Effects of peripheral and spinal κ-opioid receptor stimulation on the exercise pressor reflex in decerebrate rats

Steven W. Copp, Audrey J. Stone, Katsuya Yamauchi, and Marc P. Kaufman

Heart and Vascular Institute, Penn State College of Medicine, Hershey, Pennsylvania

Running title: κ-opioid receptors and the exercise pressor reflex

Corresponding author: Steven W. Copp

Heart and Vascular Institute

Penn State College of Medicine

500 University Dr., Hershey, PA 17033

e-mail: scopp@hmc.psu.edu
Abstract

The exercise pressor reflex is greater in rats with ligated femoral arteries than it is in rats with freely perfused femoral arteries. The exaggerated reflex in rats with ligated arteries is attenuated by stimulation of μ- and δ-opioid receptors on the peripheral endings of thin fiber muscle afferents. The effect of stimulation of κ-opioid receptors on the exercise pressor reflex is unknown. We tested the hypothesis that stimulation of κ-opioid receptors attenuates the exercise pressor reflex in rats with ligated, but not freely perfused, femoral arteries. The pressor responses to static contraction were compared before and after femoral arterial or intrathecal injection of the κ-opioid receptor agonist U62066 (1, 10, and 100 μg). Femoral arterial injection of U62066 did not attenuate the pressor responses to contraction in either group of rats. Likewise, intrathecal injection of U62066 did not attenuate the pressor response to contraction in rats with freely perfused femoral arteries. In contrast, intrathecal injection of 10 and 100 μg of U62066 attenuated the pressor response to contraction in rats with ligated femoral arteries, an effect which was blocked by prior intrathecal injection of the κ-opioid receptor antagonist nor-binaltorphimine. In rats with ligated femoral arteries, the pressor response to stimulation of peripheral chemoreceptors by sodium cyanide was not changed by intrathecal U62066 injections, indicating that these injections had no direct effect on the sympathetic outflow. We conclude that stimulation of spinal, but not peripheral, κ-opioid receptors attenuates the exaggerated exercise pressor reflex in rats with ligated femoral arteries.

Keywords: skeletal muscle afferents, exercise, peripheral artery disease, spiradoline
Activation of the exercise pressor reflex is responsible, at least in part, for the cardiovascular and ventilatory adjustments to exercise (13). The afferent arm of the reflex is comprised of group III and IV muscle afferents which respond predominately to mechanical and metabolic stimuli, respectively (14, 19). Group III and IV afferents, which together are termed thin fiber afferents, synapse onto interneurons in the dorsal horn of the spinal cord. These interneurons, in turn, project initially to neurons in the ventrolateral medulla and the nucleus of the solitary tract (9, 29), and eventually to autonomic preganglionic neurons. The functional consequences of activating the exercise pressor reflex during exercise include increases in arterial blood pressure, heart rate (HR), and ventilation, all of which contribute to the increases in exercising skeletal muscle blood flow and O₂ delivery (1, 2, 27).

In cats, dogs, and rats, acute or chronic muscle ischemia exaggerates the exercise pressor reflex (8, 19, 27, 32, 36). Our laboratory recently reported, for example, that the reflex is larger in rats whose femoral arteries were ligated 72 hours before the start of the experiment than it was in rats whose femoral arteries were freely perfused (36). When the femoral artery of rats is ligated, the collateral circulation provides sufficient blood flow to the hindlimb muscles when they are at rest, but cannot provide sufficient flow when they are exercising (30, 40). This blood flow pattern to the hindlimb muscles during both rest and exercise closely approximates the blood flow patterns to resting and exercising leg muscles of patients with peripheral arterial disease (4). In addition, the exercise pressor reflex evoked by either static or dynamic exercise is exaggerated in patients with peripheral arterial disease compared to the reflex evoked in healthy control subjects (4-6, 22).
Stimulation of opioid receptors inhibits afferent nociceptive transmission. Specifically, stimulation of both spinal and peripheral μ-, δ-, and κ-opioid receptors has been shown to exert potent anti-nociceptive effects in a variety of pain models (15, 23, 24, 26). Our laboratory has demonstrated recently that stimulation of μ- and δ-opioid receptors located on the peripheral endings of hindlimb muscle afferents attenuated the exaggerated exercise pressor reflex in rats with ligated femoral arteries (17, 37). The effects of κ-opioid receptor stimulation, however, on the exercise pressor reflex in this preparation are unknown.

Based on the findings described above, the present investigation was conducted to determine if peripheral and spinal κ-opioid receptor stimulation would attenuate the exaggerated exercise pressor reflex seen in rats whose femoral arteries were ligated. Specifically, we tested the hypothesis that femoral arterial injection as well as intrathecal injection of U62066, a selective κ-opioid receptor agonist, would attenuate the pressor responses to static contraction in decerebrate, unanesthetized rats whose femoral arteries were ligated 72 hours prior to the experiment. We also tested the hypothesis that femoral arterial or intrathecal injection of U62066 would not attenuate the pressor responses to static contraction in decerebrate, unanesthetized rats whose femoral arteries were freely perfused.

**Methods**

All procedures and protocols described in the present investigation were reviewed and approved by the Institutional Animal Care and Use Committee of the Penn State College of Medicine. Adult male Sprague–Dawley rats (n=73, body weight range 343-500 g) were used in this study. Rats were housed in a temperature controlled environment (24±1 ºC) on a 12:12 h light/dark cycle with food (standard rat chow) and tap water available ad libitum. In 39 rats, the
left femoral artery was ligated 72 h prior to the beginning of the experiment. Specifically, rats were anesthetized with 4% isoflurane (balance O₂), after which the left femoral artery was surgically exposed and ligated tightly (5-0 silk suture) just distal to the inguinal ligament. Three rats were subjected to a sham surgery, which consisted of exposing the femoral artery, and then passing a suture under the artery without tying it. Experiments described below were completed in rats whose left femoral artery was ligated 72 hours before the experiment (“ligated”, n=39), rats subjected to the sham surgery (“sham”, n=3) or in rats who were not subjected to any surgery and thus had patent femoral arteries (“freely perfused”, n=31).

*Surgical procedures.* On the day of the experiment, all rats were anesthetized with 3-4% isoflurane (balance O₂). The trachea was cannulated and the lungs were mechanically ventilated (Harvard apparatus) with the gaseous anesthetic until the decerebration procedure was completed. The right jugular vein and right carotid artery were cannulated with PE-50 catheters to inject fluids and to measure arterial blood pressure (P23 XL, Statham), respectively. Heart rate was calculated beat to beat from the arterial pressure pulse with a Gould Biotach. The left carotid artery was cannulated with either: 1) a PE-10 catheter with the tip advanced to ~3-4 mm above the bifurcation of the abdominal aorta (n=26, see *Femoral arterial injection* below); 2) a PE-50 catheter inserted retrogradely for measurement for arterial blood pressure (n=39, see *Intrathecal injection* and *Control experiments* below), or 3) a PE-50 catheter inserted antegradely with the tip advanced until ~2 mm distal to the bifurcation of the common carotid artery (n=8, see *Control experiments* below). In the 26 rats whose left carotid artery was cannulated with PE-10, reversible snares (2-0 silk suture) were placed around the right iliac artery and vein and the abdominal aorta and vena cava (above the tip of the PE-10 catheter). For all rats, arterial blood gases and pH were measured periodically throughout the experiment with
a blood gas analyzer (ABL 80 FLEX, Radiometer) and maintained within normal limits (PaCO$_2$: 35-45 mmHg, PaO$_2$: ~100 mmHg, pH: 7.35-7.45) by adjusting ventilation and/or administration of intravenous sodium bicarbonate (8.5%). Core temperature was measured by a rectal probe and maintained at ~37-38 °C by a heating lamp.

For all rats in the Femoral arterial injection and Intrathecal injection treatment groups (n=57), a laminectomy was performed to expose the lower lumbar spinal cord from L$_2$ to L$_5$. For rats in the Femoral arterial injection treatment group, a pool was formed using the skin on the back which was filled with warmed mineral oil (37.5 °C). The dura was cut from L$_2$-L$_5$ and reflected so that the L$_4$ and L$_5$ ventral spinal roots (which innervate the muscles of the hindlimb) could be isolated and then cut close to their exit from the spinal cord. For rats in the Intrathecal injection treatment group, the dura was cut at L$_3$-L$_4$ and a saline-filled PE-10 catheter was inserted intrathecally with the tip pointing rostrally and secured at the L$_2$ level with Kwik-Sil® (World Precision Instruments). The left tibial nerve was then surgically exposed and isolated.

For all rats in the Femoral arterial injection and Intrathecal injection treatment groups, the left calcaneal bone was severed and the triceps surae (gastrocnemius, soleus, and plantaris complex) muscles were exposed and isolated. The severed end of the calcaneal tendon was then linked by string to a force transducer (Grass instruments, FT10) which, in turn, was attached to a rack-and-pinion.

All rats were placed in a Kopf customized stereotaxic frame and spinal unit with clamps placed around the pelvis and rostral lumbar vertebrae. Dexamethasone (0.2 mg i.v.) was injected to minimize brainstem edema. A precollicular decerebration procedure was performed and all neural tissue rostral to the section was aspirated. Bleeding was controlled with small pieces of oxidized regenerated cellulose (Ethicon, Johnson and Johnson) and the cranial cavity was packed.
with cotton. Anesthesia was terminated and the rats were ventilated with room air and given a minimum of 60 minutes to recover and stabilize prior to the initiation of any experimental protocol. All experiments were performed in decerebrated instead of anesthetized rats given the evidence indicating that anesthesia prevents the exercise pressor reflex in this species (31).

Experimental procedures. Femoral arterial injection. The cut peripheral ends of the L4 and L5 ventral roots were placed on a shielded stimulating electrode. Baseline muscle tension was set at ~100 g by manually turning the rack and pinion. The hindlimb muscles were statically contracted for 30 seconds by electrically stimulating the cut L4 and L5 ventral roots (40 Hz, 0.1 ms pulse duration, ~2x motor threshold). Following recovery (~5-10 min), we stimulated κ-opioid receptors by injecting 1, 10 or 100 µg of the selective κ-opioid receptor agonist U62066 ((±)-[5α, 7α, 8β]-3,4-Dichloro-N-methyl-N-[7-(1-pyrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide mesylate salt; spiradoline; Sigma-Aldrich)) into the arterial supply of the left hindlimb via the left carotid artery catheter (see above). Before injecting U62066, we tightened the reversible snares around the right iliac artery and vein and the abdominal aorta and vena cava, a maneuver which both directed the injectate toward and partially trapped the injectate within the left hindlimb circulation. The snares were released 5 minutes following the injection and the hindlimb was allowed to reperfuse for 10 minutes. The static contraction protocol was then repeated as described above. Initially, rats were injected with 1 µg U62066. If the preparation was stable (e.g., if bleeding was controlled and arterial blood gasses and pH were within normal limits), serial injection of 10 and 100 µg doses of U62066 were used. In later experiments, some rats received 10 and/or 100 µg of U62066 only. The volume and vehicle for injection for all U62066 doses was 100 µl of saline. In 5 freely perfused and 5 ligated rats which were injected with 100 µg U62066, we subsequently injected 1 µg (in 100 µl of saline) of the
selective δ-opioid agonist DPDPE ([D-Pen$^{2,5}$]Enkephalin, [D-Pen$^2$, D-Pen$^5$]Enkephalin, Tocris). Our laboratory has reported recently that this DPDPE dose attenuated the exercise pressor reflex in both freely perfused and ligated rats (17). Thus, injection of DPDPE served as a positive control confirming that U62066 injected into the femoral artery had access to the endings of the thin fiber afferents innervating the hindlimb muscles. We also injected Evans blue dye (100 μl) in the same manner as that used for the drug injections. In all rats, the left triceps surae muscles were stained blue, a finding which indicated that U62066 injected into the femoral artery had access to the left hindlimb circulation in both groups of rats. Ventral root stimulation was used to initiate contraction in the Femoral arterial injection experiments in order to replicate the experimental protocols used in the previous studies from our laboratory which investigated the effects of peripheral μ- and δ-opioid receptor stimulation on the exercise pressor reflex (17, 37).

In five additional ligated rats, we investigated the effects of 100 μg of U62066 (in 100 μl of saline) injected into the jugular vein on the pressor and cardioaccelerator responses to the static contraction. Blood flow to and from the left hindlimb circulation was likely substantially reduced, but not abolished, when the abdominal and right leg snares were tightened. These experiments were performed, therefore, to determine if systemic circulation of U62066 could explain the slightly, but not significantly, lower peak pressor responses to static contraction we observed in ligated rats following femoral arterial injection of 100 μg U62066 (see results).

Intrathecal injection. The need to maintain an intact dura prevented us from evoking contraction by ventral root stimulation. In the following experiments, therefore, we used tibial nerve stimulation to evoke contraction. Rats were tilted head-up at a 15° angle which was maintained throughout the experiment to prevent the rostral migration of U62066 injected intrathecally. The left tibial nerve was placed on a shielded stimulating electrode. Baseline
muscle tension was set at ~100 g. After a minimum 30 s baseline period, we statically
contracted the triceps surae muscle for 30 s by electrically stimulating the tibial nerve (40 Hz,
0.01 ms pulse duration, ~2x motor threshold). Following recovery (~5-10 min), we stimulated κ-
opioid receptors by injecting 1, 10 or 100 μg of U62066 intrathecally. After 10 minutes, we
repeated the static contraction protocol. Initially, rats were injected with 1 μg U62066. If no
attenuation of the pressor response was observed and the preparation was stable, serial drug
injection and contraction protocols were performed with 10 and/or 100 μg of U62066. In later
experiments, some rats were injected only with the 10 and/or 100 μg of U62066. All intrathecal
U62066 doses were dissolved in 10 μl of saline and flushed with 60 μl of saline, the latter being
the dead space of the catheter. Subsequent to drug injections and contractions, all rats were
paralyzed with pancuronium bromide (0.5 mg/kg i.v.) and the tibial nerve was stimulated to
ensure that the pressor responses observed during contraction were not the result of electrical
activation of the axons of thin fiber afferents in the tibial nerve. In each of the rats used in this
protocol, 10 μl of blue dye (followed by a 60 μl flush) was injected intrathecally. Blue dye never
reached the medulla in any of the rats examined.

Control experiments. To investigate the possibility that pain or discomfort from the
surgical procedure, rather than femoral artery ligation per se, could account for either the
exaggerated exercise pressor reflex or the fact that 10 and 100 μg of U62066 injected
intrathecally attenuated the reflex in ligated rats, we measured the pressor and cardioaccelerator
responses to contraction in sham rats (n=3). In the sham experiments static contraction and
intrathecal injections of 10 and 100 μg of U62066 were performed exactly as described above for
the Intrathecal injection protocol.
In five ligated rats, we attempted to block the attenuating effects of intrathecal injection of 10 and 100 μg of U62066 that was observed in the Intrathecal injection protocol (see Results). Blockade was achieved by prior intrathecal injection of the selective κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI, Sigma-Aldrich, (28)). Nor-BNI’s antagonistic effect on κ-opioid receptors has been reported to require approximately two hours following intraperitoneal injection (10, 21). These reports prompted us to wait about 75 minutes before the first contraction was initiated. We injected 1 μg of nor-BNI intrathecally (in 10 μl of saline followed by 60 μl saline flush) immediately following placement of the intrathecal catheter, which was prior to the decerebration procedure and the 60 min post-anesthesia recovery period. Static contraction of the hindlimb muscles was evoked as described in the Intrathecal injection protocol and the pressor and cardioaccelerator responses were compared among the nor-BNI, 10 μg, and 100 μg U62066 conditions. In preliminary experiments (n=2) intrathecal injection of 1 μg nor-BNI only 10 min prior to intrathecal U62066 injections and subsequent contractions did not block the attenuating effects of either 10 or 100 μg of U62066.

To directly investigate the possibility that intrathecal injections of either 10 or 100 μg of U62066 acted within the brainstem, we activated the peripheral chemoreceptors by intra-carotid artery injection of sodium cyanide (25 μg/kg). Specifically, in 8 ligated rats whose left carotid artery was cannulated (PE-50) antegradely, we compared the pressor responses to sodium cyanide before and after intrathecal injections of 10 and 100 μg of U62066.

Data analysis. In all experiments mean arterial blood pressure (MAP, in mmHg), HR (in bpm), and muscle tension were displayed continuously in real-time with a Spike 2 data acquisition system (Cambridge Electronic Design, Cambridge, UK). Data were recorded and stored on a computer hard drive (Dell) for future off-line analysis. Baseline MAP and HR values
were determined from the 30 s baseline period that preceded contraction. The pressor (increases in MAP) and cardioaccelerator (increases in HR) responses to static contraction and sodium cyanide injection were calculated as the difference between the peak MAP and HR values obtained during contraction and the baseline values. The tension-time index (TTI; in kg·s) for static contraction was calculated by subtracting the area under the tension trace for the 30 s baseline period from the area under the tension trace for the 30 s contraction period (contraction-baseline).

All data are expressed as mean ± SE. Statistical comparisons were performed with either repeated measures or non-repeated measures ANOVAs as appropriate. When indicated by the ANOVA result, individual means were compared with Holm-Sidak post hoc tests. Significance was accepted at p<0.05.

Results

Femoral arterial injection. Consistent with our previous report (37), the peak pressor response to static contraction evoked by L4 and L5 ventral root stimulation was greater in ligated rats (26±2 mmHg) than it was in freely perfused rats (16±1 mmHg, p<0.01). Femoral arterial injection of 1, 10, and 100 μg of U62066 had no effect on baseline MAP or HR in either freely perfused or ligated rats. Most importantly, there were no effects of any dose of U62066, injected into the femoral artery, on the pressor or cardioaccelerator responses to static contraction in either freely perfused or ligated rats (Figure 1). There were no differences between the TTIs among conditions (Figure 1). Average peak tension development was ~600-700 g and it did not differ among conditions in either freely perfused (overall p=0.87) or ligated (overall p=0.87) rats.
In subsets of freely perfused and ligated rats, which received femoral arterial injections of 100 μg of U62066, 1 μg of the δ-opioid agonist DPDPE injected in the same manner as U62066 significantly attenuated the pressor responses to static contraction (Figure 2). Femoral arterial injection of 1 μg DPDPE also attenuated the cardioaccelerator responses to static contraction in ligated rats but not in freely perfused rats. The TTIs were not different between the 100 μg U62066 and 1 μg DPDPE condition (Figure 2). These findings are similar to a recent investigation from our laboratory (17) and demonstrate that U62066 injected into the femoral artery had access to the entire hindlimb circulation in both groups of rats.

To further investigate the slight attenuation of the pressor response to static contraction caused by femoral arterial injection of 100 μg of U62066 found in our experiments (see Figure 1), we injected 100 μg of the agonist into the jugular vein in five additional ligated rats. We found that 100 μg of U62066 injected into the jugular vein markedly and significantly attenuated the pressor and cardioaccelerator responses to static contraction (Figure 3). The TTIs were not different before and after 100 μg of U62066 (Figure 3). This suggested to us that the slightly lower pressor response observed following femoral arterial injection of 100 μg U62066 in ligated rats was likely explained by the κ-opioid agonist circulating to the spinal cord.

**Intrathecal injection.** Similar to our finding in the **Femoral arterial injection** protocol, the pressor response to static contraction was greater in ligated rats (37±5 mmHg) than it was in freely perfused rats (20±3 mmHg, p<0.01). Intrathecal injection of 1, 10, and 100 μg of U62066 had no effect on baseline MAP or HR in either freely perfused or ligated rats. Likewise, intrathecal injection of 1, 10, and 100 μg of U62066 had no effect on the pressor and cardioaccelerator responses to static contraction in freely perfused rats (Figure 4, left panels). Intrathecal injection of 10 and 100 μg, but not 1 μg, of U62066 significantly attenuated the
pressor response to contraction in ligated rats (Figure 4, right panels; see original tracings in Figure 5). The difference in the pressor responses between the 10 and 100 μg U62066 conditions did not reach statistical significance (p=0.27). The cardioaccelerator responses to contraction were significantly attenuated by each dose of U62066 in the ligated rats (Figure 4, right panels). There were no differences in TTIs among conditions (Figure 4). Average peak tension development was ~700-1000 g and it did not differ among conditions in either freely perfused (overall p=0.61) or ligated (overall p=0.96) rats. Neuromuscular blockade via pancuronium bromide injection abolished the pressor responses to tibial nerve stimulation in both freely perfused (1±1 mmHg, p=0.18) and ligated (1±1 mmHg, p=0.07) rats, findings which indicated that responses evoked by contraction were not caused by electrical activation of the axons of thin fiber afferents.

Control experiments. The pressor and cardioaccelerator responses to static contraction in three sham rats before U62066 injection were 20±3 mmHg and 9±2 bpm, respectively. These responses were not different from those found in the freely perfused rats (see Figure 4; pressor response: p=0.93, cardioaccelerator response: p=0.17). Likewise, the pressor and cardioaccelerator responses to contraction in sham rats were not attenuated following intrathecal injection of 10 (pressor: 20±3 mmHg, cardioaccelerator: 9±3) or 100 (pressor: 20±3 mmHg, cardioaccelerator: 7±3) μg of U62066. Pancuronium bromide injection abolished the pressor (1±1 mmHg, p=0.37) and cardioaccelerator (1±1 bpm, p=0.42) responses to tibial nerve stimulation in the sham rats.

When compared to the responses found for ligated rats before U62066 from the Intrathecal injection protocol (see Figure 4), intrathecal injection of 1 μg of the κ-opioid receptor antagonist nor-BNI had no effect on the pressor (Before U62066: 37±5 mmHg, nor-BNI: 32±8...
mmHg, $p=0.59$) or cardioaccelerator (Before U62066: 25±5 bpm, nor-BNI: 14±3 bpm, $p=0.59$) responses to static contraction. This indicates that endogenous spinal $\kappa$-opioid receptor stimulation did not modulate the exercise pressor reflex in ligated rats. Nevertheless, nor-BNI, injected intrathecally ~75 minutes before contraction, prevented the attenuating effects of intrathecal injection of 10 and 100 $\mu$g of U62066 on the pressor and cardioaccelerator responses to contraction (Figure 6). This finding confirms that the attenuating effects of intrathecal U62066 injections were mediated via $\kappa$-opioid receptors. There were no differences in the peak tensions or TTIs among conditions. Pancuronium bromide injection abolished the pressor (1±1 mmHg, $p=0.98$) and cardioaccelerator (1±1 bpm, $p=0.98$) responses to tibial nerve stimulation in the rats injected with nor-BNI.

Intrathecal injection of either 10 or 100 $\mu$g of U62066 had no effect on the pressor and cardiodecelerator responses to intra-carotid artery injection of sodium cyanide (25 $\mu$g/kg) in ligated rats (Figure 7). This finding indicates that the attenuations of the pressor responses to contraction observed following intrathecal injections of 10 and 100 $\mu$g of U62066 in ligated rats were mediated via local spinal $\kappa$-opioid receptor stimulation and were not the result of the rostral migration of the drug to have direct inhibitory effects on either thoracico-lumbar or medullary neurons controlling the sympathetic outflow.

**Discussion**

We found that intrathecal, but not femoral arterial, injection of the selective $\kappa$-opioid receptor agonist U62066 attenuated the exaggerated exercise pressor reflex in rats whose femoral arteries were ligated 72 hours before the start of the experiment. In contrast, neither intrathecal nor femoral arterial injection of U62066 had any effect on the exercise pressor reflex in rats.
whose femoral arteries were freely perfused. We also found that intrathecal injections of
U62066 had no effect on the exercise pressor reflex in sham rats. Intrathecal injection of the
selective κ-opioid receptor antagonist nor-BNI had no effect on the exaggerated exercise pressor
reflex in rats with ligated femoral arteries. Nor-BNI did, however, block the attenuating effects
of intrathecal injection of U62066 in ligated rats. Intrathecal injections of U62066 did not
attenuate the pressor responses to the cyanide-induced stimulation of arterial chemoreceptors, a
finding which supports the notion that the attenuation of the exercise pressor reflex by intrathecal
U62066 was not attributable to its migration to either the spinal sympathetic outflow or to the
medulla. Considered together, our findings demonstrate that stimulation of spinal, but not
peripheral, κ-opioid receptors attenuated the exaggerated exercise pressor reflex in rats whose
femoral arteries were ligated 72 hours before the start of our experiments.

Our laboratory has paid particular attention to the role played by opioid receptors on the
peripheral endings of thin fiber afferents in attenuating the exercise pressor reflex. Previously,
we have shown that stimulation of μ-opioid receptors on the endings of group IV afferents
attenuated the reflex in rats with ligated femoral arteries, but had no effect on the reflex in rats
with freely perfused arteries (37). We have also shown that stimulation of δ-opioid receptors on
the endings of group III afferents attenuated the reflex in both rats with ligated femoral arteries
and those with freely perfused femoral arteries (17). In contrast, we now show that stimulation
of κ opioid receptors on the peripheral endings of group III and IV afferents had no effect on the
exercise pressor reflex in both rats with ligated femoral arteries as well as in rats with freely
perfused arteries. The stimulus applied to the κ-opioid receptors in our experiments was 100 fold
greater than that used in our previous experiments in which we stimulated μ- and δ-opioid
receptors in the periphery. Consequently, we think it is unlikely that 100 μg of U62066, injected
into the femoral artery, was below threshold when we were attempting to stimulate κ-opioid
receptors on the peripheral endings of group III and IV afferents.

Our present finding that intra-arterial hindlimb injection of a κ-opioid receptor agonist
did not attenuate the exercise pressor reflex in freely perfused or ligated rats raises the possibility
that κ-opioid receptors are not found on the group III and IV muscle afferents. We are not aware
of any studies investigating the presence of κ-opioid receptors on the group III and IV afferents
involved in the exercise pressor reflex specifically. Our original hypothesis that intra-arterial
U62066 injection would attenuate the exercise pressor reflex in ligated rats was based on studies
that have reported antinociceptive effects of peripherally administered κ-opioid receptor agonists
(3, 21, 39). We should point out, however, that the antinociceptive effects of peripherally
administered κ-opioid receptor agonists are controversial. For example, some studies reported
that peripherally administered κ-opioid receptor agonists were antinociceptive, an effect which
was prevented by prior administration of a κ-opioid receptor antagonist (3, 39). In contrast,
another study reported that peripheral administration of κ-opioid receptor agonists had no effect
on nociception (38). Still another study reported that peripherally administered κ-opioid receptor
agonists were antinociceptive, but that the effect was not blocked by κ-opioid receptor
antagonists (35). The resolution to these different findings may lie in the modality, location, and
intensity of the noxious stimulus applied to the periphery (16, 21, 25).

Our finding that spinal κ-opioid receptor stimulation attenuated the exercise pressor
reflex in rats with ligated femoral arteries raises the issue of whether the κ-opioid receptors
stimulated by U62066 were located on the central endings of group III and IV afferents or were
located postsynaptically on interneurons in the dorsal horn of the spinal cord. We can only
speculate on this issue but the fact that we could not attenuate the exercise pressor reflex with
high doses of U62066 injected into the femoral artery, suggests to us that the attenuation of the reflex by intrathecal injection of this κ-opioid agonist was attributable to the inhibition of interneurons in the dorsal horn receiving input from group III and IV afferents. It seems reasonable to suggest that if femoral artery ligation had increased the number of κ-opioid receptors on the central endings of group III and IV afferents, then this increase would have also been expressed on the peripheral endings of these afferents as well. We would be surprised to find that the protein comprising the κ-opioid receptor would be transported from the cell body in only one direction, namely towards the spinal cord.

The effects of κ-opioid receptor agonists on the discharge of dorsal horn neurons have been investigated. For the most part, the findings point to an inhibitory effect of κ-opioid receptor agonists on the responses of neurons located in the deep dorsal horn to a mechanical stimulus applied to an inflamed ankle. Specifically, iontophoretic application of a κ-opioid receptor agonist decreased the responses to pressure applied to an inflamed ankle in 7 of 15 of the dorsal horn neurons tested, increased the responses to pressure in 4 of 15 of the neurons tested, and had no effect on the responses in the remaining 4 neurons (34). In contrast, superfusion of the spinal cord with κ-opioid receptor agonists has been reported to increase the responses of neurons in the superficial dorsal horn of rats to mechanical stimuli as well as to increase the size of their receptive fields, effects were thought to be hyperalgesic (12). These somewhat contradictory findings may be explained by differences between the location of the neurons tested as well as by differences between the methods of applying the κ-opioid agonists. Nevertheless, further clarification and investigation is needed.

Dynorphin A and B are the endogenous ligands for kappa opioid receptors. Although dynorphin can be found in the rat dorsal root ganglia, the amount as assessed by
radioimmunoassay per gram of tissue is much less than that found in the dorsal horn (7). In addition, the number of dynorphin cell bodies in laminae I, II and V of the rat dorsal horn has been reported to be increased by inflammatory stimuli (11, 33). The effect of femoral artery ligation on the number of dynorphin containing cells in the rat dorsal horn remains to be determined. Nevertheless, the location of dynorphin containing interneurons in the dorsal horn, namely laminae I, II, and V, is the same as that of the central terminals of group III and IV muscle afferents (10, 18, 20). This correspondence, in turn, raises the possibility that these dynorphin containing interneurons could have an inhibitory effect on input arising from the group III and IV afferents stimulated by exercise. Our data, however, does not support this possibility because nor-BNI, a selective κ opioid receptor antagonist injected intrathecally, did not increase the exercise pressor reflex, a finding which would have been expected if endogenous κ-opioid receptors were stimulated when the hindlimb muscles of ligated rats were contracted.

In conclusion, we found that stimulation of spinal, but not peripheral, κ-opioid receptors attenuated the exaggerated exercise pressor reflex to static contraction in decerebrate, unanesthetized rats whose femoral arteries were ligated 72 hours before the experiment. Given that femoral artery ligation in the rat does not impact blood flow at rest but does reduce blood flow capacity during exercise to 10-20% of normal (30, 40), our preparation offers useful parallels to the blood flow patterns to skeletal muscle in patients with peripheral arterial disease. In addition, our findings add to previous reports from our laboratory demonstrating that peripheral μ- and δ-opioid receptor stimulation in rats with ligated femoral arteries (17, 37) attenuated the exercise pressor reflex. Specifically, stimulation of peripheral μ- and δ-opioid receptors, but not peripheral κ-opioid receptors, may prove useful in the treatment of both
claudication and the exaggerated pressor responses to exercise in patients with peripheral arterial disease. Although the present investigation suggests that spinal k-opioid receptor stimulation may also relieve both claudication and the exaggerated pressor responses to exercise in these patients, our finding that U62066 has only a central action may preclude its usefulness.

**Acknowledgements**

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

**Grants**

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


opioid receptors attenuate the exercise pressor reflex. *Am J Physiol Heart Circ Physiol*

18. Light AR, and Perl ER. Spinal termination of functionally identified primary afferent

19. McCloskey DI, and Mitchell JH. Reflex cardiovascular and respiratory responses

20. Mense S, and Craig AD, III. Spinal and supraspinal terminations of primary afferent
fibers from the gastrocnemius-soleus muscle in the cat. *Neuroscience* 26: 1023-1035,

1990.

22. Muller MD, Drew RC, Blaha CA, Mast JL, Cui J, Reed AB, and Sinoway LI.
Oxidative stress contributes to the augmented exercise pressor reflex in peripheral arterial

23. Nagasaka H, Awad H, and Yaksh TL. Peripheral and spinal actions of opioids in the
blockade of the autonomic response evoked by compression of the inflamed knee joint.
*Anesthesiology* 85: 808-816, 1996.

24. Nagasaka H, and Yaksh TL. Effects of intrathecal mu, delta, and kappa agonists on
thermally evoked cardiovascular and nociceptive reflexes in halothane-anesthetized rats.


Figure captions

**Figure 1.** Effects of femoral arterial injection of 1, 10 and 100 μg of the κ-opioid receptor agonist U62066 on the pressor and cardioaccelerator responses to static contraction in freely perfused and ligated rats. Sample sizes and baseline values are indicated within mean bars for their corresponding conditions. Data are mean±SE. †p<0.05 versus Before U62066 in freely perfused rats.

**Figure 2.** Effects of femoral arterial injection of 1 μg of the δ-opioid receptor agonist DPDPE on the pressor and cardioaccelerator responses to static contraction in five freely perfused and five ligated rats. Experiments were performed in subsets of freely perfused and ligated rats that received femoral arterial injection of 100 μg of the κ-opioid receptor agonist U62066. Baseline values are indicated within mean bars for their corresponding conditions. Data are mean±SE. *p<0.05 versus 100 μg U62066.

**Figure 3.** Effects of intravenous (jugular vein) injections of 100 μg of the κ-opioid receptor agonist U62066 on the pressor and cardioaccelerator responses to static contraction in five ligated rats. Baseline values are indicated within mean bars for their corresponding conditions. Data are mean±SE. *p<0.05 versus Before U62066.

**Figure 4.** Effects of intrathecal injection of 1, 10, and 100 μg of the κ-opioid receptor agonist U62066 on the pressor and cardioaccelerator responses to static contraction in freely perfused and ligated rats. Sample sizes and baseline values are indicated within mean for their
corresponding conditions. Data are mean±SE. †p<0.05 versus Before U62066 in freely perfused rats, *p<0.05 versus Before U62066 within ligated rats.

Figure 5. An example of the pressor and cardioaccelerator responses to static contraction from a ligated rat before U62066 and after intrathecal injection of 100 μg of U62066. Note the marked attenuation of the pressor and cardioaccelerator responses to contraction following intrathecal U62066 injection. BP; blood pressure.

Figure 6. Effects of intrathecal injection of 10 and 100 μg of the κ-opioid receptor agonist U62066 on the pressor and cardioaccelerator responses to static contraction in ligated rats in the presence of prior intrathecal injection of 1 μg of the κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI). Baseline values are indicated within mean bars for their corresponding conditions. Data are mean±SE.

Figure 7. Effects of intrathecal injection of 10 and 100 μg of the κ-opioid receptor agonist U62066 on the peak pressor responses and reductions in HR following sodium cyanide injection. Sample sizes and baseline values are indicated within mean bars for their corresponding conditions. Data are mean±SE.
Figure 1.

**Freely perfused**

Overall p=0.18

**72 h ligated**

Overall p=0.38

Increase in MAP (mmHg)

Before U62066

1 μg

10 μg

100 μg

n=15 88±7

n=5 89±11

n=11 93±6

n=8 95±8

n=11 93±7

n=5 102±9

n=9 90±3

n=8 93±3

Increase in HR (bpm)

Before U62066

1 μg

10 μg

100 μg

n=15 406±24

n=5 436±21

n=11 450±26

n=13 400±13

n=9 382±9

n=18 399±18

n=15 415±15

TTI (kg/s)

Before U62066

1 μg

10 μg

100 μg

n=15 20±3

n=5 22±4

n=11 21±3

n=13 19±4

n=18 21±4

n=15 19±3

n=15 19±3

n=15 19±3
Figure 2.

**Freely perfused (n=5)**

- Increase in MAP (mmHg)
  - 100 μg U62066: 92±11
  - 1 μg DPDPE: 94±11
  - p=0.03

- Increase in HR (bpm)
  - 100 μg U62066: 437±29
  - 1 μg DPDPE: 430±34
  - p=0.60

- TTI (kg*s)
  - 100 μg U62066: 15.0±1.5
  - 1 μg DPDPE: 15.0±1.5

**72 h ligated (n=5)**

- Increase in MAP (mmHg)
  - 100 μg U62066: 93±2
  - 1 μg DPDPE: 105±4
  - p=0.04

- Increase in HR (bpm)
  - 100 μg U62066: 429±19
  - 1 μg DPDPE: 437±14
  - p=0.04

- TTI (kg*s)
  - 100 μg U62066: 15.0±1.5
  - 1 μg DPDPE: 15.0±1.5
Figure 3.

**72 h ligated (n=5)**

- **Increase in MAP (mmHg)**
  - Before U62066: 113±18
  - 100 μg (i.v.): 119±18
  - p=0.04

- **Increase in HR (bpm)**
  - Before U62066: 482±18
  - 100 μg (i.v.): 491±38
  - p=0.04

- **TIT (kg*s)**
  - Before U62066: 15±5
  - 100 μg (i.v.): 15±5
  - p=0.30
Figure 4.

**Freely perfused**

Overall $p=0.92$

<table>
<thead>
<tr>
<th>Condition</th>
<th>Before U62066</th>
<th>1 $\mu$g</th>
<th>10 $\mu$g</th>
<th>100 $\mu$g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in MAP (mmHg)</td>
<td>106±7</td>
<td>104±9</td>
<td>123±19</td>
<td>110±10</td>
</tr>
<tr>
<td>n=16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Before U62066</th>
<th>1 $\mu$g</th>
<th>10 $\mu$g</th>
<th>100 $\mu$g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in HR (bpm)</td>
<td>424±18</td>
<td>422±18</td>
<td>408±13</td>
<td></td>
</tr>
<tr>
<td>n=16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**72 h ligated**

Overall $p<0.01$

<table>
<thead>
<tr>
<th>Condition</th>
<th>Before U62066</th>
<th>1 $\mu$g</th>
<th>10 $\mu$g</th>
<th>100 $\mu$g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in MAP (mmHg)</td>
<td>111±7</td>
<td>114±10</td>
<td>116±10</td>
<td>114±19</td>
</tr>
<tr>
<td>n=10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Before U62066</th>
<th>1 $\mu$g</th>
<th>10 $\mu$g</th>
<th>100 $\mu$g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in HR (bpm)</td>
<td>437±12</td>
<td>419±11</td>
<td>403±16</td>
<td></td>
</tr>
<tr>
<td>n=7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TTI (kg*s)**

Overall $p=0.77$

<table>
<thead>
<tr>
<th>Condition</th>
<th>Before U62066</th>
<th>1 $\mu$g</th>
<th>10 $\mu$g</th>
<th>100 $\mu$g</th>
</tr>
</thead>
<tbody>
<tr>
<td>19±2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Before U62066</th>
<th>1 $\mu$g</th>
<th>10 $\mu$g</th>
<th>100 $\mu$g</th>
</tr>
</thead>
<tbody>
<tr>
<td>20±1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Before U62066</th>
<th>1 $\mu$g</th>
<th>10 $\mu$g</th>
<th>100 $\mu$g</th>
</tr>
</thead>
<tbody>
<tr>
<td>20±1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Before U62066</th>
<th>1 $\mu$g</th>
<th>10 $\mu$g</th>
<th>100 $\mu$g</th>
</tr>
</thead>
<tbody>
<tr>
<td>20±1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Before U62066

After 100 μg U62066

Tension (kg)

HR (bpm)

BP (mmHg)

30 s
Figure 6.

72 h ligated (n=5)

Overall p=0.60

Increase in MAP (mmHg)

1 μg nor-BNI

146±18

10 μg U62066

135±16

100 μg U62066

138±19

Increase in HR (bpm)

1 μg nor-BNI

437±28

10 μg U62066

449±31

100 μg U62066

431±31

Overall p=0.74

Overall p=0.62
Figure 7.

**72 h ligated**

Overall p=0.70

<table>
<thead>
<tr>
<th>Condition</th>
<th>Increase in MAP (mmHg)</th>
<th>Decrease in HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before U62066</td>
<td>144±8</td>
<td>427±20</td>
</tr>
<tr>
<td>10 μg</td>
<td>150±6</td>
<td>440±26</td>
</tr>
<tr>
<td>100 μg</td>
<td>143±6</td>
<td>405±17</td>
</tr>
</tbody>
</table>

Overall p=0.86