

IN-VIVO HYPOGASTRIC NERVE STIMULATION AND RECORDING DURING BLADDER FILLING

Hypothesis / aims of study

In our investigation of bladder rerouting strategies for reinnervation of the decentralized canine bladder, we hypothesize that the new neuronal pathways may allow sensory reinnervation of the bladder following nerve transfer. It is also possible that different anesthetics may have differential effects on nerve firing activity during bladder filling in both normal intact and reinnervated bladders. We aim to stimulate and record nerve activity during bladder filling in normal intact bladders for eventual application to monitoring sensory reinnervation of the bladder following nerve transfer. The effects of the anesthetics isoflurane versus propofol on increased detrusor pressure following hypogastric nerve electrical stimulation were also compared.

Study design, materials and methods

Electrophysiology studies were designed to perform nerve stimulation and recording in rats followed by canines.

Electrophysiology study in rats: Under isoflurane anesthesia (3-5% induction dose and 1-2% maintenance dose, inhalation), electroneurogram (ENG) recordings were performed in rats, in acute experiments. Sciatic nerves were exposed and stimulations of the hindpaw with Semmes-Weinstein monofilaments of varying forces (10g-300g) were performed. Bipolar cuff electrodes were placed on the sciatic nerve (n=7) to record sciatic afferent nerve discharges. The sciatic nerve was transected between the spinal cord and the electrode, to eliminate efferent nerve signals and record afferent fibers discharge only with hindpaw stimulations. Followed by sciatic nerves recordings, ENG recordings were performed from ureter with associated hypogastric nerves (n=7) and also on only the hypogastric nerve (n=1) during bladder filling with infusion rate of 0.5ml/min. Recordings were made of the nerve discharges with an electrode placed around the ureter with associated hypogastric nerve during bladder filling. This was confirmed by increased bladder pressure with electrical stimulation of the hypogastric nerve enclosed with the ureter in the cuff electrode. All recordings that performed from hypogastric nerve include electrical stimulation (0.2-3mA, 20Hz) of hypogastric nerve before recording. All recordings were performed using a low noise amplifier (SR560, Stanford Research Systems, Sunnyvale, CA) at 10k gain sampled at 20kHz, filtered 300Hz-10kHz, interfaced with a PowerLab Analog to Digital converter and the data was captured using LabChart software (ADInstruments, Colorado Springs CO). Bladder pressure was also recorded at a sampling rate of 10/s during filling.

Electrophysiology study in canines: Female mixed-breed hounds with intact bladder innervation, body mass of 20-25 Kg and 6-8 months of age were used. Maximum changes in detrusor pressures were determined following electrical stimulation (3-10mA, 20Hz) of the hypogastric nerves under isoflurane inhalation anesthesia at 1-3% mean alveolar concentration in oxygen (n=21). Under isoflurane anesthesia, electroneurogram (ENG) recordings were performed from the hypogastric nerves during bladder filling with an infusion rate of 30ml/min followed by using the same instrumentation used to perform recording in rats. Bipolar cuff electrodes were placed on the hypogastric nerve (n=7), to record nerve discharges. Detrusor pressures were recorded at a sampling rate of 4/s during filling. Five of these same dogs were then transitioned to propofol anesthesia administered intravenously by continuous-rate infusion (0.3-0.5 mg/kg/min). Following electrical stimulation and recording, hypogastric nerves were harvested from five dogs undergoing decentralization for other experiments. These nerves were cryosectioned and examined for expression of the adrenergic marker enzyme tyrosine hydroxylase (present in sympathetic peripheral nerves) using immunohistochemical methods.

Results

Electrophysiology test results in rats: Sciatic nerve recordings consistently showed increased afferent fibers discharge with increased size of monofilament used to stimulate the hindpaw, with the highest discharge observed with the 300g monofilament and lowest with 10g. In contrast, recording from ureter associated with hypogastric nerve showed that nerve discharges increased substantially in response to bladder filling in 2 of 7 rats, and increased moderately in 2 other rats. However, there was no response in the remaining rats, perhaps due to nerve damage during cuff placement. Whereas, recording from hypogastric nerve alone in one rat showed increase in nerve discharges during bladder filling. Electrical stimulation of hypogastric nerve caused an increase in bladder pressure before each recording.

Electrophysiology test results in canines: Electrical stimulation of the hypogastric nerve caused an increase in detrusor pressure (2 to 20 cmH₂O) under isoflurane anesthesia in 18 of 21 dogs and no response in the remaining 3. We found 2 of 5 dogs tested under propofol anesthesia showed increased in detrusor pressure of about 8 cmH₂O as compare to isoflurane that showed the increased in detrusor pressure of about 3 to 4 cmH₂O in these same dogs. One of the other 3 dogs showed detrusor pressure of about 6 cmH₂O under both propofol and isoflurane. The remaining two dogs showed an absence of bladder contractions in response to hypogastric nerve stimulation under either anesthetic. Electroneurogram recording from the hypogastric nerve decreased substantially in response to bladder filling in 1 of 7 dogs, and increased moderately in one other dog. However, there was no response in the remaining 5 dogs, perhaps due to nerve damage during cuff placement. Each collected hypogastric nerve showed positive tyrosine hydroxylase immunostaining, confirming that the nerves contain sympathetic fibers.

Interpretation of results

Because we found an increase in afferent nerve discharges during paw stimulation with increased monofilaments sizes in rats this suggest that we may be able to record a similar increase in afferent nerve discharges from the bladder when tension on the bladder wall increases. Results of hypogastric nerve stimulation indicate that the neuronally intact canine detrusor muscle

contracts under both isoflurane and propofol anesthesia. Electroneurogram recordings from the cuff electrode around the ureter-nerve complex in rats and around hypogastric nerve in canines revealed that the nerve discharges changes with an increase in bladder pressure during bladder filling. We did not isolate single fibers (units) from these whole nerve recordings, thus we are not reporting individual nerve fiber activity.

Concluding message

The results from hypogastric nerve stimulation confirm findings by de Groat and colleagues in cats {1,2} and by Elmer in rats {3} showing that electrical stimulation of hypogastric nerves in animals with intact bladder innervation elicits low-amplitude bladder contractions under different anesthetics. Based on results from sciatic nerve recording in rats and hypogastric nerve recording in both species, rats and canines, we conclude that the present technique used to record nerve activity in normal intact bladder may be appropriate to record afferent nerve activity during bladder filling in canines with surgically rerouted neural pathways. No discernible difference between isoflurane and propofol anesthesia was found.

References

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2. de Groat, W., and Ryall, R.W. (1969). J Physiol 1969 Jan;200(1):87-108.
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Disclosures

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