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The inhibitory effects of antimuscarinic autoantibodies in the sera of primary Sjogren syndrome patients on the gastrointestinal motility

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ABSTRACT

Impairment of gastrointestinal tract (GI) function, including delayed gastric emptying and colonic dysmotility, are common features of primary Sjögren's syndrome (SS). However, the pathogenesis remains largely unknown. The aim of the current study was to investigate the role of functional autoantibodies to the muscarinic receptor in mediating GI dysfunction associated with primary SS. The effect of SS or normal immunoglobulin G (IgG) on smooth muscle (SM) motility was assessed by comparing the amplitude of carbachol (CCh) or electrical field stimulation (EFS) – induced muscle contraction before and after IgG application. Muscarinic receptor type 3 (M3R) played a dominant role in both colon and gastric SM contraction, while M2R was partly involved in gastric smooth muscle contraction. Preincubation for 1 h of the colon and gastric SM strips with 1 mg/ml purified IgG from the sera of four primary SS patients (SS IgG) significantly inhibited carbachol-induced smooth muscle contraction (CISC) over a range of CCh concentrations, whereas IgG from healthy controls had little effect. Incubation of the colon SM strips with SS IgG also inhibited EFS-induced colon muscle contraction, which was mimicked by the M3R-selective blocker, 4-DAMP. SR1403330, an NK1 antagonist, had little effect on EFS-mediated colonic SM contraction. The results suggest that autoantibodies isolated from primary SS patients' sera inhibit muscarinic receptor-mediated cholinergic neurotransmission in mouse colon and stomach, which may provide clues for explaining the GI dysfunction seen in patients with primary SS.

1. Introduction

Gastrointestinal dysmotility is associated with primary Sjögren's syndrome (SS). In previous clinical studies, impaired esophageal motor function has been reported in primary SS patients using manometric assessment (Türk et al., 2005; Rosztóczy et al., 2001). A prolonged gastric emptying was also found using scintigraphy, and a considerable proportion of primary SS patients exhibited a markedly decreased gastric motility and dysfunction of the urinary tract (Kovács et al., 2003). However, the digestive tract disturbances demonstrated in primary SS patients has not been verified at tissue levels and the underlying pathogenesis remains unclear.

There is increasing evidence that autoantibodies from the sera of SS patients (SS IgG) affect the function of muscarinic receptors. For example, infusion of serum IgG from human primary SS patients to mice causes salivary gland hypofunction (Nguyen et al., 2000), and acute or chronic application of SS IgG inhibits the function of muscarinic receptors in isolated bladder strips (Waterman et al., 2000) and salivary epithelial cells (Li et al., 2004; Dawson et al., 2006). While these findings do not explain the gastrointestinal dysmotility in primary SS patients, it is known that secretion in salivary glands and contraction in smooth muscle of bladder and the gastrointestinal tract is primarily mediated by a muscarinic receptor, suggesting that these receptors are targeted by patient autoantibodies.

We hypothesized that if SS autoantibodies have anti-muscarinic activity, they will inhibit smooth muscle contraction in the gastrointestinal tract, resulting in the gastrointestinal dysmotility. Our study demonstrates that functional antibodies from patients with primary SS have an inhibitory effect on gastrointestinal smooth muscle motilities mediated by muscarinic receptor in stimulated conditions.

Abbreviations: SS, Sjögren's syndrome; CCh, Carbachol; GI, gastrointestinal tract; IgG, immunoglobulin G; SM, smooth muscle; CISC, carbachol-induced smooth muscle contraction; EFS, electrical field stimulation; M3R, muscarinic receptor type 3; 4-DAMP, 1,1-dimethyl-4-diphenyl acetoxy piperidinium iodide; PLC, phospholipase C; NK1, neurokinin 1.

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2. Materials and methods

2.1. Patients and animal

Blood samples were obtained with informed consent from patients with primary SS who fulfilled at least 4 of the 6 European consensus criteria, including seropositivity for anti-Ro/La antibodies ($n=4$), and from healthy controls ($n=6$). Purified SS IgG were positive for anti-M3R antibodies as determined by the in vitro bladder strip assay (Waterman et al., 2000), while the controls tested negative for anti-M3R antibodies. IgG was prepared using the caprylic acid precipitation technique (Jackson et al., 2008). Male BALB/c mice (ages 10–12 weeks, 20–30 g) were killed and the colons excised and suspended in a 100-ml organ bath containing Krebs-Ringer solution with 95% O_2/5% CO_2 (pH 7.4) at 37 °C. Any fecal matter present was gently flushed from the colon and the mesentery was dissected free. The study was approved by the Clinical Ethics Committee of Flinders Medical Center.

2.2. Functional assay of smooth muscle contraction induced by carbachol (CCh) or electrical field stimulation (EFS) in colon and stomach

The longitudinal smooth muscles (10 mm in length) were prepared from the central part of colon. In stomach, the circular muscle strips (10 mm in length and 2 mm in width) were obtained from the fundic regions. SM preparations were mounted longitudinally in 10-ml jacketed organ baths containing Krebs solution at 37 °C and gassed with 95% O_2/5% CO_2. Carbachol (CCh) concentration–response curves were produced by the cumulative addition of CCh from 0.1 to 10 μM. Electrical stimuli, pulse width 0.5 ms and strength 70 V, were generated with a stimulator (Grass 588, UK) and delivered using a pair of electrodes, one positioned in the organ bath and the other set on the tip of the J-shaped tissue holder. EFS for 3 s was applied at frequencies of between 10 and 70 Hz. Contractile responses to EFS or CCh stimulation were measured using isometric transducers (Letica, Spain) connected to a PowerLab/8s data acquisition system (AD Instruments, Sydney, Australia).

2.3. Experimental protocol and data analysis

The longitudinal smooth muscle and circular muscle strips were left to equilibrate for 30 min before the experiment was started. For analysis of IgG effects on smooth muscle contraction, patient or control IgG was added to give a final concentration of 1.0 mg/ml. After a 30-min incubation, consecutive smooth muscle contractions induced by CCh or EFS were recorded at 30 min intervals. The amplitudes of pre- and post-IgG smooth muscle contractions were calculated using the peak parameters – peak amplitude function of the DataPad on Chart v4.2 software (ADInstruments) and compared by unpaired Student t-test or 2-way analysis of variance using GraphPad Prism (ver 3.0a for Macintosh; GraphPad Software, San Diego, CA). P values <0.05 were considered significant.

3. Results

3.1. Subtype of muscarinic receptors expressed in mouse colon and stomach

We first investigated what subtype of muscarinic receptors play a dominant role in mouse colon and stomach SM contraction. Fig. 1A shows a typical concentration–response curve induced by carbachol (CCh) recorded from colon muscle strips.

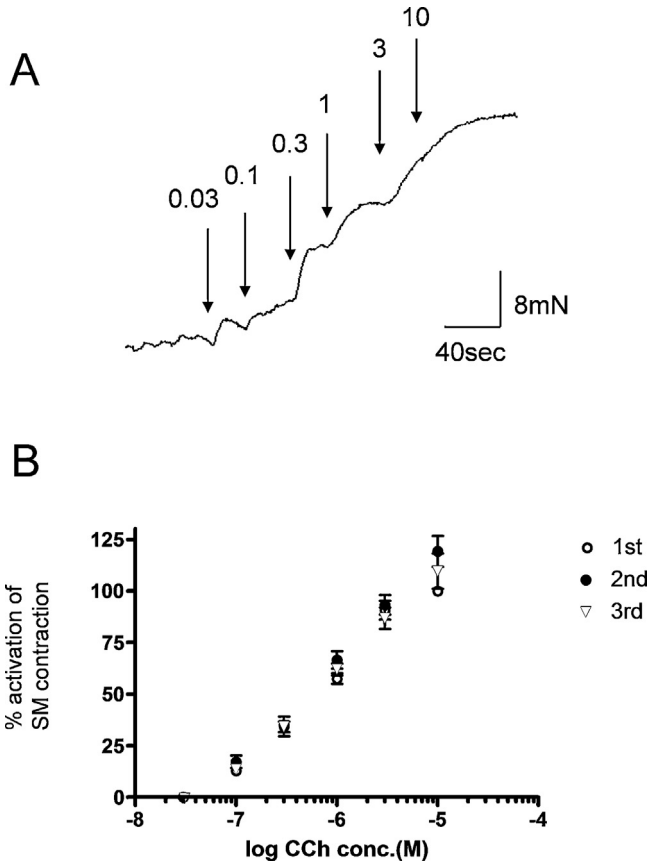

Fig. 1. (A) Representative concentration–response curve induced by carbachol (CCh) recorded from colon muscle strips. CCh concentration–response curves were produced by the cumulative addition of CCh from 0.03 to 10 μM. (B) Average CCh concentration–response curves measured at three recording points at 30 min intervals. The data are presented as mean ± S.E ($n=6$). There was no time-dependent changes in amplitudes of CCh-induced muscle contractions.

CCh concentration–response curves were produced by the cumulative addition of CCh from 0.03 to 10 μM (Fig. 1A). We repeated the experiment three times (1st, 2nd, 3rd) at 30 min intervals and confirmed there was no time-dependent changes of amplitudes in CCh-induced muscle contractions (Fig. 1B).

We then pretreated the muscle strips for 30 min with methoctramine(Tocris, Bristol, UK), a muscarinic type 2 (M2) preferring muscarinic receptor antagonist, or 1,1-dimethyl-4-diphenyl acetoxy piperidinium iodide (4-DAMP; Tocris, Bristol, UK), a muscarinic type 3 (M3) preferring muscarinic receptor antagonist, to identify the putative components involved in the CCh-induced smooth muscle contraction (CISC) in mouse colon (Fig. 2). 300 nM methoctramine exerted minimal effects on the CISC (Fig. 2A). By contrast, 30 nM 4-DAMP significantly inhibited CISC at whole range of CCh concentrations (Fig. 2B). The inhibitory effect of 4-DAMP was reversible. CISC was partly recovered 30 min after washout of 4-DAMP. Pretreatment of the muscle strips with 10 μM U73122, a PLC inhibitor, for 30 min also significantly inhibited CISC (Fig. 2C).

Similar experiments was done using gastric smooth muscle (Fig. 3). 300 nM methoctramine also inhibited CISC in gastric smooth muscle (Fig. 3A), but its inhibitory effect was much less than 4-DAMP (Fig. 3B). The inhibitory effect of 4-DAMP was also reversible in mouse gastric smooth muscle. The result demonstrates that muscarinic type 3 receptor (M3R) plays a dominant role in both colon and gastric smooth muscle, although muscarinic type 2 receptor partly plays a role in gastric smooth muscle.