Pharmacological Reports
2011, 63, 1243–1247
ISSN 1734-1140

Short communication

Digoxin increases hydrogen sulfide concentrations in brain, heart and kidney tissues in mice

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Abstract:
The interest in digoxin has recently increased due to the expanding knowledge regarding endogenous cardiac glycosides and a potential oncological application of this drug. Hydrogen sulfide (H_2S), a crucial co-modulator of various physiological processes, is involved in the pathophysiology of different disorders and may be useful in the treatment of some diseases. The interaction between cardiac glycosides and H_2S is unknown. The aim of the study is to assess the influence of digoxin on H_2S tissue concentrations in mouse brain, heart and kidney. Thirty male BALB/c mice were given intraperitoneal injections of digoxin at 0.5 mg/kg body weight (b.w.) per day (group D1, n = 10) or 1 mg/kg b.w. per day (group D2, n = 10). The control group (n = 10) received physiological saline. Free H_2S tissue concentrations were measured *via* the Siegel spectrophotometric modified method. There was a significant, progressive increase in the H_2S concentrations for both the low and high digoxin doses in the brain (7.7% and 8.5%, respectively), heart (by 6.0% and 22.1%, respectively) and kidney (by 7.6% and 13.0%, respectively). This report shows that digoxin administration is followed by an increase in the free H_2S concentrations in mouse brain, heart and kidney tissues.

Key words:
hydrogen sulfide, cardiac glycosides, digoxin, heart, mice

Abbreviations: Akt – protein kinase B, CTS – cardiotonic steroids, ERK – extracellular signal-regulated protein kinase, H_2S – hydrogen sulfide, K_{ATP} – ATP-sensitive potassium channels, NO – nitric oxide, PI3K – phosphoinositide 3-kinase, PKC – protein kinase C

Introduction

Recent studies have shifted the perspective on hydrogen sulfide (H_2S) from a dangerous industrial and environmental toxin to a crucial co-regulator of various physiological processes in mammals [15]. Moreover, H_2S has been shown to be involved in the development of different clinical disorders in many branches of medicine [18]. The importance of H_2S is so pervasive that several pharmaceutical companies are already working on H_2S-based agents to treat cardiovascular diseases and other disorders [24].

Plant extracts containing cardiac glycosides were used by the ancient Egyptians, Romans and Syrians as emetics and heart tonics, and medieval warriors added

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it to their arrows to poison targets. In the twentieth century, cardiac glycosides were established as an important agent in the treatment of heart failure [21]. In the era of evidence-based medicine, cardiac glycosides were pushed aside following the release of the Digitalis Investigation Group results [1]. However, these compounds were later resurrected with clinical *post-hoc* reanalysis (low digoxin concentrations significantly reduced mortality and hospitalizations in chronic heart failure patients) [2, 25]. They are again extensively researched with new vistas in oncology, endogenous synthesis (endogenous cardiotonic steroids – CTS) discovery and complex physiological actions explored [17, 39].

The interaction between cardiac glycosides and endogenous H_2S is unknown. The aim of this study is to assess the influence of digoxin on endogenous H_2S concentrations in mouse brain, heart and kidney tissues.

Materials and Methods

Animals

Thirty BALB/c strain male mice (8–9 week old individuals) weighing approximately 20 g were involved in the study. The animals were housed under standard laboratory conditions and had free access to water and food. They were kept at 22–24°C with a light/dark cycle of 12 h (8 am – 8 pm, and 8 pm – 8 am, respectively).

Study design

An injectable solution of purified cardiac glycoside digoxin (Digoxin WZF, Polfa Warszawa, Poland) was used. Intraperitoneal injections of 0.5 mg per kg b.w. of digoxin (group D1, n = 10) or 1 mg per kg b.w. of digoxin (group D2, n =10) were given daily for 5 consecutive days at the same time of the day (10:00 am) in 0.2 ml of saline solution. The control group (n = 10) received physiological saline at the same rate and volume. The individuals were randomly assigned to each group. The animals tolerated the applied doses of digoxin well and remained in good condition throughout the duration of the experiment. Measurements of the free H_2S concentrations were performed using the modified method of Siegel [28, 30].

The study was performed in accordance with the guidelines for the care and use of laboratory animals accepted by Bioethical Committee of the Jagiellonian University Medical College (Kraków, Poland).

Tissue samples preparation

Two hours after the last injection, the animals were killed by cervical dislocation, and their brains, hearts, and kidneys were quickly removed, and homogenized with 0.01 mol/l sodium hydroxide (NaOH) at a ratio of 1:4 for brain, 1:5 for kidney and 1:10 for heart and frozen. Then, 50% trichloroacetic acid (TCA) was added (0.5 ml to 2 g of brain samples in tight capsules of 3 ml and 0.25 ml to 1 g of heart or kidney sample in tight capsules of 2 ml), and the suspension was shaken and centrifuged. Subsequently, 1.5 ml brain and 0.75 ml heart or kidney supernatant samples were moved to 2 ml tight capsules with 0.15 ml or 0.075 ml of 0.02 mol/l N,N-dimethyl-p-phenyldiamine sulfate in 7.2 mol/l hydrochloric acid (HCl). Then, 0.15 ml or 0.075 ml portions of 0.03 mol/l iron(III) chloride ($FeCl_3$) in 1.2 mol/l HCl were added, respectively. After 20 min in darkness, the content was shaken for 1 min with 1 ml of chloroform.

H_2S tissue concentration measurements

Absorbance was measured at 650 nm with the Varian Cary 100 spectrophotometer. A standard curve was plotted with an iodometrically determined 0.0001 mol/l sodium sulfide (Na_2S) solution. For all groups of the animals, four concurrent analyses of each tissue type were performed.

Statistical analysis

Statistical analysis was performed within the R Environment using Student's *t*-test. Values of $p < 0.05$ were considered to be statistically significant.

Results and Discussion

There was a significant progressive increase in the H_2S concentration along with the increasing digoxin doses as compared to the control group in the brain (D1 by 7.7%, D2 by 8.5%), heart (D1 by 6.0%, D2 by