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Effect of rimonabant on oesophageal motor function in man

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SUMMARY

Background
Cannabinoid type 1 (CB1) receptors are implicated in the control of transient lower oesophageal sphincter relaxations (TLESRs) in animals. In man, it is unclear whether CB1 receptors are involved in the control of oesophageal function.

Aim
To study the effects of the CB1 receptor antagonist rimonabant on fasting and postprandial LES function in healthy subjects.

Methods
Twelve healthy volunteers underwent two oesophageal manometry studies with administration of wet swallows and a meal after 3 days' premedication with placebo or rimonabant 20 mg.

Results
Rimonabant did not significantly alter preprandial LES pressure (21.1 ± 4.0 vs. 17.3 ± 3.0 mmHg, N.S.), but postprandial LES pressures were significantly enhanced (9.9 ± 1.9 vs.17.1 ± 2.7 mmHg in the first and 10.0 ± 1.4 vs. 19.3 ± 3.6 mmHg in the second postprandial hour, both $P < 0.05$). Swallow-induced relaxations and amplitude of peristaltic contractions were not altered, but rimonabant significantly increased the duration of peristaltic contractions at all time points (e.g. 5.0 ± 0.3 vs. 8.0 ± 0.3 s preprandially and 5.0 ± 0.2 vs. 8.2 ± 0.3 s at 60 min postprandially, both $P < 0.01$). The number of postprandial TLESRs (3.1 ± 0.5 vs. 1.2 ± 0.5, $P < 0.05$) and acid reflux episodes (1.4 ± 0.2 vs. 0.3 ± 0.1, $P < 0.05$) were significantly lower after rimonabant.

Conclusion
The CB1 receptor antagonist rimonabant enhances postprandial LES pressure and decreases TLESRs in healthy subjects.

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INTRODUCTION

Gastro-oesophageal reflux disease (GERD) is defined as the presence of symptoms or lesions that can be attributed to the reflux of gastric contents into the oesophagus.[1, 2] The pathophysiology of GERD is multifactorial and involves several mechanisms such as failure of the antireflux barrier, impaired oesophageal clearance, the presence of offensive factors in the refluxate and defective oesophageal tissue resistance.[3] Among the dysfunctions of the antireflux barrier, transient lower oesophageal sphincter relaxations (TLESRs) are the major mechanism underlying gastro-oesophageal reflux events in normal subjects, and in most GERD patients.[4–6] TLESRs are a vago–vagal reflex, triggered by activation of stretch receptors of the proximal stomach, and organised in the brain stem.[5–7]

Pharmacological restoration of the antireflux barrier is considered a relevant target for the control of GERD.[8] Several gastroprokinetic agents, which also enhance lower oesophageal sphincter (LES) pressure and oesophageal motility have been developed and used in the treatment of GERD.[2, 8] Because of side effects or lack of efficacy, none of these drugs has led to a currently established treatment for GERD. Drugs inhibiting TLESRs are considered a novel and attractive target for GERD therapy. [9–11] Baclofen, an agonist at the gamma-aminobutyric acid receptor type B (GABA-B), has been demonstrated to inhibit considerably the occurrence of TLESRs and gastro-oesophageal reflux, with an acceptable side-effect profile.[11–14] It is currently thought that the antireflux effect of baclofen depends on peripheral inhibition of mechanosensitive gastric vagal afferents, thereby raising the threshold for action potential firing, and partly on central inhibition of vagal afferents leading to reduced transmitter release.[15]

Cannabinoid receptors (CBRs) have many similarities with GABA-B receptors. For example, agonism of both GABA-B receptors and CBRs inhibits transmitter release presynaptically and postsynaptically hyperpolarizes neurons in the central nervous system (CNS).[16, 17] Common CBR-mediated actions *in vivo* include skeletal muscle relaxation, hypothermia, and antinociception.[18] CBRs have an important role in inhibition of emesis triggered by stimuli in the gastrointestinal tract or CNS.[19–21] They have been localised to specific points along the emetic pathway in the central nervous system that receive input from gastrointestinal vagal afferents. There is considerable anatomical overlap of pathways involved in emesis and triggering of TLESRs.[22, 23] CBRs, like GABA-B receptors, belong to the G protein-coupled receptor superfamily and are subdivided into two groups, CBR1 and CBR2.[24] Delta-9- tetrahydrocannabinol (delta(9)-THC) is the principal psychoactive compound of marijuana and is well established as an agonist at CBRs. More recently, endogenous ligands (endocannabinoids) have been isolated, the most important of which are arachidonylethanolamide (anandamide) and 2-arachidonyl glycerol.[24–26] The endocannabinoids appear to act as retrograde messengers, i.e. they can be released by postsynaptic neurons and diffuse to nerve terminals where they reduce transmitter release.[25, 26] A number of synthetic ligands for CBRs have been made, and although CBR agonists are moderately subtype selective, antagonists show definitive selectivity.[26] The CB1 antagonist rimonabant was commercially available as a drug in the treatment of obesity, until being expelled from the market because of an increased prevalence of depression.[27, 28]

In dogs and in humans, delta(9)-THC significantly reduced resting LES pressure and the occurrence of TLESRs after a standard meal.[29] A study in dogs established that CB1 agonists inhibit the occurrence of TLESRs, whereas rimonabant reversed this effect.[30] The effects of rimonabant on LES function in man has not been addressed so far. Thus, the aim of the present study was to investigate the effect of rimonabant on oesophageal motility and LES function in man.

MATERIALS AND METHODS

Subjects

Studies were performed in 12 healthy volunteers (five men and seven women; mean age, 30.2 ± 1.8 years; range, 23–41 years) with a mean body weight of 66 ± 3 kg. None of the subjects had symptoms or a history of gastrointestinal disease or upper gastrointestinal surgery, nor were they taking any medication. In addition, volunteers with a history of depression were excluded. Written informed consent was obtained from each subject and the study protocol had been approved previously by the Ethics Committee of the University Hospital.

Study design

All subjects underwent two studies after 3 days' premedication with rimonabant 20 mg or matching placebo in a double-blind randomised cross-over design, at least 1 week apart. The 3 days' administration was chosen based on the SmPC information. Upon oral ingestion, maximum concentration is reached after approximately 2 h. Rimonabant's half-life is longer in obese patients

(approximately 16 days) than in non-obese subjects (9 days). Steady state is reached after 3 days.[31] On each day of measurement, subjects were studied after an overnight fast of at least 12 h and the dose of placebo or rimonabant for that day was administered in a double-blind fashion in the motility research unit. Together with a stationary manometry probe, a pH probe was passed through the mouth under topical anaesthesia and positioned with the pH electrode at 5 cm above the LES. A summary of the protocol is shown in Figure 1. After placement of the assembly, the subjects remained in a sitting position for a habituation period of 20 min. This period allowed baseline assessment of oesophageal peristalsis and LES function. Ten wet swallows of 5 mL of water were administered and followed by oral ingestion of 20 mg of rimonabant or placebo in a double-blind, randomised cross-over order. During the 30 min after administration of the drug oesophageal and lower oesophageal sphincter pressure and oesophageal pH were recorded concomitantly. Sixty minutes after drug administration, the subjects ingested a mixed liquid meal (200 mL, 300 kcal, 13% proteins, 48% carbohydrates, 39% lipids; Nutridrink, Nutricia, Bornem, Belgium) and recordings continued for 2 h after. Throughout the study, 10 wet swallows of 5 mL of water were administered at 30-min intervals. The sensations of fullness, nausea, heartburn, belching, satiety, hunger, anxiety, dizziness, sleepiness and fatigue were measured every 15 min using validated 100-mm visual analogue scales.[32]

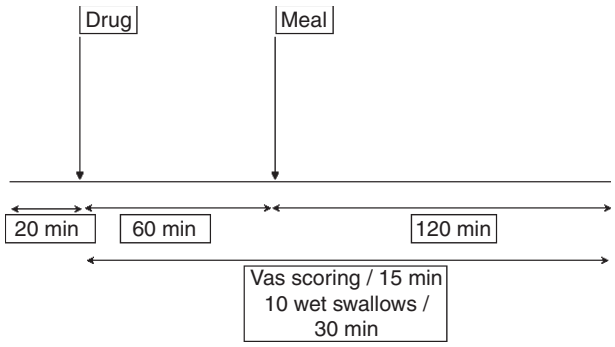

Figure 1 | Study outline. Healthy volunteers underwent oesophageal sleeve manometry and pH measurement studies after 3 days' premedication with rimonabant 20 mg or placebo. After placement of the assembly ten wet swallows of 5 mL of water will be administered, followed by ingestion of the medication. After 60 min, a standardised meal will be administered and measurements will continue for another 120 min. At 30-min intervals, 10 wet swallows will be administered. Throughout the study, at 15-min intervals the intensity of eight epigastric symptoms will be scored on visual analogue scales.

Recording methods

Following an overnight fast, an oesophageal manometric catheter fitted with a 6-cm Dent Sleeve was introduced through the mouth. Subsequently, the oesophageal catheter was positioned so that pressures could be recorded from the fundus (side hole 2 cm below the sleeve), the lower oesophageal sphincter (sleeve), oesophageal body (side holes 4, 7 and 10 cm proximal to the sleeve) and pharynx (side hole 28 cm proximal to the sleeve, to detect swallows). The oesophageal catheter was infused at a flow rate of 0.5 mL/min with distilled water using a low-compliance pneumo-hydraulic capillary infusion system (Arndorfer Medical Specialties, Milwaukee, WI, USA). The infusion system was connected to external pressure transducers, and signals were recorded on a polygraph (Synectics Medical, Stockholm, Sweden).

The oesophageal pH was measured with an antimony pH electrode (Synectics Medical, Stockholm, Sweden) positioned 5 cm above the proximal margin of the sleeve. The pH electrode was calibrated in buffers of pH 1 and pH 7 before and after each study. During the study period, the oesophageal pH was recorded continuously using an ambulatory data-logger (MicroDigitrapper; Synectics Medical).

Data analysis

Lower oesophageal motility. The tracings were analysed in a blinded fashion by one of the authors (ES) and checked by a second reader (JT). The basal lower oesophageal sphincter pressure was measured at end expiration relative to the end-expiratory intragastric pressure. The basal lower oesophageal sphincter pressure was visually determined every 3 min and averaged over 15-min intervals. The influence of drug administration on the basal lower oesophageal sphincter pressure was assessed by comparing the value of the first with the value of the third preprandial 30-min interval.

Transient lower oesophageal sphincter relaxations were defined according to criteria previously published[33] as follows: (i) absence of a swallowing signal for 4 s before to 2 s after the onset of lower oesophageal sphincter relaxation; (ii) relaxation rate of ≥1 mmHg/s; (iii) time from onset to complete relaxation of ≤10 s; and (iv) nadir pressure of ≤2 mmHg. Excluding multiple swallows, lower oesophageal sphincter pressure falls that fulfil the last three criteria, but have a duration of >10 s, can also be classified as transient lower oesophageal sphincter

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relaxations irrespective of the timing of lower oesophageal sphincter relaxation relative to swallowing.

Oesophageal pH. The percentage of time with an oesophageal pH < 4 and the number of acid reflux episodes were calculated. Acid reflux episodes were defined as a decrease in oesophageal pH to a value below pH 4 for at least 4 s or, if the basal oesophageal pH was already below pH 4, as a rapid further drop in pH of at least 1 pH unit.

Statistical analysis

Data are presented as the mean ± standard error of the mean (S.E.M.). The paired *t*-test was used for the comparison of the mean values between the periods or between the rimonabant and placebo studies. The changes in the basal lower oesophageal sphincter pressure was evaluated using analysis of variance for repeated measures. The frequency and duration of transient lower oesophageal sphincter relaxations, the number of acid reflux episodes and the symptom scores were compared using the Wilcoxon signed rank test. A *P*-value <0.05 was considered to be statistically significant. Based on previous studies, the study had an 85% power to detect 30% difference in TLESR rate at 5% significance level.[14, 32]

RESULTS

Conduct of the study

The positioning of oesophageal manometric catheter and pH probe were well tolerated, and all subjects completed both the sessions of studies. No adverse events were reported.

Lower oesophageal sphincter pressure

Prior to drug administration, and in the preprandial period prior to the meal, LES resting pressure and swallow-induced relaxations were similar for both conditions (Tables 1 and 2). In the placebo studies, ingestion of the meal was associated with a significant decrease in the LES pressure during both the first and the second postprandial hour (Figure 2). In contrast, in the rimonabant studies, no significant decrease in LES pressure occurred after the meal (Table 1).

Oesophageal motility

The amplitude of peristaltic contractions and swallow-induced relaxations were not significantly altered by rimonabant in the preprandial and postprandial periods. However, rimonabant significantly increased the duration of peristaltic contractions in the preprandial and postprandial periods (Table 1). Rimonabant did not alter the amplitude of swallow-induced relaxations, but the duration of contractions was significantly increased by rimonabant (Table 1).

Transient lower oesophageal sphincter relaxations

The numbers of transient lower oesophageal sphincter relaxations after the administration of placebo and rimonabant are summarised in Figure 2 and Table 3. After placebo, ingestion of the meal was associated with a significant increase in the rate of TLESRs during the first and the second postprandial hour. In contrast, no significant increase in the rate of TLESRs relaxations occurred postprandially in the rimonabant studies (Figure 3, Table 3). Compared to placebo, the number of TLESRs in the rimonabant studies was significantly lower for the whole postprandial period and in the first postprandial hour.

Table 1 | Oesophageal motility parameters, before and after intake of placebo or rimonabant, and before and after the meal

	LES pressure (mmHg)		Distal contraction amplitude (mmHg)		Distal contraction duration (s)	
	Placebo	Rimonabant 20 mg	Placebo	Rimonabant 20 mg	Placebo	Rimonabant 20 mg
Basal	19.5 ± 2.7	24.0 ± 2.6	76.6 ± 3.7	78.6 ± 5.8	4.8 ± 0.2	7.9 ± 0.3†
Postdrug	17.7 ± 3.2	21.2 ± 3.7	83.9 ± 5.2	80.8 ± 5.1	5.2 ± 0.3	8.0 ± 0.3†
Postprandial 1st hour	9.9 ± 1.9‡	17.1 ± 2.7*	78.0 ± 3.9	80.8 ± 4.3	5.2 ± 0.2	8.2 ± 0.3†
Postprandial 2nd hour	10.0 ± 1.4‡	19.3 ± 3.6*	77.3 ± 5.5	75.6 ± 4.3	5.2 ± 0.3	8.2 ± 0.4†

* $P < 0.05$ compared to placebo.

† $P < 0.001$ compared to placebo.

‡ $P < 0.05$ compared to basal.

Table 2 | Characteristics of swallow-induced relaxations before and after intake of placebo or rimonabant, and before and after the meal. No significant differences occurred between groups or over time

	Relaxation (%)		Duration (s)	
	Placebo	Rimonabant 20 mg	Placebo	Rimonabant 20 mg
Basal	98 ± 1	96 ± 3	11.8 ± 0.5	12.2 ± 0.4
Postdrug	97 ± 1	97 ± 1	12.5 ± 0.7	12.2 ± 0.4
Postprandial 1st hour	95 ± 2	97 ± 2	11.5 ± 0.7	12.1 ± 0.3
Postprandial 2nd hour	96 ± 2	97 ± 2	11.8 ± 0.6	12.7 ± 0.4

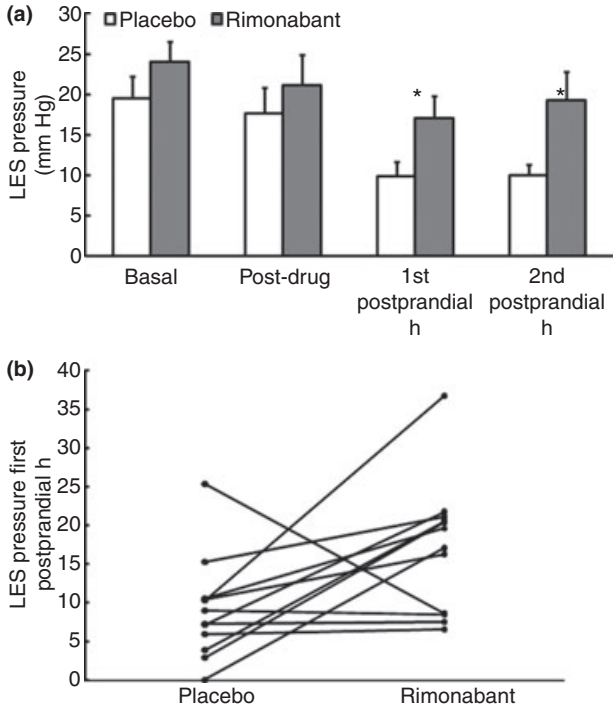

Figure 2 | Lower oesophageal sphincter pressure. (a) Basal LES pressure was comparable for both conditions. Rimonabant pre-treatment prevented the meal-induced decrease in LES pressure. *$P < 0.05$ compared to placebo. (b) Individual results for the LES pressure during the first postprandial hours.

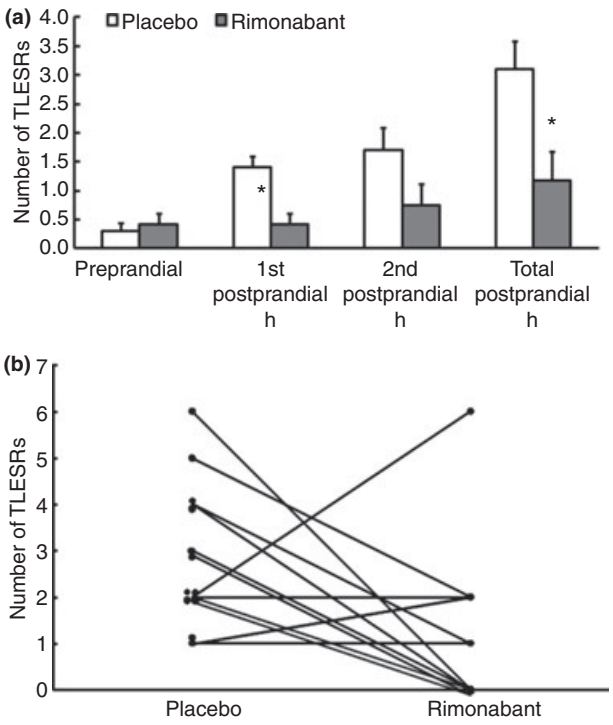

Figure 3 | TLESRs. (a) Basal LES pressure was comparable for both conditions. Rimonabant pre-treatment prevented the meal-induced decrease in LES pressure. *$P < 0.05$ compared to placebo. (b) Individual results for the total number of postprandial TLESRs.

Table 3 | Number of transient lower oesophageal sphincter relaxation

Pre-treatment	Preprandial	First postprandial hour	Second postprandial hour	Total postprandial
Placebo	0.3 ± 0.1	1.4 ± 0.2†	1.7 ± 0.4†	3.1 ± 0.5†
Rimonabant 20 mg	0.4 ± 0.2	0.4 ± 0.2*	0.8 ± 0.4	1.2 ± 0.5*

* $P < 0.05$ compared to placebo.

† $P < 0.05$ compared to preprandial.

After placebo, postprandial TLESRs lasted significantly longer than preprandial TLESRs (26.2 ± 3.4 vs. 31.5 ± 2.1 s, $P < 0.05$) while no difference was seen between pre- and postprandial TLESRs with rimonabant (19.9 ± 3.4 vs. 19.7 ± 3.3 s, N.S.). In the postprandial period, TLESR duration was significantly shorter after rimonabant compared to placebo (19.7 ± 3.3, vs. 31.5 ± 2.1 s; $P < 0.05$).

Oesophageal pH

The percentage of time pH < 4 in the oesophagus did not differ between the rimonabant and placebo studies in the preprandial (0.1 ± 0.05% vs. 0.3 ± 0.1%, N.S.) but tended to be lower after rimonabant in the postprandial period (0.1 ± 0.05 vs. 0.4 ± 0.1%; $P = 0.06$). The number of acid reflux episodes increased significantly after the meal in the placebo studies but not in the rimonabant studies (Figure 4). The number of acid reflux episodes during the postprandial period after rimonabant was significantly lower than after placebo (Figure 4).

Symptoms

No significant differences in symptom scores (calculated as area under the curve, *AUC*) during both the preprandial and postprandial periods were found between rimonabant and placebo studies (details not shown).

DISCUSSION

The aim of the present study was to investigate how suppression of endocannabinoid action by rimonabant would influence oesophageal motility, the occurrence of TLESRs elicitation and gastro-oesophageal reflux events in healthy volunteers. We found that rimonabant inhibited the decrease in LES pressure and the increase in the rate of TLESRS and reflux events after a meal, leading to a significant lower number of acid reflux events. In addition, the duration of peristaltic contractions in the distal oesophagus was prolonged after rimonabant.

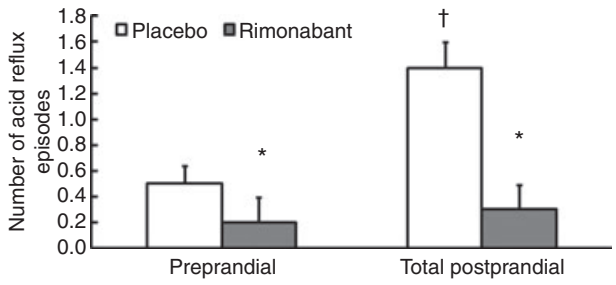

Figure 4 | Acid-reflux episodes. The number of acid-reflux episodes during both the preprandial and postprandial period was significantly lower after rimonabant. The pre-treatment with rimonabant was not associated with a postprandial increase of acid reflux episodes as after placebo. *$P < 0.05$ compared to placebo. †$P < 0.05$ compared to preprandial.

The observation that rimonabant increased LES pressure after a meal is in line with previous studies, which demonstrated that the cannabinoid agonist delta(9)-THC reduced resting LES pressure after a standard meal in man.[29] The inhibition of TLESRs and reflux events we observed is more surprising, as delta(9)-THC administration inhibited the occurrence of TLESRs after a standard meal in healthy volunteers,[29] and in the dog rimonabant enhanced the rate of TLESRs and reflux events induced by an acidified meal and intra-gastric air insufflation.[30] These observations, which indicate ongoing suppression of TLESR by a CB1 receptor in the dog,[30] were not confirmed in the present human study.

The reason for the apparent species difference between dog and man, and the mechanism underlying the inhibition of TLESRs and reflux events by rimonabant remains to be established. The dosing regimen differed strongly between the dog studies and the present studies: dogs received a unique dose of 0.22 μmol/kg while the volunteers were treated for 3 days of medication with 20 mg/day. Furthermore, binding studies show that rimonabant has an affinity for the CB1 receptor in the low nanomolar range, but in functional experiments, rimonabant is not a neutral antagonist but has rather been found to be an inverse agonist at the CB1 receptor.[34, 35] The effects of rimonabant in the present study therefore can reflect antagonist properties, inverse agonist effects, or both.

Transient lower oesophageal sphincter relaxations are controlled by a vago–vagal reflex pathway, which is triggered by gastric distention, integrated in the brainstem and induces release of nitric oxide from intrinsic nerves at the LES.[36–38] Endocannabinoids can potentially control this vago–vagal reflex pathway at many points as CB1 receptors are expressed on vagal afferents, in the brain stem, on interneurons in the wall of the gastrointestinal tract and on postganglionic fibres innervating smooth muscle in the gastrointestinal tract.[39–42] In the dog, rimonabant only increased the number of TLESRs, whereas their characteristics and oesophageal motility were unchanged, and this was considered an argument in favour of a central site of action.[30] The observation that fasting LES pressure was not affected by rimonabant in the present study, whereas the postprandial drop in LES pressure and the occurrence of TLESRs were inhibited could also argue in favour for a

central action, for instance on vago–vagal reflex pathways. Rimonabant also has the potential to inhibit the occurrence of TLESRs at the level of triggering through distention of the proximal stomach. In a gastric barostat study, we demonstrated a significant decrease in postprandial volume of the proximal stomach in healthy volunteers after rimonabant pre-treatment.[43] In the present study in man, the duration of distal oesophageal contractions was prolonged and the duration of TLESRs was shortened by rimonabant. Another important site of action for rimonabant therefore to consider is the enteric nervous system. Indeed, we and others previously provided evidence for a continuous endocannabinoid tone acting on CB1 to submit neurotransmitter release in the enteric nervous system.[39–42] Rimonabant may thus enhance the release of, for instance, acetylcholine from intrinsic motor neurons, which could contribute to the observed longer duration of peristaltic contractions and higher postprandial LES pressure. The absence of any change in symptoms from the gastrointestinal tract could also be viewed as an argument against a primarily central site of action. Further clarifying the site of action where rimonabant inhibits TLESRs and reflux would require studies with a peripherally acting CB1 antagonist, and no such drug is presently available for human studies.

Inhibition of TLESRs is a now well-established therapeutic target in GERD, and several drugs are being investigated for their ability to inhibit TLESRs, including GABA-B agonists, metabotropic glutamate-5 receptor antagonists and the mixed dopamine-2 antagonist/cholinesterase inhibitor itopride.[9–15, 32, 44–46] The magnitude of inhibition of TLESRs and reflux events observed with rimonabant is at least in the same order of magnitude as observed with these agents. On the other hand, long-term administration of rimonabant has been associated with an increased occurrence of depression,[47] and this adverse event does not seem compatible with the use of rimonabant or similar CB1 antagonists for GERD therapy. Development of peripherally acting CB1 antagonists could be considered for this and other indications, provided that the site of action in TLESR inhibition is outside the blood-brain barrier.[48, 49]

The present study has a number of limitations. First, only a single dose of rimonabant was studied, as this was the only one available for the treatment of obesity in man. Second, the number of TLESRs and reflux events was low, which may be due to the fact that we studied asymptomatic healthy volunteers, and due to the relatively small size of the test meal. Finally, reflux events were only assessed by pH monitoring. It is conceivable that the use of oesophageal impedance monitoring might have increased the number of detectable and quantifiable reflux events, especially after the meal.

In summary, in this placebo-controlled, double-blind, randomised, crossover study, we demonstrated for the first time that the CB1 receptor antagonist, rimonabant, inhibits the meal-induced increase in TLESRs, increases postprandial LES pressure leading to a lower number of acid reflux events. In addition, rimonabant increased the duration of distal oesophageal peristaltic waves. Further studies will need to address whether this effect of rimonabant has a central or a peripheral site of action.

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