Age-related smooth muscle reactivity changes in the rat bladder: an in vitro study

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Abstract

An experimental study was conducted to investigate developmental changes of the rat detrusor smooth muscle (DSM) reactivity from newborn to adult period. Urinary bladders were obtained from adult (4 months old), 1-month-old and newborn (4–7 days old) male Sprague-Dawley rats. DSM reactivity of the three groups was evaluated in organ chambers. The newborn DSM strips revealed markedly increased purinoceptor- and cholinoceptor-mediated contractions (ATP, carbachol) with increased maximum response (2.98- and 8.96-fold increase for ATP, 2.90- and 4.22-fold increase for carbachol, respectively) and sensitivity (1.65- and 1.29-fold increase for ATP when the newborn bladder compared with the 1-month-old and adult groups, respectively). Additionally the maximum contractile response to KCl in the bladder from the newborn was 1.65- and 8.96-fold increased compared to bladders of the 1-month-old and adult groups, respectively. However, no significant changes in the adrenoceptor-mediated relaxation (isoproterenol) of the rat DSM were observed among the groups. These results indicate that development alters the in vitro responsiveness of rat DSM. The newborn rat bladder gains some of the adult bladder properties within 1 month. These changes are likely to reflect the changing role of parasympathetic regulation in the DSM reactivity during development of the rat bladder.

Keywords: Detrusor smooth muscle; In vitro; Bladder; Rat

1. Introduction

Urinary bladder is a very complex organ considering morphology and innervations. Smooth muscle is an integral component of the lower urinary tract and ultimately normal voiding is dependent upon the ability of the bladder musculature to respond appropriately to stimulation. Therefore, any changes or impairment in the operative contractile capacity of the bladder smooth muscle may be reflected in voiding function. Maturation or aging affects the function of the bladder have been studied previously in human and animals [1–21]. Besides the development or aging, there are also some functional bladder disorders such as transient urodynamic dysfunction of infancy, Hinman’s syndrome, etc. that present with impaired detrusor contractility seen in infants and children which tend to improve with matura-
tion [22]. The intent of the present study was to investigate detrusor smooth muscle (DSM) reactivity changes from newborn to adult period and to find out the time for newborn DSM to gain that of adult rat properties.

2. Methods

Newborn (4–7 days old, n = 25), 1-month-old (30–35 days old, n = 34) and young adult (4 months old, n = 23) male Sprague-Dawley rats were obtained from Experimental Medical Research Unit (DETAB, Kocaeli University, Kocaeli, Turkey). These rats were housed in cages in a temperature and humidity controlled room (22 ± 3 °C and 62 ± 7%, respectively) in which a 12–12 h light–dark cycle was maintained (8:00–20:00 h light). The urinary bladder was removed from different age groups following sacrificing rats [23]. After removing adhering fat and connective tissue, the bladder was opened and divided into longitudinal strips, weighed and placed in physiological saline solution of the following composition (mmol/l): NaCl 118; KCl 4.7; CaCl2 2.5; MgSO4 1.2; KH2PO4 1.18; NaHCO3 1043-6618/ - see front matter © 2003 Elsevier Ltd. All rights reserved.

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24.88; glucose 5.55. The DSM strips were suspended in a 20-ml water-jacketed (37 °C) tissue bath, containing physiological saline solution continuously gassed with 95% O₂ and 5% CO₂, resulting in a pH of 7.4. The resting tension on the tissues was maintained at 1 g during which the solution was replaced for 15 min intervals before adding drugs. This tension was shown in preliminary experiments to be optimal for this setting. Each strip was connected to a force-displacement transducer (TDA-94 COMMAT, COMMAT Iletisim Co., Ankara, Turkey) for measuring isometric force, which was continuously displayed and recorded on-line on a computer via a four-channel transducer data acquisition system using appropriate software (Polywin 95 ver 1.0 COMMAT, COMMAT Iletisim Co., Ankara, Turkey). The compounds were added to the chamber in half-log concentration increments and cumulative concentration responses obtained. The compounds were dissolved so that for every concentration the volume added to the chamber was 50 μl. At the completion of each experiment, tissues were measured, lightly blotted and weighed.

At the end of the equilibration period, strips were stimulated with 80 mM KCl. To assess the contractile response to the muscarinic agonist carbachol (10⁻¹⁰ to 3 × 10⁻⁴ M) and to the purinergic agonist ATP (10⁻¹¹ to 10⁻³ M), cumulative concentration–response curves were constructed in a stepwise manner after the response to the previous concentration reached plateau before adding the next one. Following completion of carbachol or ATP concentration–response curves, tissues were washed for a further 30 min and precontracted with a submaximal concentration of carbachol (3 × 10⁻⁶ M). After the contraction reached plateau, cumulative concentration–response curves to isoproterenol (10⁻¹¹ to 10⁻⁴ M) were obtained. These experiments were carried out in accordance with the Declaration of Helsinki, ethical approval was granted by the Kocaeli University Ethics Committee (No. AEK-405).

2.1. Analysis of data

All data are expressed as the mean value ± standard error of different experiments. The contractile responses to carbachol and ATP were calculated as milligrams of developed tension per milligram of tissue wet weight. The relaxant effects of isoproterenol were expressed as a percentage of the contraction induced by carbachol. To evaluate the effects of agonists, maximum response (E₅₀) and pD₂ values (i.e. the negative logarithm of the concentration for the half maximal response; EC₅₀) were calculated. Agonist pD₂ values were calculated from each agonist concentration–response curve by linear regression of the linear part of the curve and taken as a measure of the sensitivity of the tissues to each agonist. The significance of differences was tested by one-way ANOVA with a post-hoc Tukey’s–Kramer test and P < 0.05 was considered to indicate significance.

2.2. Compounds

The following compounds were all obtained from Sigma Chemical Co., St. Louis, MO, USA: carbachol chloride, adenosine triphosphate, isoproterenol hydrochloride. All compounds were dissolved in distilled water and freshly prepared on the day of the experiments. In the high K⁺ solution, NaCl was exchanged for equimolar amounts of KCl. Fresh solutions were prepared on the day of the experiments.

![Fig. 1. Carbachol concentration–response curves in DSM strips. Each point is expressed as mg mg⁻¹ and is given as the mean ± S.E.M. Numbers in parentheses indicate the number of DSM strips obtained from different animals. (*) Significant difference from other age groups (P < 0.05).](image)
3. Results

DSM strips was obtained from the rats with 1 week interval from birth to 4 months old and studied in standard organ baths to determine when DSM reveals mature contractile properties in the preliminary study. In a series of preliminary experiments, 1-month-old rats were included in the study as a third group. We also observed poor response to agonist studies on the DSM strips immediately after birth but stabilized from the fourth day of life. Therefore, we studied DSM strips of the newborn group 4 days after birth.

Cumulative concentration–response curves were obtained for carbachol in DSM strips from newborn, 1-month-old and adult rats. The concentration–response curve for carbachol shifted to the left, with significantly increased $E_{\text{max}}$ values in the newborn group compared to the other groups ($P < 0.05$) (Fig. 1, Table 1). However, the contractile response curves for carbachol was not different between the 1-month-old and adult groups with similar $E_{\text{max}}$ and $pD_2$ values (Fig. 1, Table 1).

ATP produced concentration-dependent contraction in the three groups. The concentration–response curve for ATP was shifted to the left with significantly increased $E_{\text{max}}$ and $pD_2$ values in the newborn group compared to the other groups (Fig. 2, Table 1). The contractility was not different between the 1-month-old and adult groups and there were no significant changes in the $E_{\text{max}}$ and $pD_2$ values (Fig. 2, Table 1).

The maximum response to 80 mM KCl was increased in the newborn group but there was no significant difference between the adult and 1-month-old groups (Fig. 3, Table 1). Isoproterenol produced concentration-dependent relaxation in submaximally (85–90% of maximal contraction) precontracted ($3 \times 10^{-6}$ M carbachol) DSM strips obtained from each group. Similar relaxation concentration–response curves in the three groups were observed. No significant changes in the $pD_2$ and $E_{\text{max}}$ values were found (Fig. 4, Table 1).

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Neonatal bladder</th>
<th>1-month-old bladder</th>
<th>Adult bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder weight (g)</td>
<td>0.013 ± 0.001*</td>
<td>0.042 ± 0.004*</td>
<td>0.122 ± 0.015*</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (KCl)</td>
<td>173.67 ± 34.93*</td>
<td>47.94 ± 15.87</td>
<td>32.55 ± 7.4</td>
</tr>
<tr>
<td>Carbachol</td>
<td>201.33 ± 1.39*</td>
<td>69.35 ± 0.20</td>
<td>47.62 ± 0.27</td>
</tr>
<tr>
<td>ATP</td>
<td>92.6 ± 7.6*</td>
<td>10.33 ± 1.01</td>
<td>10.33 ± 1.01</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>79.12 ± 6.29</td>
<td>94.98 ± 6.49</td>
<td>98.42 ± 10.91</td>
</tr>
<tr>
<td>$pD_2$ (Carbachol)</td>
<td>7.29 ± 0.03</td>
<td>7.10 ± 0.13</td>
<td>6.41 ± 0.13</td>
</tr>
<tr>
<td>ATP</td>
<td>6.45 ± 0.32*</td>
<td>3.89 ± 0.23</td>
<td>4.90 ± 0.79</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>7.33 ± 0.35</td>
<td>6.80 ± 1.03</td>
<td>7.00 ± 0.94</td>
</tr>
</tbody>
</table>

Values are from number of DSM strips from 4 to 10 different animals in each group. * $P < 0.05$ comparing the newborn group with the other groups.

Fig. 2. ATP concentration–response curves in DSM strips. Each point is expressed as mg mg$^{-1}$ and is given as the mean±S.E.M. Numbers in parentheses indicate the number of DSM strips obtained from different animals. * Significant difference from other age groups ($P < 0.05$).
Fig. 3. Eighty millimolar KCl-induced maximal contractile responses of DSM strips. Each point is expressed as mg mg$^{-1}$ and is given as the mean ± S.E.M. Numbers in parentheses indicate the number of DSM strips obtained from different animals. (*) Significant difference from other age groups ($P < 0.05$).

Fig. 4. Isoproterenol concentration–response curves in DSM strips precontracted with carbachol $3 \times 10^{-6}$ M. Each point is expressed as a percentage of the contraction induced by carbachol and is given as the mean ± S.E.M. Numbers in parentheses indicate the number of DSM strips obtained from different animals.

4. Discussion

The development of the urinary bladder is a complex event that requires time for maturation. Many changes have been observed during maturation considering size, capacity, receptor and muscle functions [3–8,11,18]. Following maturation, aging begins and changing of the structure and function continues [1,2,9,10,20]. For instance detrusor function appears to decline with age so that there is a trend towards reduction in strength of contraction of the detrusor and impairment of emptying efficiency. This leads to larger post void residual volumes with increasing age [14]. Degenerative detrusor smooth muscle abnormalities in the bladders of old rats has been reported from the electronmicroscopic studies [15,16]. The differences in newborn and adult can be attributable to the structural changes of the urinary bladder smooth muscle. A previous study reported that changes of bladder smooth muscle myosin heavy chain
is forms during development in rats [20]. Another possibility for the age-related changes might be attributable to the metabolic events in bladder smooth muscle. The bladder preparations from old rats revealed a reduced basal metabolic rate compared to young adult bladders [1].

Parasympathetic stimulation is considered very important in bladder of most mammals including human and animals [5,8,11,17,18,24-27]. Contractile responses of the bladder are under the control of the parasympathetic nervous system. ATP is released as a contractile co-transmitter with acetylcholine from parasympathetic nerves supplying bladder [5,23,26]. Carbachol- or ATP-induced cholinergic and purinergic stimulation of rat DSM were significantly potentiated in the newborns compared to the other groups at all concentrations tested, in accordance with the previous studies [3,12]. Three different factors at least in part may cause the different contractile properties between the newborn and the adult groups. Firstly, the increased responsiveness to parasympathetic stimulation might be assumed to be attributable to the increased number of receptors or an enhanced functional response to activation of a given number of receptors [12,13,24]. Recent studies focused on the receptor properties of the bladder on humans and animals during the development and disease states. Most of these studies revealed abnormal parasympathetic innervation and receptor properties [5,11,24–26]. The purinergic receptors are present in the human and animal bladder and do not have an important role in excitatory transmission under normal circumstances [11]. However, the distribution and subclasses of purinergic receptors in the human bladder have been shown to change during postnatal development and purinergic excitatory mechanisms are upregulated in patients with detrusor instability [24]. This mechanism may play a role in children and infants with dysfunctional Voiding, vesicoureteral reflux, etc. [3,4,11–14]. Secondly, the supersensitivity to carbachol or ATP may be explained by incomplete parasympathetic innervation in the neonatal rats. In a previous study, transection of the spinal cord induced increased bladder contractions in the neonatal rat [28]. Third explanation is impaired Ca2+ influx through voltage-gated Ca2+ channels has been reported to contribute to bladder contraction during DSM stimulation so that Ca2+ release may have a role in the increased carbachol-induced contractile response in the newborn group compared to the older groups [29]. However, the increased ability of the neonatal bladders cannot be explained solely by the neurotransmitters, since the effective mechanism of KCl was non-receptor mediated. In our study, no significant differences were found in the concentration-response curve, maximum response or pD2 values for isoproterenol between the newborns and adults. Most of the previous studies also revealed no significant change with isoproterenol-induced relaxant response in the bladder of the rats from various age ranges from 5-, 7-, 18-, 22-, 24-, 29-month-old rats [10,27]. In contrast to most of the studies, decreased isoproterenol-induced DSM relaxations have been observed with aging previously when 22-month-old rats are compared with 90-day-old rats [9]. This discrepancy might be attributable to the age or method differences between the studies.

In conclusion, purinergic-, cholinergic- and non-receptor-mediated contractions of the DSM were increased whereas the adrenoceptor-mediated relaxations were not altered in the newborn rat bladder compared to the adult bladder. Additionally newborns at least in part gained adult DSM properties 1 month after birth. These changes might be attributable to altered signal transduction in addition to the altered number of cholinergic or purinergic receptors in neonatal tissues require further study.

References


