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EAST TENNESSEE STATE UNIVERSITY DEPARTMENT OF HEALTH SCIENCES

Comparative Study of Anesthesia's effect on Baroreceptor Reflex and Sympathetic Nerve Activity in Adult Rats

By

John B. Harbin

An Undergraduate Thesis Submitted in Partial Fulfillment
of the Requirements for the
University Honors Scholars Program
Honors College
and the
Honors in Health Sciences, Human Health
College of Public Health
East Tennessee State University

John B. Harbin	May 7, 2021
John B. Harbin	Date
Matthew R. Zahner	May 7, 2021
Dr. Matthew R. Zahner, Thesis Mentor	Date
Sean Fox	May 7, 2021
Dr. Sean Fox, Reader	Date

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COMPARATIVE STUDY OF ANESTHESIA'S EFFECT ON BARORECEPTOR REFLEX AND SYMPATHETIC NERVE ACTIVITY IN ADULT RATS

By

JOHN B. HARBIN

A Thesis submitted to:
Department of Health Sciences and Honors College

Degree Awarded:
Spring 2021

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ABSTRACT

Anesthesia affects the central nervous system and can suppress cardiovascular activity. In this study, we compared two anesthetics, urethane and α-chloralose, to better understand their effect on sympathetic control of blood pressure, as well as how they would affect baroreceptor response and blood pressure in adult rats. To do this we performed baroreceptor tests in adult rats under isoflurane anesthesia and then either urethane (I.V. 1.25 g/kg, n=2) or α-chloralose (100 mg/kg, n=2). We found that baroreceptor responses were not significantly different between urethane or α-chloralose anesthesia. However, significant depression of baseline blood pressure occurred under α-chloralose anesthesia compared with urethane. Additionally, we observed significant elevation of baseline renal sympathetic nerve activity (RSNA) occurred under urethane anesthesia. Ultimately, our findings suggest that both urethane and α-chloralose provided sufficient induction of anesthesia without significantly modifying baroreceptor response. However, since urethane significantly raised baseline sympathetic nerve activity, it should be avoided in studies where raised sympathetic activity could confound with the test results. α-chloralose significantly lowered baseline blood pressure by nearly 30%, and its use should be avoided in studies where lowered blood pressure may confound the results.

CHAPTER ONE

INTRODUCTION

Modern medicine has existed for thousands of years since its inception by Hippocrates, commonly known as the father of modern medicine (Grammaticos, 2008). However, it was not until the mid-1800's that physicians began using anesthetics during surgical procedures to numb pain or to induce consciousness. The first recorded use of anesthesia was at Massachusetts General Hospital in Boston by dentist William T.G. Morton and surgeon John Warren on October 16, 1846. Up to that point, patients would have to simply grit their teeth or be given opium and a shot of whisky to dull the pain (Harrah, 2015). As anesthesia has continued to grow and modernize, so have its applications. Anesthesia now has practical uses in many other animal species in addition to humans, and many different types of anesthesia now exist to accomplish different levels of pain reduction and sleep induction. In fact, under the anesthesia umbrella, exists a range of medications called sedatives, which reduce a patient's awareness and response to external stimuli while maintaining consciousness. Patients will experience symptoms ranging from pain relief (analgesia) and lapse in memory (amnesia) while maintaining consciousness. General anesthesia, which includes components of sedatives, induces unconsciousness. During the unconscious state, the patient will not feel or remember anything (Lingappan, 2021). Another distinct difference between sedatives and anesthetics is their effect on the cardiovascular system. Sedatives tend to leave cardiovascular function unimpaired particularly at low doses, while anesthetics usually impair cardiovascular function (Wilson, 2019).

The cardiovascular system is responsible for circulating oxygen and nutrient-containing blood throughout the entire body, and for this reason flow must be maintained within narrow

margins (Anatomy, 2019). This requires help from a multitude of different receptors to maintain heart rate, blood pressure, myocardial contractility, etc., at homeostatic levels. The baroreflex is a cardiovascular reflex that is important in maintaining blood pressure around a homeostatic set-point. When deviations in blood pressure occur, they are detected by receptors located in the aortic arch of the heart and carotid sinus called baroreceptors (Robertson, 1956). These receptors relay a signal via the vagus and glossopharyngeal nerves to the nucleus tractus solitarius (NTS) in the brain stem medulla. A response is then generated via the autonomic nervous system to raise or lower the blood pressure. Most general anesthetics are known for depressing baroreflex response (Robertson, 1956).

In our study, we compared the effects of two anesthetics, urethane and α-chloralose, on an adult rat's sympathetic baroreflex control of blood pressure. To do this, we recorded the blood pressure and renal sympathetic nerve activity (RSNA) before (under isoflurane as control) and after transitioning to either urethane or α-chloralose. We found that both urethane and α-chloralose significantly modified baseline blood pressure and sympathetic nerve activity.

CHAPTER TWO

LITERATURE REVIEW

Urethane

Urethane has been used as a surgical anesthetic in scientific experimentation for decades. In rodents, urethane anesthesia has a fast induction and duration, making it a useful anesthesia in biomedical research. According to Field (1993) in the book "*Laboratory Animals*", compared with chloral hydrate and pentobarbitone anesthesia in rodents, urethane had a significantly faster onset time of anesthesia (~1minute) when injected intraperitoneally (1.2-1.5 g/kg IP) as well as a duration that exceeded the testing parameters of 24 hours. Field (1993) also reported in his review of literature that a urethane dosage of 1.2-1.5 g/kg IP produces a significant analgesic effect that maintains anesthesia through invasive procedures. This analgesic effect urethane has is also excellent for preserving activity of neural pathways in various areas of the central and peripheral nervous systems. This makes it an attractive anesthetic to maintain various reflex responses (Maggi, 1986). Urethane is also excellent at minimizing the depression of the cardiovascular and respiratory systems and maintaining spinal reflexes (Fish, 1997). Urethane's minimal effect on the cardiovascular system is due to minimal inhibition of the sympathetic nervous system and increased catecholamine output (Fish, 1997). Increased SNS activity increases catecholamine secretion which helps prevent a depression of cardiac output. Respiratory function is also very stable during urethane induced anesthesia. In decerebrate rats, both respiratory frequency and tidal volume were maintained with the introduction of urethane anesthesia. Since the rat is decerebrate, urethane's lack of effect on the respiratory system indicates a lack of effect on brainstem or spinal activity. When compared with pentobarbital, chloralose, and ketamine anesthesia, only urethane anesthesia maintained respiratory rate (Sapru, 1979).

Urethane's Effect on Autonomic Nervous System

Urethane has a complex effect on the autonomic nervous system, and its mechanism is not entirely understood. When testing the effect of urethane on the following receptors: $GABA_A$, glycine, NMDA and AMPA, test results illustrated that urethane anesthesia produced a different effect on each receptor. These receptors are ion channels located on post-synaptic neurons in the central nervous system. When the neurotransmitter gamma amino butyric acid (GABA) binds to the $GABA_A$ receptors it hyperpolarizes the membrane and reduces action potential firing. Conversely, when glutamate channels NMDA and AMPA are potentiated, the membrane is pushed towards depolarization and triggering action potentials. One way that urethane anesthesia suppresses neuronal firing is by augmenting the activity of the inhibitory neurotransmitter GABA. Urethane (concentration of 10mM) was tested on $GABA_A$ and glycine receptors, which resulted in an excitatory response of 23% ± 4% and 33% ± 4% respectively (Hara, 2002). Excitation occurred with a decrease in action potential firing. In contrast, urethane inhibited NMDA and AMPA receptors 10% ± 3% and 18% ± 2% respectfully (Hara, 2002). In the presence of agonist, inhibitory effects were unchanged, suggesting noncompetitive inhibition. Urethane effectively inhibited NMDA and AMPA receptors while potentiating $GABA_A$ and glycine receptors. As opposed to effecting one or two neurotransmitters to induce anesthesia, urethane appeared to act non-selectively on multiple neurotransmitters to lower action potential firing and induce anesthesia (Lee, 2018). Urethane's activation of $GABA_A$ channels was supported by results of separate testing where urethane doubled the duration of GABAergic post-synaptic conductance in guinea pigs. The doubling of duration of GABAergic conductance led to depressed synaptic activity and action potential firing. This extended duration of inhibition caused induction of anesthesia (Scholfield, 1980). Conversely, when urethane anesthesia was tested in rats against pentobarbital and

chloralose, it did not suppress activity of the postganglionic sympathetic neurons (Shimokawa, 1998).

Urethane's effect on blood pressure, heart rate, and RSNA has yet to be fully understood. Compared with chloralose (50 mg/kg IV) and pentobarbital (30 mg/kg IV), urethane (800 mg/kg IV) increased blood pressure, heart rate, and RSNA in adult rats (Shimokawa, 1998). As reviewed by Field (1988) urethane, pentobarbitone, and chloral hydrate show that urethane (1.2-1.5 g/kg IP) anesthesia produced dose-related decrease in blood pressure and heart rate. The effects of combined chloralose-urethane anesthesia on renin angiotensin system (a homeostatic regulator of blood pressure), SNS, and nitric oxide (a vasodilator) production during blood pressure decrease were examined in anesthetized wistar rats (Bencze, 2013). The anesthesia produced substantial decrease in blood pressure due to modifying RAS, SNS, and NO contributions in blood pressure regulation. Blood pressure was lowered due to greater RAS contribution and decreased SNS and NO contribution (Bencze, 2013).

Drawbacks of Urethane Anesthesia

Urethane is considered a moderate carcinogen, thus urethane as a surgical anesthetic should only be used when the surgical patient is undergoing a non-recovery procedure according to a review by Field (1988). The anesthetic was caused liver cancer in adult rats when injected interperitoneally, as well as malignant lymphoma in female rats via drinking water (NTP, 2021). Urethane's carcinogenic properties eliminate it from being considered ethical as human anesthesia; however, no experimental testing of the relationship between urethane exposure and human cancer has been conducted. In adult rats, suggested optimal dosage range for anesthesia during testing was found to be between 1.2g/kg -1.5g/kg IP. However, according to Field (1993), at the upper end of the dosage range, tests found that urethane had a 25% mortality rate on the rats, and toxicity

in multiple organs in the digestive system. Rats were prone to heart failure and displayed "significant depression of heart rate and blood pressure" with a high dosage. Therefore, while urethane induces anesthesia rapidly and sustains it for a long period of time, there is marginal error that can be made in administering the correct dosage.

Sympathetic Nervous System Cardiovascular Control

Baroreceptors are receptors located within the carotid sinus and the aortic arch (Guyenet, 2006). They relay deviations in blood pressure to the nucleus of the tractus solitarius in the brain stem and cause subsequent activation of the baroreflex. The baroreflex provides short-term homeostatic control of the blood pressure levels via a negative-feedback loop. This negative feedback loop maintains sympathetic activity to keep blood pressure around a set point. If the individual is hypertensive, baroreflex activation is increased and heart rate and blood pressure are lowered. The baroreflex arc occurs when baroreceptors, located in the aortic arch, act as afferent mechanoreceptors which detect a rise in blood pressure due to stretching of the arterial wall (Guyenet, 2006). The signal is relayed to the central nervous system, a response is generated to decrease sympathetic nerve outflow, and activation of the parasympathetic branch of the autonomic nervous system occurs. Response of the parasympathetic nervous system is mediated by a cholinergic response with the release of the neurotransmitter acetylcholine, leading to reflex bradycardia and weaker myocardial contraction. This decreased heart rate and contraction causes depressed blood pressure.

Renal sympathetic nerve activity is responsible for raising low blood pressure to homeostatic levels. Detected systemic hypotension results in increased RSNA, as well as decreased baroreflex activation, which increases the low blood pressure (Sun, 2014). Afferent receptors in the peripheral nervous system detect deviating low blood pressure and send a signal

to the central nervous system. This results in an efferent, sympathetic response from the autonomic nervous system that is mediated by increased catecholamine levels (Kannan, 1998). The response results in reflex tachycardia and increased myocardial contraction, causing raised arterial pressure. The sympathetic response that is generated also simultaneously decreases the firing rate of baroreceptors which lessens its parasympathetic effect.

Alpha Chloralose

α-chloralose is another anesthetic that is frequently used when conducting invasive procedures on rats. According to the book "*Laboratory Animal Anaesthesia",* It produces stable, light anesthesia that lasts from 8-10 hours (Flecknell, 2009). Its lack of cardiorespiratory interference is what makes it an attractive option in studies where minimal effect on cardiovascular and respiratory systems is desired. Additionally, autonomic reflexes are left unimpaired by α-chloralose (Suckow, 2005). Flecknell (2009) states in his book that if used at higher dosages (100 mg/kg IP), α-chloralose is capable of inducing anesthesia in adult rats on its own. However, at a dosage of around 50 mg/kg, α-chloralose produces immobilizing effects that are more typical of a sedative or paralytic than anesthesia. Therefore, α-chloralose is often paired with either stronger anesthetic (such as urethane) to maintain sleep, or a temporary anesthetic to induce sleep before letting a-chloralose be introduced (Flecknell, 2009). Field (1988) found in his review of literature that urethane, when used in conjunction with α-chloralose, produced stable, high-dose anesthesia for approximately 6 hours when using 0.25-0.40 g/kg IP of urethane followed by 0.035-0.040 mg/kg IP of α-chloralose approximately 30 minutes later. When combined with α−chloralose, urethane modified chloralose to deter typical properties of α-chloralose anesthetic such as

excessive central nervous system stimulation and muscle reflex, while maintaining α-chloralose's beneficial cardiorespiratory stability.

Alpha-Chloralose's Effect on Sympathetic Nervous System

Like urethane, the central mechanism of action of α-chloralose as an anesthetic remains relatively unknown. Results of comparative testing between sodium pentobarbital and α-chloralose on baroreflex control in rats, show that α-chloralose produced greater baroreflex sensitivity for tachycardic response than for bradycardic response. α-chloralose anesthesia left the baroreflex sensitivity to bradycardic responses uninhibited, but depressed baroreflex sensitivity to tachycardic responses by 35% (Bedran-de-Castro, 1990). This response suggests that α-chloralose anesthesia is best used in experimentation when either no effect on bradycardic baroreflex sensitivity or is desired. This result was challenged by the result of a separate comparative test between urethane, α-chloralose, and sodium pentobarbital on rat baroreceptor reflex function. This comparative test found that α-chloralose attenuated the function of the baroreflex 3-4-fold, a result that was supported with decreased bradycardic response (Fluckiger, 2002). Therefore, it is not entirely certain whether or not α-chloralose anesthesia suppresses the bradycardic baroreflex response, based upon the conflicting test results. One proposed mechanism of anesthesia for α-chloralose is its effect on α-chloralose is allosteric regulation of $GABA_A$ receptor chloride conductance. After introducing α-chloralose (100 μM) into frog oocytes, GABA affinity on $GABA_A$ receptors was increased 5x its original values (Garrett, 1998). This potentiation of GABA-inducted current suggests a possible mechanism of action for α-chloralose anesthesia. Although results of α-chloralose's effect on sympathetic nervous system have been drawn in numerous tests, the lack of a consistent conclusion and dose dependency indicates that further testing is necessary.

Drawbacks of Alpha-Chloralose Anesthesia

Despite α-chloralose being an excellent anesthetic for certain testing parameters, there are several drawbacks which warrant consideration. First, α-chloralose is created by heating equal amounts of glucose and chloral hydrate, producing two isomers. When correctly prepared, α-chloralose is a reliable anesthetic. However, if prepared improperly, a-chloralose racemizes to the convulsant B-chloralose. B-chloralose is an isomer with very negative effects and can induce muscle pain (Clarke, 2014). Therefore, it is critical that caution is exercised when forming α-chloralose. Additionally, results of numerous tests have shown that α-chloralose in low dosages around 50mg/kg does not provide sufficient anesthesia on its own (Flecknell, 2009; Kohn, 1998). Instead of acting as an anesthetic with analgesic properties, under-dosed α-chloralose behaves similarly to a sedative with immobilizing effects or a paralytic (Holtzgrefe, 1987). This issue raises ethical concerns because the rat may not be anesthetized and perceiving pain, is paralyzed and unable to produce a withdrawal reflex. Rats sedated by α-chloralose were often found to also behave erratically and react involuntarily during the recovery period post-operation (Shimokawa, 1998; Flecknell, 2009). However, this could be due to the rats mistakenly being dosed with the convulsant β-chloralose.

CHAPTER 3

METHODS

Experimental procedures were conducted on male Sprague-Dawley rats that were supplied through East Tennessee State University (ETSU). All described experimental procedures were approved by the University Committee on Animal Care at ETSU and National Institute of Health. Internal temperature was monitored with a rectal probe and regulated at approximately 38^0 with a heating pad. Catheters were then placed in a femoral artery and vein. The rate was transitioning from isoflurane to either alpha-chloralose (100mg/kg IV) or urethane (1.3 g/kg iv, with supplementary doses of 0.1 g/kg iv if required) depending upon experimental procedure. Nociceptive stimulation to the rat's paw with a lack of withdrawal response verified the effectiveness of anesthesia. A tracheotomy was performed, allowing the rats to be artificially ventilated. Bilateral jugular vein cannulations facilitated the administration of vasoactive drugs (McDowall, 2006). RSNA, average RSNA, heart rate (bpm), and arterial pressure (AP, mmHg) were recording using CED Spike 2 Charting software on a computer. This software was used for all charting procedures and data collection, and baseline and reflex curves were recorded continuously for each of the four anesthesia variants.

Experimental procedure began with anesthetizing the rats with 2-3% isoflurane in oxygen enriched air. Once the rats had been sedated, and the RSNA and blood pressure values reached homeostatic levels, baseline values were recorded on CED Spike 2 software. Baroreflex curves were created by measuring the changes in arterial pressure and RSNA values in response to infusion of sodium nitroprusside (SNP, 50 μg/ml), a vasodilator, and phenylephrine hydrochloride (PE, 125 μg/m), a vasoconstrictor. SNP and PE were administered via the jugular vein initially at 2.5 ml/h, and then the rate was increased every 30 seconds by an additional 2.5 ml/h, to a maximum

of 25 ml/h (McDowall, 2006). The maximum volume infused was ~1.2 ml, but the infusion was halted if it no longer produced a change in heart rate or RSNA or if blood pressure reached the minimum threshold of 50 mmHg or the maximum threshold value of 200 mmHg).

Data Analysis

To analyze the data for the baseline and baroreflex tests, we sampled data points for blood pressure and RSNA/average RSNA at 5000 hz and binned into 1s averages, and the data points were imported into Microsoft Excel spreadsheets. The data points for baseline and the data points for baroreflex were grouped separately, and this process was repeated for each of the anesthesia tests. The average sympathetic nerve activity was also calculated for each baseline and reflex section. For each baseline and reflex, blood pressure and RSNA was then binned into consecutive blocks categorized by blood pressure value, starting at 60 and increasing by a value of 10 to 150. The average RSNA during the same blocks were also determined, and then above process was repeated to achieve the % Baseline RSNA values of the binned data. At the end of the recording the proximal end of the nerve was cut and the subsequent activity subtracted. The data sets were then analyzed using Prism GraphPad software (version 4.0). Baroreflex data were fit to the following equation to create baroreflex curves:

$$y = A_1 / \{1 + \exp[A_2(x - A_3)]\} + A_4$$

The equation is represented where y is RNSA, x is blood pressure, A1 is the y range (y at the top plateau − y at the bottom plateau), A2 is the gain coefficient, A3 is blood pressure at the midpoint (which is also the point of maximum gain), and A4 is y at the bottom plateau (McDowall, 2006). The threshold and saturation values for blood pressure were defined as the values of blood pressure that corresponded to the points where y was 5% (of the y range) below and above the upper and

lower plateaus, respectively. To compare the values of Gain (Delta % RSNA), blood pressure, upper and lower plateaus of RSNA values (% baseline), and range of RSNA values (% baseline) between the four anesthesia tests, one-way ANOVA tests were used. After the ANOVA test, an unpaired t-test of Baseline RSNA values was run between a-Chloralose and urethane. Sample size was n=2. $P < 0.05$ was regarded as statistically significant and significant results were marked on the corresponding graph with an asterisk. *Values are means ± SE, which varied based upon study performed.

CHAPTER 4
RESULTS

Effect of urethane on the baroreflex.

To determine the effect of urethane anesthesia on baroreflex regulation of RSNA, we performed baroreflex experiments under isoflurane anesthesia and then urethane (1.25g/kg). Figure 1 shows the comparative baroreflex responses during isoflurane and urethane anesthesia. Baseline blood pressure under isoflurane was 99.2 ± 7.2 mmHg. After transition to urethane anesthesia, baseline blood pressure was 104.1 ± 7.2 mmHg, and not significantly reduced compared with baseline. Urethane significantly increased baseline RSNA to 148.3 ± 9.1% ($P<0.05$, n = 2) of baseline RSNA under isoflurane.

Baroreceptor-induced increases in RSNA upon decreasing blood pressure were not affected significantly by urethane ($P> 0.05$, n=2, Fig. 2D). In the control baroreceptor responses, under isoflurane anesthesia, a decrease in blood pressure to 60-mmHg increased RSNA to a maximum plateau of 122.6 ± 14.8%. After transition to α-chloralose the decrease in blood pressure to 60 mmHg increased RSNA to a maximum plateau of 113.3 ± 14.8% relative to control baseline RSNA.

Baroreceptor-induced decreases in RSNA, upon increasing blood pressure, were also not affected significantly by urethane ($P> 0.05$, n=2, Fig. 2E). In the control baroreceptor responses, under isoflurane anesthesia, an increase in blood pressure to 150-mmHg decreased RSNA to a minimum plateau of 19.2 ± 13.6% of baseline RSNA. After transition to α-chloralose the increase in $P> 0.05$, n=2, to 150 mmHg decreased RSNA to a maximum plateau of 21.1 ± 13.6% relative to control baseline RSNA.

Urethane had no significant effect (P> 0.05) on the maximum gain of the baroreflex. In control experiments, under isoflurane anesthesia, the maximum baroreflex gain was −2.3 ± 0.4 Δ%RSNA/ΔAP. After transition to a-chloralose the maximum gain was −1.9 ± 0.4 Δ%RSNA/ΔAP (P > 0.05 Fig. 2F).

Effect of alpha chloralose on the baroreflex.

Alpha chloralose significantly affected baseline blood pressure. Figure 2C shows the effect of α-chloralose on baseline blood pressure. Baseline blood pressure under isoflurane anesthesia was 116.5 ± 7.2 mmHg. After transition to α-chloralose, baseline blood pressure was significantly reduced to 83.1± 7.2 mmHg (n = 2, P < 0.05). However, α-chloralose did not significantly decrease baseline RSNA (Fig. 2A.; 69.4 ± 9.1%).

Baroreceptor-induced increases in RSNA upon decreasing blood pressure were not affected significantly by α–chloralose (P> 0.05, n=2, Fig. 2D). In the control baroreceptor responses, under isoflurane anesthesia, a decrease in blood pressure to 60-mmHg increased RSNA to a maximum plateau of 133.2 ± 15% of baseline RSNA. After transition to α–chloralose the decrease in blood pressure to 60 mmHg increased RSNA to a maximum plateau of 133.2 ± 15% relative to control baseline RSNA.

Baroreceptor-induced decreases in RSNA upon increasing blood pressure were also not affected significantly by α-chloralose (Fig. 2E). In the control baroreceptor responses, under isoflurane anesthesia, an increase in blood pressure to 150-mmHg decreased RSNA to a minimum plateau of 35.6 ± 13.6% of baseline RSNA. After transition to α-chloralose the xx mmHg increase in blood pressure decreased RSNA to a minimum plateau of 45.0 ± 13.6% relative to control baseline RSNA.

α-chloralose had no significant effect on the maximum gain of the baroreflex. In control experiments, under isoflurane anesthesia, the maximum baroreflex gain was −1.9 ± 0.4 Δ%RSNA/ΔAP. After transition to a-chloralose the maximum gain was −1.3 ± 0.4 Δ%RSNA/ΔAP ($P > 0.05$ Fig. 2F).

CHAPTER 5

DISCUSSION

This study provides new physiological observations on the effects of urethane and α-chloralose on baroreceptor control of blood pressure and RSNA in adult rats. We were interested in better understanding how baroreceptor control and RSNA would be individually modified by different variants of anesthesia. We were also determining if there was any significant impact to RSNA or blood pressure values.

Urethane anesthesia is well known for providing deep anesthetization that is long lasting. While inducing anesthesia, urethane manages to preserve neural pathways in the peripheral nervous system and areas of the central nervous system which makes it excellent for studying various autonomic reflex responses (Maggi, 1986). Urethane has a mild effect on the cardiovascular system due to minimal inhibition of SNS and catecholamine output. Attenuated reduction of SNS activity prevents depression of cardiac output (Fish, 1997). However, tests of urethane anesthesia on the cardiovascular system results in mixed conclusions. One test of urethane on the cardiovascular system of an adult rat found that it increased blood pressure and RSNA levels (Shimokawa, 1998). Conversely, two other tests concluded that urethane potentiated depression of blood pressure and sympathetic nerve activity. One test concluded that this was due to a dose-dependent decrease of blood pressure, while the other found that decreased blood pressure was due to decreased sympathetic nervous system contribution (Field, 1988; Bencze, 2013). It is apparent that there is not true conclusive evidence as to the effect of urethane on the cardiovascular system.

In our study, we found that urethane increase baseline RSNA compared with isoflurane (urethane) (Fig. 2A.). This baseline RSNA value is the reading taken prior to administration of sodium-nitroprusside, and subsequent depression of blood pressure and elevation of RSNA levels (of % baseline). The mean value of baseline RSNA for urethane was compared in % of baseline to the baseline of our control anesthetic, isoflurane (urethane). This significant increase in baseline RSNA further supports findings of separate testing of urethane on sympathetic activity, which also found that baseline RSNA values were increased under urethane anesthesia (Shimokawa, 1998). Our findings of significantly increased baseline RSNA also provides further evidence that urethane maintains cardiovascular activity by enhancing SNS activity (Fish, 1997). Additionally, we found that baseline blood pressure under urethane anesthesia was also increased from the control, although not significantly (Fig. 2C.). The increased baseline RSNA refutes conclusions that urethane anesthesia lowers RSNA in adult rat. However, we are unable to confidently refute the claim that urethane also lowers blood pressure, due to our lack of a statistically significant increase in blood pressure (Field, 1988; Benzce, 2013).

Baroreceptor control was not modified significantly from our control anesthetic under urethane anesthesia. We reported urethane having a marginally depressed upper plateau of RSNA (% baseline) as can be seen in Figures 1 & 2D. This upper plateau value is taken after administration of sodium-nitroprusside and once blood pressure falls to 60mmHG. Additionally, the lower plateau RSNA values (% baseline) taken after administration of phenylephrine and once blood pressure rose to 150mmHG, were only slightly higher under urethane anesthesia than the control (Fig. 2E.). This resulted in urethane having marginally lower gain (Δ %RSNA/ΔmmHg) and range of RSNA values (% baseline) than isoflurane (urethane) (Fig. 2A & 2B.). However, we

cannot confidently claim that this conclusion can support or refute claims made in other testing about urethane's effect on baroreceptor control, due to our lack of statistical significance.

α-chloralose is best known for providing long-lasting, mild anesthesia. If used at higher dosages (100 mg/kg IP), α-chloralose is capable of inducing anesthesia in adult rats on its own (Flecknell, 2009). However, at lower dosages, α-chloralose produces effects more typical of sedation than of anesthesia. α-chloralose is therefore often paired with a stronger anesthetic (urethane) to quickly induce anesthesia, before being switched over to α-chloralose to maintain cardiovascular stability (Field, 1988). Although the mechanism of anesthesia for α-chloralose is not well documented, frogs anesthetized by α-chloralose exhibited increased GABA affinity for $GABA_A$ receptors by 5x original values (Garrett, 1998). Results of testing would suggest that activation of inhibitory $GABA_A$ channels would reduce action potential propagation and sympathetic activity, which explains a potential mechanism of anesthesia. α-chloralose's specific effects on the CNS or PNS have not been well documented and its effect on the baroreflex response is not fully understood. One test of baroreceptor function in adult rats under α-chloralose anesthesia found that the baroreceptor's bradycardic response was reduced 3-4x, while separate testing found that α-chloralose left bradycardic responses uninhibited (Fluckiger, 2002; Bedran-de-Castro, 1990). Ultimately then, one goal of our study was to quantify if there was any significant effect that α-chloralose anesthesia had on the function of the baroreflex control and RSNA.

In our study, α-chloralose depressed baseline RSNA compared to the control anesthetic isoflurane (chloralose), although not significantly. Interestingly, α-chloralose anesthesia resulted in lowered baseline RSNA from control values while urethane significantly raised baseline RSNA from control values. Differing effects on baseline RSNA could be one way that the anesthetics

affect the cardiovascular system differently. However, we cannot confidently make this claim due to a lack of significance in our baseline RSNA value under α-chloralose anesthesia. We also found that rats anesthetized by α-chloralose had significantly reduced mean baseline blood pressure (mmHg) than the control, isoflurane (chloralose). As shown in Figure 2C., α-chloralose attenuated baseline blood pressure by nearly 30%. Such a substantial depression in baseline blood pressure would prove significant in studies where baseline blood pressure must remain unaffected. This significant depression in baseline blood pressure is surprising because α-chloralose is well known for excess muscle contraction in rats during slow induction of and recovering from anesthesia (Shimokawa, 1998; Flecknell, 2009).

Mean upper plateau value of RSNA (% baseline) in the α-chloralose anesthetized rat was much lower than the control value from isoflurane (chloralose) (Figures 1 & 2D.). This upper plateau value is taken after administration of sodium-nitroprusside and once blood pressure falls to 60mmHG. The lower plateau RSNA values (% baseline) taken after administration of phenylephrine and once blood pressure rose to 150mmHG, were greatly increased under α-chloralose anesthesia from the control (Figure 2E.). However, we were not able to claim significance due to standard error being considered. Depressed upper plateau of RSNA (% baseline) and elevated lower plateau of RSNA (% baseline) values resulted in α-chloralose also having lower gain (Δ %RSNA/ΔmmHg) and range of RSNA values (% baseline) than the control (Figures 2A & 2B.). Furthermore, we found that α-chloralose had lower range and gain values than urethane or isoflurane (urethane). The low gain under α-chloralose anesthesia results in a baroreceptor response that produces a slower rise in blood pressure back to homeostatic levels. Additionally, our low range value under α-chloralose indicates a decrease in RSNA sensitivity to

depressed blood pressure. However, our lack of statistical significance means we cannot confidently say that these conclusions about range and gain are due to α-chloralose.

We realize that one pitfall in this study is that it was statistically underpowered. Power analysis, appropriate to avoid a type II statistical error recommends a sample size of 10-12 rats per group. The small sampling size was due to time constraints which limited the depth of the study. As such these findings are preliminary but also provide good evidence that we will detect important differences between these two anesthetics. Additionally, the scope of this study could also be expanded to include other variants of anesthesia. Other known anesthetics with documented use in adult rats include sodium-pentobarbital, chloral-hydrate, and ketamine-xylazine. Including more variants of anesthesia in future studies would allow us to draw further comparisons about the advantages of using one anesthetic over another. It will also allow us to draw greater conclusions about particular benefits that an anesthetic may have on cardiovascular control.

Conclusion

Based on the results, both urethane and α-chloralose anesthesia produced mild effects on adult rat baroreceptor control compared to the control anesthesia isoflurane. α-chloralose and urethane anesthesia both produced a depressed range of RSNA (% baseline) and gain (Δ %RSNA/ΔmmHg) during the baroreflex, which suggests a depression of baroreceptor sensitivity compared to control isoflurane. However, those conclusions are not statistically significant and so we cannot say that those effects were the result of the anesthesia used. The most notable differences in effects of urethane and α-chloralose on the cardiovascular system lie in their significant modification of either baseline blood pressure or baseline RSNA. Urethane produced both elevated baseline blood pressure and RSNA compared with control isoflurane (urethane). α-chloralose

produced depressed baseline blood pressure* and RSNA compared with control isoflurane (chloralose). Ultimately, our findings suggest that both urethane and α-chloralose provided sufficient induction of anesthesia without significantly modifying baroreceptor response. However, since urethane significantly raised baseline sympathetic nerve activity, it should be avoided in studies where raised sympathetic activity could confound with the test results. α-chloralose significantly lowered baseline blood pressure by nearly 30%, and its use should be avoided in studies where lowered blood pressure may confound the results.

APPENDIX

FIGURE 1.
BAROREFLEX CURVES

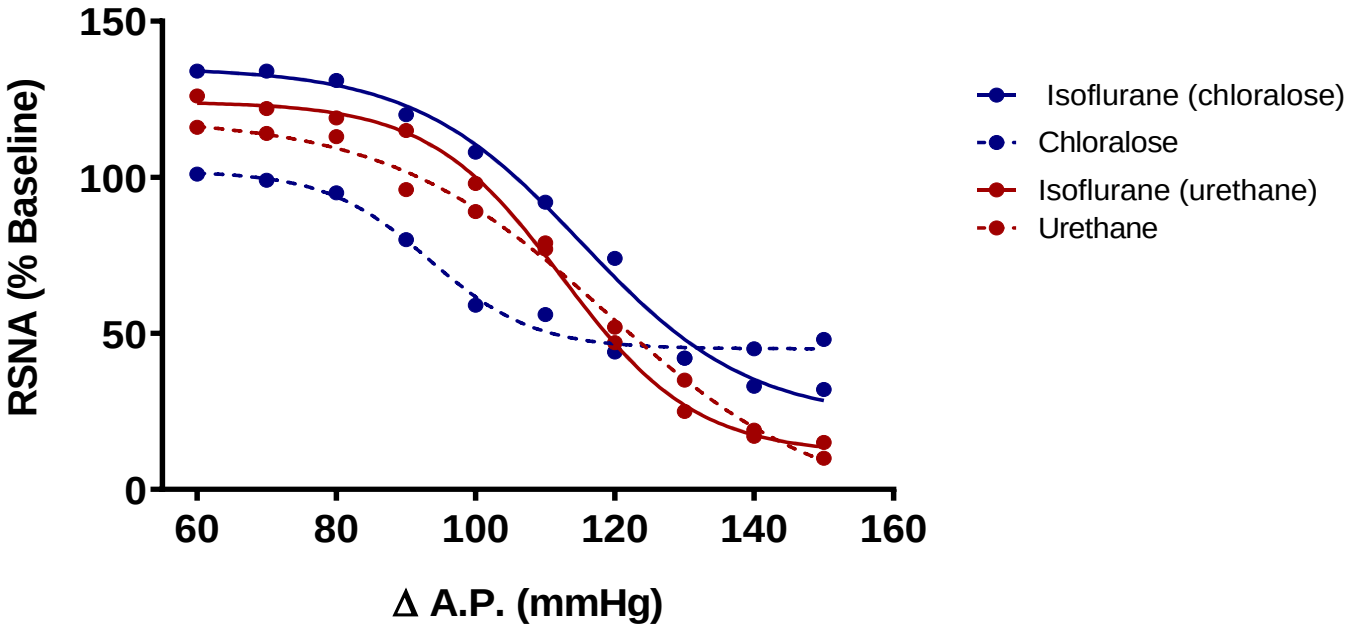
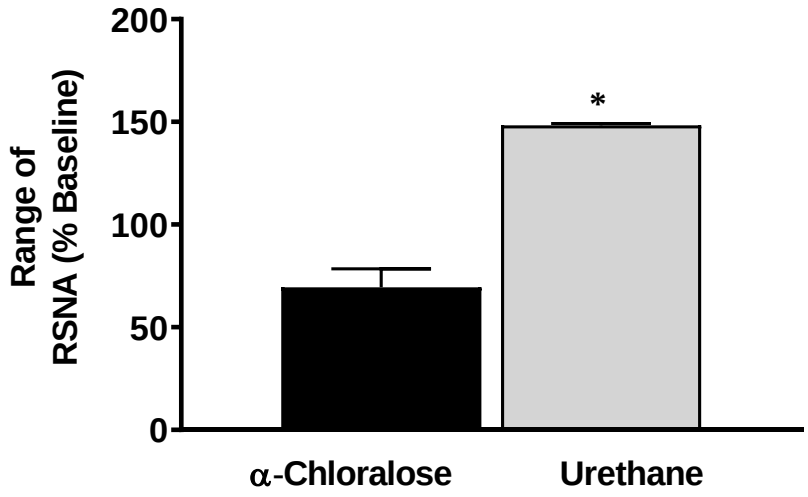
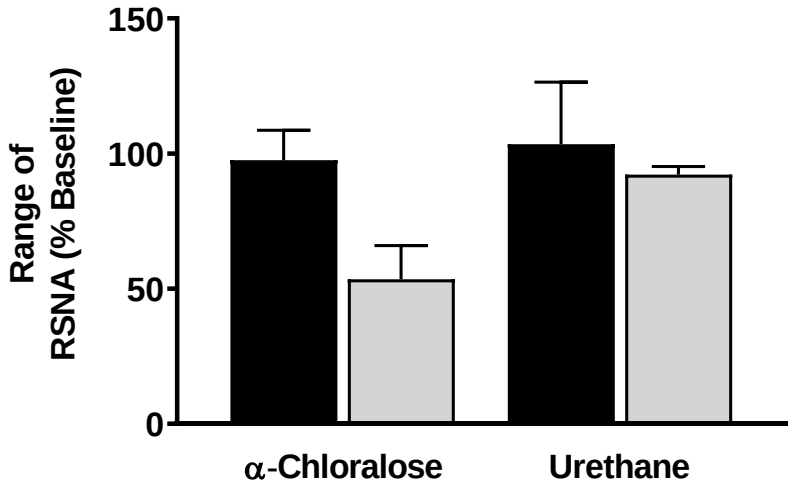


FIGURE 2.

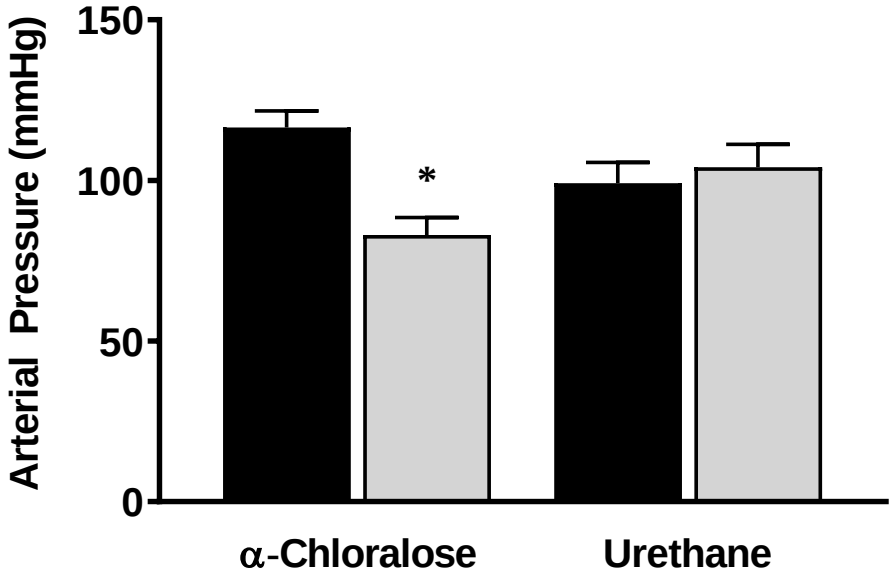
A. BASELINE SYMPATHETIC ACTIVITY



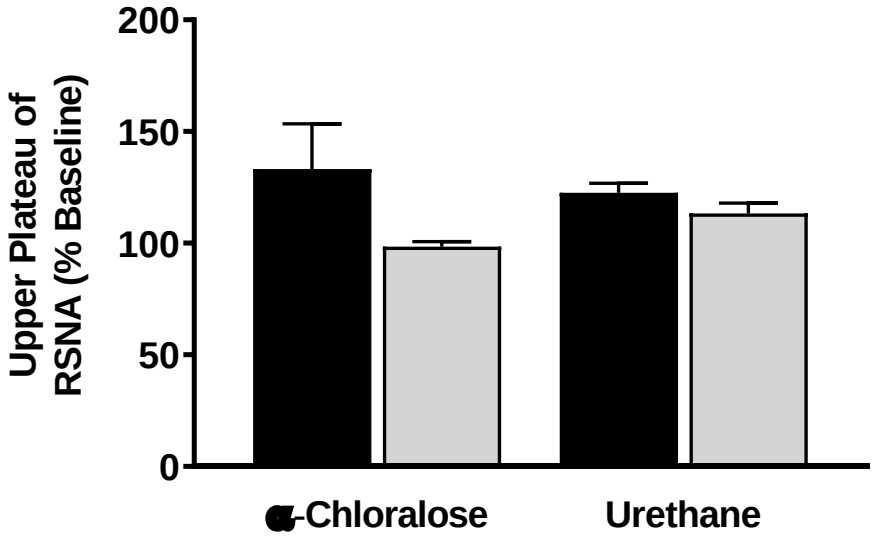
B. RANGE OF SYMPATHETIC ACTIVITY



C. BASELINE BLOOD PRESSURE



D. UPPER PLATEAU OF SYMPATHETIC ACTIVITY



E. LOWER PLATEAU OF SYMPATHETIC ACTIVITY

F. GAIN OF SYMPATHETIC ACTIVITY

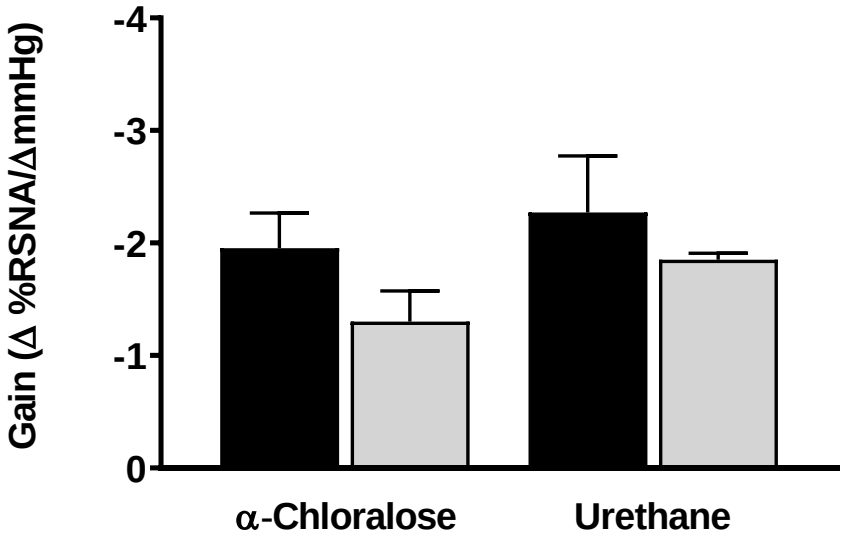


Figure legends

Figure 1. Represents baroreflex relationship of blood pressure and renal sympathetic nerve activity (RSNA). The curves were produced during isoflurane (control) and then transitioned to either urethane or α-chloralose.

Figure 2. Group data showing the Baseline Sympathetic Activity (A) Range of Sympathetic Activity (B) Baseline Blood Pressure (C) Upper Plateau of Sympathetic Activity (D) Lower Plateau of Sympathetic Activity (E) and Gain of Sympathetic Activity (F). Data are represented as means ± SE. Data were analyzed using one-way ANOVAs with group Tukey post-test. N=2 per group and * indicates significance.

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