Cardiovascular and Intravesical Pressure Responses during Natural Micturition in Conscious Rats

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Abstract: Urinary bladder distension is known to influence the cardiovascular system under a pathophysiological condition such as spinal cord injury, hypertension, and arteriosclerosis. A reflex due to bladder distension and/or contraction is considered as one reason for the cardiovascular disturbance associated with micturition. However, it has remained unknown how much intravesical pressure (IVP) rises during micturition in daily life and to what extent mean arterial blood pressure (MAP) and heart rate (HR) respond at that time. To answer these questions, we attempted to examine the direct changes in IVP, MAP, and HR during natural micturition in freely moving conscious rats. IVP increased from the baseline value of 4 ± 0.2 mmHg to 14 ± 0.5 mmHg during natural micturition. Although MAP and HR began to increase before micturition, the increases in MAP and HR became significant 1–4 s before its onset. The peak increases in MAP and HR (7 ± 0.8 mmHg and 14 ± 3 beats/min, respectively) were delayed by 2 s from the peak IVP. Following an administration of xylocaine into the urinary bladder, the increases in MAP and HR during micturition were significantly blunted to 5 ± 2 mmHg and 8 ± 3 beats/min, although IVP increased the same as it did during micturition without xylocaine. Moreover, the relationship between IVP and MAP or HR during natural micturition resembled that between IVP and the vesico-cardiovascular reflex responses during isovolumic bladder contraction in anesthetized rats. Therefore it is concluded that natural micturition in freely moving conscious rats accompanies the significant cardiovascular responses despite a limited increase in intravesical pressure, to which a reflex from the urinary bladder may substantially contribute. [The Japanese Journal of Physiology 54: 567–574, 2004]

Key words: natural micturition, intravesical pressure, arterial blood pressure, heart rate, reflex from the urinary bladder.

A cardiovascular symptom such as transient nocturnal micturition syncope, angina pectoris, or cerebrovascular accident in hypertensive patients or autonomic dysreflexia with thoracic spinal cord lesion has been frequently observed during micturition behavior [1–5]. As one possible reason for the cardiovascular disturbance associated with micturition behavior, a reflex from the urinary bladder elicited by bladder distension and/or contraction may be considered. When intravesical pressure and volume are largely increased by an infusion of saline into the urinary bladder through a urethral catheter, the bladder distension causes reflex increases in external carotid, cardiac, renal, adrenal, and splenic sympathetic nerve activities in anesthetized rats, cats, and dogs [6–12]. In previous studies that used anesthetized animals, intravesical pressure was raised to more than 50 mmHg. Therefore an activation of vesical mechanosensitive afferents leads to a generalized excitation of the sympathetic nervous system, which may induce a pressor
response and tachycardia. However, it is unknown whether such high vesical pressures as those used in acute animal experiments and cystometrogram studies in humans are developed during natural micturition in conscious animals and humans. An increase in intravesical pressure may be limited because urine is excreted through the urethra pathway during natural micturition. If so, the cardiovascular responses associated with micturition will lessen under the natural condition.

To clarify the cardiovascular responses and intravesical pressure (IVP) during natural micturition, we attempted to simultaneously measure arterial blood pressure (AP), heart rate (HR), and IVP by using freely moving conscious rats. Furthermore, to determine whether a reflex from the urinary bladder mainly evoked the cardiovascular responses to natural micturition, we examined the effect of xylocaine injected into the urinary bladder on the cardiovascular responses during natural micturition.

METHODS

The experiments were performed on eight Sprague-Dawley male rats (3 conscious, 5 anesthetized), weighing 311 ± 34 g, in accordance with the “Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences” approved by the Physiological Society of Japan. The present experimental protocols were also approved by the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development, Hiroshima University.

Preparations. The rats were anesthetized by inhaling a mixture of 4% halothane, N₂O, and O₂ for the implantation of catheters and electrodes. Atropine sulfate (0.03 mg) was injected intramuscularly as a preanesthetic medication to reduce salivation and bronchial secretion. The rats breathed the gas mixture spontaneously through a mask during surgery. The electrocardiogram (ECG), HR, and respiration were continuously monitored. To maintain the level of surgical anesthesia, we increased the concentration of halothane 1.0–1.5% if we observed an increase in HR and/or respiration and/or a withdrawal of the limb in response to noxious stimulus, such as a pinch of the paw and/or surgical procedure. Rectal temperature was maintained by a heating pad at 37–38°C during surgery.

Polyvinyl catheters were inserted into the left external jugular vein for administering drugs and into the left carotid artery for measuring AP. A pair of stainless steel wire electrodes was implanted under the skin of the left chest for monitoring ECG. The urinary bladder wall was pierced with a 20 G needle, and a polyvinyl catheter was inserted and fixed on the wall with a drop of vet bond (No. 1469, 3M Animal Care Product, St. Paul, WI, USA). The urinary bladder catheter was connected to a three-way stopcock for measuring IVP or infusing 2% xylocaine. IVP was not measured during drug infusion. The arterial, venous, and intravesical catheters were tunneled subcutaneously and exteriorized at the back of the neck. The rats were housed in their cages and warmed with a heating pad, and they were recovered from anesthesia soon after surgery. We confirmed urine flow through the urethra. An antibiotic (penicillin G, 10,000 units, MEIJI SEIKA, Tokyo, Japan) was given intramuscularly for 2–3 days following the operation. When the rats were killed and dissected after the experiments, we confirmed no leakage of urine from the bladder wall.

Recording of data. The AP and IVP were measured through the carotid artery and intravesical catheters connected to pressure transducers (DPTIII, Baxter, Tokyo, Japan). HR was derived from the R-wave of ECG by a tachometer. AP, IVP, HR, and ECG were simultaneously recorded on an 8-channel penwriting recorder (8K23, NEC Sanei, Tokyo, Japan) and sampled at 200 Hz with an analog–digital converter (MP100, BIOPAC systems; Santa Barbara, CA, USA). Mean arterial blood pressure (MAP), IVP, and HR over 1 s were calculated by means of a conventional software program (Acqknowledge 3.7.0; BIOPAC systems; Santa Barbara, CA, USA) in offline analysis.

Experimental protocols

I. Changes in IVP, MAP, and HR during natural micturition. We conducted the experiments with conscious rats over a period of 1–3 days after implantation surgery. They could move freely in a transparent plastic cage, eat food, and drink water ad libitum. IVP, MAP, and HR were continuously measured throughout the experiments. The rats spontaneously micturated without artificial stimulation. A total of 47 trials of natural micturition were observed in 3 conscious rats, and the changes in IVP, MAP, and HR during micturition were analyzed. To estimate urinary volume during natural micturition in conscious rats, the urinary bladder was emptied, then distended by an infusion of warm saline (volume, 0.6 ± 0.1 ml) in 28 trials. Immediately after this infusion, the saline was not expelled. All rats began to urinate in about 10 s, suggesting that urine volume excreted during natural micturition may be close to 0.6 ml.
To examine whether a reflex from urinary bladder afferents induced the cardiovascular responses during natural micturition, 2% xylocaine (volume, 0.2 ± 0.1 ml) was administered into the urinary bladder; it was retained for 30 min without excretion. An introduction of micturition with an injection of warm saline then washed out the xylocaine. During the subsequent two hours, the rats micturated spontaneously without any infusion (n = 5 trials in 3 conscious rats). The changes in IVP, MAP, and HR during micturition were analyzed between the absence and presence of xylocaine.

II. Reflex responses in MAP and HR during isovolumic bladder contraction. The rats (n = 5) were anesthetized with sodium pentobarbital (35 mg/kg, I.P.). The urinary bladder was exposed with a middle incision of the abdominal area. An intravesical catheter was inserted through the urethra for measuring IVP, and the ureters, which were isolated from the urinary bladder, were cannulated. Rectal temperature was maintained at 37–38°C with a heating pad. The IVP, MAP, ECG, and HR were recorded with the same procedures as those used for conscious rats. When saline (volume, 1.0 ± 0.2 ml) was infused into the urinary bladder, a series of isovolumic bladder contractions were evoked at intervals of 40–110 s, and the reflex responses in MAP and HR during the bladder contractions were identified (n = 43 trials in 5 anesthetized rats).

Data treatment. The responses in the IVP, MAP, and HR in each trial of micturition were viewed on a computer display. The data points that contained a distinct noise were excluded from further analysis. The IVP signal was differentiated as shown in Fig. 1. When the differentiated IVP (dVP/dt) reached the maximum value (denoted [dVP/dt]_{max}), spontaneous micturition usually started. On the other hand, when the dVP/dt reached the minimum value (denoted [dVP/dt]_{min}), spontaneous micturition ended. Because the rats showed unique micturition behavior (raising the tail before urination), it was easy to visually confirm the start of urinary excretion, which was manually marked by turning on an electric switch. When the marker signal and the (dVP/dt)_{max} were compared in off-line analysis, they were virtually coincident, indicating that the (dVP/dt)_{max} represented the micturition onset. Similarly, the (dVP/dt)_{min} seemed to represent the end of micturition. In an individual trial, the average values of IVP, MAP, and HR obtained 20–70 s before the (dVP/dt)_{max} were defined as the baseline levels. Their changes from the premicturition baseline levels were aligned at the time point of

![Fig. 1. The responses in intravesical pressure (IVP), arterial blood pressure (AP), heart rate (HR), and the differential wave of IVP (dVP/dt) during natural micturition in a conscious rat. The maximum and minimum values of dVP/dt, denoted as (dVP/dt)_{max} and (dVP/dt)_{min}, are marked by upward (↑) and downward (↓) arrows. The (dVP/dt)_{max} was almost identical to the start of spontaneous micturition. The (dVP/dt)_{min} was almost identical to the end of micturition.](image-url)
the \((dVP/dt)_{\text{max}}\) or the \((dVP/dt)_{\text{min}}\) and further averaged over the trials.

**Statistical analysis.** The changes in IVP, MAP, and HR during natural micturition were statistically analyzed by the use of a one-way analysis of variance (ANOVA). When a significant F-value in the main effect was present, a Dunnett post hoc test was performed to see if there was a significant difference between the baseline control and the values at a given time. The peak responses in IVP, MAP, and HR during natural micturition were compared by using an unpaired \(t\)-test between the absence and presence of xylocaine injected into the urinary bladder. The level of statistical significance was defined as \(P < 0.05\). All data are expressed as mean ± SE.

**RESULTS**

**Changes in IVP, MAP, and HR during natural micturition.** The changes in IVP, AP, HR, and \(dVP/dt\) during natural micturition in a conscious rat are exemplified in Fig. 1. A gradual increase in IVP was observed before micturition. When the increase in IVP was rapidly accelerated, the rat began to urinate; the \((dVP/dt)_{\text{max}}\) was almost identical to the start of spontaneous micturition. IVP started to decline during micturition, and the \((dVP/dt)_{\text{min}}\) was almost identical to what it was at the end of micturition, after which the elevated IVP returned to the baseline level within approximately 10 s. AP and HR increased in parallel with the changes in IVP during natural micturition. The increases in AP and HR reached the peak with a time delay of 2–3 s from the peak of IVP. With a decline in IVP following micturition, the AP recovered quickly to the baseline level and the HR returned to it slowly.

The time courses of the average changes in IVP, MAP, and HR during natural micturition in conscious rats are shown in Fig. 2. The baseline value of IVP was 4 ± 0.2 mmHg. Before micturition, IVP gradually developed to 7 ± 0.3 mmHg over approximately 20 s, at which the increase in IVP was abruptly accelerated; it appeared to be a threshold for initiating micturition. The IVP reached the peak value of 14 ± 0.5 mmHg; its peak rise during natural micturition was 10 ± 0.5 mmHg from the baseline level. The elevated IVP began to decrease before the \((dVP/dt)_{\text{min}}\), and it returned to the baseline level within 15 s. The baseline MAP was 110 ± 3 mmHg and the baseline HR 455 ± 12 beats/min. Although MAP and HR began to increase before micturition, their increase became significant \((p < 0.05)\) 1 to 4 s just before the \((dVP/dt)_{\text{max}}\). The peak increases \((7 ± 0.8 \text{ mmHg and} \)

\[\text{(dVP/dt)}_{\text{max}}\text{ or (dVP/dt)}_{\text{min}}\]

\[\text{Fig. 2. The time courses of the average changes in IVP, mean arterial blood pressure (MAP), and HR before, during, and after natural micturition in 3 conscious rats. A total of 47 micturition trials were obtained. The IVP and the changes in MAP and HR from the premicturition control values were aligned at the (dVP/dt)}_{\text{max}}(A) \text{or at the (dVP/dt)}_{\text{min}}(B) \text{in an individual trial and further averaged over the trials. The data are shown as mean ± SE.}\]
14 ± 3 beats/min) were delayed by 2 s from the peak IVP. Immediately after the (dVP/dt)_{min}, MAP showed a quick drop and fell to the baseline level, whereas HR recovered slowly for more than 30 s toward the baseline level.

Following an administration of xylocaine into the urinary bladder, the cardiovascular responses during natural micturition were observed to examine a possible role of the vesical reflex. Figure 3 compares the peak responses in IVP, MAP, and HR during natural micturition between the absence and presence of xylocaine. The increases in MAP and HR during micturition significantly (p < 0.05) reduced to 5 ± 2 mmHg and 8 ± 3 beats/min in the presence of xylocaine, although IVP increased to 15 ± 2 mmHg with the same time course and magnitude as IVP during micturition without xylocaine.

**Reflex responses in IVP, MAP, and HR during isovolumic bladder contraction.** When we infused saline into the urinary bladder of anesthetized rats, the baseline IVP increased to 13 ± 2 mmHg, from 3 ± 1 mmHg. A series of isovolumic bladder contractions were spontaneously evoked, resulting in a further increase in IVP to 36 ± 2 mmHg. Figure 4 shows the time courses of the average changes in IVP, MAP, and HR during isovolumic bladder contraction. MAP and HR respectively increased 17 ± 0.8 mmHg and 23 ± 2 beats/min in proportion to the rise in IVP in the anesthetized condition.

The relationships between IVP and the cardiovascular responses during isovolumic bladder contraction in anesthetized rats and during natural micturition in conscious rats are superimposed in Fig. 5. The slope of the regression line in the IVP–MAP relationship was similar between the two conditions (0.51 for natural micturition and 0.68 for isovolumic bladder contraction), though the ranges of IVP and MAP were quite different; the same tendency was observed in the IVP–HR relationship.

**DISCUSSION**

It has been reported that urinary bladder distension influences the cardiovascular system under pathophysiological conditions in humans such as spinal cord injury, hypertension, and arteriosclerosis [1–5]. Furthermore, the reflex change in sympathetic nerve activity in response to excessive bladder distension has been well documented with the use of anesthetized animals [6–12]. However, it remains unknown how much IVP increases during natural micturition in daily life and to what extent MAP and HR respond at that time. To answer these questions, we have attempted to examine the changes in IVP, MAP, and HR during natural micturition in freely moving conscious rats. We found that IVP increased to 14 mmHg during natural micturition, which was much smaller than the pressure reported during isovolumic bladder contraction in anesthetized animals or during a cystometrogram in humans, and that MAP and HR increased 7 mmHg and 14 beats/min in parallel with the rise in IVP. The increases in MAP and HR during natural
micturition were significantly blunted by a prior administration of xylocaine into the urinary bladder, suggesting that a reflex from the urinary bladder may evoke the cardiovascular responses during natural micturition in conscious rats.

The slight increase in IVP during natural micturition in conscious rats can be explained because urine could be excreted through the urethra pathway in the natural condition. Despite such a small increase in IVP, we found significant increases in MAP and HR. It is known that vesical afferents are quiescent during increases in volume, but they become active when a certain tension threshold is reached [13–15]. Furthermore, the threshold of IVP for the activation of some hypogastric and pelvic mechanosensitive afferents is as low as 5–20 mmHg [16–18]. This range of IVP corresponds to the change in IVP observed during natural micturition in this study. Therefore it is conceivable that the change in IVP during natural micturition is large enough to activate mechanosensitive afferents in the urinary bladder.

The stimulation of bladder afferents may cause the reflex cardiovascular responses to natural micturition, since it has been reported that the sympathetic nervous system is reflexly activated by the stimulation of mechanosensitive afferents in the urinary bladder in anesthetized animals [6–12]. To test this hypothesis, we examined the effect of xylocaine injected into the urinary bladder on the cardiovascular responses during natural micturition. The increases in MAP and HR during micturition significantly reduced in the presence of xylocaine, suggesting that a reflex from the urinary bladder may contribute to the cardiovascular responses. Furthermore, the peak increase in IVP preceded the peak increases in MAP and HR by 2 s, suggesting that it may trigger the cardiovascular responses. The relationship between IVP and MAP during natural micturition in conscious rats resembled the relationship observed during isovolumic bladder contraction in anesthetized rats that was reflexly induced (Fig. 5). Taken together, it is quite likely that a substantial part of the increases in MAP and HR during natural micturition is caused by a reflex associated with the stimulation of mechanosensitive receptors in the urinary bladder.

It is noteworthy that although an intravesical application of xylocaine attenuated the cardiovascular responses during natural micturition, it did not block detrusor muscle contraction. During natural micturition with xylocaine, IVP increased to 15 mmHg with the same time course and magnitude as the IVP response during micturition without xylocaine. This is because the mammalian bladder functions as a
reservoir for hypertonic urine, and the structural feature of the urothelium must be impermeable to urine [13]. It is very likely that xylocaine may not diffuse into the smooth muscle layer underneath the urothelium. Moreover, not only smooth muscles, but also various receptors in the muscular and adventitial layers of the urinary bladder wall are considered to be preserved from an influence of xylocaine. On the contrary, it is quite likely that the vesical afferents in the mucosa of the bladder should be affected by xylocaine. Indeed, many C-afferent fibers that respond to slow bladder distension of the viscus with physiological volumes are found in the mucosa of the rat urinary bladder [19, 20]. The finding that the increases in MAP and HR were blunted by a prior infusion of xylocaine suggests that the mechanosensitive receptors in the mucosa of the urinary bladder play a role in evoking the cardiovascular responses during natural micturition.

It is of interest that MAP and HR start to increase before the obvious rise in IVP in conscious rats (Figs. 1 and 2). These gradual cardiovascular responses before micturition may be initiated by a descending feed-forward signal from the higher central nervous system, based on cortical sensation related to a slight rise in intravesical pressure and/or an increase in vesical volume. This is supported by a previous microneurographic study demonstrating that when the urge to urinate was pronounced, muscle sympathetic nerve activity and blood pressure in humans increased significantly [21]. However, such gradual increases in MAP and HR were also observed during isovolumic bladder contraction in anesthetized rats (Fig. 4), suggesting another possibility that some subcortical structures, which receive bladder afferent information, may produce the gradual increases in MAP and HR preceding micturition even in an anesthetized condition. Recently, Sasaki [22, 23] reported that a group of neurons in Barrington’s nucleus projecting to the spinal cord displayed a ramp increase in firing before micturition contraction and further increased firing with bladder contraction in anesthetized cats. Neurons in Barrington’s nucleus receive information from the urinary bladder and have divergent projections to the locus coeruleus and to the sacral parasympathetic nucleus [24], suggesting that this nucleus may operate as a link between parasympathetic regulation and cardiovascular regulation during micturition. Either possibility remains to be examined in a future comprehensive study.

In conclusion, natural micturition accompanies the significant cardiovascular responses despite a limited increase in intravesical pressure. If bladder outlet obstruction, including benign prostatic hyperplasia or urethral stricture, is evoked in humans, bladder overdistension causes a greater elevation in intravesical pressure, which in turn may lead to exaggerated cardiovascular responses during micturition.

**Fig. 5. The relationships between IVP and the changes in MAP (A) or HR (B) during natural micturition in conscious rats (solid circles) and during isovolumic bladder contraction in anesthetized rats (open squares).** The same data shown in Fig. 2 (conscious rats) and in Fig. 4 (anesthetized rats) are plotted. The slope of the linear regression line (dotted line) of the IVP–MAP relationship was 0.51 for conscious rats and 0.68 for anesthetized rats. Also, the slope of the linear regression line of the IVP–HR relationship was 1.20 beats/min/mmHg for conscious rats and 0.98 beats/min/mmHg for anesthetized rats, respectively. Arrows indicate the trajectories during bladder contractions. The responses of MAP and HR to the change in IVP seem to be similar between the conscious and anesthetized conditions, although the ranges of IVP were quite different.

**Japanese Journal of Physiology** Vol. 54, No. 6, 2004 573
This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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