Published in final edited form as:

Neuroscience. 2014 September 26; 277: 26–35. doi:10.1016/j.neuroscience.2014.06.061.

The role of prostaglandins in spinal transmission of the exercise pressor reflex in decerebrate rats

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Abstract

Previous studies found that prostaglandins in skeletal muscle play a role in evoking the exercise pressor reflex; however the role played by prostaglandins in the spinal transmission of the reflex is not known. We determined, therefore, whether or not spinal blockade of cyclooxygenase (COX) activity and/or spinal blockade of endoperoxide receptor (EP) 2 or EP4 receptors attenuated the exercise pressor reflex in decerebrate rats. We first established that intrathecal doses of a non-specific COX inhibitor Ketorolac (100ug in 10ul), a COX-2 specific inhibitor Celecoxib (100μg in 10μl), an EP2 antagonist PF-04418948 (10μg in 10μl), and an EP4 antagonist L-161,982 (4μg in 10μl) effectively attenuated the pressor responses to intrathecal injections of Arachidonic Acid (100μg in 10μl), EP2 agonist Butaprost (4ng in 10 μl), and EP4 agonist TCS 2510 (6.25μg in 2.5 μl), respectively. Once effective doses were established, we statically contracted the hindlimb before and after intrathecal injections of Ketorolac, Celecoxib, the EP2 antagonist and the EP4 antagonist. We found that Ketorolac significantly attenuated the pressor response to static contraction (before Ketorolac: 23±5 mmHg, after Ketorolac 14±5 mmHg; p<0.05) whereas Celecoxib had no effect. We also found that 8μg of L-161,982, but not 4 μg of L-161,982, significantly attenuated the pressor response to static contraction (before L-161,982: 21±4 mmHg, after L-161,982 12±3 mmHg; p<0.05), whereas PF-04418948 (10μg) had no effect. We conclude that spinal COX-1, but not COX-2, plays a role in evoking the exercise pressor reflex, and that the spinal prostaglandins produced by this enzyme are most likely activating spinal EP4 receptors, but not EP2 receptors.

Keywords

static contraction; thin fiber muscle afferents; cyclooxygenase; endoperoxide receptors; sympathetic nervous system

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Introduction

The cardiovascular adjustments to exercise include increases in arterial pressure, heart rate and ventilation. In part, these increases have been shown to be caused by a reflex arising from contracting skeletal muscles (Coote et al., 1971, McCloskey and Mitchell, 1972, Smith et al., 2001). The functional significance of this reflex, aptly named the exercise pressor reflex (Mitchell et al., 1983), is that it has been shown to increase arterial blood flow to contracting muscles in both humans (Amann et al., 2011) and animals (O'Leary et al., 1999). The afferent arm of the exercise pressor reflex is comprised of thinly myelinated group III afferents as well as unmyelinated group IV afferents (McCloskey and Mitchell, 1972). Group I and II muscle afferents have been shown to play no role in evoking the exercise pressor reflex (McCloskey et al., 1972, Waldrop et al., 1984).

Group III and IV muscle afferents terminate in laminae I, II and V of the dorsal horn (Mense and Craig, 1988), where they are thought to release glutamate and substance P as their neurotransmitters and neuromodulators, respectively (Kaufman et al., 1985, Hill et al., 1992, Adreani et al., 1996). Intrathecal injection of NMDA, a glutamate analog, and substance P have in turn been shown to increase spinal cord concentrations of prostaglandin E2 (PGE2) (Dirig and Yaksh, 1999, Hua et al., 1999), which is a cyclooxygenase metabolite of arachidonic acid. There are two forms of cyclooxygenase (COX), namely I and II. Biochemical and immunocytochemical evidence suggest that both are expressed constitutively in the spinal cord (Beiche et al., 1996, Ebersberger et al., 1997, Willingale et al., 1997).

Prostaglandin E2 stimulates the endoperoxide receptor (EP), which, in turn, is coupled to G proteins. There are four types of EP receptors, termed EP1-4, and each is found in the spinal cord (Oida et al., 1995, Kawamura et al., 1997, Harvey et al., 2004, Johansson et al., 2011, Natura et al., 2013). The available evidence suggests that EP2 and EP4 receptors are the most likely to mediate the spinal cord effects of PGE2 release by incoming traffic from group III and IV muscle afferents (Vanegas and Schaible, 2001).

These findings, considered together, raised the possibility that PGE2 production played a role in the spinal transmission of the exercise pressor reflex. We were therefore prompted to test the hypothesis that spinal blockade of cyclooxygenase attenuated the exercise pressor reflex in decerebrated rats. We were also prompted to test the hypothesis that spinal blockade of either EP2 or EP4 receptors, both of which are stimulated by PGE2, attenuated the reflex.

Experimental Procedures

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University, Hershey Medical Center. Adult male Sprague-Dawley rats (n=85; average weight was 430 ± 4 g) were used in these experiments. The rats were housed in a temperature controlled room ($24 \pm 1^\circ\text{C}$) with a 12:12 h light-dark cycle and fed a standard diet and tap water *ad libitum*.

Surgical Preparation

On the day of the experiment, rats were anesthetized with isoflurane gas (2-3%) in oxygen. The trachea was cannulated and the lungs were ventilated mechanically (Harvard Apparatus) with the gas anesthetic. Both carotid arteries and the jugular vein were cannulated (PE-50). One carotid arterial catheter was connected to a pressure transducer (model P23 LX, Statham) to measure blood pressure; heart rate was calculated beat to beat from the arterial pulse pressure (Gould Biotach). The venous catheter was used to administer drugs and fluids. Arterial blood gases and pH were monitored using an automated blood gas analyzer (ABL 80 FLEX, Radiometer). P_{CO_2} and arterial pH were maintained within normal ranges by adjusting ventilation and oxygen or through an intravenous administration of sodium bicarbonate (8.5%). Body temperature was maintained between 36.5 and 38.0°C by an isothermal heating pad and lamp.

A laminectomy was performed from L₃ to L₅ to expose the spinal cord and the lower lumbar roots. The rats were then secured in a Kopf customized spinal frame by clamps placed on the pelvis. The dura was opened from L₄ to L₃ and a catheter (PE-10) was inserted with its tip pointing towards the head. The tip was positioned so that it was at the level of the L₄ and L₅ roots' exit points from the spinal cord because L₄ and L₅ dorsal roots relay sensory input from hind limb skeletal muscle. The catheter was then glued in place with WPI Kwik-Sil™. The left calcaneal bone was sectioned and attached to a force transducer (FT-10, Grass) to measure developed tension when statically contracting the triceps surae muscles. The sciatic nerve was isolated for placement of the stimulating electrode.

A pre-collicular decerebration was performed by sectioning the brain less than 1 mm anterior to the superior colliculi. All neural tissue rostral to the section was removed. To minimize bleeding, small pieces of oxidized regenerated cellulose (Ethicon, Johnson & Johnson) were placed on the internal skull surface and the cranial cavity was packed with gauze. Immediately after pre-collicular transection, gas anesthesia was discontinued and the rats' lungs were ventilated mechanically with room air. The rat was tilted head-up at an angle of 18°. After decerebration, the rats were allowed to stabilize for at least one hour before any experimental protocol was initiated.

Experimental Protocols

The first protocol determined that intrathecal injections of Ketorolac (100 µg in 10 µl), a non-selective COX-1 and COX-2 inhibitor, and Celecoxib (100 µg in 10 µl), a selective COX-2 inhibitor, effectively blocked the activity of cyclooxygenase. This was accomplished by measuring the pressor response to arachidonic acid (100 µg in 10 µl), injected intrathecally through the catheter placed at the L4/L5 level of the spinal cord, both before and after intrathecal injections of either Ketorolac (n=5) or Celecoxib (n=9). The time between the first injection of arachidonic acid and either COX antagonist was approximately 10 minutes. The time between injecting the COX inhibitor and the second injection of arachidonic acid was 25 minutes. Previous studies have shown that intrathecal injections of both Ketorolac and Celecoxib at these concentrations reach peak effect 25 minutes after giving the drug (Lee and Seo, 2008). Vehicle control experiments were also performed. Specifically, we measured the pressor responses to arachidonic acid (100 µg in 10 µl),

injected intrathecally, before and after intrathecal injections of saline (10 μ l) as well as 70% DMSO and 30% saline (10 μ l), the vehicles for Ketorolac (n=4) and Celecoxib (n=8) respectively.

The effects of these COX inhibitors on the exercise pressor reflex were next examined. The hindlimb muscles were statically contracted for 30 seconds by stimulating the sciatic nerve (1-2 times motor threshold, 0.01 ms pulse duration, 30-40 Hz) before and after injecting either Ketorolac (n=6) or Celecoxib (n=5) intrathecally. The time between the first contraction and injecting the COX inhibitor was 10 minutes. The time between injecting the COX inhibitor and the second contraction was 25 minutes. This experiment was repeated with intrathecal injections of the vehicle controls for Ketorolac and Celecoxib (see above) (both n=4).

To investigate the effect of Ketorolac on the sympathetic outflow arising from the intermediolateral horn of the thoracic and upper spinal cord a catheter was placed into the carotid artery with its tip positioned near the carotid sinus. The pressor response to carotid arterial injection of sodium cyanide (25 μ g/kg), which stimulated the carotid chemoreceptors was then measured. Twenty five minutes after Ketorolac was injected intrathecally (100 μ g in 10 μ l) sodium cyanide (25 μ g/kg) was injected again and the pressor responses were measured.

The second protocol determined whether the pressor responses to static contraction of the hindlimb muscles were due to activation of specific spinal EP receptors by presumptive COX metabolites of arachidonic acid. Intrathecal injections of EP2 and EP4 receptor antagonists were administered in attempt to attenuate the exercise pressor reflex. The efficacy of the blockade needed to be established before the effects of EP antagonists on the exercise pressor reflex could be examined. The cannula used to make these intrathecal injections was placed and positioned in the same manner as that described for the first protocol. Likewise assessment of the effectiveness of the blockade was patterned after the protocol previously described for the cyclooxygenase inhibitors. Specifically, the pressor response to an intrathecal injection of Butaprost (4 ng/10 μ l), an EP2 receptor agonist, was measured before and after an intrathecal injection of PF-04418948 (10 μ g in 10 μ l), an EP2 receptor antagonist. The second dose of Butaprost was injected 90 minutes after injecting PF-04418948 because that is when this EP2 antagonist exerts its peak effect (af Forselles et al., 2011). In a separate group of rats, the pressor response to TCS 2510 (6.25 μ g/2.5 μ l), an EP4 receptor agonist, was measured before and after intrathecal injection of L-161,982 (4 μ g in 10 μ l), an EP4 receptor antagonist. The second dose of L-161,982 was injected 25 minutes after injecting TCS 2510 because that is when this antagonist exerts its peak effect (Yamauchi et al., 2013). This experiment was repeated with 100% DMSO (10 μ l), the vehicle for PF-04418948 (10 μ g in 10 μ l) and 77% DMSO (10 μ l), the vehicles for L-161,982 (4 μ g in 10 μ l) for the EP2 and EP4 receptor antagonists, respectively.

The effects of these EP receptor antagonists on the exercise pressor reflex were then examined. To do this the hind limb muscles were statically contracted for 30 seconds by stimulating the sciatic nerve (1-2 times motor threshold, 0.01 ms pulse duration, 30-40 Hz) before and 90 minutes after injecting PF-04418948 (10 μ g in 10 μ l, n=6) as well as before and

25 minutes after injecting L-161,982 (4 μ g in 10 μ l, n=4 or 8 μ g in 20 μ l, n=5) intrathecally. This experiment was repeated with 77% DMSO (20 μ l), the vehicle for L-161,982 (8 μ g in 20 μ l). It was not repeated with the vehicle for PF-04418948 (see Results).

In the rats in which the sciatic nerve was stimulated electrically to induce static contraction, at the end of each experiment, pancuronium bromide (500 μ g) was injected intravenously and then the sciatic nerve was stimulated again. The pulse durations, current intensities and frequencies of stimulation were the same after paralysis with pancuronium as those before paralysis. Sciatic nerve stimulation after paralysis with pancuronium did not increase either arterial pressure or heart rate in 32 of the 33 rats tested. The findings from the one rat in which sciatic nerve stimulation after paralysis evoked pressor-tachycardia responses were discarded because the stimulus causing them could be attributed to the electrical stimulation of the thin fiber axons traveling in this nerve.

Data Analysis

In all experiments, baseline as well as peak changes in mean arterial pressure, heart rate, and developed tension were recorded continuously with a Spike 2 data acquisition system (CED, Cambridge) and stored on a computer hard drive (Dell). The initial 30 seconds prior to stimuli were taken as the baseline values. Mean arterial pressure (MAP) is expressed in millimeters mercury (mmHg) and heart rate (HR) is in beats per minute (bpm). The tension-time index (TTI) was calculated by integrating the area between the tension trace and the baseline level and is expressed in kilogram seconds (kg·s). All values are expressed as means $\pm$ standard error (S.E.M.). Statistical comparisons were performed with paired t-tests. The criterion for statistical significance was set at $p < 0.05$.

Results

Inhibition of COX-1 and COX-2

Ketorolac (100 μ g in 10 μ l) significantly attenuated the pressor response to arachidonic acid. Although Ketorolac attenuated the cardioaccelerator response to arachidonic acid, it was not significant (n= 5; Figure 1 A & B). Saline, the vehicle for Ketorolac, had no effect on the pressor or cardioaccelerator responses to arachidonic acid (before vehicle: 77 $\pm$ 20 mmHg, 23 $\pm$ 3 bpm; after vehicle: 86 $\pm$ 9 mmHg, 28 $\pm$ 5 bpm; n=4; $p > 0.05$). Baseline values for MAP were similar before and after injection of Ketorolac, but were significantly greater after injection of the vehicle (i.e., saline), an increase which was presumably caused by a long lasting effect caused by the conversion of arachidonic acid to cyclooxygenase metabolites.

Intrathecal injections of Ketorolac (100 μ g in 10 μ l) significantly attenuated the pressor response to static contraction of the hind limb muscles though the cardioaccelerator response was not altered (Figure 2 A-C). In another group of rats, the vehicle for Ketorolac, namely saline, had no effect on the pressor or cardioaccelerator response to static contraction (before vehicle: 23 $\pm$ 7 mmHg, 37 $\pm$ 15 bpm, 18 $\pm$ 3 kg·s; after vehicle: 23 $\pm$ 3 mmHg, 30 $\pm$ 8 bpm, 15 $\pm$ 3 kg·s; n=4; $p > 0.05$). Baseline values for MAP and HR were similar before and after injecting either Ketorolac or saline.

When sodium cyanide was injected into the carotid artery of four rats before and after injecting intrathecally Ketorolac neither the pressor (before Ketorolac: $+63 \pm 15$ mmHg; after Ketorolac: $+77 \pm 9$ mmHg) nor heart rate (before Ketorolac: -4 ± 8 bpm; after Ketorolac: -14 ± 20 bpm) responses to sodium cyanide were attenuated (both $p > 0.05$).

Inhibition of COX-2

The dose of Celecoxib (100 μ g in 10 μ l) used in our experiments effectively blocked the pressor response to intrathecal injection of arachidonic acid. Celecoxib significantly attenuated the pressor and cardioaccelerator responses to arachidonic acid (Figure 1 C & D). In a separate group of rats 70% DMSO, the vehicle for Celecoxib, had no effect on the pressor or cardioaccelerator responses to arachidonic acid (before vehicle: 74 ± 9 mmHg, 20 ± 3 bpm; after vehicle: 62 ± 15 mmHg, 16 ± 6 bpm; $n=8$; $p>0.05$). Baseline values of MAP were lower before Celecoxib than afterwards, and this could be due to a long lasting effect of metabolites of arachidonic acid, which could still be produced by COX-1. Baseline HR was similar before and after injection of Celecoxib, but was higher after injection of the vehicle ($p<0.05$).

Intrathecal injection of Celecoxib (100 μ g in 10 μ l) had no effect on the pressor or cardioaccelerator response to static contraction of the hind limb muscles (Figure 2 D-F).

Inhibition of EP2 receptors

PF-04418948, the EP2 receptor antagonist, injected intrathecally, significantly attenuated the pressor response, but not the cardioaccelerator response, to Butaprost, the EP2 receptor agonist (Figure 3 A & B). In a separate group of rats, DMSO, the vehicle for PF-04418948, had no effect on the pressor or cardioaccelerator responses to Butaprost (before vehicle: 25 ± 6 mmHg, 6 ± 3 bpm; after vehicle: 28 ± 4 mmHg, 4 ± 1 bpm; $n=4$; $p>0.05$).

Intrathecal injection of PF-04418948 did not attenuate the pressor response to static contraction; however, it did attenuate the cardioaccelerator response (Figure 4 A-C), an effect whose magnitude was small although it was statistically significant. At least in part, the attenuation of the cardioaccelerator response to contraction by PF-04418948 may have been secondary to an increased baroreflex, which in turn was evoked by the increased pressor response to contraction.

Inhibition of EP4 receptors

The EP4 antagonist, L-161,982 (4 μ g in 10 μ l), significantly attenuated the pressor and cardioaccelerator responses to the EP4 agonist, TCS 2510 (Figure 3 C & D). In a separate group of rats, 77% DMSO (10 μ l), the vehicle for L-161,982, had no effect on the pressor or cardioaccelerator response to TCS 2510 (before vehicle: 29 ± 6 mmHg, 16 ± 3 bpm; after vehicle: 52 ± 7 mmHg, 15 ± 4 bpm; $n=4$; $p>0.05$).

In four rats, intrathecal injection of L-161,982 (4 μ g in 10 μ l), the same dose that significantly attenuated the pressor response to EP4 agonist, TCS 2510, had no effect on the pressor or cardioaccelerator responses to static contraction of the hind limb muscles (Figure 5 A-C). When twice the dose of L-161,982 (8 μ g in 20 μ l) was injected in a separate group

of five rats the pressor response to contraction was attenuated but the cardioaccelerator response was not (Figure 5 D-F). In a separate group of four rats 77% DMSO (20 μ l), the vehicle for L-161,982, had no effect on the pressor or cardioaccelerator response to static contraction of the hindlimb (before vehicle: 20 ± 4 mmHg, 11 ± 1 bpm, 18 ± 3 kg·s; after vehicle: 22 ± 3 mmHg, 14 ± 6 bpm, 20 ± 4 kg·s; $n=4$; $p>0.05$).

In a separate group of three rats, L-161,982 (8 μ g in 20 μ l) did not significantly attenuate the pressor (before: $+52 \pm 10$ mmHg, after: $+52 \pm 19$ mmHg) or heart rate (before: -8 ± 13 bpm, after: -12 ± 14 bpm) responses to sodium cyanide (both $p > 0.05$). Consequently, the larger dose of L-161,982 did not attenuate the exercise pressor reflex by exerting a direct action on the sympathetic outflow arising from the intermediolateral horn of the upper lumbar and thoracic spinal cord.

Discussion

The current study showed for the first time that cyclooxygenase (COX) products of arachidonic acid metabolism play a role in the spinal transmission of the exercise pressor reflex. In decerebrated unanesthetized rats, Ketorolac-induced inhibition of COX-1 and COX-2 in the lumbar spinal cord attenuated the exercise pressor reflex, whereas Celecoxib-induced inhibition of COX-2 in the lumbar cord did not attenuate the reflex. Although both forms of the cyclooxygenase enzyme are constitutive to the rat spinal cord (Yaksh et al., 2001, Ghilardi et al., 2004), only COX-1 appeared to play a role in the spinal transmission of the exercise pressor reflex in our experiments. Although Ketorolac has been shown to be more selective in blocking the activity of COX-1 than it is in blocking the activity of COX-2, the difference is not profound, resulting in this agent being categorized as non-selective for the two forms of the enzyme (Pallapies et al., 1995, Warner et al., 1999). This relative lack of selectivity prompted us to determine the effect of Celecoxib, a selective COX-2 inhibitor (DeWitt, 1999), on the exercise pressor reflex. Despite the fact that Celecoxib had no effect on the reflex, its effectiveness as a COX-2 inhibitor was demonstrated by the finding that it attenuated the pressor response to intrathecal injection of arachidonic acid by almost 75%.

The majority of the evidence suggests that spinal COX-2, but not spinal COX-1, was responsible for the cyclooxygenase component causing allodynia, hyperalgesia or neuropathic pain arising from inflamed or injured tissues. For example, intrathecal injections of COX-2 selective inhibitors, such as Celecoxib and L-745337, as well as intrathecal injections of non-selective COX inhibitors, such as Ketorolac, decreased spinal cord PGE2 concentrations induced by inflammation or injury; likewise both types of inhibitors decreased behavioral measures of hyperalgesia (Telleria-Diaz et al., 2010). In contrast, COX-1 selective inhibitors, injected intrathecally, had no effect on behavioral measures of hyperalgesia (Lee and Seo, 2008). In addition, maneuvers which caused neuropathic pain, induced by spinal nerve ligation, or inflammation, induced by injection of Complete Freund's Adjuvant into the foot, markedly increased spinal levels of COX-2, but had no effect on spinal levels of COX-1 (Feng et al., 1993, Beiche et al., 1996, Nishiyama, 2006). Considered together, these findings lead to the conclusion that COX-2, but not COX-1, played a critical role in the spinal transmission of nociceptive input causing neuropathic pain

or hyperalgesia. This conclusion, however, is not held universally. For example, Hefferan et al (2003) reported that constitutively expressed COX-1 in the spinal cord played a critical role in the development of mechanical allodynia 4-8 hours following spinal nerve ligation. Specifically, they found that SC-560, a COX-1 and COX-2 inhibitor, but not SC-236, a selective COX-2 inhibitor, prevented the emergence of allodynia following spinal nerve ligation (Hefferan et al., 2003). It is difficult to draw parallels between these findings, which focused on the role played by spinal cyclooxygenases in the reflex control of the sympathetic outflow, and other findings, which focused on the role played by spinal cyclooxygenases in nociception. The reason for this difficulty is that these experiments assessed the role played by cyclooxygenase in the spinal transmission of thin fiber afferent input arising from muscles that were presumably healthy and unchallenged by inflammation or nerve injury, whereas the latter experiments assessed the role played by cyclooxygenase in the spinal transmission of thin fiber afferent input arising from inflamed tissues or injured nerves.

The current experiments showed that EP4, but not EP2 receptors, played a role in the spinal transmission of the exercise pressor reflex. The available evidence suggests that in the rat both EP2 and EP4 receptors are found postsynaptically on interneurons in the dorsal horn, whereas only EP4 receptors are found presynaptically on the terminals of thin fiber afferents synapsing onto interneurons (Kawamura et al., 1997, Bar et al., 2004). EP2 receptors may be more plentiful and/or potent than EP4 receptors on dorsal horn interneurons because the concentration of agonist needed to increase the responses of these interneurons to mechanical stimulation is much less for the EP2 receptor than for the EP4 receptor (Bar et al., 2004). In our experiments, antagonism of EP2 receptors by PF-04418948 had no effect on the exercise pressor reflex. Nevertheless, the dose of PF-04418948 used was sufficient to attenuate by more than half the pressor response to intrathecal injection of Butaprost, an EP2 receptor agonist. Consequently, it is unlikely that the inability of this EP2 receptor antagonist to attenuate the exercise pressor reflex was because its concentration was below threshold. Findings from the current study suggest that EP4 receptors alone play a major role in the spinal transmission of the exercise pressor reflex in healthy rats.

Intrathecal injections of antagonists to EP1 and EP3 receptors were not investigated. The reason for this involves previous findings suggesting that neither of the two endoperoxide receptors were likely to play a role in the spinal transmission of the exercise pressor reflex in freely perfused, healthy muscles. For example, EP1 receptor knockout mice display mechanical and pain sensitivities that were the same as those of their wild type counterparts (Johansson et al., 2011). Likewise, EP1 receptor knockout mice display normal nociceptive responses to a formalin test (Minami et al., 2001). In addition, topical application of a selective EP3 receptor agonist to the spinal cord attenuated, rather than facilitated, the responses of dorsal horn neurons to nociceptive input arising from an inflamed knee joint of a rat (Natura et al., 2013). Considered together, these findings suggested that EP1 and EP3 receptors do not play a major role in the spinal transmission of the reflex.

Two mechanisms could explain the finding that intrathecal EP4 receptor blockade attenuated the exercise pressor reflex. The first mechanism is that PGE2 production induced the central terminals of group III and IV afferents to increase their release of glutamate and substance P

when these thin fiber afferents were stimulated by contraction of the hindlimb muscles (Kaufman et al., 1985, Hill et al., 1992, Adreani et al., 1996). The second mechanism is that PGE2 production, induced by the release of glutamate and substance P, increased the activity of interneurons in the dorsal horn playing a role in the spinal transmission of the reflex. An important limitation of our findings is that they do not distinguish between the two mechanisms.

Spinal cord cyclooxygenase is responsible for synthesizing several arachidonic acid metabolites in addition to PGE2. Prostacyclin (PGI₂), for example, is one of these metabolites whose presence has been reported in the dorsal horn. The mRNA for the PGI₂ receptor (IP) has been shown to be weakly expressed in the lumbar spinal cord of healthy untreated mice (Doi et al., 2002). The levels of both mRNA translating the IP receptor, as well as the protein comprising it, are substantially increased by inflammation (Doi et al., 2002, Schuh et al., 2014). Due to the relatively weak expression of the mRNA translating the IP receptor in the lumbar cord of healthy non-inflamed mice, its role in the spinal transmission of the exercise pressor reflex was not investigated. The thromboxane (TP) receptor is stimulated by thromboxanes, which are also cyclooxygenase products of arachidonic acid metabolism. Although this receptor has been shown to play a role in stimulating the peripheral endings of group III and IV afferents during static contraction of hindlimb muscles (Leal et al., 2011), its role in the spinal transmission of the exercise pressor reflex is unknown.

In addition to their effects in the spinal cord, prostaglandins play a role in either sensitizing or stimulating the peripheral endings of the group III and IV afferents evoking the exercise pressor reflex (Mense, 1981, Rotto et al., 1990a, Rotto et al., 1990b, Kaufman, 2012). For example, injection of non-selective COX inhibitors into the arterial supply of hindlimb skeletal muscle has been shown to attenuate the reflex (Stebbins et al., 1986). In addition, static contraction of the triceps surae muscles has been shown to result in the intramuscular production of both arachidonic acid (Rotto et al., 1989) as well as PGE2 (Symons et al., 1991, McCord et al., 2008). Furthermore, blockade of the EP4 receptor on the peripheral endings of group III and IV muscle afferents has been shown to attenuate the exercise pressor reflex (Yamauchi et al., 2013). The present finding that intrathecal blockade of the EP4 receptor attenuated the exercise pressor reflex suggests that this endoperoxide receptor functions on both the central and peripheral terminals of the group III and IV afferents when they are stimulated by contraction of freely perfused muscles that are neither inflamed nor injured.

In conclusion, our findings indicate that cyclooxygenase-1, but not cyclooxygenase-2, plays a role in producing the prostanoids participating in the spinal transmission of the exercise pressor reflex in freely perfused hindlimb muscles of decerebrated unanesthetized rats. Our findings also indicate that the EP4 receptor, but not the EP2 receptor, plays a role in this spinal transmission of the reflex. We stress that our findings are applicable to healthy animals only. The role played by both COX-2 and EP2 receptors in the spinal cord in pathophysiological situations, such as when the muscles have been inflamed or have had their afferent nerve supply damaged or irritated, might be quite different from that described in our experiments which have focused on the normal physiological situation.

Acknowledgments

We would like to thank Joyce Kim for excellent technical assistance.

Grants

This work was supported by the National Institutes of Health Grants HL-096570 and AR-059397.

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Highlights

- Spinal cyclooxygenase plays a role in transmitting thin fiber afferent input from healthy contracting muscles
- COX-1, not COX-2, plays a role in producing prostanoids participating in spinal transmission of the exercise pressor reflex
- The EP4 receptor, but not the EP2 receptor, plays a role in this spinal transmission of the exercise pressor reflex

Arachidonic Acid injection

Figure 1.

Effects of intrathecal injection of Ketorolac (100μg in 10μl) or Celecoxib (100μg in 10μl) on the pressor (A and C) and cardioaccelerator (B and D) responses evoked by intrathecal injection of Arachidonic Acid (100μg in 10μl). Baseline values are given within mean bars for their corresponding conditions. Asterisks (*) denotes significantly smaller pressor response after Ketorolac or Celecoxib than before, $p < 0.05$.

Figure 2.

Peak pressor and cardioaccelerator responses to static contraction before and after intrathecal injection of Ketorolac (A and B) or Celecoxib (D and E). Developed tension was similar before and after Ketorolac and Celecoxib (C and F). Baseline values are given within mean bars for their corresponding conditions. Asterisks (*) denotes significantly smaller pressor response after Ketorolac than before.

Figure 3.

Effects of intrathecal injection of PF-04418948 (10 μ g in 10 μ l) or L-161,982 (4 μ g in 10 μ l) on the pressor (A and C) and cardioaccelerator (B and D) responses evoked by intrathecal injection of Butaprost (4ng in 10 μ l) or TCS 2510 (6.25 μ g in 2.5 μ l), respectively. Baseline values are given within mean bars for their corresponding conditions. Asterisks (*) denotes significantly smaller pressor response after PF-04418948 or L-161,982 than before, $p < 0.05$.

Figure 4.

Peak pressor (A) and cardioaccelerator (B) responses to static contraction before and after intrathecal injection of PF-04418948 (10 μg in 10 μl). Developed tension was similar before and after PF-04418948 (C). Baseline values are given within mean bars for their corresponding conditions. Asterisks (*) above mean bars denotes significantly smaller cardioaccelerator response after PF-04418948 than before, $p < 0.05$. Cross (†) following baseline values within mean bars denotes significantly different baseline values after drug or vehicle than before, $p < 0.05$.

Figure 5.

Peak pressor (A) and cardioaccelerator (B) responses to static contraction before and after intrathecal injection of L-161,982 (4 µg in 10 µl). Peak pressor (D) and cardioaccelerator (E) responses to static contraction before and after intrathecal injection of L-161,982 (8 µg in 20 µl). Developed tension was similar before and after L-161,982 (C and F). Baseline values are given within mean bars for their corresponding conditions. Asterisks (*) denotes significantly smaller pressor response after L-161,982 than before.