



ELSEVIER

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Localization of muscarinic acetylcholine receptor 2 to the intestinal crypt stem cell compartment

Eleanor D. Muise, Neeru Gandotra, John J. Tackett,
Michaela C. Bamdad, Robert A. Cowles*

Department of Surgery, Section of Pediatric Surgery, Yale School of Medicine, USA

ARTICLE INFO

Article history:

Received 17 November 2016

Received in revised form

13 December 2016

Accepted 15 December 2016

Available online 21 December 2016

Keywords:

Enteric nervous system

Muscarinic acetylcholine receptor

Stem cell compartment

Intestinal crypt

Paneth cell

ABSTRACT

The data presented in this article are related to the research article entitled “Distribution of muscarinic acetylcholine receptor subtypes in the murine small intestine” (E.D. Muise, N. Gandotra, J.J. Tackett, M.C. Bamdad, R.A. Cowles, 2016) [1]. We recently demonstrated that neuronal serotonin stimulates intestinal crypt cell division, and induces villus growth and crypt depth (E.R. Gross, M.D. Gershon, K.G. Margolis, Z.V. Gertsberg, Z. Li, R.A. Cowles, 2012; M.D. Gershon, 2013) [2,3]. Scopolamine, a nonspecific muscarinic receptor antagonist, inhibited serotonin-induced intestinal mucosal growth [2]. Here we provide data regarding the localization of muscarinic acetylcholine receptor 2 to the intestinal crypt stem cell compartment.

© 2016 Published by Elsevier Inc. This is an open access article under the CC BY license

(<http://creativecommons.org/licenses/by/4.0/>).

Specifications Table

Subject area	Biology
More specific subject area	Muscarinic acetylcholine receptors
Type of data	Figures
How data was acquired	RNA extraction, RT-PCR, Immunofluorescence, Microscopy (Zeiss 510 Laser Scanning Confocal microscope, Carl Zeiss International, Germany)
Data format	Raw

DOI of original article: <http://dx.doi.org/10.1016/j.ifs.2016.10.030>

* Corresponding author.

E-mail address: robert.cowles@yale.edu (R.A. Cowles).

<http://dx.doi.org/10.1016/j.dib.2016.12.032>

2352-3409/© 2016 Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Experimental factors	No pretreatment
Experimental features	Muscarinic acetylcholine receptor subtype presence determined by RT-PCR, and localization determined by immunofluorescence and confocal microscopy
Data source location	Yale University College of Medicine, New Haven, CT
Data accessibility	The data are supplied within this article

Value of the data

- Muscarinic receptors are ubiquitous throughout the gastrointestinal tract.
- Nonspecific blockade of muscarinic receptors blunts neuronal serotonin stimulated growth.
- Muscarinic acetylcholine receptor subtype 2 localizes to Paneth cells within the intestinal crypt stem cell compartment.

1. Data

Data show all five muscarinic acetylcholine receptor subtypes (mAChR) were present in the mouse duodenum, jejunum, and ileum across all ages of mice by RT-PCR (Fig. 1). Mouse ileum was further imaged with immunofluorescence microscopy and highlighted mAChR2 within intestinal crypts (Fig. 2). mAChR2 was localized to the crypt stem cell compartment (Fig. 3), and further co-localized within Paneth cells in the intestinal crypt with lysozyme (Fig. 4).

2. Experimental design, materials and methods

2.1. Animals

This study was approved by the Institutional Animal Care and Use Committee, and animals were maintained in accordance with guidelines from this committee on the care and use of laboratory animals, and described in detail previously [1].

2.2. Real-time polymerase chain reaction (RT-PCR)

RNA isolation was performed using RNeasy Kit (Qiagen) and concentration measured using Nanodrop (Thermo Scientific). Two micrograms of RNA was used as a template for cDNA synthesis, and reverse transcription was performed with KAPA Mouse Genotyping Kit (Kapa Biosystems). Subtype specific primers for mAChR 1–5 (Table 1) were used for amplification under the following conditions: 3 min @95 °C, 30 s @95 °C, 50 s @60 °C, 30 s @72 °C x30 cycles, with 10 min @72 °C extension and held at 12 °C. The products were separated by gel electrophoresis (1.5% agarose gel with ethidium bromide).



Fig. 1. mAChR RT-PCR. Photo of mAChRs extracted from jejunum of 6 week-old C57BL/6 mouse, mAChRs 1–5 flanked by ladder (L) in 1.5% agarose gel with ethidium bromide.

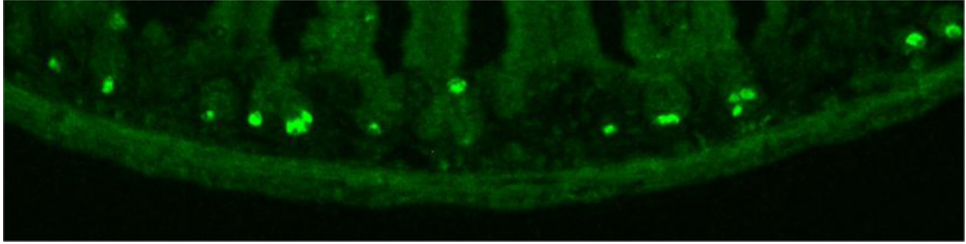


Fig. 2. Immunofluorescence staining for subtype specific mAChR2 localized to intestinal crypts. 10x.

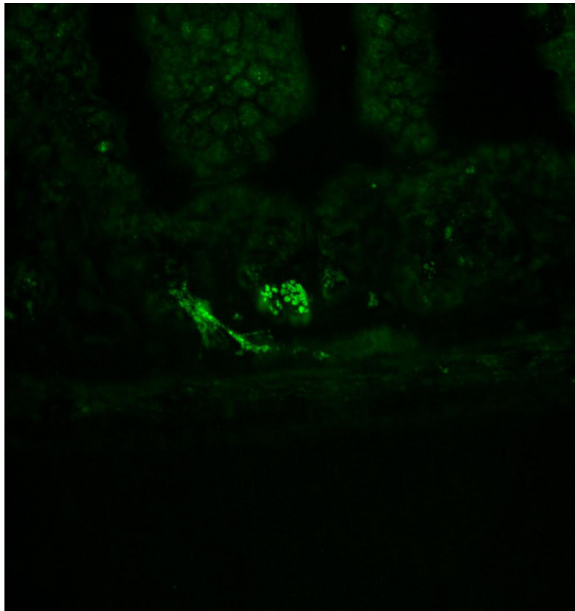


Fig. 3. mAChR2 localized to intestinal crypt stem cell compartment. mAChR2 labeling illuminated on channel 488 demonstrates a discrete granular pattern within the intestinal crypt stem cell compartment.

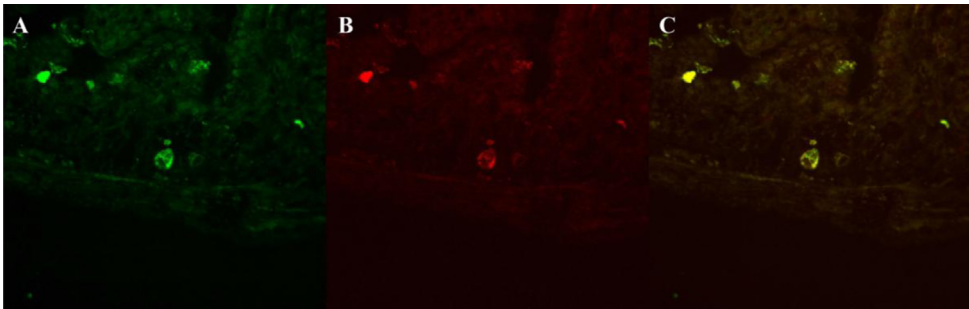


Fig. 4. mAChR and Lysozyme co-localize to Paneth cells in the intestinal crypt. A demonstrates mAChR2 on channel 488 with an intracellular granular pattern in the center of the image. B captures the same slide on channel 594, stained for lysozyme, a Paneth cell marker, which highlights the characteristic granules. C illustrates the merge of both images with yellow color representing areas of overlapping signal.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
mAChR subtype specific primer sequences for RT-PCR.

Gene	Sense	Antisense
<i>mAChR1</i>	5'-GCACAGGCACCCACCAAGCAG-3'	5'-AGAGCAGCAGCAGCGGAACG-3'
<i>mAChR2</i>	5'-GGCAAGCAAGAGTAGAATAAA-3'	5'-GCCAACAGGATAGCCAAGATT-3'
<i>mAChR3</i>	5'-GTCTGGCTTGGGTCATCTCT-3'	5'-TTCGTGCCTTGTCTGTAG-3'
<i>mAChR4</i>	5'-AGTGCTTCATCCAGTCTTGTTCCA-3'	5'-CACATTCATTGCCTGTCTGCTTTG-3'
<i>mAChR5</i>	5'-CTCATCATTGGCATCTTCTCCA-3'	5'-GGTCCTTGGTTCGCTTCTGT-3'

Table 2
mAChR and lysozyme primary and secondary antibodies.

Antigen	Host species	Dilution	Company
Primary antibodies			
<i>mAChR1</i>	Rabbit	1/20	Millipore (AB5164)
<i>mAChR2</i>	Rabbit	1/20	Millipore (AB5166)
<i>mAChR3</i>	Rabbit	1/20	CosmoBio Co (YCUPSM3)
<i>mAChR4</i>	Mouse	1/20	Millipore (MAB1576)
<i>mAChR5</i>	Rabbit	1/40	Abcam (AB41171)
Lysozyme	Goat	1/200	SCBT (SC27958)
Secondary antibodies			
<i>anti Rabbit 488</i>	Goat	1/200	Life Technologies (a11034)
<i>anti Rabbit 488</i>	Donkey	1/200	Life Technologies (a21206)
<i>anti Mouse 488</i>	Goat	1/200	Life Technologies (a11029)
<i>anti Goat 594</i>	Donkey	1/200	Life Technologies (a11058)

2.3. Immunohistochemistry

Ileum from 2 week-old mice was flushed and fixed with Nakane fixative, washed with tris-buffered saline (TBS), dehydrated with ethyl alcohol, and subsequently placed in propylene oxide. Fixed dehydrated tissue was embedded in epon (Electron Microscopy Sciences) then sectioned at 1–2 μ m.

Etching was performed with potassium hydroxide in methanol and propylene oxide to dissolve the plastic, and subsequently rinsed with methanol then TBS. Antigen retrieval was performed with heated citrate buffer.

Slides for were quenched with ammonium chloride prior to blocking. Primary and secondary immunofluorescence antibodies for mAChR1-5 and lysozyme are given in Table 2. Slides were mounted with Vectashield (Vector Laboratories), and kept at -20°C .

2.4. Imaging

Images were obtained with a Zeiss 510 Laser Scanning Confocal microscope (Carl Zeiss International, Germany). No spectral wavelength overlap was observed between Alexa 488 and Alexa 594 channels.

Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2016.12.032>.

References

- [1] E.D. Muise, N. Gandotra, J.J. Tackett, M.C. Bamdad, R.A. Cowles, Distribution of muscarinic acetylcholine receptor subtypes in the murine small intestine, *Life Sci.* (2016).
- [2] E.R. Gross, M.D. Gershon, K.G. Margolis, Z.V. Gertsberg, Z. Li, R.A. Cowles, Neuronal serotonin regulates growth of the intestinal mucosa in mice, *Gastroenterology* 143 (408–17) (2012) e2.
- [3] M.D. Gershon, 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract, *Curr. Opin. Endocrinol. Diabetes Obes.* 20 (2013) 14–21.