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ROLE OF PROSTAGLANDINS IN SPINAL TRANSMISSION OF THE EXERCISE PRESSOR REFLEX IN DECEREBRATED 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 (EP) 2 or 4 receptors attenuated the exercise pressor reflex in decerebrated rats. We first established that intrathecal doses of a non-specific COX inhibitor Ketorolac (100 μg in 10 μl), a COX-2-specific inhibitor Celecoxib (100 μg in 10 μl), an EP2 antagonist PF-04418948 (10 μg in 10 μl), and an EP4 antagonist L-161,982 (4 μg in 10 μl) effectively attenuated the pressor responses to intrathecal injections of arachidonic acid (100 μg in 10 μl), EP2 agonist Butaprost (4 ng in 10 μl), and EP4 agonist TCS 2510 (6.25 μg in 2.5 μl), respectively. Once effective doses were established, we statically contracted the hind limb 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 8 μg 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 (10 μg) 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.

Key words: static contraction, thin fiber muscle afferents, cyclooxygenase, endoperoxide receptors, sympathetic nervous system.

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 N-methyl-D-aspartate receptor (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).

PGE2 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

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Abbreviations: bpm, beats per minute; COX, cyclooxygenase; DMSO, dimethyl sulfoxide; EP, endoperoxide receptor; HR, heart rate; kg s, kilogram seconds; mmHg, millimeters mercury; MAP, mean arterial pressure; PGE2, prostaglandin E2; TTI, tension–time index.

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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 ± 1 °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, Hollistan, MA, USA) 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, Cleveland, OH, USA). 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). Pco_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 L_3 to L_5 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 L_4 to L_3 and a catheter (PE-10) was inserted with its tip pointing toward the head. The tip was positioned so that it was at the level of the L_4 and L_5 roots' exit points from the spinal cord because L_4 and L_5 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 L_4/L_5 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 min. The time between injecting the COX inhibitor and the second injection of arachidonic acid was 25 min. Previous studies have shown that intrathecal injections of both Ketorolac and Celecoxib at these concentrations reach peak effect 25 min 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 μl) as well as 70% dimethyl sulfoxide (DMSO) and 30% saline (10 μl), 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 hind limb muscles were statically contracted for 30 s 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 min. The time between injecting the COX inhibitor and the second contraction was 25 min. 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 μg/kg), which stimulated the carotid chemoreceptors was then measured. Twenty-five minutes after Ketorolac was injected intrathecally (100 μg in 10 μl) sodium cyanide (25 μg/kg) was injected again and the pressor responses were measured.

The second protocol determined whether the pressor responses to static contraction of the hind limb 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 an 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