毛地黃對 Na,K-ATPase 異構酵素在量與分佈的影響

Effect of digitalis treatment on the abundance and distribution of subunit isoforms of Na,K-ATPase

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一、中文摘要

毛地黃 (digitalis) **長期以來，用來治療心臟衰竭的病人，可以增強心肌的收縮力，其機制是經由抑制** Na, K-ATPase**，使細胞內鈉離子的濃度增加，進而抑制鈣-鈉離子的交換，使肌細胞質內的鈣離子濃度增加，促使肌細胞收縮。這個計劃的目的即在了解長期使用毛地黃對** Na,K-ATPase **異構酵素** (isoforms) **的影響。我們過去的研究顯示老鼠的紅血球母細胞**(erythroblast) **在去核過程中，使細胞內的鈉離子濃度增加而造成細胞質內的** Na, K-ATPase **異構酵素，**α3 isoform **增加很多。這與毛地黃抑制** Na,K-ATPase **會使細胞內的鈉離子濃度增加的機制相似。因此在這個研究計劃裡，我們將滲透壓膠囊**(Alzet mini-osmotic pump, model 2002) **植入老鼠腹部皮下。以** 30 μg/kg/day **的速率，將毛地黃注入老鼠體內，以觀察其** Na,K-ATPase **異構酵素，在腎臟細胞的變化。結果發現植入含有毛地黃的滲透壓膠囊的老鼠，其** α1 **異構酵素**(α1 isoform) **在遠曲小管**(distal convoluted tubule)**有明顯增加，近曲小管**(proximal convoluted tubule)**則無。其他如：**α2**，** α3 **異構酵素則沒有太大的改變。我們同時觀察到紅血球的** α1**異構酵素，也有增加的現象。目前，我們正在檢測其他段的腎小管褪心肌細胞是否有相同的變化。**

關鍵字：毛地黃，Na, K-ATPase **異構酵素，滲透壓膠囊，腎臟細胞**

二、英文摘要

Digitalis is a drug commonly used to improve cardiac contractility in patients with congestive heart. The mechanism involved is the inhibition of Na,K-ATPase by digitalis resulting in a decrease in the Ca/Na exchange and an accumulation of the intracellular concentration of the cardiotonic Ca ions. The goal of this project is to study the long term effect of digitalis treatment on the subunit isoforms of the Na,K-ATPase. In a previous study, we have observed an increase in the intracellular Na ions in erythroblasts during denucleation and an increase in the α3 isoform of this Na transport enzyme. Inhibition of this enzyme by digitalis will also cause an increase in intracellular Na concentration. In this project, we have used a mini-osmotic pump (Alzet mini-osmotic pump, model 2002) implanted subcutaneously in the abdomen area of rats to provide continuous infusion of the drug at a rate of 30 μg/kg/day. We have examined the effect of this long term infusion on the Na,K-ATPase in the renal tubular cells. We found that the treatment with digitalis causes the α1 isoform subunit to increase in the distal

convoluted tubule (DCT), whereas no such increase is observed in proximal convoluted tubule (PCT). There are no significant changes in the α2 and α3 isoforms. In addition, we also have observed a similar increase in the α1 isoform in the red blood cells of the treated rats. The sensitivity of the α1 to digitalis is different from that of α2 or α3. We are presently attempting to understand whether this observation is related to the different requirement of transport activity at various parts of the nephron. In addition we are examining to see if similar changes occur in cardiac cells.

Introduction:

The goal of this project is to study the effect of long term treatment with digitalis on modification of subunit isoforms of Na,K-ATPase (sodium pump). To improve the contractility of the heart, digitalis, a cardiac glycoside and a potent inhibitor of the sodium pump is frequently administered in congestive heart failure. Digitalis exerts a positive inotropic effect due to its inhibition of Na,K-ATPase with the accumulation of intracellular sodium, and enhanced calcium availability due to the ***increased*** exchange of Ca/Na (Doursout et al., 1992; Blaustein, 1993).

Na,K-ATPase is a ubiquitous membrane-bound enzyme, responsible for the translocation of sodium out and potassium into the cell. It is important for the cell to maintain its volume and generate action potential in excitable cell. This pump protein consists of a catalytic α-subunit, glycosylated β-subunit and a γ-subunit. The α-subunit is a multispanning membrane protein with a molecular mass of 112 kDa that is responsible for the most known physiological function of the enzyme. It contains the binding sites for the cation, ATP, and the specific inhibitor, ouabain. Three isoforms of the α subunit (α1, 2,α3) are found in vertebrates (Sweadner,1989). Recently, a fourth α-isoform (α4) was found in rat testis (Shamraj, 1994). It has been also reported that chronic ouabain treatment activates the synthesis of the pump protein (Rayson, 1989). The long-term treatment induces hypertension (Manuta et al., 1994, Yuan et al., 1993). Since sensitivity of the Na,K-ATPase to ouabain affects the inotropic and toxic responses to the drug, and different isoforms of the α subunit have different ouabain sensitivity, it is important to determine which isoforms are produced during the development of the pathology and what changes occur after treatment with digitalis.

Therefore, to investigate changes and distribution of the subunit isoforms of the Na,K-ATPase, we used a mini-osomotic pump (Alzet mini-osmotic pump, model 2002) implanted subcutaneously in the abdomen area of rats to provide continuous infusion of digitalis at a rate of 30 μg/kg/day. For the control group, the mini-osmotic pump was implanted in the same area of rats but containing only normal saline. Electron microscopic immunocytochemistry was used to identify and examine the

distribution of the varions isoforms of the α subunit of the Na,K-ATPase.

Materials and Methods:

1. Experimental animals

Sprague-Dawley rats were ordered from the animal center of National Science Conuncil, Taiwan, ROC. These rats were putting in a room with 12hr light/12 hr dark day cycle and fed with standard rat chew. Rats were randomly assigned to receive either ouabain or vehicle (0.9% NaCl) with implanted mini-osmotic pump (model 2002, Alza Co., CA.) subcutaneously in the abdomen area of rats for 4 weeks. Ouabain (Sigma Chemical Co., St Louis, MO) were dissolved in 50% alcohol and filled in the mini-osmotic pump to provide continuous infusion at a rate of 30 mg/kg/day for 4 weeks. Control animals were given vehicle only. At the end of the treatment period both group of animals were sacrificed to obtain kidney and cardiac muscle for immunocytochemistry.

2. Immunocytochemistry

Tissues were cut into small pieces and fixed in a fixative containing 2% parafomaldehyde and 0.2% glutaraldehyde, pH 7.4, at 4 ^{o}C for 2 hours and then, fixed 2% paraformaldehyde at 4 ^{o}C overnight with gentle rock. After fixation, tissue blocks were washed with phosphate buffer and dehydrated with 70% alcohol and penetrated with LR White. After penetration, the tissue blocks were embedded in gel capsules and polymerized in a vacuum oven at 50 ^{o}C for 24 hours.

Sections were cut by Ultracut E and immunolabeled with polyclonal antisera. Polyclonal rabbit antisera against the α1, α2 and α3 isoforms of the α subunit and the β1 and β2 isoforms of the β subunit of the rat Na,K-ATPase were purchased from UBI (Upstate Biotechnology). 10nm-gold labeled goat anti rabbit IgG was purchased from Amersham. Sections were collected on formvar coated 100-mesh nickel grids and treated with 5% H_2O_2 for 10 minutes and blocked with 2% BSA and labeled with antisera at a dilution of 1:50 overnight at 4^{o}C. Sections were then washed with phosphate buffer saline and incubated with 10 nm gold labeled goat anti rabbit IgG (1:100) at room temperature for 2 hours. Control sections will be processed in parallel and included omission of antibody from the labeling sequence, as well as substitution of the antibody with non-immune rabbit serum or an irrelevant antibody. After wash and air dry, sections were observed under a transmission electron microscope (JEOL 1200 EX).

Results and Discussion:

Treatment with digitalis for 4 weeks causes an up regulation of α1 isoform of Na,K-ATPase. In the rat kidney we found the increase in the α1 isoform subunit to confine in the distal convoluted tubule (DCT) only. There was no such increase in proximal convoluted tubule (PCT). No significant changes in the α2 and α3 isoforms were observed. In addition, we also have observed a similar increase in the

α1 isoform in the red blood cells of the treated rats. This indicates that the up-regulation of the α1 isoform can respond to the physiological need. The sensitivities of the various isoforms to digitalis differ. The α1 isoform is less sensitive to digitalis than α2 or α3 isoforms. Our results seem to indicate that the phyiological response to pump inhibition is to synthesize subunit isoform that is less sensitive to inhibition. We are presently attempting to understand whether this observation is related to the different requirement of transport activity at various parts of the nephron. In addition we are examining to see if similar changes occur in cardiac cells.

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