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Young rats exhibit an age- and sex-dependent bladder response to alpha-antagonists but not beta-agonists

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Summary

Introduction
Previous studies have suggested that the onset of alpha- (α) and beta- (β) adrenergic receptor activity is delayed in young animals. The use of alpha1- (α1-) antagonists for dysfunctional voiding, and beta3- (β3-) agonists for overactive bladder in younger children may not be indicated if maturation is required before bladder and urethral adrenergic receptors are active.

Objective
To determine the sex- and age-dependent responses of the bladder and external urethral sphincter (EUS) to α- and β-adrenergic agents in neonatal and young adult rats.

Materials and methods
A total of 72 naïve Sprague-Dawley rats (36 female, 36 male) and 15 bladder-reduced (BR) female Sprague-Dawley rats underwent cystometry and EUS electromyography at 3, 6, and 9 weeks of life. Following administration of WAY 100,635 (0.3 mg/kg, serotonergic receptor antagonist), the non-selective α-agonist phenylephrine (0.3 mg/kg), α-antagonist phentolamine (1–3 mg/kg), β-agonist isoprenaline (3 mg/kg) and β-antagonist propranolol (3 mg/kg) were delivered intravenously. The maximum intravesical pressure (IVP), pressure threshold (PT), intermicturition interval (IMI), contraction duration (CD), burst amplitude and burst frequency were compared after each drug.

Results
The α-antagonist phentolamine lowered the IVP in 9-week-old males without lowering the PT. In contrast, the β-agonist isoprenaline lowered the IVP in both males and females of all ages, also without affecting the PT. Isoprenaline was also effective at shortening the CD in females, suggesting more effective bladder emptying. The α-agonist phenylephrine increased the IVP in 3-week-old and 6-week-old females and 3-week-old males, but this effect was blocked by pretreatment with phentolamine. The β-antagonist propranolol increased the PT in both males and females, and shortened the IMI in females, which was consistent with retention. Phenylephrine increased the burst duration in 9-week-old naïve females, while isoprenaline increased the burst amplitude and duration in 9-week-old BR females.

Conclusions
In the neonatal rat, both α- and β-adrenergic receptors actively regulate bladder function by 3 weeks of life, but the desired effect of decreasing IVP by α-antagonists was delayed until 9 weeks in male rats. In contrast, β-agonists were effective at decreasing IVP in both male and female rats of all ages, which suggests that they are better agents for enhancing bladder emptying in female and young male rats.

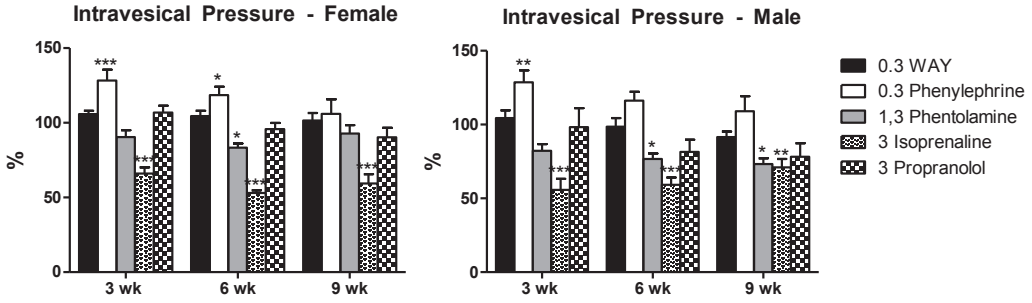

Figure Voiding pressure response to alpha- and beta-adrenergic agents.

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Introduction

The use of alpha1- (α1-) adrenergic antagonists for the treatment of pediatric dysfunctional voiding has yielded mixed results [1—4]. While post-void residuals have been shown to decrease in patients with elevated post-void residuals [1,4,5], a randomized trial of the α1-antagonist doxazosin failed to show a difference in the number of incontinent episodes or post-void residuals compared with placebo, although parents felt that their children were symptomatically improved [2]. While the location of the α1-adrenergic receptor at the bladder neck would suggest that the child with bladder neck dysfunction would benefit more from an α1 blockade than a child with a difficulty in relaxing the pelvic floor [5], previous experiments have suggested that the activity of α- and β-adrenergic receptors are delayed in rabbits until 6 weeks of life [6]. If this were true in humans, then the α1-adrenergic antagonists and β3-adrenergic agonists may not be effective in younger children due to an immature adrenergic system. The present study aimed to determine the timing for the onset of function of the α- and β-adrenergic systems that regulate the rat bladder and external urethral sphincter (EUS), and also determine if the adrenergic agents could enhance bladder emptying in an established rat model of EUS dysfunction — bladder reduction (BR) [7].

It was expected that the α-antagonists would decrease intravesical pressure (IVP) as a result of relaxation of the bladder neck, and if there was an effect on the EUS, a shortening of the contraction duration (CD) would be found, along with an increase in EUS burst amplitude or duration. During voiding, rats have repetitive bursts of EUS activity that draw urine through the urethra, therefore an increase in EUS bursting activity is a marker of improved emptying, not detrusor-sphincter dyssynergia. Administration of an α-agonist should increase IVP and CD, and decrease EUS bursting. Since β-agonists were designed to treat overactive bladders, it was anticipated that they would not change the IVP or pressure threshold (PT), but would increase the intermicturition interval (IMI), which is a marker for bladder capacity. If there were basal β-agonist signaling in the bladder, β-antagonists would be expected to shorten IMI and cause urinary frequency.

Materials and methods

Bladder reduction

The Stanford University Administrative Panel on Laboratory Animal Care approved all procedures. Fifteen 1-week-old female Sprague-Dawley rats underwent BR under 2% isoflurane anesthesia. The animals were placed on a 37 °C water-circulating heating pad. Using sterile technique, a lower midline abdominal incision was made to expose the bladder. The top half of the bladder was stitched with 5-0 Vicryl sutures and tied externally with a 5-0 PDS suture. Lidocaine (0.2%, IP, Hospira, IL) and cefazolin (25 mg/kg, IM, Hospira, IL) were administered. The abdominal muscle and skin were closed using 5-0 Vicryl. The animals were closely monitored until recovery and then returned to the mother. Thereafter, they were monitored daily for 1 week for possible complications and proper weight gain.

Cystometrogram and external urethral sphincter-electromyography

Cystometrogram and external urethral sphincter-electromyography (EMG) were recorded for BR and naïve animals at 3 weeks (BR: five females, naïve: 12 males, 12 females), 6 weeks (BR: five females, naïve: 12 males, 12 females), and 9 weeks (BR: five females, naïve: 12 males, 12 females) of life using a transvesical isotonic preparation. These time points were chosen because rats develop a mature voiding reflex at 3 weeks and no longer require perigenital stimulation by the mother rat to void, are sexually mature at 6 weeks, and attain 80% of adult body weight by 9 weeks. Overflow retention was found in two additional BR females and four additional naïve males at 3 weeks; their data were not included in the analysis. All animals received urethane (1.2 g/kg, subcutaneously) 1 h before surgery, and were placed on a 37 °C water-circulating heating pad. The bladder was exposed via a lower midline incision and a flared-tip PE 50 tubing (BD Intramedic, NJ) was inserted into the top of the bladder dome. The catheter was secured using 5-0 silk. Anesthetized rats were chosen as the preparation since urethane exerts the least inhibition on the voiding reflex, and rats are large enough for accurate recording of the EUS. Cystometry via a suprapubic tube more closely approximates non-catheterized voiding pressures than a transurethral catheter [8]. Two 25-μm Teflon® coated platinum-iridium wire electrodes (A-M Systems, WA) were hooked at the tip of a 25-gauge needle and inserted percutaneously into both lateral sides of the EUS at the middle portion of the urethra. The needle was withdrawn and the electrode was then left embedded in the muscle to record EUS-EMG activity. The bladder catheter was connected to a three-way stopcock that was connected to an infusion pump (World Precision Instruments Inc., FL), and to a pressure sensor (Biopac Systems Inc., CA). The infusion rate was set between 0.05 and 0.10 ml/min. The EUS-EMG electrodes were connected to the amplifier (Biopac Systems Inc., CA) and a grounding cable was placed subcutaneously. Gain was set at 1 k; with a sampling rate of 1250 Hz. High and low pass filters were set at 10 Hz and 500 Hz, respectively. A length of PE-10 tubing was placed in the right external jugular vein for drug administration. Initial cystometry was carried out for 1 h to establish baseline bladder and EUS function. For the first set of experiments, six naïve animals of each sex were used at each time point. WAY 100,635 (*N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate, a 5-HT_{1A} (serotonergic) receptor antagonist, (0.3 mg/kg, IV, Sigma, MO)) was given to naïve animals to block any partial 5-HT_{1A} agonist contribution by the α antagonist [9]. The agonist was given first, followed by the antagonist: phenylephrine hydrochloride (α1-agonist, 0.3 mg/kg, IV, Sigma, MO), phentolamine hydrochloride (α1-, α2-antagonist, 1 mg/kg for 3-week-old animals, 3 mg/kg for all others, IV, Sigma, MO. The 1 mg/kg concentration of phentolamine was used for 3-week-old animals to avoid hypotension), isoprenaline hydrochloride, (non selective β-