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COMPARISONS BETWEEN VOLUNTARY BEHAVIOUR AND REFLEX RESPONSE IN CONTROL OF THE LOWER URINARY TRACT FUNCTION OF MICE

Hypothesis / aims of study

In studies of the lower urinary tract (LUT) function, experiments using conscious animals previously acclimatized in metabolic cages are the most suitable for examining natural "micturition *behaviour*" of the species, although the information derived from the evaluation are somewhat limited. On the other hand, to obtain a detailed profile of "*reflex* micturition" activity without an influence of animal's mood, studies using *in-vivo* cystometrograms (CMGs) in anaesthetized or decerebrate unanaesthetized animals are necessary [1]. Furthermore, a combination of these manners, which covers methodological demerits of each, is expected to be more effective for exploring the mechanism underlying the LUT function. Thus, present studies using both of these two methods were conducted to determine an involvement of the forebrain in control of the LUT function by comparing between “voluntary *behaviour*” (under conscious states) and “*reflex* response” (under decerebrate unanaesthetized conditions) and to further examine differences between female and male mice in the mechanism.

Study design, materials and methods

Thirty-six C57BL/6 mice (12-13 week-old, n=18 for each sex) were used. For evaluating micturition *behaviour*, conscious mice were individually placed in newly-designed metabolic cages, which were improved for precise collection of voided urine, for consecutive 5 days, and data of voided urine and water-intake were continuously recorded into a computer. For CMG study, mice were decerebrated under sevoflurane anaesthesia and intravesical pressure was recorded *via* a PE-50 tube inserted into the bladder dome. CMG recordings were conducted under unanaesthetized conditions by continuously infusing saline (10 μl/min) at room temperature.

Evaluated parameters are: water intake (μl/day), voided urine volume (μl/day), urine volume/void (μl), voiding time/void (s), number of voids(/day), mean uroflow rate (μl/s) in metabolic cage study using conscious mice (i.e., micturition *behaviour*); and maximal voiding pressure (MVP, mmHg), closing peak pressure (CPP, mmHg) which is the peak pressure during nonvoiding phase of a bladder contraction, bladder compliance (BCP, μl/mmHg), voided volume (VV, μl), volume threshold for micturition (VT, μl), voiding efficiency (VE, %) in CMG study using decerebrate unanaesthetized mice (i.e., *reflex* micturition).

All values are expressed as mean ± S.E.M. Statistical analyses were made using unpaired *t*-test: $p < 0.05$ was considered significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Results

Values in parameters associated with involuntary micturition *behaviour* in conscious mice and those associated with *reflex* micturition in decerebrate unanaesthetized mice are presented in Tables 1 and 2, respectively. Urine volume/void in conscious mice is associated with VV in decerebrate unanaesthetized animals, however the former is significantly greater than the latter (female, $p<0.05$; male, $p<0.0001$).

Table 1 Voluntary micturition *behaviour* in conscious mice

	Water intake (μl)/day	Urine volume (μl)/day	Urine volume (μl)/void	Voiding time (s)	Number of voids/day	Mean uroflow rate (μl/s)
Female	2892 ± 320	1640 ± 126	255.4 ± 38.6	2.9 ± 0.4	7.3 ± 0.8	89.3 ± 6.4
Male	2446 ± 197	1498 ± 89	425.4 ± 51.8*	5.7 ± 0.6**	3.9 ± 0.5**	73.6 ± 3.4*

Female, *n*=8; male, *n*=8. Asterisk(s): significantly different between female and male.

Table 2 *Reflex* micturition during CMGs in decerebrate unanesthetized mice

	MVP (mmHg)	CPP (mmHg)	BCP (μl /mmHg)	VV (μl)	VT (μl)	VE (%)
Female	27.3 ± 1.1	11.9 ± 0.5	26.4 ± 3.3	162.6 ± 12.7	167 ± 12.4	97.2 ± 0.6
Male	23.3 ± 1.0*	8.3 ± 0.6***	31.9 ± 2.5	146.5 ± 8.8	150.3 ± 9.0	97.4 ± 0.4

Female, *n*=10; male, *n*=10. Asterisk(s): significantly different between female and male.

Interpretation of results

In conscious mice there were sex differences in *behaviours* of both voiding (i.e., voiding time, mean uroflow rate) and urine storage (i.e., urine volume/void, and number of voids/day), whereas in decerebrate unanaesthetized mice sex differences were found in parameters associated with bladder contraction pressures (i.e., MVP, CPP) but not in those with urine storage (i.e., BCP, VT). VV in decerebrate unanaesthetized mice are significantly smaller than urine volume/void in conscious mice, suggesting the possibility that the forebrain has regulatory projections to the brainstem (i.e., at least, inhibitory to the bladder) that can suppress a neural circuit of the reflex micturition and facilitate urine storage. Under conscious states (i.e., “*voluntary*”), male mice held larger urine volume than female mice did, although in decerebrate unanaesthetized conditions (i.e., “*reflex*”) there was no differences between the female and the male in the VV and VT (i.e., functional bladder capacity).

Concluding message

The metabolic cage experiment using conscious animals is advantageous to examine "*voluntary behaviour*" of micturition and the *in-vivo* CMG using decerebrate unanaesthetized animals is useful to determine "*reflex* activity" of the LUT. Applying both methods for evaluations makes it possible to distinguish "*reflex*" from "*voluntary*" activity and to clarify roles of the forebrain in the control of the LUT function. The present study revealed that the tonic control from the forebrain to the *reflex* micturition circuit can significantly facilitate urine storage and that in the storage period male mice tend to hold urine volume larger than female mice do under conscious states.

References

1. Yoshiyama M et al., Am J Physiol 295:R954-R960 (2008)

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