Endomorphins potentiate ASIC currents and enhance the lactic acid-mediated increase in arterial blood pressure-effects amplified in hindlimb ischemia

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Introduction:
Intermittent claudication, a manifestation of limb ischemia and atherosclerosis in people with peripheral artery disease (PAD), is characterized as leg pain when walking that is typically alleviated by rest. Tissue acidosis of ischemic muscle is associated with the release of inflammatory mediators such as lactic acid, H+ ions, substance P, arachidonic acid, ATP, and serotonin, collectively known as an “inflammatory soup”. Lactic acid evokes the exercise pressor reflex (EPR) that arises from contracting skeletal muscle and serves to readjust the cardiovascular system via increases in arterial blood pressure, heart rate and ventilation. It is known that the build-up of lactic acid and H+ ions stimulates acid-sensing ion channels (ASICs) of thin muscle afferents and induces cell depolarization via this chemoreflex. ASICs are members of the epithelial Na+ channel/degenerin (ENaC/DEG) family of ion channels. ASIC1-3 are expressed mainly in central and peripheral nervous systems. Dorsal root ganglion (DRG) neurons express primarily ASIC1a and ASIC3 subunits. Ischemia not only causes the release of inflammatory mediators, but also can increase local opioid neuropeptide release. The naturally occurring opioid peptides, endomorphin 1 (E-1) and 2 (E-2). Given the release of endomorphins at ischemic sites and lactic acid-mediated pressor effects, the purpose of the present study was to determine whether these endogenous opioid peptides modulate ASIC currents and thereby, enhance the lactic acid-evoked muscle chemoreflex in rats with either freely perfused or ligated femoral arteries.

Given the opioid abuse epidemic, it is crucial to further study whether clinical opiates also exert similar effects on ASIC3 channel subtype.

Methods:
DRG neuron labeling, isolation and femoral artery ligation:
Femoral ligation of adult male Sprague-Dawley rats was performed under anesthesia three days prior to DRG neuron isolation. Additionally, DRG neurons innervating triceps surae muscle were retrogradely labeled by injecting the muscle with 15-20 μl of Dil (3% in DMSO). Three days postligation, rats were anesthetized with CO2 and quickly decapitated with a laboratory guillotine. The lumbar (L4-L6) DRG tissue was removed and enzymatically dispersed. The dissociated neurons were then plated onto polystyrene culture dishes and stored in a humidified atmosphere containing 5% CO2/95% air at 35Â°C. In some experiments, DRG neurons were pretreated overnight with 0.5 μg/μl pertussis toxin (PTX) in the supplemented MEM.
Electrophysiology:

ASIC currents were recorded from Dil-labeled DRG neurons with an Axopatch 200B amplifier and acquired with custom-designed F6 software written by Stephen R. Ikeda (NIH/NIAAA) employing IGOR Pro (WaveMetrics, Inc.). ASIC currents were evoked by switching external recording solutions with a pH of 7.4 to 6.0. After full recovery at pH 7.40, the cells were preincubated for a minimum of 5 min in the control solution containing the test compound (i.e. E-1 or E-2). Following the preincubation period, the external solution was switched to one that ranged from 6.0 to 7.3. We chose the last sec duration of the test pulse to measure current amplitude as this time period appeared relatively stable.

Data analysis:

Electrophysiological recordings were analyzed with IgorPro and statistical analysis was performed with Prism. All data are expressed as mean ± standard error (SEM). Statistical significance between two groups was performed employing paired and unpaired t-test. Data containing more than 2 groups was evaluated with one-way analysis of variance with repeated measures followed by Tukey’s post-hoc test. A p value <0.05 was considered statistically significant. The concentration-response relationships were determined by the sequential application of endomorphins in increasing concentrations.

Results:

Potentiation of sustained ASIC currents by endomorphins in DRG neurons of freely perfused (FP) and ligated (LIG) rats:

The present study examined the modulation of ASIC currents by endomorphins, especially E-2, under normal and ischemic conditions in DRG neurons. ASIC currents were activated after exposure to an external solution of pH 6.0 in a DRG neuron isolated from a control rat with FP hindlimbs. The ASIC current displayed the typical sustained current under acidic conditions. After return to baseline, the neuron was pre-exposed to an external solution (pH 7.4) containing 10 μM E-2 for 5 min. Thereafter, the solution was switched to one with a pH of 6.0 + 10 μM E-2. Under these conditions, the average enhancement of sustained currents was about 112 ± 35 % (n=17). On the other hand, DRG neurons from rats with ligated femoral arteries showed a 243.7 ± 59% (n=9) enhancement of the sustained current following exposure to 10 μM E-2 + pH 6.0. Which is significantly higher than the FP controls (P=0.025). Similar to E-2, the sustained ASIC currents were potentiated by E-1 exposure, with a more pronounced effect observed in DRG neurons from the LIG group.

To determine whether the enhancement of sustained ASIC currents by endomorphins involved MOR signaling pathways, a separate group of DRG neurons was pretreated with PTX to uncouple MOR from heterotrimeric G proteins. Exposure of PTX-treated DRG neurons to E-2 (10 μM, pH 6.0) resulted in potentiation of sustained ASIC currents. The mean % increase ±SEM of ASIC currents was 114 ± 39% (n=5), a value that was not significantly different (p = 0.757) from DRG neurons of FP rats not treated with PTX (112 ± 35%, n=17). These results suggest that the endomorphin-mediated enhancement of sustained ASIC currents was independent of MOR stimulation.
Desensitization time constants (tau) of the rapidly inactivating ASIC3 and ASIC1a currents in the continued presence of protons are approximately 0.3 sec for the former and vary for the latter ranging from 1.2 to 3.4 sec. We measured $I_s$ in DRG neurons, which express both ASIC3 and ASIC1a, and compared these values to $I_s$ in ASIC3- and ASIC1a-expressing L-cells. The mean tau values from FP and LIG DRG neurons were not significantly different ($p=0.166$) for both groups. Since a functional ASIC is composed of three channel isoforms, the scatter observed in DRG neurons is likely the result of varying ASIC3 and ASIC1a ratios that constitute the whole-cell ASIC current.

Summary:

1-Exposure of DRG neurons to either E-2 or E-1 led to a potentiation of the sustained ASIC currents that was independent of MOR activation since PTX pretreatment did not alter the opioid-mediated current enhancement.

2-The endomorphin-mediated potentiation was significantly greater in DRG neurons isolated from rats with ligated femoral arteries when compared to the control group.