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Electroejaculation functions primarily by direct activation of pelvic musculature: Perspectives from a porcine model

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ABSTRACT

Ejaculatory dysfunction is a significant cause of infertility in men that have incurred spinal cord injury or iatrogenic lesions to the sympathetic nerves in the retroperitoneum. For such patients, electroejaculation – whereby a voltage is applied transrectally under general anesthesia – is a highly-effective procedure to obtain ejaculate. At present, however, there remains uncertainty as to the physiological mechanism by which electroejaculation prompts seminal emission in males with neurogenic anejaculation. Thus, in the present study, we aimed to determine, for the first time, whether electroejaculation functions by mimicking a neurophysiological response, or by directly activating local pelvic musculature. Using electroejaculation in a novel porcine model, we monitored the strength of contraction of the internal urethral sphincter (a smooth muscle involved in ejaculation) before and after lesioning its sympathetic innervation with a combination of progressively-worsening surgical and pharmacological insults in three anesthetized boars (46.1 ± 7.4 kg). Importantly, prior to this investigation, we confirmed the comparative structural anatomy of the porcine model to humans through gross dissection and histological analysis of the infrarenal retroperitoneal sympathetic nerves and ganglia in 18 unembalmed boars. Prior to sacrifice, three of these boars underwent functional testing to confirm control of the internal urethral sphincter by the hypogastric nerves. Our results demonstrate that electroejaculation-induced contraction of the internal urethral sphincter was preserved following each progressive neural insult compared to the control state ($p > 0.05$). In contrast, these same insults resulted in paralysis/paresis of the internal urethral sphincter when its sympathetic innervation was directly stimulated with bipolar electrodes ($p < 0.05$). Taken together, our results provide the first empirical evidence to suggest that electroejaculation does not initiate ejaculation through neural mechanisms, but rather activates pelvic musculature in an independent and non-physiological manner.

1. Introduction

Ejaculatory dysfunction is a significant cause of male infertility in patients that have incurred spinal cord injury [1–4] or iatrogenic lesions to the preaortic sympathetic nerves in the retroperitoneum [5,6]. Consequently, a variety of compensatory techniques have been developed for these patients that aim to permit ejaculation (and thus semen/spermatozoa collection), such as phosphodiesterase type-5 inhibitors [7,8] or α_1-adrenergic agonists [9,10], penile vibratory stimulation (PVS) [11–13] and electroejaculation (EEJ) [14–16]. Although use of PVS fortified with adjunct pharmacological treatment is typically the first choice to treat neurogenic anejaculation [4,15], this approach requires all components of the neural ejaculatory reflex to be intact (i.e., the spinal ejaculation generator, sacral efferents and afferents and thoracolumbar sympathetic outflow) [3,11,17–21]. Thus, EEJ — whereby a voltage is applied transrectally under general anesthesia — remains an essential tool given its success in obtaining ejaculate from spinal cord injured patients or those with injury to the neural

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ejaculatory reflex loop.

Despite its frequency of use and high success rate, there remains uncertainty as to the physiological mechanism by which EEJ prompts seminal emission in males with neurogenic anejaculation. For example, numerous studies have suggested that EEJ activates relevant adrenergic fibers to simulate a response similar to the normal neurological or afferently-induced ejaculatory reflex [5,14,22–28]. In contrast, it has been proposed that EEJ enables seminal emission by directly stimulating the smooth musculature of the pelvic sexual organs [29,30]. Moreover, additional reports on sexual function following spinal cord injury have acknowledged that EEJ may very well stimulate either/both nerve and smooth muscle tissues responsible for ejaculation [31–33].

Given this lack of consensus regarding the putative physiological mechanism(s) of EEJ, the present study aimed to provide the first empirical investigation into whether EEJ functions by mimicking a neurophysiological response, or by directly activating relevant musculature. Using a novel porcine model, we monitored the strength of EEJ-induced contractions of the internal urethral sphincter (IUS; a smooth muscle involved in ejaculation) before and after lesioning its sympathetic innervation during a series of incrementally-worsening insults — a lesion-and-test paradigm that would reveal the underlying mechanism of EEJ. Prior to this investigation, we conducted a series of experiments to first confirm the comparative structural and functional anatomy of the porcine model to humans.

2. Material and methods

All experimental procedures were approved by Western University's Animal Care Committee (AUS Approval No. 2015-037) in accordance with the guidelines established by the Canadian Council on Animal Care and Ontario Animals in Research Act. A total of 21 boars were used in the present study ($\mu_{body\ mass}$ = 35.4 ± 6.5 kg, save one animal that weighed 130 kg; Yorkshire Landrace X Duroc; Caughell Farms [n = 18]/Thalen Livestock [n = 3]). The animals were obtained by purchase as living, uncastrated boars (n = 9; $\mu_{body\ mass}$ = 35.9 ± 10.6 kg, save one animal that weighed 130 kg), or through donation to our urogenital research program as fresh (not embalmed) cadaveric material, having been sacrificed as part of a separate biomedical research program conducted at the Canadian Surgical Technologies & Advanced Robotics (CSTAR) lab at Western University (n = 12; $\mu_{body\ mass}$ = ~35 kg; castrated). Moreover, because the present study sought to evaluate the functional mechanism of EEJ and was not concerned with measures of fertility (i.e., viability of sperm etc.), we favoured the use of boars that were a more manageable size for surgery rather than requiring animals that had reached sexual maturity. Boars were excluded from the present study if there was evidence of previous surgery/dissection that involved the periaortic retroperitoneum.

2.1. Validation of the boar as a suitable large animal model for ejaculatory research

To examine the role of the efferent sympathetic innervation during EEJ, we ultimately examined the effect of progressively-worsening neural lesions on EEJ-induced muscular contractility. The IUS was chosen as a representative smooth muscle because of its distinct location, ease of access and integral role in antegrade ejaculation. However, to ensure translation of the progressively-worsening neural lesions, it was necessary to use a suitable model that was comparable to the known retroperitoneal anatomy of humans. Pigs have been identified as a suitable model for urogenital studies because of their anatomical and functional resemblance to humans [34–38]. However, no literature exists to specifically describe the anatomy of the efferent innervation to the IUS (i.e., the infrarenal aortic plexus and hypogastric nerves) in comparison to humans.

2.1.1. Evaluation of porcine retroperitoneal sympathetic plexuses

To evaluate structural consistency of the efferent sympathetic innervation to the IUS in the boar, the neuroanatomical organization of the infrarenal aortic plexus and hypogastric nerves was examined in 18 unembalmed specimens by gross cadaveric dissection. The arrangement of the infrarenal preaortic sympathetic nerves was examined from the lumbar sympathetic chains to the hypogastric nerves, and mapped according to our previous work in humans [39]. Tissue samples from suspected ganglia, or regions uncertain to be classified as ganglia, were excised for histological confirmation. To confirm the presence of ganglia, 5 µm sections were stained with Haematoxylin and Eosin (H&E) using standard regressive procedures [40], and inspected for the presence of neuron cell bodies. If no neuron cell bodies were observed, subsequent serial sections were examined throughout the tissue. Additionally, using a previously published protocol [39], immunohistochemical staining with anti-tyrosine hydroxylase antibody verified the adrenergic nature of the neurons in one boar. Refer to Supplemental Methods - Histology for further details.

2.1.2. Role of the hypogastric nerves in IUS contractility

To model a complete (bilateral) and/or partial (unilateral) neural lesion of the efferent supply to the IUS in the boar, we needed to verify the presumed control of the IUS by the right and left hypogastric nerves (the caudal continuation of the aortic plexus). This was examined using an intraoperative bipolar electrostimulation protocol combined with a lesion-and-test approach modified from Ando et al. (1993) in three anesthetized boars ($\mu_{body\ mass}$ = 33.9 ± 3.5 kg) [41]. The detailed protocol can be found in Supplemental Methods - Anesthesia. To summarize, IUS contractility was monitored by a pressure-transducer (Product No. MLT844, ADInstruments, Colorado Springs CO, USA) connected to the 3 mL balloon (inflated to a stable pressure between 250 and 400 mmHg) of a 8-10Fr Foley catheter placed securely in the bladder neck, while bipolar electrostimulation was applied (10 Hz, 30 mA, 1 m s pulse width, 200 V, 30s). To access the bladder neck, a small transvesicular incision was made at the apex of the bladder and urine was drained using suction. Subsequently, the apical incision was extended inferiorly along the avascular plane to a point approximately 1–2 cm above the internal urethral orifice — the pressure-sensing catheter was then inserted into the urethra from this superior approach. Three bipolar stimulations (separated by at least 3 min of rest to limit possible muscle fatigue) were performed to obtain the average contractile strength (mean ± SD) for each experimental state. Fig. 1 shows a general schematic depicting the experimental setup for our lesion-and-test approach, with further details of the protocol found in Supplemental Methods - Bipolar Electrostimulation.

2.2. Uncovering the mechanism of EEJ

To address whether EEJ functions by mimicking a neurophysiological response or by directly activating local pelvic musculature in an uncoordinated manner, three living boars ($\mu_{body\ mass}$ = 46.1 ± 7.4 kg) underwent a non-recoverable surgery (Refer to Supplemental Methods - Anesthesia for a detailed protocol) where EEJ-induced contraction of the IUS was monitored during four progressively-worsening insults to its efferent innervation: *(i)* unilateral (right) HN lesion; *(ii)* complete adrenergic nerve block (xylazine); *(iii)* bilateral HN lesion, and; *(iv)* post-mortem with bilateral lesion. The unilateral lesion was performed by transecting the R-HN. Next, a pharmacological nerve block was performed to abolish the remaining sympathetic innervation to the IUS by systemic administration of xylazine (2.4–4.0 mg/kg; ~80% of initial dose). This pharmacological block was implemented prior to physically transecting the remaining L-HN to ensure elimination of any accessory or collateral sympathetic innervation that may also aid in IUS contraction, if present. To verify the neural impairment for each of the aforementioned lesion states, direct bipolar electrostimulation on the efferent nerve (as described in the previous subsection) was performed.