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RESEARCH PAPER

Neuromedin U can exert colon-specific, enteric nerve-mediated prokinetic activity, via a pathway involving NMU_1 receptor activation

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Background and purpose: The neuromedin U (NMU) receptors, NMU_1 and NMU_2, are expressed in the gut but their functions are unclear. This study explores the role of NMU in gastrointestinal motility.
Experimental approach: The effects of NMU were examined in the forestomach and colon isolated from NMU_2R wild-type and NMU_2R-/- (knockout) mice, looking for changes in muscle tension and in nerve-mediated responses evoked by electrical field stimulation (EFS), and in models of peristalsis in mouse colon and faecal pellet transit in guinea-pig colon.
Key results: In the mouse forestomach, NMU (1 nM-10 μM) concentration-dependently induced muscle contraction, in the presence of tetrodotoxin and atropine, in preparations from both wild-type and NMU_2R-/- mice (pEC_{50}: 7.9, 7.6, E_{max}: 0.26, 0.20g tension, respectively, $n=8$ each concentration). The same concentrations of NMU had no consistent effects on the responses to EFS ($n=8$). In the mouse colon, NMU (0.1 nM-1 μM) had no significant effect on baseline muscle tension ($n=8$), but concentration-dependently potentiated EFS-evoked contractions in preparations from both wild-type and NMU_2R-/- mice, pEC_{50}: 8.1, 7.8, E_{max}: 24%, 21%, respectively, $n=6$-11. NMU (0.01 nM-0.1 μM, $n=5$-7) concentration-dependently decreased the interval between waves of peristalsis in the mouse colon (pEC_{50}: 8.8) and increased the rate at which a faecal pellet moved along the guinea-pig colon.
Conclusions and implications: These results demonstrate that NMU exerts colon-specific, nerve-mediated, prokinetic activity, via a pathway involving activation of NMU_1 receptors. This suggests that this receptor may represent a molecular target for the treatment of intestinal motility disorders.
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Keywords: enteric nervous system; neuromedin U; prokinetic; motility; peristalsis; $NMU_2R-/-$

Abbreviations: E_{max}, maximally effective concentration; EFS, electrical field stimulation

Introduction

Neuromedin U (NMU) was first recognized as a potent contractile substance in genitourinary (Minamino *et al*., 1985; Westfall *et al*., 2002; Prendergast *et al*., 2006), gastrointestinal (GI, Maggi *et al*., 1990; Benito-Orfila *et al*., 1991; Westfall *et al*., 2002; Prendergast *et al*., 2006) and vascular (Minamino *et al*., 1985; Sumi *et al*., 1987) smooth muscle. However, there are notable exceptions where the ability of NMU to contract different smooth muscle preparations shows marked species-, regional- or organ-dependency (Minamino *et al*., 1985; Brown and Quito, 1988; Maggi *et al*., 1990; Westfall *et al*., 2002; Prendergast *et al*., 2006). NMU-like immunoreactivity (-LI) has been detected in the GI tract (Domin *et al*., 1986; Ballesta *et al*., 1988; Honzawa *et al*., 1990) and shown to be present within functionally distinct enteric neurones (Augood *et al*., 1988; Brown and Quito, 1988; Furness *et al*., 1989; Timmermans *et al*., 1989). The discrete localisation of NMU in the mucosal and submucosal enteric neurones of the pig small intestine (Timmermans *et al*., 1989) suggests a role in the regulation of mucosal function such as ion transport (Brown and Quito, 1988).

Fujii *et al*. (2000) and Szekeres *et al*. (2000) demonstrated that NMU was the cognate ligand for the orphan G-protein-coupled receptor FM-3, now designated the NMU_1 receptor (NMU_1R). NMU is also the ligand for FM-4 (Howard *et al*., 2000) and TGR-1 (Hosoya *et al*., 2000), now designated the NMU_2 receptor (NMU_2R). NMU_1R mRNA is preferentially expressed in human peripheral tissues (Fujii *et al*., 2000; Hedrick *et al*., 2000; Hosoya *et al*., 2000; Howard *et al*., 2000; Raddatz *et al*., 2000; Szekeres *et al*., 2000). In contrast,

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NMU_2R mRNA has limited GI tract expression with much greater levels in the CNS (Howard *et al*., 2000; Raddatz *et al*., 2000, Shan *et al*., 2000). In the gut, the presence of both receptors has been detected (Fujii *et al*., 2000; Hedrick *et al*., 2000; Raddatz *et al*., 2000; Shan *et al*., 2000; Szekeres *et al*., 2000). Consequently, in this study we determined the effects of NMU on both muscle tension and neuronally-mediated responses evoked by electrical field stimulation (EFS) in isolated stomach and colon preparations from wild-type and $NMU_2R-/-$ (receptor knockout) mice, so as to obtain evidence of GI prokinetic-like activity. Subsequently, the effects of NMU on peristaltic motility and faecal pellet transit were further investigated in the mouse and guinea-pig isolated colon. Our results show, for the first time, a marked colonic prokinetic activity of NMU, which may be mediated via NMU_1 receptors expressed within the nerves of the gut wall.

Methods

Generation of $NMU_2R-/-$ mice

These were obtained from Deltagen (San Carlos, CA, USA). A 6.93 kb IRES-lacZ reporter and neomycin resistance cassette (IRES-lacZ-neo) was subcloned into a 5.9 kb fragment isolated from a mouse genomic phage library, such that 159 base pairs coding for the NMU_2R protein were replaced by IRES-LacZ-neo. After electroporation into 129/OlaHsd mouse embryonic stem (ES) cells, the cells were selected for G418 resistance, and colonies carrying the homologously integrated neo DNA were identified by PCR amplification using a 5′ neo-specific primer (5′-CCGGGAAGCTTGA GTTTGTCCTCAA-3′) paired with a primer located outside the target homology arms on the 5′ side (GS1: 5′-CAAGCACA TTCCCAGTATGCAGATC-3′). The homologous recombination event was confirmed on the 3′ side using a 3′ neo-specific primer (5′-ACGTACTCGGATGGAAGCCGGTCTT-3′) paired with a primer located outside the target homology arm on the 3′ side (GS2: 5′-TCTGCCTTCACTGCTGTATA TCTGTC-3′). Colonies that gave rise to the correct size PCR product were confirmed by Southern blot analysis using a probe adjacent to the 5′ region of homology. The presence of a single neo cassette was confirmed by Southern blot analysis using a neo gene fragment as a probe. Male chimeric mice were generated by injection of the target ES cells into C57Bl/6J blastocysts. Chimeric mice were bred with C57Bl/6J mice to produce F1 heterozygotes. Initial germline heterozygotes were tested for the homologous recombination event using the primers described above (located outside of the target construct). Following confirmation of the target event in animals, subsequent genotyping tracked transmission of the target construct. F1 heterozygous males and females were mated to produce F2 wild-type, heterozygous ($NMU_2R+/-$) and homozygous null mutant ($NMU_2R-/-$) animals. Mice were backcrossed with C57BL/6J mice and all phenotypic analysis was performed in a hybrid C57Bl/6J/129 background (75/25%, respectively). Mice were maintained in a temperature-controlled (23°C) barrier facility with a 12-h light/dark cycle and had access to water and regular rodent chow *ad libitum*.

Mouse isolated forestomach and colon EFS studies

Adult male mice (25–30 g) were killed by blow to the head followed by cervical dislocation. All efforts were made to minimize the number of animals used and culling was performed in accordance with the UK Animals (Scientific Procedures) Act 1986. Following a midline incision, whole stomachs and descending colons were blunt-dissected and placed immediately in Krebs solution (NaCl 121.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 5.6 mM) previously equilibrated with 5% CO_2 in O_2.

Full wall thickness strips of the forestomach and distal colon ($\sim 10 \times \sim 5$ mm) were cut parallel to the circular muscle and were suspended under 10 mN of tension for isometric recording between two parallel platinum ring electrodes in 5 ml tissue baths containing Krebs solution bubbled with 5% CO_2/95% O_2, maintained at pH ~7.4 and 37°C. Tension was measured using Pioden dynamometer UF1 force–displacement transducers (Pioden Control Ltd, UK). Data acquisition and analysis were performed using MP100 hardware and AcqKnowledge software (Biopac Systems, Inc., Santa Barbara, CA, USA). Tissues were allowed to equilibrate for at least 60 min during which time bath solutions were changed every 15 min. EFS was achieved using monophasic square-wave pulses of 0.2 (0.5) ms pulse width, 5 Hz frequency at a maximally effective voltage (70–80 V; Digitimer, Welwyn Garden City, UK) for 10 (30) s at 2 (3) min intervals for 30 min periods, each period was separated by a 5-min interval in which the bath solutions were changed. The values in parentheses are EFS parameters for forestomach preparations. These parameters of EFS were selected as those that consistently evoked nerve-mediated responses with a good signal-to-noise ratio over background spontaneous muscle activity. For EFS studies, the effects of pharmacological agents were expressed as the percentage change in amplitude compared to the mean amplitude of at least three pre-drug, post-EFS contractile responses. Direct muscle contractions were expressed in *g* tension generated. pEC_{50} is the negative logarithm to base 10 of the EC_{50} value, which is the concentration of the agonist that produces 50% of the maximal response. Maximally effective concentration (E_{max}) denotes the maximal response achieved by the drug.

Mouse isolated proximal colon peristalsis studies

Adult, male BKW mice (25–40 g) were killed by cervical dislocation. The schematic diagram of the apparatus used to study peristalsis is shown in Figure 1. A segment of proximal colon (~3 cm), cut ~1 cm distal to the caecum, was dissected and placed in a 10 ml tissue bath (3 cm diameter, 1.5 cm high) containing Krebs solution, bubbled with 5% CO_2/95% O_2, maintained at pH ~7.4 and 37°C. After the mesentery had been carefully removed, the colon was secured horizontally and the oral end was cannulated and connected to a 50 ml reservoir of Krebs solution. The intraluminal content of the colon was allowed to expel naturally via peristalsis, induced by raising the height of the reservoir to 4 cm above the level of the fluid in the bath. Once the intraluminal contents had been removed, the connection to the reservoir was closed and the anal end cannulated and connected to the drain and a vertical

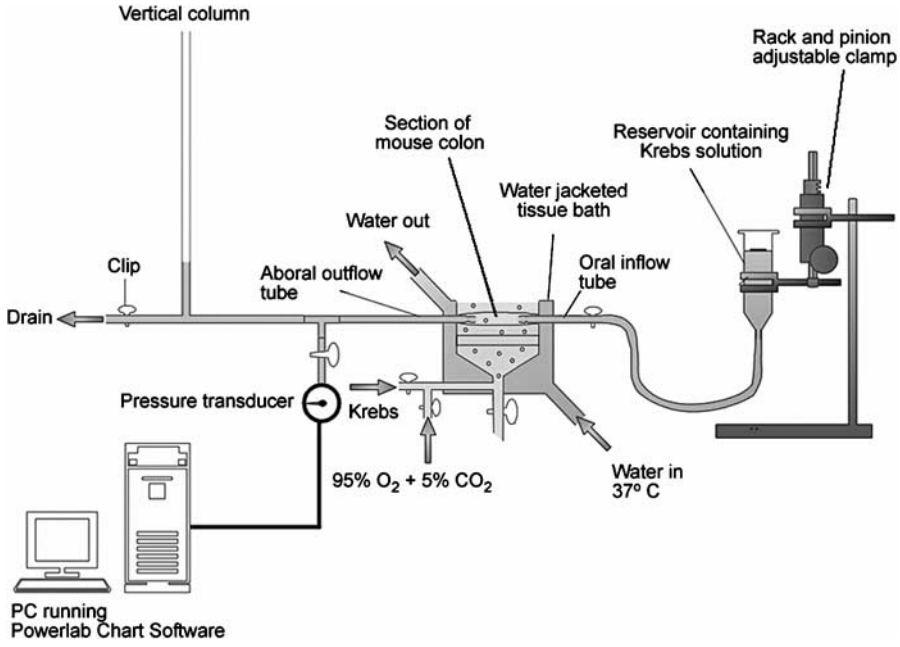

Figure 1 Schematic diagram of the apparatus used to induce and measure the peristaltic reflex in the mouse isolated proximal colon. The colon was cannulated and secured horizontally in a bath containing Krebs solution. The glass tubes to which oral and anal ends of the colon were cannulated were held in position via a screwcap assembly. The oral cannula was connected to the reservoir (50 ml; open to the atmosphere; internal diameter 29 mm; adjustable clamp) containing Krebs solution using flexible tubing. The anal cannula was connected to the drain and a vertical column (2.5 mm internal diameter; open to the atmosphere) that could be opened or closed. Changes in the volume of the fluid propelled into the vertical tube during peristalsis were measured as pressure changes.

column (2.5 mm internal diameter open to the atmosphere) and the tissues washed 2–3 times with fresh Krebs solution. Peristalsis was induced by closing the clip to the drain and opening the connection to the reservoir (resulting in an intraluminal pressure of 4 cmH_2O). The height of the reservoir was further adjusted, if required, so that regular peristalsis with a rate around one peristaltic contraction every 2 min was obtained. Peristaltic movements were characterised by a highly visible circular muscle contraction starting at the oral end and travelling to the anal end; at the end of each peristaltic contraction, the colon relaxed and fluid flowed back into the colon. Peristalsis became regular within 10–15 min. Changes in the volume of the fluid propelled into the vertical tube during the peristalsis were measured as pressure changes (1 ml = 19 cm H_2O) and were recorded using pressure transducers (P75 Type 379, Hugo Sachs, Germany) connected to a quad bridge amplifier and PowerLab system using Chart v4.1.2 software for Windows (ADInstruments, Castle Hill, New South Wales, Australia). This arrangement ensures that only a propulsive (peristaltic) contraction, characterised by closure of the lumen and subsequent movement of fluid in the anal direction, can displace fluid within the vertical tube. Thus, any nonpropulsive increases in circular muscle tone have negligible effect on the fluid height in the vertical tube because under these conditions, the fluid preferentially moves into the larger volume reservoir; equal heights of fluid are maintained in both the reservoir and the narrow tube. NMU (0.01 nM–0.1 μM) or vehicle (0.01% bovine serum albumin (BSA) 0.1% of the bath volume) was added serosally. Only a single concentration of NMU was tested in any one tissue. The interval between peristaltic contractions was measured as the distance between the maximum pressure points in each subsequent wave. The effects of NMU on peristalsis are presented as the percentage decrease in the minimum interval between each wave of peristalsis in the 5 min period before and after the addition of NMU.

Guinea-pig isolated colon faecal pellet transit studies

This *in vitro* colonic motility model was adapted from that employed by Jin *et al*. (1999). Briefly, recently fed, adult male Dunkin–Hartley guinea-pigs (200–250 g) were killed by increasing the concentration of CO_2 followed by cervical dislocation. A 15–20 cm length of colon was removed and divided equally into two. One segment was used to test the effects of drugs on pellet transit times and the other served as a time-matched, vehicle control. In separate studies, no difference was observed in pellet transit times between any of the colon segments (data not shown). Colon segments were pinned out horizontally, under slight tension, in a 70 ml tissue bath perfused with Krebs solution (11 mM glucose, 1 ml min^{-1}), bubbled with 5% CO_2/95% O_2 and maintained at pH ~7.4 and 37°C. The expulsion of pellets was allowed to occur spontaneously over a 30-min period after which each segment was perfused intraluminally at a rate of 0.25 ml min^{-1} for 15 min with an oxygenated glucose–saline solution (0.1 and 0.9%, respectively) using a PE-10 catheter inserted through the caudal end and advanced ~4 cm. After this equilibration period, dried, lacquered faecal pellets were gently inserted into the oral end and allowed to travel along the length of the colon. The time taken for each pellet to traverse 3 cm of the mid-portion of the colon was measured. Upon expulsion, the expelled

catheter was immediately returned to its original position and the pellet was reinserted after a 5-min delay. After at least three consistent control transit measurements (±3 s), at 5 min intervals, a single concentration of NMU or vehicle was administered into the lumen via the catheter and transit times measured at 5 min intervals over a further 20-min period. The data observed are expressed graphically as means±s.e.m. measured in seconds.

Data analysis and statistical procedures

All data are expressed as means±s.e.m.; n indicates the number of animals. For EFS studies, statistical differences were determined using Student's t-test for paired or unpaired data; $P<0.05$ is considered as statistically significant. Comparison of pEC_{50} values was conducted using a repeated measures analysis of variance (ANOVA). For pellet transit studies, analyses were performed on the percentage change from basal transit (mean of the three control pellets) using a one-way repeated-measures ANOVA followed by planned comparison of LS means; the effects of NMU were compared with those of vehicle alone.

Drugs and chemical reagents used

All drugs were freshly prepared before use. Rat NMU-23 was obtained from Bachem (UK) Ltd, and was prepared in 0.9% NaCl containing 0.01% BSA (Sigma, Gillingham, UK). Peptidase inhibitors were not used in this study as they were previously shown to have no effect on the potency of exogenous NMU in isolated preparations (Westfall *et al*., 2002). The muscarinic receptor agonist and antagonist, carbachol and atropine (Sigma, UK), respectively, and the nerve toxin tetrodotoxin (Tocris, Bristol, UK) were all dissolved in distilled H_2O.

Results

Effects of NMU on baseline tension in wild-type and $NMU_2R-/-$ mouse stomach and colon

In the absence of EFS, and in the continued presence of tetrodotoxin (1 μM) and atropine (1 μM), NMU (1 nM–10 μM) concentration-dependently contracted the forestomach from wild-type and $NMU_2R-/-$ mice, with pEC_{50} values of 7.9±0.3, 7.6±0.3 and E_{max} values of 0.26±0.05, 0.20±0.03 g tension ($n=8$ at each concentration), respectively; there was no significant differences between either the pEC_{50} or E_{max} values (Figure 2). By comparison, the carbachol (1 nM–100 μM) concentration–response curve produced a pEC_{50} value of 6.9±0.3 and an E_{max} value of 2.25±0.4 g tension, $n=4$ at each concentration (Figure 2). NMU (0.1–10 μM) had no significant ability to cause a contraction in either wild-type or $NMU_2R-/-$ colons, $n=8$ at each concentration (data not shown).

Characterisation of responses evoked by EFS in mouse stomach and colon

Responses to EFS were variants of a compound triphasic response, characterised by an initial contraction during EFS, which was either sustained or gave way to a subsequent relaxation or was characterised by a simple relaxation during EFS. Regardless of the pattern of response evoked during EFS, termination of electrical stimulation immediately evoked a large-amplitude 'post-EFS' contraction. In the forestomach, the initial phasic contraction was sustained during EFS in 35% of preparations whereas in 65% it was followed by muscle relaxation. These were 28±6% ($n=6$) and −85±18% ($n=9$) of the amplitude of the post-EFS contractions, respectively. In the distal colon, EFS evoked relaxation in 80% of the preparations; in the remaining 20% a contraction occurred during EFS. These were −8±1% ($n=8$) and 46±28% ($n=2$) of the amplitude of the post-EFS contractions, respectively (Figure 3a).

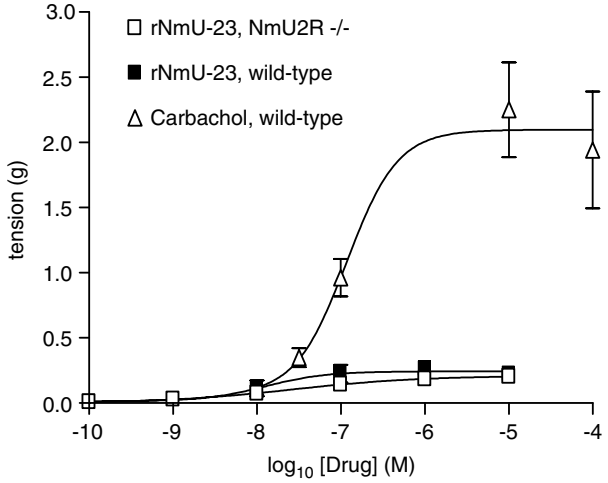

Figure 2 Concentration–response curves to rat NMU-23, in the presence of tetrodotoxin (1 μM) and atropine (1 μM), in isolated forestomach from wild-type and $NMU_2R-/-$ mice. NMU concentration-dependently contracted forestomach preparations from both wild-type and $NMU_2R-/-$ mice ($n=8$ at each concentration). The NMU-induced contractions in the forestomach were small when compared to the carbachol concentration–response curve ($n=4$). Data are expressed as g of tension generated. All values are means±s.e.m. (vertical lines).

All responses to EFS, in all tissue strips examined were prevented by tetrodotoxin (1 μM, 20 min contact, $n=4$ forestomach and colon, data not shown) demonstrating the neurogenic nature of the response. The addition of atropine (1 μM, 20 min contact) to forestomach and colon preparations prevented any contraction evoked during EFS and substantially increased the amplitude of the relaxation response by 164±61% ($n=6$) and 156±44% ($n=4$), whereas reducing the amplitude of the post-EFS contraction by 63±3% ($n=6$) and 83±16% ($n=5$), respectively.

Effects of NMU on EFS-evoked responses in wild-type and $NMU_2R-/-$ mouse stomach and colon

In preparations from both regions of the gut, the application of NMU did not appear to affect the responses evoked during EFS, but these were not quantified owing to their inconsistent nature (see above). In forestomachs from wild-type and $NMU_2R-/-$ mice, NMU (0.1 nM–1 μM, $n=6$–9 at each concentration) had no consistent effect on the post-EFS

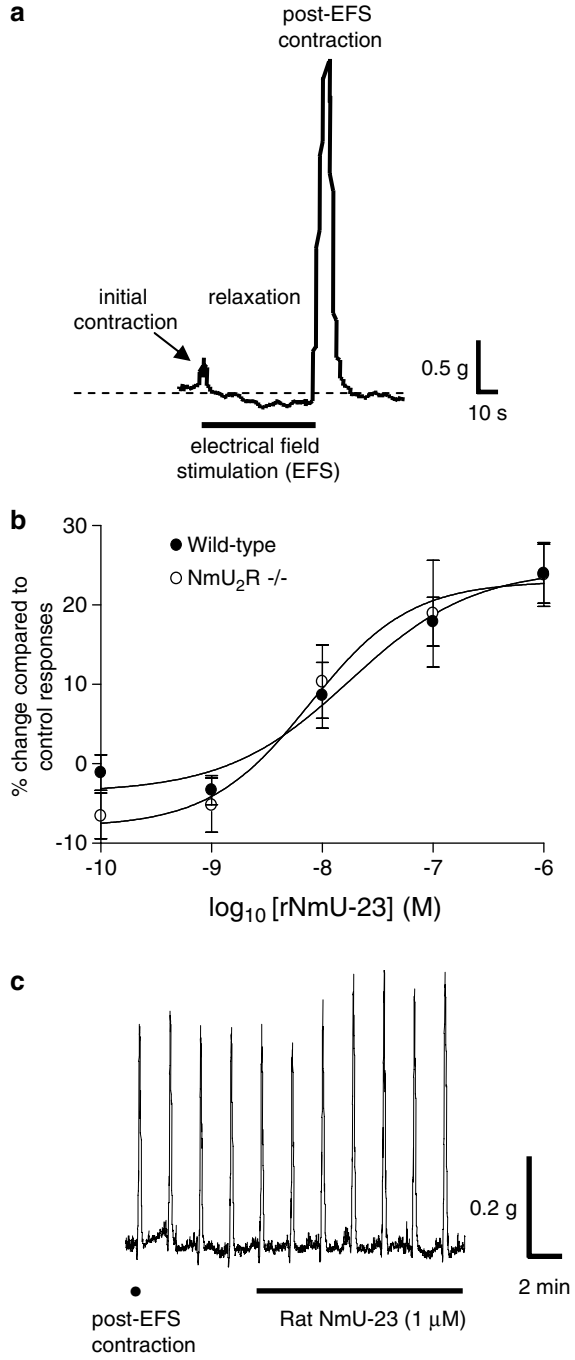

Figure 3 (**a**) EFS (70–80 V, 5 Hz, 0.2 ms, 10 s over 2 min) of mouse isolated distal colon evoked variants of a compound triphasic response as illustrated on an expanded time base. This was prevented by tetrodotoxin (1 μM, 20 min contact). (**b**) Rat NMU-23 ($n=6$–9 at each concentration) concentration-dependently increased electrically stimulated, nerve-mediated, post-EFS contractions in the isolated distal colon of wild-type and $NMU_2R-/-$ mice. (**c**) Experimental trace, on contracted time base, demonstrating the potentiating effect of NMU on post-EFS contractions in the mouse isolated distal colon. Data are expressed as the percentage change in the amplitude of the nerve-evoked, post-EFS contractions as compared to the mean of three pre-drug post-EFS contractions. All values are means$\pm$s.e.m. (vertical lines).

contractions. For example, changes in the amplitudes of the post-EFS contractions following the application of NMU 0.1 μM or vehicle were, respectively, 16 ± 11 and 24 ± 26% in NMU_2R wildtype mice ($n=8$, $P=0.8$) and 32 ± 10 and 18 ± 15% in $NMU_2R-/-$ mice ($n=8$, $P=0.5$). However, in colons from both wild-type and $NMU_2R-/-$ mice, NMU (0.1 nM–1 μM, $n=6$–11 at each concentration) concentration-dependently increased the amplitude of the post-EFS contractions, with significant effects at 10 nM–1 μM compared to vehicle controls ($P<0.05$; changes in the presence of vehicle were between -5 ± 4 and 3 ± 2%, respectively, depending on the concentration of NMU tested). The pEC_{50} values were 8.1 ± 0.1, 7.8 ± 0.2 and E_{max} values were 24 ± 3, 21 ± 4% for wild-type and NMU2R$-/-$, respectively (Figure 3b and c); there was no significant difference between either the pEC_{50} or E_{max} values. The threshold concentration was between 1 and 10 nM and the maximum effect was between 0.1 and 1 μM.

In separate experiments using wild-type colon preparations, the effect of NMU (10 nM; approximately the EC_{50} concentration in the previous experiment with EFS) was determined against contractions induced by carbachol, at a concentration previously determined to be submaximally effective (0.1 μM, 30 s contact). NMU had no effect on the amplitude of the carbachol-induced contractions; 2 ± 5% change compared with vehicle controls, 3 ± 4% ($P>0.05$; $n=4$).

Effects of NMU on peristalsis in mouse isolated colon

The typical peristalsis motor activity and the effects of addition of vehicle, the lowest (0.01 nM), middle (1 nM) and the highest concentration of NMU (100 nM) are shown in Figure 4a. The addition of vehicle had no effect on peristalsis (the minimum interval between peristaltic contractions before and after vehicle were 125 ± 9 and 108 ± 12 s, respectively) but NMU (0.01 nM–0.1 μM, $n=5$–7) concentration-dependently decreased the interval between successive waves of peristalsis (Figure 4b), the pEC_{50} for the concentration–response curve was 8.84.

Effects of NMU on faecal pellet transit in guinea-pig isolated colon

NMU (0.1 and 1 μM) reduced the faecal pellet transit time measured over the mid 3 cm of the colon, as compared to time-matched vehicle controls ($n=4$, 6 and 4 for the saline controls and the NMU 0.1 and 1 μM concentration, respectively; $P<0.005$ at 0.1 μM and $P<0.01$ at 1 μM; Figure 5). The maximum effect was observed at $\sim$20 min post-drug application and showed a $\sim$30% reduction in transit time.

Discussion and conclusions

NMU is highly expressed in the enteric nervous system (Domin *et al*., 1986; Augood *et al*., 1988; Ballesta *et al*., 1988; Furness *et al*., 1989; Timmermans *et al*., 1989), yet the effects of NMU on GI motility has not been adequately addressed, other than demonstrating that it is potent at contracting GI smooth muscle (Maggi *et al*., 1990; Westfall *et al*., 2002;

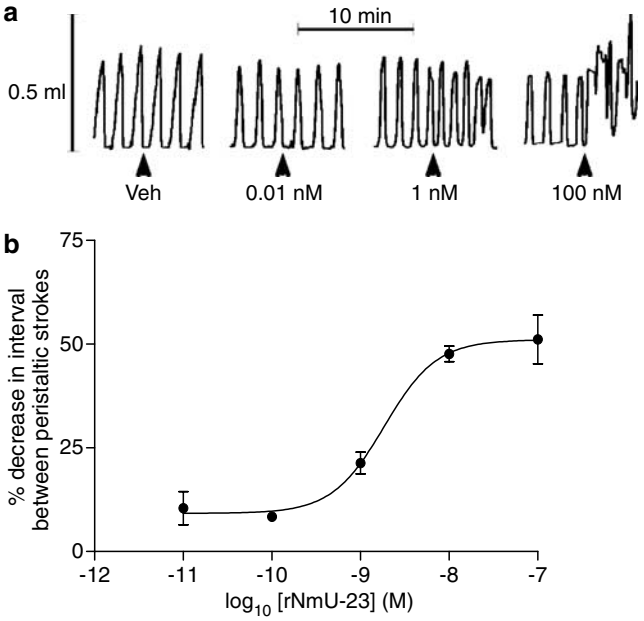

Figure 4 (**a**) Typical traces illustrating the peristaltic motor activity in the mouse isolated proximal colon and the effects of vehicle (Veh), the lowest (0.01 nM), middle (1 nM) and highest (100 nM) concentrations of rat NMU-23. (**b**) The effects of rat NMU-23 on peristaltic movements in the mouse isolated proximal colon. NMU concentration-dependently decreased the interval between the peristaltic strokes ($n=5$–7 at each concentration). The percentage reduction is the change between the mean intervals between the peristaltic strokes in the 5 min period before and after addition of NMU. All values are means ± s.e.m. (vertical lines).

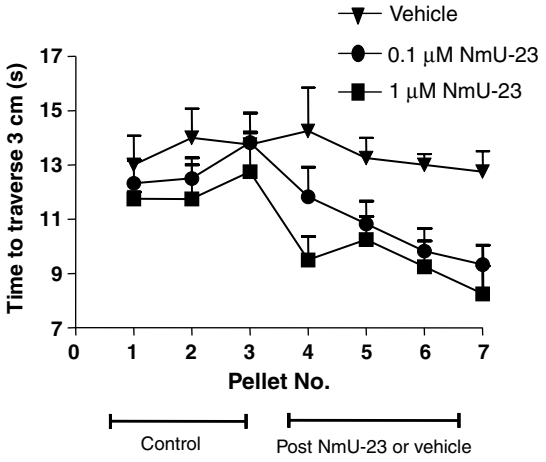

Figure 5 Effects of rat NMU-23 on faecal pellet transit in the guinea-pig isolated colon. NMU reduced the time taken for faecal pellets to traverse 3 cm, as compared to time-matched vehicle controls ($n=4$, 6 and 4 for the saline controls and the NMU 0.1 and 1 μM concentration, respectively). The maximum effect was observed at ~20 min post-drug application and showed a ~30% reduction in transit time. Data are expressed as changes from basal transit, measured in seconds. The overall changes following NMU or vehicle treatment were analysed using one-way repeated-measures ANOVA followed by *post hoc* planned comparisons of the LS means; compared with vehicle controls, $P<0.01$ at 0.1 and 1 μM. All values are means ± s.e.m. (vertical lines).

Prendergast *et al*., 2006). We have also shown that NMU has the ability to contract the muscle of mouse, isolated from forestomach, directly. In this tissue, the potency of NMU was found to be consistent with that reported by others (Benito-Orfila *et al*., 1991; Prendergast *et al*., 2006). Nevertheless, the contractions induced by NMU were small; the E_{max} value was ~10-fold lower than that obtained from the carbachol concentration–response curve. Further, there was no significant difference in the amplitude of NMU-induced contractions between forestomachs of wild-type and $NMU_2R-/-$ mice, suggesting that they were mediated via NMU_1R. This observation is consistent with the loss of NMU-induced contractile activity found in $NMU_1R-/-$ mouse forestomach (Prendergast *et al*., 2006). However, of particular interest was the observation that NMU failed to contract colonic smooth muscle. This regionally dependent difference in the ability of NMU to contract mouse GI smooth muscle is consistent with the findings of Benito-Orfila *et al*. (1991), who observed that NMU was able to contract the circular muscle of stomach, but not that of the ileum or colon, and those of Prendergast *et al*. (2006), who reported a much lower potency of NMU to contract the longitudinal muscle of rat ileum.

A significant and novel finding of this study was that NMU potentiated post-EFS contractions in the mouse colon, suggesting that in this region of the gut, NMU exerts prokinetic activity. Similar changes were not clearly observed in the forestomach, although it is possible that any effects of NMU on neuronally-mediated contractions may have been obscured by the marked potency of NMU at contracting the muscle and increasing the variability of the responses to EFS. As NMU did not increase the amplitude of carbachol-induced contractions, the clear ability of NMU to increase neuronally mediated contractions of the colon is most likely owing to a prejunctional effect within the enteric ganglia that directly or indirectly modulates cholinergic and/or noncholinergic excitatory or inhibitory transmitter release. As there was no significant difference in the magnitude of the responses to NMU in the wild-type and $NMU_2R-/-$ colons, this neuromodulatory effect of NMU may be mediated via NMU_1 receptors. Additionally, the concentrations of NMU that facilitated the EFS-evoked contractions were similar to those found to be active in human NMU_1R-transfected HEK 293 cells (Hedrick *et al*., 2000). These data are consistent with the expression of NMU_1R mRNA in the GI tract, where it may be more predominant in the small intestines than the large intestines (Fujii *et al*., 2000; Hedrick *et al*., 2000; Raddatz *et al*., 2000; Szekeres *et al*., 2000; Westfall *et al*., 2002).

The increase in neuronally-mediated contractions of the colon caused by NMU is indicative of an ability to exert prokinetic activity. The increase in the rate of peristaltic movements in mouse isolated colon segments induced by NMU is consistent with it possessing prokinetic activity. This was confirmed in the guinea-pig colon, where NMU was found to be propulsive as it increased the transit rate of faecal pellets. Interestingly, NMU appeared to be more potent at increasing peristalsis in the mouse colon (approximately 10-fold) than at facilitating electrically evoked contractions in the same region of the gut. The explanation for this difference is not known and could be suggestive of an additional role for the NMU_2R in the mechanisms by which NMU affects peristalsis. However, it is also possible that, in

contrast to the experiments in which motor nerve responses were predominantly measured in response to EFS, both motor and sensory nerves play a role in the peristaltic reflex, creating the potential for amplification of the excitatory responses to NMU in this preparation. A comparison between the mouse and guinea-pig tissues is considered to be valid, as the bioactive COOH-terminal region of NMU is almost completely conserved across all species and human, porcine and rat NMU show similar potencies at stimulating calcium release in human NMU_1R- and NMU_2R-transfected COS-7 cells (Raddatz *et al*., 2000).

In summary, our data demonstrate that the enteric peptide NMU acts to regulate GI motility in a regionally specific manner. It was potent at inducing a small contraction of the mouse forestomach muscle, but not in the colon. However, a novel finding of this study is that in contrast to the forestomach, NMU acts via the nerves within the colon to induce prokinetic activity; further experiments are now required to determine the precise nerve phenotypes sensitive to the effects of NMU. These effects may be mediated predominantly via NMU_1R. Additionally, given the potential ability of NMU to regulate mucosal ion transport (Brown and Quito, 1988), the role of NMU and of the NMU_1 receptor in particular, warrants further investigation as a potential target for the treatment of intestinal motility and secretory disorders.

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Conflict of interest

The authors state no conflict of interest.

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