Relative Contributions of the Thalamus and the Paraventricular Nucleus of the Hypothalamus to the Cardiac Sympathetic Afferent Reflex

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Running head: Thalamus and PVN regulating sympathetic activity

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Abstract

The cardiac sympathetic afferent reflex (CSAR) is induced by stimulating the cardiac sympathetic afferents which evokes increases in sympathetic outflow and arterial pressure. In the present study, we attempted to identify the contribution of thalamic and hypothalamic nuclei involved in the CSAR. First, we observed that there was an increase in the number of c-fos labeled cells in the paraventricular nucleus (PVN) (190 ± 18 vs. 101±15; P<0.05), the paraventricular nucleus of the thalamus (PVT) (239 ± 23 vs.151 ± 15; P<0.05) and in the mediodorsal thalamic nucleus (MD) (92 ± 9 vs. 63 ± 6; P<0.05) following epicardial application of bradykinin (BK) compared to the control group (P<0.05). Second, using extracellular single-unit recording, we found 25% of spontaneously active neurons in the thalamus were stimulated by epicardial application of BK or capsaicin in intact rats. However, 24% of spontaneously active neurons in the thalamus were still stimulated by epicardial application of BK or capsaicin despite vagotomy and sinoaortic denervation. None of the neurons in the thalamus responded to baroreflex changes in arterial pressure, induced by I.V. injection of phenylephrine or sodium nitroprusside. The CSAR was inhibited by microinjection of muscimol or lidocaine into the PVN. However it was not inhibited or blocked by microinjection muscimol or lidocaine into the thalamus. Taken together, these data suggest that the thalamus while activated is not critical for autonomic adjustments in response to activation of the CSAR. On the other hand, the PVN is critically involved in the central pathway of the CSAR.

Key words: thalamus, sympathetic activity, cardiovascular, paraventricular nucleus
Introduction

It has been well established that the cardiac sympathetic afferent reflex (CSAR) is a sympatho-excitatory reflex, which can be initiated by the increases in cardiac pressure and volume as well as various substances such as adenosine, bradykinin (BK), and hydrogen peroxide released in the myocardium in the state of myocardial ischemia or chronic heart failure (CHF)(11, 29). The CSAR induced by stimulating cardiac sympathetic afferents increases sympathetic outflow and arterial pressure. Sensitization of the CSAR may contribute, in part, to the sympatho-excitation in CHF(45, 57) and renovascular hypertension(59). Thus, it is important to understand the mechanism(s) involved in elaboration of the CSAR. Some brainstem nuclei, such as the nucleus tractus solitarii (NTS) and the rostral ventrolateral medulla (RVLM), are known to be involved in modulating the CSAR(24, 47, 58). The hypothalamic paraventricular nucleus (PVN) is another important component in several cardiovascular reflex pathways involved in the control of the CSAR and sympathetic outflow(52, 56).

The activity of cardiac sympathetic afferent fibers is increased during myocardial ischemia(28, 41). Anginal pain often results from ischemic episodes that excite chemosensitive and mechanosensitive receptors in the heart(13, 14, 42). Ischemic episodes release a number of chemicals, including adenosine and BK, which in turn, activate chemosensitive and mechanosensitive receptors in the heart, eliciting activation of sympathetic and vagal afferent pathways(15, 41). Ascending pain signals from these fibers result in the activation of brain centers, which are
involved in the perception, and integration of cardiac pain (15, 41). Previous studies in humans indicated that the central structures activated constitute the pathways for perception of anginal pain and that the thalamus may act as a gateway to afferent pain signals, with cortical activation being necessary for the sensation of pain (36, 37). In addition, bilateral activation of the thalamus occurs in both angina and silent ischemia (36, 37). Subnuclei in the thalamus are connected with nuclei that are associated with central autonomic regulation (18, 32, 35). For example, the medial portion of the mediodorsal nucleus of the thalamus (MD) is connected to nuclei that include the NTS, insular cortex, anterior cingulate cortex, amygdala, lateral hypothalamus, and periaqueductal gray (18, 32, 35). In addition, the paraventricular nucleus of the thalamus (PVT) receives inputs from homeostatic control regions of the brain including the NTS (33) and the parabrachial nucleus (22) as well as from many regions in the hypothalamus (5, 7, 22, 32). Taken together, these data suggest that the thalamus may contribute to the regulation of excitatory cardiovascular reflexes.

However, it is not known whether the thalamus is involved in the CSAR, as the role of specific thalamic nuclei involved in the regulation of autonomic outflow induced by activation of cardiac sympathetic afferents has not been systematically studied. We hypothesized that the thalamus may be a critical component of the central neurocircuitry for sympathetic outflow in the CSAR. Thus, first we examined if the thalamus is activated in response to stimulation of cardiac sympathetic afferents as is the PVN. Secondly, we determined if the CSAR could be blocked by inhibition of the thalamus and/or the PVN.
Methods

General Procedures

Male Sprague-Dawley rats weighing between 250 and 300 g were obtained from SASCO Breeding Laboratories (Omaha, NE). This study was approved by the Institutional Animal Care and Use Committee of the University of Nebraska and was carried out under the guidelines of the American Physiological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Each rat was anesthetized by intraperitoneal injection of urethane (0.75 g/kg i.p.) and α-chloralose (70 mg/kg i.p.). Adequate depth of anesthesia was assessed by the absence of corneal reflexes and paw withdrawal response to a noxious pinch. Supplemental doses of anesthesia were administered to maintain an adequate depth of anesthesia during the experiment. The trachea was cannulated for mechanical ventilation using a rodent ventilator (SAR-830, CWE, USA). The right femoral artery and femoral vein were cannulated for the recording of arterial blood pressure and administration of chemicals, respectively. The mean arterial pressure (MAP) and heart rate (HR) were simultaneously recorded on a PowerLab data acquisition system (8SP, ADInstruments. USA). A constant infusion of 60 μL/min infusion of saline was instituted to maintain the hydration status of the animal. With this regimen there are no change in RSNA, MAP or HR over the usual ~6 hrs of experimental protocol (26, 50). Body temperature was maintained at about 37 ℃ with an animal temperature controller (ATC 1000, World Precision Instruments, USA).
Experimental Protocols

First, c-fos gene expression was determined following epicardial application of BK (0.3 nmol in 2.0 µl, repeat 6 times at intervals of 20 min) in 6 rats with vagotomy and sinoaortic denervation. Six applications of epicardial stimulation were chosen based on previous studies (19, 25). It has been reported that c-Fos gene expression is induced 30 min after neuronal activation, and reaches maximal levels in 60–90 min. The c-Fos protein then remains elevated for the next 2–5 h (23). We assume that single application of BK or Cap can activate c-Fos via activation of CSAR, however, in order to get maximal c-Fos protein expression, in this study we chose to repeat epicardial application/stimulation (6 times) at intervals of 20 min during 2 hours (25, 40).

Second, the effects of epicardial application of saline, BK or capsaicin on the activity of thalamic neurons were randomly investigated in 10 intact rats. Successive applications of chemicals were separated by at least 15 min for the complete recovery of the neuron discharge and MAP. The activity changes of the BK sensitive, insensitive and inhibitory response neurons were respectively investigated. In order to exclude the possibility that the increased activity of the BK sensitive neurons in the thalamus was secondary to the elevation of arterial pressure induced by epicardial application of the chemicals and investigate whether these neurons involve the baroreflex, the response of the thalamic neurons to transient changes in MAP induced by the PE (5–25 µg kg\(^{-1}\)) and sodium nitroprusside (SNP; 4–20 µg kg\(^{-1}\)) were determined.
Third, the effects of epicardial application of saline, capsaicin or BK, on the activity of the thalamic neurons were randomly investigated in 8 rats with vagotomy and sinoaortic denervation.

Lastly, to determine if the thalamus is an important component for the cardiogenic sympatho-excitatory response, we determined if the CSAR could be blocked or inhibited by microinjection of lidocaine (8.5 nmol/side) or the GABA_A agonist muscimol (100 pmol/side) into the MD (bilateral microinjection, n=6), PVN (bilateral microinjection n=6) or PVT (unilateral, n=6). The PVN was injected since it is known to be involved in regulating sympathetic outflow(52, 56).

**CSAR Induced by Epicardial Application of Chemicals**

A limited left lateral thoracotomy was performed to expose the heart and the pericardium was removed. The cardiac afferents were activated by application of a piece of filter paper (3×3 mm) saturated with capsaicin (1.0 nmol in 2.0 µl) or BK (0.3 nmol in 2.0 µl) to the epicardial surface of the anterior wall of the left ventricle(27, 56, 57). In the control group a piece of filter paper saturated with saline (2.0 µl) was used. Each piece of filter paper was removed 1 minute later, and the epicardium was rinsed three times with 10 ml of warm normal saline (38ºC). Successive applications of chemicals were separated by at least 15 min. The CSAR was evaluated by the responses of the MAP or RSNA to epicardial chemical stimulation.

**c-Fos Immunohistochemical Labeling**

Animals were placed randomly into BK-treated and vehicle-treated (0.9% saline) groups. The CSAR was induced by epicardial application of BK. c-fos gene
expressions were determined following epicardial application of BK (0.3 nmol in 2.0 µl, repeated 6 times at intervals of 20 min) in 6 rats with vagotomy and sinoaortic denervation. Ninety minutes after the last application of BK or 0.9% saline, animals were deeply anesthetized and perfused transcardially with 100 ml saline, followed by ice cold 4% paraformaldehyde (Sigma) in 0.1 M phosphate buffer (pH 7.2). Then brains were removed and cryoprotected by storing overnight at 4°C in 0.1 M phosphate buffer (pH 7.2) containing 30% sucrose. The brains were frozen on dry ice and sectioned coronally at 30 µm on a sliding microtome.

The avidin–biotin–peroxidase complex (ABC) method was used for staining of c-Fos protein, as described in our previous studies(55). Briefly, after treatment with 0.5% hydrogen peroxide and 1% normal goat serum (Vector ABC Kit, Vector Laboratories, Burlingame, CA, USA), brain sections were incubated in a primary polyclonal rabbit anti-c-Fos antibody (1:5,000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C for 24 h, and in biotinylated anti-rabbit IgG (Vector Kit, 1:200) for 60 min. Subsequently, the sections were placed in ABC solution (Vector Kit, 1:50) for 30 min, and then incubated in 30% hydrogen peroxide and 3,3′-diaminobenzidine (DAB) for 5–8 min. The c-Fos protein was visualized by the DAB reaction products as dark-brown staining.

Quantitative analysis of the c-fos staining in control and BK-treated rats was performed by counting the number of cells staining for c-fos in each area of nuclei. Tissue sections from control and BK-treated rats were carefully matched in order to compare as closely as possible identical fields or nuclei in the brain. These sections were processed simultaneously for immunocytochemistry in order to minimize
differences in staining intensity due to daily variations in the method. The fields were analyzed on a Leica light microscope. Three representative sections, which most closely matched the standard stereotaxic planes of the Paxinos & Watson’s atlas (2005)(34) were chosen. Bilateral c-Fos positive cells were counted by treatment-blinded analyzer using NIH Image/ImageJ software particle analysis function with equivalent detection thresholds across conditions. The average number of c-fos-stained cells was taken to represent the number of activated cells within a given nucleus.

**Vagotomy and Sinoaortic Denervation**

Bilateral cervical vagal denervation and arterial baroreceptor denervation were carried out. First, the carotid sinus area was exposed bilaterally, each carotid sinus nerve was identified and cut, and all other nerve fibers that were visible in this area were also cut. The carotid bifurcation and the common carotid arteries were stripped of adventitial tissues from 4 mm below the bifurcation to 4 mm above. Then, the vessels were painted with 10% phenol solution to destroy any remaining nerve fibers in this area. Each vagus was then identified in the neck, tied and sectioned. The effectiveness of the denervation was determined by recording the change in HR to intravenous injection of phenylephrine (PE, 20 µg kg⁻¹). This dose of PE evoked an increase in MAP between 25 and 40 mmHg. The arterial baroreceptor denervation was assumed to be completed if the change in the HR was less than 5 beats min⁻¹ in response to this MAP challenge.

**Extracellular Single-unit Recording in Vivo**

Rats were placed in a stereotaxic apparatus (Model 620, Stoelting, Chicago).
The stereotaxic coordinates for the thalamus were determined according to the atlas of Paxinos & Watson (2005)(34). Typically, three tracks were explored for extracellular recording in each rat, from -1.7 to -2.2 mm caudal to bregma, 0.2-0.6 mm lateral (right side) to the midline and a depth of 5.4-6.8 mm ventral to the dorsal surface. Extracellular single-unit recording was carried out using a single micropipette (resistance 5–15 MΩ) filled with 0.5 M sodium acetate containing 2 % pontamine sky blue. The glass micropipettes were advanced using a microdrive controller (Type 860, Hugo Sachs Elektronik, Germany) into the thalamus. The spontaneous activity of neurons was amplified (gain: 1000) with an AC/DC differential amplifier (Model IX1, Dagan Corporation, USA) with a low-frequency cutoff at 30 Hz and a high-frequency cutoff at 3 kHz. The neuronal discharge was recorded on a PowerLab data acquisition system (8/30, ADInstruments, Australia). The frequency of the neuronal discharge was analyzed with special software (SpikeHistogram, ADInstruments, Australia). The pontamine sky blue was iontophoresed (−15 μA, 10 min) to mark the site of the last recorded neuron, and other recording sites were extrapolated from the marked point according to Paxinos & Watson’s atlas (2005)(34).

In spontaneously active neurons in the thalamus, the neurons sensitive to epicardial application of BK were determined. The BK sensitive neurons were identified by at least 30% increases in their peak discharge frequency above baseline following the application of BK.

**Renal Sympathetic Nerve Activity Recording**

The RSNA was recorded as previously described(54). Briefly, a branch of the left
renal nerve was identified through a retroperitoneal incision and the nerve-electrode junction was insulated electrically from the surrounding tissues with gel (Wacker, St. Louis, MO). The electrical signal from the electrode was linked via a high-impedance probe (H1P5) to a Grass P511 band-pass amplifier (gain, 10,000) with high- and low-frequency cutoffs of 1,000 Hz and 100 Hz. The rectified output (RC filtered, time constant, 0.5 s) was then recorded and integrated using PowerLab (8si, ADInstruments, Sydney, Australia). Efferent RSNA at the beginning of the experiment was defined as basal nerve discharge. The RSNA recorded at the end of the experiment (after the rat was injected with hexamethonium, 30 mg/kg iv) was defined as background noise. The value of RSNA was calculated by subtracting the background noise from the actual recorded value, and changes found in integration of the nerve discharge during the experiment were expressed as a percentage of the basal value. Responses of MAP and HR were expressed as the difference between the basal value and the value after each dose of a drug.

Microinjections

The anesthetized rat was placed in a stereotaxic apparatus (David Kopf Instruments, Tujanga, CA). A longitudinal incision was made on the head and bregma was exposed. A small burr hole was made in the skull to allow access to the thalamus. The coordinates for the PVT, determined using the Paxinos and Watson atlas, were 1.8 mm posterior to bregma, at the midline, and 5.5 mm ventral to the dura. The coordinates for the MD were 1.8 mm posterior to bregma, 0.5 mm lateral to midline (bilateral), and 5.3 mm ventral to the dura. The coordinates for the
PVN were 1.8 mm posterior to bregma, 0.4 mm lateral to midline (bilateral), and 7.9 mm ventral to the dura. A thin needle (0.2 mm OD) connected to a 0.5 μl microsyringe (Hamilton) was lowered into the thalamus. The effects on RSNA, MAP and HR were observed after microinjection of artificial spinal fluid (CSF), lidocaine (8.5 nmol/side) or muscimol (100 pmol/side). The volume of the microinjection in each PVN was 100 nl, and the microinjection was completed within 1 min. At the end of the experiment, 100 nL of Chicago blue dye (Sigma-Aldrich, St. Louis, MO) was injected into each microinjection site for histological identification.

**Statistical Analysis**

Due to the large variability in the baseline neuronal discharge rate, the percent change was used for comparison before and after treatments. The thalamic neurons were considered to be responsive if their peak discharge frequency after treatment changed >30 % from baseline. The baseline pulse pressures, systolic arterial pressures, MAP and HR were averaged over 2 minutes before treatment. Maximum RSNA, discharge of neurons and blood pressure responses were measured ~30 s after BK or Capsaicin application. Data are presented as mean ± SE. Differences between groups were determined by a two-way ANOVA followed by the Newman-Keuls test for post hoc analysis of significance (StatView II; Berkeley, CA). \( P<0.05 \) was considered statistically significant.

**Results**

* c-Fos Staining in the Thalamus of Rats Following Stimulation of Heart with BK
There was an increased number of c-fos stained neurons in the thalamus and the PVN of rats with epicardial application of BK, shown in Figure 1. Figure 2 shows the mean numbers per section of c-fos-labeled neurons in specific nuclei of the thalamus. Compared to the animals in the control group (n=6), the numbers of c-fos stained neurons were significantly (P<0.05) increased in several areas in the thalamus following epicardial application of BK compared with control group, which include the PVT (151 ± 15 vs. 239 ± 23, n=6 P<0.05) and MD (63 ± 6 vs. 92 ± 9, n=6 P<0.05). In addition, the numbers of c-fos stained neurons were also significantly increased in the PVN (101 ± 15 vs. 190 ± 18, n=6 P<0.05). There were no significant differences (P>0.05) in the number of labeled neurons in the median preoptic area (MnPO), subfornical organ (SFO), central medial thalamic nucleus (CM) and paratenial thalamic nucleus (PT).

**Extracellular Single-unit Recording in the Thalamus of Rats Following Epicardial Application of BK**

A total of 32 spontaneously active neurons were recorded from 10 intact rats in this study. Eight of 32 (25%) spontaneous discharging neurons from the thalamus, were sensitive to epicardial application of BK by exhibiting increases in their discharges rate by greater than 30%. The discharge activity of one neuron was inhibited after BK application, and the remaining 24 cells showed no response to epicardially application BK. Figure 3 schematically shows the approximate locations of neurons in the thalamus which were sensitive to epicardial application of BK, and neurons in the thalamus which were not activated by epicardial application of BK.
Neuronal discharge rate and MAP was increased after BK application, while neuronal discharge and MAP were unchanged following vehicle application (epicardial 0.9% saline) (Fig 4.). All the neurons which were sensitive to epicardially application of BK were also sensitive to epicardially application of capsaicin. As shown in figure 5, the mean MAP and neuronal discharge rate were significantly increased by epicardially application both capsaicin and BK, but not saline. HR was not significantly changed after BK, capsaicin or vehicle application (data not shown). None of the neurons in the thalamus responded to baroreflex changes in arterial pressure, induced by I.V. injection of PE or SNP (Fig. 6).

In addition, the effects of epicardial application of saline, capsaicin or BK, on the activity of the thalamic neurons, were randomly investigated in 8 rats with vagotomy and sinoaortic denervation to exclude the effects of vagal afferents or baroreceptor afferent input. MAP after epicardial application of BK (79.1±3.8 vs. 87.8±4.0 mmHg, P<0.05) or capsaicin (79.8±2.6 vs. 90.2±3.0 mmHg, P<0.05) were significantly increased compared to before treatment in rats with vagotomy and sinoaortic denervation. Seven of 29 spontaneous discharging neurons (24%) from the thalamus were activated by epicardial application of BK (2.2±0.2 vs. 6.6±1.0 spikes/s, P<0.05) or capsaicin (2.3±0.1 vs. 7.4±0.7 spikes/s, P<0.05) in rats with vagotomy and sinoaortic denervation. Application of saline had no change.

**Effects of Blood Pressure and RSNA Responses to Epicardial BK**

Representative raw tracing of RSNA and blood pressure responses to epicardial BK pretreatment before and after microinjection of lidocaine into the PVT
and PVN are shown in Fig. 7. Microinjection of lidocaine into the PVT, which inhibits the activity of neurons (12, 38), did not abolish the CSAR evoked by epicardial application of BK (Fig. 7A and B), however the CSAR was blocked by microinjection of lidocaine into the PVN (Fig. 7C and D). Bilateral microinjection of lidocaine into the MD, also did not affect the CSAR evoked by epicardial application of BK. Microinjection of lidocaine or muscimol into PVT or MD had no significant effect on baseline RSNA and MAP.

In contrast to the CSAR being inhibited by microinjection lidocaine or muscimol into the PVN, the CSAR could not be inhibited or blocked by microinjection of lidocaine or muscimol into the PVT or MD (Fig. 8). This dose and volume of lidocaine and muscimol has been used in the past to block transmission in various areas in the brain (17, 39). Furthermore, doubling the dose of lidocaine (17.0 nmol, 200 nl) injected into the MD and PVT in two rats, did not even attenuate the reflex, suggesting that the lack of response in MD and PVT was not due to “incomplete blockade”. The baseline MAP (-15.4 ± 4.6 vs. -1.2 ± 2.9, n=6; P<0.05) and RSNA (-25.9 ± 7.7 vs. -0.9 ± 1.9, n=6; P<0.05) were decreased after microinjection of muscimol into the PVN compared with microinjection of aCSF, but not after microinjection of lidocaine. Microinjection of aCSF had no significant effect on the RSNA, MAP or the CSAR.

Discussion

In the present study, we found that there are increased numbers of c-fos labeled cells in the thalamus, which include the PVT and the MD, and in the PVN of
the hypothalamus following epicardial application of BK. To confirm this, using extracellular single-unit recording, we found 25% of the neurons in the thalamus were activated by epicardial application of BK or capsaicin in the intact rats. However, neurons in the thalamus were also activated by epicardial application of BK despite vagotomy and sinoaortic denervation. Additionally, none of neurons in the thalamus responded to baroreflex changes in arterial pressure in intact animals. These results indicate that specific regions in the thalamus, including the PVT and MD, are activated by stimulation of cardiac sympathetic afferents, but are independent of vagal and baroreceptor input. Finally, the renal sympatho-excitatory response activated by stimulation of cardiac sympathetic afferents is mediated via the PVN but not the thalamic PVT and MD.

The PVT is a unique midline intralaminar nucleus that has been implicated in the regulation of autonomic and visceral functions(2, 44). PVT neurons also respond to stress and to peripherally administered psychostimulants cocaine and amphetamine(3, 9). In addition, PVT receives inputs from homeostatic control regions of the NTS(33). Previous studies have shown that the NTS receives sensory inputs from cardiac receptors, and is an important area for the central transmission of the CSAR(47, 49, 51). Chemical stimulation of cardiac sympathetic afferents induces c-fos expression in the NTS, while dorsal rhizotomy virtually eliminated the c-fos expression (19, 21). An electrophysiological study has shown that NTS neurons can be excited by stimulation of cardiac sympathetic afferents (48). These results suggest that cardiac sympathetic afferents terminate in the NTS, which is involved in this sympatho-excitatory cardiovascular reflex. In
addition, spinal cord stimulation increases c-fos and heat shock protein 72 expressions in the PVT, PVN and NTS, both known to be involved in regulation of pain and emotions(8). In this respect, it is possible that the PVT may integrate cardiac sympathetic afferent input that passes through the dorsal horn of the spinal cord to the pons(8). The MD also has connections with the insular cortex and the hypothalamus, which are associated with central autonomic regulation(18, 30, 32).

Our results show that c-fos stained neurons were significantly increased in several areas in the thalamus following epicardial application of BK, which include MD and PVT. Furthermore, using extracellular single-unit recording, we found most neurons, which were activated by epicardial application of BK or capsaicin were localized in the MD and PVT. C-fos has been widely used as a tool for the study of neural correlates of nociception(4, 6, 20), and its expression in the brain in response to specific afferent input is specific for neurons, but not for glial, ependymal or endothelial cells(31). The c-fos immunoreactivity mapping technique allows examination of large numbers of cells in the brain in a single animal after noxious stimulation and/or tissue injury. Extracellular single unit recording *in vivo* from selected brain regions is a classic technique for studies of responses of central neurons to sensory stimulation. The interactions between the CSAR and the arterial baro- or chemoreflex were investigated with relatively few neurons in the NTS(48). Similarly, relatively few neurons in the RVLM dictate sympatho-excitation as well(1).

Thus, it is possible that seemingly small proportion of activated neurons may play an important role on the CSAR regulation. These results suggest that, specific regions in the thalamus, including the PVT and the MD, are activated by stimulation
of cardiac sympathetic afferents.

Both cardiac sympathetic afferents and cardiac vagal afferents can be activated during myocardial ischemia or epicardial application of some chemicals (29). In the present study, 7/29 (24%) neurons in the thalamus were activated by epicardial application of BK or capsaicin in rats with vagotomy and sinoaortic denervation. Similarly, 25% of the neurons were activated in intact rats suggesting that the sympathetic afferent nerves contribute the majority of the afferent input to the thalamus from the heart related to CSAR. Consistent with these observations, none of the neurons in thalamus responded to baroreflex changes in arterial pressure in intact rats, suggesting that this effect was not secondary to change in blood pressure induced by epicardial applications.

It is well known that the CSAR induced by stimulating the cardiac sympathetic afferents increases sympathetic outflow and arterial pressure. Our results show that bilateral microinjection of an anesthetic, lidocaine, into the PVN abolished the CSAR. Furthermore, microinjection of muscimol, a GABA<sub>A</sub> receptor agonist, into the PVN significantly attenuated the CSAR. These results are consistent with previous studies(53, 56). Interestingly, in contrast to microinjection of muscimol or lidocaine into the PVN, the CSAR could not be inhibited or abolished by microinjection of muscimol or lidocaine into the MD or PVT. It is known that lidocaine is a local anesthetic, which blocks voltage-gated sodium channels. Microinjections of lidocaine in discrete brain areas have been widely used as a tool for reversible functional inactivation(12, 38). Both the reversible inhibition of the PVN with lidocaine and the irreversible lesion of the PVN with direct current abolished the
CSAR, indicating the necessity of the PVN area for the expression of the CSAR(56).

On the other hand, microinjection of muscimol into the PVN significantly decreased baseline RSNA and MAP, while microinjection of muscimol into the PVT or MD had no significant effects on baseline RSNA and MAP. There are two possible explanations for the paradox that thalamic neurons are evoked by epicardial BK, while the CSAR cannot be inhibited or abolished by microinjection of muscimol or lidocaine into the thalamus. One is that the present study was done in anesthetized rats and that both the MD and the PVT have connections with the insular cortex which may have been inhibited by the anesthetic(10, 43). Another possible explanation is that the MD and PVT received sympathetic afferent nerve information, but do not play a direct role in modulating sympathetic efferent nerves. Previous studies have indicated that the thalamus may act as a gate to afferent pain signals, with cortical activation being necessary for the sensation of pain(36, 37). Bilateral activation of the thalamus has been shown in both angina and silent ischemia(36, 37). Thus, compared to the PVN that play a critical role on CSAR, the thalamus may be considered to be involved in pain pathways, including processing cardiac pain in response to input from the heart, but not as a crucial component of the central neurocircuitry of the CSAR.

The CSAR is an excitatory sympathetic reflex, which can be initiated by myocardial ischemia and in CHF(11). It has been shown that BK activates the kinin B2 receptors on cardiac capsaicin-sensitive afferents to produce a sympatho-excitatory reflex response(15, 29). The CSAR is enhanced in rats and dogs with CHF(11, 29), which at least partially contributes to the over excitation of
the sympathetic nervous system(16, 46). Also, the cardiogenic sympathetic reflex involves predominantly brain stem nuclei(15). Although the brainstem nuclei play a critical role in generation and regulation of sympathetic activity, the present study indicated that areas in the thalamus such as the PVT and MD are activated by stimulation of cardiac sympathetic afferents. However, the physiologic role of the thalamus on the CSAR needs further investigation.

In summary, although some specific regions in the thalamus, including the PVT and the MD, are activated by stimulation of cardiac sympathetic afferents, specific blockade of the activity within these nucleus fail to block the CSAR. These data also confirm and validate the critical role of the PVN in the CSAR. Taken together, these data indicate that the thalamus, although considered to be involved in pain pathways, including processing cardiac pain in response to input from the heart, is not a critical component of the central neurocircuitry, like the PVN that dictates autonomic outflow in response to activation of the CSAR.

Acknowledgements

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References


Figure Legends

Figure 1. Representative images of c-Fos immunoreactivity in the thalamus and the paraventricular nucleus (PVN) in a control rat following epicardial application of saline (A and B) and a rat following epicardial application of bradykinin (BK) (C and D). The paraventricular nucleus of the thalamus, PVT; the mediodorsal thalamic nucleus, MD; third ventricle, 3V; dorsal 3rd ventricle, D3V

Figure 2. Number of c-fos positive cells in the paraventricular nucleus (PVN), median preoptic area (MnPO), subfornical organ (SFO), the paraventricular nucleus of the thalamus (PVT), the mediodorsal thalamic nucleus (MD), central medial thalamic nucleus (CM) and paratenial thalamic nucleus (PT) in control rats and rats with epicardial application of bradykinin (BK). Values are mean ± SE (n=6). * p<0.05 compared with control.

Figure 3. A–C: Approximate locations of neurons in the thalamus which were sensitive to epicardial application of bradykinin (diamonds), and neurons in the thalamus which were not activated by epicardial application of bradykinin (filled circles) in intact and denervation rats. The distance (in mm) posterior to bregma is shown for each section. AH, anterior hypothalamic nucleus; f, fornix; 3V, third ventricle; OX, optic tract; SO, supraoptic nucleus.

Figure 4. Original recordings of blood pressure, spikes and discharge rate (left) and
spike discriminator window demonstrating single-unit discharge (right) from the same neuron in the paraventricular nucleus of the thalamus (PVT) in response to bradykinin (BK) (A) or Saline (B), respectively.

Figure 5. Effects of epicardial application of saline, capsaicin (Cap) and bradykinin (BK) on the activity of capsaicin sensitive neurons in the thalamus which including the paraventricular nucleus of the thalamus (PVT) and the mediodorsal thalamic nucleus (MD), and MAP in intact rats. Values are mean ± SE (n=8). * p<0.05 compared with saline.

Figure 6. Representative tracings and histogram showing the effects of i.v. injection of phenylephrine (A) or sodium nitroprusside (B) on the arterial pressure and the activity of a capsaicin sensitive neuron in the thalamus of a rat. C: Mean arterial pressure (MAP) and mean firing rate in response to phenylephrine or sodium nitroprusside (i.v.) (n=8). Values are presented as mean ± SE. * p<0.05 compared with before treatment.

Figure 7. Tracing showing the effects of microinjection of lidocaine (8.5 nmol/side) into the PVT and the PVN on the CSAR induced by epicardial application of bradykinin (BK) in a rat with vagotomy and sinoaortic denervation. The CSAR was evaluated by the renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) response to epicardial application of bradykinin (BK). Microinjection of lidocaine into the PVN abolished the CSAR. Microinjection of
lidocaine into the PVT had no effect on the CSAR.

Figure 8. Effects of microinjection of aCSF, lidocaine and GABAA agonist muscimol into the MD, PVT or PVN on the CSAR. The CSAR was evaluated by the renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) response to epicardial application of bradykinin (BK) in rats with vagotomy and sinoaortic denervation. Microinjection lidocaine and GABAA agonist muscimol into the PVN abolished CSAR. Values are mean ± SE (n=6). *P<0.05 compared aCSF.
Figure 1
Figure 2

Number of c-Fos Positive Cells

Control
Bradykinin

PVN  MnPO  SFO  PVT  MD  CM  PT

0  50  100  150  200  250  300

*
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8