Development changes esophageal smooth muscle reactivity: an in vitro study

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Abstract

This study was designed to determine if age related changes occur in esophageal smooth muscle reactivity in developing rats. Esophageal smooth muscle strips were obtained from three groups of rats: neonatal (1–3 days old), 1-month-old (30–35 days old) and young adults (4 months old). Esophageal smooth muscle reactivity was assessed in vitro by organ chamber experiments. Concentration–response curves were analyzed for differences in smooth muscle reactivity. Contractile response to cholinergic agonist carbachol increased in the neonatal group with increased maximum contraction compared to the other groups. However, there was no change in $pD_2$ value among the groups. Maximum contraction evoked by KCl was also significantly increased in the neonatal group compared to the adult group. Relaxation responses elicited by beta adrenoceptor agonist isoproterenol and 5-hydroxytryptamine (5-HT) receptor agonist serotonin were increased in the neonatal group with increased $E_{max}$ and $pD_2$ values compared to the other groups. The present study indicates that the neonatal esophageal smooth muscle responds to serotonergic, adrenergic and cholinergic agonists to a significantly greater degree than the adult esophageal smooth muscle.

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1. Introduction

Esophageal function begins in-utero with fetal ingestion of amniotic fluid during fourth month of gestation [1]. Anatomic and physiologic esophageal properties start to differentiate after birth in humans [2–4]. For instance, the normal velocity of the peristaltic wave in the distal esophagus increases after first week of life and normal pressure changes slightly with age [3]. Lower esophageal sphincter is only 0.5–1 cm in length at birth and gradually increases to 2.5–3 cm by 3 months of age [3]. Length of the manometric sphincter is approximately 1 cm in infants and 2–4 cm in adults [3]. Immature motor co-ordination of esophageal and sphincteric function may be encountered in the healthy newborn and spontaneously resolved by 1 month of age [4]. Since these observations suggest that esophageal properties changes with maturation; smooth muscle reactivity was evaluated in neonatal, 1-month-old and adult rats.

2. Materials and methods

2.1. Animals

The Kocaeli University Ethics Committee (Kocaeli, Turkey) (No: REC-162/10) granted the ethical approval. Sprague Dawley rats were divided into three groups according to age: neonatal (1–3 days old, n = 30), 1-month-old (30–35 days old, n = 32), adult (4 months old, n = 29). All rats were obtained from Experimental Medical Research Unit (DETAB, Kocaeli University Medical Faculty, Kocaeli, Turkey), housed individual solid bottom plastic cages on sawdust bedding at a temperature and humidity controlled room (22±3°C and 62±7%, respectively) in which a 12/12 h light–dark cycle was maintained (08:00–20:00 h light). Standard laboratory rat food and water were freely available.

2.2. Organ chamber experiments

The rats were harvested by decapitation and almost 1.5 cm thoracic portion of the esophagus was excised. Esophageal smooth muscle strips were prepared and mounted in 20 ml
organ chambers for isometric tension measurement [5]. The organ chambers contained Tyrode’s solution composed of (mmol l\(^{-1}\): NaCl 136.0; KCl 2.7; CaCl\(_2\) 1.8; MgCl\(_2\) 1.05; NaH\(_2\)PO\(_4\) H\(_2\)O 0.42; NaHCO\(_3\) 11.9; glucose 5.5. The solution was gassed with 95% O\(_2\) and 5% CO\(_2\) during the study and the temperature was maintained at 37 °C by a thermo regulated water circuit. The strips were connected to a force transducer (FDT 10A, COMMAT Iletisim Co., Ankara, Turkey) for the measurement of isometric force, which was continuously displayed and recorded on an online computer via a four channel transducer data acquisition system (TDA-94 COMMAT, COMMAT Iletisim Co., Ankara, Turkey) using a software (Polywin 95 ver. 1.0 COMMAT, COMMAT Iletisim Co. Ankara, Turkey), which also had the capacity to analyze the data. After mounting, the strips were allowed to equilibrate under a basal tension of 0.5 g for 90 min. During this period the bath fluid was routinely changed every 15 min. After equilibration, the strips were lightly blotted and weighted. Isometric tension was measured in a organ bath. At the completion of each experiment, tissues were allowed to equilibrate under a basal tension of 0.5 g for 60 min. During this period the bath fluid was routinely changed every 15 min. After contraction, the tissues were washed for a further, 60 min and pre-contracted with a submaximal concentration of carbachol (3 × 10\(^{-6}\) mol l\(^{-1}\)). Concentration–response relationships for serotonin, isoproterenol, carbachol (carbamylcholine chloride), isoproterenol (isoproterenol bitartrate), serotonin (serotonin creatinine sulfate), papaverine (papaverine hydrochloride), KCl (potassium chloride). In the high K\(^+\) solution NaCl was exchanged for equimolar amounts of KCl. Compounds were prepared daily in distilled water and kept in ice during the course of experiments.

2.4. Statistical analysis

Significance was tested by one-way analysis of variance (ANOVA) with a post-hoc Tukey’s Kramer test. Probabilities of less than 5% (P < 0.05) were considered significant.

2.5. Compounds

The following compounds were used (Sigma Chemical Co., St. Louis, MO): carbachol (carbamylcholine chloride), isoproterenol (isoproterenol bitartrate), serotonin (serotonin creatinine sulfate), papaverine (papaverine hydrochloride), KCl (potassium chloride). In the high K\(^+\) solution NaCl was exchanged for equimolar amounts of KCl. Compounds were prepared daily in distilled water and kept in ice during the course of experiments.

3. Results

Esophageal strip weight significantly changed in three groups of rats (Table 1). All contractile force was normalized to muscle weight and expressed as mg mg\(^{-1}\). Cumulative addition of carbachol produced significantly increased concentration-dependent contractions with increased \(E_{\text{max}}\) value of esophageal strips in the neonatal group compared to the other groups (Table 1, Fig. 1). Maximum contraction response to carbachol in esophageal strips from the neonatal group was 46.68 and 50.10% greater than response in esophageal strips from the 1-month-old and adult groups, respectively. In the adult group (200 mg mg\(^{-1}\) weight) the number of preparations used from different animals. No significant change in \(pD_2\) value was observed.

<table>
<thead>
<tr>
<th>Characteristics of rats in the three groups</th>
<th>Neonatal (n = 38)</th>
<th>1-month-old (n = 32)</th>
<th>Adult (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>1.85 ± 0.26</td>
<td>3.58 ± 0.37</td>
<td>183 ± 15.73</td>
</tr>
<tr>
<td>Esophageal strips weight (mg)</td>
<td>0.0052 ± 0.0003*</td>
<td>0.043 ± 0.0025*</td>
<td>0.089 ± 0.006</td>
</tr>
<tr>
<td>(E_{\text{max}})</td>
<td>16.27 ± 6.48*</td>
<td>9.24 ± 1.59</td>
<td>6.21 ± 0.88</td>
</tr>
<tr>
<td>KCl</td>
<td>30.68 ± 0.06*</td>
<td>16.36 ± 0.01</td>
<td>15.31 ± 1.55</td>
</tr>
<tr>
<td>Carbachol</td>
<td>98.83 ± 8.55*</td>
<td>84.89 ± 2.29</td>
<td>73.52 ± 1.46</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>96.57 ± 2.16*</td>
<td>89.25 ± 1.22</td>
<td>83.47 ± 1.23</td>
</tr>
<tr>
<td>(pD_2)</td>
<td>6.95 ± 0.02</td>
<td>6.91 ± 0.08</td>
<td>6.96 ± 0.98</td>
</tr>
<tr>
<td>Serotonin</td>
<td>7.61 ± 0.32*</td>
<td>6.51 ± 0.03</td>
<td>6.49 ± 1.32</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>8.36 ± 0.09*</td>
<td>7.21 ± 0.04</td>
<td>6.81 ± 0.16</td>
</tr>
</tbody>
</table>

Values are arithmetic mean ± S.E.M., n the number of preparations used from different animals. (P < 0.05, statistically different from the response of strips from the adult group. \(pD_2\) values for carbachol, \(E_{\text{max}}\) (% of 10\(^{-3}\)M carbachol) and \(pD_2\) values for serotonin, isoproterenol, \(E_{\text{max}}\) (% of 80mM KCl) and \(pD_2\) values for carbachol, \(E_{\text{max}}\) (mg) values for KCl.
Contraction elicited by KCl was significantly increased in the neonatal group. Maximum contraction response to KCl in esophageal strips from the neonatal group was 43.21 and 61.83% greater than response in esophageal strips from the 1-month-old and adult groups, respectively (Table 1, Fig. 2). Serotonin produced concentration-dependent relaxation in submaximally (55–60% of maximal contraction) precontracted (3 × 10^{-6} mol l^{-1} carbachol) esophageal strips obtained from each group. Relaxation in response to serotonin was significantly increased in the neonatal group compared to the other groups (Table 1, Fig. 3). Maximum relaxation response to serotonin in esophageal strips from

![Carbachol concentration-response curves in esophageal strips](image1)

![KCl-induced maximal contractile responses of esophageal strips](image2)
the neonatal group was 14.10 and 25.60% greater than response in esophageal strips from the 1-month-old and adult groups, respectively. Moreover pD2 value of the neonatal group was slightly but significantly increased compared to the other groups. In precontracted (3 × 10^{-6} mol l^{-1} carbachol) esophageal strips, isoproterenol produced concentration dependent relaxations. Relaxation in response to isoproterenol was significantly increased with increased pD2 values in the neonatal group compared to the other groups (Table 1, Fig. 4). Maximum relaxation response to isoproterenol in esophageal strips from the neonatal group was 7.57 and 13.56% greater than response in esophageal strips from the 1-month-old and adult groups, respectively. No significan
t differences were found for either E_{max} or pD2 values for papaverine (10^{-6} to 10^{-4} mol l^{-1}) acting on esophageal strips from three different age groups (data not shown).

4. Discussion

Feeding difficulties and gastroesophageal reflux (GER) are common problems in infancy. The factors underlying these problems remain unsettled but changes of esophageal motor activity may contribute. It is well known that smooth muscle plays an important role in esophageal peristalsis. The combined effects of inhibitory and excitatory nerves
contraction. Greater contractile response of the ally calcium is recognized as a regulator in smooth muscle reactivity.

Increased esophageal smooth muscle reactivity in the neonatal esophagus has been indicated previously. Increased relaxation relaxes esophageal smooth muscle by enhancing cAMP cellular calcium by the neonatal esophagus. Isoproterenol channels, suggesting an increased reliance on extracellular calcium through voltage dependent calcium diated by nonspecific depolarization. KCl leads to contraction during esophageal peristalsis in human and animal. In this present study, increased carbachol-induced contractile response was found in the neonatal group compared to the adult group. Ach-induced contraction requires influx of extracellular calcium and may be linked to the phosphatidylinositol metabolism, production of diacylglycerol and arachidonic acid, and activation of a protein kinase C-dependent pathway. Thus, we can assume that the increased esophageal smooth muscle reactivity in the neonatal group might be partly related to postreceptor mechanisms of the contraction.

The smooth muscles contractions distributed in many organs are modulated by various cellular signals; generally calcium is recognized as a regulator in smooth muscle contraction. Greater contractile response of the neonatal strips to KCl was found in the study. Thus, the increased smooth muscle reactivity of the neonatal esophagus to generate tension cannot be explained solely by the neurotransmitters, since the effects of KCl are mediated by nonspecific depolarization. KCl leads to contraction through membrane depolarization and influx of extracellular calcium through voltage dependent calcium channels, suggesting an increased reliance on extracellular calcium by the neonatal esophagus. Isooproterenol relaxes esophageal smooth muscle by enhancing cAMP has been indicated previously. Increased relaxation response was demonstrated in the neonatal esophageal smooth muscle compared to the adult esophageal smooth muscle. Therefore, it could be assumed that the decreased responsiveness to beta-adrenergic receptor stimulation in esophageal tissues from adult rats might be attributable to alteration in adenylate cyclase-cAMP pathway. In rat esophagus, serotonin induce a relaxation response which mediated by activation of 5-HT receptors. The receptor activation causes increased tissue cAMP content over basal level in esophageal smooth muscle. Therefore decreased responsiveness to 5-HT receptor stimulation might be attributable to changing signal transduction or number of 5-HT receptors in the adult esophagus. This observation would suggest a possible common pathophysiological mechanism of alteration in the adenylate cyclase-cAMP pathway. Since we did not perform experiments with an adenylate cyclase activator such as forskolin, we cannot comment on this issue. The role of changes in G protein, another intermediate indispensable for signal transduction between 5-HT receptors and adenylate cyclase, remains to be investigated.

5. Conclusions

Developmental esophageal smooth muscle reactivity changes were demonstrated in the present study. Esophageal muscle strips of the neonatal group generated more active force than the adult group. Most of the changes were occurred within 1 month after birth. These changes might be attributable to altered signal transduction or receptor properties require further study.

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References


