased nuclear Ca^{2+}, which in turn initiates the expression of pro-apoptotic genes and the fragmentation of nuclear DNA and programmed cell death [4,80].

As cell membranes of neural tissue contain three iso-enzymes of digitalis receptors (alpha 1, alpha 2, alpha 3) [31], CGs can bind to and inhibit the action of the Na^+/K^+ pump in neural tissue [40,42,91,119]. Furthermore, CGs may also affect other neural cells, e.g., astrocytes. While astrocytes do not generate action potentials upon membrane depolarization [9,107], voltage-sensitive Na^+ channels are expressed in their membranes [107]: thus, CGs can inhibit active extrusion of Na^+ via their action on Na^+, K^+-ATPase, thereby enhancing intracellular Na^+ accumulation and causing significant cell death [113].

Because CGs affect the conducting function of neurons via the $Na^+–K^+$ pumps, they should ultimately also affect neurotransmission. Vaillend and co-workers in 2002 studied the effects of partial inhibition of $Na^+–K^+$-ATPases on neuronal hyperexcitability by the low-affinity CG, dihydroouabain (DHO) [120]. They found that the CG reversibly induced interictal-like epileptiform bursting activity in the CA1 region of the brain, due to the fact that the CG disturbed the function of GABA, an inhibitory neurotransmitter. Furthermore, burst-firing was correlated with inhibition of the pumps, and it was found that DHO induced a transient depolarization followed by a long-lasting hyperpolarization in CA1 pyramidal neurons. This was accompanied by a 30% decrease in resting input resistance. The authors also found reduced GABAergic potentials and enhanced excitatory postsynaptic potentials and spike firing. They concluded that this is the primary mechanisms underlying the hyperexcitability (due to dysfunction of the inhibitory function of GABA) associated with impaired $Na^+–K^+$ pump activity [120].

However, we also suggest that a second mechanism is prevalent here, namely CG's effects on astrocytes, their function in GABAergic pathways and, ultimately, in the glutamate (excitory neurotransmitter) pathway. Glutamine, glutamate and GABA are essential amino acids indispensable for brain metabolism and function. Astrocytic-derived glutamine is the precursor of *both* glutamate and GABA. Glutamate is produced as neurotransmitter by the neurons, but because it is neurotoxic after it has fulfilled its neurotransmission function, it needs to be removed fast and efficiently by the astrocytes [102]. In the astrocytes it is converted to glutamine, which is shunted back to the neurons to again produce glutamate as well as GABA. However, glutamate is neurotoxic if it is not removed adequately by astrocytes and converted to glutamine. In addition to the neurotransmitter roles of glutamate and GABA, they act as alternative metabolic substrates that enable metabolic communication between astrocytes and neurons [108]. Because CGs may cause astrocyte death by inhibiting active extrusion of Na^+ by $Na^+–K^+$-ATPase, glutamate will not be efficiently converted to glutamine in the astrocytes [113]. As it is neurotoxic, both the GABAergic and glutamate pathways will be affected. Elevated concentrations of glutamate in the synaptic clefts because of inability of astrocytes to remove it adequately will additionally result in the excessive activation of the glutamate receptors, also resulting in neuronal death [21,52,114]. Furthermore, the dominant astrocytic glutamate transporter, GLT-1, is Na^+-dependent [95,116]. Disruption of this transporter may contribute to neuronal damage in conditions like stroke, trauma, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease [14,22,26,29,44,52,68,77,95,96,102,112,113]. This GLT-1 transporter is also closely involved in protecting neurons [52]. Researchers found that treatment of the neural and astrocytic cell