ABSTRACT

GABA Stimulates Aldosterone Production in Intact Zona Glomerulosa Cells in Fetal Bovine Adrenal Glands

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Aldosterone is a steroid hormone produced in the adrenal cortex. It is mainly used in regulating ion movement such as $Na^+$ and $K^+$ via $Na^+/K^+$ pumps and thus it regulates mean arterial pressure. GABA (Gamma Amino Butyric Acid) is an inhibitory neurotransmitter that is found predominately in the central nervous system (CNS). This study aims to understand the effects of GABA on the aldosterone production in intact zona glomerulosa cells in fetal bovine adrenal glands. An Enzyme-Linked Immunosorbent Assay (ELISA) test was used to assess the effects of GABA on aldosterone production. GABA was given to an experimental group in varying concentrations of: 1.4 micromoles/ml, 2.8 micromoles/ml, and 5.6 micromoles/ml. It was observed that the aldosterone production increased at each concentration: 1.4 micromoles/ml increased by 7.85752% (with p=0.00014), 2.8 micromoles/ml increased by 4.81063% (with p=0.00012), and 5.6 micromoles/ml increased by 3.82624% (with p=0.00364).

The data indicate that a dose dependent curve relationship exists as the GABA concentration increased and the aldosterone production increased.

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# GABA STIMULATES ALDOSTERONE PRODUCTION IN INTACT ZONA GLOMERULOSA CELLS IN FETAL BOVINE ADRENAL GLANDS

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By

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# DEDICATION

I would like to dedicate this thesis to my mentor, Dr. Hillis, who allowed me to work in his laboratory as a freshman. I have learned a tremendous amount and am forever thankful to you. Also I would like to dedicate this thesis to my parents, my friends, and advisors for their encouragement and words of wisdom.

# CHAPTER 1

# Introduction

## *Hypertension*

Hypertension is a chronic medical condition that affects individuals of all ages, but primarily occurs in adults. [1] The prevalence increases dramatically with age, and in many populations 50% of people over 60 have hypertension; however, the increase in prevalence occurs more rapidly in women. [2] Hypertension is a major risk factor for stroke, coronary heart disease, and kidney problems. [1] This condition affects about 1 billion people worldwide and contributes to 51% of deaths due to stroke and 45% of deaths due to coronary heart disease. [3] Both genetic and environmental factors contribute to the variation of blood pressure and hypertension found across different regions and races. [1] These factors account for as much as 30% of the variation in blood pressure in populations. [3] Though some specific genetic mutations very rarely cause hypertension, the kidneys have been indicated to be the likely site where the tendency to develop hypertension occurs. [4] Without routine checkups, individuals might not realize that they have hypertension as it is often symptomless, but they realize that they are hypertensive only after the onset of a serious complication. [5]

## *Physiology/Pathophysiology of Hypertension*

Hypertension is influenced by two major factors: cardiac output and peripheral resistance. Cardiac output is the result of stroke volume and heart rate. Stroke volume is further related to the walls of the ventricles and the end diastolic volume of the heart chambers. [6] Peripheral resistance is affected by anatomic and physiologic changes in the arteries and arterioles. In fact, the contraction of the smooth muscle for an extended period of time causes structural changes in the thickening of the arteriolar walls through the help of angiotensin which causes an irreversible rise in peripheral resistance. [4] Figure 1 illustrates this complex relationship. [5]

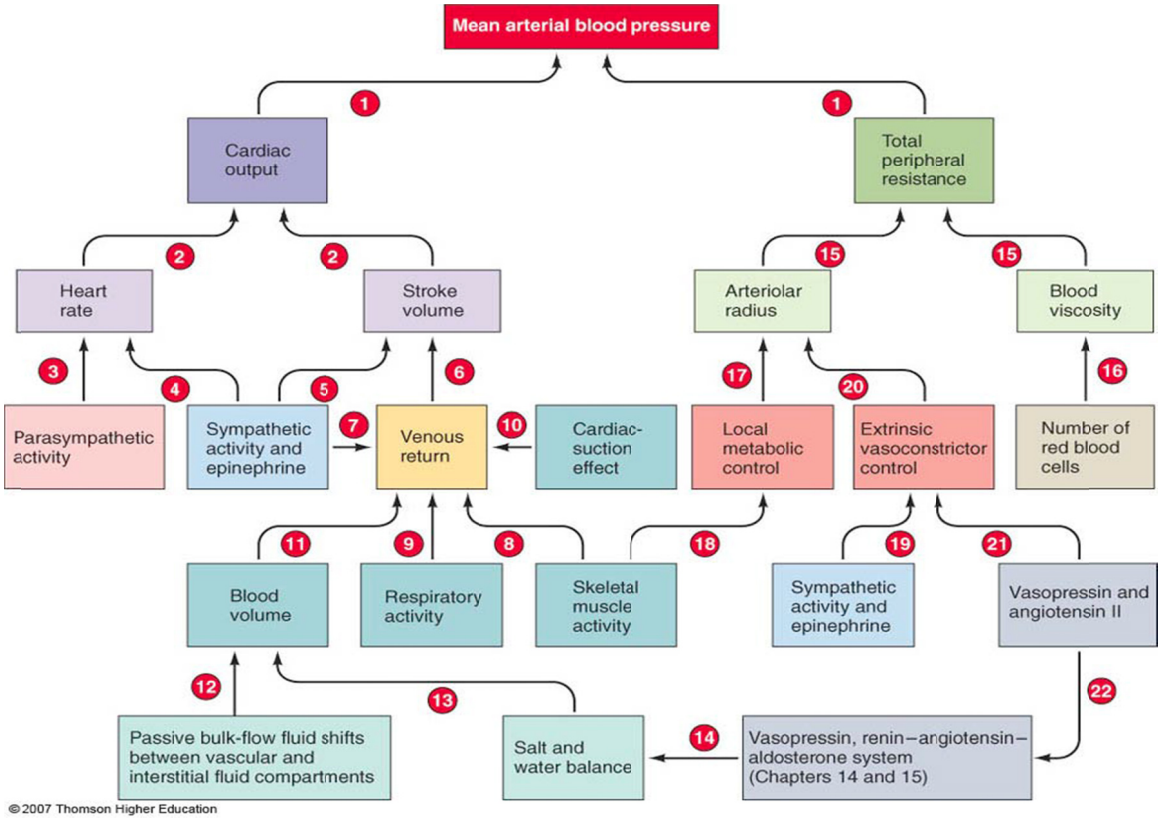


Figure 1: Factors involved in mean arterial blood pressure

Many forms of hypertension exist, but the forms that will be mentioned specifically are: pre-hypertension, primary hypertension, and secondary hypertension. Pre-hypertension, according to recent National Institutes of Health guidelines, is defined as the range of blood pressures between 120/80 and 140/90. Prehypertensive patients can be treated through a change in diet and exercise. The main goal in managing prehypertensive patients is to act before the blood pressure rises to hypertensive levels. [5] Primary hypertension ('essential' hypertension) is a heterogeneous disorder resulting from dysregulation of hormones, proteins, and neurogenic factors involved in blood pressure regulation as well as factors of diet and activity level. [1] Essential hypertension is mainly controlled/treated through use of medications and alterations of lifestyle. Secondary hypertension occurs secondarily to underlying kidney or endocrine condition or to exogenous substances and constitutes about 10% of the cases of hypertension. [1] The treatment of secondary hypertension involves addressing and controlling the conditions that lead to the onset of disorder and with medications. [7]

*Hyperkalemia*

Hyperkalemia is a disorder that pertains to a serum potassium level higher than 5 milliequivalents/liter (normal range: 3.5-5.0 milliequivalents/liter). Potassium is a crucial intracellular cation involved in the maintenance of cell metabolism, cell survival, proper electric signal transmission, and proper muscle function. Depending on the severity of hyperkalemia, the functioning of heart rhythms can vary from changes in the EKG readings caused by mild hyperkalemia to an inhibition of the electric activity in the heart

which can potentially lead to the heart in stopping beating in the case of severe hyperkalemia. Hyperkalemia can result from kidney dysfunction, adrenal gland diseases, potassium movement out of cells into the bloodstream, or medications. In terms of adrenal diseases, hypoaldosteronism has been found to be a major factor in hyperkalemia [8,9,10].

*Kidney*

The kidneys are organs that are located in the retroperitoneal space and are composed of an outer cortex and an inner medulla. The renal glomeruli are located in the outer renal cortex and within Bowman's capsules. The medulla, on the other hand, is more centralized and consists of ten to eighteen structures called medullary pyramids; the medullary rays of the pyramids penetrate the cortex. The nephron is the kidney's functional unit and can be divided into vascular and tubular elements. The vascular elements include the afferent arteriole, the glomerulus, the efferent arteriole, the peritubular capillaries, and the vasa recta. The tubular elements include Bowman's space, the proximal tubule, the loop of Henle composed of the thin and thick limbs of the descending loops of Henle (where passive diffusion occurs) and the thick and thin limbs of the ascending loop of Henle (where active transport occurs), the distal convoluted tubule, and the collecting ducts and tubules. [11] Nephrons are further differentiated based on their anatomic location. The majority of nephrons are called cortical nephrons because they reside in the cortex. There are some nephrons, called the juxtamedullary nephrons, located near the medulla, which begin at the loop of Henle and extend into the medulla.

The main functions of the kidneys include filtration, secretion, reabsorption, and production of erythropoietin. Filtration moves fluid from the blood into the lumen of the tubule, reabsorption (further divided into passive and active components) moves material from the tubule into the blood, secretion selectively moves molecules form the blood into the tubule, and the kidneys also produce erythropoietin. The principal effect of erythropoietin, an exocrine hormone, is to regulate the production of red blood cells through the differentiation and modification of erythroid progenitor cells. [12] Many hormones act upon the kidney, one of which is aldosterone.

*Aldosterone*

Aldosterone is a steroid hormone that is produced in the zona glomerulosa portion of the adrenal cortex. As a steroid hormone, aldosterone is transported through the blood using a mineralocorticoid receptor and can enter the cell and further enter the nucleus where it functions as a transcription factor as it directly interacts with DNA regulatory elements. Other characteristics of steroid hormones include that in comparison to peptide hormones they are slower and have long-terms effects. Figure 2, known as the steroid biosynthetic pathway, illustrates the formation of aldosterone from cholesterol. [13]

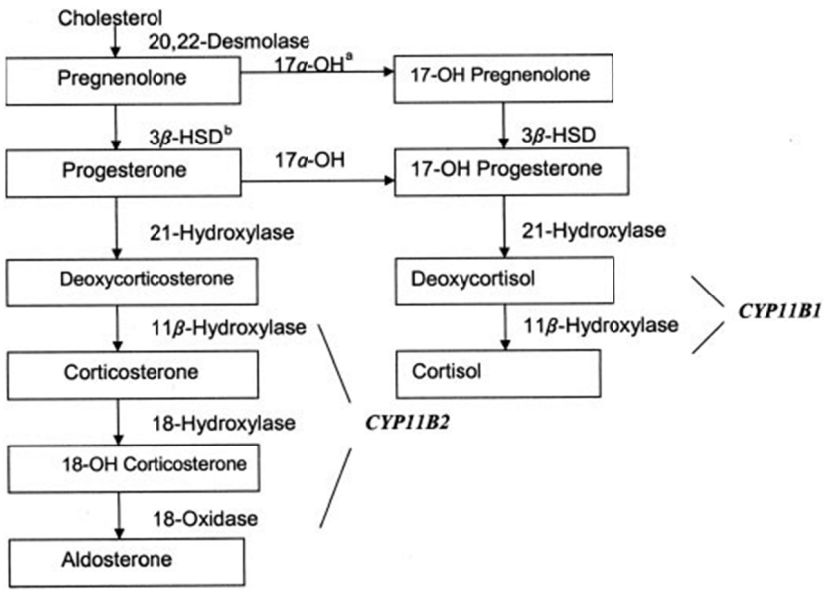


Figure 2: Adrenal Cortisol Pathway for the formation of Aldosterone (Steroid Biosynthetic Pathway)

Aldosterone is involved in sodium reabsorption and potassium secretion in the distal renal tubule, which thereby causes an increase blood volume and an increase in blood pressure. Aldosterone binds to principal cells, a type of cell, found in the kidney. [6,13] Principal cells ensure that the sodium ion channels are open on the apical membrane of the distal renal tubule. Aldosterone increases the number of open sodium and potassium channels on the apical side of the membrane by either opening existing channels or stimulating the synthesis of new channels; it also stimulates the synthesis of sodium and potassium ATPase pumps in the basolateral membranes. It is through these effects of aldosterone that it can affect the simultaneous reabsorption of sodium and secretion of potassium. The renin-angiotensin-aldosterone-system regulates aldosterone secretion and allows for aldosterone to carry out its task effectively [6].

Research over the past years indicates that about 5%-13% of hypertension cases result from hyperaldosteronism (high levels of circulating aldosterone). It has been noted that mineralocorticoid receptors in the heart and in the sympathetic nervous system can induce hypertension and inflammatory responses. Also hypoaldosteronism predisposes to the development of hyperkalemia. [9] The application of these findings in a clinical setting suggests that physicians might want to reconsider the importance of aldosterone and testing for hyperaldosteronism and hyperkalemia by getting a plasma aldosterone concentration to plasma renin activity ratio, because hyperaldosteronism can be a crucial player in many hypertensive cases and hypoaldosteronism can be a major contributor to hyperkalemia cases. [14,9] Methods of controlling adrenal aldosterone secretions involve regulating the renin angiotensin aldosterone system, potassium levels, and ACTH levels. These factors can either directly or indirectly influence aldosterone secretions. The renin-angiotensin-aldosterone system will be discussed in detail below. Potassium ion can either directly stimulate aldosterone secretion or indirectly by activating the local renin-angiotensin system in the zona glomerulosa. Physiological amounts of ACTH stimulate aldosterone secretion acutely, but the ACTH being administered in a pulsatile fashion maintains this action. It is found that both stimulatory and inhibitory factors also regulate aldosterone secretion. [1]

*Renin-Angiotensin-Aldosterone-System*

The renin-angiotensin system controls extracellular fluid volume via regulation of aldosterone secretion. In fact, the renin-angiotensin system maintains the circulating blood volume constant by causing aldosterone-induced sodium retention during volume

deficiency and by decreasing aldosterone-dependent sodium retention where volume is ample. Renin a proteolytic enzyme that cleaves angiotensinogen is synthesized in juxtaglomerular cells, the cells of the muscular walls of the renal afferent arteriole. After renin is secreted many steps are initiated: angiotensin I (a decapeptide) is cleaved from the renin substrate angiotensinogen made in the liver. Angiotensin I is further cleaved into angiotensin II, an octapeptide). The process is catalyzed by angiotensin-converting enzyme (ACE) found principally in the lungs. Angiotensin II aids in vasoconstriction and sodium and water retention through its stimulation of enhanced aldosterone synthesis. Both of these actions increase blood pressure and reverse the effects of low blood pressure. [6,11] There has been increasing evidence indicating that important non-circulating 'local' renin-angiotensin epicrine or paracrine systems in the kidneys, the heart, and the arterial tree control blood pressure.

*GABA*

Gamma amino butyric acid is an inhibitory neurotransmitter that is often administered to patients who are suffering from anxiety or fear and is a common treatment for disorders such as epilepsy. [15] GABA is found in increased concentrations in the central nervous system (CNS) i.e. brain and spinal cord, but in trace amounts in the peripheral tissues. [16] The fact that GABA is a neurotransmitter is validated by the fact that GABA is neither an essential metabolite nor incorporated into protein. Studies indicate that almost 30%-40% of neurons use GABA as their primary neurotransmitter. [17] Since GABA is so widespread in the CNS, it has been difficult to define its CNS role, but it is known that GABA is one of the most important inhibitory neurotransmitters in

the brain. GABA is released via a calcium ion-dependent mechanism. [18] Glucose is the predominant precursor for GABA's synthesis and glutamic acid decarboxylase, found exclusively in the GABAergic neurons, catalyzes the conversion of glutamate to GABA. GAD requires a cofactor, pyroidoxal phosphate that is derived from vitamin $B_6$, for activity. Figure 3 illustrates the synthesis of GABA from glutamate in a detailed manner. [17]

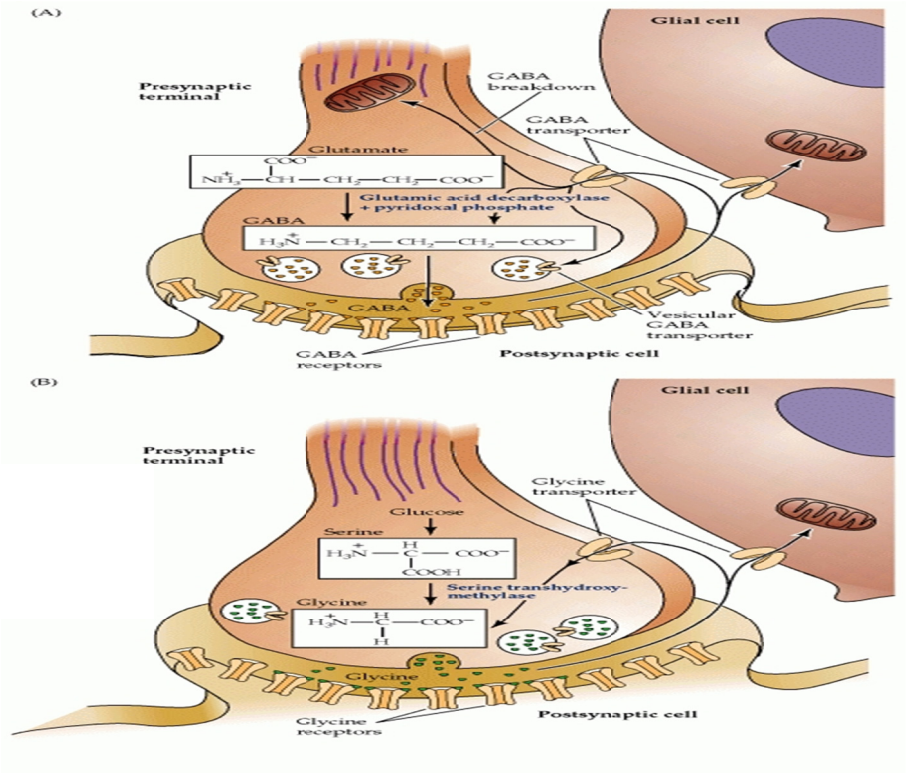


Figure 3: GABA synthesis

There are two types of receptor types of GABA: $GABA_A$ and $GABA_B$. The differences between these two types of receptors are electrophysical, pharmacological, and

biochemical in nature. Another differentiating characteristic between $GABA_A$ and $GABA_B$ is the sensitivity of the $GABA_A$ receptor and the lack of sensitivity of the $GABA_B$ receptor to bicuculline, an agonist of $GABA_A$. [16]

Analyzing $GABA_A$ receptors from an electrophysical point of view, $GABA_A$ increases membrane conductance with the equilibrium potential near resting level of -70 mV. Membrane hyperpolarization increases conductance, resulting in an increase in the firing threshold thereby causing a decrease in the probability of action potential initiation, targeting the periventricular nucleus which is responsible for neuronal inhibition. The chloride ion influx is GABA-dependent via a receptor-associated channel, and GABA is directly responsible for opening the chloride ion channel. [17, 19] GABA has been shown to control the excitability of the brain, the modulation of anxiety, feeding and drinking behavior, cognition, vigilance memory and learning. [17, 20]

$GABA_B$ on the other hand is not well known in comparison to $GABA_A$, which could be attributed to the limited number of pharmacological agents selective for this site. The receptor works indirectly with potassium ion channels. Both these receptors decrease the calcium ion conductance and inhibit cAMP production via a G protein mechanism. They also regulate both presynaptic and postsynaptic inhibition. [17] There is evidence that GABA inhibits ACTH (adrenocortical trophic hormone) by inhibiting the release of CRF (cortisol releasing factor). [20]

The experiment described herein is intended to analyze the effects of GABA on the aldosterone production in fetal adrenal glands and in turn to examine whether there might be significant therapeutic effects that GABA might have in treating hyperkalemia

due to hypoaldosteronism or hypertension that is related to hyperaldosteronism, without generating any significant side effects.

# CHAPTER 2

## Materials and Methods

### *Preparation of Rat Collagen*

In order to prepare the adrenal explants for more profuse and better-maintained outgrowth, a reconstituted rat tail collagen substrate was utilized. These rat-tails were harvested from healthy adult rats that were sacrificed for other experimental purposes, cleaned, and put in the freezer at -20$^{o}$C until further usage. When they were deemed ready for use, they were thawed and soaked in 70% isopropyl alcohol for about 15 minutes. After the 15 minutes, collagen was extracted from them under a sterilized hood using sterilized Kelly clamps, scissors, and various other instruments according to the methodology proposed by Ehrmaan and Gey. [21] The extracted collagen strands were placed in a petri dish with sterilized distilled water. A solution of 1:1000 glacial acetic acid in sterilized distilled water solution was prepared. One hundred fifty ml of this solution and the extracted collagen strands were placed in a sterile centrifuge bottle and refrigerated for 48 hours. During these 48 hours, the collagen of the rats became swollen, and the solution took on a cloudy appearance. The solution was centrifuged at 2300 revolutions per minute at 4$^{o}$C for 2 hours. After the centrifugation, 40 ml of the solution was aseptically pipetted into another sterile tube and labeled S1. Thirty ml of the original acetic acid solution was pipetted into the collagen-acetic acid solution. Both the S1 and the collagen-acetic acid solution were placed in the refrigerator. After 24 hours, the collagen-acetic acid solution was centrifuged again at 2300 revolutions per minute for 2

hours at 4°C. After centrifuging, 70 ml were extracted and placed in another sterile tube labeled S2, and refrigerated at -20°C. The original solution was then shaken with 2 ml of 1:1000 acetic acid: water solution and stored away at -20°C for 24 hours. After 24 hours, the original solution was centrifuged for the last time at 2300 revolutions per minute for 2 hours. After the centrifugation was done, 50 ml of the solution was extracted and placed in another sterilized tube and labeled S3. After the S1, S2, S3 were all prepared, they were combined and placed in the refrigerator at -20°C.

*Dialysis and Reconstitution of the Collagen Explants*

The combined collagen extracts were then dialyzed in approximately 8-inch dialysis tubes which came from the Spectra/Por molecularporous membrane tubing. The dialysis bags were weighted on one end with a section of glass tubing, closed off with silicon rubber stoppers, and sterilized in the autoclave. After the autoclaving process, about 45 mls of the collagen extract was taken from the S1, S2, and S3 and pipetted into the dialysis bag and dialyzed with approximately a liter of distilled water at room temperature every 8 hours for 24 hours. After the last dialysis, the collagen extract was aseptically pipetted into forty sterile storage test tubes each containing 1 ml of collagen extract. Sterilized cotton balls each containing one or two drops of 28% ammonium hydroxide (28 ml of sodium hydroxide: 100 ml of water) were placed in the test tubes and the test tubes were slanted at about 5° for at least two hours. Afterwards, the test tubes were assessed to see if the collagen solidified and had slanted at about 5°. Cotton balls were then removed and water washing began to remove any excess ammonium hydroxide.

The washing process involved pipetting 2 ml of sterilized distilled water into tubes and placing the tubes into the roller drum at a speed of about 2 revolutions per minute at a temperature of 37 $^{o}$ Celsius for 24 hours with changing the water every 8 hours[21]. The old water was extracted carefully in order to keep the slanted collagen intact. After the last water washings, Basal Medium of Eagle (BME) washings were done. These washings were done by adding 2 ml of BME to each tube and then rotating the tubes in the roller drum at the same settings as the water washings and the procedure was similar to that of the water washings.

*Preparation of 1X BME solution*

The 10X BME solution was prepared from BME components which were provided by Cellgro to which sterile water was added in a 1 liter sterile volumetric flask. After the contents were mixed thoroughly, they were filtered into a sterile flask. The flasks were provided by Nalgene Filtration Products and contained a membrane capable of removing all possible bacterial and fungal contamination. The solution was then divided into two sterile 500 ml containers that are stored in the refrigerator. A 1X BME solution was then made from the 10X BME solution. One hundred ml of the 10X BME solution was aseptically pipetted into a 1000 ml volumetric flask, and 10 ml of penicillin and streptomycin mixture (provided by Cellgro) was added to the flask. After this, 29.4 ml of sterile sodium bicarbonate solution was added to the flask, and then sterile distilled water was added to the 1000 cc mark of the volumetric flask. The complete BME was then filtered in a sterile flask (Nalgene Filtration Products) containing a membrane capable of removing all possible bacterial and fungal contamination. This filtration

required the use of the vacuum line supplied to the laboratory attached to the side arm of the filtering flask. The filtered BME was then labeled 1X BME and stored at 4$^{o}$C in the refrigerator.

*Dissection of Adrenal Glands*

After the washings were complete, the adrenal glands were harvested from fetal calves from the vendor, Sierra for Medical Science Inc in Los Angeles. The adrenal glands were transferred within hours of harvest to a Petri dish containing fetal bovine serum, which was provided by Atlanta Biologicals. The way in which the adrenal glands were transferred into the petri dish was as follows: two sterile scalpels in a scissor like fashion were used to cut the adrenal glands. After removing the connective tissue covering the glands and removing fat and any other non-adrenal tissue, the outer cortex was cut into 1mm*1mm*0.5mm fragments and then transferred to the Petri dish. Before the process could be continued, the total quantity of complete BME [BME with 10% (by volume) FBS] medium for the cultures was aseptically prepared before the explants were placed upon the reconstituted collagen. Sterile Pasteur pipettes were then used to take some of the explants, placed on the collagen near the bottom of the culture tubes, and the explants were separated by 1 cm with a maximum of 4 explants per culture tube. The explants were placed in such a position that they were nourished by the growth medium when the culture was placed in the slanted (5$^{o}$) test tube rack. An hour was given for the explants to be fixed on the collagen substrates, so that they were anchored thoroughly before the medium was added. Once they were properly anchored, the growth medium of 90% BME

and 10% FBS (Fetal Bovine Serum) was added. The culture tubes were then placed in a $CO_2$ incubator with settings of 35$^{o}$C and 5% $CO_2$ for 24 hours.

*Preparation of GABA dilutions and Addition of GABA to the Adrenal Explants*

About 21 hours later, the concentrations of the GABA were made. The dilutions of GABA were made in the following way: GABA was diluted in 14 ml of PBS solution to ensure that it was completely diluted. In the next 3 hours, the 2 ml (of the total 56 ml) of the FBS/BME solution were placed into separate tubes and placed into a refrigerator. These 56 ml were considered the baseline control measurements. After that, the tubes were divided into 4 groups of 7 tubes to represent the control group and the three GABA concentrations. Fourteen mls of the control (FBS/BME solution), 1.4 concentration, 2.8 concentration, and 5.6 concentration were added to the tubes and placed in the $CO_2$ incubator for another 24 hours. [22] After 24 hours, the 14 mls of each group (in total 56 mls) wer extracted, placed into separate test tubes, placed into separate cups with differing concentrations, and frozen at -20$^{o}$C.

*ELISA*

An ELISA (Enzyme-Linked Immunosorbent Assay) is performed in order to see what effect GABA had on aldosterone production by the adrenal explants [21]. The Aldosterone ELISA kit were purchased from the ALPCO Immunoassays Company. The use of the kit is to have a direct quantitative analysis of the aldosterone concentration in human serum. The basic idea behind the assay is that of competitive binding. Essentially

the unlabeled antigen, present in the standards, controls, and samples competes with an enzyme labeled antigen conjugate for a set number of antibody binding sites on the microwell plate. Washing and drying allows for any unbound substances to be eliminated. After the washing process, the enzyme substrate is added. The stop solution is used to end the reaction. The microliter plate reader measures the absorbance and the intensity of color is inversely related to the concentration of aldosterone in the sample. Fifty microliters of the calibrator, controls, and samples are added into the ninety-six well plate in duplicates. After that, 100 microliters of the conjugate (avidin-horse radish peroxidase) is diluted so that 240 microliters were added to 12 ml of water in a separate container. The plate is then incubated for one hour on a plate shaker that is set at 8 revolutions per minute. The plate is then washed with 300 microliters of water of a diluted wash buffer (50 ml of the wash buffer concentrate in 450 mls of water) three times and tapped each time to ensure that all of the liquid is removed. Afterwards, 150 microliters of TMB (tetramethylbenzidine and hydrogen peroxide in a non-DMF/non-DMSO containing buffer) is added into the wells every 20 seconds until the plate is full. The plate is then incubated on the plate shaker at 8 revolutions per minute for 10 minutes. Fifty microliters of the stop solution is pipetted into the plate every 20 seconds. After adding the stop solution, the plate was read on a plate reader at 450 nm using the program Gen5 1.09. The anti-aldosterone antibody used in these experiments was coupled chemically with the enzyme, Aldosterone-Biotin, an Avidin-horse radish peroxidase (HRP). The enzyme generates a dark blue to light blue color, which is detected by a colorimeter at 495 nm wavelength; the amount of the colored product is proportional to the amount of anti-antibody interacting with the aldosterone produced in each

experimental and control group culture tube. Known concentrations of aldosterone are provided in the aldosterone kits for the creation of a standard curve, the kits having been attained from the ALPCO Immunoassays Company.

# CHAPTER 3

## Results

The ELISA determinations provided optical densities for each sample. Each sample was determined in duplicate in order to get an average optical density value. Using the program, Gen5 1.09, a graph was created comparing the optical densities that were determined experimentally to the known values of aldosterone levels that came with the ELISA assay. After running the program, a 5 parametered equation (R=0.999) that gave the aldosterone concentration was determined for tube 1 and tube 2 for each sample and control (Figure 4 and Table 5).

The nature of the experiment was to determine the exact number of individual adrenal cells in each test tube but it was not possible to determine the type of each adrenal cell (glomerulosa, retucularis, or fasiculata). The percent decrease of aldosterone level for each culture tube ostensibly determined the effect of varying GABA concentrations on aldosterone production. The formula to calculate the percent increase was:

$$\%\ increase = \left(\frac{tube\ 1 - tube2}{tube\ 1}\right) * 100$$

The aldosterone level in the control group increased by 19.62%, had a standard deviation of 10.26%, and a sample size of 8. The aldosterone level in the 1.4 microliters concentration of GABA increased by 7.86%, had a standard deviation of 10.9142, and a

sample size of 8; the aldosterone level in the 2.8 microliters concentration of GABA increased by 4.81%, had a standard deviation of 8.15, and a sample size of 8. The aldosterone level in the 5.6 microliters concentration of GABA increased by 3.83%, had a standard deviation of 15.242, and a sample size of 8. The values were calculated after eliminating an extreme outlier that was noted in the test tubes of trial 2 in the [5.6] GABA concentration.

The Student T test was used to interpret the data. It is a two-tailed test that is used to compare mean values between two groups: the control and the individual concentrations of GABA and the test. The following equation was used:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

N represents the number of trials, s represents the standard deviations, X is the sample mean, and t is the $n_1+n_2-2$ degrees of freedom. In order for the differences in the means to be considered significant, t must be less than 0.95. Another datum that is obtained from the t test or confidence interval is the p-value and the data are considered significant if $p < 0.05$.

With the Student t-test, it was deemed that the p value was less than .05 ([1.4] was 0.00014, [2.8] GABA concentration was 0.00012, and [5.6] GABA concentration was 0.00364) which indicated with a high level of statistical significance that as the GABA concentration increased in the experimental groups the aldosterone production increased in a decreasing fashion. Table 6 displays the percent change of

aldosterone production as the GABA concentrations varied, and Figure 7 graphically represents the same information.

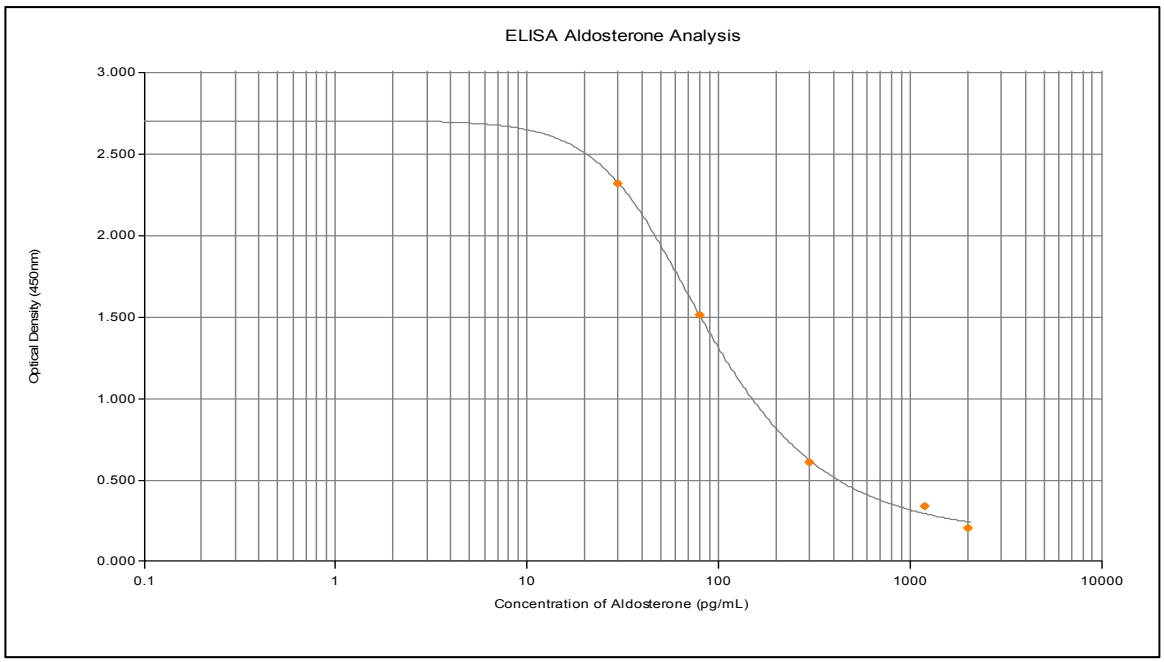


Figure 4: The graphical representation that takes the optical densities from the ELISA test and represents them as aldosterone concentrations

| Curve Name | Curve Formula | Parameter | Value | Std. Error | 95% CI min | 95% CI max |
|---|---|---|---|---|---|---|
| Curve | Y = (A-D)/(1+(X/C)^B)^E + D | A | 2.71 | 0.0648 | 1.88 | 3.53 |
| | | B | 1.98 | 0.637 | -6.12 | 10.1 |
| | | C | 46 | 21.1 | -222 | 314 |
| | | D | 0.16 | 0.126 | -1.44 | 1.76 |
| | | E | 0.457 | 0.313 | -3.52 | 4.43 |

Table 5: The 5 parametered equation that is used to display the optical densities as aldosterone concentrations

| Trials | Control:<br>% increase | 1.4:<br>% increase | 2.8:<br>% increase | 5.6:<br>% increase |
|---|---|---|---|---|
| 1 | -33.3 | -4.64 | 16.5 | -1.31 |
| 2 | -24.6 | 21.9 | 9.07 | 609* |
| 3 | -30.0 | 15.2 | 4.77 | -2.58 |
| 4 | -15.9 | 3.25 | 4.53 | -4.07 |
| 5 | -10.4 | 4.92 | 0.01 | 7.03 |
| 6 | -5.83 | 3.05 | 4.96 | -7.20 |
| 7 | -10.5 | 23.08 | 9.91 | 37.0 |
| 8 | -26.5 | -3.98 | -11.4 | -2.04 |

Table 6: The table with percent increases shown (three significant figures) in the control groups and the experimental groups. (*) marks the outlier that was disregarded while performing the Student T test.

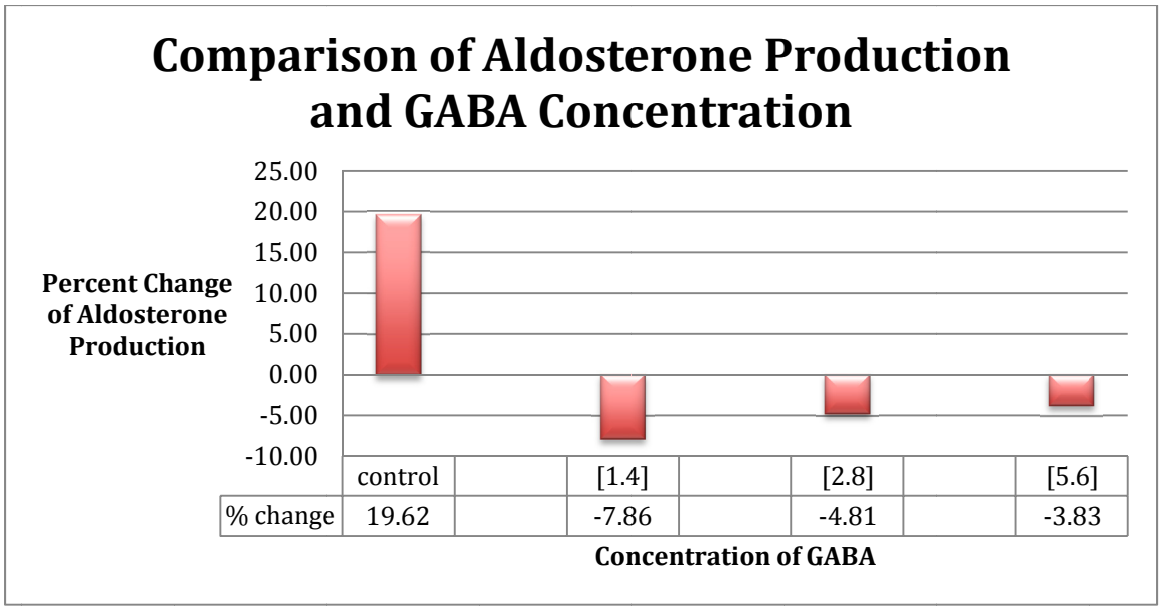


Figure 7: Graphical representation of the percent change among the control and experimental group displaying the effect of GABA in fetal bovine adrenal glands

# CHAPTER 4

## Discussion

The results indicate that the control group experiences a percent decrease in aldosterone production while the experimental groups experience an inverse percent increase in aldosterone production in comparison to the control group. It is seen that the control group on average experienced a 19.62% decrease in aldosterone production, the 1.4 micromoles/L concentration of GABA experienced a 7.86% increase, the 2.8 micromoles/L concentration of GABA experienced a 4.81% increase, and the 5.6 micromoles/L concentration of GABA experienced a 3.83% increase in aldosterone production. This information is consistent with previous studies that point out that GABA is excitatory in immature. The study points at the possibility of GABA being excitatory in immature adrenal glands as well as an increase of aldosterone production.

Research indicates that the manner in which GABA switches form being an excitatory neurotransmitter to an inhibitory neurotransmitter is by an ongoing release of GABA. In fact many studies point to the fact that miniature postsynaptic currents which are created by the independent release of GABA can aid in the expression of $K^+/Cl^-$ co-transporters that can negatively feedback. As a result, GABA negatively feedbacks $GABA_A$ and switches the functioning of it from excitatory to inhibitory. [23] This notion can explain the gradual decrease in aldosterone production as the concentration of GABA increased because a higher concentration of GABA can potentially mimic the effect of ongoing GABA release.

This in vitro study indicates that GABA stimulates aldosterone production and that it is more excitatory in smaller concentrations in fetal adrenal glands. The results are contrary to what was hypothesized, as the original assumption was that GABA would function as an inhibitory neurotransmitter thus inhibiting aldosterone production. However with GABA working as an excitatory neurotransmitter, it would be not conducive to deem GABA as an agent for a possible anti-hypertension therapy through the simply this study.

Some curious things that were noted included the extreme outlier that was found in the 5.6 GABA concentration. The percent decrease was seen to be -609% which was disregarded while calculations were made. Potential reasons for this extreme value to be an outlier would include that there was a solid particulate in the microwell plate, which was picked up by the plate reader as an optically dense substance. Another reasoning would include the notion that during the dissection instead of cutting simply the zona glomerulosa potentially some of the other layers were cut as an explant as well.

Future direction from this study would include comparing the effects of GABA on aldosterone production in adult adrenal glands as a way to see if the effects are in fact inhibitory as expected. Also though the average (overall) percentages of change amongst the experimental groups indicated that there was a percent increase in a diminishing fashion, the individual trials did not indicate such a definite trend thus it would be beneficial to further look in the trials and as a way to understand what could have potentially been the reasoning behind not obtaining a clear trend.

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