UCI Office of Research
Research Management System

Animal Use Protocol

Title

General Information

Version: 25.0

Project Overview

Provide a non-technical, lay-language summary of your project.
Uncheck this box to remove all text from the box below

Metabolites (e,g, bradykinin) produced during myocardial ischemia (i.e., lack of blood supply to the heart) activate sympathetic sensory nerve located in the heart, which lead to chest pain (angina pectoris) and reflex cardiovascular responses, like increase in blood pressure. The aim of this project is to investigate mechanisms underlying the role of central nervous system (CNS) in regulation of blood pressure responses during myocardial ischemia. Previously, have demonstrated that some brain areas, such as , paraventricular nucleus (PVN) in hypothalamus and rostral ventrolateral medulla (rVLM) in the brain stem are activated by stimulation of sympathetic sensory nerve originated from the heart. However, it is unknown about the function of these brain regions in central processing cardiovascular reflexes in response to the stimulation of the heart and the underlying mechanisms of their actions. Therefore, the goal of this study is to evaluate the role of the and PVN in processing cardiovascular reflexes during the stimulation of the heart and the interaction between /PVN and rVLM. As such, the present proposal will provide new and distinctive information about mechanisms underlying cardiovascular responses following heart stimulation, which will directly lead to the development of new methods to manage ischemic events of the heart, like heart attack.

Study Characteristics

Animal Biosafety Level
ABSL-1

Will animals be physically restrained for more than brief periods without the use of sedation or anesthesia?
No

Will paralytic agents be used in live animals under this protocol?
Yes

Will surgical procedures be performed under this protocol?
Yes

Type of surgical procedure(s) to be performed (check all that apply):
Major

Will animals recover from surgical procedures (survival surgery)?
No (Terminal Surgery Only)

Project Continuation

Is this application a three-year de-novo renewal of a previously approved protocol?
Yes

Total # of animals used in the last 3 years
Did any adverse or unanticipated events with animal health, behavior or well-being occur during the last 3 year period of this study, that was not previously reported to the IACUC?
Progress Summary
Justification for Continuing the Project
Adverse events and unanticipated problems:

None to date within the last three year approval period.

Justification for Continuing the Project

Myocardial ischemia through actions of multiple ischemic metabolites evokes excitatory cardiovascular reflex responses, some of which can be lethal in the setting of a heart attack or following bypass surgery. have shown that a number of ischemic metabolites in an interactive and multifactorial fashion stimulate cardiac afferents and hence generate cardiac afferent input through the central nervous system (CNS) processing leading to excitatory cardiovascular reflex responses, the mechanisms including CNS processing underlying ischemia-mediated cardiovascular reflex responses remain unclear and represent our current investigation. This long-term research project has been funded by the NIH since The current studies will investigate the role of several central cardiovascular centers (i.e., brain nuclei) including nucleus located in hypothalamus, midbrain and medulla in processing of cardiac afferent inputs during ischemia. For these nuclei there are no data in the literature that speak to their importance in regulation of cardiovascular reflexes during myocardial ischemia and hypertension.

List the previously approved protocol number

Summarize the progress made on the project during the last 3-year period.

During the last three years, that cardiovascular neurons are present in the external lateral parabrachial nucleus (elPBN) within the pons and their activity is increased by activation of cardiac afferents following chemical and electrical stimulation of heart. that the elPBN cardiovascular neurons regulate rostral ventrolateral medulla (rVLM) activity through excitatory elPBN-rVLM projections during cardiac sympathetic afferent stimulation. These data suggest that eth elPBN and the elPBN-rVLM neural pathways are important in regulation of excitatory cardiac-cardiovascular reflex responses, which contribute to the central-processing sympathoexcitatory reflexes induced by stimulation of cardiac spinal afferent, particularly during myocardial ischemia.

Publication

1.

Abstract

1.

Total Number of Animals Used in the last 3 years

6 cats and 68 rats.

Funding Source	Funding Status	Award/Proposal #	Billing Account #
	Pending		

Funding & Billing Information

Other Funding Sources (not captured in table above)

Has the project undergone peer-review by an extramural sponsor/funding agency (e.g., NIH study section)?
Yes

PI Home Department

Species

Cat
Rat

Cat

Species Justification

Explain why the proposed species is the most appropriate model for this research.
Uncheck this box to remove all text from the box below

For this study, we propose that the use of cat and rat species would be the most appropriate model. have demonstrated that the neural control of the cat and rat's cardiovascular system is similar to that of humans. have shown that it is possible to study cardiac reflexes induced by chemical stimulation of heart in the rat and cat, but we will use rat for studying the reflex responses to chemical stimulation of heart so as to minimize the number of cats used. However, we will use cat for studying the role of brain nuclei in regulation of reflexes to myocardial ischemia to cardiac stimulation because of the advantage of cats' head size, which facilitates placement of multiple or closely spaced recording and stimulation electrode. The following reasons also show why both rat and cat are the best species for the study:

1) Many laboratories around the world use rats and cats to conduct cardiac-related studies. This indicates that the rat and cat are frequently used animals for this kind of study. Furthermore, we can easily obtain information from previous studies regarding experimental methods to refine our techniques and procedures to minimize pain, distress, and discomfort in the animals.

2) The brain regions in the rat and cat have been well studied and mapped. Therefore, the rat and cat are reliable species for this study.

3) has established an excellent rat model to obtain consistent pressor (increase in blood pressure and heart rate) responses induced by stimulation in the heart. Studies using this rat model have been published in several well-known scientific journals. It suggests that this rat model is an appropriate model for the proposed study. In addition, not changing the animal model will save additional animals that would be needed to establish a new experimental model.

4) No evidence has yet shown that other small species of animals can be used for this study. In respect to this, neither guinea pigs nor swine serve as adequate models to study cardiovascular responses caused by stimulation of the heart as we normally perform in our laboratory. In addition, a swine cannot be used as an animal model because the specific brain regions in swine have not yet been clearly defined.

Animal Characteristics

List the specific strains that will be used.

Phenotypic Abnormalities or Special Health Conditions

Additional Information about Species/Strains

Rat

Species Justification

Explain why the proposed species is the most appropriate model for this research.
Uncheck this box to remove all text from the box below

For this study, we propose that the use of cat and rat species would be the most appropriate model. have demonstrated that the neural control of the cat and rat's cardiovascular system is similar to that of humans. have shown that it is possible to study cardiac reflexes induced by chemical stimulation of heart in the rat and cat, but we will use rat for studying the reflex responses to chemical stimulation of heart so as to minimize the number of cats used. However, we will use cat for studying the role of brain nuclei in regulation of reflexes to myocardial ischemia to cardiac stimulation because of the advantage of cats' head size, which facilitates placement of multiple or closely spaced recording and stimulation electrode.

Animal Characteristics

List the specific strains that will be used.
Uncheck this box to remove all text from the box below

Sprague-Dawley and Wistar-Kyoto rat

Phenotypic Abnormalities or Special Health Conditions

Additional Information about Species/Strains

Rationale & Alternatives

Search Results

Date of Search	Time period from	Time period to	Database	# of Results	Keywords
Dec-30-2016	Jan-01-1966	Dec-30-2016	PubMed	3	myocardial ischemia, cardiac afferent stimulation, cardiovascular reflexes, in vivo neuronal recording, cardiovascular model, cat alternative, rat altenative, sympathetic nerve activity recording, PVN, and rVLM
Dec-30-2016	Jan-01-1966	Dec-30-2016	Web of Science	3	myocardial ischemia, cardiac afferent stimulation, cardiovascular reflexes, in vivo neuronal recording, cardiovascular model, cat alternative, rat altenative, sympathetic nerve activity recording, PVN, and rVLM

Database Searches

Discussion of Search Results

Uncheck this box to remove all text from the box below

We are investigating cardiovascular responses to stimulation of cardiac sympathetic sensory nerves. and always strive to reduce the number of animals and to refine the experimental design and procedures. As suggested, we have conducted literature search again. However, there is no similar study to ours, which has been reported before. Thus, our study is not an unnecessary duplicate of any other previous work.

Other Sources Used to Consider Alternatives

Replacement

Discuss efforts to partially or fully replace live animals with in vitro models, (i.e. cell culture), computer simulation, or use of a less-sentient species (e.g. insects).

Uncheck this box to remove all text from the box below

As discussed above, a vertebrate animal model is necessary to study reflex cardiovascular responses to nerve fiber stimulation. A fully connected circular and nervous system needs to be intact in order to observe the full affects and true nature of cardiac reflexes during myocardial ischemia. The pathway for these reflex responses involves both cardiovascular and nervous system and thus non-animal models will not suffice.

Reduction

Describe the steps you have taken to reduce the number of animals to the minimum required to obtain scientifically valid data.

Answer

The literature search does not help significantly with reducing numbers of animals, since our group currently is main research team in the world in respect with studying the mechanisms of the cardiovascular reflexes evoked by stimulation of cardiac sympathetic afferents. In order to reduce the number of animals used, more than one protocol will be performed on some animals used in this project. Also, we will minimize the number of surgeries performed and reduce the animal use count.

Refinement

Explain how the experimental design and procedures have been refined to improve efficiency and minimize pain and distress.

Uncheck this box to remove all text from the box below:

We are constantly refining our experimental procedures to limit the surgical time and the extensiveness of the surgery. We invite outside investigators to our laboratory and we send our staff to other laboratories to learn the latest techniques that might apply to our studies. We encourage the veterinary staff and any member of IACUC to come visit our laboratory to view our procedures.

Study Segments

Experimental Design	Species
Study 1	Cat
Study 2	Rat

Study 1

Species

Cat

Experimental Design Summary

Does this study segment tab describe the establishment and maintenance of a breeding colony?

Describe the rationale behind the experiment or the hypothesis being tested in this study segment.
Uncheck this box to remove all text from the box below

Study 1 will examine that: 1) the brain nuclei such as PVN and rVLM processes ischemia-induced cardiac afferent inputs through glutamate mechanisms leading to CSR responses; 2) Excitatory glutamatergic and PVN-rVLM projections participate in processing CSR; and 3) GABA modulates both PVN processing as well as and PVN-rVLM excitatory projections through $GABA_A$ receptors.

Abbreviations used in the text:

BP	blood pressure
CNS	central nervous system
CSR	cardiac sympathoexcitatory reflex
PVN	paraventricular nucleus
HR	heart rate
RSNA	renal sympathetic nerve activity
rVLM	rostral ventrolateral medulla

Rationale The and PVN are involved in regulation of cardiovascular function including following stimulation of chemoreceptors. However, their role in processing reflexes, particularly cardiac-cardiovascular reflexes, has not been evaluated. Our anatomical studies have shown that PVN, and rVLM neurons, specifically glutamatergic neurons, are activated following stimulation of cardiac spinal afferents. Other studies have shown that inhibition of rVLM by microinjection of a $GABA_A$ agonist reduces the increase in RSNA and BP evoked by activation of the PVN These data imply that the PVN participates in processing CSR through glutamatergic mechanisms.

Experimental protocols *(please refer to Section 5 for surgical procedures in detail)*

The following receptor agonists, antagonists and vehicle control will be used:

- Bradykinin (BK) – to induce pressor reflex by stimulation of the heart
- Kynurenic acid (Kyn) - inhibits action of glutamate, a ubiquitous excitatory neurotransmitter in the brain.
- D,L-Homocysteic acid (DLH) – glutamate agonist
- AP-5 - NMDA glutamate receptor antagonist
- NBQX - AMPA glutamate receptor antagonist.
- Gabazine - $GABA_A$ receptor antagonist
- Normal saline (vehicle control for agonists and antagonists)

Experimental protocols *(please refer to Section 5 for surgical procedures in detail)*

Four groups of cats will be utilized incorporating microdialysis with HPLC neurochemical analysis, cardiovascular reflex response and electrophysiological recordings.

Group A: In denervated cats, we will assess extracellular concentrations of glutamate and GABA in the PVN during cardiac sympathetic afferent stimulation using microdialysis and HPLC and will evaluate modulation of glutamate concentrations by GABA through $GABA_A$ receptor blockade. Animals will be assigned to groups PVN. The fluid samples (dialysates) following stereotaxic implantation of a microdialysis probe in this region will be collected from PVN respectively. In each group *thee subgroups* of cats will be studied: *1) cardiac stimulation + perfusion of vehicle* through microdialysis probe in the PVN, *2) cardiac stimulation + perfusion with gabazine* ($GABA_A$ antagonist), and *3) Time control* without drugs in perfusate and cardiac stimulation.

Group B: In vagotomized cats, we will determine if the PVN processes CSR responses through a glutamatergic ionotropic NMDA and AMPA receptor mechanism that involves GABA through $GABA_A$ receptor stimulation. Animals will be assigned to groups PVN. After placing a pipette into the PVN, responses of blood pressure (BP), heart rate (HR), and RSNA to repeated cardiac afferents stimulation during myocardial ischemia and BK application will be

evaluated separately before and after unilateral (or if necessary, bilateral) microinjection of the following drugs (50 nl) in five subgroups: non-selective glutamate receptor blockade (Kyn, 50 nM), NMDA blockade (AP-5, 25 mM), AMPA blockade (NBQX, 20 mM), $GABA_A$ receptor blockade (gabazine, 27 mM) and vehicle (saline) serving as controls.

Group C: We will evaluate the roles of glutamate and GABA and their receptors in regulation of PVN sympathoexcitatory neuronal activity during separate stimulation of cardiac sympathetic afferents with ischemia and epicardial BK. Animals will be assigned to groups: PVN. Recording electrodes will be inserted into the PVN of cats. After identifying a neuron responsive to ischemia and epicardial BK, the cardiac sympathetic nerve (CN) will be stimulated electrically to quantitatively assess evoked activity. PVN activity and RSNA will be recorded simultaneously. PVN neurons also will be evaluated for cardiovascular-related activity. CN-evokec PVN activity will be further evaluated in five subgroups in each group before and after administration (iontophoresis) of the following drugs into the PVN: Kyn (25 mM), AP5 (25 mM) NBQX (20 mM), gabazine (27 mM) or saline control. Efficacy of GABA receptor blockade will be assessed with GABA (1.25 M).

Group D: We will determine if excitatory or PVN-rVLM glutamatergic pathways participate in sympathoexcitatory responses induced by cardiac stimulation, are modulated by GABA. Animals will be assigned to two groups: nd PVN-rVLM. In vagatomized cats, micropipettes will be placed in the or PVN, and rVLM for microinjection and neuronal recording, respectively. A stimulating electrode will be placed in the rVLM to antidromically identify or PVN-rVLM axonal projections in separate cats. Similar to recording studies in the after characterization of presympathetic rVLM or PVN neurons we will further study these rVLM neurons in the following protocols: 1) we will examine the CN-evoked rVLM activity before and after unilateral microinjection of Kyn (25 mM, 50 nl), gabazine (27 mM) or vehicle control into the or PVN in three subgroups; and 2) we will evaluate the response of CN-evoked rVLM activity induced by microinjected of DLH into the before and after iontophoresis of Kyn, AP5, NBQX or vehicle into the rVLM in four subgroups. Consideration of three years time course, we will investigate group first. Therefore, total 7 subgroups of cats will be used in the first groups. If we could finish first group within 3-years, we will conduct the second group of PVN-rVLM with another 7 subgroups.

Anticipated results we expect to observe increases in extracellular [Glu] (glutamate) and [GABA] in the PVN during cardiac sympathetic afferent stimulation with ischemia and BK. Moreover, elevated [Glu] during epicardial stimulation will be augmented after pre-treatment with $GABA_A$ antagonists. Reinforcing the microdialysis data, we expect that inhibition of NMDA or AMPA glutamate receptors in the PVN will reduce the CN-evoked rVLM activity as well as ischemia and BK-induced CSR responses. In contrast, inhibition of $GABA_A$ receptors in the PVN will enhance CN-evokec PVN and rVLM activity and the excitatory CSR. Finally, iontophoresis of Kyn, AP5 or NBQX into the rVLM will reduce the enhancement of the CN-evoked rVLM activity induced by administration of DLH into the PVN.

Alternative protocols If we could not obtain the results that we expect, we would examine the role(s) of other excitatory neurotransmitters such as angiotensin II. Previous studies reported that angiotensin II functions as an excitatory neurotransmitter in the PVN. We will also have the option to examine the roles of $GABA_B$ receptors and/or other inhibitory neurotransmitters like opioids. It is possible that we may not observe enhancement of the CN-evoked rVLM neuron responses following blockade of $GABA_A$ receptors. If so, we will investigate other inhibitory neurotransmitters such as opioids that may modulate the excitatory pathway between the PVN and the rVLM.

Our current success rate in this study is approximately 85%. We take into consideration the difficulty in accurate placement of injection and recording sites and biological variability of neuronal responses from animals to determine our success rates of the experiments. Ten successful animals are necessary for statistical significance.

10 cats /85% = 12 cats, so12 cats total for each group

The number of groups = Group A + B + C + D = 3 + 10 + 10 + 7 = 30 groups

30 groups x 12 animals = 360 cats needed

Thus, for study #1 360 cats total needed.

Does this study segment describe the establishment and maintenance of a breeding colony?
No

Animal Monitoring

Species	Monitoring Parameters	Monitoring Frequency	Responsible Person
Cat	heart rate & BP	every 15 min	experimentor
Cat	corneal reflex	every 15 min	experimentor
Cat	body temperature	every 15 min	experimentor

Animal Monitoring Details

What, if any, clinical signs or symptoms are expected in response to the procedures/manipulations in this study?
Uncheck this box to remove all text from the box below

Heart rate and blood pressure (BP) and body temperature are expected to be stable as well as no corneal reflex throughout the experiment, indicating a good sedation level. Change in stability of heart rate and BP may indicate pain or distress. Response to corneal probing also indicate that the animal is coming out of anesthesia. In this case, supplemental anesthetics will be given as needed.

Management Plan for Animal Monitoring

Documentation of Animal Monitoring

Endpoints

Experimental Endpoints

Humane Endpoints

What are the experimental endpoints for this study segment?
Uncheck this box to remove all text from the box below

All protocols in this study involve terminal procedures components. Animals will be kept under anesthesia for about 9 hours. After the final collection of data, animals will be given an overdoses of alpha-chloralose for deep anesthesia, thereafter euthanized with intravenous injection of saturated KCl.

What clinical signs or other criteria will be used to determine that an animal must be removed from the study ahead of schedule (Humane Endpoint)?
Provide additional details about any checked items, or any other endpoint criteria:

Blood pressure will be monitored and will be used to determine whether the experiment must be terminated ahead of schedule.

If the blood pressure of experimental animal become unstable and below 50/30 mmHg after treatment for rescuing the animal under anesthesia, the experiment have to be terminated ahead of schedule.

Euthanasia

Will all research animals be euthanized at the conclusion of this experiment/study segment?
Yes

Indicate the euthanasia methods that will be used on the animals from these experiments - Check all that apply
You MUST...

Animal number calculation for experimental part Study 1

Cat		
	Max	Description
	360	30
	360	**Cat**

Copy IACUC SOP templates

Procedure	Species
surgical procedures	Cat
surgical endpoints and Euthanasia	Cat
non-surgical procedures	Cat

Procedure Descriptions

1. The surgical procedures on the cat will be described as following:

All protocols in the study #1-2 involve terminal procedure components. Surgical instruments are cleaned before each use. Autoclavable materials are sterilized by autoclaving. A glass bead sterilizer may be used to re-sterilize instrument tips. Fur is removed from the surgical site. The surgical site is wiped with three alternating applications each of antiseptic (chlorhexidine or povidone-iodine) and 70% alcohol, working outwards from the center of the surgical field.

For cats, following initial induction of anesthesia by subcutaneous injection of ketamine, intravenous injection of alpha chloralose will be administered through the cephalic vein in the front leg before cannulation. After observing no response to corneal or conjunctival probing and muscle movement or trembling indicating an adequate anesthesia, an incision will be made over the femoral artery located in the upper thigh. The femoral artery and vein each of the animal will be cannulated via a small incision into the blood vessel. The animal will be intubated with a cuffed endotracheal tube. Then, the animals constantly will be monitored for stable heart rate and blood pressure, response to corneal or conjunctival probing and muscle movement will be assessed every 15 min during surgery. Additional dosage of anesthetic will be given when observing presence of corneal or conjunctival reflex, muscle movement, or unstable heart rate and blood pressure.

A sinoaortic barodenervation and vagotomy will be performed. A midline incision will be made over the throat on the neck. Both left and right common carotid arteries and vagus nerves will be isolated. The carotid sinus nerve is isolated from internal carotid artery and transected to achieve carotid sinus denervation. Similarly, Aortic depressor nerve will be isolated from common carotid artery and transected to achieve aortic barodenervation. 10% phenol will be applied locally around the sinoaortic nerve to deactivate their activities. Successful barodenervation will be confirmed by absence of a normal decrease of heart rate in response to an increase in arterial blood pressure induced by administration of phenylephrine and/or nitroglycerine (i.v.; please see section 7 for dosages). The cervical vagus nerve on both sides will be transected to achieve the bilateral vagotomy. Body temperature will be measured by rectal probe and maintained at 37°C by a circulating water heating pad and heat lamp.

Cardiac Stimulation: The chest will be opened through the midline sternotomy or in the 4-5th intercostal space and the anterior surface or lateral surface of the heart will be exposed, respectively. To achieve midline sternotomy, an incision will be made over the midline of the sternum. The muscles and connective tissue will be removed from over the sternum which is then cut along the midline. For the thoracotomy procedure, an incision of about one inch will be made over the 4-5th intercostal space, perpendicular to the ribs. Intercostals muscles are carefully removed to provide access to the anterior surface of the heart. The incision will be covered with moistened gauze, which will only be removed during application of BK to the surface of the heart. Needle will be inserted through parietal pericardium for catheter access into pericardial space. Catheter is sutured onto parietal pericardium for purpose of BK application onto the visceral pericardium. Under a surgical microscope, a ligature will be placed around a coronary artery and tightened to cause the coronary artery occlusion that leads to myocardial ischemia.

A craniotomy will be performed to expose the rVLM in the medulla, PVN in the hypothalamus. Depending on the protocol, the exposure may be on either dorsal (spinal) or ventral (stomach) sides of the body. For majority of the studies dorsal approach will be sufficed. Animals will be placed in a stereotaxic head frame and the head flexed to an angle of 45°. The medulla will be exposed by midline incision of the skin, separation of the overlying muscles and a removal of occipital plate. We have found in our preliminary studies that it is necessary to remove the cerebellum to be able to accurately identify the correct surface landmarks. A single or three-barrel micropipette will be inserted into the rVLM, and /or PVN.

Microinjection of some chemicals, such as kainic acid, GABA, DL-Homocysteric, among others, will be injected into rVLM, and /or PVN to help identify nuclei excitatory or inhibitory role in regulating blood pressure. At the end of experiment, Chicago sky blue will be microinjected to identify the site of microinjection (please see section 7 for dosages).

Recording of renal sympathetic nerve activity (RSNA): The left or right kidney will be exposed through an incision made into the right, or left lower abdominal quadrant. The kidney is identified, and the peritoneum, overlying the kidney is incised to gain access into the retroperitoneal space and kidney. A branch of the renal nerve will be separated from the renal plexus and surrounding connective tissues. The distal end of the renal nerve will be crushed to eliminate afferent discharge and the proximal end placed on bipolar silver recording electrodes. The site will be covered with vinyl polysiloxane dental impression material or mineral oil to secure the nerve-electrode contact or prevent damage and desiccation of the nerve, respectively. RSNA will be recorded. A gauze sponge moistened with saline will be placed in the incision to prevent desiccation. Hexamethonium and gallamine triethiodide (i.v.) will be used to identify basal renal nerve activity and inhibit spontaneous muscle activity, respectively (please see section 7 for dosages) and this is to minimize the influence of muscle contraction-induced noise and movements on the recorded renal sympathetic nerve activity and/or central neuronal activity in both cat and rat studies. specifically, gallammine will be given 15 min before RSNA and/or central neuronal activity will be recorded in both Studies. Adequate anesthesia of animal will be assessed by observing and monitoring the absence of a corneal or conjunctival reflex before and during administration of gallamine. Additional dosages of anesthetic will be administered when observing the presence of a conjunctive reflex response.

For the cardiac reflex studies, a sinoaortic barodenervation and cervical vagotomy will be performed on both sides of the neck. A midline incision will be made on the front side of the neck. Muscle and connective tissues will be dissected and separated from the common carotid artery. The left and right cervical vagus nerves will be isolated from adjacent the common carotid artery and transected (vagotomy). The carotid sinus nerve will be isolated from the internal carotid artery and transected to achieve carotid sinus denervation. The aortic depressor nerve will be also isolated from the common carotid artery and transected to achieve aortic barodenervation. The sinoaortic barodenervation will be accomplished in order to prevent secondary buffering of cardiac-cardiovascular reflexes by arterial baroreceptors. Vagotomy will be performed to allow full manifestation of the excitatory cardiac-cardiovascular reflexes during ischemia and/or chemical stimuli. Successful barodenervation will be confirmed by absence of a normal decrease of heart rate in response to an increase in arterial blood pressure induced by administration of phenylephrine and or nitroglycerine.

For the cardiac reflex studies, a sternotomy is not required. Instead, a small incision will be made across the left chest over the 5^{th} or 6^{th} rib. The ribs will be separated and held apart by a retractor to expose the heart. The pericardium will be pinched and a tiny incision is made to allow insertion of a small catheter. The pericardium will be sewn cinched around the catheter. The catheter will be used to inject and withdraw chemicals that will be applied to the surface of the heart. After withdrawing the chemicals, saline will be used to flush and wash off excess chemicals on the surface of the heart in the same manner.

2. Surgical endpoints and Euthanasia

Terminal surgeries will take about 3 hours. The entire experiment will last for approximately 8 hours. At the end of the study, 2% Chicago blue dye will be injected into the brain regions where recording or microinjection took place. The animals will be deeply anesthetized with a large dose of pentobarbital (200 mg/kg, i.v.) and then euthanized with 1M potassium chloride (1 ml/kg, i.v.). The cranium will be removed to expose the brain, which will be harvested and sliced into coronal sections for identifying injection and recording sites.

In some rat experiments without brain stimulation or brain injection, the cranium will be not open and the thorax will be opened to produce a pneumothorax and confirm death.

Cat

Study 2

Species

Rat

Experimental Design Summary

Does this study segment tab describe the establishment and maintenance of a breeding colony?

Describe the rationale behind the experiment or the hypothesis being tested in this study segment.

Uncheck this box to remove all text from the box below

Study #2 we will examine if cardiac excitatory reflexes associated with ischemia are enhanced in sustained hypertension. The at least in part, through an IL-1β mechanism.

Rationale Compared to patients without hypertension, patients with ischemic heart disease and hypertension have significantly higher mortality and morbidity rate associated with the augmented sympathetic nerve activity during acute myocardial ischemia or exercise. The contribution of cardiac-cardiovascular reflexes to these events is uncertain since there are no studies available on cardiac reflex responses in hypertensive animal or central neural mechanisms that process afferent input. Thus, this study is unique.

Experimental protocols

Using anatomical, molecular, hemodynamic and electrophysiological approaches, four groups of studies will be conducted in hypertensive rats, i.e., cold-induced hypertension (CI-HTN), spontaneously hypertensive (SHR) and rats as well as in normotensive Sprague-Dawley (SD) and Wistar-Kyoto (WKY) rats.

Group A We will determine if by examining responses of blood pressure (BP) and RSNA to chemical stimulation of the heart in hyper- and normotensive rats. Thus we will examine responses to intrapericardial α,β-meATP, at different doses (31 - 125 nmol) or vehicle, in five subgroups of vagotomized rats including CI-HTN, SHR and their respective controls SD and WKY.

Group B We will determine the role of with three protocols. *Protocol 1,* using immunohistochemical staining, we will determine if cardiac afferent stimulation with α,β-meATP activates more neurons containing IL-1β in hypertensive vs. normotensive rats in five subgroups. *Protocol 2,* using ELISA, we will and normotensive rats following intrapericardial α,β-meATP in five subgroups. In both protocols hyper- and normotensive rats without cardiac stimulation will serve as baseline controls in other 10 subgroups (five subgroups x two protocols). *Protocol 3,* we will examine the normotensive rats in five subgroups. Thus, total 25 subgroups of rats will be used in this group of study.

Group C We will evaluate the role of After placing a pipette into the responses of BP and RSNA to repeated stimulation of cardiac ischemia-sensitive afferents with intrapericardial α,β-meATP will be examined before and after unilateral (or if necessary bilateral and normotensive rats in five subgroups.

Group D We will examine the influence of IL-1Rs on enhanced activity of rVLM sympathoexcitatory neurons in hypertension. Micropipettes will be placed in the and rVLM for microinjection and neuronal recording, respectively. A stimulating electrode will be placed in the rVLM to antidromically in additional rats. After characterization of rVLM neurons (see Study #2 Group D), we will measure evoked rVLM activity induced by intrapericardial α,β-meATP before and after unilateral microinjection of IL-1R antagonist (2 μg/ml) or vehicle (0.1% BSA, as control) into the in the three and their respective controls in total 10 subgroups (five subgroups x two chemicals used for microinjection).

Anticipated results: We anticipate that relative to normotensive SD and WKY rats during chemical stimulation of ischemically sensitive cardiac afferents. Cardiac afferent stimulation activates than in normotensive rats. than in normotensive rats. and rVLM neuronal activity much more in hypertensive than normotensive rats.

Alternative protocols: We may not observe that stimulation of cardiac afferents in a larger group and there may be little expression of IL-1R mRNA and protein in the If this is the case, we have the option to examine if cardiac stimulation in the PVN in hypertensive rats than in normotensive rats. If the PVN~IL-1β works, we will focus on studying the role of PVN processes the exaggerated instead of the Additionally, we can investigate the role of other cytokines such as rats than in normotensive rats.

Our current success rate in this study is approximately 85%. We take into consideration the difficulty in accurate placement of injection and recording sites and biological variability of neuronal responses from animals to determine our success rates of the experiments. Ten successful animals are necessary for statistical significance.

10 rats /85% = 12 rats, so12 rats for each group

The number of groups = Group A + B + C + D = 5 + 25 + 5 + 10 = 45 groups

45 groups x 12 animals, so 540 rats needed

Thus, for study #2 540 rats in total needed.

Does this study segment describe the establishment and maintenance of a breeding colony?

No

Animal Monitoring

Species	Monitoring Parameters	Monitoring Frequency	Responsible Person
Rat	heart rate & BP	every 15 min	experimentor
Rat	corneal reflex	every 15 min	experimentor
Rat	body temperature	every 15 min	experimentor

Animal Monitoring Details

What, if any, clinical signs or symptoms are expected in response to the procedures/manipulations in this study?
Uncheck this box to remove all text from the box below

Heart rate and blood pressure (BP) and body temperature are expected to be stable as well as no corneal reflex throughout the experiment, indicating a good sedation level. Change in stability of heart rate and BP may indicate pain or distress. Response to corneal probing also indicate that the animal is coming out of anesthesia. In this case, supplemental anesthetics will be given as needed.

Management Plan for Animal Monitoring

Documentation of Animal Monitoring

Endpoints

Experimental Endpoints

Humane Endpoints

What are the experimental endpoints for this study segment?
Uncheck this box to remove all text from the box below

All protocols in this study involve terminal procedures components. Animals will be kept under anesthesia for about 9 hours. After the final collection of data, animals will be given an overdoses of alpha-chloralose for deep anesthesia, thereafter euthanized with intravenous injection of potassium chloride (1 M, 1 ml/kg).

What clinical signs or other criteria will be used to determine that an animal must be removed from the study ahead of schedule (Humane Endpoint)?
Provide additional details about any checked items, or any other endpoint criteria:

For Studies #1 and #2, blood pressure of experimental animal will be monitored. If the blood pressure of experimental animal become unstable and below 50/30 mmHg after treatment for rescuing the animal under anesthesia, the experiment have to be terminated ahead of schedule.

Euthanasia

Will all research animals be euthanized at the conclusion of this experiment/study segment?

Yes

Indicate the euthanasia methods that will be used on the animals from these experiments - Check all that apply

You MUST...

Animal number calculation for experimental part Study 2

Rat

Max	Description
540	45
540	**Rat**

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Procedure Descriptions

The surgical procedures on the rat will be described as following:

Rats will be anesthetized with intramuscular injection of ketamine and xylazine. After observing no response to corneal or conjunctival probing and muscle movement or trembling indicating an adequate anesthesia, an incision will be made over the femoral artery located in the upper thigh. The femoral artery and vein each of the animal will be cannulated via a small incision into the blood vessel. The animal will be intubated with a cuffed endotracheal tube. Then, the animals constantly will be monitored for stable heart rate and blood pressure, response to corneal or conjunctival probing and muscle movement will be assessed every 15 min during surgery. Supplemental anesthesia (alpha-chloralose) is also given intravenously when observing presence of corneal or conjunctival reflex, muscle movement, or unstable heart rate and blood pressure.

A sinoaortic barodenervation and vagotomy will be performed. A midline incision will be made over the throat on the neck. Both left and right common carotid arteries and vagus nerves will be isolated. The carotid sinus nerve is isolated from internal carotid artery and transected to achieve carotid sinus denervation. Similarly, Aortic depressor nerve will be isolated from common carotid artery and transected to achieve aortic barodenervation. 10% phenol will be applied locally around the sinoaortic nerve to deactivate their activities. Successful barodenervation will be confirmed by absence of a normal decrease of heart rate in response to an increase in arterial blood pressure induced by administration of phenylephrine and/or nitroglycerine (i.v.; please see section 7 for dosages). The cervical vagus nerve on both sides will be transected to achieve the bilateral vagotomy. Body temperature will be measured by rectal probe and maintained at 37°C by a circulating water heating pad and heat lamp.

Cardiac Stimulation: The chest will be opened through the midline sternotomy or in the 4-5th intercostal space and the anterior surface or lateral surface of the heart will be exposed, respectively. To achieve midline sternotomy, an incision will be made over the midline of the sternum. The muscles and connective tissue will be removed from over the sternum which is then cut along the midline. For the thoracotomy procedure, an incision of about one inch will be made over the 4-5th intercostal space, perpendicular to the ribs. Intercostals muscles are carefully removed to provide access to the anterior surface of the heart. The incision will be covered with moistened gauze, which will only be removed during application of α,β-meATP to the surface of the heart. Needle will be inserted through parietal pericardium for catheter access into pericardial space. Catheter is sutured onto parietal pericardium for purpose of α,β-meATP application onto the visceral pericardium. Under a surgical microscope, a ligature will be placed around a coronary artery and tightened to cause the coronary artery occlusion that leads to myocardial ischemia.

A craniotomy will be performed to expose the rVLM in the medulla and/or in the hypothalamus. Depending on the protocol, the exposure may be on either dorsal (spinal) or ventral (stomach) sides of the body. For majority of the studies dorsal approach will be sufficed. Animals will be placed in a stereotaxic head frame and the head flexed to an angle of 45°. The medulla will be exposed by midline incision of the skin, separation of the overlying muscles and a removal of occipital plate. We have found in our preliminary studies that it is necessary to remove the cerebellum to be able to accurately identify the correct surface landmarks. A single or three-barrel micropipette will be inserted into the rVLM and /c

Microinjection of some chemicals, such as kainic acid or DL-Homocysteric, will be injected into rVLM and /or to help identify nuclei excitatory or inhibitory role in regulating blood pressure. At the end of experiment, 2% Chicago sky blue will be microinjected to identify the site of microinjection (please see section 7 for dosages).

Recording of The left or right kidney will be exposed through an incision made into the right, or left lower abdominal quadrant. The kidney is identified, and the peritoneum, overlying the kidney is incised to gain access into the retroperitoneal space and kidney. A branch of the renal nerve will be separated from the renal plexus and surrounding connective tissues. The distal end of the renal nerve will be crushed to eliminate afferent discharge and the proximal end placed on bipolar silver recording electrodes. The site will be covered with vinyl polysiloxane dental impression material or mineral oil to secure the nerve-electrode contact or prevent damage and desiccation of the nerve, respectively. will be recorded. A gauze sponge moistened with saline will be placed in the incision to prevent desiccation. Hexamethonium and gallamine triethiodide (i.v.) will be used to identify basal renal nerve activity and inhibit spontaneous muscle activity, respectively (please see section 7 for dosages) and using gallamine is to minimize the influence of muscle contraction-induced noise and movements on the recorded and/or central neuronal activity in both cat and rat studies. specifically, gallammine will be given 15 min before and/or central neuronal activity will be recorded in both Studies. Adequate anesthesia of animal will be assessed by observing and monitoring the absence of a corneal or conjunctival reflex before and during administration of gallamine. Additional dosages of anesthetic will be administered when observing the presence of a conjunctive reflex response.

For the cardiac reflex studies, a sinoaortic barodenervation and cervical vagotomy will be performed on both sides of the neck. A midline incision will be made on the front side of the neck. Muscle and connective tissues will be dissected and separated from the common carotid artery. The left and right cervical vagus nerves will be isolated from adjacent the common carotid artery and transected (vagotomy). The carotid sinus nerve will be isolated from the internal carotid artery and transected to achieve carotid sinus denervation. The aortic depressor nerve will be also isolated from the common carotid artery and transected to achieve aortic barodenervation. The sinoaortic barodenervation will be accomplished in order to prevent secondary buffering of cardiac-cardiovascular reflexes by arterial baroreceptors. Vagotomy will be performed to allow full manifestation of the excitatory cardiac-cardiovascular reflexes during ischemia and/or chemical stimuli. Successful barodenervation will be confirmed by absence of a normal decrease of heart rate in response to an increase in arterial blood pressure induced by administration of phenylephrine and/or nitroglycerine.

For the cardiac reflex studies, a sternotomy is not required. Instead, a small incision will be made across the left chest over the 5^{th} or 6^{th} rib. The ribs will be separated and held apart by a retractor to expose the heart. The pericardium will be pinched and a tiny incision is made to allow insertion of a small catheter. The pericardium will be sewn cinched around the catheter. The catheter will be used to inject and withdraw chemicals that will be applied to the surface of the heart. After withdrawing the chemicals, saline will be used to flush and wash off excess chemicals on the surface of the heart in the same manner.

Non-surgical procedures

Cold temperature (mild cold at 6 °C) will be exposed to some of live rats for at least 7 weeks to make cold-induced hypertension model in study #2. These rats are free to access to food and water. The rats will be paired housed like regular rats housed, no special requirement needed for housing.

Rat

Total number of animals

Species	Max
Cat	360
Rat	540

USDA Pain Category	Species	Number of Animals	Number of Animals
USDA Category D	Cat	360	0
USDA Category D	Rat	540	0

Animal Numbers Justification

Explain how the animal numbers were determined.

Will animals experience unrelieved pain/distress (category E procedures)?

Provide the scientific justification for the number of animals required for these studies.

Uncheck this box to remove all text from the box below

In order to achieve statistical significance with power of 80%, significance level of 0.05, average 12 (11-14) cats/group will be required to detect meaningful difference (>30% differences among groups) in concentrations of neurotransmitters collected from brain nuclei, mean blood pressure (MAP), renal sympathetic nerve activity (RSNA), brain nuclei activity with a power of 80% and significant level of 0.05 on 85% success rate and our previous studies [redacted]. A one-way ANOVA followed by Tukey's *post hoc* test will be used to compare changes of neurotransmitter level, MAP, RSNA, and brain nuclei activity (impulses/30 stimuli) among multiple groups/subgroups.

Similar to cat study, average 12 (11-14) rats/group will be required to detect meaningful difference (>30% differences among groups) in MAP and RSNA, numbers of activated neurons in brain nuclei, interleukins concentrations, and brain nuclei activity with a power of 80% and significant level of 0.05 on 85% success rate and our previous studies ([redacted]). A one-way ANOVA followed by Tukey's *post hoc* test will be used to compare changes of MAP, RSNA, interleukin level, and brain nuclei activity (impulses/30 stimuli) among multiple groups/subgroups.

Will animals experience unrelieved pain/distress (category E procedures)?

No

Drugs & Agents

Anesthesia

Will animals receive anesthesia agents?
Yes

Species	Name of Drug/Agent	Dose Range *(list dose in mg/kg body weight)*	Route *(SQ, IP, IV etc.)*	Frequency *(how often given?)*	Duration of Treatment *(how long will treatment last?)*
Cat	Ketamine	30 mg/kg	SQ	once	2-4 hours
Rat	Ketamine	75-100 mg/kg	SQ	once	2-4 hours
Rat	Xylazine	5-10 mg/kg	SQ	once	2-4 hours
Cat	Alpha-chloralose	60-100 mg/kg	IV	once	3-5 hours
Cat	Alpha-chloralose	5-10 mg/kg	IV	as needed	2-3 hours
Rat	Ketamine	25 mg/kg	SQ	as needed	1-2 hours
Rat	xylazine	2-3 mg/kg	SQ	as needed	1-2 hours

How will the anesthesia be administered to the animals?

Will anesthetic gases be used in this project?
No

Analgesia

Will animals receive analgesics, sedatives, or other therapeutic agents (e.g. antibiotics, supplemental fluids, etc.)?
Yes

Species	Name of Drug/Agent	Dose Range *(list dose in mg/kg body weight)*	Route *(SQ, IP, IV etc.)*	Frequency *(how often given?)*	Duration of Treatment *(how long will treatment last?)*
Cat, Rat	6% Dextran	1-2 ml/kg	IV	as needed	1-2 hours
Cat, Rat	0.9% saline	1-2 ml/kg	IV	as needed	1 hour
Cat, Rat	8% bicarbonate	0.5 ml/kg	IV	as needed	1-2 hours
Cat, Rat	Gallamine triethiodide	4 mg/kg	IV	as needed	2-4 hours

Experimental & Other Agents

Will other agents be administered to animals?
Yes

Species	Name of Drug/Agent	Dose Range *(list dose in mg/kg body weight)*	Route *(SQ, IP, IV etc.)*	Frequency *(how often given?)*	Duration of Treatment *(how long will treatment last?)*
Cat, Rat	Bradykinin	0.0005 - 0.001 mg/kg	other	procedural	5-10 min
Rat		0.001 - 0.006 mg/kg	other	procedural	5-10 min
Cat, rat	Phenylephrine	0.01 mg/kg	IV	procedural	10-20 min
Cat, rat	Nitroglycerine	0.03 mg/kg	IV	procedural	10-20 min
Cat, rat	Kyurenic acid (Kyn)	1 mM, 50 nl	other	procedural	10-15 min
Cat, rat	D,L-homocysteic acid (DLH)	4-8 nM, 50 nl	other	procedural	10-15 min

Cat, rat	Kainic acid (KA)	1 mM, 50 nl	other	procedural	10-15 min
Cat, rat	AP5	25 mM, 50 nl	other	procedural	10-20 min
Cat, rat	NBQX	10 mM, 50 nl	other	procedural	once
Cat, rat	GABA	1.25 M, 50 nl	other	procedural	10-20 min
Cat, rat	Gabazine	27 mM, 50 nl	other	procedural	once
Cat, rat		0.001-0.002mg/ml, 50 nl	other	procedural	once
Cat, rat	2% Chicago blue dye	100 nl	other	once	once
Cat, rat	Potassium chloride	1 M, 1 ml/kg	IV	once	once

Will Controlled Substances (as defined by the U.S. Drug Enforcement Administration) be used in the study?
Yes - list CSUA number:

CSUA number

Indicate which controlled substances will be used - Check all that apply:
CSUA #:

Will non-pharmaceutical grade (i.e. chemical grade) agents be used in live animals
Yes

Provide justification for the use of non-pharmaceutical grade drugs in live animals.
Describe how the drug will be prepared before being given to the animals.
Describe how the drug will be prepared for administration in live animals (e.g., reconstitution, filtering, etc.)

for preparing the non-pharmaceutical grade drugs used in live animals, the drug(s) will be dissolved in normal saline (0.9% NaCl) or artificial cerebrospinal fluid (aCSF) solution and then filtered through a 0.2 µm NYL filter.

Animal Locations & Husbandry

Food or Water Variations

Indicate the food or water variations that will be implemented in this protocol - Check all that apply:

Justification for the Food or Water Variations

Do the experiments or procedures require any special dietary requirements or restrictions, or additions to the normal drinking water?
Yes - describe below

For making [redacted] we will provide high salt food for one group of rats.

Will food or fluid be restricted on this protocol?
No

Animal Husbandry Variations

Indicate the animal husbandry variations that will occur - Check all that apply:

Justification for the Animal Husbandry Variations

Will there be deviations from standard environmental enrichment on this protocol?
No

Will wire-bottom caging be used?
No

Researcher-Maintained Animals

Will LAB STAFF provide basic routine husbandry and care for the animals?

Will animals be held in the lab (outside of the vivarium) for more than 12 hours?

An Emergency Plan is REQUIRED for the following scenarios:

Will animals be held outside of ULAR animal facilities (e.g. in the researcher's laboratory) for more than 12 hours?
No

Will the research team provide routine husbandry and care for animals housed in ULAR animal facilities?
No

Other Husbandry/Housing Variations

Describe any other variations or special considerations (not already captured in the sections above).

Will there be other husbandry deviations or special considerations regarding the animal use in this protocol?
Yes

Describe the deviation in detail:

The high salt diet is 4% NaCl diet that is commercially available product. These rats will be housing same as regular rats for 10- 15 weeks and no additional water is required.

For the cold housing of rats, young SD rats (about 150 gram) will be put in regular cage and placed into a cold room (6 °C) located in for 10-15 weeks and provided with standard rodent chow and tap water *ad libitum*. No other special requirement for these rats.

Animal Locations

Indicate all locations where live animal procedures and/or housing will take place - Check all that apply:

Will any live animals (owned by UCI) be taken to offsite (non-UCI) locations for procedures?

Will any non-UCI site(s) and/or offsite locations be used for any procedures on UCI-owned animals?
No

Hazards & Safety

Chemical Hazards

Requirements for the Use of Potentially Hazardous Chemicals or Agents:

Chemical Name	Hazard Type	SDS	Containment Level	Special Precautions
Kainic Acid				

Biological Materials, Primary Cells or Cell Lines

Requirements for Use of Biological Materials:

Infectious Agents

Requirements for the Use of Infectious Agents:

Recombinant DNA

Preparation & Use of rDNA

Radioactive Hazards

Radiation Use Authorization (RUA) #:

Removal of Radioactive Waste & Monitoring of Radioactivity

Creation of New Transgenic Animals

Are you creating a NEW strain of transgenic animal by crossbreeding 2 different strains?

Animal Biosafety Levels

Indicate the animal biosafety levels - Check all that apply:

Other Hazards or Safety Considerations

Other Protocol Information

Other Protocol Information
Uncheck this box to remove all text from the box below:

For the rats will be kept in the cold housing, routine housing and animal husbandry is provided by ULAR. Animal behavior will be monitored by research team member twice per week and weight once per week after these rats placed into a cold room (6 ^{o}C) during the 10-15 weeks period. Systolic and diastolic blood pressure and heart rates in conscious rats also will be measured non-invasively by researcher with a volume pressure recording sensor and an occlusion tail-cuff (CODA system, Kent Scientific) once per week after these rats placed into a cold room (6 ^{o}C).

Personnel

Principal Investigator (PI)

Faculty Sponsor

Co-Investigator/Senior Researcher

Research Personnel

Other Research Personnel

Additional Personnel Information

Emergency Contact Information
Emergency Contact:

Add New User

Training Requirements

PI Qualifications

Description of PI Qualifications

Training Plan for Study Team Members

Describe the Principal Investigator's Qualifications to perform or oversee the research.
Uncheck this box to remove all text from the box below

is the lead researcher and the principal investigators on this protocol. is trained as an MD and a PhD in the area of internal medicine, cardiovascular medicine, cardiovascular physiology, autonomic neuroscience and pharmacology. has over years of experience in conducting both anesthetized and conscious survival animal experiments. These are documented in original publications review articles, chapters and textbooks, and by continuous NIH funding for years, including the current NIH grants on which serves as principal investigator. cv can be provided upon request. directs all experimental procedures in laboratories, including the ones contained in these protocols has regular laboratory meetings at which investigators in laboratory present the results of experiments and discuss experimental approaches, problems and solutions. As such, assumes responsibility for all studies conducted in this animal protocol.

Links to Other Protocols

Other Regulatory Review Requirements

Indicate if other regulatory reviews are required for this protocol - Check all that apply:

If additional details need to be discussed regarding any of the links above, describe them below.

Does this research involve the use of human adult or embryonic stem cells (including induced pluripotent stem cells) in live animals?
No

Is Institutional Biosafety Committee review/approval needed for any part of this research?
No

Is Institutional Review Board (IRB) review/approval needed for any part of this research?
No

Number	Protocol Title	Document Template
		Animal Use Protocol (AUP)

PI Certification

PI Certification

I hereby acknowledge and assure the following:
By clicking this button, I certify that the above statements are understood and will be followed by all research team members.