Effects of Intravesically Administered Anticholinergics, β-Adrenergic Stimulant and α-Adrenergic Blocker on Bladder Function in Unanesthetized Rats

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UKIMURA, O. Effects of Intravesically Administered Anticholinergics, β-Adrenergic Stimulant and α-Adrenergic Blocker on Bladder Function in Unanesthetized Rats. Tohoku J. Exp. Med., 1993, 170 (4), 251-260 —— Comparative analysis of the effects of intravesical instillation of drugs on urodynamic parameters (MVP, maximum intravesical pressure; RR, residual rate; BC, bladder capacity) was performed using an experimental model in unanesthetized rats. The drugs investigated in this study were atropine (7.2 x 10^{-4} - 7.2 x 10^{-2} M), propantheline (7.2 x 10^{-3} - 2.2 x 10^{-2} M), oxybutynin (2.5 x 10^{-3} - 2.5 x 10^{-2} M), isoproterenol (5 x 10^{-2} - 10^{-1} M) and prazosin (5 x 10^{-4} M). Of the anticholinergics, propantheline and oxybutynin showed a remarkable suppression of MVP accompanied with a consistent increase of RR and BC in a dose-dependent manner. Atropine showed, however, no suppression of MVP in spite of a significant change of RR and BC. Isoproterenol suppressed MVP with an increase of RR and BC in a dose-dependent manner at a relatively high concentration. Prazosin increased BC and RR at a relatively low concentration. This study revealed that these intravesical drugs have the ability to suppress spontaneous bladder contraction in unanesthetized rats and to change the micturition function in the urinary filling and storage phases. It is expected that intravesical instillation therapy for detrusor hyperreflexia will be improved in the future based upon the data obtained.

intravesical instillation; urodynamics; anticholinergics; β-adrenergic stimulant; α-adrenergic blocker

Although pharmacotherapy for patients with neurogenic bladder, particularly of those suffering from urinary incontinence due to detrusor hyperreflexia, exclusively comprises an oral intake of anticholinergic agents, sufficient clinical effects are not always obtained. The oral dosage of anticholinergics is often limited by systemic side effects, so that alternatives capable of more effectively controlling detrusor hyperreflexia with fewer systemic side effects have been looked for.

Intravesical instillation therapy has been thought to be an attractive therapeutic alternative through which a high concentration of drug could be achieved

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in the bladder with possibly fewer systemic side effects. Effects on urodynamic parameters by intravesical instillation have been investigated in human using several kinds of drug such as calcium antagonists (verapamil: Mattiasson et al. 1989), anticholinergics (atropine: Ekström et al. 1993), combined anticholinergic and calcium antagonistic properties (oxybutynin: Brendler et al. 1989; Madersbacher and Jilg 1991; terodiline: Ekström et al. 1992) or α-adrenoceptor antagonists (phentolamine: Ekström et al. 1993). Although the capacity of the bladder in detrusor hyperreflexia was reported to increase significantly with these drugs, distinct changes in other urodynamic parameters, particularly in maximum intravesical pressure, were not always recognized in the clinical trials.

It is, therefore, necessary to characterize the pharmacological property of each drug on the bladder function when administered intravesically using an experimental model, with the aim of developing more effective intravesical instillation therapy for detrusor hyperreflexia. There have been, however, only a few experimental papers using in vivo or in vitro whole bladder experimental models (Gotoh et al. 1986; Kato et al. 1989) in this field.

The aim of the present study is to compare the effects of various drugs administered intravesically at different concentrations on the bladder function using an experimental model in unanesthetized rats (Ukimura et al. 1992). In this model evaluation of the pharmacological effects of intravesically administered drugs on spontaneous physiological micturition are possible and interference by anesthetics on the bladder function is avoidable.

**Material and Methods**

A total of fifty male Wistar rats weighing 300–500 g were used in the present study. Under pentobarbital anesthesia, the bladder was exposed through an abdominal median incision. An Intramedic PE50 polyethylene tube filled with saline was then inserted into the bladder and was fixed at the apex. The tube passing through a subcutaneous tunnel was externalized at the vertex of the head and connected to a cannula swivel (Instech Laboratory Inc., Horsham, PA, USA) through a protective coil. A second tube attached to the other end of the cannula swivel was connected to a pressure transducer and an injection pump.

The day following the operation, cystometry was performed using a tube connected to a cannula swivel with the subject in unanesthetized condition. A sterilized saline solution at room temperature was infused intravesically at a rate of 0.2 ml/min and the change in intravesical pressure was recorded using a Polygraph system (Nihon Kohden, Tokyo). From the cystometrogram recorded, the bladder capacity (BC) and the maximum intravesical pressure (MVP) were calculated. Following spontaneous micturition, the fluid remaining in the bladder was aspirated through the tube, and the residual rate (RR) was calculated by dividing the volume of residual fluid by that of the fluid infused. These three parameters were calculated each time cystometry was performed in the following experiments.

As a control study, saline containing no drugs and at half the volume of BC was administered intravesically and left in the bladder for 30 min. Cystometry was then performed after the elimination of the fluid from the bladder. Next, saline at half the volume of BC, containing atropine sulfate (Nacalai Tesque Inc., Kyoto), propantheline
hydrobromide (Dainihon Pharmaceutical Co., Osaka), oxybutynin hydrochloride (Kodama Pharmaceutical Co., Tokyo), isoproterenol hydrochloride (Nacalai Tesque Inc., Kyoto) or prazosin hydrochloride (Phaizer-Taito Pharmaceutical Co., Tokyo) was administered intravesically and left in the bladder for 30 min. Cystometry was repeated every 30 min after the elimination of the fluid from the bladder. The experiment was continued for at most 300 min, or until the MVP recovered to a level of more than 90% of the control in the cases of propantheline, oxybutynin and isoproterenol, and until the RR recovered to a level of less than 10% of the control in the cases of atropine and prazosin. The time taken for the recovery of MVP or RR was recorded as the effective time. When spontaneous micturition did not occur in spite of infusing saline at a volume of more than twice the BC in the case of propantheline, oxybutynin and isoproterenol, cystometry was discontinued, judging that urinary retention had occurred.

The MVP and BC data were presented as percentages of the control values. For statistical analysis paired t-test (on the results between saline containing no drug and saline containing a drug) or the Mann-Whitney U-test (on the results between two drugs) was employed. A probability level of less than 0.05 was accepted for significance.

RESULTS

The experimental results are summarized in Table 1.

When the bladder was filled with infused saline, spontaneous excretion of fluid occurred with a sharp increase in intravesical pressure (Figs. 1-5). In the control study (n=50), the mean and s.e. of MVP, RR and BC were 12.7±4.1 mmHg, 1.7±4.5%, 0.49±0.22 ml, respectively.

Changes in urodynamic parameters caused by intravesical instillation of each drug were as follows.

Atropine (Fig. 1). No suppressive effects of MVP was found in any group.

Table 1. Summary of experimental results

<table>
<thead>
<tr>
<th>Drug</th>
<th>MVP (%)</th>
<th>RR (%)</th>
<th>BC (%)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine 7.2×10^{-4} M</td>
<td>0.5 mg/ml (n=5)</td>
<td>89±16</td>
<td>*49±13</td>
<td>112±17</td>
</tr>
<tr>
<td>Atropine 7.2×10^{-3} M</td>
<td>5.0 mg/ml (n=5)</td>
<td>101±12</td>
<td>*52±17</td>
<td>132±22</td>
</tr>
<tr>
<td>Atropine 7.2×10^{-2} M</td>
<td>50 mg/ml (n=4)</td>
<td>*126±11</td>
<td>*59±10</td>
<td>206±31</td>
</tr>
<tr>
<td>Propantheline 7.2×10^{-3} M</td>
<td>3.2 mg/ml (n=5)</td>
<td>75±9</td>
<td>41±20</td>
<td>97±14</td>
</tr>
<tr>
<td>Propantheline 2.2×10^{-2} M</td>
<td>10 mg/ml (n=4)</td>
<td>18±5</td>
<td>100±0</td>
<td>* &gt;200</td>
</tr>
<tr>
<td>Oxybutynin 2.5×10^{-3} M</td>
<td>1.0 mg/ml (n=5)</td>
<td>51±17</td>
<td>35±13</td>
<td>111±22</td>
</tr>
<tr>
<td>Oxybutynin 7.2×10^{-3} M</td>
<td>2.8 mg/ml (n=5)</td>
<td>45±14</td>
<td>45±18</td>
<td>163±26</td>
</tr>
<tr>
<td>Oxybutynin 2.5×10^{-2} M</td>
<td>10 mg/ml (n=4)</td>
<td>38±11</td>
<td>100±0</td>
<td>* &gt;200</td>
</tr>
<tr>
<td>Isoproterenol 5×10^{-2} M</td>
<td>12.5 mg/ml (n=4)</td>
<td>*61±13</td>
<td>*51±28</td>
<td>91±26</td>
</tr>
<tr>
<td>Isoproterenol 1×10^{-1} M</td>
<td>25 mg/ml (n=4)</td>
<td>*26±11</td>
<td>*100±0</td>
<td>* &gt;200</td>
</tr>
<tr>
<td>Prazosin 5×10^{-4} M</td>
<td>0.2 mg/ml (n=5)</td>
<td>82±10</td>
<td>*23±14</td>
<td>*182±35</td>
</tr>
</tbody>
</table>

The data are given as mean±s.e. MVP, maximum intravesical pressure; RR, residual rate; BC, bladder capacity; Time, effective time. *p<0.05, Statistically different from the corresponding value with saline.
However, RR, BC and effective time increased significantly in a dose-dependent manner.

Propantheline (Fig. 2). MVP was suppressed in a dose-dependent manner. RR and effective time increased in a dose-dependent manner. In the group of higher concentration \((2.2 \times 10^{-2} \text{ M}; n=4)\) BC increased remarkably resulting in urinary retention, but no change of BC was found in the group with a concentration of \(7.2 \times 10^{-3} \text{ M} (n=5)\).

Oxybutynin (Fig. 3). MVP was suppressed in a dose-dependent manner. RR, BC and effective time increased in a dose-dependent manner. In the group with a concentration of \(2.5 \times 10^{-2} \text{ M} (n=4)\) urinary retention was found.

Isoproterenol (Fig. 4). MVP was suppressed in a dose-dependent manner. RR and effective time increased in a dose-dependent manner. In the group with a concentration of \(1 \times 10^{-2} \text{ M} (n=4)\) BC increased with urinary retention, but no change of BC was found in the group with a concentration of \(5 \times 10^{-2} \text{ M} (n=4)\).

Prazosin (Fig. 5). MVP was not significantly changed, but BC increased remarkably and there was a slight increase of RR.

Statistical analysis (Mann-Whitney U-test) on the results in the concentration \(7.2 \times 10^{-3} \text{ M}\) revealed that of the anticholinergics oxybutynin was a more potent

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**Atropine**

![Atropine Graphs](image)

**Fig. 1.** Cystometrograms of the control and after intravesical administration of atropine in different concentrations.
suppressive agent on MVP than propantheline \((p < 0.05)\). As for BC, from the results in the concentration \(7.2 \times 10^{-3} \text{ M}\), oxybutynin was a more effective agent of the anticholinergics for increasing BC than atropine \((p < 0.05)\). Prazosin \((5 \times 10^{-4} \text{ M})\) was also a more effective agent for increasing BC than atropine \((7.2 \times 10^{-4} \text{ M})\) \((p < 0.05)\).

**DISCUSSION**

A large number of laboratory experiments dealing with pharmacological investigations of bladder contractility by drugs administered systematically have been performed. There have been, however, only a few papers available which investigated the responses of bladder to drugs administered intravesically. Gotoh et al. (1986) and Kato et al. (1989) reported a significant decrease of bladder contractility evoked by cholinergic agents or direct electric stimulation by intravesical verapamil in rabbit bladders in vivo and in vitro. In addition, we have previously shown that intravesical verapamil caused significant suppression of MVP accompanied with an increase of residual urine in a dose-dependent manner using an experimental model for unanesthetized rats (Ukimura et al. 1992).

The experimental model for unanesthetized rats used in the present study is superior to other models using in vivo or in vitro bladder, because pharmacological
effects of intravesical drugs on spontaneous bladder contraction accompanied with fluid excretion through the urethra, like a normal micturition, can be evaluable in this system. Furthermore, possible suppression of spinal reflexes involved in bladder contraction by general anesthetics can be deleted completely. Accordingly, data obtained from this model are thought to be readily applicable to the clinical stage.

In the present study comparative analysis on the potencies of drugs affecting on each urodynamic parameter could be performed. The most striking result revealed in this analysis was the absence of suppressive effects on MVP by intravesical atropine even in high concentration such as 0.5–50 mg/ml (7.2 × 10^{-4}–7.2 × 10^{-2} M). Among the anticholinergics, on the other hand, propantheline and oxybutynin showed remarkable suppression of MVP accompanied with the consistent increase of RR and BC in a dose-dependent manner. The rat bladder is well known to possess the characteristic of atropine-resistant contractility of the detrusor (Ambache and Zar 1970; Carpenter 1977). The results obtained in this study appeared to be consistent with this particular function of the rat bladder. The atropine-resistant contraction recognized in the present study was, however, different from that occurring in normal physiological micturition, because RR and
BC increased significantly in spite of there being no suppression of MVP.

Pharmacologically, three different subtypes of muscarinic receptors (M1, M2, M3) are distinguished, and M3 is known to be the dominant receptor in the detrusor in humans and rats (Doods et al. 1987; Lepor et al. 1989; Morita et al. 1991). Among the anticholinergics, propantheline and oxybutynin were reported
to be relatively selective to M3. On the other hand, atropine is not selective to any subtypes (Noronha-Blob et al. 1989, 1991; Noronha-Blob and Kachur 1991). The difference in responses of the rat bladder to atropine and to the other anticholinergics may be due to the difference in selectivity to muscarinic receptor subtypes.

As for other urodynamic parameters, BC was influenced most remarkably by oxybutynin, followed by atropine and propantheline in a concentration of $7.2 \times 10^{-3}$ M. Furthermore, oxybutynin suppressed MVP significantly longer than propantheline and atropine. Oxybutynin has a calcium antagonistic property in addition to an anticholinergic one, and these results may be attributable to this additional property. As for RR no distinct differences in the effects of intravesical drugs were observed among anticholinergics.

As widely known, the urinary bladder receives both cholinergic and adrenergic innervation, and adrenergic stimulation results in a contraction of the bladder base (α response) and a relaxation of the bladder body (β response) (Levin et al. 1988). Although adrenergic agents have not been used popularly for oral treatment of detrusor hyperreflexia, they are also expected to have practical ability for intravesical instillation.

β-Adrenoceptor agonists were reported to have suppressive effects on bladder contraction using in vitro or in vivo animal experiments (Sjögren and Anderson 1979; Levin et al. 1983). The present study is the first report to show that intravesical isoproterenol could interfere the physiological micturition reflex in unanesthetized animal resulting in significant decrease in MVP and increases in RR and BC. The concentration of isoproterenol used in the present study ($12.5-25$ mg/ml or $5 \times 10^{-2}-5 \times 10^{-1}$ M) is so high that a practical application as a treatment of detrusor hyperreflexia is not available readily, because its side effects on the cardiac function may be serious if a large quantity of the drug be absorbed into the general circulation through the bladder epithelium.

Prazosin, one of the specific postsynaptic α-adrenoceptor blocking agents, was reported to increase BC markedly and diminish the uninhibited detrusor contraction in neurogenic bladder when administered orally (Jensen 1981). The present study also suggested that prazosin could be available to control detrusor hyperreflexia by intravesical instillation.

The effects of intravesical instillation of terodiline on urodynamic parameters in normal human bladders were reported by Ekström et al. (1992). The study using healthy volunteers revealed that instillation of terodiline had no effects on normal bladders using an even higher concentration than that used in responders with neurogenic detrusor hyperactivity. Additionally, low sensitivities to intravesical drugs in patients with neurogenic or nonneurogenic detrusor hyperactivity were reported using verapamil (Mattisson et al. 1989), terodiline (Ekström et al. 1992) and atropine and phentolamine (Ekström et al. 1993). It is, accordingly, supposed that intravesical drugs at a lower concentration than that
used in this study might have the potency to suppress the detrusor uninhibited contraction in patients with neurogenic detrusor hyperactivity.

For the increasing number of patients with neurogenic bladder using clean intermittent catheterization, the clinical application of intravesical instillation therapy is desired. Intravesical anticholinergics, α-adrenoceptor blocking agents and β-adrenoceptor agonists were shown to have the potency to change the micturition function in the urinary filling and storage phase. The data obtained experimentally in this study will contribute to the advancement of intravesical instillation therapy. Particularly, in selecting drugs for such therapy great attention should be paid to the pharmacological property of each drug.

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References


