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Endogenously released opioids mediate meal-induced gastric relaxation via peripheral mu-opioid receptors

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SUMMARY

Background
The centrally acting mu-opioid receptor antagonist naloxone inhibits meal-induced gastric accommodation.

Aim
To study the role of peripheral mu-opioid receptors in the regulation of gastric tone and food intake by comparing the effects of naloxone with the peripherally restricted mu-opioid receptor antagonist methylnaltrexone.

Methods
Methylnaltrexone (12 mg s.c.), naloxone (20 μg/kg/h intravenous infusion after 0.4 mg bolus) and placebo were studied in 23 healthy volunteers. Gastric volume was recorded using an intragastric bag held at constant pressure connected to a barostat, with administration of a nutrient drink after 30 min. Pressure in the stomach was measured during intragastric nutrient drink infusion until the volunteers scored maximal satiation.

Results
Methylnaltrexone inhibited significantly the volume increase after food intake as assessed with the barostat ($P < 0.01$). During nutrient drink infusion the intragastric pressure significantly decreased as compared with the preprandial pressure after placebo treatment. Both methylnaltrexone and naloxone significantly inhibited this intragastric pressure decrease ($P < 0.001$ and $P < 0.05$, respectively). Volunteers scored maximal satiation after 979 $\pm$ 96, 958 $\pm$ 84 and 1124 $\pm$ 107 mL nutrient drink infused (for naloxone, methylnaltrexone and placebo treatment, respectively; $P < 0.05$).

Conclusions
These results indicate that endogenous opioids mediate gastric accommodation and satiation via peripheral mu-opioid receptors. Effects were less pronounced after naloxone treatment, which indicates that centrally involved mu-opioid receptors mediate an opposing effect.

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INTRODUCTION

During food intake smooth muscle of the stomach relaxes so that the stomach can accommodate sometimes large quantities of the food we eat without major increase in intragastric pressure (IGP).[1] This reflex relaxation, also referred to as gastric accommodation is likely to play a part in the control of food intake.[2, 3]

Delta-, kappa- and mu-opioid receptors are found throughout the enteric and central nervous system and influence gastrointestinal motility and sensation.[4–7] Only a limited number of studies indicate a complex role for opioids in the control of proximal stomach motility: in different barostat studies, opioid receptor agonists morphine and remifentanil have both increased and decreased gastric tone[8–10] whereas the centrally acting mu-opioid receptor antagonist naloxone inhibited meal-induced gastric accommodation.[11] Also the role of opioids in the regulation of food intake is complex: in general opioid agonists enhance feeding and opioid antagonists decrease feeding[12, 13] but these effects are dependent for example on the treatment duration and whether normal weight, low weight (anorexia patients) or obese volunteers/patients are selected.[5]

The various and sometimes opposing findings with opioid agonists and antagonists in search for the role of opioids in the control of food intake and gastric motility could be explained by the fact that central and peripheral opioid receptors mediate various and sometimes opposing effects e.g. in the control of intestinal motility in rats,[14] gastric relaxation to a meal in dogs[15] or gastric emptying in humans.[16] Recently, the peripherally restricted and selective mu-opioid receptor antagonist methylnaltrexone was approved for the treatment of opioid-induced constipation.[17] In order to study the role of peripheral mu-opioid receptors in the regulation of gastric tone and food intake we investigated the effect of methylnaltrexone on gastric sensitivity, gastric compliance and gastric relaxation after a meal in a barostat study.

Recently the barostat has been criticised: the inflated balloon distends the proximal stomach, might exaggerate gastric accommodation and hamper physiological responses to food intake.[18–20] We therefore developed a new technique to assess gastric accommodation during food intake by measuring the IGP.[21] In this study we investigated the effect of methylnaltrexone in a barostat study of which the protocol was identical to the one in which we previously investigated the effect of naloxone on gastric sensorimotor function.[11] In order to confirm the barostat findings we performed separate experiments that investigated the effect of both naloxone and methylnaltrexone on gastric tone and accommodation to a meal by measuring the IGP during food intake.

METHODS

Ethical approval

All study procedures were approved by the Ethics Committee of the Leuven University Hospital, Belgium. Written, informed consent was obtained from all subjects and studies conformed to the Declaration of Helsinki.

Study subjects

A total of 23 healthy volunteers (HV's, 12 men, age: 28 ± 2 years, body mass index: 21.7 ± 0.4 kg/m^2) participated in the studies. None of the HV's had symptoms or a history of gastrointestinal disease, other significant diseases, psychological disorders or drug allergies; none were taking any medication or had a drug history. HV's were asked to refrain from alcohol, tea and coffee at least 12 h before participation, and refrain from smoking cigarettes at least 1 h before the start of the experiment. Study populations differed between the different experiments (isobaric barostat distension to determine compliance and sensitivity, barostat experiment to determine gastric accommodation after meal intake, IGP during food intake) although some subjects participated in more than one experiment. Between two experiments with the same subject at least 1 week washout was respected.

Barostat studies

After an overnight fast of at least 12 h, a double lumen polyvinyl tube (Salem sump tube 14 Ch; Sherwood Medical, Petit Rechain, Belgium) with an adherent polyethylene bag (maximal volume 1200 mL; 17 cm maximal diameter) was introduced through the mouth and secured to the subject's chin with adhesive tape. The correct position of the bag in the gastric fundus was checked fluoroscopically. The polyvinyl tube was then connected to a programmable barostat device (Synectics Visceral Stimulator, Stockholm, Sweden). To unfold the bag, it was inflated with a fixed volume of 300 mL for 2 min with the subject in a recumbent position and again deflated completely. The subjects were then positioned in a comfortable sitting position with the knees bent (80°) and the trunk upright in a specifically designed bed. After a 30-min adaptation period, the minimal distending pressure (MDP) was determined by increasing the intra-bag pressure with 1 mmHg every 3 min, until a volume of 30 mL or more was reached.[22] Hereafter, either compliance and sensitivity or gastric accommodation to a meal was determined.

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Compliance and sensitivity to distension. Thirteen HV's (7 men, age: 31 ± 2 years, body mass index: 21.9 ± 0.6 kg/m^2) were enrolled for this experiment. 15 min after treatment with either methylnaltrexone [12 mg subcutaneous injection (0.6 mL)] or placebo (subcutaneous injection of 0.6 mL saline) isobaric distensions were performed in stepwise increments of 2 mmHg starting from MDP, each lasting for 2 min, while the corresponding intragastric volume was recorded. Subjects were instructed to score their perception of upper abdominal sensations at the end of every distending step. They used a global graphic rating scale that combined verbal descriptors on a scale graded 0–6.[22] The end point of this distension sequence was established at an intra-bag volume of 1500 mL, or when the subjects reported discomfort or pain (score 5 or 6). Hereafter, the volunteers could leave the hospital.

For each 2 min isobaric distending period, the intragastric volume was calculated by averaging the recording. The thresholds for perception and discomfort were computed after the experiments by analysing the perception score corresponding to each distension step. Perception threshold was defined as the lowest pressure relative to MDP that evoked a perception score of 1 or more, and the corresponding volume. Discomfort threshold was defined as the lowest pressure relative to MDP and the corresponding volume that provoked a score of 5 or more. Pressure-volume and pressure-perception curves were obtained from the stepwise distensions and fitted with a linear regression model as previously described.[22, 23] Gastric compliance was calculated as the slope and the intercept of the pressure–volume curve obtained during the isobaric distensions.

Gastric accommodation to a meal. Eleven HV's (6 men, age: 29 ± 2 years, body mass index: 22.1 ± 0.7 kg/m^2) were enrolled for this experiment. After positioning of the barostat bag, the pressure level was set at MDP + 2 mmHg. The intra-bag volume at this pressure was recorded for 15 min, and then either placebo or methylnaltrexone was subcutaneously administered. Intra-bag volume was continuously monitored for 90 min, after the first 30 min, a standardised liquid meal (200 mL, 300 kcal; 13% proteins, 48% carbohydrates, 39% lipids; Nutridrink; Nutricia, Bornem, Belgium) was administered. Barostat measurements continued for 60 min after the start of meal ingestion.

To evaluate gastric tone before and after administration of the meal, mean intra-balloon volume was calculated over consecutive 5-min intervals of the measurement.

Statistical analysis. Paired Student's *t*-tests were used to assess whether the mean values of two parameters differ between both treatment groups. Gastric accommodation was compared using a repeated measured ANOVA as the mean area under the curve in the hour after the liquid meal intake. All data are presented as the mean ± standard error of the mean (S.E.M.). A P-value < 0.05 was considered to be statistically significant (N.S.: not significant).

Intragastric pressure measurement during nutrient drink infusion

Eleven HV's (5 men, age: 28 ± 2 years, body mass index: 22.2 ± 0.6 kg/m^2) were enrolled for this experiment. After an overnight fast of at least 12 h, a high-resolution solid-state manometer system (36 channels, 1 cm in between each channel, Manoscan 360, Sierra Scientific Instruments, Los Angeles, USA, Manoview analysis software v2.0.1) was positioned through the nose so that at least one sensor was positioned in the lower oesophageal sphincter (LES; detected as a clearly elevated pressure zone compared with oral and aboral areas), while IGP was measured using the average pressure of the first five pressure channels that were clearly positioned below the LES or the pressure area influenced by the LES (in most cases this was approximately 3–8 cm under the LES). A second infusion catheter (Flocare, Nutricia) was positioned in the stomach through the mouth. The tip of the infusion catheter was positioned approximately 5 cm under the LES and its position was verified by fluoroscopy.

The catheters were fixed to the subjects chin and the HV's were asked to take a comfortable position on a bed. Volunteers were randomly assigned to one of three treatment regimens:

(i) placebo methylnaltrexone (0.6 mL saline subcutaneous) and placebo naloxone (intravenous saline 100 mL/h after a 1 mL bolus injection).

(ii) methylnaltrexone [12 mg subcutaneous injection (0.6 mL)] with placebo naloxone.

(iii) placebo methylnaltrexone with naloxone (infusion rate of 20 μg/kg/h after a 0.4 mg bolus injection; Narcan, Bristol-Myers Squibb Pharma, Braine-l'Alleud, Belgium).

Treatment was applied after a stabilisation period of at least 15 min and 15 min hereafter nutrient drink (Nutridrink, Nutricia, Zoetmeer, The Netherlands; 630 KJ, 6 g

proteins, 18.4 g carbohydrates, and 5.8 g lipids per 100 mL) was infused directly in the stomach at a constant speed of 60 mL/min determined by an automated system using a peristaltic pump (Infusomat SpaceP; Braun Medical, Diegem, Belgium). Starting 5 min before nutrient drink ingestion HV's were asked to score their satiation at 1-min intervals, using a graphic rating scale that combines verbal descriptors on a scale graded 0–5 (1, threshold; 5, maximum satiation). Intragastric infusion was stopped as soon as a score of 5 was reached on their satiation scores, hereafter the experiment was terminated.

The original data were imported from the recording software to an Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet. We were primarily interested in slow IGP changes that could reflect changes in gastric muscle tone. Therefore, and in order to avoid influence from movement artefacts as well as artefacts caused by coughing, sneezing, moving or swallowing a moving minimum was calculated per channel from the original data (minimum value over 30 s of original data) followed by a moving median (median value over 120 s of the moving minimum data). Data were analysed and represented per minute as the average value of the five measurement channels that were clearly positioned below the LES as described above.

Statistical analysis. To compare the results a linear mixed model of a completely randomised design with repeated measures and factors treatment and time was constructed. All analyses were performed with SAS 9.2 (SAS Institute Inc., Cary, NC, USA). When the data before the start of the nutrient drink infusion was modelled, no treatment main effect ($P = 0.081$) or treatment-by-time interaction effect ($P = 0.98$) was observed. Therefore, and in order to compare the effect of a treatment on the IGP during nutrient drink infusion a baseline value (calculated as the average pressure in the last 5 min before nutrient drink infusion started) was subtracted from the data before comparison. Data are presented as mean ± S.E.M. Relations between the IGP increase from nadir and the corresponding satiation score increases were examined by correlation analysis ($P < 0.05$ is considered significant).

RESULTS

Influence of methylnaltrexone on gastric compliance and sensitivity to distension, measured with the barostat

The mean MDP on the day of saline and methylnaltrexone administration was not significantly different (6.7 ± 0.3 vs. 6.9 ± 0.3 mmHg; N.S.). During isobaric distensions, gastric compliance remained unaltered after saline and methylnaltrexone administration (43.6 ± 4.9 vs. 43.6 ± 4.8 mL/mmHg; N.S.; Figure 1a). Also, methylnaltrexone administration had no significant influence on the pressure-perception curves (Figure 1b; *AUC* 5.4 ± 0.3 vs. 5.4 ± 0.4; N.S.). The pressures needed to induce first perception (10.0 ± 0.6 vs. 9.9 ± 0.6 mmHg; N.S.) or discomfort (16.0 ± 0.6 vs. 17.0 ± 0.4 mmHg; N.S.) and the corresponding intra-balloon volumes, (respectively, 246 ± 33 vs. 223 ± 22 and 514 ± 45 vs. 490 ± 48 mL; N.S.) did not differ between saline and methylnaltrexone.

Influence of methylnaltrexone on gastric accommodation to a meal, measured with the barostat

Preprandial intragastric volumes were similar in both saline and methylnaltrexone condition (143 ± 16 vs. 124 ± 23 mL; N.S.). Ingestion of the meal caused an immediate relaxation of the proximal stomach in all subjects, reflected by an increase in the balloon volume (Figure 2). During the administration of methylnaltrexone, the gastric accommodation was significantly inhibited ($P < 0.01$).

Influence of methylnaltrexone and naloxone on intragastric pressure during nutrient drink infusion, measured with high resolution manometry

No significant treatment effect ($P = 0.081$) or treatment-by-time effect ($P = 0.98$) could be detected before the start of the nutrient drink infusion. When comparing the IGP during nutrient drink infusion a clear treatment effect ($P < 0.005$), time effect ($P < 0.0001$) and treatment-by-time effect ($P < 0.01$) could be observed. Significant differences between treatments at individual time points are shown in Figure 3. Healthy volunteers scored maximal satiation after 1124 ± 107, 979 ± 96 and 958 ± 84 mL nutrient drink infused (for placebo, naloxone and methylnaltrexone treatment, respectively; $P < 0.05$ between placebo and methylnaltrexone treatment).

DISCUSSION

The role of opioids in the control of proximal stomach motility is seemingly complex: it has been shown that the centrally acting mu-opioid receptor agonists morphine and remifentanil both increased and decreased gastric tone depending on the study protocol and centre.[8–10] In a previous barostat study that we performed the used methods were the same as in this study the

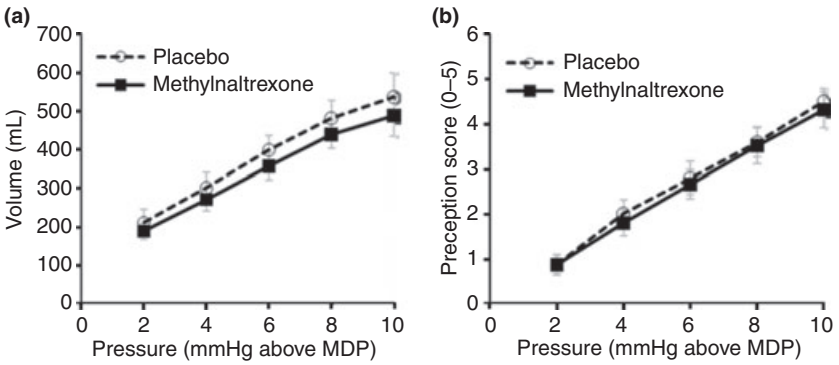

Figure 1 | (a) Pressure-volume curves obtained by gradually increasing isobaric gastric distensions. Gastric compliance, which was calculated as the slope of the pressure-volume curves, was not different after methylnaltrexone treatment. (b) Pressure-perception curves obtained by gradually increasing isobaric gastric distensions. The area under the pressure-perception curves did not differ between saline and methylnaltrexone. MDP, minimal distending pressure.

centrally acting mu-opioid receptor antagonist naloxone had no effect on basal gastric tone but inhibited meal-induced gastric accommodation, although the significance of the latter finding was borderline.[11] In this study we showed that although methylnaltrexone did not affect basal gastric tone, the effect of methylnaltrexone on gastric accommodation was more pronounced than that of naloxone.

The effect on gastric accommodation was assessed using the barostat technique and an alternative technique to assess gastric accommodation. Indeed, although the barostat is still considered the gold standard to assess gastric tone and accommodation, the nature of this measurement is regarded as unphysiological: the inflated balloon distends the proximal stomach, might exaggerate gastric accommodation and hampers physiologic responses to food intake.[18–20] We therefore developed a new technique to assess gastric relaxation during food intake in a more physiological manner, by measuring the IGP during food intake.[21] Indeed, it is generally accepted that the tone of the stomach muscles decrease during food intake, to allow storage of the meal without a rise in IGP.[24, 25] Using this principle to assess gastric accommodation, we found that during nutrient drink ingestion, the IGP decreased rapidly to gradually increase thereafter

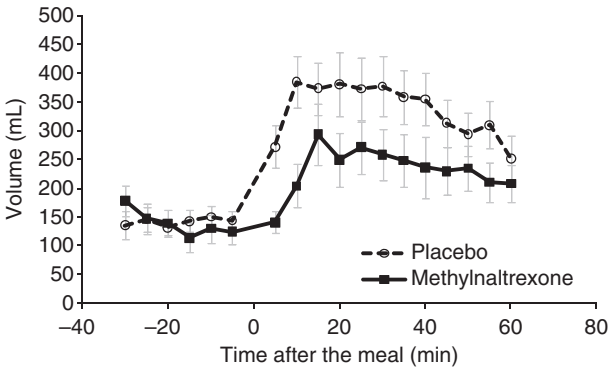

Figure 2 | Mean (±S.E.M.) intragastric volume at 5-min intervals as measured by gastric barostat in 11 healthy volunteers before and after administration of a liquid meal. The postprandial volume increase was significantly lower after methylnaltrexone treatment ($P < 0.01$).

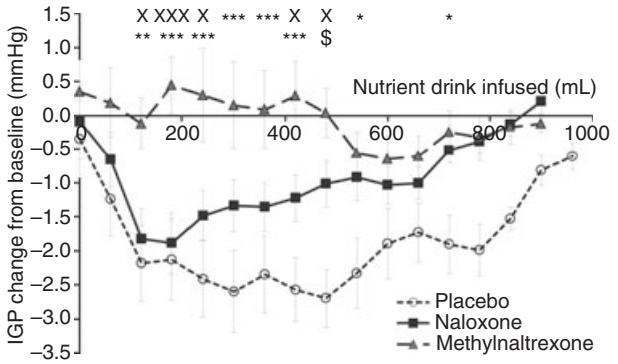

Figure 3 | Intragastric pressure (IGP) change from baseline during intragastric nutrient drink infusion and after treatment with placebo, naloxone or methylnaltrexone. Results presented as mean ± S.E.M. ($n = 11$) until the average ingested volume was reached. $^{x,*,\$}P < 0.05$, $^{**}P < 0.01$, $^{xxx,***}P < 0.001$ where ^{x} and ^{xxx} represent a significant difference between methylnaltrexone and naloxone, *,*** and *** represent a significant difference between methylnaltrexone and placebo and $^{\$}$ represents a significant difference between naloxone and placebo.

while nutrient drink ingestion is continued. When we impaired gastric accommodation by treating the volunteers with the nitric oxide synthase inhibitor N^G-monomethyl-l-arginine the IGP during nutrient drink ingestion was significantly increased, supporting the hypothesis that IGP reflects gastric accommodation.[21] In this study, barostat and IGP measurements both confirmed impaired accommodation after methylnaltrexone, indicating that both are valid to assess gastric accommodation.

Despite the shortcomings of the barostat technique it is at present the only technique that can assess the effect of a treatment on sensation to distension and compliance. After methylnaltrexone treatment stepwise increase in intraballoon pressure did not change perception as compared with placebo. Moreover, pressures needed to induce first perception and discomfort were not influenced by methylnaltrexone. These findings argue against a role for peripheral mu-opioid receptor activation in the control of sensitivity to gastric distension and confirm our previous findings with naloxone.[11] As argued in the latter article our findings do not exclude efficacy of exogenously applied agonists in the control of visceral sensitivity. Indeed, opioids are well-known for their antinociceptive effects in somatic but also visceral pain.[26] A possible limitation of our experimental design is that we only distended until the volunteers reported discomfort although it could be argued that endogenous opioids are only released when truly painful sensations are elicited. On the other hand, in similar barostat studies we performed with functional dyspeptic patients the same range of pressure values was used to show that these patients were hypersensitive to gastric distension.[22, 27]

Gastric accommodation is a reflex relaxation of the stomach muscles. In short, this reflex consists of vagal afferent neurons that synapse in the nucleus tractus solitarii (NTS) and the dorsal motor nucleus of the vagus and can activate vagal efferent neurons that in turn activate inhibitory motor neurons (mainly nitrergic in nature) in the enteric nervous system of the stomach to finally decrease the gastric muscle tone.[28] Endogenous opioids might be involved in several ways, hereafter we will discuss two options:

(i) Mu-opioid receptors have been identified on nerve terminals in the myenteric plexus of the stomach.[29] In rats it has been shown that (mu-) opioid receptor agonists inhibit vagal stimulation-induced acetylcholine release in isolated, vascular perfused stomachs.[30] Although endogenous release of opioids in the enteric nervous system during the gastric accommodation reflex has not been described it is plausible that opioid receptors on acetylcholine releasing nerves are activated during the gastric accommodation reflex inhibiting acetylcholine release which could result in a decreased gastric tone. Indeed, in isolated rat stomachs endogenous opioids are released upon vagal nerve stimulation,[31] and enkephalins are likely to be the predominant endogenous opioids that activate neuronal mu opioid receptors in the gut.[6]

(ii) In the medial subnucleus of the tractus solitarii (mNTS) mu-opioid receptors are localised[32] whereas the presence of endogenous mu receptor agonists as endomorphin-1 and 2 has been described.[33] Herman *et al.* performed a series of experiments in rats that showed that microinjection of mu opioid receptor agonists in the mNTS reduced the gastric tone whereas injection of mu-opioid receptor antagonists *per se* had no effect on the gastric tone. However, oesophageal distension-induced gastric tone decrease was blocked by injection of mu-opioid receptor antagonists in the NTS.[34] The authors proposed that upon activation of the vago-vagal reflex pathway that leads to gastric tone decrease, vagal afferents release opioids (possibly endomorphine-2) that inhibit GABA interneurons in the mNTS. Once the influence of GABA signalling is depressed, glutamate released from the vagal afferents can excite second-order NTS neurons and the vago-vagal reflex pathway that inhibits the gastric tone is activated.[34] This hypothesis could well explain our findings: we did not observe an effect of methylnaltrexone on basal gastric tone but the food-induced decrease in gastric tone was blocked by methylnaltrexone.

The question remains why the effect of the peripherally restricted mu-opioid receptor antagonist methylnaltrexone was more pronounced than that of the centrally-acting antagonist naloxone. Both methylnaltrexone and naloxone can reach the NTS,[34] however, naloxone can also influence higher brain areas that might cancel the effects it has on the NTS or the enteric nervous system out. Opioids are well-known to inhibit GABA release also in the higher centres of the central nervous system,[35] opioid antagonists such as naloxone could disinhibit GABAergic transmission in higher central nervous system areas and counteract the effects in the NTS, although this remains speculation with regard to the gastric accommodation reflex.

In this study we also showed that methylnaltrexone significantly increased satiation during food intake, whereas no significant effect of naloxone could be observed. The apparent difference with our previous

study where naloxone significantly enhanced meal-induced satiation,[11] is probably attributable to the relatively small size of the effect of naloxone and the limited sample size. Also in the literature there is some controversy with regard to the role of opioids in the control of food intake: in general opioid agonists enhance feeding and opioid antagonists decrease feeding.[12, 13] It has, however, been shown that this effect is dependent, for example, on the treatment duration and whether normal weight, low weight (anorexia patients) or obese patients are selected.[5] Similar to the effects on proximal stomach motility this complexity can be explained by the fact that different opioid receptors have different effects at the peripherally and at the central level. It has previously been suggested that gastric accommodation is a determinant of satiation both in healthy volunteers and FD patients with weight loss using the barostat, IGP measurements and imaging techniques.[2, 36, 37] Also in this study impaired gastric accommodation after methylnaltrexone treatment might explain why volunteers drank less.

Methylnaltrexone and naloxone were administered in a different dose and route of administration. Moreover, both drugs have different binding affinities to the mu receptor[38, 39] and their pharmacokinetic profiles are different.[40, 41] Although direct comparison is difficult we have dosed the drugs as they are prescribed in routine clinical practice.[11, 17] We therefore assume that they are similarly effective as mu-opioid receptor antagonists and that observed differences can be attributed to the fact that methylnaltrexone has no described effects centrally whereas naloxone passes the blood brain barrier.

In conclusion, we have shown that although the peripherally restricted mu-opioid receptor antagonist methylnaltrexone has no effect on gastric sensation to distension or compliance, gastric accommodation to a meal was impaired and feelings of satiation were enhanced. These results indicate that endogenous opioids mediate gastric accommodation and satiation via peripheral mu-opioid receptors. The fact that the effects were less pronounced or absent after treatment with the centrally acting mu-opioid receptor antagonist naloxone indicate that centrally involved mu-opioid receptors may mediate an opposing effect on gastric accommodation.

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