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Potential Application of the Synchronization Modulation Technique on Clinical Aspects

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By John Quick

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Dedication

This senior thesis project is dedicated to my late mother, Kathryn James Quick who passed away January 8th, 2011 from metastatic cancer. She was the best mother in every way I can imagine. I love you mom.

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I – Introduction

Objective

I will be taking on a senior project in Dr. Wei Chen's Molecular Biophysics lab. This lab houses a variety of technology including X81 computer-controlled confocal microscope, spectrofluorometer, patch clamp, tissue slicers, spectrophotometer, microinjection facilities and more. Dr. Wei Chen (PhD) alongside his post-doctoral lab staff have developed a technique in which they can control the turnover rate of the sodium potassium pump (NaK ATPase). The sodium potassium pump is a membrane bound protein found in all cells including heart, brain, nerve, and muscle tissue. Its role is specialized in these various tissues, but the homeostasis and balance of the proper concentration of charged species is a fundamental function in all cells. In-vitro, the NaK ATPase within a given cell operate independently and out of synchronization with each other. Dr. Wei Chen's Synchronization Modulation technique induces a synchronization frequency that modifies the various pumps to become synchronized in the exact same phase to one another. Then, this frequency is varied in order to increase the exchange rate of the pump. The pump is a sodium potassium antiporter that exchanges three sodium ions for two potassium ions across the cells plasma membrane via active transport and consumption of adenosine triphosphate (ATP). It is apparent that being able to increase the exchange frequency of the pump can have an astounding effect on cellular concentration of sodium, potassium, charge gradient and separation of charge that can influence most tissue, organ and cellular functions of humans.

Why am I doing this? This technique shows promise on certain biological systems, and due to the abundance of the NaK ATPase throughout the body, exploration of further possibilities for clinical treatment of disease is possible. As a senior approaching graduation with interests in attending medical school, this project has great application to aiding in my understanding of

human biological systems, tissue and organ function, and treatment of disease. The objective of this thesis is to research and compile various publications of diseases and predict potential clinical applications using the Synchronization Modulation technique.

The Sodium Potassium Pump

The Sodium Potassium Pump (Na^+/K^+ ATPase) is a protein found in the membrane of cells that transport sodium out and potassium in the cell in a 3:2 ratio. This is facilitated by the use of 1 ATP per cycle in the form of primary active transport. This keeps the concentration gradients of these two elements relatively high. Potassium is more concentrated inside the cell, and sodium outside. The pump also creates an electric potential across the membrane, where the inside has a more negative character. The great importance of this machinery in all cells is evident by the relative abundance (800,000 – 30 million pumps) and the fact it consumes (hydrolyses) 25% up to 70% of the cytoplasmic ATP. The chemical and electrical potential is beneficial and can be used to do work. The electrical potential in nerve and muscle cells allows for excitability. The chemical gradient of sodium amassed outside of the cell provide the energy for passive (facilitated) transport of glucose, amino acids and more into the cell through channel proteins/ion channels (Raven, et al, 2008). The electrolyte movement across epithelial sheets plays a big role as well. Translocation of sodium across an epithelium creates an osmotic gradient that drives water absorption. This is seen in the kidney as well as the lumen of the small intestine. The distribution of pumps can be even, or concentrated

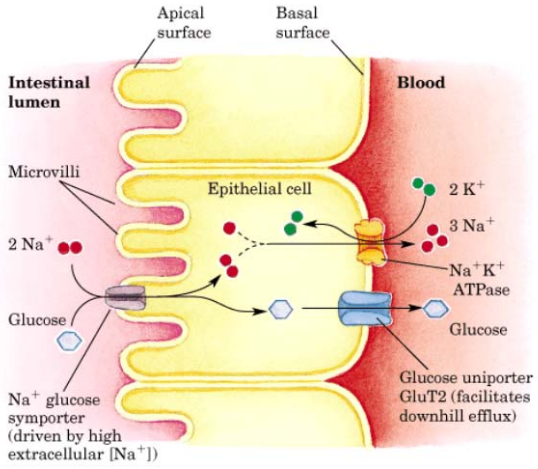

Figure 1: Showing polarity of intestinal epithelium. Ziegler, M. (2003).

in certain membrane domains like the polarized epithelial cells of the kidney. Apical membrane of a polarized cell faces the lumen. The basolateral membrane is the surface of the membrane that forms its basal and lateral surfaces. Polarity is maintained in the cell by targeting to or withdrawing from a specific cell pole and prevention of diffusion of membrane constituents between apical and basolateral domains by specific proteins in intercellular junctional complexes (Junqueria, et al., 2005). Na/K ATPase is exclusively located on the basolateral membrane while the sodium gradient created by the pump between intra/extracellular compartments diffuses mainly through the apical membrane (Demonstrated by Figure 1, above). Pump abnormalities have been discovered to contribute to hypertension (the over production of Aldosterone, the hormone that regulates sodium reabsorption into blood) and heart failure. Na/K ATPase is composed of two subunits Alpha (larger) and Beta (smaller). The Alpha catalytic subunit is where Sodium and Potassium bind and phosphorylation occurs. The Beta glycoprotein subunit appears to only be necessary for the activity of the complex (Figure 2). Studies have indicated 4 separate isoforms of alpha and 3 betas. Isoform 1 is the most abundant, and almost, if not the only type in the kidney. Isoform 2 is predominantly expressed in the heart, skeletal muscle, and the brain. Alpha-3 is primarily expressed in neural tissue and the ovaries. Iso-4 is restricted to the testis. One way this pump is regulated in the renal proximal tubule is by controlling the conductance/concentration of K^+ by ATP dependent K^+ channels (Negative

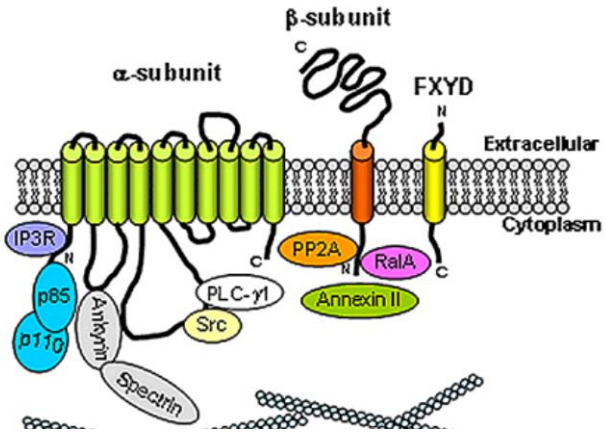

Figure 2: Diagram showing the composition of NaKATPase. Rajasekaran, Sigrid (2009).

feedback). The concentration of intracellular ATP will regulate the efficiency of these channels for potassium recycling. Na^+ is the rate limiting factor for Na/K ATPase. Having a very high affinity for sodium allows tight regulation of the pump function by the intracellular concentration. This is the fundamental aspect of sodium electrolyte homeostasis. A slight rise in intracellular concentration of sodium speeds up a pump's function, while a reduction slows it down. Additionally any process that changes the sodium affinity also alters pump activity. Selectivity of molecules in the potassium portion of the pump is somewhat variable. Potassium can be replaced by ammonium and Rubidium. The switching of ammonium occurs a lot in the kidney medulla to participate in ammonium recycling. This "P-type" Enzyme has two main conformation states that can be unphosphorylated or phosphorylated (E-1, E-2 and E-1P, E-2P). The two conformations of ATPase are characterative by their respective affinities for sodium, potassium and ATP. The E-1 conformer has a high affinity for sodium and ATP and low affinity for potassium and is open to the intracellular side. The E-2 phase is open to the exterior and display low affinity for sodium and high for potassium (Figure 3). The site of ATP binding domain is located between adjacent membrane spanning loops in the extracellular region. Another regulatory aspect of this enzyme is control of the phosphorylation

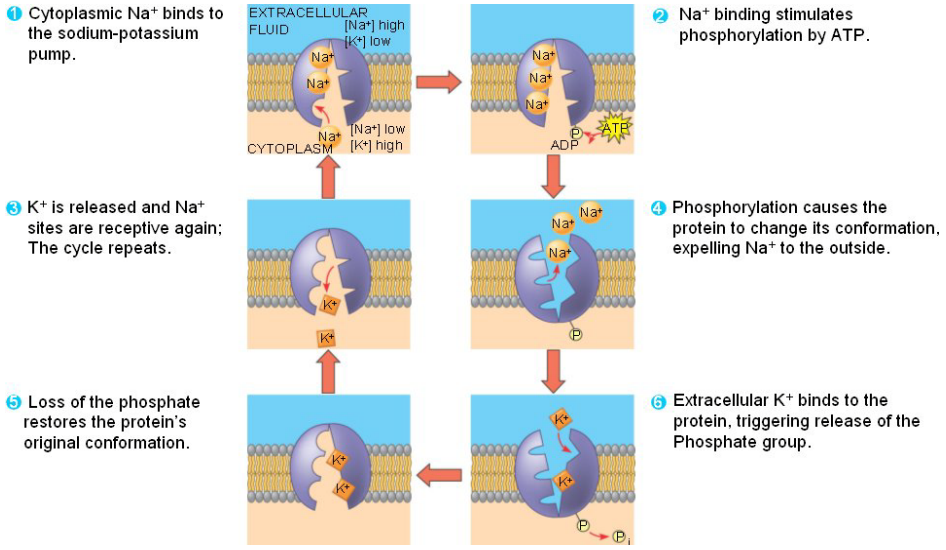

Figure 3: Sodium-Potassium Pump Conformations. Chen, P. (2009).

by protein kinases. This pump is so abundant in neurons that it consumes two-thirds of the cells expended energy. When ATP is bound to the Sodium Potassium Pump, three intracellular sodium cations bind. This triggers the NaK ATPase to hydrolyze the attached ATP molecule converting it to an ADP (Adenosine Diphosphate) molecule and releasing energy that is used to change the conformation of the pump. This change exposes the bound sodium cations to the outside where they are released and binds two extracellular potassium cations. Once the potassium binds the pump releases the still bound ADP molecule restoring itself to its original conformation, which in so doing transports the potassium cations into the cell and completes a pump cycle. This exchange of three sodium cations (with a combined charge of 3^+) with two potassium cations (with a combined charge of 2^+) are not electroneutral. This means that for every cycle of the pump the cell becomes increasingly more negative as the outside becomes increasingly positive. This charge separation creates an electric dipole along the cell membrane as well as an electro gradient (Feraille, E. et al., 2001).

Techniques & Methods

Previous work during the 1980's tested the effects of applying an electric field to sodium potassium pumps and observing the result. This included activating pump function in tissues such as red blood cells (Teissie & Tong, 1980). Additionally others observed and increase in pump function at specific frequencies (Tsong & Austumian, 1986-87). Since the sodium-potassium pump is an antiporter, the voltage dependence of the pump inverts between the sodium and potassium phases. This essentially means, that a membrane potential change that results in either a hyper- or depolarization would only facilitate one transport and inhibit the other (Chen, 2005). Chen & Zhang (2006) then tested using an oscillating electric field (train of squared pulses) with a frequency comparable to the pumps natural turnover rate, which resulted in significantly increasing the pumping rate. The Synchronization Modulation technique involves applying an electric field to a tissue in order to control Sodium Potassium pump function. The AC field facilitates the transport of both ions across the cell and is important for the reversal of the pump and its overall function. This provides electrical energy along with ATP. A DC field facilitates only one ions transport while inhibiting the other. The first part of the technique is called the synchronization step. The thousands of pumps in the tissue are active and working at the same time but they may be running at different rates and pumping phases. Synchronization brings these pumps into phase with one another so that they are working in the same conformations at the same time. Unsynchronized pumps carry a net outwork current of sodium, however once synchronized the pump function increases 6 fold. The AC field in the Synchronization step is first applied at the cell's natural turnover rate, 50 Hertz. Chen (2008) showed that individual pump molecules can be individually synchronized, so that they share the natural turnover rate as the field oscillation, and all export Na ions during the positive half-cycle and import K ions in the negative half-cycle.

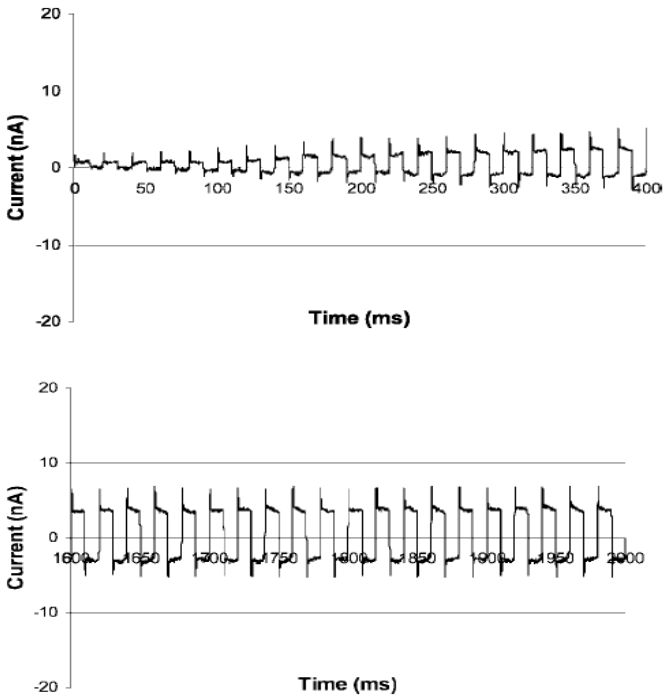

Figure 4: Comparison of pump currents between the first 20 pulses (upper) and 4th set of 20 pulses (bottom).

After this stage completes, the modulation starts. This is where the stimulation frequency varies with time in a step-wise fashion, increasing turnover rate even more. Starting with the 50 Hz from the synchronization step, the frequency is gradually increased up to 200 Hz in approximately 2 minutes duration (Chen, W., et al., 2007). The mechanism essentially involves increasing the efficiency of the NaK ATPase in order to modulate the exchange of Sodium for Potassium. The technique can also inhibit this transfer, or run the process in reverse if needed. The net flow of sodium and potassium can be modified to correct for pathological abnormalities where pump function and the amount of pumps present have changed.

Demonstration of Technique Used In Lab

-Rat Kidney

In experiments involving Rat Kidneys, the stop flow technique is where oil is injected into the lumen of a tubule (specifically lumen of nephron) blocking the flow of native filtrate. Often times Castor Oil and high viscosity silicon oil are used. Oils will move according to the luminal pressure. If one desires a stable and permanent obstruction, wax is employed such as paraffin. The micropipette is positioned by the micromanipulator to pierce the renal capsule lumen near the surface (Figure 8). Fluid is then introduced into the lumen and separated from the native filtrate by upstream and downstream oil blocks. Despite the blockage, transporters in the epithelia will continue to function, voltage can be measured, and magnitude/direction of electrochemical gradient for each ion can be determined. Often times in these experiments double barreled pipettes are employed, one barrel to perfuse the oil, and the other to insert the experimental solution. In our lab we used a variant of this method called the shrinking drop method, to measure rates of fluid reabsorption. The experimental solution is applied between the oil drops and the rate at which the oil drops converge back together (Windhager, E. 1992). This can be done at the same time an electrical field is applied to test the effects of increasing sodium-potassium pump turnover rates on the reabsorption of ions in the proximal tubule.

Free-Flow micropuncture is also a method employed by our lab. A downstream oil block is used to prevent downstream flow of filtrate. Oil is placed in the tip prior to performing the micropuncture. Then a syringe filled with fluid is compressed pushing the oil into the lumen. After the oil has been placed in the lumen, negative pressure on syringe is applied to promote uptake of filtrate. After collection the tip is removed and stored for later analysis. Continuous microperfusion is employed in proximal and distal tubules and also the Loop of Henle. This free-flow micropuncture derivative refers to pumping experimental fluid through nephron at a rate determined by experimenter. During this time electrical measurements can be made (Windhager, E. 1992).

Another method for measuring the activity of the Sodium Potassium pump is to measure a baseline of ion concentrations and then applying the NaK ATPase Inhibitor (Ouabain) and then measuring the concentrations after the pumps have been shut off. This technique is useful to compare the relative effectiveness of various experiments on NaK ATPase dependent tissues and organs (Chen, W., et al., 2007).

II - My Laboratory Work

My role in the lab with Dr. Wei Chen and graduate student Clausell Mathis was to help create computer programs in MatLab to aid in data analysis. The raw data retrieved from various experiments is prone to a lot of electromagnetic interference. Programs were designed to aid in data analysis to remove the noise, correct for baseline shifts, smoothing of the graphed data, and other tweaks to make the analysis and presentation of the data as straight forward as possible.

Electromagnetic interference (noise) is caused by the inherent disturbances from other electromagnetic sources including electrical circuits, the sun, and anything that can give off electromagnetic radiation. A common example would be the interference seen on old TVs with analog input signals in the form of lines moving across the screen. To eliminate the interference from our data we designed a program that took our recording and averaged together two traces

and two templates at precise increments that allowed the noise to destructively interfere and cancel out. This, of course, required that we record a template, followed by a trace, followed by another template and then a second trace in a specific increment so that the electromagnetic interference would be 180° out of phase to cancel out (Figure 5).

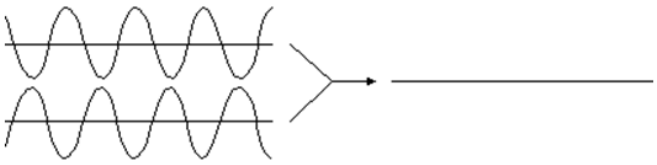

Figure 5: Diagram showing destructive interference. World Hovercraft Organization (2007).

The necessity for the baseline shift is due to the need to correct the baseline due to the (poor) health of the muscle fiber. The P-Clamp software in the lab has a built in baseline shift feature, however we wanted to design a similar program to use in MatLab for data output. MatLab has the linespace function and we just had to create the coding to identify beginning and ending locations and loops to repeat the shift for each trace. Figure 6 below shows an example of a baseline shift.

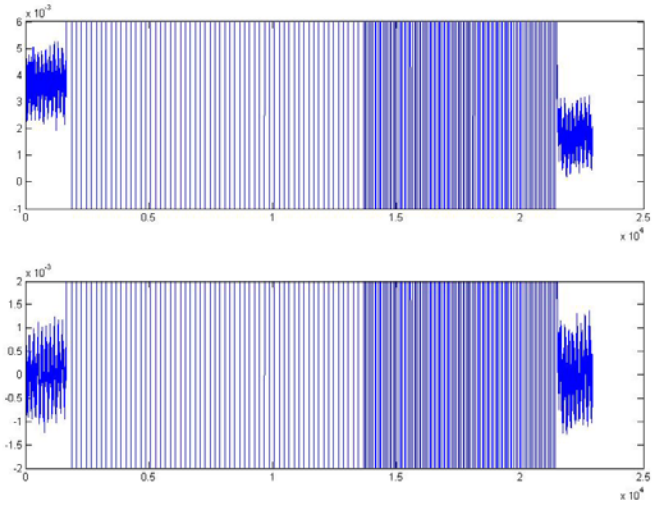

Figure 6: Example of Baseline Shift.

The smoothing of the data was a much more difficult task. In order to smooth the points we had to average adjacent points and replace the first point with that average, then move one point over and repeat the process. We decided on averaging three consecutive points for each point, however averaging five, seven or more points would increase the effectiveness of the smoothing. The following figure shows the before and after when applying the smoothing technique.

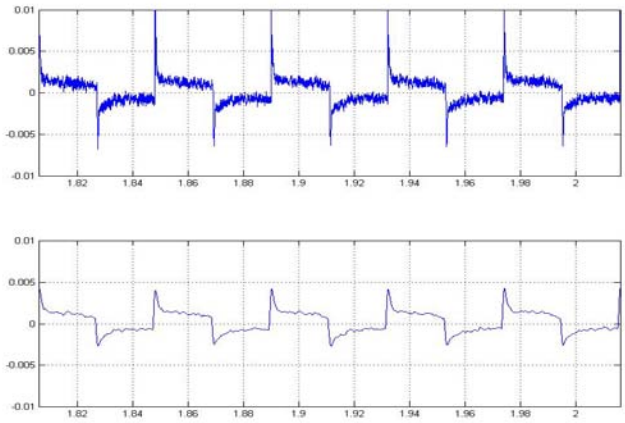

Figure 7: Top shows before smoothing. Bottom is afterwards. Mathis, C. 2011.

We also added to our program to include the baselines at the very beginning and very end of our plotted data. This was accomplished by retrieving a few hundred data points from the beginning of one of our stored variables, and averaging them, then plotting this value for a few hundred data points before the plot. This was repeated, but for several hundred points near the end of the stored variables.

Some example of the programming and data output is shown below:

```
function osinm0()
%
clear
datafile1=input('Enter input data file name:','s');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%initialization
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%p4hi=p4hd+Amp/4;

data1=load(datafile1);
n=length(data1(:,1));




v1(1:n)=data1(1:n,2);%*1e+3;
v2(1:n)=data1(1:n,2);%*1e+3;
%z=v1(n)
%a=v1(n-(n-1));
%b=v1(n-(n-2));
%c=v1(n-(n-3));


for j=1:2
 for i=1:1:n-2
    if v1(i) < v1(i+1) > v1(i+2)
        v1(i:i+2)= (v1(i) + v1(i+1) + v1(i+2))/3;
        %v1(i)= (v1(i) + v1(i+1) + v1(i+2))/3;
    end

    if v1(i) > v1(i+1) < v1(i+2)
        v1(i:i+2)= (v1(i) + v1(i+1) + v1(i+2))/3;
        %v1(i)= (v1(i) + v1(i+1) + v1(i+2))/3;
    end



 end

end


t=(1:1:n)/10000;

savedata=[t' v1'];
save([datafile1 '_spike_removed'],'savedata','-ascii','-tabs');

```

Figure 8: Source Code of MatLab program. Mathis C. and John Quick, 2011.

III – Potential Clinical Applications

This paper served to describe the background necessary to provide potential clinical applications. Various diseases, their cause and/or their symptoms can be impacted directly or indirectly by sodium-potassium pump function. Increasing the turnover rate by 10 times or more has strong implications in the physiology of the organ. The osmotic gradient is directly related to the concentration of sodium; water follows the sodium gradient. Chloride ion also to a certain extent follows sodium to balance the charge. The concentration of Sodium or lack thereof, indirectly affects other secondary active transporters such as the Sodium-Glucose Symporter, the Sodium Hydrogen Antiporter, and Sodium channels to name a few. In the following paragraphs, a brief introduction to an organ systems physiology will be provided, followed by the introduction of associated diseases and finally how this technique can positively affect these conditions.

Kidney – (Physiology)

The kidney is an important organ in the body of an organism. It filters the waste from the blood while reabsorbing beneficial metabolites into the bloodstream. The kidneys regulate fluid osmolarity and electrolytes. The basic functional unit of the Kidney is called the Nephron. Figure 14 shows the topology of the Nephron. Some key sections of the nephron are listed in abbreviated forms. These include Proximal Tubule (PT), Thin Descending Loop of Henle(tDL), Thin Ascending Loop(tAL), Thick Ascending Loops(TAL), Distal Convoluted Tubule (DCT), and Collecting Duct (CD). The basolateral membrane has multiple infoldings increasing surface area for transport. Minimal cellular junctions make the epithelial tissue leaky (Feraille, E. et al., 2001). The cortical portion of the nephron is near the exterior surface of the kidneys, and includes the PT, DCT and Macula Densa. The Medullary section of the nephron consists of the inner portions of the kidneys and the bottom portion of figure 9 (Henle's Loop and Collecting Ducts) fall into this area. The Macula Densa is the area where the DCT comes into close proximity to the glomerulus (the socket shaped portion before PT). These specialized cells provide feedback mechanisms to regulate filtration rate and helps stimulate release of the hormone renin. Blood pressure is measured in the juxtaglomerular apparatus, which causes the secretion of Renin. The apparatus is comprised of specialized cells that synthesize store and secrete renin and are attached to the afferent arteriole that supplies blood to

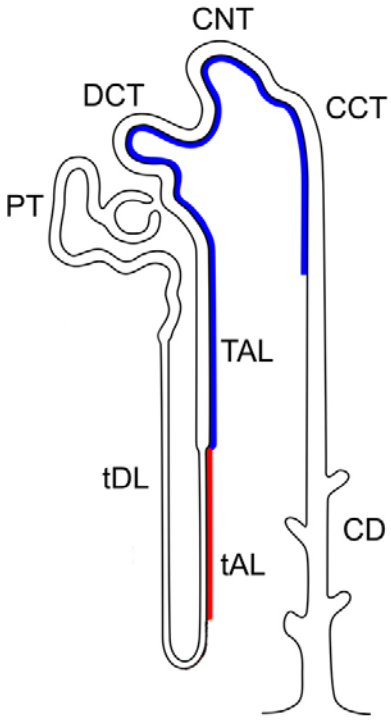

Figure 9: Diagram of Nephron. Fahkle, et al. (2010).

the glomerulus. Renin converts Angiotensinogen to Angiotensen I, then into Angiotensen II (in Lung) and finally into Angiotensen III which signals the release of Aldosterone from the Adrenal Glands (Feraille, et al., 2001). Aldosterone, a mineralocorticoid, increases the reabsorption of sodium ions (followed by water) in the collecting ducts and distal convoluted tubules in the nephron. Aldosterone accomplishes sodium reabsorption through Na transporters such as NaK ATPase, Sodium-Hydrogen Exchanger, Glucose-Sodium Symporter, and Na selective channels. The hormone is released in response to decrease in NaCl in distal tubule and Low blood pressure. Blood osmolarity is measured by osmoreceptors in the hypothalamus. So it acts to raise blood pressure upon it's' release. The reabsorption of water in the distal straight tubule is modulated by antidiuretic hormone (ADH) which causes the permeability of water to increase; removing water from the collecting ducts (water is conserved). If the body fluids are hyperosmotic, ADH hormone is released, if it is hypoosmotic it is not released. If ADH isn't released diuresis occurs and excess water is excreted in urine. Alcohol inhibits the release of ADH (Alcohol is known to cause dehydration), while pain and fear enhances its release. ADH also controls the sensation of thirst.

On a cellular level, the cells that comprise the nephritic tubules are highly polarized. The Sodium Potassium Pumps serves as a primary active transport pump that provides the necessary gradients for other secondary active transport pumps to function. The NaK ATPase faces the interstitial (basolateral) side of the cell, driving out sodium. This brings in more sodium into the cell down its concentration gradient either exchanging itself for hydrogen (Antiporter) or with Glucose (Synporter). The charged solutes provide the electrochemical gradients that drive passive diffusion of electrolytes through their ion channels (Feraille, E. et al., 2001).

Renal Failure

In the often fatal disease condition of kidney (renal) failure, the nephrons in the kidney become overworked and over-exhausted due to dietary and other causative factors leading to damage. This severely hinders the kidney's overall ability to adequately filter blood. Dialysis is a painful treatment where patients are hooked to a machine that filters metabolic waste from their blood. Kidney transplants are also a viable cure for this condition in the most extreme cases. The technique applied in this scenario could increase the efficiency of the remaining nephrons many times. The stimulation of NaK ATPase by the electrical field prevents the need for ATP consumption and therefore preserves the low energy charged often associated with this condition to be conversed for other vital processes. The technique will directly aid in reabsorption of sodium and water, and through secondary active transport mechanisms also bicarbonate and glucose. This could more than compensate for kidney damage or lack of function and return the organs to a state where they are able to filter the blood effectively during the treatment. This would prevent the need for transplants or dialysis, and offer a less invasive, less painful alternative.

Hypertension

Since the kidneys play a formidable role in blood pressure homeostasis, this disease is listed here. Hypertension is characterized by persistently high arterial blood pressure. This disease increases risk for heart attacks, heart failure, strokes and kidney failure. It has been suggested that hypertension can be caused by the decreased contractibility of blood vessels due to decreased expression/function of NaK ATPase. The lack of functionality prevents the proper sodium homeostasis. Constriction of vasculature in kidneys causes salt and water retention raising blood pressure. Additionally an endogenous ouabain-like molecule is proposed to be

connected to this disease by inhibiting (via hormone) NaK pump function (Rose, et al., 1994). Chen's technique could be applied to offset the effects of decreased expression/function of pumps in this condition as well, restoring the water/sodium homeostasis required for a normal blood pressure.

Pancreas – (Physiology)

The Pancreas is one of the organs in the body that is both an exocrine and an endocrine gland. The Islet of Langerhans region of the pancreas contains specialized cells that secrete hormone (Figure 10). Among these cells are Alpha-Cells which produce glucagon and Beta-Cells which are responsible for producing Insulin. These islets appear as clusters of cells embedded within the exocrine portion of pancreatic tissue. The human pancreas has around one million islets embedded throughout the pancreas. The body's sole endogenous source of insulin is produced by the Beta Cells. The exocrine portion of the pancreas provides digestive enzymes that aid in the digestion of food in the small intestine (Junqueira, et al., 2005).

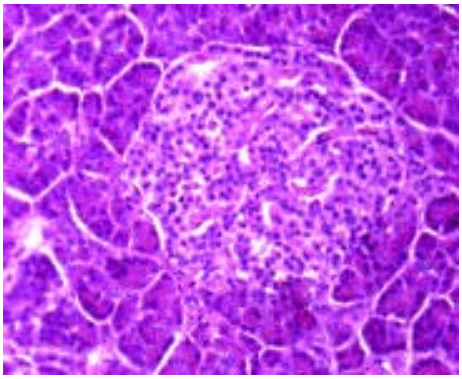

Figure 10: Pancreatic Islet. (Wick, 1997).

Diabetes

Diabetes Mellitus is an increasingly common disease in 'first world' countries. This condition is caused by either a lack of insulin production due to autoimmunal destruction of pancreatic beta cells (Type I) or the unresponsiveness of insulin on target tissues (Type II). Type I is commonly inherited genetically and presents itself at a young age. Type II can present itself at any time, but is often seen in adults. Obesity increases your risk factor for type II. The disease is characterized

by destabilization of blood glucose homeostasis which can lead to acute difficulties such as coma or death. Long term health risks include cardiovascular disease, chronic renal failure and retinal damage. Insulin is an endocrine signaling molecule that is indicative of the fed state. Insulin binds to cellular surface receptors and promotes the uptake of glucose by the cells and promotes an increase of glucose transporter expression. Untreated diabetes shows a trend of decreased NaK pump expression in skeletal muscles. Insulin promotes the expression of Na,K ATPase in muscle tissues sometimes higher than the control group (Schmidt, et al., 1994). Additionally, Weidmann et al. (1991) found that both types of diabetes intracellular sodium retention was present. Sodium has been known to increase the glucose transporters affinity for binding glucose. Our technique therefore, would expel excess intracellular sodium, and to a lesser extent positively modulate the uptake of glucose. This has the potential at low-moderate levels above normal blood glucose concentrations to bypass the need for insulin. At moderate-high levels above normal this could quickly facilitate the uptake of glucose synergistically with insulin.

Skeletal Muscles - (Physiology)

Each skeletal muscle is comprised of individual components known as fibers. These fibers are long, cylindrical and multinucleated composed of myosin and actin which give the striated appearance. Myosin (thick) and Actin (thin) comprise the repeating functional unit of muscle called the sarcomere (Figure 11). The interaction between these two filaments is responsible for contraction of muscle. The mechanism of contraction is primarily dependent on sodium and calcium which trigger various depolarizing events and bind to gated channels. Ultimately when the Calcium ion is released from the sarcoplasmic reticulum, it interacts with a protein associated with Actin called troponin and binds to it. Troponin changes conformation and directly affects tropomyosin another associated protein which then changes position allowing the myosin binding sites on the thin (actin) filaments to be available for binding. The Myosin then binds to the thin filaments consuming ATP, and the filaments slide past each other creating movement. When the muscle is called into action it may lengthen, shorten or remain the same size creating tension. In experiments in our laboratory, the muscle was held tightly with the sarcomere fully extended. When stimulation is applied the muscle only twitches, it does not actually contract. Figure 20 also shows how the myosin and actin form characteristic bands. The sarcomere spans from one z-line to the next (Junqueira, et al).

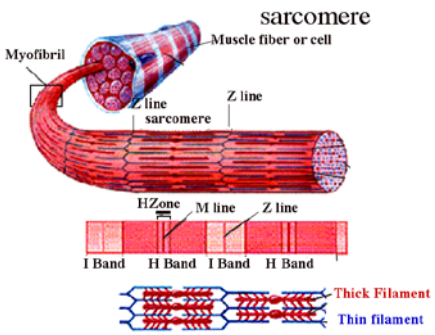

Figure 11: Diagram of Sarcomere. Casalotti, S. (2011).

Myotonic Dystrophy

Myotonic Dystrophy is part of a group of genetically inherited disorders referred to as muscular dystrophies and it is a disease characterized by muscle wasting, muscle weakness, fatigue, cataracts, heart conduction defects, among others. Persons with this disorder may suffer from the inability to relax certain muscles after use (myotonia) such as locked jaw. Lichtein, et al. (1992) found that there were 3-6 times lower concentration of sodium potassium pumps present in muscles of patients with this disease compared to similarly aged controls. It is clear that the lack of sodium homeostasis due to the deficiency in pump concentrations plays a vital role in this disease. This lower concentration of pumps can account for the increased intracellular concentration of sodium and the hyperkalemia conditions in blood plasma post exercise. Application of our technique would promote the uptake of potassium while expelling the sodium, restoring the muscle to a normal state. Those suffering this disease could enjoy moderate exercise without experiencing the after effects of fatigue.

McArdle Disease

McArdle Disease is an autosomal recessive genetically inherited glycogen storage disease caused by deficiency in a muscle phosphorylase enzyme. Phosphorylase is required for the breakdown of glycogen stores in tissues. Without the ability to tap into this vital resource, individuals quickly use up their ATP and creatine stores, and become fatigued. Often these individuals suffer pain and weakness after exercise. This is attributed to the extracellular hyperkalemic conditions. The concentration of sodium potassium pumps in the muscles of these individuals were 30% lower than normal which accounts for the inadequate reuptake of potassium (Haller, et al., 1998). Chen's technique can be applied here as well. Ramping up the turnover rate of the sodium potassium pump can cure the hyperkalemic conditions, and restore metabolite homeostasis. This would reverse the muscle fatigue and weakness associated with this condition.

Heart – (Physiology)

The cardiac muscle is a unique muscle type only found in the heart. Like skeletal muscles, striations appear in the fibers; however muscle control is involuntary.

Unlike the other muscle types, cardiac muscles have intercalated discs that connect the individual myocytes together (figure 12). This allows an action potential to be easily spread to all cells of the heart through gap junctions. This is vital for the synchronized contraction of the heart muscle. The contraction of the heart is controlled by sympathetic and parasympathetic regulation from the central nervous system. In the human heart, there are four chambers, each pushing blood into the next chamber, to the lungs or throughout the body.

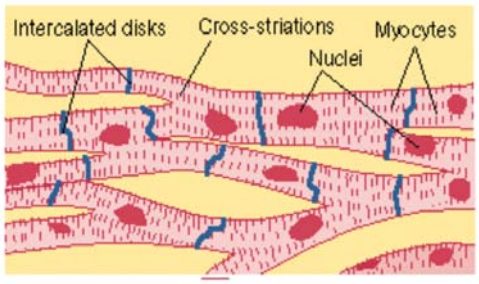

Figure 12: Cardiac Myocytes. (Casalotti, S 2011)

Heart Failure

Cardiac failure is a disease of the heart muscle, where the tissue becomes enlarged, in an attempt to compensate for inadequate pumping ability. This causes more strain on the muscles, which lead to a failure when the heart can no longer pump enough blood to the rest of the body. Secondary to the lack of proper blood flow, is that fluid is retained in other areas of the body causing edema in the arms and legs as well as fluid in lungs, liver and gastrointestinal tract. Primary causes of this condition are coronary heart disease (narrowing of the coronary arteries starving the heart tissue) and abnormal heart rhythms among others. Studies show that in the hypertrophied ventricles of cardiac failure exhibit about 41% lower expression of NaK ATPase compared to those with normal ventricular function (Allen et al., 1992). This is thought to impact the ability for the muscle to contract efficiently due to the interaction of sodium and calcium involved in muscle contraction and signal transduction. Our technique could be applied to

provide the correct sodium levels for optimal muscle contraction to reverse or at least prevent progression of this disease to its terminal stages.

Conclusion

In summary, the synchronization modulation technique developed and refined by Dr. Chen and his lab has the potential for many therapeutic uses. The Sodium Potassium Pump (Na,K ATPase) is a protein that is embedded in the membranes of every cell. Concentrations of these pumps can reach a substantial level in various cells, and consume upwards of 60% of the available energy supply. The pump transports 2 potassium ions into the cell in exchange for expelling three sodium ions consuming one molecule of ATP per cycle. At the organismal level, this pump has a role in metabolite homeostasis, water reabsorption, signal transduction, muscle contractions and more.

The technique uses an oscillating electric field to synchronize the pumps with a 100 pulses at 50Hz (the Na,K ATPase natural turnover rate). This results in about a six-fold increase in net movement of sodium and potassium across the cell. Once the pumps are synchronized and working in the same phase with respect to one another, the frequency is adjusted in stepwise increments (modulation) which increase the pump turnover rate upwards of ten-fold. Applying this technique to various organ systems such as the kidney, skeletal muscles and heart has the potential to offset or cure disease states. Restoring metabolite balance could compensate for renal failure and high blood pressure. Increasing the turnover rate of the pumps could help restore muscle function and cure arrhythmias and contraction issues associated with the heart.

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