SAPHENOUS NERVE STIMULATION: A NOVEL BLADDER-INHIBITORY REFLEX PATHWAY EVOKED IN ANESTHETIZED RATS

Hypothesis / aims of study

Overactive bladder (OAB) is a chronic urinary disorder that affects approximately 15% of the adult population. Current treatment options include drugs and sacral (S3) nerve stimulation. Despite various limitations – e.g., side-effects or unintended stimulation-evoked responses – both methods achieve effective treatment of OAB symptoms. Posterior tibial nerve stimulation (PTNS) therapy has recently emerged as a markedly less-invasive alternative to sacral neuromodulation. In lieu of a permanently implanted neurostimulation device, PTNS therapy is achieved by repeated (e.g., weekly) nerve stimulation sessions using a percutaneous needle electrode. The stimulation amplitude of PTNS therapy is typically set at the threshold for eliciting a motor response (e.g., foot twitch). And, depending on the placement of the stimulating electrode within the lower leg, the threshold amplitude can range from 0.5 - 2 mA [1]. However, recent animal studies in both our lab [2] and others have shown that reflex bladder inhibition achieved by PTNS requires very large stimulation amplitudes. In anesthetized rats, bladder-inhibitory responses were evoked by PTNS applied between 3 and 6 times the foot twitch threshold; while similar studies in cats demonstrated bladder inhibition by PTNS applied at 2 to 4 times the motor threshold. Based on these findings, we hypothesized the presence of a secondary sensory pathway that is concomitantly activated by percutaneous PTNS. We tested this hypothesis by investigating the effects of electrically stimulating the saphenous nerve in anesthetized rats. The goal of this study was to characterize the input-output relationship of this reflex pathway by applying low-amplitude electrical pulses at varying stimulation frequencies.

Study design, materials and methods

With approval obtained from the local animal use committee, we conducted a study involving six female Sprague-Dawley rats (250-300g). Each rat was initially anesthetized under isoflurane (1.5 – 3 %) and subsequently switched to urethane (2 IP injections, 1.2g/kg) following completion of all surgical procedures [2]. The bladder dome was catheterized with PE50 tubing, which was connected in series to a pressure transducer (Utah Med, Midvale, UT, USA) and an infusion pump (Harvard Apparatus, Holliston, MA, USA). An incision along the medial aspect of the lower leg provided access to the saphenous nerve (SAFN), caudal to the knee joint. The SAFN was dissected and implanted with a bipolar stimulating nerve cuff electrode. Electrical pulses were provided by an external pulse generator (A-M Systems, Carlsborg, WA, USA). A pair of de-insulated stainless steel wire electrodes was inserted into the external urethral sphincter (EUS) muscle using a perineal approach. Both the bladder pressure signal and EUS electromyogram (EMG) were filtered and amplified. The bladder was emptied and then continuously filled with saline (infusion rate = 0.08 - 0.1ml/min) throughout the remainder of the experiment. Repeated bladder contractions were confirmed by rapid increases in pressure with concomitant bursting EUS activity. The experimental protocol involved an initial baseline period (no stimulation, 10 minute duration) that was followed by alternating 10-minute periods of nerve stimulation (acute response) and no stimulation (prolonged response).

The amplitude of all stimulation trials was set at the predicted threshold for activating large-diameter A-fibres (25 μA, 200μs pulse width) [3], while the stimulation frequency of each trial was varied randomly between 2 Hz and 100 Hz. All data was acquired digitally (sample rate = 20 kHz) using PowerLab 16/35 (AD Instruments Inc.) and analysed post hoc using the LabChart software (AD Instruments Inc.). Bladder function was quantified by calculating the bladder contraction rate (BCR-contractions/min) during each 10-minute interval (e.g., acute inhibition); while bladder inhibition was defined by a minimum 10% decrease in the BCR. Mann-Whitney U test (non-parametric test), and multiple comparison tests were used to analyse the effects of stimulation parameters on bladder function. All data were summarized as the mean ± standard error.

Results

Across all experiments (n = 6 rats), we measured a BCR of 0.6 ± 0.1 contractions per minute during the baseline period and discovered that low-amplitude (25 μA) SAFN stimulation could consistently evoke a bladder-inhibitory response.

- **Acute bladder inhibition** – as defined by a decrease in BCR during SAFN stimulation – was observed in response to trains of electrical pulses applied at frequencies between 5Hz and 100Hz. The extent to which SAFN stimulation inhibited bladder function also varied with respect to the stimulation frequency. The overall response to SAFN stimulation (averaged across all frequencies) resulted in a 31.5 ± 7.11% reduction in the BCR, which ranged from 3.25% to 56.2%. Peak inhibition of acute bladder function was observed during 20 Hz SAFN stimulation; whereas the weakest response was observed during 2 Hz SAFN stimulation. For this data set, statistically significant inhibition was achieved at 20 Hz (p <0.05).

- **Prolonged bladder inhibition** – as defined by a decrease in BCR following SAFN stimulation – was evoked in response to stimulation trials applied at frequencies between 5Hz and 50Hz. The overall prolonged response to SAFN stimulation was a 12.5 ± 11.7 % reduction in BCR (range = 5.0 % to 32.6%). Maximum prolonged bladder inhibition was observed following SAFN stimulation at 10 Hz; whereas the weakest response was observed following 2 Hz SAFN stimulation. For this data set, statistically significant inhibition was achieved at 10 Hz (p <0.05).

Interpretation of results

Our study provides experimental evidence that characterize a bladder-inhibitory reflex mediated by large-diameter SAFN afferents in anesthetized rats. Despite the low stimulation amplitude, the robust manner in which the bladder-inhibitory responses were evoked across all experiments suggests a strong and highly-sensitive reflex pathway. As a direct comparison, reflex bladder inhibition by PTNS in anesthetized rats required the activation of small-diameter (Aδ) fibres, which was 3 to 6 times the amplitude for evoking a foot twitch [1].
Concluding message
The results of this study suggest the presence of a secondary sensory pathway which could potentially contribute to the treatment of OAB symptoms by PTNS therapy. Further work is needed to characterize this reflex pathway and also potentially translate these findings in OAB patients.

References

Disclosures
Funding: University of Toronto - Connaught New Investigator Award Clinical Trial: No Subjects: ANIMAL Species: Sprague-Dawley Rats Ethics Committee: University of Toronto Animal Care Committee