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Short communication

Agonists of proteinase-activated receptor 2 excite guinea pig ileal myenteric neurons

David R. Linden [a,1], Brian P. Manning [a,1], Nigel W. Bunnett [b], Gary M. Mawe [a,*]

[a] *Given C 423, Department of Anatomy and Neurobiology, The University of Vermont College of Medicine, Burlington, VT 05405, USA*
[b] *Departments of Surgery and Physiology, University of California, San Francisco, CA 94143, USA*

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Abstract

The effects of proteinase-activated receptor 2 (PAR2) agonists on the electrical properties of intact guinea pig ileal myenteric neurons were measured with intracellular microelectrodes. Approximately 52% of AH neurons and 41% of S neurons responded to pressure ejection of SLIGRL-NH_2 or trypsin with a prolonged depolarization that was often accompanied by increased excitability. When added to the bathing solution, trypsin caused a concentration-dependent depolarization of responding neurons with an estimated EC_{50} value of 87 nM. Collectively, these novel observations indicate that PAR2 excites a proportion of myenteric neurons, which may contribute to dysmotility during intestinal inflammation.

Keywords: Euteric nervous system; Neuroimmune interaction; Electrophysiology; Thrombin receptor; Trypsin

1. Introduction

Proteinase-activated receptor 2 (PAR2) is a member of a family of G-protein coupled receptors that is activated through the proteolytic cleavage of the extracellular amino terminus by serine proteases. The resulting new amino terminus then becomes a tethered ligand that binds to and activates the receptor. PAR2 is cleaved by trypsin, a digestive serine protease derived from pancreatic trypsinogen, and tryptase, a serine protease that is stored in and released from mast cell granules (Dery et al., 1998; Vergnolle, 2000).

A potential target for these serine proteases is the myenteric plexus of the gastrointestinal tract, which contains neurons that are responsible for regulating motor activities of the bowel (Furness and Costa, 1987). A subset of ileal myenteric neurons express PAR2 and respond to receptor activation with an increase in intracellular calcium concentration (Corvera et al., 1999). Although functional PAR2 exists on myenteric neurons, nothing is known about the effect of PAR2 activation on the electrical properties of myenteric neurons. To investigate this issue, intracellular recordings were obtained from individual guinea pig ileal myenteric plexus neurons in intact whole mount preparations exposed to exogenously applied activators of PAR2.

2. Materials and methods

2.1. Tissue preparation

All methods used in this study were approved by the University of Vermont Animal Care and Use Committee. Adult guinea pigs (either sex, 250 to 300 g) were anesthetized with isoflurane and exsanguinated. The terminal ileum was removed and placed in iced Krebs solution (in mM: NaCl, 121; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25; NaH_2PO_4, 1.2; and glucose, 8; aerated with a 95%O_2:5%CO_2). Nifedipine (5 mM) and atropine (200 nM) were also added to eliminate smooth muscle contraction. The mucosa, submucosa and circular muscle of the ileum were removed exposing the myenteric plexus on the longitudinal smooth muscle. The preparation was then moved to a recording chamber.

* Corresponding author. Tel.: +1-802-656-8257; fax: +1-802-656-8704.
E-mail address: gmawe@zoo.uvm.edu (G.M. Mawe).
[1] Contributed equally to this work.

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2.2. Electrophysiological recordings

The methods used for electrophysiological recording were similar to those previously described (Manning and Mawe, 2001). Briefly, preparations were maintained at 37 °C, and individual myenteric neurons were impaled with glass microelectrodes filled with 2.0 M KCl that had resistances in the range of 50–100 MΩ. Transmembrane potential was measured with an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA, USA) and electrical signals were acquired and analyzed using MacLab Chart software (AD Instruments, Castle Hills, Australia). A cell was excluded from the study if the input resistance was below 50 MΩ or had an action potential peak less than 0 mV. Compounds to be tested were applied either by pressure microejection through glass micropipettes (15–20-μm tip diameter) placed 50–100 μm from the target ganglia with pulses of nitrogen gas (300 kg/cm^2; 100–1000 ms) or by addition to the circulating Krebs solution.

2.3. Materials

All solutions and compounds were obtained from Sigma (St. Louis, MO) with the exception of SLIGRL-NH$_2$, which was synthesized by solid phase methods and purified by reverse-phase high-pressure liquid chromatography (N.W.B.'s laboratory).

3. Results

The effects of PAR2 agonists on passive and active electrical properties were measured in a total of 92 neurons from 64 preparations. Using the criteria described previously (Bornstein et al., 1994; Wood, 1994), the neurons were classified as AH [35 neurons; resting membrane potential (RMP): -68 ± 3 mV; input resistance: 105 ± 10 MΩ] or S (57 neurons; RMP: -56 ± 2 mV; input resistance: 141 ± 13 MΩ). In responding cells, activation of PAR2 with SLIGRL-NH$_2$ or trypsin elicited a prolonged depolarization (Fig. 1). Local microejection of a synthetic peptide that corresponds to the sequence of the PAR2 tethered ligand, SLIGRL-NH$_2$ (100 μM in pipette, 900-ms duration), elicited a mean depolarization of 7 ± 1 mV ($n = 6$) in 50% (6/12) of AH neurons from an RMP of -67 ± 6 mV ($n = 6$) with a duration (measured to 50% repolarization) of 42 ± 7 s ($n = 6$). SLIGRL-NH$_2$ elicited a depolarization of 10 ± 2 mV ($n = 12$) in 39% (12/31) of S neurons from an RMP of -55 ± 5 mV ($n = 12$) with a duration of 45 ± 15 s ($n = 12$). In all responding neurons, the membrane potential returned to the original resting level.

To test whether the PAR2 agonist-induced depolarization was due to a direct action of SLIGRL-NH$_2$ on the impaled neuron, SLIGRL-NH$_2$ was applied to myenteric neurons in the presence of bath-applied tetrodotoxin

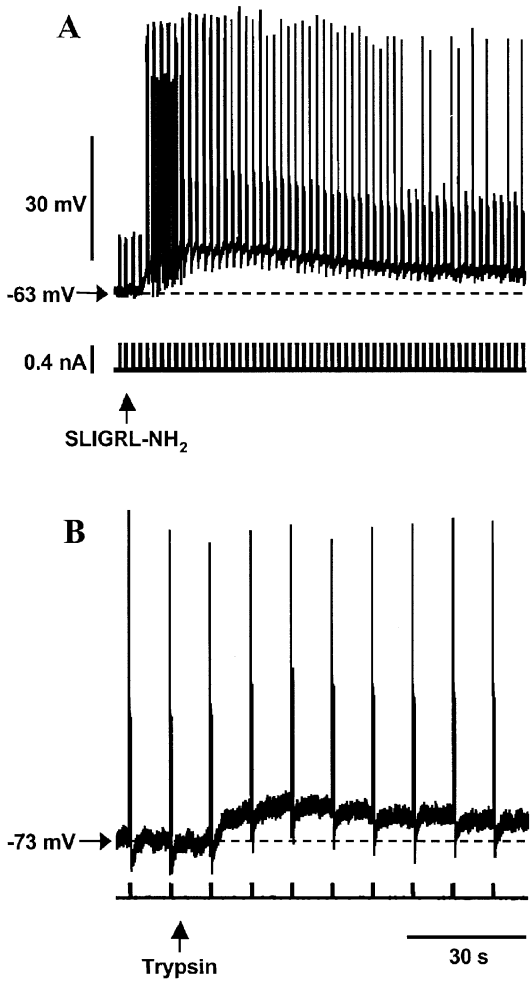

Fig. 1. SLIGRL-NH$_2$ and trypsin depolarize and excite myenteric neurons. Representative traces showing that pressure ejection of (A) SLIGRL-NH$_2$ and (B) trypsin depolarize and excite ileal myenteric neurons. (A) Recording from a neuron with S-type electrical properties that responded to SLIGRL-NH$_2$ not only with a membrane depolarization, but also with an increase in the number of action potentials elicited by depolarizing current pulses and spontaneous activty. (B) Recording from a neuron with AH-type electrical properties that responded to trypsin with a comparable depolarization. Both traces are on the same voltage, current and time scale.

(0.5 μM), a voltage-activated Na^+ channel blocker, which inhibits action potential conduction. Tetrodotoxin did not alter the SLIGRL-NH$_2$-induced depolarization (control, 6.4 ± 0.9 mV; tetrodotoxin, 5.3 ± 0.6 mV; $n = 6$; $P > 0.05$ paired t-test).

The endogenous PAR2 agonist, trypsin, caused a depolarization that was comparable to the SLIGRL-NH$_2$-induced response. Although trypsin also activates PAR4 receptors, no evidence currently exists that PAR4 receptors are expressed in the gastrointestinal tract. Local microejection of trypsin (100 μM in pipette, 900-ms duration), elicited a depolarization of 11 ± 2 mV ($n = 7$) in 54% (7/13) of AH neurons from an RMP of -66 ± 7 mV ($n = 7$) with a duration of 65 ± 9 s ($n = 6$). Trypsin elicited a depolarization of 7 ± 2 mV ($n = 8$) in 44%

(8/18) of S neurons from an RMP of -52 ± 6 mV ($n = 8$) with a duration of 36 ± 6 s ($n = 8$). In all responding neurons, the membrane potential returned to the original resting level. There were no differences in the passive membrane properties of responding versus nonresponding neurons, in response to either agonist. For AH neurons, the RMP (responding: -66 ± 4 mV, $n = 13$; nonresponding: -64 ± 4 mV, $n = 12$) and input resistance (responding: 104 ± 20 MΩ, $n = 13$; nonresponding: 118 ± 13 MΩ, $n = 12$) were not different between the two groups. Likewise, for S neurons, the RMP (responding: -54 ± 3 mV, $n = 20$; nonresponding: -57 ± 2 mV, $n = 29$) and input resistance (responding: 165 ± 24 MΩ, $n = 20$; nonresponding: 138 ± 18 MΩ, $n = 29$) were not different between the two groups ($P > 0.05$ unpaired *t*-test).

The depolarizations in response to SLIGRL-NH_2 or trypsin were associated with changes in membrane input resistance. The depolarizations to PAR2 agonists were associated with an increase in input resistance in 77% (10/13) of AH neurons (control, 113 ± 26 MΩ; agonist, 129 ± 25 MΩ; $P < 0.05$ paired *t*-test; mean increase of 21 ± 10 %; $n = 10$) and a decrease in input resistance in 23% (3/13) of AH neurons (control, 73 ± 14 MΩ; agonist, 62 ± 13 MΩ; $P < 0.05$ paired *t*-test; mean decrease of 16 ± 2 %; $n = 3$). In S neurons, the depolarizations to PAR2 agonists were associated with an increase in input resistance in 45% (9/20) of neurons (control, 156 ± 31 MΩ; agonist, 188 ± 39 MΩ; $P < 0.05$ paired *t*-test; mean increase of 17 ± 6 %; $n = 9$) and a decrease in input resistance in 55% (11/20) of S neurons (control, 178 ± 41 MΩ; agonist, 157 ± 34 MΩ; $P < 0.05$ paired *t*-test; mean decrease of 12 ± 4 %; $n = 11$). No differences in RMP or input resistance were observed between neurons responding with an increased or decreased input resistance for each cell type ($P > 0.05$ unpaired *t*-test). Because an increase or decrease in input resistance is associated with a net closing or opening of ion channels, respectively, it is likely that a complex intracellular signaling cascade underlies the PAR2-mediated depolarization.

Concurrent with changes in passive membrane properties, PAR2 agonists occasionally caused an increase in the number of action potentials elicited during a depolarizing current pulse. This occurred in 46% (6/13) of responding AH neurons and 40% (8/20) of responding S neurons. Spontaneous action potentials occurred following application of SLIGRL-NH_2 or trypsin in 23% (3/13) of responding AH neurons and 15% (3/20) of responding S neurons. No differences in RMP or input resistance were observed between responding neurons with or without increased excitability ($P > 0.05$ unpaired *t*-test).

To assess the concentration–effect relationship for the trypsin, varying concentrations of the protease (0.03–1 μM) were added directly to the bathing solution for approximately 1.5 min. Because of the likelihood of receptor desensitization, trypsin was bath-applied only once for each preparation. Trypsin caused a concentration-dependent depolarization of myenteric neurons with an estimated concentration that elicits a half-maximal response (EC_{50} value) of 87 nM (Fig. 2). This value was determined by a least-squares nonlinear regression analysis of the data ($R^2 = 0.98$; Prism v. 3.0a for Macintosh, GraphPad Software, La Jolla, CA).

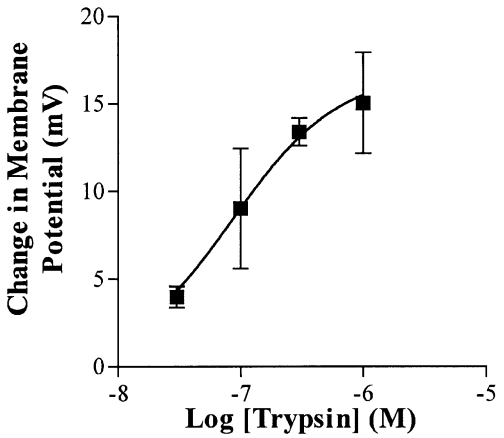

Fig. 2. Trypsin concentration-dependently depolarizes myenteric neurons. Bath-applied trypsin concentration-dependently depolarized myenteric neurons with an estimated EC_{50} value of 87 nM. Data are mean ± S.E. for $n = 3–6$ neurons.

4. Discussion

The data presented here indicate that activation of PAR2 elicits a prolonged depolarization and excitation of myenteric neurons in the guinea pig ileum. This observation is novel, and expands our understanding of the role that PAR2 plays in the physiology and pathophysiology of the alimentary canal. Because PAR2 is expressed on a subset of myenteric neurons (Corvera et al., 1999) and tetrodotoxin does not alter the response to SLIGRL-NH_2, it is likely that the action of this synthetic peptide is directly on the neurons that express the receptor. Further support for this hypothesis is the correlation between the proportion of myenteric neurons that express PAR2 (~ 60%), respond to PAR2 activation with an increase in intracellular calcium concentration (~ 50%) (Corvera et al., 1999), and the proportion of neurons that respond in the current study (~ 45%).

The physiological significance of PAR2-mediated excitation may be related to inflammatory states of the intestine. During inflammation, the tight junctions that separate luminal content from the extracellular fluid of the gut wall break down, increasing paracellular macromolecular permeability (Casellas et al., 1986; Resnick et al., 1990). It is only when the mucosal barrier is compromised that trypsin would be allowed to activate PAR2 expressed on myenteric neurons. Likewise, tryptase, another agonist of PAR2, would only be released from mast cells resident in the gut wall during inflammation (Metcalfe et al., 1997).

Prolonged depolarization and excitation of myenteric neurons may lead to dysmotility of the ileum. Although proteases may act directly on intestinal smooth muscle (Corvera et al., 1997; Saifeddine et al., 1996) and enterocytes (Kawabata et al., 1999) to alter local motility patterns, PAR2 excitation of myenteric neurons may underlie changes in intestinal motility at sites remote from the inflammatory injury. This concept is supported by the observation that colonic antigen challenge in the rat generates a myoelectric disturbance in the jejunum that is dependent on colonic mast cell degranulation and neural activation (Oliver et al., 1997). Thus, the role of PAR2 activation of myenteric neurons may be to exacerbate focal inflammation to a more global dysmotility through neural pathways.

Acknowledgements

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