Muscarinic acetylcholine receptors of the small intestine and pancreas of the rat: distribution and the effect of vagotomy

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Summary

1. The distribution of muscarinic cholinergic receptors (mAChR), detected by atropine-inhibitable binding of [3H]quinuclidinyl benzilate, was examined in membrane fractions of pancreas, small intestinal muscle, mucosa, villi and crypts of sham-operated and vagotomized rats.

2. Specific (atropine inhibitable) [3H]quinuclidinyl benzilate binding was greater to the ileal mucosa than to jejunal mucosa or to duodenal mucosa, but binding to crypt and villus fractions was not significantly different. This distribution of specific [3H]quinuclidinyl benzilate binding suggests that cholinergic mucosal innervation is more important in the ileum than the jejunum.

3. Vagotomy produced a decrease in the amount of specific [3H]quinuclidinyl benzilate binding to duodenal mucosa only, suggesting that parasympathetic denervation of the small intestine does not cause mucosal hypersensitivity to acetylcholine by an increase in mAChR.

Key words: muscarinic cholinergic receptors, pancreas, parasympathetic nervous system, small intestine, vagotomy.

Abbreviations: EDTA, ethylenediaminetetraacetic acid; mAChR, muscarinic cholinergic receptors.

Introduction

Cholinergic nerve fibres are abundant in the submucosal plexus of human small intestine and have terminals close to the epithelial basement membrane [1]. In addition, specialized neuro-effector junctions (synapses) between nerve fibres and epithelial cells have been described [2]. Villus motility [3], crypt cell turnover [4] and mucosal electrolyte secretion are stimulated by cholinergic agents [5–7], suggesting that cholinergic nerves may influence these functions. The fact that these effects are blocked by atropine shows the involvement of muscarinic acetylcholine receptors (mAChR), although their location cannot be attributed on this evidence to any single cell type, neural or otherwise. Responses of the intestinal mucosa to muscarinic cholinergic stimulation could be due to a direct stimulation of the epithelial cells by a neurotransmitter or hormone released from a cholinergically stimulated intermediary cell.

If a direct cholinergic innervation of the epithelial cells exists, then these cells will be equipped with mAChR, membrane structures exhibiting specific high-affinity binding of muscarinic agonists and antagonists. In the latter category are the benzilic acid derivatives whose affinity for the mAChR of brain is very high, e.g. propylbenzilylcholine mustard [8], N-methyl-4-piperidyl benzilate [9] and quinuclidinyl benzilate [10]. In the present report we describe the distribution of mAChR in the small intestine and pancreas of the rat shown by the specific (i.e. atropine-inhibited) binding of [3H]quinuclidinyl benzilate. The binding of quinuclidinyl benzilate to cells or cell membrane components has been shown in a variety of tissues to displace a hierarchy of cholinergic agonists in the correct sequence of their physiological potency, being displaced itself only by very high-affinity muscarinic antagonists [11].

The number and distribution of nicotinic acetylcholine receptors on skeletal muscle cells
are markedly influenced by denervation [12]. Parasympathetic denervation of the intestine might also lead to changes in mAChR, particularly, if the receptors identified were indeed intimately connected with the parasympathetic innervation of the gut. The effects of vagotomy on [3H]quinuclidinyl benzilate binding to intestine were therefore also examined.

Methods

Vagotomized and paired control rats

Pairs of fasting male Sprague–Dawley strain rats (Simonsen Laboratories Inc., Gilroy, U.S.A.) given standard laboratory chow and housed in a 12 h light–dark cycle were anaesthetized with ether. Through a midline incision the stomach was retracted downwards. In the vagotomized animals the left gastric artery, identifiable vagal trunks and all other neurovascular tissue around the oesophagus at the diaphragmatic hiatus were divided. In the sham-operated control rats the stomach was merely retracted. The abdominal wound was closed with clips and the operated animals were allowed unlimited water immediately and food after 24 h. There was no mortality; one vagotomized animal developed a superficial infection of the wound. The vagotomized control rats of an experimental pair were therefore also examined.

Vagotomized animals were distended with food. The distension with a solution of ethylenediamine-tetra-acetic acid (EDTA; 10 mmol/l) in saline, pH 7.0, clamped at both ends and immersed in a phosphate-buffered saline at 4°C for 20 min. The segment was drained, gently blotted and then rolled between two horizontal polyethylene rollers (the lower 18 mm and the upper 7 mm in diameter covered with soft rubber tube 2 mm thick). By means of a stirrup, a 150 g load could be applied to the upper roller, which was rotated with a crank handle to allow the segment through without pulling on it. This yielded a large portion of the intestinal mucosa, which microscopically was seen to comprise sheets of epithelium and clumps of enterocytes.

The segment was gently rinsed with phosphate-buffered saline, drained, blotted and passed between the rollers under a load of 350 g, which yielded the remaining mucosa of the segment, which was seen to comprise only isolated crypt fragments. Histological section of the remaining mucosa showed the mucosal skeleton of fibroblasts, connective tissue and blood vessels denuded of epithelial cells.

[3H]Quinuclidinyl benzilate binding assay

All tissues were weighed and homogenized in 10 vol. of ice-cold sucrose (0.3 mol/l) with a rotating-blade homogenizer (Polytron, Brinkmann Instruments) twice for 5 s at setting 6. The homogenates were filtered through cheesecloth and centrifuged at 1000 g for 10 min. The supernatant, a crude membrane preparation, was assayed for [3H]quinuclidinyl benzilate binding immediately.

The method of Yamamura & Snyder [14], first described for the assay of [3H]quinuclidinyl benzilate binding to rat brain, was used with minor modification. Crude membrane fraction (50 μl) and 10 μl of water or 10 μl of atropine (10⁻³ mol/l) were incubated in a borosilicate glass tube (12 mm × 75 mm) for 5 min at 37°C. A solution of [3H]quinuclidinyl benzilate in phosphate-buffered saline (10 μl) containing 1.58 pmol of [3H]quinuclidinyl benzilate, specific radioactivity 1.59 KBq/pmol (The Radiochemical Centre, Amersham, U.K.), was added with thorough mixing and the incubation continued in a shaking water bath for 15 min at 37°C. The incubate was then poured on to pre-wetted glass-fibre filters (Whatman GF/B) in a vacuum filtration manifold (Millipore Corp.) and the tubes were rinsed on to the filters with three 1 ml washes of ice-cold phosphate-buffered saline. The filters were directly washed with a further 3 ml and the vacuum was kept on until the
filters appeared dry. Drying was then completed with 15 min at 85°C in a hot-air oven and the filters were placed in 5 ml of scintillation cocktail [TritonX-100 (1 l)/toluene (2 l)/Omnifluor (New England Nuclear, 12 g)] and counted for radioactivity in a scintillation counter (LS 8100, Beckman Instruments) with high efficiency for tritium and automatic quench correction.

By this method, a blank incubation without cell membranes gave a radioactivity count similar to the background reading. Specific [3H]quinuclidinyl benzilate binding was the mean total binding minus the mean binding in the presence of atropine, both being performed in duplicate.

To determine the saturability of [3H]quinuclidinyl benzilate binding a mucosal scrape was prepared from the whole length of the small intestine of a fasted rat. Specific radioactivity of the labelled quinuclidinyl benzilate was lowered with unlabelled pure quinuclidinyl benzilate supplied by Hoffmann-La Roche (Nutley, NJ, U.S.A.) and the assay performed as described.

All the tissue fractions from either a vagotomized animal or from its paired control were measured in a single assay, which included an aliquot of a rat brain homogenate as a check of assay variability.

Sucrase activity was measured in jejunal villus and crypt fractions by the method of Dahlqvist [15], protein was measured by the Lowry method [16] with bovine serum albumin as standard and specific [3H]quinuclidinyl benzilate binding was expressed as femtomoles per milligram of protein.

Statistical evaluation of results was made by Student's t-test and analysis of variance [17].

Results

The sucrase activity of the villus cell homogenate (80.2 ± 9.4 units/mg of protein) was significantly higher than that of the crypt cell homogenate (63.6 ± 11.7, P < 0.05) and did not alter after vagotomy. Similarly, total protein obtained from each of the tissue homogenate was unaffected by vagotomy.

The specific binding of [3H]quinuclidinyl benzilate to the crude membrane preparation of scraped jejunal mucosa is shown in Fig. 1. Saturation of binding occurs at a concentration of above 20 mmol/l, the maximum binding being about 30 fmol/mg of protein. For the experimental preparations the binding of [3H]quinuclidinyl benzilate alone, in the presence of atropine (10⁻⁶ mol/l; non-specific binding) and their difference (specific binding) are shown in Table 1. Clearly a large proportion of the total binding is nonspecific, this proportion being greatest in the pancreatic preparation. Specific [3H]quinuclidinyl benzilate binding to the ileal mucosa (52 ± 3.0 fmol/mg of protein) was greater than that to either jejunal mucosa (26 ± 2.0 fmol/mg of protein, t = 3.08, P < 0.02) or duodenal mucosa (29 ± 2.4 fmol/mg of protein, t = 2.72, P < 0.05). These differences were also present after vagotomy.

![Fig. 1. Saturability of quinuclidinyl benzilate binding to jejunal mucosal membranes. Each point is the mean of three observations.](image)

**TABLE 1. Binding of [3H]quinuclidinyl benzilate to gut tissues of sham-operated and vagotomized rats**

Results are means ± SEM from 10 rats. Specific binding is the difference between total binding and that occurring in the presence of atropine (1·4 × 10⁻⁶ mol/l). * Different from ileal mucosa, ** different from control; P < 0.05.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control Total + Atropine</th>
<th>Specific</th>
<th>Postvagotomy Total + Atropine</th>
<th>Specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenal mucosa</td>
<td>132 ± 7·9</td>
<td>103 ± 13</td>
<td>29 ± 2·4</td>
<td>109 ± 14</td>
</tr>
<tr>
<td>Jejunal mucosa</td>
<td>93 ± 14</td>
<td>67 ± 12</td>
<td>26 ± 2·0</td>
<td>79 ± 9</td>
</tr>
<tr>
<td>Jejunal villi</td>
<td>181 ± 10</td>
<td>141 ± 14</td>
<td>40 ± 3·1</td>
<td>147 ± 16</td>
</tr>
<tr>
<td>Jejunal crypts</td>
<td>163 ± 11</td>
<td>110 ± 8·2</td>
<td>53 ± 3·0</td>
<td>152 ± 13</td>
</tr>
<tr>
<td>Jejunal muscle</td>
<td>92 ± 8</td>
<td>64 ± 10</td>
<td>27 ± 4·1</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>Ileal mucosa</td>
<td>143 ± 12</td>
<td>91 ± 11</td>
<td>52 ± 3·0</td>
<td>125 ± 17</td>
</tr>
<tr>
<td>Pancreas</td>
<td>210 ± 22</td>
<td>172 ± 12</td>
<td>38 ± 8·0</td>
<td>216 ± 30</td>
</tr>
</tbody>
</table>
There was a tendency for specific 

\[ ^3H \text{quinuclidinyl benzilate binding to be lower in the postvagotomy preparations than in their paired controls, although this was statistically significant only for the duodenal mucosa where the specific } ^3H \text{quinuclidinyl benzilate binding was reduced from } 3.3 \pm 2.4 \text{ to } 1.3 \pm 0.9 \text{ fmol/mg of protein } (F = 5.377, P < 0.05). 

Discussion

In the present study specific and saturable binding of \[ ^3H \text{quinuclidinyl benzilate to membrane preparations of the intestinal mucosa of the rat has been demonstrated. The amount of specific binding, between 26 and 52 fmol/mg of protein, is comparable with reported values for regions of mouse brain of 40–100 fmol/mg of protein [18]. This might suggest that there exists a direct cholinergic innervation of the mucosal epithelial cells, although some other considerations must be made. Firstly, the existence of mAChR on the membrane of non-innervated cells such as leucocytes [11] and erythrocytes [19] shows that the demonstration of receptors is not evidence itself of cholinergic innervation. Secondly, the presence of the receptors in the preparation could be due to its contamination with other cells such as neuron fragments or blood cells. Inevitably some contamination of a mucosal scrape with blood cells will occur but is unlikely to contribute more than 2–3% of the mucosal mass, and nerve fibres even less. Because the receptor density of these cells is similar to that of the mucosal scrapes and EDTA-released cell preparations, their contribution will not influence the overall result.

The distribution of mAChR between villus and crypt cells is apparently uniform on the basis of receptor density referred to protein. This could, however, indicate a smaller number of receptors per crypt cell, whose individual protein content is less than that of the larger villus cell. It is thus unlikely that the crypt cells have a denser population of mAChR than have the villus cells.

Thus, purely on the basis of mAChR distribution, the secretory response to cholinergic stimulation need not necessarily be localized to the crypts.

It may be noted that the jejunal villus and crypt cell preparations which were treated with EDTA had a higher binding than did scraped jejunal mucosa, although addition of EDTA to a final concentration of 5 mmol/l in the assay had no effect on the total binding. Possibly this greater activity of the EDTA preparations is due to inhibition by EDTA of proteinases or to production of a smaller particle size of membranes, allowing greater access of the ligand to the binding sites.

Ileal mucosal scrape cells had a significantly higher \[ ^3H \text{quinuclidinyl benzilate binding than duodenal or jejunal scrapes. This suggests that the ileal mucosa has a richer cholinergic innervation and indeed, in human small intestine, muscarinic blockade with atropine has more effect on water and electrolyte transport [20]. However, morphological differences between the jejunum and ileum, namely the more sparse and short villi and the greater population of goblet cells, could conceivably increase the mAChR population there.

The specific \[ ^3H \text{quinuclidinyl benzilate binding to muscle was less than that to the mucosal fractions and did not change with vagotomy. Binding to pancreas membranes was similar to that found by Ng et al. [21], but was limited by the very high non-specific binding in the tissue. This tissue was the only one of those examined which showed an increase in specific binding after vagotomy, although this failed to reach statistical significance.

Vagotomy was followed by a significant decline in the specific \[ ^3H \text{quinuclidinyl benzilate binding in the duodenal mucosa. This was surprising as it had been expected that, by analogy with skeletal muscle, denervation might increase the number of acetylcholine receptors [12]. Alternatively, as has been suggested by Hirschowitz et al. [22] on the basis of pharmacological studies of the denervated stomach, a change in receptor affinity could occur. Denervation clearly has different effects upon acetylcholine receptors in skeletal muscle as opposed to smooth muscle. In both systems postdenervation hypersensitivity to neurotransmitters occurs, but more recently Sachs et al. [23] using cat iris and Burt [24] using sympathetic ganglia have demonstrated that deafferentation did not affect mAChR activity in these tissues, whereas Rotter et al. [25] have demonstrated that, after denervation of the hypoglossal nucleus, the earliest change detected is a decrease in mAChR followed by a reduction of synaptic contacts. The present findings appear to confirm that in the gut parasympathetic denervation is followed by a fall in mAChR.

Is there then a receptor basis for denervation hypersensitivity? Clearly measurement of the total specific \[ ^3H \text{quinuclidinyl benzilate binding may fail to detect changes in a number of subpopulations of mAChR, only one of which might be the physiologically important receptor and could be increased in numbers or in affinity for acetylcholine after denervation. This could
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only be analysed by more subtle kinetic studies of the mAChR of normal and denervated epithelia. In the present study only one of three described approaches [11] to the characterization of a putative mAChR has been completed, namely the demonstration of saturable and specific binding; pharmacological specificity studies have yet to be performed on the intestinal mucosal mAChR and the final stage of localization in situ and quantification of the mAChR in epithelia should further elucidate the distribution of these receptors at a cellular level.

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References