# Vasoaction of the blood vessels of the Red-spotted newt in response to topical application of pharmaceuticals used to treat hypertension

Presented to the faculty of Lycoming College in partial fulfillment of the requirements for departmental Honors in Biology

By
Julie D. Neff
Lycoming College
April 24, 2001

Approved by:

Dr. Robert Zaccaria, Department of Biology, Lycoming College

Dr. Robert Angstadt, Department of Biology, Lycoming College

Dr. David Haley, Department of Mathematics, Lycoming College

Dr. Holly Bendorf, Department of Chemistry, Lycoming College

# Vasoaction of the blood vessels of the Red-spotted newt in response to topical application of pharmaceuticals used to treat hypertension

Julie D. Neff
Lycoming College
Honors Project-Spring 2001

# Abstract

The purpose of this study was to test the vasodilatory effects of various pharmaceuticals on the circulatory system of the red-spotted newt. The drugs were diluted using aged tap water (ATW) and were applied topically to the skin of the newt using a syringe. Red blood cells passing through capillaries in the skin on the ventral side of the newt are visible using a dissecting microscope. The numbers of red blood cells passing through a given capillary were counted before application of various test substances and at 1, 2, 5, and 10 minutes following application. The percent increases in the number of red blood cells passing through selected capillaries were calculated for each newt. Statistical tests were performed to identify any significant differences in blood flow between capillaries receiving experimental drug solutions and those receiving ATW as a control. The drugs used in this study are commonly prescribed for human circulatory problems. Hydralazine hydrochloride was the first drug tested. It does not belong to a specific class because the exact mechanism of action is not known. It showed a statistically significant increase from the ATW control at the 33.2mg/ml concentration. Other drugs tested include metoprolol, diltiazem, benazepril, and nitroglycerine. Significant increases from the control were also found for diltiazem and nitroglycerine. The highest percent increase in blood flow was produced by diltiazem at 523.9%. The drugs for which the mechanism of action directly acts on the microvasculature produced a significant increase in the blood flow of the newt. Those drugs that affect blood pressure by other systemic mechanisms did not produce significant increases in this system. Therefore, with further studies the vasculature of the newt could possibly be used as a model for the response of the human circulatory system to vasodilators.

# Introduction

The proper functioning of the circulatory system is essential to sustaining life in all highly developed organisms. Of course humans are no exception. Problems with circulation cause some of our most serious health problems. One type of drug used to treat circulatory problems is a vasodilator. This type of drug relaxes the smooth muscle in blood vessel walls allowing an increase in blood flow. Increased blood flow is important to treating ailments such as hypertension, migraines, and impotence. Drugs with this action can also be used to stimulate hair growth and to treat cardiac problems. Vasodilators are pharmaceutically important to the well being of many people.

One of the overall goals of this study was to see if the circulatory system of the red-spotted newt could be used as a possible model for the human circulatory system in terms of the response of blood vessels to vasodilators. The red-spotted newt was chosen for this study because its cutaneous blood vessels can be easily seen on its light yellow colored ventral side using a dissecting microscope. Its transparent epidermis makes it possible to actually see individual red blood cells passing through the capillaries. Little is known specifically about the kind of response the circulatory system of the red-spotted newt might show to vasodilatory drugs.

The red-spotted newt was also chosen because of previous research of this nature done in this lab (Kindlimann and Zaccaria, 1999). That research focused on the response of the cutaneous blood vessels of the newt to herbal treatment. The herbs used were garlic, *Gingko biloba*, and *Aloe vera.* Each herb was applied topically to the newt and responses were recorded at 1, 2, 5, and 10 minutes. Each herb was found to have vasoactive properties. Garlic had the greatest vasodilatory effects. At its one hundred

percent concentration, or full concentration, it increased blood flow by 518%. Since garlic had this dramatic effect, work has been done to develop a dose response curve. The success of the study on herbs has spurred interest to see if pharmaceuticals that have known vasodilatory properties in humans have as profound an effect on the vasculature of the newt as the herbs have.

A pharmaceutical vasodilator, hydralazine hydrochloride, was chosen based upon chemical properties that made it easy to work with. It is available in crystalline form and is highly soluble in water. Thus, it can be easily diluted to the desired concentration. Hydralazine is usually administered orally alone or in combination with other agents to treat hypertension (Drug Facts and Comparisons, 2000). It has been used clinically since 1950 (Dubois, Schmid, et al. 1987). In humans the dosage should be maintained at the lowest effective level and not exceed 300mg/day in usual cases.

Hydralazine was the first in a series of drugs to be tested in the present study. Following the hydralazine study, additional studies were conducted on drugs from each major class of pharmaceuticals used to treat hypertension. The major classes are beta-blockers, angiotensin converting enzyme (ACE) inhibitors, and calcium channel blockers. Some experimental work with nitroglycerine is also presented here. The second study conducted used the beta-blocker metoprolol as the drug. The third study was done with diltiazem, which is a calcium channel blocker. The fourth study used the ACE inhibitor benazepril as the drug and the fifth study focused on nitroglycerine.

## Methods and Materials

(modified for each drug tested)

The crystalline hydralazine was used as received from Sigma-Aldrich biochemical company. The initial concentration used, 0.02mg/ml, was very low and was based on a calculated tissue level concentration (100mg oral dose in a human being/5 liters of blood in the body). After several trials, this concentration was increased five fold and then by larger degrees in accordance with the previous herb study, which used significantly higher concentrations that were not based on tissue level availability. The same types of elevated concentrations (3.32mg/ml and 33.2mg/ml) were then tried with hydralazine. The desired concentrations were prepared using appropriate amounts of hydralazine and aged tap water (ATW).

Crystalline diltiazem and metoprolol were also obtained from Sigma-Aldrich. Diltiazem was in the form of its hydrochloride salt and metoprolol was in the form of a tartrate salt. Benazepril tablets were purchased from a local pharmacy after the project was explained to the pharmacist. The concentrations used for these three drugs were calculated based on the demonstrated optimal hydralazine concentration. This concentration was approximately 1500 times the tissue level concentration that was originally calculated. Therefore, the tissue level concentration was calculated using the recommended dosages of each of these drugs and then this value was multiplied by 1500. Experimental trials for these drugs were limited to a single concentration because of time constraints on the study. Sublingual nitroglycerine tablets were obtained from an alumnus who works in the medical field and who was interested in this project. The concentrations used for this were based on the solubility of the drug and the likely

concentration delivered sublingually. Three different concentrations of nitroglycerine were tested so a dose response curve could be plotted. All drugs mentioned above were mixed with ATW to obtain the appropriate concentrations.

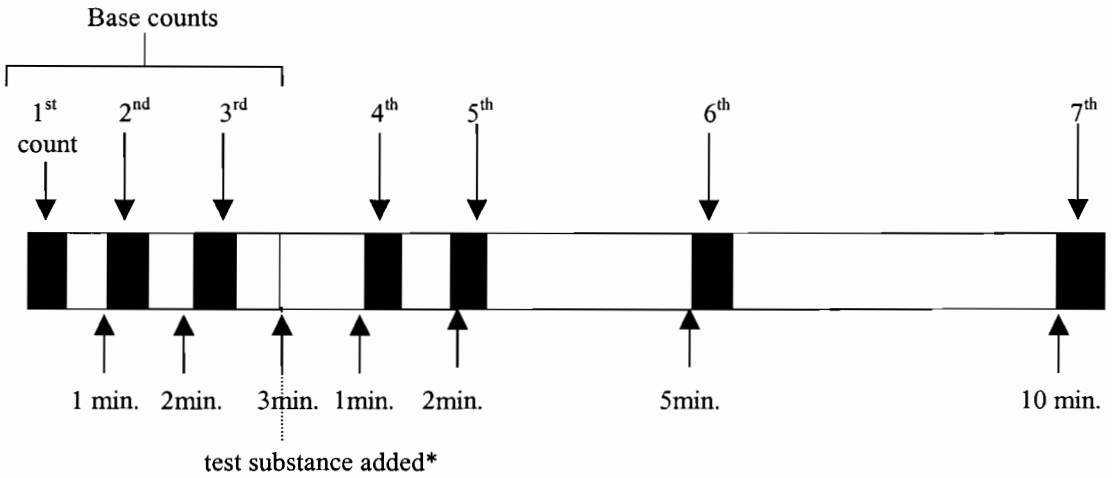
As mentioned earlier, the red-spotted newt, *Notopthalamus viridescens*, was the animal used in this study. These newts are easy to work with and easy to care for in the lab. They have a thin epidermal layer covering their entire body, which makes viewing the cutaneous capillaries possible. Their coloration consists of a green dorsal side with orange-red spots and a yellow ventral side with small black spots. Each newt has a unique spot pattern that can be used for identification. These features can be seen in Figure 1.

**Figure 1: The Red-spotted newt**

The first step in the data collection procedure was to anesthetize the newt. The newt's entire body was placed in a dish containing 0.2% MS222 anesthetic until the animal was completely anesthetized. It was then placed ventral side up on a wax tablet on the platform of a dissecting microscope. The magnification of this microscope was 45x using 15x eyepieces. Two lamps were used as light sources to view the capillaries of the animal. After placing the newt so that blood cells flowing through the cutaneous

capillary preferably had only a few red blood cells flowing through it. The number of cells could be anywhere from 2 to the mid-30s in a span of 30 seconds. Three 30-second base counts were taken using the chosen capillary in a span of about 3 minutes. After the base counts, the substance being tested was applied topically. Some preliminary trials were done using garlic to familiarize the experimenter with the procedure. Control trials were done using aged tap water (ATW), and the study trials were done using various concentrations of each test substance mixed with ATW. The test substance was applied using a 1.0cc syringe. After 1 minute, the number of red blood cells moving through the capillary were counted for 30-seconds, and the same was done after 2, 5, and 10 minutes. Figure 2 is a timeline of these events.

**Figure 2: Procedural timeline**




In Figure 2, the shaded regions represent 30-second intervals during which the number of red blood cells that passed through the capillary were counted. Following all the necessary counts, the newt was placed in a bath of ATW until it was awake and active and was then placed back in the tank where the newts were housed. Each trial was conducted by following the procedural timeline (Fig. 2) and observing an individual capillary in each of six different newts. For each animal the percentage increase in blood

flow (red blood cells counted) from the average of the base counts was calculated. This calculation was repeated for each of the observation times. That is, separate calculations were done for each individual newt at each observation time (1, 2, 5, and 10 minutes). Thus, calculations were done as follows. For each animal, the three base counts were averaged together and then the number of red blood cells at 1 minute was divided by this average. A value of 1 was then subtracted from each quotient to account for the number replacing itself. The remaining number was multiplied by a factor of 100 to get the actual percent increase in number of red blood cells and therefore the increase in blood flow. This was then repeated for each individual newt at each observation time.

**Sample Calculation:** Newt #1 average of three base counts = 15.3
Observed number of RBC's at 1 minute after applying ATW = 21
21/15.3 = 1.373; 1.373-1 = 0.373; 0.373x100 = 37.3% increase

These results were analyzed in various ways. First, time responses were produced for each concentration tested (including the ATW control) by plotting the average percent increase in the number of red blood cells at each time over time. Second, a dose response curve was plotted for the average percent increases at all times combined for hydralazine and nitroglycerine since more than one concentration was tested. Last, the data was analyzed statistically using independent sample t-tests. This test compares the mean percent increase for each particular experimental condition to the ATW control mean that corresponds to it. In addition, the same data was subjected to a non-parametric statistical test called a Mann-Whitney test. This test uses the median for each set of percent increases to determine significance. All significances found using the t-tests were confirmed by the Mann-Whitney test.

# Results

The results presented here are for five studies. Vasodilatory responses over time after application are shown for metoprolol (study #2), diltiazem (study #3), and benazepril (study #4). Vasodilatory responses to various concentrations of hydralazine (study #1) and nitroglycerine (study #5) are shown in the form of dose response curves. For all studies the data are reported as average percent increases from the baseline (pre-application counts) for six individual newts. These numbers are represented in graphs for each study and were also used in comparison to the ATW control for statistical analysis.

Study #1: Response to hydralazine administration

The results of this study showed the basic vasodilatory effects that were expected from the hydralazine. The expected results were an increase of some magnitude in blood flow. The peak increase for the control and the 33.2mg/ml concentration was at 5 minutes (see Tables 1 and 2). Responses to both the 0.10mg/ml and 3.32mg/ml concentrations peaked at 10 minutes. These values are summarized first by numbers of red blood cells in Table 1 and second by percent increases in Table 2. The percentages in Table 2 correspond with the actual numbers of red blood cells recorded in Table 1. However, Table 2 shows averages of the individual percent increases and therefore these values are not the same as if percent increases were calculated using the average numbers of red blood cells from Table 1. The numbers expressed in Table 2 are the basis of statistical tests used to analyze this data. For hydralazine, analysis was done comparing the averages of the average percent increases for all times combined to the ATW control. The asterisk (*) in Table 2 denotes the largest average percent increase observed for

hydralazine. This 361.8% increase represents approximately a four and one half-fold increase in the original number of red blood cells.

**Table 1: The average number of red blood cells that passed through a chosen capillary in each of six newts at a given time and concentration of hydralazine.** (All RBC counts are averages for observations on six newts.)

| Concentration (mg/ml) | Average of 3 baseline values (# of RBCs) | 1 minute (# of RBCs) | 2 minutes (# of RBCs) | 5 minutes (# of RBCs) | 10 minutes (# of RBCs) |
|---|---|---|---|---|---|
| Control (ATW) | 19.5 | 33.3 | 40.0 | 41.7 | 38.5 |
| 0.02 | 22.4 | 36.6 | 41.4 | 36.4 | 41.4 |
| 0.10 | 22.9 | 28.0 | 28.0 | 36.5 | 43.5 |
| 3.32 | 16.3 | 33.2 | 38.3 | 55.0 | 66.0 |
| 33.2 | 21.2 | 58.5 | 67.5 | 78.7 | 64.5 |

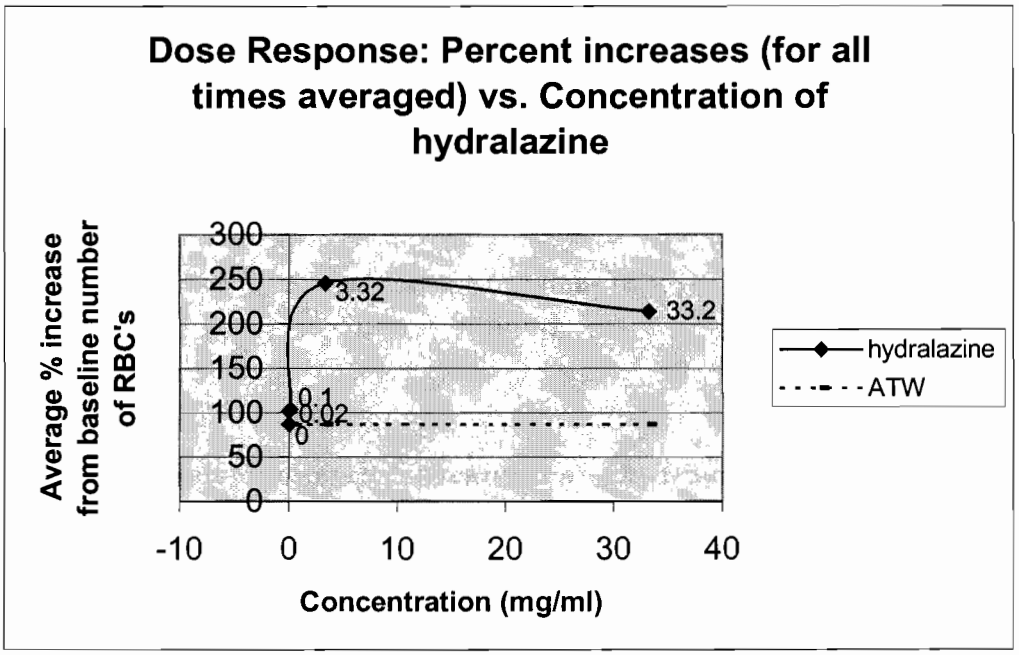
**Table 2: The average of individual percent increases in numbers of red blood cells for each time and concentration of hydralazine.**

| Concentration (mg/ml) | Average % increase at 1 minute | 2 minutes | 5 minutes | 10 minutes | Avg. % increase: all times averaged |
|---|---|---|---|---|---|
| Control (ATW) | 64.2 | 97.2 | 104.6 | 82.9 | 87.2 |
| 0.02 | 63.1 | 118.7 | 119.1 | 105.7 | 101.7 |
| 0.10 | 16.3 | 47.3 | 96.6 | 253.7 | 103.5 |
| 3.32 | 137.7 | 183.7 | 300.5 | 361.8* | 245.9 |
| 33.2 | 159.8 | 212.2 | 257.8 | 226.2 | 214.0 |

By examining Table 1, an increased blood flow for the ATW control can be seen. This can tell us what the normal response of the newt is to the heat, anesthesia, and other physical parameters inherent in the experiment. For example, it might suggest that there is a natural reaction to simply applying fluid to the skin of the newt. The concentration of 3.32mg/ml elicited results that were very different from the control pattern. At this concentration the characteristic increase between 1 and 2 minutes was present, but the number of red blood cells continued to increase between 2 and 5 minutes and between 5 and 10 minutes. This concentration, though it was not the highest tested, produced the

largest percent increase for all concentrations of hydralazine that were tested in the study. The percent increase at 33.2mg/ml is slightly less than the percent increase at 3.32mg/ml possibly because the drug crystallized out more at this concentration and did not reach the tissues. Figure 3 shows the overall dose response curve

**Figure 3: Dose response for hydralazine: average for all times observed**




This dose response figure (Fig.3) shows the relationship between the percent increase and concentration at which it was observed. It shows the greatest increase from the 0.10mg/ml to the 3.32mg/ml concentration. It also shows a slight decrease from the 3.32mg/ml to the 33.2mg/ml concentration. The dotted line represents the average percent increase of the ATW control over all times observed. The smaller concentrations are difficult to interpret from this scale so the square root of each concentration was calculated and plotted. This scale was used for an additional representation and is shown in Figure 4.

**Figure 4: Modified dose response curve for hydralazine: averages for all times observed**

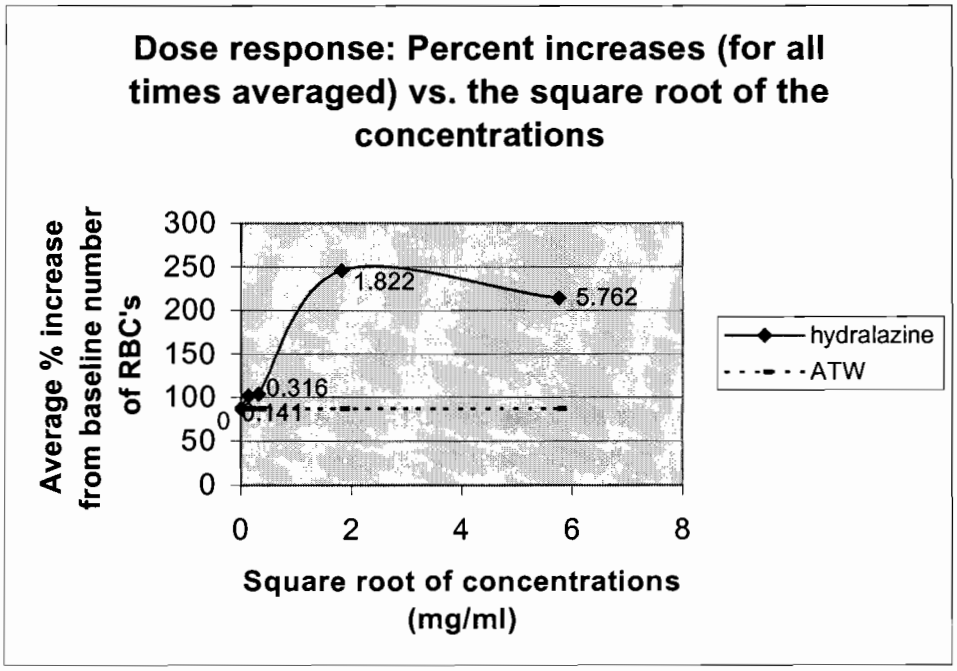



**Table 3: Significance values for each concentration of hydralazine in comparison to the ATW control**

| Concentration (mg/ml) | Sq. root of conc. | Significance (p value) |
|---|---|---|
| 0.02 | 0.141 | .398 |
| 0.10 | 0.316 | .402 |
| 3.32 | 1.822 | .065 |
| 33.2 | 5.762 | ***.011*** |

The results of statistical analysis for the response to hydralzine are shown in Table 3. Independent sample t-tests were performed to compare the difference between each concentration and the ATW control. The significance value in bold italic shows a significant difference at the $p<0.05$ significance level for the 33.2mg/ml concentration. This shows that the only significant difference was reached at the highest concentration used. The percent increase for 3.32mg/ml was not significantly different than the ATW control even though its overall value is higher than the percent increase for 33.2mg/ml.

The reason for this is that the standard deviation for the 3.32mg/ml concentration was much higher than the standard deviation for the 33.2mg/ml concentration. Because of the high level of variance, the p-value for the 3.32mg/ml concentration cannot be considered significant.

Study #2: Response to metoprolol administration

**Figure 5: Time response for metoprolol administration**

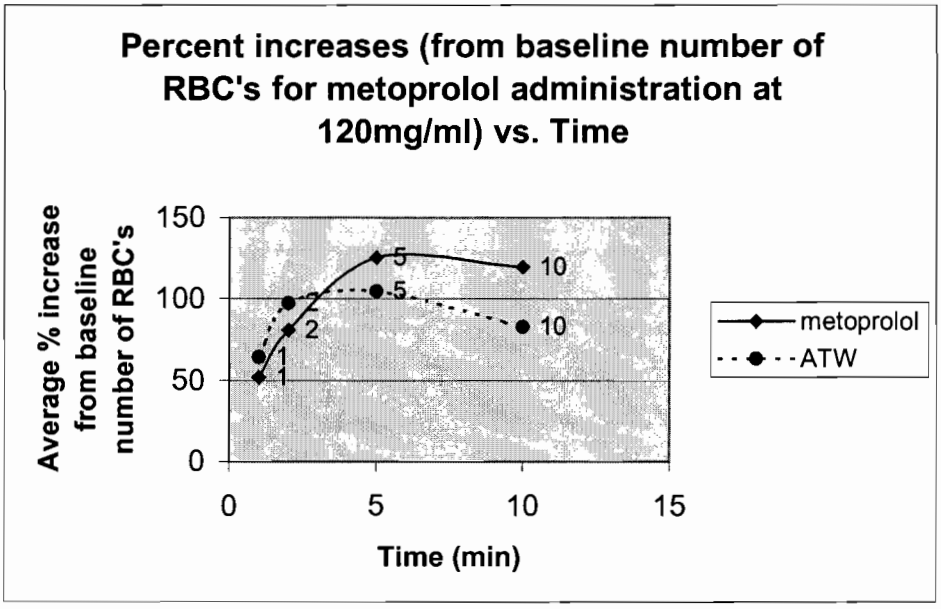



Figure 5 shows the time response to one concentration of the beta-blocker metoprolol. It shows the highest percent increase at the five minute time. The dotted line shows the time response curve for ATW so it can be used as a reference. No dose response curve was produced for metoprolol since only one concentration was tested. Table 4 shows the significance values for each time. According to these values, metoprolol produced no significant difference from the ATW control at each of the times. These results were expected from metoprolol for reasons discussed in a later section.

**Table 4: Significance values for metoprolol at each time of observation**

| Time (min) | Significance value (p value) |
|---|---|
| 1 | .354 |
| 2 | .399 |
| 5 | .385 |
| 10 | .319 |

Study #3: Response to diltiazem administration

**Figure 6: Time response for diltiazem administration**

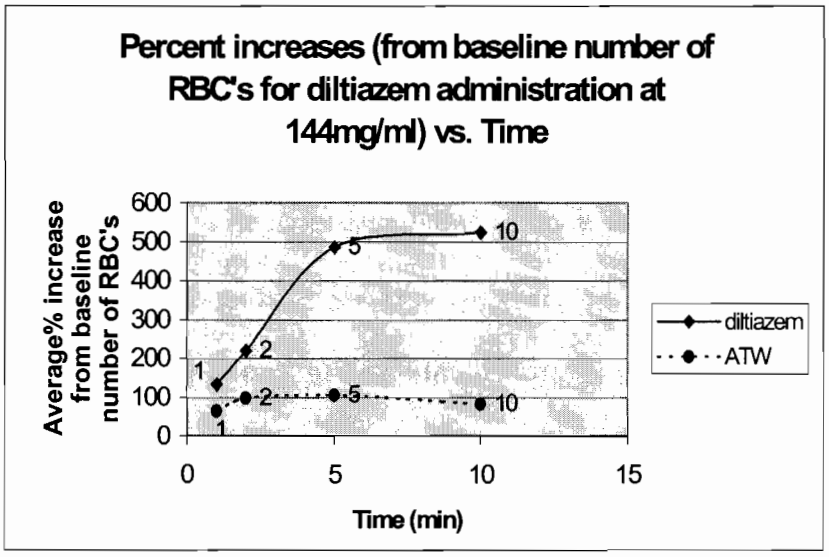



Figure 6 shows the time response for one concentration of the calcium channel blocker diltiazem. The ATW curve is again shown as a reference. This drug produced its highest percent increase at the 10 minute time. Again, no dose response curve was plotted because only one concentration was tested. Statistical analysis showed a much higher significant difference from the ATW control than was found for metoprolol under similar conditions. The significance values are shown in Table 5. The values shown in bold italic are significantly different ($p<0.05$) from the ATW control at those times.

These results were expected because of the mechanism of action of the drug. This will be discussed in a later section.

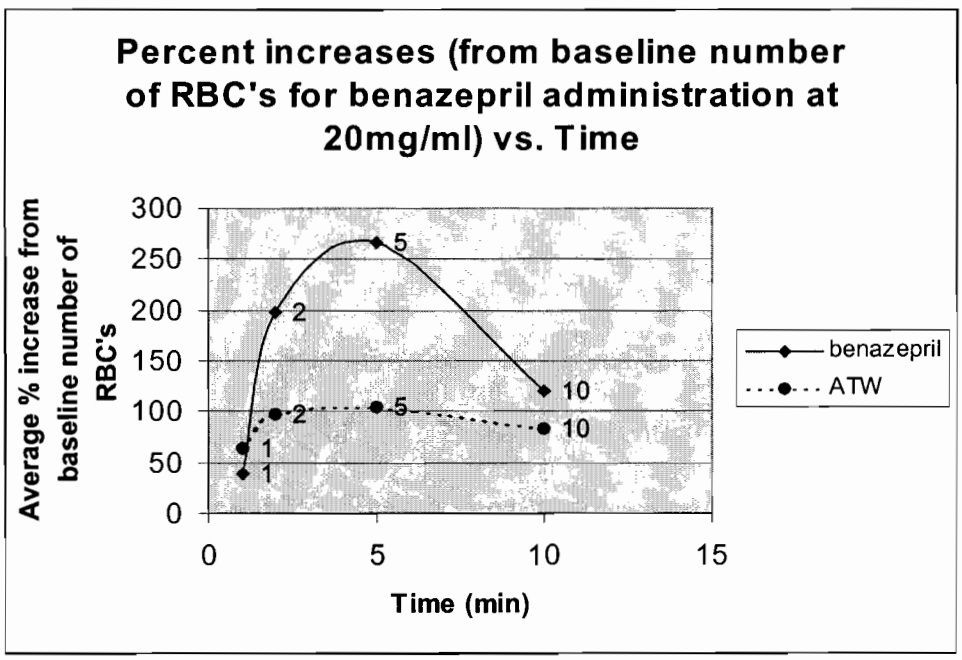
**Table 5: Significance values for diltiazem at each time of observation**

| Time (min) | Significance value (p value) |
|---|---|
| 1 | .076 |
| 2 | ***.013*** |
| 5 | ***.013*** |
| 10 | ***.006*** |

Study #4: Response to benazepril administration

Figure 7 shows the time response for the ACE inhibitor benazepril. The peak percent increase in number of red blood cells was at the 5 minute observation. Once again, data were recorded for a single concentration so no dose response curve was plotted. The ATW time response is shown as a reference.

**Figure 7: Time response for benazepril administration**



Statistically none of the percent increases were significantly higher than the ATW control. Significance values are shown in Table 6. Again, this response was expected because of the nature of the drug and will be discussed later.

**Table 6: Significance values for benazepril at each time of observation**

| Time (min) | Significance value (p value) |
|---|---|
| 1 | .287 |
| 2 | .057 |
| 5 | .067 |
| 10 | .316 |

Study #5: Response to nitroglycerine

Several concentrations of nitroglycerine were tested, so a dose response curve was plotted rather than time responses. The concentrations of nitroglycerine tested were 0.015mg/ml, 0.625mg/ml, and 1.25mg/ml. These concentrations, used for topical administration, were based on solubility of the drug and concentrations of the drug that are delivered via its normal route of sublingual administration. Figure 8 shows the dose response as well as the average percent increase of the ATW control.

From the concentrations tested, the highest concentration of nitroglycerine clearly had the largest impact on blood flow. The lower concentrations also showed an impressive increase from the baseline number of red blood cells as well as from the ATW control. Independent sample t-tests were also done for this data. Significance values are reported in Table7. The 0.015mg/ml and 1.25mg/ml concentrations were significantly different than the control ($p < 0.05$). The 0.625mg/ml concentration was very nearly significant with a p value of 0.054. This concentration is not significantly different because it has a higher standard deviation than the other two concentrations tested.

**Figure 8: Dose response for nitroglycerine administration**

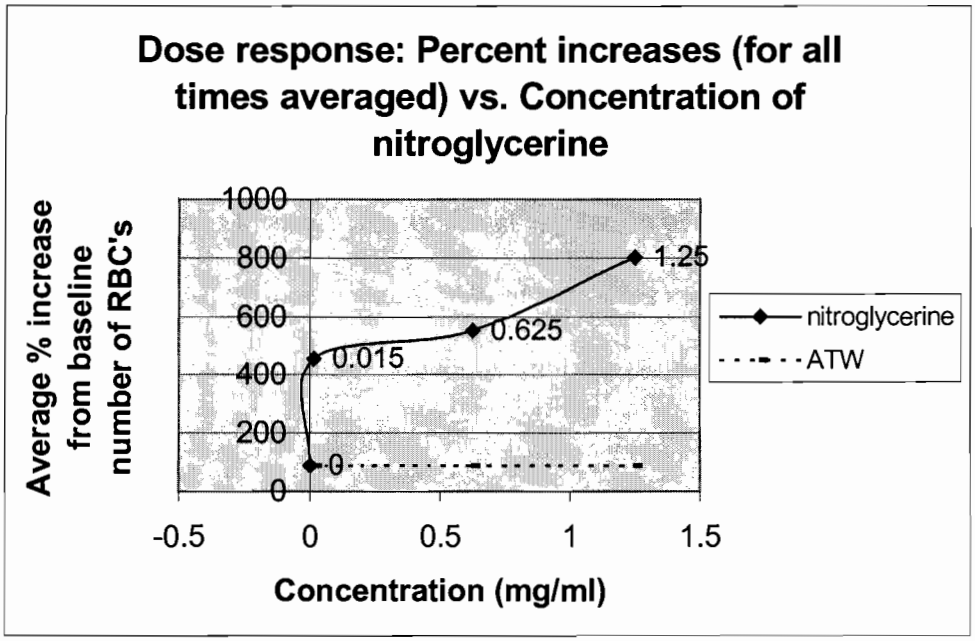



**Table 7: Significance values for nitroglycerine comparing test values to the ATW control**

| Concentration (mg/ml) | Significance (p value) |
|---|---|
| 0.015 | ***.020*** |
| 0.625 | .054 |
| 1.250 | ***.014*** |

In summary, hydralazine (study #1), diltiazem (study #3), and nitroglycerine (study #4) showed percent increases in the numbers of red blood cells from the baseline value that were significantly higher than the percent increases of the ATW control according to statistical testing. Metoprolol (study #2) and benazepril (study #4) did not produce statistically significant results. Incidentally, the non-parametric Mann-Whitney test confirmed the significances found using the independent sample t-tests. This is important because with the Mann-Whitney test there are no assumptions of normalcy on

the sampling variables as there are with the t-test. Since this test simply reinforced the current results, the significance values were not reported.

## Discussion

The action of hydralazine includes direct relaxation of vascular smooth muscle because it interferes with cellular calcium movement. This movement of calcium is responsible for initiating and maintaining the contractile state of the vascular smooth muscle. These effects result in peripheral vasodilation that in turn causes decreased peripheral resistance and a decrease in arterial blood pressure. Hydralazine preferentially dilates arterioles instead of veins, thus minimizing postural hypotension and promoting increased cardiac output (Drug Facts and Comparisons, 2000). Hydralazine is extensively metabolized in the liver and is excreted as active drug and metabolites in the urine (Drug Facts and Comparisons, 2000). These metabolites are mainly the products of acetylation and hydroxylation (Dubois, Schmid, et al. 1987). Hydralazine has not been put in a specific class of drugs because the exact mechanism of action has not been completely mastered. With this information about hydralazine, the present study then set out to test the receptivity of blood vessels in the skin of the newt to the drug. The development of a dose response curve for hydralazine was the initial objective.

Most previous studies involving hydralazine have used rats as test animals. A pharmacological study published in 1992 (Grichois, Blanc, et al. 1992) looked at short-term variability of blood pressure and heart rate in rats treated with hydralazine and also some rats treated with the ACE inhibitor enalapril. After acute administration, these drugs caused variably decreased blood pressure. This led the authors to suggest that the

rats have reflex responses that mediate vascular sympathetic activation. Heart rate was also variably increased indicating a reflex mediated cardiac sympathetic activation (Grichois, Blanc, et al. 1992). These findings show that rats are a good model of the human response to hydralazine since humans also have the cardiac reflex.

Another study focused on the effects of hydralazine on sleeping rats. That study found a lowered mean blood pressure and an increased respiration during all sleep stages for the rats. This may be possible because hydralazine can cross the blood-brain barrier in the form of metabolites. Hydralazine also potentially causes the formation of nitric oxide within the vasculature that can freely diffuse across the blood-brain barrier to increase the concentration at the brain stem to stimulate respiration (Carley, Trbovic, et al., 1997). In a more recent study, it was shown that antihypertensive drugs regulate tissue antioxidant enzyme expression through transcriptional control mechanisms. This was found by comparing mRNA levels with tissue enzyme specific activities for drugs such as hydralazine. Significant results were found in myocardial tissues, but liver tissue differences proved to be insignificant (Ma and Johnson, 1999). Through studies such as these, the mechanisms of hydralazine are slowly being revealed along with all of its effects on the body.

The newt model proposed in the study of hydralazine produced the expected results based on the mechanisms of the drugs tested, but has some areas of high variability. Of course when working with animals there will be individual variability, but there were also some additional factors worth considering. Since the hydralazine was dissolved in water, it was not always easy to know exactly where a drop of the test solution remained. If the animal was too wet, it would simply run off and may never

have reached the cutaneous vasculature. This was realized early in the study, so some steps were taken to eliminate as much of this as possible. For example, the skin of each newt was allowed to dry out more than usual so that the hydralazine solution would definitely be absorbed into the skin. This allowed the hydralazine-ATW mixture to bead up on the skin so that exact placement was known. Another problem that was encountered was the crystallization of the solution at high concentrations. Air currents in the room and heat from the dissecting microscope lamps may have been the cause of the crystallization. When this occurred, it was almost impossible to see the capillaries and therefore count the red blood cells. It also prevented the drug from being completely absorbed into the tissues. This problem kept us from reaching one of the objectives of the study, which was to test a concentration comparable to the garlic concentration that was used in the previous studies on herbs. This target concentration would have been 333.2mg/ml hydralazine, but no data could be recorded. So, the procedure with the newts must be adapted to accommodate whatever substance is being worked with.

Two additional sources of variability may have been the drug and animal in relation to one another. Hydralazine has some common adverse reactions, especially with higher doses. In humans, cardiovascular reactions may include palpitations, tachycardia, or angina pectoris. Headache, dizziness, tremors, or depression and anxiety are some central nervous system reactions. Hydralazine may also cause gastric problems such as constipation, vomiting, nausea, or diarrhea. Rash, fever, chills, nasal congestion, edema, and muscle cramps are also possible effects. Most of these can be eliminated by lowering the dosage (Drug Facts and Comparisons, 2000). This being known, it is hard to say what type of reactions may occur in the newt. Also, it is known that amphibian systems

are highly variable from species to species. This is evident in Duellman's Biology of Amphibians, since he chose two representative species throughout the book to describe each system (Duellman, 1986).

The success of the work with hydralazine stimulated an interest to look at other classes of drugs. As mentioned previously, the three major classes of pharmaceuticals used to treat hypertension are beta-blockers, calcium channel blockers, and angiotensin converting enzyme (ACE) inhibitors. It is necessary to know the characteristics of these classes to fully understand the results of this study. The first class studied were the beta-blockers. These drugs produce antihypertensive effects by decreasing cardiac output or decreasing peripheral vascular resistance. They get their name because they block $beta_1$ and $beta_2$ adrenoreceptors in several proposed locations (Katzung, 1998). Blockades are thought to occur in the brain, kidney, and at peripheral adrenergic neurons. When beta-receptors are blocked, they inhibit the production of renin. Renin can lead to vasoconstriction and increased blood pressure when present (Katzung, 1998).

As a representative of this class metoprolol is relatively cardioselective, which means it selectively blocks beta-receptors in the heart and vascular smooth muscle (Katzung, 1998). In the present study, the results for metoprolol were not statistically significant because of the mechanism of the drug and the topical method of administration used here. The beta-blocking mechanism has a systemic effect and therefore is not likely to be locally effective and may have to be administered orally or intravenously to produce an effect. Since this study used topical application and the drug does not act directly on the smooth muscle of the blood vessels, one would not expect

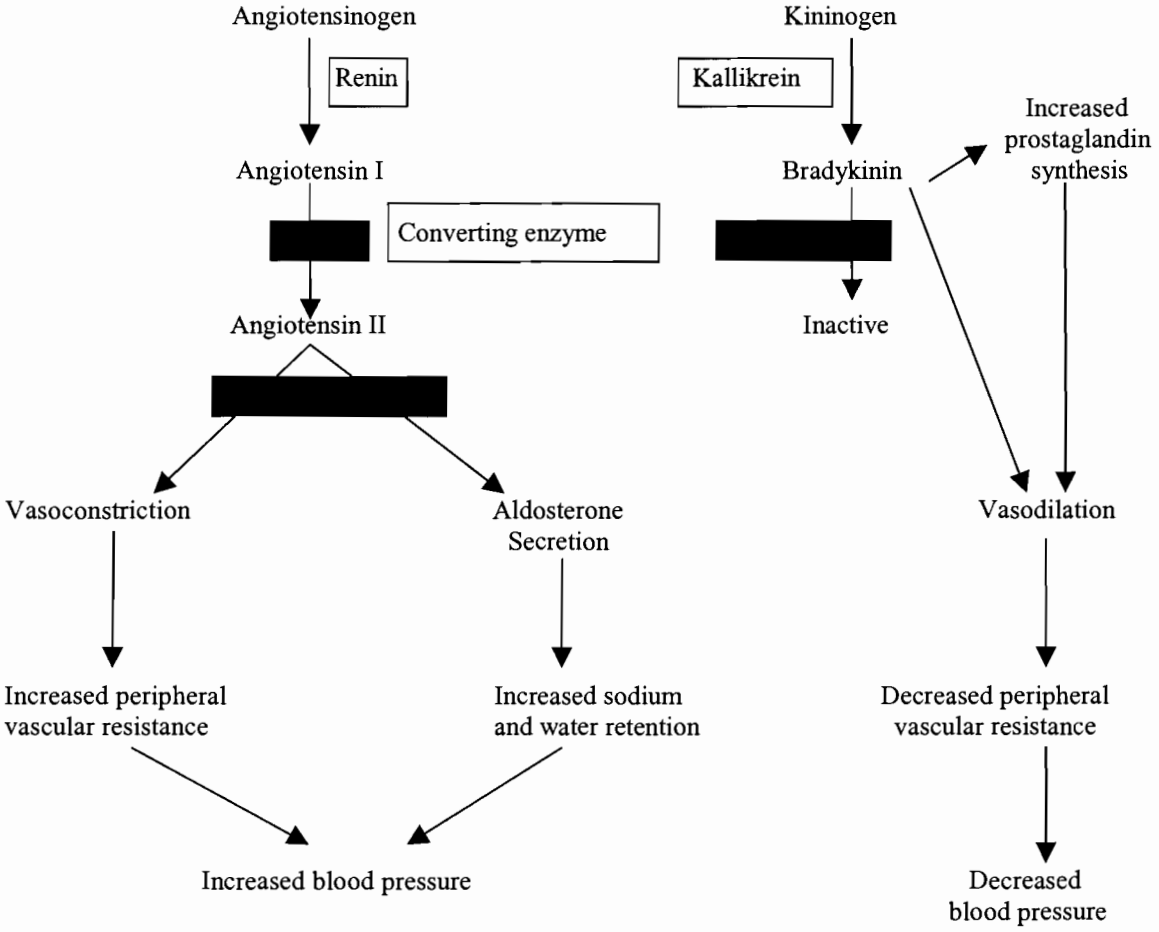
vasodilation to result and indeed the results were not significantly different from the ATW control.

Calcium channel blockers were the second class of drugs that were studied. These drugs inhibit calcium influx into arteriole smooth muscle (Katzung, 1998). This prevents contraction of the muscle and therefore does not allow the arteriole to be constricted. A reduced rate of calcium ion diffusion into cardiac muscle cells can control the force of contractions and reduce arrhythmia, tachycardia, and hypertension (Katzung, 1998). In smooth muscle cells, this action can dilate blood vessels to increase blood flow (Katzung, 1998).

The representative for this class was a drug called diltiazem. The results for the test concentration chosen were very statistically significant. This is believed to be because this class of drug has a more direct effect on the blood vessels. The mechanism is not systemic like the beta-blockers. The direct effect is ideal with the topical application because it can act at the specific point of application. Parenthetically, it should be noted that the representative for this class was supposed to be a drug called nifedipine because it is more selective as a vasodilator (Katzung, 1998). Unfortunately it was only soluble in very volatile solvents. An ethanol control was tried and showed no apparent adverse effects to the animal and the test concentration was calculated the same as the other drugs. When it was mixed with the drug and applied to the newt, however, there was a harmful reaction. Two newts died and one nearly died because of this application. This is when diltiazem was chosen to replace nifedipine, and it produced very good results.

The third class studied was the ACE inhibitor class. The mechanism for these drugs is shown in Figure 9. The blockage of the converting enzyme does not allow the production of angiotensin II, therefore vasoconstriction is decreased and there is a hypotensive effect on blood pressure. Blocking the converting enzyme also allows bradykinin to be active and vasodilation is promoted. As with the beta-blockers, this is a very systemic mechanism.

**Figure 9: ACE Inhibitor Mechanism of Action**




The representative for this class was a drug named benazepril. It was not expected to produce statistically significant differences from the ATW control since the mechanism would not be expected to act locally in the present system. As expected, it did not show a statistically significant difference from the ATW control at any of the times.

The final study focused nitroglycerine, which slightly strayed from the main goal of testing the three main classes of hypotensive drugs. Nevertheless, since nitroglycerine is a reliable and potent vasodilator it was decided to include a test of its effectiveness on the newt vasculature. Nitroglycerine is commonly used to treat angina and is in a class of drugs called the nitrates. The action of nitroglycerine is usually selective to smooth muscle cells (Katzung, 1998). It is denitrated and releases a free nitrite ion. Subsequently, nitric oxide is released due to an unknown enzymatic reaction and functions as a potent vasodilator (Katzung, 1998). A problem with nitroglycerine is that cells can become tolerant to it and it loses its effect. The cause of this is not known for certain and it is difficult to reverse (Katzung, 1998).

The nitroglycerine used in this study produced impressive results. At all trial concentrations it produced a statistically significant increase in vasodilation from the ATW control. This would be expected due to the nature of the drug. The test concentrations were mixed using sublingual tablets. These tablets are intended for application to tissue that is similar to the skin of the newt. The stratified squamous epithelium of the human mouth is similar to the epithelium covering the newt's body. So, the nature of the drug and the direct local mechanism produced very good results, as expected, with the testing procedure of this study.

The drugs used in this study are simply representatives for each clinical class of hypotensives. One would expect that the drugs that directly effect the smooth muscle cells of the blood vessels would be effective in this system. This is the case for hydralazine, diltiazem, and nitroglycerine. Drugs with a more systemic effect would not be expected to increase the number of red blood cells flowing through individual vessels.

In fact, metoprolol and benazepril did not produce significant vasodilation in this system. Therefore this test system, using the newt vasculature, shows promise as a model for the human cardiovascular system in relation to vasodilators.

# Literature Cited

Carley, D.W.; Trbovic, S.M.; Bozanich, A. Cardiopulmonary control in sleeping Sprague-Dawley rats treated with hydralazine. *Journal of Applied Physiology*. 83(6): 1954-1961; 1997

Drug Facts and Comparisons. Facts and Comparisons: January 2000

Dubois, J.P.; Schmid, K.; Reiss, W. Metabolism of hydralazine in man. Part II: Investigation of features relevant to drug safety. *Arzneimittelforschung*. 37(2): 189-93; 1987

Duellman, W.E.; Trueb, L. Biology of Amphibians. New York: McGraw-Hill Book Company; 1986

Grichois, M.L.; Blanc, J.; Deckert, V.; Elghozi, J.L. Differential effects of enalapril and hydralazine on short-term variability of blood pressure and heart rate in rats. *Journal of Cardiovascular Pharmacology*. 19(6): 863-9; 1992

Katzung, Bertram G. Basic and Clinical Pharmacology. Stamford, CT : Appleton and Lange ; 1998

Kindlimann, Ashley R. ; Zaccaria, Robert. The demonstration of vasoactive properties of selected herbs on the cutaneous vasculature of the Red spotted newt. Unpublished report. 1999

Ma, Lin; Johnson, P. Antihypertensive drug therapy and antioxidant enzyme mRNA levels in spontaneously hypertensive (SHR) rats. *Comparative Biochemistry and Physiology B*. 122(1): 119-126; 1999