Original Paper

The Role Played by Adenosine in Modulating Reflex Sympathetic and Pressor Responses Evoked by Stimulation of TRPV1 in Muscle Afferents

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Key Words

Adenosine • TRPV1 • Muscle sensory nerves • Sympathetic activity • Blood pressure

Abstract

Background/Aims: Activation of metabolite-sensitive transient receptor potential vanilloid type 1 (TRPV1) receptors (capsaicin receptors) in afferent nerves of the hindlimb muscles of rats increases renal sympathetic nerve activity (RSNA) and blood pressure (BP) via a reflex mechanism. The purpose of this study was to examine the role of adenosine in modulating the reflex RSNA and BP responses to stimulation of TRPV1. **Methods:** RSNA and BP responses were recorded in rats. Immunofluorescence and patch-clamp methods were employed to examine the receptor mechanisms responsible for the effects of adenosine. **Results:** Adenosine, in the concentration of 100 μ M, injected into the femoral artery had an inhibitory effect on the reflex RSNA and BP responses induced by capsaicin. Likewise, arterial injection of adenosine analogue CGS21680 (A_{2A} subtype receptor agonist, 10 μ M and 100 μ M) also attenuated the reflex responses. In addition, co-existence of A_{2A} and TRPV1 was observed in the dorsal root ganglion neurons. The prior application of adenosine or CGS21680 inhibited the magnitude of capsaicin-induced currents in muscle sensory neurons. **Conclusion:** Adenosine contributes to muscle afferent TRPV1-engaged reflex sympathetic and pressor responses. It is likely that TRPV1 response is impaired as the levels of adenosine are increased in the hindlimb muscles under diseased conditions.

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Introduction

The metabolic by-products accompanying the inflammatory process (lactic acid, H^+) and inflammatory mediators themselves (histamine, serotonin, prostaglandin E₂) have

been identified as potential endogenous ligands for the C fiber transient receptor potential vanilloid type 1 (TRPV1) [1]. Hydrogen ions (H^+) in general and lactic acid in particular have been shown to activate C fiber afferents similar to the effect seen with capsaicin [2, 3]. *In vitro* studies have demonstrated that H^+ inhibits the binding of the capsaicin analogue resiniferatoxin to vanilloid receptors, a finding attributed to competition for the same binding site [1].

The levels of metabolite such as lactic acid and acid phosphate are elevated in active muscle during exercise. Activation of thin fiber muscle afferent nerves' metabolic receptors increases arterial blood pressure (BP) and heart rate (HR) via a reflex muscle mechanism [4, 5]. When capsaicin is injected into the arterial supply of the dog hindlimb to stimulate TRPV1 receptors, BP rises by 20%, an effect abolished by sectioning afferent nerves [6]. The reflex pressor response is likely due to the stimulation of both Group III and IV fibers since capsaicin stimulates 71% of Group IV and 26% of Group III dog hindlimb muscle afferent fibers [7]. It is known that TRPV1 receptor appears preferentially on chemically sensitive thin fiber muscle afferent nerves [8]. In our prior studies, we observed that when capsaicin is injected into the arterial supply of the hindlimb muscles of rats, sympathetic nervous activity (SNA) and BP increase and the effects are mediated via the engagement of TRPV1 receptors on muscle afferents [9].

Data have been published demonstrating that interstitial adenosine and adenine nucleotides (ATP, ADP and AMP) levels are elevated in active muscle during exercise in human subjects as well as in animals [10–12]. Prior work has been performed to examine the role played by adenosine in evoking the reflex responses of SNA and BP to exercise. For example, reports have shown that adenosine, injected in the brachial artery, evokes the muscle reflex [13, 14]. In addition, femoral artery injection of adenosine increases SNA and BP only if the purine is allowed to re-circulate to the systemic circulation [15]. In contrast, the muscle reflex evoked by handgrip exercise is accentuated by injection into the brachial artery of theophylline, an adenosine receptor antagonist [16]. Other studies reported that adenosine does not lead to increases in SNA and BP and to evoke the muscle reflex [17, 18].

A prior work suggests that stimulation of adenosine receptors on peripheral sensory fibers inhibits its discharge and this reduces pain [19]. Notably, an *in vitro* experiment further provides evidence that adenosine inhibits TRPV1 activity by directly interacting with the receptor protein [20]. Therefore, the purpose of our current study was to shed light on the role played by adenosine in regulating the reflex SNA and BP responses to stimulation of muscle afferents via TRPV1. We hypothesized that adenosine attenuates muscle afferent TRPV1-engaged reflex SNA and BP responses via A_{2A} subtype receptor. Furthermore, our experiments were designed to use the immunofluorescence method to examine the co-existence of A_{2A} and TRPV1 in the dorsal root ganglion (DRG) neurons; and the whole cell patch-clamp method to examine whether the current magnitude of the DRG neuron response induced by capsaicin was affected by the prior application of adenosine.

Materials and Methods

RSNA and cardiovascular responses

All procedures outlined in this study were approved by the Animal Care Committee of this institution. The twenty-two male rats weighting 200–250 g were anesthetized by inhalation of an isoflurane oxygen mixture (2–5% isoflurane in 100% oxygen). An endotracheal tube was inserted and attached to a ventilator. The animals were artificially ventilated, and end tidal CO_2 was monitored by a respiratory gas monitor and maintained within normal ranges as previously described [9]. Polyethylene (PE-50) catheters were inserted into an external jugular vein and the carotid arteries for the purposes of drug administration and measurement of BP, respectively. PE-10 catheters were inserted into the femoral arteries so drugs could be injected into the arterial blood supply of the hindlimb muscles of the leg. The skin covering the triceps surae muscle and femoral region was surgically separated from the muscle below to eliminate inputs from

cutaneous afferents in the hindlimb. During the experiment, fluid balance, basal BP and body temperature were maintained [9].

BP was measured by connecting the carotid arterial catheter to a pressure transducer (model P23 ID, Statham). Mean arterial pressure (MAP) was obtained by integrating the arterial signal with a time constant of 4 s. HR was determined from the arterial pressure pulse. The renal SNA (RSNA) was recorded as previously described [21, 22]. Briefly, a bundle of the renal nerves were carefully dissected from other connective tissues. A piece of laboratory film was placed under the isolated nerves, and two tips of a bipolar electrode to record neural activity were placed between the nerves and the film. These were embedded in a silicone gel. Once the gel was hardened, the silicone rubber was fixed to the surrounding tissue. The RSNA signal was amplified with an amplifier (P511, Grass Instruments) with a band-pass filter of 300 Hz in low-cut frequency and of 3 kHz in high-cut frequency and made audible.

Decerebration was performed as previously described [21, 22]. A transverse section was made anterior to the superior colliculus and extending ventrally to the mamillary bodies. The brain rostral to the section was then removed. This approach afforded the opportunity to examine the effect of arterial injection of capsaicin on blood pressure without considering the confounding effects of anesthesia. Once the decerebration was complete, anesthesia was removed from the inhaled mixture. A recovery period of 60 min after decerebration was employed to allow sufficient time for elimination of the effects of anesthesia gas from the preparation.

On the day of experiment, a stock solution of 250 µg/ml of capsaicin was diluted in saline to make the concentrations of 1.0 µg/kg body weight [9]. Then, capsaicin (volume: 0.1–0.15 ml) was injected into the femoral artery. The duration of the injections was 1 min. At least 30 min were allowed between injections. To examine effects of adenosine on TRPV1 responses, adenosine (0.5, 5 and 50 nmol/kg) and adenosine analogue CGS21680 (0.5, 5 and 50 nmol/kg) were infused into the femoral artery 20 min before capsaicin application, respectively. In additional protocol, 50 nmol/kg of adenosine or 50 nmol/kg of CGS21680 was intravenously given and RSNA and BP responses induced by capsaicin were examined.

Immunohistochemistry

L4–6 DRGs of four rats were immediately dissected after they were anesthetized and then transcardially perfused. The tissues were cut using a cryostat to obtain DRG sections (10 µm). The sections were washed with PBS and incubated with the rabbit anti-A_{2A} antibody (1:100, Abcam) overnight at 4 °C. After being washed in PBS, the sections were incubated with the goat anti-rabbit fluorescein isothiocyanate (FITC)-labeled secondary antibody (1:200, NERUOMICS) for 2 hours at room temperature. To examine co-localization of A_{2A} and TRPV1 within DRG neurons, sections were incubated with the second primary antibody (guinea pig anti-TRPV1 at 1:100, NERUOMICS) overnight. Then, the sections were washed and incubated for 1 hour at room temperature with a secondary antibody (Alexa Fluor-594 conjugated goat anti-guinea pig IgG, 1:200) for 2 hours at room temperature. After that, the sections were washed in PBS, and coverslipped. FITC- and Alexa Fluor-594-labeled DRG neurons were examined using a Nikon microscope with appropriate filters, and the images were stored digitally on a computer.

Capsaicin-Induced Current of DRG Neurons

The fourteen rats were anesthetized by inhalation of an isoflurane-oxygen mixture. A fluorescent retrograde tracer, 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine percholate (DiI; 60 mg/ml; Molecular Probes, Eugene, OR), was injected into the white portion of the gastrocnemius muscle [22, 23]. Four to five days were allowed before electrophysiological recordings were performed. At the end of each experiment, the gastrocnemius muscle was dissected to confirm locations of DiI. The data were included in this experiment if DiI was in the white portion of the gastrocnemius muscle.

The rats were anesthetized and decapitated. Then, DRGs at lumbar levels 4–6 were removed, and immediately placed into Dulbecco's modified Eagle's medium. The DRG tissues were then processed [22, 23] and plated onto a 35-mm culture dish containing poly-L-lysine-precoated coverslips and maintained for at least 60 min before electrophysiological recordings.

Whole cell recordings were made using fire-polished glass filled with the internal solution as described previously [22, 23]. The recording chamber was continuously perfused with artificial cerebral spinal fluid (aCSF). DiI-labeled DRG neurons were identified using a combination of fluorescence illumination and differential interference contrast (DIC; x20–40) optics on a Nikon TE2000 inverted microscope. Under DIC

images of cells were displayed on a video monitor. Size of cell soma was estimated by calculating the mean of the longest and shortest cross-sectional diameters with the aid of a calibrated eyepiece reticle. The DRG neurons with diameter $< 35 \mu\text{m}$ were recorded in this study [22, 23].

For all chemical tests with capsaicin, solutions were applied locally and rapidly (2 s duration) to the neurons. The tip of each syringe was placed $100 \mu\text{m}$ from the cell soma using a manipulator. The gravity-fed solutions were controlled using manual switching of one-way stopcock valves. For capsaicin responsiveness experiments, capsaicin, adenosine, CGS21680 and MSX-3 were prepared fresh each day from a 1 mM of respective stock solution. To determine the effect of adenosine and CGS21680 exposure on capsaicin responsiveness, $1 \mu\text{M}$ of adenosine and $0.1\text{--}10 \mu\text{M}$ of CGS21680 was applied 2 min before capsaicin was given, respectively. In additional experiment, $10 \mu\text{M}$ of MSX-3, an antagonist to A_{2A} receptor, was given before adenosine and then capsaicin was applied.

Whole cell configuration was maintained at -60 mV . An equilibration period of 5–10 min was allowed after whole cell access was established and the recording reached a steady state. The recording was then made to measure changes in inward currents evoked by chemical stimuli. Signals were recorded and saved in a PC-based computer using pClamp 10.1 and the magnitude of inward current was determined using Clampfit 10.1 (Axon Instruments). Neurons were considered to be capsaicin sensitive if an evoked inward current was $>50 \text{ pA}$ in peak amplitude.

Data Analysis

The data of responses of RSNA, MAP and HR as well as amplitude of capsaicin-evoked currents were analyzed using a one-way repeated-measure analysis of variance. As appropriate, Tukey post hoc tests were utilized. Values are presented as means $\pm$ SE. For all analyses, differences were considered significant at $P < 0.05$. All statistical analyses were performed by using SPSS for Windows version 15.0 (SPSS, Chicago, IL).

Results

Effects of adenosine and CGS21680 on RSNA and BP responses induced by capsaicin

Adenosine ($0.5\text{--}50 \text{ nmol/kg}$) and adenosine analogue CGS21680 ($0.5\text{--}50 \text{ nmol/kg}$) infused into the femoral artery did not significantly alter RSNA, MAP and HR. There were no significant differences in basal MAP and HR among all the groups before injections ($P > 0.05$). In experiment of adenosine infusion, basal MAP (mm Hg)/HR (beats/min): $97 \pm 6/392 \pm 12$ (control); $93 \pm 5/387 \pm 10$ (0.5 nmol); $91 \pm 5/399 \pm 15$ (5 nmol); $98 \pm 7/388 \pm 12$ (50 nmol); and $101 \pm 6/390 \pm 14$ (recovery), respectively. In experiment of CGS21680 infusion, basal MAP (mm Hg)/HR (beats/min): $92 \pm 7/390 \pm 11$ (control); $98 \pm 9/399 \pm 12$ (0.5 nmol); $95 \pm 5/405 \pm 14$ (5 nmol); $97 \pm 9/389 \pm 13$ (50 nmol); and $91 \pm 8/395 \pm 12$ (recovery), respectively. In control and recovery experiments, the saline solution was infused into the arterial line and then capsaicin was injected. Figure 1 further shows that 50 nmol of adenosine, 5 nmol and 50 nmol of CGS21680 significantly attenuated RSNA, MAP and HR responses induced by $1.0 \mu\text{g/kg}$ of capsaicin ($n=12$ for adenosine and $n=10$ for CGS21680). It is noted that 50 nmol of CGS21680 had a greater attenuation on the reflex responses than the same amount of adenosine.

In addition, 50 nmol of adenosine and 50 nmol of CGS21680 ($n=6$ in each group) were intravenously given and the responses of RSNA, MAP and HR evoked by $1.0 \mu\text{g/kg}$ of capsaicin were examined. Intravenous administration of adenosine failed to significantly alter capsaicin-induced reflex responses. i.e., changes of RSNA, MAP and HR were $119 \pm 21\%$, $39 \pm 9 \text{ mmHg}$ and $22 \pm 5 \text{ bpm}$ in control; $121 \pm 18\%$, $42 \pm 10 \text{ mmHg}$ and $20 \pm 6 \text{ bpm}$ after adenosine ($P > 0.05$ vs. respective controls) and $115 \pm 18\%$, $36 \pm 8 \text{ mmHg}$ and $19 \pm 4 \text{ bpm}$ after CGS21680 ($P > 0.05$ vs. respective controls).

Co-existence of A_{2A} and TRPV1 in DRG neurons

Dual immunofluorescence techniques were used to examine co-localization of fluorescent A_{2A} and TRPV1 immunoreactivity in DRG neurons. Figure 2 illustrates that the appearance of A_{2A} and TRPV1 within DRG neurons is characterized by fluorescent green and

Fig. 1. Top panel: Effects of adenosine on responses of the renal sympathetic nervous activity (RSNA), mean arterial pressure (MAP) and heart rate (HR) induced by capsaicin (1.0 μ g/kg body weight) injected into the femoral artery. $P < 0.05$ vs. control and recovery. Number of animals = 12. There were no significant differences in baseline MAP and HR among groups ($P > 0.05$). Bottom panel: Effects of adenosine analogue CGS21680 on responses of the RSNA, MAP and HR induced by capsaicin (1.0 μ g/kg body weight) injected into the femoral artery. $P < 0.05$ vs. control and recovery. Number of animals = 10.

Fig. 2. Immunofluorescence was employed to examine double-labeling for A_{2A} and TRPV1. Representative photomicrographs show that A_{2A} and TRPV1 staining were presented in the same DRG neurons. Arrows indicate representative cells positive for both A_{2A} and TRPV1 after they were merged. Scale bar = 50 μ m.

red color, respectively. A_{2A} and TRPV1 are largely presented in small size of DRG neurons. The photomicrographs of Figure 2 also show that both A_{2A} and TRPV1 staining appear in the same DRG neurons.

Fig. 3. (A): Effects of adenosine on capsaicin-induced currents in DRG neurons. Top panel: Original traces of DRG neuron response to 1 μM capsaicin. Bottom panel: Average data show that adenosine (1 μM) attenuated peak amplitude of inward currents in DRG neurons as compared with only capsaicin application. Number of neurons = 19. * $P < 0.05$ vs. capsaicin. (B): Effects of adenosine analogue CGS21680 on capsaicin-induced currents in DRG neurons. Top panel: Original traces of DRG neuron response to 0.1–10 μM CGS21680. Bottom panel: Average data show that CGS21680 attenuated peak amplitude of inward currents in DRG neurons as compared with only capsaicin application. Number of neurons = 26. * $P < 0.05$ vs. capsaicin. (C): The prior application of 10 μM of MSX-3 attenuated the effects of adenosine (1 μM) on capsaicin-currents in 9 DRG neurons ($P > 0.05$ vs. capsaicin alone).

Effects of adenosine CGS21680 on current of DRG neurons evoked with TRPV1 activation

Capsaicin-induced currents in the DRG neurons innervating muscles were further examined in this experiment. Figure 3 (A&B) show that the prior application of adenosine and CGS21680 significantly attenuated the magnitude of the DRG neuron TRPV1 response. i.e., the current amplitude was 2.37 ± 0.35 nA in 19 neurons after capsaicin; and 0.56 ± 0.15 nA in 19 neurons with 1 μM of adenosine application of ($P < 0.05$ vs. capsaicin alone). Also, 0.1, 1.0 and 10 μM of CGS21680 significantly attenuated the magnitude of TRPV1-induced currents in the DRG neurons. i.e., the current amplitude was 2.35 ± 0.31 nA in 26 neurons after capsaicin; and 0.23 ± 0.10 nA in 21 neurons with 1 μM of CGS21680 application of ($P < 0.05$ vs. capsaicin alone). Figure 3 (C) further shows that the prior application of 10 μM of MSX-3 attenuated the effects of adenosine on capsaicin-currents ($P > 0.05$ vs. capsaicin alone, in 9 DRG neurons).

Discussion

In general, adenosine A_1 , A_{2A} , A_{2B} , and A_3 receptors (ARs) are present at the peripheral sites on sensory afferent nerve terminals (A_1 R and A_{2A} R) or on adjacent cell types that can potentially influence sensory inputs such as nociception under certain conditions (A_{2A} R, A_{2B} R, and A_3 R) [24]. Among these adenosine receptor subtypes, adenosine A_{2B} and A_3 receptors have not been identified directly on sensory neurons, but their localization on mast cells and ability to contribute to aspects of inflammation make them potential indirect contributors to inflammatory pain via release of mediators from such cells [24]. A_{2A} Rs are present in DRG [25] and local peripheral administration of A_{2A} R agonists leads to mechanical hyperalgesia and increases flinching with formalin [26, 27]. Hyperalgesia evoked by A_{2A} R activation is mediated by increases in cAMP in the sensory nerve terminal, activation of PKA, phosphorylation of Na⁺ channels, increased inward currents, and sensory afferent activation [28]. A_1 Rs have the similar function in regulating sensory inputs; however, it has been reported that A_{2A} Rs have a direct effect on TRPV1 channel protein [20]. Thus, in the current, we examined the role played by adenosine and its subtype A_{2A} Rs in modulating SNA and BP responses evoked by activation of TRPV1 in muscle afferent nerves.

Data of our current study demonstrate that arterial injection of adenosine significantly attenuated the reflex SNA and BP responses induced by stimulation of muscle afferents' TRPV1 in rats. Inhibition on the TRPV1-mediated reflex SNA and BP responses was also observed after activation of A_{2A} by CGS21680. Our additional study shows co-existence of A_{2A} and TRPV1 within DRG neurons, and that the prior application of adenosine or CGS21680 attenuated the magnitude of capsaicin-induced currents in muscle DRG neurons.

Prior studies have demonstrated that arterial injection of adenosine increases SNA and BP in humans [13, 14]. However, the effects of adenosine on SNA and BP differ with species and route of administration [13–15, 17, 18]. Thus, in a control group of the current study, we intravenously injected the same amount of adenosine and CGS21680 and then examined their respective effects on the SNA and BP responses evoked by capsaicin. It was found that intravenous injection of adenosine or CGS21680 failed to significantly alter the reflex SNA and BP responses, suggesting that the likelihood of systemic effects caused by adenosine and CGS21680 was minimal.

Adenosine, a metabolic by-product of ATP utilization, has potent vasodilator effects in cardiac and skeletal muscle [29]. Previous studies have demonstrated that adenosine is present in the muscle interstitium during exercise [10, 12, 30]. Adenosine is involved in the regulation of numerous important processes in human skeletal muscle during exercise, such as blood flow and local vasodilation in response to ischemia [31]. Aminophylline, a nonspecific adenosine receptor antagonist, has been used to block adenosine receptors in a previous study, suggesting that adenosine receptor antagonism in the exercising muscles accentuates muscle SNA during fatiguing exercise [16]. Nonetheless, the mechanisms responsible for accentuated SNA response seen after aminophylline remain unknown. It is assumed that adenosine is likely to directly inhibit muscle afferent activity. Results of our current study provide evidence demonstrating adenosine and adenosine A_{2A} analogue inhibit SNA and BP responses evoked by stimulation of muscle afferents' TRPV1. It should be noted that stimulation of muscle afferent nerves' TRPV1 is involved in modifying SNA and BP responses in rats [9, 22]. Also, it has been reported that TRPV1 mediates the exercise pressor reflex in rats [32]. In contrast, a prior study using cats suggests that TRPV1 is unlikely to evoke the muscle reflex under normal physiological conditions [33]. Note that there are distinct differences in the make-up of afferent neurons when compared to different species [34].

The role of adenosine in regulating TRPV1 may become more important as TRPV1 is downregulated and its response is impaired in some cardiovascular diseases such as heart failure due to the hindlimb ischemia [35, 36]. Thus, it is speculated that adenosine or stimulation of its A_{2A} receptors may be beneficial to the abnormalities in sympathetic responsiveness observed in heart failure due to its inhibitory effects on TRPV1.

In addition, adenosine plays a role as a regulator of neuronal survival [37]. Effects of adenosine on target cells are influenced by the subtype and density of the adenosine receptors that are present on those cells [38]. In this regard, primary neurons derived from the superior cervical ganglion (SCG) were previously used to study since it represents a well-characterized model for neurotrophin-dependent sympathetic neuronal apoptosis [39]. A prior study has revealed that addition of exogenous adenosine deaminase (ADA), to nerve growth factor (NGF) maintained SCG cultures results in a dramatic increase in apoptosis, suggest that the presence of endogenous adenosine is necessary for the survival of sympathetic neurons [40]. It is also found that A_{2A} receptors are predominant adenosine receptor subtype expressed in primary rat SCG neurons [40]. Consistently with this, the survival of NGF-deprived SCG neurons is enhanced by the addition of an A_{2A} R specific agonist CGS21680, and this effect is reversed when cells were exposed to an A_{2A} R antagonist [40]. These findings suggest that adenosine is likely to play an important role in the regulation of cell survival in the peripheral nervous system and that it may synergize with NGF in contributing to the neuronal survival.

Our prior study demonstrated that the levels of NGF in sympathetic neurons and DRG neurons of rats are impaired after heart failure induced by ligation of the coronary artery [41]. NGF can affect the response of TRPV1 in DRG neurons [42]. Infusion of NGF in the muscles or addition of NGF to the culture dish containing DRG neurons increases the magnitude of TRPV1 response in IB_4 -negative DRG neurons (expressing trkA receptors for NGF, depends on NGF for survival) [42]. It is postulated that impaired NGF seen in heart failure attenuates TRPV1 expression and activity thereby leading to less SNA and BP responses to TRPV1 stimulation in rats with heart failure. As adenosine is increased in the circulation of heart failure [43], this may restrict TRPV1 and play a beneficial role in this disease.

In patients with heart failure, the level of plasma adenosine is ~200 nmol/L (nM) and ~60 nM in healthy subjects [43]. In rats with pressure overload hypertrophy, the level of coronary effluent adenosine is ~2000 pmol/min/g wet heart tissue (2 nmol) and ~1 nmol in control animals [44]. Nevertheless, the concentrations of adenosine used in our current study were 0.5–50 nmol/kg body weight, and they were injected into the arterial blood supply of the hindlimb muscles. Considering that a rat weighed 200–250g and muscles weigh of the injected leg was ~10–20g, muscle tissues would receive adenosine at ~0.05–5 nmol/g tissue. These levels of adenosine are likely relevant to heart failure. This may also suggest that the increased levels of adenosine in heart failure are likely to lead to the blunted metaboreflex [35, 36].

With respect to the effects of adenosine in the presence of a TRPV1 antagonist, capsaicin-currents were largely attenuated as a TRPV1 antagonist (such as capsazepine) was applied [22], by which we did not have the opportunity observing the effects of adenosine on capsaicin-currents in the presence of a TRPV1 antagonist in the current study. *In vivo* part of the current study, adenosine or CGS21680 did not alter RSNA and BP per se. Likewise, we did not examine the effects of a TRPV1 antagonist as adenosine was given.

Conclusions

Adenosine and activation of its A_{2A} receptor inhibit muscle afferent TRPV1-engaged reflex SNA and BP responses. There are receptor mechanisms responsible for the role of adenosine. First, the co-existence of A_{2A} and TRPV1 in DRG neurons is observed in the current study. Second, the prior application of adenosine and its A_{2A} subtype receptor agonist decreases the current magnitude of the DRG neuron response induced by capsaicin. Thus, it is likely that TRPV1 response is inhibited as the levels of adenosine are increased in the hindlimb muscles under diseased conditions such as heart failure.

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Disclosure Statement

None.

References

- 1 Szallasi A, Blumberg PM: Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacol Rev* 1999;51:159-212.
- 2 Bevan S, Geppetti P: Protons: small stimulants of capsaicin-sensitive sensory nerves. *Trends Neurosci* 1994;17:509-512.
- 3 Stahl GL, Longhurst JC: Ischemically sensitive visceral afferents: importance of H⁺ derived from lactic acid and hypercapnia. *Am J Physiol Heart Circ Physiol* 1992;262:H748-H753.
- 4 Kaufman MP, Forster H: Reflexes controlling circulatory, ventilatory, and airway responses to exercise. *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*. 1996, Vol 12, pp 381-447.
- 5 Kaufman MP, Longhurst JC, Rybicki KJ, Wallach JH, Mitchell JH: Effects of static muscular contraction on impulse activity of groups III and IV afferents in cats. *J Appl Physiol* 1983;55:105-112.
- 6 Crayton SC, Mitchell JH, Payne FC, 3rd: Reflex cardiovascular response during injection of capsaicin into skeletal muscle. *Am J Physiol* 1981;240:H315-H319.
- 7 Kaufman MP, Iwamoto GA, Longhurst JC, Mitchell JH: Effects of capsaicin and bradykinin on afferent fibers with ending in skeletal muscle. *Cir Res* 1982;50:133-139.
- 8 Ma QP: Vanilloid receptor homologue, VRL1, is expressed by both A- and C-fiber sensory neurons. *Neuroreport* 2001;12:3693-3695.
- 9 Li J, Maile MD, Sinoway AN, Sinoway LI: Muscle pressor reflex: potential role of vanilloid type 1 receptor and acid-sensing ion channel. *J Appl Physiol* 2004;97:1709-1714.
- 10 Hellsten Y, Maclean D, Radegran G, Saltin B, Bangsbo J: Adenosine concentrations in the interstitium of resting and contracting human skeletal muscle. *Circulation* 1998;98:6-8.
- 11 Li J, King NC, Sinoway LI: Interstitial ATP and norepinephrine concentrations in active muscle. *Circulation* 2005;111:2748-2751.
- 12 Mo FM, Ballard HJ: The effect of systemic hypoxia on interstitial and blood adenosine, AMP, ADP and ATP in dog skeletal muscle. *J Physiol* 2001;536:593-603.
- 13 Costa F, Biaggioni I: Role of adenosine in the sympathetic activation produced by isometric exercise in humans. *J Clin Invest* 1994;93:1654-1660.
- 14 Costa F, Diedrich A, Johnson B, Sulur P, Farley G, Biaggioni I: Adenosine, a metabolic trigger of the exercise pressor reflex in humans. *Hypertension* 2001;37:917-922.
- 15 MacLean DA, Saltin B, Radegran G, Sinoway L: Femoral arterial injection of adenosine in humans elevates MSNA via central but not peripheral mechanisms. *J Appl Physiol* 1997;83:1045-1053.
- 16 Cui J, Leuenberger UA, Blaha C, Yoder J, Gao Z, Sinoway LI: Local adenosine receptor blockade accentuates the sympathetic responses to fatiguing exercise. *Am J Physiol Heart Circ Physiol* 2010;298:H2130-2137.
- 17 MacLean DA, Vickery LM, Sinoway LI: Elevated interstitial adenosine concentrations do not activate the muscle reflex. *Am J Physiol Heart Circ Physiol* 2001;280:H546-H553.
- 18 Riksen NP, van Ginneken EE, van den Broek PH, Smits P, Rongen GA: In vivo evidence against a role for adenosine in the exercise pressor reflex in humans. *J Appl Physiol* 2005;99:522-527.
- 19 Lynch ME, Clark AJ, Sawynok J: Intravenous adenosine alleviates neuropathic pain: a double blind placebo controlled crossover trial using an enriched enrolment design. *Pain* 2003;103:111-117.
- 20 Puntambekar P, Van Buren J, Raisinghani M, Premkumar LS, Ramkumar V: Direct interaction of adenosine with the TRPV1 channel protein. *J Neurosci* 2004;24:3663-3671.

- 21 Lu J, Xing J, Li J: Role for NGF in augmented sympathetic responses to activation of mechanically and metabolically sensitive muscle afferents in rats with femoral artery occlusion. *J Appl Physiol* 2012;113:1311-1322.
- 22 Xing J, Gao Z, Lu J, Sinoway LI, Li J: Femoral artery occlusion augments TRPV1-mediated sympathetic responsiveness. *Am J Physiol Heart Circ Physiol* 2008;295:H1262-H1269.
- 23 Xing J, Lu J, Li J: ASIC3 function and immunolabeling increases in skeletal muscle sensory neurons following femoral artery occlusion. *J Physiol* 2012;590:1261-1272.
- 24 Burnstock G, Sawynok J: Adenosine Triphosphate and Adenosine Receptors and Pain; in Beaulieu P, Lussier D, Porreca F, and Dickenson AH (eds) Seattle, IASP, 2010, pp 303-326.
- 25 Kaelin-Lang A, Lauterburg T, Burgunder JM: Expression of adenosine A2a receptor gene in rat dorsal root and autonomic ganglia. *Neurosci Lett* 1998;246:21-24.
- 26 Doak GJ, Sawynok J: Complex role of peripheral adenosine in the genesis of the response to subcutaneous formalin in the rat. *Eur J Pharmacol* 1995;281:311-318.
- 27 Taiwo YO, Levine JD: Direct cutaneous hyperalgesia induced by adenosine. *Neuroscience* 1990;38:757-762.
- 28 Gold MS, Reichling DB, Shuster MJ, Levine JD: Hyperalgesic agents increase a tetrodotoxin-resistant Na⁺ current in nociceptors. *Proc Natl Acad Sci U S A* 1996;93:1108-1112.
- 29 Collis MG: The vasodilator role of adenosine. *Pharmacol Ther* 1989;41:143-162.
- 30 Li J, King NC, Sinoway LI: ATP concentrations and muscle tension increase linearly with muscle contraction. *J Appl Physiol* 2003;95:577-583.
- 31 Delyani JA, Van Wylen DG: Endocardial and epicardial interstitial purines and lactate during graded ischemia. *Am J Physiol* 1994;266:H1019-1026.
- 32 Smith SA, Leal AK, Williams MA, Murphy MN, Mitchell JH, Garry MG: The TRPV1 receptor is a mediator of the exercise pressor reflex in rats. *J Physiol* 2010;588:1179-1189.
- 33 Kindig AE, Heller TB, Kaufman MP: VR-1 receptor blockade attenuates the pressor response to capsaicin but has no effect on the pressor response to contraction in cats. *Am J Physiol Heart Circ Physiol* 2005;288:H1867-H1873.
- 34 Smith ES, Lewin GR: Nociceptors: a phylogenetic view. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2009;195:1089-1106.
- 35 Li J, Sinoway AN, Gao Z, Maile MD, Pu M, Sinoway LI: Muscle mechanoreflex and metaboreflex responses after myocardial infarction in rats. *Circulation* 2004;110:3049-3054.
- 36 Smith SA, Williams MA, Mitchell JH, Mammen PP, Garry MG: The capsaicin-sensitive afferent neuron in skeletal muscle is abnormal in heart failure. *Circulation* 2005;111:2056-2065.
- 37 Ribeiro JA, Sebastiao AM, de Mendonca A: Adenosine receptors in the nervous system: pathophysiological implications. *Prog Neurobiol* 2002;68:377-392.
- 38 Sebastiao AM, Ribeiro JA: Fine-tuning neuromodulation by adenosine. *Trends Pharmacol Sci* 2000;21:341-346.
- 39 Ham J, Eilers A, Whitfield J, Neame SJ, Shah B: c-Jun and the transcriptional control of neuronal apoptosis. *Biochem Pharmacol* 2000;60:1015-1021.
- 40 Ramirez SH, Fan S, Maguire CA, Perry S, Hardiek K, Ramkumar V, Gelbard HA, Dewhurst S, Maggirwar SB: Activation of adenosine A2A receptor protects sympathetic neurons against nerve growth factor withdrawal. *J Neurosci Res* 2004;77:258-269.
- 41 Xing J, Lu J, Li J: Nerve growth factor decreases in sympathetic and sensory nerves of rats with chronic heart failure. *Neurochem Res* 2014;39:1564-1570.
- 42 Xing J, Lu J, Li J: Contribution of nerve growth factor to augmented TRPV1 responses of muscle sensory neurons by femoral artery occlusion. *Am J Physiol Heart Circ Physiol* 2009;296:H1380-1387.
- 43 Funaya H, Kitakaze M, Node K, Minamino T, Komamura K, Hori M: Plasma adenosine levels increase in patients with chronic heart failure. *Circulation* 1997;95:1363-1365.
- 44 Chung ES, Perlini S, Aurigemma GP, Fenton RA, Dobson JG, Jr, Meyer TE: Effects of chronic adenosine uptake blockade on adrenergic responsiveness and left ventricular chamber function in pressure overload hypertrophy in the rat. *J Hypertens* 1998;16:1813-1822.