Effects of Duloxetine on Urethral Continence Reflex and Bladder Activity in Rats with Cerebral Infarction

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Abbreviations and Acronyms

A-URS = urethral pressure response amplitude during sneezing
BP = baseline pressure
CI = cerebral infarction
ICI = intercontraction interval
LPP = leak point pressure
Pabd = abdominal pressure
PT = pressure threshold
SUI = stress urinary incontinence
UBP = urethral baseline pressure
VC = voiding bladder contraction pressure

Accepted for publication March 11, 2015.

Study received approval from the University of Ryukyus and University of Pittsburgh institutional animal care and use committees.

Supported by grants from the Institute for Food and Health Science, Yazuya and Mitsui Life Social Welfare Foundation, Japan, and National Institutes of Health Grant NIH DK067226.

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Purpose: We investigated the effect of duloxetine, a norepinephrine and serotonin reuptake inhibitor, on the sneeze induced continence reflex and on bladder function in rats with cerebral infarction.

Materials and Methods: Using urethane anesthesia the effect of duloxetine (1 mg/kg intravenously) on the amplitude of urethral responses during sneezing as well as urethral baseline pressure at the mid urethra was evaluated in normal female adult rats and cerebral infarction rats. Tilt leak point pressure was also measured. In normal and cerebral infarction rats continuous cystometry was evaluated before and after duloxetine injection.

Results: In cerebral infarction rats urethral baseline pressure was 43% lower than in normal rats but the amplitude of urethral responses during sneezing did not differ in the 2 groups. Duloxetine increased the amplitude of urethral responses during sneezing and urethral baseline pressure by 31% and 21%, respectively, in normal rats but did not affect either in cerebral infarction rats. Also, in cerebral infarction rats leak point pressure was 29% lower compared with normal rats. Duloxetine increased leak point pressure in normal rats but not in cerebral infarction rats. Cerebral infarction reduced intercontraction intervals without affecting the amplitude of bladder contractions compared with normal rats. Duloxetine prolonged intercontraction intervals in cerebral infarction rats but not in normal rats.

Conclusions: These results suggest that cerebral infarction induces not only bladder overactivity but also stress urinary incontinence, which may account for mixed incontinence in patients with cerebral infarction. After cerebral infarction duloxetine reduced bladder overactivity but failed to enhance active urethral closure mechanisms during sneezing, suggesting that disorganization of the brain network after cerebral infarction might influence the effect of duloxetine on lower urinary tract function.

Key Words: urinary bladder; urethra; urinary incontinence, stress; cerebral infarction; duloxetine

Stress urinary incontinence, the most common type of urinary incontinence in women after middle age, can be caused by 2 impaired closure mechanisms of the urethra (ie urethral hypermobility due to a loss of bladder neck/proximal urethra support and intrinsic sphincter deficiency).[1] In CI

0022-5347/15/1943-0842/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2015.03.091
Vol. 194, 842-847, September 2015
Printed in U.S.A.

survivors there is also a high prevalence of urinary incontinence varying from 12% to 79% depending on time after CI.[2,3] Although urinary incontinence has an important negative impact on quality of life,[4] the mechanisms underlying urinary incontinence after CI have not been studied in detail. In CI the neurological dysfunction is caused by focal brain damage due to ischemia and/or hemorrhage. When brain damage is located in a small area in the right frontal region of the cerebrum, which is involved in the control of micturition, it may result in bladder overactivity and urge urinary incontinence.[5] In addition, it has been reported that CI patients can also show SUI or mixed incontinence.[6] Thus, we hypothesized that CI might be a pathophysiological condition not only of urge incontinence but also of SUI.

Norepinephrine and serotonin reuptake inhibitors such as duloxetine have demonstrated clinical efficacy in the treatment of SUI[7] as well as overactive bladder.[8] We have previously established a rat model that can be used to examine sneeze induced active urethral closure mechanisms that are mediated by somatic nerve induced reflex contractions of the external urethral sphincter and the pelvic floor striated muscles.[9] We have also reported that duloxetine enhances these mechanisms via activation of spinal noradrenergic and serotonergic systems in normal rats.[10,11] However, to our knowledge it has not been investigated whether these mechanisms are affected by CI.

Therefore, we examined the effect of duloxetine on the sneeze induced continence by using urethral microtransducer tipped catheter methods and LPP measurements in normal rats and rats with CI. To clarify the effect of CI on bladder function we also investigated the effect of duloxetine on continuous cystometrograms using normal and CI rats.

MATERIALS AND METHODS

Animals

A total of 44 adult female Sprague Dawley® rats weighing 238 to 270 gm were studied using experimental protocols approved by the University of Ryukyus and University of Pittsburgh institutional animal care and use committees. Experiments were performed in normal rats and rats with CI.

Cerebral Infarction

CI was produced according to methods described previously.[12] Briefly, rats were anesthetized with 2% isoflurane and the right carotid bifurcation was exposed through a midline incision in the neck. The common carotid artery was occluded and the branches of the external carotid artery were dissected and divided. The pterygopalatine branch was identified and ligated close to its origin. A 4-zero monofilament nylon thread with the tip rounded by exposure to a flame was introduced into the internal carotid artery and advanced 17 mm from the carotid bifurcation to the origin of the middle cerebral artery, where it occluded blood flow and, thus, induced infarction on the right side of the brain. After suturing the neck incision the rats were placed in a restraining cage and allowed to recover from isoflurane anesthesia. All rats showed postoperative neurological deficits characterized by left hemiparesis and eyelid ptosis. Experiments were done 3 days after CI induction

Sneeze Reflex and Sneeze Induced Urethral Continence Reflex

The sneeze reflex, which is a highly coordinated reflex evoked by irritation of the nasal mucosa, removes irritations and cleans the airway. In this study the sneeze reflex induced by a rat whisker cut and inserted gently in the nostril while under urethane anesthesia was used to increase abdominal pressure.

Duloxetine Effect Experiments

1) Mid Urethral Pressure Responses and Baseline Pressure. A total of 12 normal and 6 CI rats were examined. The bladder was emptied. A 3.5Fr SPR-524 nylon catheter (Millar Instruments, Houston, Texas) with a side mounted microtransducer located 1 mm from the catheter tip was inserted in the urethra from the urethral orifice according to previously described methods.[9,10,13]

In normal and CI rats the sneeze reflex was induced, and A-URS and UBP were measured. A-URS was determined as the maximal pressure change in cm H_2O from baseline. Average UBP was obtained from a plateau section of pressure recordings with a 1 to 2-minute duration just before the sneeze response according to our previous reports.[10,13] Sneeze induced responses were measured before and after intravenous injection of duloxetine (1 mg/kg, Kemprotec, Middlesbrough, United Kingdom). Sneeze reflexes were evoked repeatedly to obtain at least 10 measurable responses before and after duloxetine treatment.

To evaluate the intensity of the induced sneeze, which varied among sneeze events, Pabd increases during sneezing were also measured via an intra-abdominal balloon catheter inserted through the rectum because it was not possible to measure intravesical pressure in the emptied bladder. Sneeze induced increases in Pabd were determined as pressure values in cm H_2O measured from baseline to peak of the pressure responses according to our previous reports.[10,13]

2) Tilt LPP. In 8 normal and 6 CI rats urethral function was measured using the vertical tilt/intravesical pressure clamp method.[14] Intravesical pressure was increased in 1 to 2 cm H_2O increments via the suprapubic tube by raising a reservoir with saline solution containing Evans blue (100 μg/ml, Sigma®). The pressure at which visible leakage from the urethral meatus occurred was defined as LPP. Mean values of 3 or 4 estimates were analyzed. After control LPPs were obtained duloxetine (1 mg/kg) was injected intravenously and intravesical pressure was increased again to evaluate the effect of the drug on LPP.