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NEUROMODULATION IN A RAT MODEL OF THE ISOVOLUMETRIC BLADDER CONTRACTION: EVIDENCE FOR AN OPIOID MECHANISM

Hypothesis / aims of study

Neuromodulation of spinal nerve stimulation inhibits the bladder micturition reflex in the rat (1). The aim of this study was to evaluate the role of endogenous opioid system in the neural control of the urinary bladder and in the inhibition of the micturition reflex by spinal nerve stimulation.

Study design, materials and methods

In anesthetized female rats (urethane, i.p. 1.2g/kg), a bared portion of teflon-coated, 40-guage, stainless steel wire (Cooner Wire Co., Chatsworth, CA) was placed under each side of the L6 spinal nerve bilaterally. One jugular vein was cannulated with polyethylene tubing for intravenous (i.v.) administration of tested drugs. A cannula was placed into the bladder via the urethra and the urethra was ligated to ensure an isovolumetric bladder. The urethral cannula was linked with a pressure transducer and the signal was amplified through a DC amplifier. Saline infusion induced the isovolumetric bladder contraction. Frequency/interval of the bladder contraction was analysed to evaluate effects of opioid receptor-selective antagonists on bladder inhibitory response evoked by SN stimulation (pulse-width 0.1 ms, 10 Hz). After a 10 min control period, either saline or opioid receptor antagonists (i.v.) were administered for 5 min before the start of spinal nerve stimulation. Nerve stimulation was applied for total of 15 min and 5 min per intensity tested at 0.8*Tmot (0.8 times motor threshold intensity), 1*Tmot and 2*Tmot. The BRC was recorded for 20 minutes post stimulation.

Results

There was no significant change in the isovolumetric bladder contractions when no electrical stimulation was applied (n=7) during a 50 min recording or to intravenous injections of any of the opioid receptor antagonists. Mu-opioid receptor antagonist naloxone (0.3 mg/kg), kappa- opioid receptor antagonist nor-BNI (2mg/kg) and delta-opioid receptor antagonist naltrindole (5 mg/kg) did not alter the frequency of contractions to 133 ± 30%, 90 ± 12%, and 96 ± 4% of controls, respectively, which are not significantly different from saline injection (110 ± 6% of control, $p>0.05$).

To examine whether endogenous opioid system is involved in neuromodulation of bladder function, nerve stimulation was applied. Following saline injection, spinal nerve stimulation attenuated bladder contraction frequency, directly proportional to the current intensity. 0.8*Tmot, 1*Tmot (0.20 ± 0.02 mA) and 2*Tmot stimulation significantly ($p<0.05$, n=14 vs. control) decreased the frequency of contractions during stimulation to 46.09 ± 10%, 46.02 ± 13%, and 10.28 ± 5% of controls, respectively. Neither kappa-(nor-BNI, 2 mg/kg, i.v.) nor delta- (naltrindole, 5 mg/kg, i.v.) opioid receptor antagonists affected bladder inhibitory responses to spinal nerve stimulation (figure 1A).

In contrast, mu-opioid receptor antagonist (naloxone 0.3 mg/kg, iv, n=7) attenuated bladder inhibitory responses evoked by spinal nerve stimulation at therapeutic current intensity of 0.8*Tmot and 1*Tmot (0.17 ± 0.03 mA). Following naloxone injection, 0.8*Tmot and 1*Tmot stimulations did not change the frequency of contractions to 152 ± 31% and 163 ± 61% of controls, respectively. Student's t-test analysis demonstrates that effects of neuromodulation (0.8*Tmot and 1* Tmot) in naloxone treated group are significantly different from that in saline treated group ($p<0.05$). However, naloxone failed to attenuate the inhibition of the contraction frequency to higher intensity of 2*Tmot stimulation, to 30 ± 15% of control (figure 1B).

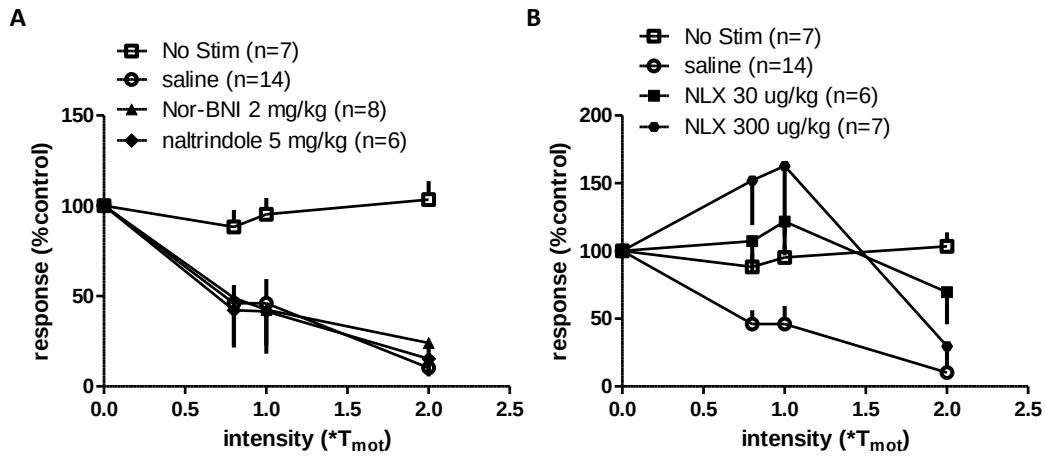

Figure 1. Intensity-dependent effects of spinal nerve stimulation on frequency of isovolumetric bladder contractions following intravenous administration of kappa opioid receptor antagonist nor-BNI, delta-opioid receptor antagonist naltrindole. (**A**) or mu-opioid receptor antagonist naloxone. (**B**) Tmot: motor threshold intensity.

Interpretation of results

It has been reported that electrical stimulation of the spinal nerve produced an intensity-dependent attenuation of the frequency of bladder contractions in the rat, which may be mediated by both afferent and efferent mechanisms. Lower intensities of stimulation may activate large, fast-conducting fibers and actions through the afferent limb of the micturition reflex arc and higher intensities may additionally act through the efferent limb(1). Similarly, in the present study, attenuation of bladder contraction frequency was directly proportional to the current intensity of the spinal nerve stimulation. In addition, intravenous administration of mu-, but neither kappa- nor delta-opioid receptor antagonists attenuated bladder inhibitory responses to low intensities of spinal nerve stimulation. However responses to high intensity (2*Tmot) of stimulation were not attenuated by any of the opioid receptor antagonists. Therefore, the present results demonstrate that the bladder inhibitory effect of neuromodulation at the therapeutic range of intensities occurs by promoting endogenous opioid release and at a mu opioid receptor associated with bladder micturition control. Non-opioid mechanisms are involved in neuromodulation to high intensity of spinal nerve stimulation. In those experiments where antagonists were administered before neuromodulation, we noted no change in frequency of bladder contractions, suggesting that opioid tone was minimally present or contributed to outcomes.

Concluding message

The inhibitory effects on bladder contraction to spinal nerve stimulation may be mediated by both opioid and non-opioid mechanisms. Lower intensities of stimulation may action through the afferent limb of the micturition reflex arc by stimulating endogenous opioid release. Higher intensity stimulation may additionally act through the efferent limb of a non-opioid mechanism. While the specific locations of these opioid mechanisms remain unknown, these data provide evidence for their critical involvement in neuromodulation therapy.

References

1. Neuromodulation in a rat model of the bladder micturition reflex. Am J Physiol Renal Physiol 302: F477–F486, 2012.

Disclosures

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