The Neurophysiologic Mechanisms of Tourniquet Pain

The Activity of Neurons in the Rostroventral Medulla in the Rat

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Background: Tourniquet pain frequently complicates the use of pneumatic tourniquets during surgical procedures involving the extremities. The mechanisms of tourniquet pain are not understood and therefore the treatment is nonspecific. An animal model was sought to provide an ethical means to study the neurophysiologic and neuropharmacologic mechanisms of tourniquet pain. Neurons in the rostral ventromedial medulla are involved in the nociceptive- and circulatory-responsive neuronal networks. The goal of this study was to determine the activity of neurons in the rostral ventromedial medulla in response to the maintenance of tourniquet inflation in the rat as a component of an investigation of the neurophysiologic mechanisms of tourniquet-induced pain.

Methods: In 18 male, pentobarbital-anesthetized rats, heart rate, systolic blood pressure (SBP), and cell firing rates (CFR) of nociceptive-responsive rostral ventromedial medulla neurons, characterized as having an OFF or ON cell response to noxious heat, were monitored. CFR was monitored continuously at baseline, during an IV infusion of phenylephrine sufficient to increase SBP by 50%, during the application of a pneumatic tourniquet to the hind limb thigh and inflated to 300 mmHg for a period of 60 min, and during a 30-min recovery period.

Results: Phenylephrine-induced hypertension resulted in an increase in OFF CFR. Maintenance of tourniquet inflation resulted in a progressive decrease in OFF CFR and a progressive increase in ON CFR. An increase in SBP in response to tourniquet pain paralleled the changes in CFR. Mean SBP at 5 min preinflation, 5 min postinflation, 55 min postinflation, and 10 min postdeflation were 101 ± 11, 105 ± 9, 118 ± 14, and 103 ± 12 mmHg, respectively.

Conclusions: The changes in SBP and CFR observed during tourniquet inflation were consistent with previously reported responses to nociceptive stimuli. Phenylephrine-induced hypertension caused an opposite effect on the CFR of rostral ventromedial medulla neurons as compared with a noxious stimulus such as heat or maintenance of tourniquet inflation. This experimental design is presented as an animal model to study the neurophysiologic and neuropharmacologic aspects of tourniquet pain. (Key words: Brain, medulla; nucleus raphe magnus; rostral ventromedial medulla. Pain: tourniquet.)

NERVE compression is a common etiologic feature of a variety of clinical pain problems. One such problem, related to compression and ischemia of a segment of nerve, is the pain associated with the maintenance of inflation of a pneumatic tourniquet on an extremity of a patient undergoing distal extremity surgical procedures. This sensation has been described as tourniquet pain.1 Tourniquet pain is characterized by a severe, dull, aching sensation at the site of the tourniquet or distal extremity and may develop despite otherwise adequate anesthesia for the surgical procedure.2 Tourniquet pain may also be manifested by a gradual, progressive increase in heart rate and blood pressure.3

Animal models and an in vitro nerve preparation have been reported to present neurophysiologic and circulatory responses to the maintenance of tourniquet inflation.4-6 A limitation of these models is that noception, per se, in response to the maintenance of tourniquet inflation, could not be detected and monitored. Three physiologically defined classes of neurons have been previously described in the rostral ventromedial medulla (RVM).7 These cells have been characterized according to their cell firing rate (CFR) immediately preceding the tail-flick response to a noxious heat stimulus. "ON" cells show an increase in CFR imme-
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diately before tail flick. “OFF” cells show a decrease or pause in CFR immediately before tail flick. “Neutral” cells show no change in CFR before a tail-flick response. These cells have also been reported as being responsive to induced changes in blood pressure.8

The goals of this study were to establish the maintenance of tourniquet inflation as a nociceptive stimulus in this animal model based upon the CFR responses of RVM ON and OFF cells and to observe the response of RVM ON and OFF cells to phenylephrine-induced hypertension and the prolonged nociceptive stimulus and increase in arterial blood pressure associated with tourniquet pain. The response of RVM neurons to such a prolonged, continuous nociceptive stimulus has not been previously reported.

Materials and Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Cincinnati College of Medicine. Male Sprague-Dawley rats (Zivic-Miller) weighing between 250 and 350 grams were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). Arterial and venous cannulae were placed in the (L) femoral vessels for hemodynamic monitoring and medication and fluid administration, respectively. Anesthesia was maintained throughout the experiment by a continuous intravenous infusion of pentobarbital at 8–10 mg/h. This depth of anesthesia was adequate to maintain spontaneous respirations and changes in CFR indicative of a nociceptive response to heat without movement or other physical signs of escape behavior in the animals.

The animals were placed in a stereotactic head frame in the prone position. Body temperature was measured rectally (YSI Model 44TA; Yellow Springs Instrument, Yellow Springs, OH) and maintained at 36–38°C with an insulated circulating water plate placed beneath the animal. The cranium was excised to expose the dura at a point 8–10 mm caudal to the bregma. A tungsten steel electrode (A–M Systems, Everett, WA) was placed via a micromanipulator into the RVM, nucleus raphe magnus, or nucleus gigantocellularis, at the following stereotactic coordinates: nucleus raphe magnus at 10.8 mm caudal to the bregma, 0.0–0.2 mm lateral to the midline, and 8.7–8.9 mm below the surface of the skull, and nucleus gigantocellularis at 9.5 mm caudal to bregma, 0.0–0.7 mm lateral to midline, and 7.5–8.5 mm below the surface for the skull. Heart rate and blood pressure were monitored continuously and recorded on a strip chart recorder (model 7D polygraph; Grass, Quincy, MA).

After a 30–45-min period for stabilization, a 1×9 cm commercially manufactured tourniquet (Hokanson, Seattle, WA) was placed on the (R) hind limb thigh. Cells in the RVM were identified and characterized as having an ON or OFF cell response to a noxious heat stimulus to the tail (model 33 Tail Flick Analgesia Meter, III TC Life Sciences, Woodland Hills, CA) and noxious pinch of the hind limb. RVM neuron firing activity was monitored using an AC amplifier/window discriminator with a high impedance head stage (WDR-420, Fintronics, Orange, CT). The signal was amplified 1,000–5,000 times and filtered by a band pass filter with a low frequency cutoff of 400 Hz and a high frequency cutoff of 8 kHz. The window discriminator section produced a 0.1-ms, 5-V pulse for each action potential. The output of the amplifier/window discriminator was connected to a signal processor board (TecMar Labmaster DMA, Scientific Solutions, Solon, OH) using on-line data acquisition software (Cognitech, Cincinnati, OH) within a personal computer.

After a 15–30-min period to establish a baseline, CFR was monitored in response to an intravenous infusion of phenylephrine 25 µg/ml at a rate of 2.5 µg/min (prepared from phenylephrine hydrochloride 1% stock solution, lot 2597, American Regent Laboratories, Shirley, NY). This infusion resulted in a 50% increase in systolic blood pressure (SBP) and was maintained for 5 min. After discontinuation of the phenylephrine infusion, the CFR and blood pressure were allowed 15–30 min to return to prephenylephrine administration baseline values. The tourniquet was inflated and maintained at a pressure of 300 mmHg (approximately 2.5 times SBP). The tourniquet inflation pressure of 300 mmHg was based on (1) pilot data in our laboratory using laser Doppler flow measurements to demonstrate that this pressure produced occlusion of blood flow in the limb and (2) the tourniquet inflation pressure previously used in other investigations.5,6 SBP and CFR were monitored for a 60-min tourniquet inflation period and a 30-min postdeflation recovery period. Only one experimental procedure (one cell profile) was performed per animal.

At the conclusion of the experiment, the location of the electrode was marked by electrical current (20 mA for 10 s) passed through the electrode (Lesion Maker LM4, Grass). The animals were deeply anesthetized and perfused intracardially with 500 ml
normal saline followed by 500 ml 10% formalin solution. The brain was removed and 80-μm sections were stained with neutral red dye for histologic verification of electrode placement.

**Statistical Analysis**

CFRs (spikes per second) in 60-s epochs during preinflation baseline and postdeflation periods were randomly selected for analysis. These periods were compared with a 60-s epoch at the time of maximum decrease (OFF cells) or increase (ON cells) in CFR during the tourniquet inflation period. Statistical analysis of changes in CFR with phenylephrine infusion and tourniquet inflation, as compared with preinflation baseline were performed using Student's t test. Changes in heart rate and SBP were analyzed by analysis of variance and the Student-Newman-Keuls test for multiple comparisons (Biosatistics Version 3, Stanton A. Glantz, McGraw-Hill, Health Professions Division). The minimum level of significance to reject the null hypothesis was considered to be $P < 0.05$.

**Results**

Thirty-four cells characterized as having a nociceptive response to noxious heat applied to the tail were isolated. A total of 18 cells (12 OFF cells and 6 ON cells) that maintained stable baseline activity and constant spike height (signal to noise ratio greater than 5:1) throughout the 2-h recording period were used for analysis (fig. 1).
Response to Phenylephrine Infusion

The phenylephrine infusion resulted in a 9.4 ± 0.3% decrease in heart rate (P = 0.2) and a 59 ± 18% increase in SBP (P < 0.05) as compared with preinfusion baseline values. Eight of the 12 OFF cells responded to phenylephrine-induced hypertension with a mean 41% increase in CFR (range 7–144%) as compared with preinfusion baseline (P < 0.05). The increase in CFR continued for the duration of the infusion and then gradually returned to baseline after discontinuation of the infusion (fig. 2). Changes in CFR of ON cells in response to phenylephrine-induced hypertension could not be characterized as having any consistent pattern of response.

Response to Maintenance of Tourniquet Inflation

All animals demonstrated a gradual, progressive increase in SBP in response to maintenance of tourniquet inflation. Mean SBP at 5 min preinflation, 5 min postinflation, 55 min postinflation, and 10 min postdeflation were 101 ± 11, 103 ± 9, 118 ± 14, and 103 ± 12, respectively. SBP at the 55 min after tourniquet inflation time point was significantly higher as compared with the 5-min preinflation, 5-min postinflation, and 10-min postdeflation values (P < 0.05). No significant change in heart rate was noted.

Ten of the 12 OFF cells (83%) responded to maintenance of tourniquet inflation with a progressive decrease in CFR as compared with preinflation baseline and a gradual return to baseline after deflation of the tourniquet (fig. 3). The CFR of the OFF cells at the time of maximum change was 40 ± 33% (range 0.3–93%) of control as compared with preinflation baseline CFR (P < 0.05). Five of the 6 ON cells responded to maintenance of tourniquet inflation with a progressive increase in CFR as compared with preinflation baseline and a gradual return to baseline after deflation of the tourniquet (fig. 4). The CFR of the ON cells at the time of maximum change in was 470 ± 402% (range 137–1,000%) of control as compared with preinflation baseline CFR (P < 0.05). Mean time to maximum change in CFR during tourniquet inflation was 42 ± 11 min. These changes in CFR, indicative of a progressive nociceptive response to the maintenance of tourniquet inflation, paralleled the changes in SBP reported above. Two OFF cells and one ON cell did not demonstrate a change in CFR during phenylephrine infusion or maintenance of tourniquet inflation despite an appropriate change in CFR to noxious heat and noxious pinch. Representative OFF cell and ON cell CFR and SBP histograms are presented in figures 5 and 6, respectively.

All cells were located within the boundaries of the RVM in and adjacent to nucleus raphe magnus and nucleus reticularis paragigantocellularis, as verified by histologic examination of recording electrode lesions.

Discussion

Pain resulting from compression and ischemia of a segment of nerve is poorly understood and therefore difficult to treat. One such pain problem of importance to the practice of clinical anesthesia is the pain associated with maintenance of inflation of a pneumatic tourniquet on the extremity of patients undergoing distal surgical procedures. Tourniquet pain may be relatively unresponsive to supplemental analgesic administration and may occur during otherwise adequate regional or general anesthetic administration. The neurophysiologic basis for the relative resistance of tourniquet pain to analgesic and anesthetic administration is unknown.

An anesthetized animal model is needed to provide an ethical means to investigate tourniquet-induced
pain. Previously published reports of the response to maintenance of tourniquet inflation in animals have demonstrated a circulatory response felt to be representative of a nociceptive response but nociception, per se, could not be established.

The results of this study demonstrate that after 40–45 min of tourniquet inflation a nociceptive stimulus was detected by a progressive change in the CFR of RVM neurons. The maintenance of tourniquet inflation was also associated with a 20% increase in SBP. The changes in CFR of RVM neurons observed in the animals during the maintenance of tourniquet inflation in this study clearly represents a nociceptive response rather than a hypertension-related effect, as demonstrated by the opposite CFR response to phenylephrine-induced hypertension as compared with the CFR response to the noxious heat stimulus.

The results obtained in this study support previously reported observations of the response to maintenance of tourniquet inflation in animals. Chabal and colleagues reported spontaneous neuronal discharges (consistent with C fiber conduction velocities) in a proximal segment of the sciatic nerve and a 20% increase in mean arterial pressure after 55 ± 16 min of tourniquet inflation on the hind limb in the rat.4 A 30% increase in SBP after 30–45 min of tourniquet inflation and an increase in serum cortisol and norepinephrine were reported in an observation of the response to maintenance of tourniquet inflation in the monkey.3

Maclver and Tanelian reported an increase in C fiber action potential frequency in an in vitro corneal nerve preparation in response to hypoxia and the combination of hypoxia and hypoglycemia. These changes were noted after a 20-min latency period with peak effect 25–30 min after exposure to the experimental conditions.6

Neurons in the RVM receive primary input from the periaqueductal gray matter and from the adjacent midbrain reticular formation and the dorsolateral pontine tegmentum.9–11 These RVM neurons project to spinal cord dorsal horn laminae I, II, and V via the dorsolateral funiculus as a descending inhibitory pain pathway and maintain a modulatory effect on nociceptive dorsal horn neurons12–14 (Fig. 7). Fields and coworkers7,12,15,16 and Mason17 have presented evidence that OFF cells maintain a tonic inhibitory effect on nociceptive transmission, whereas ON cells are hypothesized to facilitate nociception.

Several investigators have examined the relation between cardiovascular and pain regulatory systems.8,18,19 Acute hypertension, representing a manifestation of a stressor state, has been demonstrated to have an effect on nociceptive processing producing a state of analgesia.8,19 It is well established that both the periaqueductal gray matter and the RVM receive afferent input from nucleus tractus solitarius.20 Because nucleus tractus solitarius neurons are activated by sinoatrial baroreceptors,21 increases in blood pressure activate nucleus tractus solitarius neurons, which may then activate periaqueductal gray matter and RVM thereby activating spinal descending analgesic systems.22
Phenylephrine-induced hypertension has been demonstrated to produce analgesic effects in the unanesthetized rat as demonstrated by an increase in tail flick latency in response to noxious heat. In our studies, systemic administration of phenylephrine increased blood pressure and also increased the CFR of RVM OFF cells. An increase in the CFR of RVM OFF cells in response to phenylephrine-induced hypertension suggests a mechanism for an indirect analgesic effect of this drug.

Assimilating the information above, it is hypothesized that compression and ischemia of the segment of nerve beneath the tourniquet results in hypoxia and hypoglycemia, which have been demonstrated to cause spontaneous activation of C fibers. The C fibers transmit the nociceptive stimulus to the spinal cord dorsal horn. This nociceptive response results in activation of central nociceptive nuclei (thalamic, hypothalamic, midbrain, and pontine nuclei), triggering a sympathetic response (increase in heart rate, blood pressure, circulating cortisol and norepinephrine), and the perception of pain.

The knowledge of RVM circuitry is far from complete. Our observation that the maintenance of tourniquet inflation is associated with a decrease in the CFR of RVM OFF cells and an increase in the CFR of RVM ON cells suggests that transmission of this nociceptive stimulus may be facilitated by a reduction in the tonic

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**Fig. 5.** Representative OFF cell histogram demonstrating cell firing rate (CFR) and systolic blood pressure response to tail flick (TF) stimulus (noxious heat resulting in TF response) and maintenance of tourniquet inflation. T(UP) = tourniquet inflation; T(DN) = tourniquet deflation. CFR spike represents mean CFR during each 1-min epoch. CFR of RVM OFF cell decreased in response to TF stimulus and to tourniquet pain.

**Fig. 6.** Representative ON cell histogram demonstrating cell firing rate (CFR) and systolic blood pressure response to tail flick (TF) stimulus (noxious heat resulting in TF response) and maintenance of tourniquet inflation. T(UP) = tourniquet inflation; T(DN) = tourniquet deflation. CFR spike represents mean CFR during each 1-min epoch. CFR of RVM ON cell increased in response to TF stimulus and to tourniquet pain.

**Fig. 7.** Simplified representation of the circuitry of the rostral ventromedial medulla (RVM) with input from the hypothalamus (HYPO) and the periaqueductal gray matter (PAG) and descending projections to the spinal cord dorsal horn via the dorsolateral funiculus. (+) = excitatory input; (−) = inhibitory input.
Descending inhibitory influence from the RVM on spinal cord nociceptive neurons. Because this descending system maintains an inhibitory influence on dorsal horn neurons throughout the entire rostral-caudal extent of the spinal cord, the decrease in activity within this system may enhance the transmission of nociceptive information through the spinal cord dorsal horn in response to tourniquet pain. Irrespective of these hypothetical modulatory effects of the RVM on nociceptive transmission in response to tourniquet pain, this manuscript presents evidence that the changes in CFR of RVM neurons can be used as an indicator of nociceptive activity in response to the maintenance of tourniquet inflation in this model.

Although many aspects of the phenomenon of tourniquet pain remain unanswered, a model for further investigation is presented herein. Future investigation will use this model and the CFR response of RVM neurons in determination of the effects of various analgesic substances on the response to the maintenance of tourniquet inflation.

References