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Effects of intestinal inflammation on specific subgroups of guinea-pig celiac ganglion neurons

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ABSTRACT

The consequences of inflammation of a short region of the guinea-pig ileum on the properties of neurons in the celiac ganglia were investigated. Inflammation (ileitis) was induced in 5–8 cm of intestine by the intralumenal injection of trinitrobenzene sulfonate, 6–7 days before tissue was taken. Celiac ganglion neurons were investigated using intracellular microelectrodes and the cells were filled with dye from the recording electrode, to determine their morphologies. Tonic and phasic neurons were identified. In ganglia from normal guinea-pigs and from guinea-pigs with ileitis, cell bodies of tonic neurons were larger and their dendrites were longer and more numerous than those of phasic neurons. Tonic neurons were selectively affected by intestinal inflammation. The number of action potentials elicited by the same intensity of depolarizing current for neurons after ileal inflammation was twice that of neurons from control animals, the threshold current to evoke action potentials was about half, and some of the neurons were spontaneously active. Neurons from untreated or sham-operated animals were never spontaneously active. Many more neurons were affected than project to the 5–8 cm of intestine that was inflamed. We conclude that inflammation of a segment of the ileum causes a selective, humorally mediated, increase in excitability of tonic neurons in the celiac ganglion that control motility and secretion, but not of phasic neurons that project to the intestinal vasculature and other targets.

Inflammation of the intestine triggers substantial changes in the excitability of enteric neurons within the gut wall, and of extrinsic primary afferent neurons that innervate the gut. Within the gut, specific subgroups of neurons become hyperexcitable and changes in both motility and secretion occur [1,12,13,20,21]. The cell bodies of extrinsic primary afferent neurons in dorsal root ganglia also become more excitable, and pain signaling from the gut is potentiated [2,3,24]. Changes outlast the period of inflammation [14]. Similarly, increases in pain and discomfort and pathological alterations in patterns of motility, associated with the irritable bowel syndrome (IBS), are observed for many years after severe gastroenteritis [19,23,27].

The neurons in which post-inflammatory changes have been investigated (enteric and dorsal root ganglion neurons) are in direct connection with the sites of inflammation. Nerve endings of post-ganglionic sympathetic neurons also enter the wall of the intestine and come in close proximity to enteric neurons and the mucosa [6], which are both affected by inflammation. Thus, it might be anticipated that sympathetic neurons are also influenced by intestinal inflammation. In fact, induction of c-FOS occurs in some sympathetic post-ganglionic neurons of the inferior mesenteric ganglia following inflammation of the colon [22]. However, pre-vertebral sympathetic ganglia are heterogeneous, and in the inferior mesenteric ganglia only some neurons supply gastrointestinal targets. Thus, whether the neurons affected were those specifically associated with the intestine is unknown.

We chose the guinea-pig celiac ganglia to investigate the electrophysiological properties of neurons following inflammation of the small intestine because the electrophysiological characterization of neuron types, the projections of neurons from the ganglia to different targets, and the connections made by nerve endings in the ganglia are well established [8,11,15].

Experiments were performed on guinea-pigs (150–275 g) of either sex from the inbred Hartley strain colony of the Department of Anatomy and Cell Biology at the University of Melbourne. Experimental procedures were conducted according to the National Institute of Health guide for the care and use of laboratory animals, and were approved by the University of Melbourne Animal Experimentation Ethics Committee.

Inflammation was induced as previously described [20]. Guinea-pigs were anaesthetized by intramuscular injection of a mixture of 20 mg/kg xylazine and 100 mg/kg ketamine (Troy Laboratories,

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Table 1
Resting membrane potentials, input resistances and rheobases for celiac ganglion neurons from control, TNBS-treated and sham-operated guinea-pigs

Parameters	Groups	Tonic	Phasic-S	Phasic-LAH
RMP (mV)	Control	−56.1 ± 0.9 (34)	−59.8 ± 1.1 (21)	−60.2 ± 1.6 (16)
	Inflamed	−57.8 ± 0.9 (42)	−59.2 ± 1.8 (16)	−58.3 ± 1.2 (9)
	Sham	−58.0 ± 3.1 (11)		
R_{in} (MΩ)	Control	102 ± 2 (34)	112 ± 6 (22)	117 ± 21 (16)
	Inflamed	*148 ± 6 (42)	116 ± 9 (16)	123 ± 16 (9)
	Sham	98 ± 21 (11)		
Rheobase (threshold for AP, pA)	Control	140 ± 6 (34)	239 ± 21 (27)	315 ± 41 (16)
	Inflamed	*79 ± 8 (42)	207 ± 11 (16)	300 ± 21 (9)
	Sham	142 ± 25 (11)		

Values are means ± S.E.M. with numbers of observations in parentheses. *$P < 0.05$ statistically significant differences compared to control.

Melbourne, Australia). The abdomen was opened by a mid-line incision, the distal ileum was exteriorized and TNBS (2,4,6-trinitrobenzenesulfonic acid, Wako Industries, Nagoya, Japan), 30 mg/kg in 1 mL of 30% ethanol, was injected into the lumen of the small intestine, 8–10 cm proximal to the ileocaecal junction. The intestine was occluded just distal to the injection site during the injection and for a further minute following injection. This restricted the region of inflammation to 5–8 cm of intestine proximal to the injection. The intestine was returned to the abdominal cavity, the abdominal wall was closed, and guinea-pigs were allowed to recover from anaesthesia. Animals were taken at 6 and 7 days after TNBS injection.

Control data are from guinea-pigs of the same colony that were in the same age range, but were not subjected to surgery. In addition, changes were investigated in tissue from sham-operated animals. These animals underwent identical surgery, except for the omission of TNBS. Tissue was taken 6 and 7 days later.

For electrophysiology, the celiac ganglia were removed and placed in physiological saline (composition in mM: NaCl 118, KCl 4.8, $NaHCO_3$ 25, NaH_2PO_4 1.0, $MgSO_4$ 1.2, glucose 11.1, $CaCl_2$ 2.5; equilibrated with 95% O_2/5% CO_2). Ganglia were pinned to the silgard elastomer base of a recording dish (volume 1 mL) which was placed on the stage of an inverted microscope and continuously superfused (4 mL/min) with physiological saline at a bath temperature of 34–35 °C. Neurons were impaled with conventional borosilicate glass microelectrodes filled with 1% biocytin (Sigma–Aldrich, Sydney, Australia) in 1 M KCl. Electrode resistances were 100–170 MΩ. Recordings were made using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA, USA) in current clamp mode. Signals were digitized at 1–10 kHz, using a Digidata 1200 interface (Axon Instruments) and stored using PC-based data acquisition software (Axoscope 8.1, Axon Instruments). Data were taken from neurons that fired action potentials (APs) and had resting membrane potentials (RMPs) more negative than −40 mV. Small intracellular hyperpolarizing current pulses (duration 500 ms, intensity 30 pA, yielding voltage shifts of 5–10 mV) were used to determine input resistance (R_{in}). Excitability was assessed by injecting 0.5–2 s depolarizing current pulses, at an intensity of 50–400 pA at 20 s intervals, through the recording electrode, while a holding current was used to maintain the RMP at −60 mV. RMP, R_{in}, AP threshold (rheobase) and numbers of APs in response to depolarizing pulses were determined using in-house analysis routines written in Igor Pro 4.0 analysis software (WaveMetrics, USA).

Biocytin was passed from the recording electrodes into the neurons during impalement. Once a neuron had been injected with biocytin, a diagram of the impalement position within the ganglion was prepared so that the neuron could be identified later. To take further recordings, the electrode was moved to a new position to avoid ambiguity of cell identity. At the end of each experiment, the tissue was fixed overnight in 2% formaldehyde plus 0.2% picric acid in 0.1 M sodium phosphate buffer (pH 7.0), cleared in DMSO (3× 10 min changes), and washed in PBS (3× 10 min). Ganglia were embedded flat in low melt OCT compound (Tissue Tek, Elkhart, IN, USA) and cut in 60 μm sections using a freezing microtome. Sections were incubated in streptavidin coupled to Texas Red (Amersham Biosciences, Melbourne, Australia) at a dilution of 1:400 for 1 h and then washed three times with PBS. Sections were mounted on slides using non-fluorescent mounting medium (Dako, Glostrup, Denmark).

Sections were examined on a Zeiss Axioplan microscope, fitted for fluorescence confocal imaging (Bio-Rad 1024; Bio-Rad, Melbourne, Australia). Serial images were collected and the morphology of each neuron was categorized by viewing the three-dimensional reconstructions. Quantitative morphological data were obtained using ImageJ software (http://rsb.info.nih.gov/ij/). Images were processed using CorelDraw software (Corel Corporation, Dublin, Ireland). The number of dendrites for each cell was counted as the number of intercepts with a concentric circle of 100 μm diameter centred on the soma.

Electrophysiology experiments utilized celiac ganglia from 61 guinea-pigs with TNBS-induced ileitis, from 13 sham-operated animals and from 81 untreated animals. All neurons were characterized electrophysiologically, labeled by intracellular injection of biocytin during recording, and were later analyzed morphologically. In total, 67 neurons from guinea-pigs with ileitis, 11 from sham-operated animals and 86 from tissues of untreated animals were included in the analysis.

Data are presented as mean ± S.E.M. Statistical differences were determined by Student's *t*-test or ANOVA, as appropriate. Differences were considered statistically significant at $P < 0.05$.

TNBS caused inflammation of 5–8 cm of the ileum that was characterized by small regions of vascular dilatation, haemorrhagic foci, mucosal ulceration, flattening of the mucosa and muscle thickening, as has been previously reported following luminal application of TNBS in the guinea-pig [16,20].

Three types of neurons were encountered in ganglia from untreated, sham-operated and TNBS-treated guinea-pigs (Fig. 1): tonic neurons, phasic-S neurons that lacked a late post-action potential hyperpolarization (late AHP), and phasic neurons in which a single action potential was followed by a late AHP (phasic-LAH neurons), as described previously [4,11]. Morphological investigation of the biocytin-filled neurons confirmed previous observations (see below) that tonic neurons have larger dendritic arborisations, when compared to phasic-S or phasic-LAH neurons (Fig. 2), and that they have larger cell bodies. The number of processes of tonic neurons was about twice that of phasic neurons.

Tonic, but not phasic, neurons of the celiac ganglia were more excitable 6–7 days after injection of TNBS into the distal ileum (Figs. 1 and 3). Hyperexcitability after TNBS treatment was revealed