Temporal Effect of Varying Doses of Clonidine on the Fasting Blood Glucose Levels of Sprague Dawley Rats

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Citation

Abstract
Background Primarily used as a centrally-acting anti-hypertensive drug, clonidine has also been shown to affect blood glucose determinants by acting on peripheral α-2 adrenoceptors. However, the studies which investigate the effect of clonidine on blood glucose levels yielded conflicting results depending on conditions applied. Methods Thirty-five (35) adult male Sprague-Dawley rats weighing 140-330 grams were randomly and equally assigned to five treatment groups of varying clonidine doses: 1, 2, 4, 7µg/kg and 0.9% NaCl (control). The doses were administered intra-peritoneally after fasting the rats for 18 hours. Whole blood samples (at least 1 µl) were obtained via tail pricking/tail lancing and measured for glucose levels using a commercially available glucometer (One-touch Ultra®) 1 hour before injection to get baseline blood glucose levels and every hour for 8 hours after injection to measure changes in blood glucose levels. The rats remained fasted until after the 8-hour monitoring period. Data were analyzed using t-test, Analysis of Variance(ANOVA) and Tukey LSD at 95% confidence interval (α=0.05). Area under the curve (AUC) reflecting the cumulative blood glucose level within the first 8-hour monitoring period for each dosage was computed. Results Significant differences among fasting blood glucose levels of 2, 4, 7 µg/kg and control groups were observed at the 1st to 4th hour after injection, with the treatment groups having higher glucose levels than the control group. No significant difference was observed between the 1 µg/kg group and control group. At the 5th hour onwards, no significant difference was noted among treatment means. Cumulative dose effect of clonidine (AUC) showed a significant rise in glucose at 4 and 7 µg/kg with apparent saturation at 4 µg/kg when plotted in a dose-response curve. Conclusions Intra-peritoneal administration of clonidine significantly increases blood glucose levels when administered at 2, 4 and 7 µg/kg with peak effect at 1st to 3rd hour post injection and no significant difference starting at 5th hour post-injection onwards. Optimal dosage is found to be at 4 µg/kg.

INTRODUCTION

BACKGROUND
Clonidine is a centrally-acting alpha-adrenergic receptor agonist that is primarily used to decrease blood pressure (Katzung, 2007). Aside from its hypotensive effects, it has also been shown to affect the plasma levels of insulin, glucagon, cortisol, epinephrine, norepinephrine and glucose through different mechanisms on peripheral adrenergic receptors. Studies have been conducted to prove the effect of clonidine on these glucose determinants. However, most did not relate these findings to resulting blood glucose levels and did not quantitatively characterize the effect in relation to experimental conditions applied. In addition, studies which investigated the effect of clonidine on blood glucose levels yielded different results depending on the dosage administered and whether or not clonidine was administered as a premedication drug for surgery or stress.

OBJECTIVE
This study aims to investigate the temporal effect of single administration of varying intra-peritoneal doses of clonidine on baseline blood glucose levels of fasted, minimally-stressed Sprague-Dawley rats.

SCOPE AND LIMITATIONS
This study has a double blind, randomized controlled study design, investigating the temporal effects of clonidine on the
blood sugar levels of thirty-five (35), male, Sprague-Dawley rats weighing 140-330 grams (average 215 grams), aged 4-6 weeks. Non-diseased, minimally-stressed rats were used; thus, results of the study cannot be directly applied to diseased states such as hyperglycemia, hypertension, insulin resistance, etc. The results can only be strictly applied to the members of the same population with the same health condition. Generalization to the human population, healthy or diseased, is also beyond the scope of this study.

The subjects were given a single administration of varied doses of clonidine. The results of this study are therefore limited to the acute effects of a single administration of clonidine. Chronic use or long-term effects of clonidine are beyond the scope of this study.

The outcome measured was the hourly change in blood sugar levels of the rats, as measured by a hand-held glucometer. Other parameters such as blood pressure, insulin levels or drug concentration were not measured.

This study aims to establish whether clonidine has an effect on blood sugar levels of minimally-stressed rats. It does not aim to establish the underlying mechanism behind said effect.

**SIGNIFICANCE**

Clonidine is an alpha-2 adrenoreceptor agonist and is presently used to control high blood pressure. Studies have shown that clonidine also has an effect on blood sugar levels. However, results of these studies were conflicting. Some studies showed an increase in blood glucose levels after administration of clonidine (Ditullio et al., 1984; Skoglund, 1987; Gotoh et al., 1988; Lattermann et al., 2001; Saitoh et al., 2001; Doyle & Egan, 2003); while others showed a decrease in expected blood glucose levels after administration of clonidine (Belhoula et al., 2003; Nishina et al., 1998; Gaumann et al., 1991; Munoz et al., 2000; Pasquali et al., 2000; Schneemilch et al., 2006). Some studies even showed both an increase and decrease in blood glucose levels (Veliquette & Ernsberger, 2003).

Latterman, Shricker, Giorgieff, and Shreiber (2001) administered clonidine to patients prior to abdominal hysterectomy. Measurement of blood glucose levels after 30 minutes showed higher intra-operative plasma glucose levels and lower plasma insulin levels in patients given 1 µg/kg of clonidine. Gaumann, Tassonyi, Rivest, Fathi, and Reverdin (1991) administered clonidine premedication (300 µg) prior to craniectomy in 9 patients and compared it with placebo administered to 10 patients. Their study showed that patients given clonidine premedication had lower plasma glucose concentrations compared to those given placebo (although both were still increased compared to baseline). Nishina, Mikawa, Maekawa, Shiga, and Obara, (1998) administered 4µg/kg premedication of clonidine to children (3-13 years old) prior to minor surgery and found that clonidine attenuates the hyperglycemic response to surgery. Belhoula, et al. (2003), administered clonidine to patients with Type 2 Diabetes Mellitus prior to ophthalmic surgery and noted a decrease in intra-operative and post-operative glucose levels (up to 6 hours) after administration of 225 µg (for < 55 kg patients), 300 µg (for 55-74 kg patients) and 375 µg (if 75 kg or more) of clonidine. Another study, done by Gotoh, Iguchi, and Sakomoto, (1988), showed an increase in blood glucose levels post-intravenous or intraventricular administration of clonidine (5, 50 and 100 nmol) in rats.

These studies suggest that clonidine has a dose-dependent effect on blood glucose levels in both humans and rats. However, this effect has not been adequately investigated. The exact relationship between dose and glycemic response to clonidine has not been established. Further information about the effect of clonidine on blood glucose levels is important because clonidine, as an anti-hypertensive agent, is being used by patients who may concomitantly have problems in glycemic control. In addition, clonidine also has other indications that are associated with glycemic control. Clonidine is used in the management of diabetic painful neuropathy as stated in the article of Neal (2009). Thus, it is essential to investigate clonidine’s effect on blood glucose levels.

This study aims to address the present gap in knowledge by investigating the temporal effect of various doses of clonidine on blood glucose levels in rats. This study was designed to minimize the confounding effects of stress (which took the form of surgery in the previous studies) and disease on the effect of clonidine. Other factors, such as age, diet, and gender, which could potentially affect blood glucose levels, were also standardized. A homogenous population (male, healthy, Sprague-Dawley rats) was used in order to accurately measure the effect of clonidine on the blood glucose levels of the rats.

**METHODOLOGY**
STUDY POPULATION
Thirty-five (35) adult male Sprague-Dawley rats weighing 140-330 grams (ave. 215 grams) were procured from the Bureau of Food and Drugs in Alabang, Muntinlupa City, Philippines for the experiments. They were kept in individual steel cages at the Animal House, 2nd Floor, Paz Mendoza Building, College of Medicine under a 12-hour dark/light cycle at a temperature of 22-24°C. The rats were provided access to food (standard rat pellets) and water ad libitum. The rats were subjected to a two-week acclimatization period prior to the administration of treatments. Utmost care for the animals was ensured for the study following the guidelines of proper animal handling according to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources, National Research Council U.S.A. (1996).

MATERIALS
GLUCOMETER
The primary outcome of interest in the study is the blood glucose level of the rats. This parameter was measured using a commercially available hand-held glucometer. The glucometer used in this study is the OneTouch® Ultra™ Blood Glucose Monitoring System manufactured by Lifescan, Johnson & Johnson (USA). This equipment needs only one microliter of whole blood, and it shows the results within 5 seconds (Luo et al, 2005). Previous studies that measured rat glucose levels used the same type of equipment (Blankenhorn et al, 2005; Carroll, Zenebe and Strange, 2006). In a study done by the Diabetes Research in Children Network Study Group (2003) on the accuracy of the OneTouch® Ultra™ Blood Glucose Monitoring System in type 1 diabetic children, the results showed that the equipment consistently obtained accurate blood glucose readings based on the criteria set by the International Standardization Organization (ISO) in 2003. The ISO 15197 criteria states that the minimum acceptable accuracy for results produced by a glucose monitoring system shall be that 95% of the individual glucose meter results fall within ±15 mg/dL (0.83 mmol/L) of the reference method at glucose concentrations < 75 mg/dL (4.2 mmol/L) and within ±20% at glucose concentrations ≥ 75 mg/dL (4.2 mmol/L) (Kristensen, 2008).

VALIDITY OF GLUCOMETER USE IN RATS
A study by Luo, et al in 2005 entitled: Evaluation of Portable Glucometers for Use in Rats compared plasma and whole blood glucose levels measured by commercial laboratories to levels measured through two brands of portable glucometers (Ascensia ELITE® by Bayer HealthCare and One Touch Ultra® by LifeScan). The study also investigated the effect of heparin on blood glucose measurements.

Results from this study showed that heparin had no significant effect on blood glucose measurement. However, glucometers may underestimate blood glucose concentration when compared to commercial measurements. The investigators attributed this discrepancy to the fact that glucometers usually use whole blood samples while commercial labs usually use plasma samples. In a previous study (Luo, et al., 2005) it was shown that whole blood glucose levels are lower than plasma glucose levels when measured in systems that allowed the use of both plasma and whole blood. This difference is attributed to hematocrit which is a factor in whole blood. The researchers therefore recommended that the following correction factors be employed: One Touch Ultra® reading x 1.38=commercial lab value. It was concluded in the study that glucometers are advantageous for use in rats due to the small sample required (1µL for One Touch Ultra®).

These results support the use of a glucometer to measure blood glucose levels in our study. Also, since the design entails the use of whole blood samples, the One Touch Ultra® glucometer is the most appropriate since it is more accurate in measuring whole blood glucose levels in rats.

TREATMENT PREPARATION
EXTRAPOLATION OF HUMAN TO RAT TREATMENT DOSES OF CLONIDINE
Absolute human doses of 1µg/kg, 2µg/kg, 4µg/kg, and 7µg/kg (Latterman et. al, 2001) of the drug were extrapolated to rat doses on the basis of surface-area relationship using the method suggested by Laurence and Bacharach:

Absolute dose in human (µg) = dose by weight (µg/kg) x body weight (70kg)
PREPARATION OF DOSES OF CLONIDINE

A stock solution of 7.5 µg/ml (w/v) of Clonidine hydrochloride (Catapres®) tablet in 0.9% NaCl solution was prepared by dissolving ten (10) 75µg tablets in 0.9% NaCl and diluting to a volume of 100ml using a volumetric flask of the same capacity. Individual volumes containing the individual extrapolated doses for each rat in a particular treatment were obtained from the stock solution and diluted to 2ml using 0.9% NaCl as diluent. All of the volumes were administered to the rats using 3cc Terumo® Syringes with Gauge 25 needles.

EXPERIMENTATION PROPER

TREATMENT ASSIGNMENT

One day prior to the administration of treatments, seven rats were randomly assigned to each of the five treatment groups: (1) 1µg/kg clonidine dose, (2) 2µg/kg clonidine dose, (3) 4µg/kg clonidine dose, (4) 7µg/kg clonidine dose, (5) 2ml 0.9% NaCl (control). Treatments 1 to 4 were all extrapolated human to rat doses as stated previously. The rats were also fasted for 18 hours without restriction to water before the treatments were administered. They remained fasted without restriction to water 8 hours after the treatments were administered. Treatments were administered intra-peritoneally. Prior to the experiment proper, the investigators underwent training on rat handling, intra-peritoneal injection and blood extraction through tailprick. The investigators, data collectors, and data analysts were all blinded during the conduct of the study.

OUTCOME MEASUREMENT

Whole blood samples (at least 1 µl) were obtained via tail pricking/tail lancing method. Immediately after the blood samples were drawn, they were applied onto the test strips of the glucometer according to the protocol specified by the unit. Blood glucose concentration readings were then recorded.

Blood glucose concentration measurements were done an hour before the administration of treatments to establish the baseline blood glucose levels for all the treatments. Measurements were carried on an hour after the treatments were administered and successively for an interval of 1 hour thereafter until the 8th hour after treatment administration.

STATISTICAL ANALYSIS

Differences in the blood glucose levels of the rats in a particular treatment group were analyzed using t-test. Individual group treatment means were also calculated over the period of eight (8) hours. Trend on the dosage administration and blood glucose was also assessed among the different treatment groups.

Blood glucose levels among the treatment groups were analyzed using t-test. Differences among means of treatment effects were determined using analysis of variance (ANOVA). Post hoc analyses were employed using Tukey LSD to determine which pairs of treatment effects are significantly different from each other.

A probability of less than 5% was accepted as significant and statistical results for means were reported in terms of 95% confidence interval.

A nonlinear curve was obtained for each treatment group relating time and blood glucose level. Cumulative blood glucose levels were computed over the 8-hour period for each treatment group. Dose-response relationship was established between dosage and blood glucose levels using nonlinear regression analysis.

RESULTS

ANALYSIS OF INDIVIDUAL TREATMENT GROUPS

Analysis of variance of the mean blood glucose levels of the seven rats administered intraperitoneally with 1µg/kg of clonidine shows no significant statistical difference over the 8-hour period monitoring (p=0.62).

The graph below illustrates the behavior and variability of the hourly blood glucose levels of the seven rats and the group treatment mean over the 8-hour period when 1 µg/kg of clonidine was administered intraperitoneally.
Figure 2
Figure 1. Composite graph for all seven blood glucose levels with group treatment mean

The hourly means of blood glucose levels of the rats when a 2 µg/kg of clonidine was administered intraperitoneally show no significant statistical difference (p=0.06) over eight hours after employing the analysis of variance.

The extent of blood glucose variability among the seven rats and the group treatment mean is reflected by the graph below when the monitoring was done over the 8-hour period after 2µg/kg of clonidine was administered intraperitoneally.

Figure 3
Figure 2. Composite graph for all seven blood glucose levels with group treatment mean

After the intraperitoneal administration of 4µg/kg of clonidine among six rats, the hourly monitoring of the blood glucose levels show a highly significant statistical difference (p<0.01) over the 8-hour period. There were only six rats included in this group due to the death of one subject after the administration of the drug.

A pictorial and qualitative description of the behavior of the hourly blood glucose levels of the six rats and the group treatment mean over the 8-hour period of monitoring after 4µg/kg of clonidine was administered intraperitoneally is illustrated by the graph below.

Figure 4
Figure 3. Composite graph for all six blood glucose levels with group treatment mean

Investigation on the differences of hourly means of blood glucose levels of the seven rats belonging to the 7µg/kg treatment group was done using analysis of variance. Results show that mean blood glucose levels significantly differ every hour over the 8-hour interval after intraperitoneal administration of clonidine (p<0.01).

The graph below describes the behavior of the hourly blood glucose levels of the seven rats and the group treatment mean over the 8-hour period monitored every hour after intraperitoneal administration of 7µg/kg of clonidine.

Figure 5
Figure 4. Composite graph for all seven blood glucose levels with group treatment mean

Comparisons on the hourly means of blood glucose levels of the rats in the control group show no statistical difference among the blood glucose means (p=0.97) over the 8-hour period monitored every hour.

The hourly behavior of the blood glucose levels of the seven rats and the group treatment mean in the control group over the 8-hour period is described by the graph below.
Analysis of the individual treatment groups shows that the hourly blood glucose level means over the 8-hour period of monitoring are not significantly different in the 0.9% NaCl solution, 1µg/kg and 2µg/kg treatment groups. However, a high statistical significance on the difference among hourly blood glucose level means among the 4µg/kg and 7µg/kg treatment groups over the 8-hour period was observed.

**ANALYSIS AMONG TREATMENT GROUPS ON AN HOURLY BASIS**

The hourly mean blood glucose level values among the treatment groups over the 8-hour period are summarized in the table below.

The behavior of the hourly blood glucose level values among the treatment groups within the 8-hour period is described by the following graph.

It is important to note that the hourly blood glucose level values exhibit an increasing trend as the dose is increased from 1 µg/kg to 4 µg/kg. However, the trend did not persist within the second to fourth hour for the 4 µg/kg and 7 µg/kg, that is, the blood glucose levels in 4 µg/kg are greater than the blood glucose levels in 7 µg/kg.

Sensitivity analysis was performed between 4 µg/kg and 7 µg/kg treatment groups to assess the possible presence of an outlier observation that lowers the mean value of 7 µg/kg treatment group. Upon removal of the extremely low values, there is no significant improvement in the mean blood glucose value of 7 µg/kg compared to 4 µg/kg.

Analysis of variance (ANOVA) was employed to determine if there is a significant difference among group treatment means at a specific hour of monitoring. At baseline, ANOVA shows that there is no significant difference among group treatment means (p=0.914). However, highly statistically significant differences were noted on the mean blood glucose levels among the different treatment groups from the first to fourth hour after the intraperitoneal administration of clonidine.

One hour after the intraperitoneal administration of clonidine, ANOVA shows that there is significant difference among group treatment means (p=0.004). Post hoc analysis was performed to further assess which pair of means differ. Using Tukey LSD method of pairwise comparison, results show that 1 µg/kg treatment group significantly differs with 7 µg/kg treatment group (p=.032); 2 µg/kg treatment group significantly differs with the control group (p=.005). The 4 µg/kg treatment group significantly differs with the control group (p=.006); and 7 µg/kg treatment group significantly differs with the control group (p=.000). The 1 µg/kg treatment group does not significantly differ with the control
Temporal Effect of Varying Doses of Clonidine on the Fasting Blood Glucose Levels of Sprague Dawley Rats

group (p=.085).

On the second hour of monitoring, analysis of variance showed a statistically significant difference among treatment groups (p<0.01). Using Tukey LSD method of pairwise comparison, results show that 1 µg/kg treatment group significantly differs with 2 µg/kg, 4 µg/kg and 7 µg/kg treatment groups (p=.008, p=.000 and p=.004). Control group significantly differs with 2 µg/kg, 4 µg/kg and 7 µg/kg treatment groups (all p-values less than 0.01). The 1 µg/kg treatment group does not significantly differ with the control group (p=.094).

On the third hour, analysis of variance showed that there is a significant statistical difference among treatment groups (p<0.01). Using Tukey LSD method of pairwise comparison, results show that 4 µg/kg treatment group significantly differs with 1 µg/kg, 2 µg/kg and control groups (all p-values less than 0.01). The 7 µg/kg treatment group significantly differs with 1 µg/kg, 2 µg/kg and control groups (p-values less than 0.01). The control group differs with 4 µg/kg and 7 µg/kg treatment groups (p-values less than 0.01). The 4 µg/kg treatment group does not significantly differ with 7 µg/kg treatment group (p=.113).

On the fourth hour of monitoring, analysis of variance showed that there is a significant statistical difference among groups (p=.011). Using Tukey LSD method of pairwise comparison, results show that 4 µg/kg treatment group significantly differs with 1 µg/kg, 2 µg/kg and control groups (p=.010, p=.023 and p=.002). The control group significantly differs with 4 µg/kg and 7 µg/kg treatment groups (p=.002 and p=.014). The 2µg/kg treatment group significantly differs with 4 µg/kg treatment group (p=.023).

On the fifth to the eight hour monitoring, no significant statistical difference was noted on the mean blood glucose levels among treatment groups (p=.053, p=.620, p=.790, and p=.850).

DOSE-RESPONSE RELATIONSHIP
DETERMINATION OF FUNCTIONAL RELATIONSHIP BETWEEN TIME AND RESPONSE (BLOOD GLUCOSE LEVEL) AMONG TREATMENT GROUPS

The functional equation between time and the response to clonidine (blood glucose levels) was determined using least squares method for nonlinear relationship. A quartic polynomial was an appropriate choice of fitting the data points. Best-fit curve is chosen with the highest R-square value.

1/µG/KG TREATMENT GROUP

Figure 9
Table 2. Blood glucose level means of 1µg/kg treatment group

<table>
<thead>
<tr>
<th>TIME</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose Level Mean</td>
<td>52.714</td>
<td>74.605</td>
<td>11.143</td>
<td>67.429</td>
<td>58.145</td>
<td>69.057</td>
<td>61.429</td>
<td>65.429</td>
<td>68.395</td>
</tr>
</tbody>
</table>

Figure 10
Figure 7. Best-fit curve for dose response relationship of 1µg/kg treatment group

The equation of the best-fit curve generated is:

\[ y = -0.0527x^4 + 1.1145x^3 - 7.4385x^2 + 15.877x + 62.714 \]

with an \( R^2 = 0.8009 \).

2/µG/KG TREATMENT GROUP

Figure 11
Table 3. Blood glucose level means of 2µg/kg treatment group

<table>
<thead>
<tr>
<th>TIME</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose Level Mean</td>
<td>50.714</td>
<td>53.600</td>
<td>67.429</td>
<td>75.657</td>
<td>71.057</td>
<td>76.429</td>
<td>60.071</td>
<td>62.597</td>
<td>64.057</td>
</tr>
</tbody>
</table>

The equation of the best-fit curve is:

[Your Equation Here]
Temporal Effect of Varying Doses of Clonidine on the Fasting Blood Glucose Levels of Sprague Dawley Rats

Figure 12
Figure 8. Best-fit curve for dose response relationship of 2µg/kg treatment group

The equation of the best-fit curve generated is:
\[ y = -0.1225x^4 + 2.4309x^3 - 16.147x^2 + 36.961x + 58.714 \]
with an \( R^2 = 0.9353 \).

4µG/KG TREATMENT GROUP

Figure 13
Table 4. Blood glucose level means of 4µg/kg treatment group

Figure 14
Figure 9. Best-fit curve for dose response relationship of 4µg/kg treatment group

The equation of the best-fit curve generated is:
\[ y = -0.0141x^4 + 0.8221x^3 - 9.9298x^2 + 34.35x + 62.5 \]
with an \( R^2 = 0.9453 \).

0.9% NACL SOLUTION(CONTROL GROUP)

Figure 15
Table 5. Blood glucose level means of 7µg/kg treatment group

Figure 16
Figure 10. Best-fit curve for dose response relationship of 7µg/kg treatment group

The equation of the best-fit curve generated is:
\[ y = -0.074x^4 + 1.6835x^3 - 13.245x^2 + 37.511x + 59.429 \]
with an \( R^2 = 0.898 \).

7µG/KG TREATMENT GROUP

Figure 17
Table 6: Blood glucose level means of 0.9% NaCl Solution

Figure 18
Figure 11. Best-fit curve for dose response relationship of 0.9% NaCl Solution

The equation of the best-fit curve generated is:
\[ y = -0.007x^4 - 0.0343x^3 + 1.0923x^2 - 2.1307x + 62.143 \]
Temporal Effect of Varying Doses of Clonidine on the Fasting Blood Glucose Levels of Sprague Dawley Rats

with an $R^2 = 0.6845$.

**COMPUTATION OF AREA UNDER THE CURVE (AUC)**

Area under the curve is defined as the cumulative response (blood glucose levels) over the 8-hour monitoring period. Using the obtained equation for a given treatment group, AUC was computed by integrating the function over the 1st to 8th hour time interval. Calculations were employed using Maple v6.0.

The table below summarizes the respective AUC values among the different treatment groups.

**Figure 19**  
Table 7: Summary of AUC values

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>DOSE(µg/kg)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>0</td>
<td>534.3824</td>
</tr>
<tr>
<td>1µg/kg</td>
<td>1</td>
<td>538.1453</td>
</tr>
<tr>
<td>2µg/kg</td>
<td>2</td>
<td>583.1349</td>
</tr>
<tr>
<td>4µg/kg</td>
<td>4</td>
<td>653.9388</td>
</tr>
<tr>
<td>7µg/kg</td>
<td>7</td>
<td>654.2516</td>
</tr>
</tbody>
</table>

Results show that there is a very high AUC value, or over-all glucose level increase, in the treatment groups receiving 4µg/kg and 7µg/kg. Furthermore, the AUC values of these two treatment groups are very similar to each other.

**DOSE-RESPONSE RELATIONSHIP**

To demonstrate the dose-response relationship, a scatter-plot of the dose and response (blood glucose levels) is given below. A nonlinear functional relationship between dose and response apparently exists. A hyperbolic curve can be fitted over these data points. It is important to note that from 0 µg/kg to 1 µg/kg dose of clonidine there was no significant change in the AUC value. However, there is a significant increase in the AUC value from 1 µg/kg to 4 µg/kg dose interval. The curve reflects that when the dose changes from 4 µg/kg to 7 µg/kg, it yields a non-significant increment in the AUC values. Thus, response plateau is obtained starting at dose 4 µg/kg.

**DISCUSSION**

Experimental results reflect clonidine’s sympathomimetic effects, showing a steady increase in blood glucose levels as a response to a single dose administration. The homogeneity between treatment means at baseline and the statistically significant difference in the fasting blood glucose of rats between the treatment groups reflects that the significant difference in glucose levels is attributable to the experimental treatment. From the 1st to 4th hour, the blood glucose levels of the 4 µg/kg and 7 µg/kg treatment group, increased significantly from the control group. In addition, administration of clonidine at a dose higher than 4 µg/kg did not produce a significant change in the dose response curve. The leveling off of the dose response curve may be attributable to the presence of a threshold or ceiling dose.
brought about by saturation kinetics.

Clonidine, a centrally acting β-agonist used for hypertension, acts by both peripheral and central mechanisms to affect the blood pressure. Peripherally, it stimulates β-adrenergic receptors to produce vasoconstriction, producing a brief period of hypertension. It also acts centrally to inhibit the sympathetic tone via pre-synaptic α2 adrenalineceptor innervation (Hardman, J. & Limbird, L. 2001, Delgado, J. & Remers L. 1991) and cause hypotension that is of much longer duration than the initial hypertensive effect. This illustrates that the administration of clonidine produces a biphasic response in blood pressure, beginning with a brief hypertensive effect and followed by a hypotensive effect that persists for about four hours. This biphasic response is altered by dose only, in that larger doses produce a greater hypertensive effect and delay the onset of the hypotensive properties of the drug. This hypertensive effect is observed when clonidine is administered parenterally and is generally not seen when the drug is given orally.

It is proposed that the pancreas responds similarly to clonidine. The sympathetic innervation of the pancreatic β-cell membrane islets involves both pre and post-synaptic α2-adrenerceptors. The post-synaptic α2-adrenoeceptor in the pancreatic islets mediates inhibition of the glucose-induced release of insulin (Langer SZ & Angel I 1991) while the pre-synaptic α2-adrenoeceptor in the pancreatic islets mediates inhibition of release of norepinephrine by the post-ganglionic neuron on the peripheral nerve endings (Hardman, J. & Limbird, L. 1991). In the discussion by Abder-Zaho et. al., it was proposed that when the post-synaptic α2-adrenoeceptor in the pancreatic islets are stimulated, the release of insulin is inhibited. Similarly, it also stated that when the post-synaptic α2-adrenoeceptor is blocked, it will result in the release of insulin. Stimulation of the pre-synaptic α2-adrenoeceptor in the pancreatic islets will in turn inhibit the release of norepinephrine by the post-ganglionic neuron. This blocks the activation of the post-synaptic α2-adrenoeceptor resulting in insulin release from the pancreatic islets. This mechanism defines clonidine’s sympathomimetic and sympatholytic actions in response to appropriate α2-adrenoeceptor stimulation.

The results of the present study are similar to that found by Gotoh et al, (1988) where increased blood glucose levels were measured after intravenous and intraventricular administration of 5, 50 and 100 nmol of clonidine in rats. However, it is significantly different from other studies such as those by Latterman et al (2001), Nishina et al, (1998) and Belhoula et al (2003).

Of the treatment groups, only the 1µg/kg dose did not produce a significant difference in blood sugar. These results are incongruent with Latterman et. al’s (2001) findings on hyperglycemia in surgery where a 1 µg/kg clonidine dose increased the hyperglycemic response observed. It was purported in the study that clonidine had a role in accentuating the hyperglycemic response by inhibiting insulin secretion. Several reasons are proposed as to why this was not true for the current study. These reasons include the difference in stress response to surgery versus non stressful conditions, and the intraperitoneal mode of administration in the present study versus the intravenous mode. Furthermore, it is possible that a different mechanism was responsible for their study results. Stress was reported as the major factor in their study and was in the form of surgery. It triggered a catecholamine response where stimulation of the sympathetic nervous system promoted an increase in serum glucose. This is further supported by the study of Doyle and Egan (2003) where it is stated that glucose induced insulin secretion is actually inhibited by ephinephrine, a catecholamine that is released during stress. This study, combined with the fact that Latterman’s study concludes that clonidine failed to inhibit the surgery-induced stimulation of sympathoadrenergic pathways (Hypothalamo-Pituitary-Adrenal Axis) at a dose of 1µg/kg clonidine, supports the proposal that serum glucose in their study is primarily controlled by the HPA axis changes.

Furthermore, the results of the present study- wherein a hyperglycemic response was seen in 2, 4, and 7 µg/kg doses – is notably different from the results of the study by Nishina et al, (1998) and Belhoula et al (2003) where they found a decreased glycemic response at doses 3-6 µg/kg. This difference may be explained by the differences in population and measurement of outcomes. The latter studies used patients that were about to undergo surgery and they measured the differences in blood glucose levels during/post surgery. The surgical stress may be one important factor for the difference in results.

In the present study, treatment groups that received 4µg/kg and 7µg/kg significantly differed from the control group during 1st to 4th hour, while the treatment group which received 2µg/kg significantly differed from control at 1st hour. After computing for total body response using AUC however, it was found that the 4µg/kg and 7µg/kg doses
produced a highly significant increase in blood glucose throughout the experiment. This accentuation of blood glucose levels may be caused by the activation of the post-synaptic $\alpha_2$ adrenoreceptors in the pancreas. The interaction of clonidine with the post-synaptic $\alpha_2$ adrenoreceptors could have produced a more predominating effect, compared with the interaction of clonidine with other receptors such as the presynaptic adrenoreceptors. The response of any cell or organ, the pancreas in particular, to sympathomimetics like clonidine is affected by the density and proportion of $\alpha_2$ receptors (Hardman, J. & Limbird, L., 2001). In the article by Veliquette & Ernsberger (2003), they confirmed the $\alpha_2$ receptors agonist properties of clonidine. They described the control of insulin secretion by the presence of Imidazoline (1, R) receptors and $\alpha_2$ receptors in the pancreas. Imidazoline (1, R) receptor stimulation by clonidine was found to increase the secretion of insulin only when $\alpha_2$ receptors are effectively blocked. This fact supports the hypothesis that there is increased distribution and density of $\alpha_2$ receptors in the pancreas.

Another possible mechanism for the accentuated blood glucose levels observed is the lower threshold concentration for the activation of post-synaptic $\alpha_2$-adrenoreceptors compared to that of the pre-synaptic $\alpha_2$-adrenoreceptors in the $\beta$ cells of the pancreas (Hardman, J. & Limbird, L., 2001).

Studies in humans cite 3-5 hours as the time to reach peak plasma concentrations for clonidine, with a half life of 12 hours (Katzung 2007). In the present study conducted, treatment groups with significant results differed slightly on peak glucose reading times. Groups receiving 4 µg/kg and 7µg/kg showed peak glucose readings at the 3rd hour. The group which received 2µg/kg showed peak glucose readings at 2nd hour. This may be explained by the mode of administration of clonidine. Oral administration of clonidine would have introduced more variability in the experiment, since some rats can still vomit out the drug. An intra-peritoneal administration of a drug would cause the drug to be absorbed more quickly, thereby accounting for the shorter peak effect in our study compared to the values obtained from human studies which used oral administration of clonidine.

The observed effects of 2µg/kg, 4µg/kg, and 7µg/kg treatments may show a correspondence to the Plasma concentration vs. Time curves of clonidine for the particular doses when administered intraperitoneally. At present, no published studies that show Plasma concentration of clonidine vs. Time curve, from which $T_{max}$ and half-life of clonidine can be derived, when clonidine is administered intraperitoneally can be found.

All of the curves of treatments 2µg/kg, 4µg/kg, and 7µg/kg show peaks at which maximum blood glucose levels were measured. Computations for best fit line show that experimental glucose values produce a consistent pattern, with a rise at the fist hour and steady increase showing a dose response relationship. A hypothesis on why the 4µg/kg, and 7µg/kg curves, in particular, show very similar tapering curves is that there is a saturation of receptors at the 4 µg/kg; thus, an increase in dose no longer produces a favorable response. This is further supported by the AUC computation which reflects the cumulative body response to drug administration in the form of an increase in blood glucose.

A possible explanation as to why the curves immediately decline after reaching their respective glucose level peaks is that the clonidine-induced increase in blood glucose stimulated the pancreas to secrete insulin by a mechanism other than adrenergic control. The study by Aizawa (1992) supports this by saying that the ability of glucose to stimulate the release of insulin is sometimes mediated by a separate mechanism which can be either K* ATP channel dependent or K+ ATP channel independent.

Finally, the curve of the treatment group receiving 4µg/kg is seen to have slower rise to maximal blood glucose levels and slower decline. It is proposed that while this may be a normal variation in treatment, this dose of clonidine may have significantly unique properties. An increase in population size of the current experiment may better improve the curve distribution.

From the 5th to 8th hour, the blood glucose levels found among the treatment groups were not significant from each other. This suggests that at around the 5th hour, the sympathomimetic effects of clonidine may have already declined and that the time to maximum concentration of clonidine may be found within the first five hours after clonidine administration, regardless of dose.

**CONCLUSION**

Intra-peritoneal administration of clonidine increases blood glucose levels when given at varying doses from 1 – 7 µg/kg. Statistically significant increases in blood glucose were noted from 1st to 4th hour after the drug administration ($p=0.004<.05$; $p= 4.097e^-6<.05$; $p= 2.408e^-6<.05$; $p=...$
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0.011<0.05) for treatment groups receiving 2, 4 and 7 µg/kg clonidine. The peak effect of clonidine was observed within 1 to 3 hours post administration, with no significant difference in blood glucose levels starting at 5 th hour post injection. The apparent optimal dose based on the blood glucose levels is 4 µg/kg.

Moreover, the blood glucose levels follow a similar time distribution curve but do not appear to produce a response gradient. Purported mechanisms include a smaller number of pre-synaptic $\alpha_2$-adrenoceptors in the sympathetic innervation of $\beta$ cells of the pancreas compared to the post-synaptic $\alpha_2$-adrenoceptors, lower threshold concentration for activation of post-synaptic $\alpha_2$-adrenoceptors and that the clonidine-induced increase in the blood glucose levels stimulates the $\beta$ cells to secrete insulin, as reflected in the curves of the different treatment groups.

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