The acid pocket, hiatal hernia and TLESRs: essential players in the pathogenesis of gastro-esophageal reflux disease

Beaumont, H.

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Effect of delta-9-tetra-hydrocannabinol, a cannabinoid receptor agonist on the triggering of TLESRs in dogs and healthy subjects

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Hanneke Beaumont
Jörgen Jensen
Anita Carlsson
Magnus Ruth
Anders Lehmann
Guy Boeckxstaens
Abstract

Introduction: Transient Lower Esophageal Sphincter Relaxations (TLESRs) are the main mechanism underlying gastroesophageal reflux and are a potential target for the treatment of gastroesophageal reflux. Previous animal experiments have shown that activation of cannabinoid 1 (CB1) receptors reduces the occurrence of TLESRs. We evaluated the effect of the CB1/CB2 receptor agonist delta-9-tetra-hydrocannabinol (Δ9-THC) on TLESRs in a dog model. Based on these findings, the effect of THC on meal-induced TLESRs was studied in healthy volunteers.

Methods: In 10 dogs, a sleeve catheter was introduced through a cervical esophagostomy to evaluate the effect of Δ9-THC in the presence and absence of the CB1 receptor antagonist SR141716A on TLESRs induced by intragastric infusion of a liquid nutrient followed by insufflation of air. Secondly, the effect of 10 and 20 mg THC was studied in 18 healthy volunteers in a placebo-controlled study. All subjects underwent esophageal manometry before and during 3 hours after meal ingestion on three different occasions. Samples for plasma concentration measurements were collected. For statistical analysis, a mixed model using pairwise comparisons and paired Student’s t-tests were used in human and dogs, respectively, and data is presented as mean ± S.E.M.

Results: In dogs, Δ9-THC dose-dependently inhibited TLESRs and reduced the number of acid reflux episodes. The CB1 receptor antagonist SR141716A significantly reversed the effects of Δ9-THC on TLESRs in dogs. Similarly, in healthy volunteers, Δ9-THC significantly reduced the number of TLESRs and caused a non-significant reduction of acid reflux episodes in the first postprandial hour. In addition, basal LES pressure and spontaneous swallowing were significantly reduced by Δ9-THC. After intake of 20 mg, half of the subjects experienced nausea and vomiting leading to premature termination of the study. Other side effects were hypotension, tachycardia and central side effects.

Conclusion: The present study demonstrates that Δ9-THC both in dogs and humans significantly inhibits the increase in TLESRs evoked by meal ingestion and reduces spontaneous swallowing. In addition, Δ9-THC significantly reduced basal LES pressure in humans. These findings confirm previous observations in dogs and indicate that CB receptors are also involved in the triggering of TLESRs in humans.
Introduction

Gastroesophageal reflux disease is a common disorder with symptoms like heartburn, regurgitation and retrosternal pain\(^1\), resulting from prolonged acid exposure of the esophageal mucosa. The lower esophageal sphincter (LES) plays a central role in regulating flow across the gastroesophageal junction by generating a tonic pressure to prevent reflux of gastric contents into the esophagus. Transient lower esophageal sphincter relaxations (TLESRs), occurring in the absence of a swallow, are the main mechanism underlying reflux in healthy volunteers and in patients with gastroesophageal reflux disease (GERD).\(^2\)\(^-\)\(^4\) Therefore, pharmacological inhibition of TLESRs is a potential target to treat GERD.

TLESRs are triggered by gastric distension resulting from an increased activation of gastric mechanoreceptors in the subcardiac region of the stomach. This leads to activation of a reflex pathway involving gastric vagal afferents, brainstem integrative circuits, and efferent inhibitory pathways to the LES and crural diaphragm.\(^5\) Vagal cooling and cervical vagotomy abolish the triggering of TLESRs, indicating that TLESRs are mediated by a vago-vagal pathway.\(^6\)\(^,\)\(^7\) The insight in the neural pathway and the neurotransmitters involved in this reflex has increased enormously.\(^8\) To date the most potent pharmacological agents to reduce the rate of TLESRs are \(\gamma\)-aminobutyric acid type B (GABA\(_B\)) receptor agonists. The GABA\(_B\) receptor agonist baclofen reduces the rate of TLESRs and the number of acid reflux episodes in normal subjects\(^9\) and in GERD patients.\(^10\)-\(^12\) This effect most likely depends on inhibition of mechanosensitive gastric vagal afferents and their central synaptic connections with brain stem neurons, leading to a raised threshold for action potential firing and reduction of transmitter release, respectively.\(^13\)

Cannabinoid (CB) receptors, like GABA\(_B\) receptors, belong to the superfamily of G protein-coupled receptors.\(^14\),\(^15\) There are two types of cannabinoid receptors, the CB1 and CB2 receptor. CB1 receptors are mainly localised in the central nervous system whereas CB2 receptors are particularly associated with the immune system. Interestingly, CB1 receptors have been localised in brain areas involved in the triggering of TLESRs as well as in the nodose ganglion from which vagal afferents emanate.\(^16\),\(^17\) Moreover a study in dogs showed that the CB receptor agonist WIN 55,212-2 reduced the occurrence of TLESRs in response to gastric distension by 80%.\(^18\) A study in ferrets confirmed the involvement of CB1 receptors in the central regulation of LES relaxation and showed the presence of CB1 receptors in the brain centres involved in the triggering of TLESRs.\(^16\) These data indicate that CB1 agonists may be clinically useful to reduce TLESRs in humans and may have the potential to be used as reflux inhibitors.

To date, no specific CB1 agonists are available for human use. However, marijuana and its main psychoactive component delta\(^9\)-tetrahydrocannabinol (\(\Delta^9\)-THC) are frequently used as anti-emetics\(^19\),\(^20\) or as an appetite stimulator for example in patients who fail to respond adequately to conventional therapies, in particular cancer patients receiving chemo- or radiotherapy, or patients undergoing HIV therapy.\(^20\)-\(^22\) \(\Delta^9\)-THC has approximately equal affinity for the CB1 and CB2 receptor, but its efficacy is less at the CB2 receptor.\(^23\) In the present study, therefore, we first evaluated whether \(\Delta^9\)-THC (dronabinol, Marinol\(^\text{®}\)) had an
inhibitory effect in dogs on the triggering of TLESRs, as previously shown for the specific CB1 agonist WIN 55,212-2. The pharmacodynamic/kinetic relationship in dogs was then used to design a study in healthy volunteers evaluating its effect on the occurrence of meal-induced TLESRs.

**Materials and Methods**

**Dog study**

**Animals**

Ten adult male and female Labrador retrievers were used in the experiments. Cervical esophagostomies were made and after recovery from surgery the dogs were accustomed to rest in a Pavlov stand. Before each experiment, the dogs were fasted overnight with free access to water. A washout of at least three days was allowed between experiments. All procedures were approved by the Ethical Committee for Animal Experiments of the Göteborg region.

**Protocol**

The dogs were intubated with a water-perfused Dentsleeve multilumen catheter to record gastric, lower esophageal sphincter (LES) and esophageal pressures. An antimony pH electrode was placed 3 cm above the lower esophageal sphincter to measure acid reflux episodes and a water-perfused catheter was placed in the hypopharynx to measure swallows.

TLESRs were stimulated by infusion into the stomach through the central lumen of the assembly of an acidified liquid nutrient (30 ml kg⁻¹; 100 ml min⁻¹) followed by air insufflation (500 ml min⁻¹) to maintain gastric pressure between 10 ± 1 mmHg.

The number of TLESRs was measured during a 45 minute period starting from the infusion of the liquid. TLESRs were defined by a rapid decrease of LES pressure (> 1 mmHg s⁻¹) to a pressure < 2 mmHg above gastric pressure and a duration of > 1s, without any pharyngeal signal < 2s before onset.²⁴

Δ⁹-THC was administered intragastrically (2 ml kg⁻¹), 30 min before start of the experiment (T=0). In experiments with the CB1 antagonist SR141716A, the compound was administered intragastrically (2 ml kg⁻¹) 45 min before start of measurements, followed by Δ⁹-THC 15 min later. Except for TLESRs and acid reflux episodes, basal LES pressure, latency time from start to first TLESR and swallowing rate were determined. Basal LES pressure was defined as the average pressure during the 45 minute experimental period. Reflux episodes were defined as a drop in pH > 1 unit within 5 seconds. The average pH in the 15 seconds following nadir pH should be < 4.

**Plasma sampling and analysis**

Blood samples were taken from a foreleg vein from two dogs at each dose. After separation of the plasma the samples were stored at -18°C until analysis. The analysis of the plasma
was performed using MS/MS detection after liquid liquid extraction and chromatographic separation. 100 µl plasma was extracted by shaking with 700 µl methyl-tert-buthyl ether/hexane 50/50 v/v for 30 minutes. 450 µl of the organic phase was evaporated to dryness and reconstituted in 150 µl 30 % acetonitrile, 0.2% formic acid. 10 µl was injected onto a reversed phase C18 column, eluted by a fast gradient and detected by electrospray tandem mass spectrometry in positive ion mode.

**Drugs**

Δ⁹-THC was purchased from Sigma Aldrich, Stockholm, Sweden, and was diluted in 5% ethanol, 80% polyethylene glycol and 15% physiological saline (0.9% NaCl). The intragastrically used doses of Δ⁹-THC were 0.25 µmol kg⁻¹ (0.079 mg kg⁻¹), 0.40 µmol kg⁻¹ (0.126 mg kg⁻¹), and 0.60 µmol kg⁻¹ (0.189 mg kg⁻¹). SR141716A was dissolved in 5% ethanol, 60% polyethylene glycol and 35% physiological saline (0.9% NaCl). The intragastrically used dose of SR141716A was 0.22 µmol kg⁻¹ (0.1 mg kg⁻¹).

**Data analysis and statistics**

Each dog served as its own control. All parameters were calculated with regard to the mean of five preceding control experiments for each dog. Data are presented as mean ± SEM. Statistical analysis was done using paired Student’s t-tests. A p-value < 0.05 was regarded as statistically significant.

**Human study**

**Subjects**

Studies were performed in 18 healthy volunteers (M), ranging in age from 19 – 51 years (median 21.5). Subjects were free of any gastrointestinal symptoms, had no history of gastrointestinal surgery and did not take any medication known to influence gastrointestinal motility. They had no history of cannabis use or any other drug abuse for at least two months prior to the study and frequent cannabis users were excluded. Urine samples were taken to check for any recent drug abuse. Each volunteer gave written informed consent, and the study was approved by the Medical Ethical Committee of the Academic Medical Centre.

**Study design**

The effect of Δ⁹-THC (Marinol ® 10 and 20 mg, p.o.) was evaluated in a randomized double blind placebo controlled study. On three different study days, all with a minimum interval of one week, subjects underwent combined esophageal manometry and pH metry. Intake of alcohol and nicotine was not allowed 24 hours prior to and during the study. The studies were performed after an overnight fast. A cannula was inserted into a forearm vein for blood sampling. The manometric and pH catheter were introduced through an anaesthetised nostril and positioned so that the sleeve straddled the LES. The pH electrode was located 5 cm above the proximal margin of the LES. All studies were performed in the sitting position. Recording started just after dosing (T=0). After 45 minutes of basal recording, the subjects received a meal (550 kcal), consisting of 2 pancakes (2x100 g) with jam (2x15 g) and orange
juice (200 ml) to trigger the occurrence of TLESRs. After the meal (T=60), patients remained upright and manometric/pH recordings were obtained for three more hours. Blood pressure and heart rate were recorded before dosing, every 15 minutes during the first two hours of the study, and every 30 minutes during the next two hours. Blood samples were taken before dosing, at T = 30, 60, 120, 240 and 480 minutes. After the recording period, subjects had to stay for 4 more hours in the hospital for further monitoring of blood pressure and pulse once every hour. In order to screen for drug side effects, symptoms of dizziness, nausea and sleepiness were assessed during the total study period.

**Recording methods**

Esophageal manometry was performed using a 10 lumen assembly (Dentsleeve, Adelaide, Australia) with a sleeve sensor incorporated at its distal end to monitor LES pressure. Side holes monitored pressure in the stomach (1 cm below the distal margin of the sleeve) and at 2, 5, 8, 11, 14, 17 and 20 cm above the LES. A side hole in the pharynx monitored swallows. The sideholes and the sleeve were perfused with degassed distilled water at 0.3 ml min⁻¹ and 0.6 ml min⁻¹ respectively, using a pneumohydraulic capillary perfusion pump (Dentsleeve Pty, Belair, South Australia). The pharyngeal side hole was perfused with air at 0.6 ml min⁻¹ to reduce pharyngeal triggering of TLESRs. Pressures were sensed by external transducers connected to a polygraph (Synectics Medical, Stockholm, Sweden). To measure acid reflux, pH was recorded with an antimony electrode with built-in reference, positioned 5 cm above the proximal margin of the LES. Before and after the study the pH electrode was calibrated at 37 °C using pH 1.0 and 7.0 buffer solutions (Medtronic, Skovlunde, Denmark). Signals were digitalised, computer-processed, stored and analysed using commercially available software (Polygram, Synectics Medical, Stockholm, Sweden).

**Data analysis**

Basal LES pressure was measured at end-expiration relative to intragastric pressure, and was determined as visual means of one-minute periods every 15 minutes.

TLESRs were evaluated according to previously published criteria²⁵: 1) absence of swallowing for 4 s before to 2 s after the onset of LES relaxation, 2) relaxation rate of ≥ 1 mmHg s⁻¹, 3) time from onset to complete relaxation of ≤ 10 s, and 4) nadir pressure of ≤ 2 mmHg. LES relaxations associated with a swallow and fulfilling the above mentioned criteria 2, 3 and 4 that lasted more than 10 seconds were included as TLESR. TLESRs were counted for each subject during the preprandial period and the 3 postprandial hours. In addition, the percentage of TLESRs accompanied by an acid reflux episode was determined, as well as the proportion of acid reflux episodes associated with a TLESR and the total amount of acid reflux episodes and total acid exposure time.

Acid reflux episodes were defined as a decrease in esophageal pH below 4 for more than 5 seconds with the pH drop > 1 pH unit or, if basal esophageal pH was already below 4, as a further decrease in pH of at least 1 pH unit. Slow drifts of pH below 4 were included in the analysis of the total duration of esophageal acid exposure but were not scored as acid reflux.
delta-9-tetrahydrocannabinol and TLESRs episodes. The rate of spontaneous swallowing was determined by counting the pharyngeal pressure waves.

**Plasma sampling and analysis**

Blood samples were taken from a forearm vein at 6 time points during each study day. After separation of plasma, samples were stored at -20 °C until analysis. Metabolism of Δ⁹-THC occurs mainly in the liver by hydroxylation and oxidation. Nearly 100 metabolites have been identified for Δ⁹-THC. The 2 major metabolites are 11-hydroxy-THC and 11-nor-9-carboxy-THC. Analysis of Δ⁹-THC, 11-hydroxy-THC and 11-nor-9-carboxy-THC was done by Pharma Bio Research (Assen, The Netherlands) through high performance liquid chromatography with tandem mass spectrometric detection, in compliance with the principles of Good Laboratory Practice (GLP). Only the results of Δ⁹-THC plasma concentration will be discussed in this study.

**Statistical analysis**

Data are presented as the mean ± S.E.M. For statistical analysis, paired Student’s t-test and a mixed model using pairwise comparisons were used. P < 0.05 was considered to be significant. Based on previous studies and on the assumption that a reduction in TLESRs of 40% would occur between placebo and active drug, a sample size of 18 subjects was estimated to be required for detection of this difference at the 5% significance level with 80% power.

**Results**

**Dog study**

**Plasma THC levels**

Δ⁹-THC was introduced intragastrically at 3 different doses: 0.25 µmol kg⁻¹ (0.079 mg kg⁻¹), 0.40 µmol kg⁻¹ (0.126 mg kg⁻¹), and 0.60 µmol kg⁻¹ (0.189 mg kg⁻¹). Maximum Δ⁹-THC plasma levels were reached after 30-40 min at all doses of Δ⁹-THC (C_max 6.3 ± 1.9, 18.4 ± 2.4 and 17.6 ± 8.2 ng ml⁻¹ at 0.25, 0.4 and 0.6 µmol kg⁻¹ Δ⁹-THC, respectively; Figure 1A). No apparent differences in plasma exposure were seen between 0.4 and 0.6 µmol kg⁻¹. A higher dose, 0.8 µmol kg⁻¹, induced emesis in one out of two dogs and no further experiments were done at this dose.

**TLESRs and reflux episodes**

The average number of TLESRs in all control experiments was 9.5 ± 0.4 (10 dogs, n = 68). Δ⁹-THC produced a dose-dependent inhibition of TLESRs (7.9 ± 5.4%, 40.5 ± 6.1% and 38.9 ± 5.9% at 0.25, 0.4 and 0.6 µmol kg⁻¹, respectively; n = 5-6; Figure 2). At the highest dose, the rate of TLESRs was significantly reduced from 10.2 ± 0.6 in control experiments to 6.0 ± 0.6 during Δ⁹-THC (Figure 3A). Similarly, the number of reflux episodes was significantly
reduced by the two highest doses of $\Delta^9$-THC (55.7 ± 12.6% and 54.3 ± 19.9% at 0.4 and 0.6 µmol kg$^{-1}$, respectively; Figure 3B). The latency time to the occurrence of the first TLESR in the experiments was significantly increased at 0.4 µmol kg$^{-1}$ (Figure 4C).

Administration of the selective CB1 antagonist SR141716A (0.22 µmol kg$^{-1}$) prevented the inhibitory response on TLESRs produced by $\Delta^9$-THC, 0.4 µmol kg$^{-1}$ (n=5). The overall response was a significant increase of the number of TLESRs (49.2 ± 12.6 % increase) (Figure 2).

Basal LES pressure, swallowing rate
Basal LES pressure in control conditions was 45.4 ± 1.6 mmHg. As shown in Figure 4B, $\Delta^9$-THC slightly lowered basal LES pressure, but this effect did not reach statistical significance (48.1 ± 2.4 mmHg, 41.6 ± 7.0 mmHg and 34.8 ± 4.0 mmHg at 0.25, 0.4 and 0.6 µmol kg$^{-1}$, respectively). In contrast, the rate of swallowing was significantly reduced by $\Delta^9$-THC at 0.4 and 0.6 µmol kg$^{-1}$ (68.4 ± 5.1% and 56.6 ± 7.2%, respectively; Figure 4A). SR141716A blocked the reduction of reflux episodes and swallows induced by $\Delta^9$-THC 0.4 µmol kg$^{-1}$.
Figure 3. A) TLESRs in dogs. Δ⁹-THC produced a dose-dependent inhibition of TLESRs. At the highest dose, the rate of TLESRs was significantly reduced from 10.2 ± 0.6 in control experiments to 6.0 ± 0.6 during Δ⁹-THC. SR141716A reversed the inhibitory effect of Δ⁹-THC. B) The number of reflux episodes was significantly reduced by Δ⁹-THC at the two highest doses. * P<0.05 compared to controls (paired Student’s t-test).

Figure 4. A) Swallowing rate in dogs was significantly reduced by Δ⁹-THC at 0.4 and 0.6 µmol kg⁻¹. B) No significant effect was seen on basal LES pressure in dogs. C) Latency time to the first TLESR was significantly increased at the dose of 0.4 µmol kg⁻¹. * P<0.05 compared to controls (paired Student’s t-test).

Human study

Plasma THC levels

Maximum Δ⁹-THC plasma levels were reached at T=60 min during both doses (Δ⁹-THC 10mg 3.6 ± 0.7 ng ml⁻¹; Δ⁹-THC 20mg 8.1 ± 1.6 ng ml⁻¹), declining rapidly with time (Figure 1B). Compared to Δ⁹-THC 10mg, Δ⁹-THC 20mg reached higher plasma levels and was detected in the plasma for a longer period of time; Δ⁹-THC 10mg plasma levels were detectable till T=240 min, whereas Δ⁹-THC 20mg plasma levels were present till T=480 min. There was a large interindividual variation in plasma concentrations, with a range in peak concentrations of 11.8 ng ml⁻¹ during Δ⁹-THC 10mg and 13.6 ng ml⁻¹ during Δ⁹-THC 20mg.

Side effects

All 18 subjects tolerated the lowest Δ⁹-THC dose. However, due to nausea and vomiting in the first hour after meal intake, only 9 subjects completed the Δ⁹-THC 20mg session. Other reported side effects were dizziness, loss of concentration, sleepiness and confusion. Less frequently reported were a dry mouth and headache. Manifestation of side effects started approximately at T=60 min, i.e. at the start of the first postprandial hr and coinciding with peak plasma levels. The side effects were maximal around T=120 min and lasted for 3-4 hrs.
TLESRs and reflux

Before meal intake, the basal rate of TLESRs was comparable in the 3 different groups. During placebo, basal rate was 2.7 ± 0.3 hr⁻¹, compared to 2.7 ± 0.4 hr⁻¹ during Δ⁹-THC 10mg and 2.9 ± 0.4 hr⁻¹ during THC 20mg (Figure 4C).

During placebo, meal intake resulted in an increase of TLESRs (5.6 ± 0.4 hr⁻¹). This increase was most pronounced in the 1st postprandial hr (7.7 ± 0.5 hr⁻¹ (p<0.001), whereas in the 2nd and 3rd hr, the number of TLESRs was only marginally increased (2nd: 4.7 ± 0.6 hr⁻¹ (p<0.004); 3rd: 4.2 ± 0.5 hr⁻¹ (p<0.02); Figure 4A). Treatment with Δ⁹-THC dose-dependently reduced meal-induced increase of TLESRs during the 1st postprandial hr (placebo 7.7 ± 0.5 hr⁻¹; Δ⁹-THC 10mg 5.5 ± 0.7 hr⁻¹ (p<0.004); Δ⁹-THC 20mg 3.7 ± 1.2 hr⁻¹ (p<0.001), Figure 5.

**Figure 5.** TLESRs in human. A) Meal induced increase in TLESRs was most pronounced during the first postprandial hour. B) Δ⁹-THC 20mg, but not Δ⁹-THC 10mg, significantly reduced the rate of TLESRs during the total three postprandial hours (mixed model using pairwise comparisons). C) Δ⁹-THC dose dependently decreased the rate of TLESRs during the first postprandial hour (placebo 7.7 ± 0.5; Δ⁹-THC 10mg 5.5 ± 0.7; Δ⁹-THC 20mg 3.7 ± 1.2) (mixed model using pairwise comparisons). No reduction in TLESRs was seen during the second and third postprandial hours * P<0.05 compared to the preprandial period during placebo (paired Student’s t-test).

This corresponds to an inhibition by Δ⁹-THC 10 and 20mg of 28.5 ± 6.7% and 52.5 ± 15.3%, respectively (Figure 5). In line with this, latency time to the first TLESR was significantly increased by Δ⁹-THC 20mg (placebo 5.1 ± 1.5 min; Δ⁹-THC 10mg 10.8 ± 3.4 min (ns); Δ⁹-THC 20mg 37.6 ± 17.0 min (p<0.002); Figure 7A). During the 2nd and 3rd postprandial hr, however, TLESR rate almost returned to preprandial level and did not differ between the
three groups (2nd: placebo 4.7 ± 0.6 hr⁻¹; Δ⁹-THC 10mg 4.4 ± 0.7 hr⁻¹ (ns); Δ⁹-THC 20mg 4.6 ± 1.1 hr⁻¹ (ns); 3rd: placebo 4.2 ± 0.5 hr⁻¹; Δ⁹-THC 10mg 5.0 ± 0.6 hr⁻¹ (ns); Δ⁹-THC 20mg 3.9 ± 0.7 hr⁻¹ (ns); Figure 4C).

During basal recordings, acid reflux episodes did not occur in either of the study groups. Similar to the number of TLESRs, meal ingestion resulted in an increase in acid reflux episodes (3.5 ± 0.9 per 3 hrs) and an acid exposure time (2.9 ± 1.2 %) during placebo. Δ⁹-THC did neither reduce the total number of postprandial acid reflux episodes (Δ⁹-THC 10mg 3.8 ± 0.9 per 3 hrs (ns); Δ⁹-THC 20mg 3.6 ± 1.1 per 3 hrs (ns); Figure 6), nor total acid exposure time (Δ⁹-THC 10mg 3.2 ± 0.8% (ns); Δ⁹-THC 20mg 4.4 ± 1.5% (ns)). During the first postprandial hr, however, the reflux episode rate was decreased from 1.83 ± 0.5 hr⁻¹ after placebo to 0.67 ± 0.4 hr⁻¹ after Δ⁹-THC 20mg (ns; Figure 6). Due to the very low number of episodes involved, this did not reach statistical significance. The percentage of TLESRs accompanied by gastroesophageal reflux did not significantly differ between the groups (placebo 17%; Δ⁹-THC 10mg 20%; Δ⁹-THC 20mg 23%). Acid reflux occurred during a TLESR in 84% of all reflux episodes in the placebo group, compared to 79% in the Δ⁹-THC 10mg group (ns) and 87% in the Δ⁹-THC 20mg (ns).

![Figure 6](image)

**Figure 6.** Acid reflux episodes in human. Regarding the total postprandial period there is no difference in reflux episode rate. During the first postprandial hour the amount of reflux episodes was decreased from 1.83 ± 0.5 (placebo) to 0.67 ± 0.4 (Δ⁹-THC 20mg). This difference, however, was not significant, most likely due to the very few episodes detected.

**Basal LES pressure**

Basal LES pressure was comparable in all three groups. Meal ingestion resulted in a reduction of LES pressure. This effect was maximal directly after the meal at T=60 min, and returned to baseline around T=150 min. Δ⁹-THC induced a further decrease in LES pressure after meal intake compared to placebo. This further decrease started at T=45 min, was maximal around T=100 min, and recovered slowly with time (Figure 8). No difference in LES pressure between Δ⁹-THC 10mg and 20mg was observed.
Chapter 7

Swallowing rate

Total swallowing rate was significantly decreased by Δ⁹-THC 20mg compared to placebo (placebo 251 ± 31.1; Δ⁹-THC 10mg 208 ± 32.6 (ns); Δ⁹-THC 20mg 135 ± 28.4 (p<0.05), Figure 7B). This decrease was most pronounced during the 2nd and 3rd postprandial hr.

Blood pressure and pulse

Diastolic and systolic blood pressure were decreased significantly only at T=45 min and T=60 min (T=60; placebo:130 (111-143)/79 (63-94) mmHg; Δ⁹-THC 10mg: 124 (112-140)/ 73 (59-89) mmHg; Δ⁹-THC 20mg: 125 (114-140)/ 71 (66-77) mmHg (p=0.003). Tachycardia occurred in both Δ⁹-THC groups, but was more pronounced after Δ⁹-THC 20mg. Tachycardia started at T=45 min (placebo 58 ± 1.7 beats min⁻¹; Δ⁹-THC 10mg 63 ± 3.5 beats min⁻¹ (p<0.05); Δ⁹-THC 20mg 69 ± 4.4 beats min⁻¹ (p<0.002)), and reached its maximum around T=90 min (placebo 61 ± 1.7 beats min⁻¹; Δ⁹-THC 10mg 74 ± 3.8 beats min⁻¹ (p<0.001); Δ⁹-THC 20mg 79 ± 5.1 beats min⁻¹ (p<0.001)). After Δ⁹-THC 10mg the

Figure 7. A) latency time in human increased significantly from 5.1 ± 1.5 minutes during placebo to 37.7 ± 17.0 minutes during Δ⁹-THC 20mg (p<0.002). No significant effect was seen with Δ⁹-THC 10mg compared to placebo. B) mean swallowing rate in human. Δ⁹-THC 20mg induces a significant inhibition of swallowing rate (placebo 251 ± 31.1; Δ⁹-THC 10mg 208 ± 32.6 (ns); Δ⁹-THC 20mg 135 ± 28.4 (p<0.04)) (mixed model using pairwise comparisons).

Figure 8. Effect of Δ⁹-THC on basal LES pressure in human. Both Δ⁹-THC 10mg and 20mg significantly decreased basal LES pressure starting around T=60 and recovering slowly with time (p<0.05) (mixed model using pairwise comparisons).
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tachycardia lasted till 210 minutes, whereas after Δ9-THC 20mg, tachycardia lasted until the end of the experiment (Figure 9).

Discussion

TLESRs are the main mechanism underlying gastroesophageal reflux, and as such may represent an important potential target for pharmacological treatment of GERD.2, 3 Insight in the neurotransmitters mediating TLESRs is therefore of crucial importance. Recently, Lehmann et al. showed that exogenous and endogenous activation of CB1 receptors inhibits TLESRs in dogs18; the CB1 agonist WIN 55,212-2 reduced the occurrence of TLESRs with 80%. In line with this, our study showed that a single dose of the mixed CB1/CB2 agonist Δ9-THC caused a 40% reduction in TLESRs in the same dog model, increased the latency time to the first TLESR and reduced the number of acid reflux episodes. Whether there is a true difference in maximal effect between WIN 55,212-2 and Δ9-THC cannot be ascertained from the present study. Based on these findings, the active dosage was estimated for a human study in which we studied the effect of 2 different doses of Δ9-THC on the occurrence of meal-induced TLESRs in healthy subjects. Although the peak plasma levels obtained after a single oral dose of 10 and 20 mg Δ9-THC in healthy subjects was only half of that reached in dogs, TLESRs were significantly inhibited by Δ9-THC. Up to 52% of the TLESRs were inhibited by 20 mg Δ9-THC, but this effect was only observed during the first postprandial hour. It should be emphasized though that in our study, meal intake failed to increase the number of TLESRs during second and third postprandial hour making it difficult to demonstrate a reduction. Alternatively, the lack of effect on TLESRs in the second and third postprandial hour could result from insufficient serum levels of Δ9-THC. The peak Δ9-THC plasma concentration indeed occurred at the start of the first postprandial hour and declined rapidly with time. However, basal LES pressure was significantly reduced during the entire study, arguing against this possibility. Alternatively, basal LES pressure is more sensitive to Δ9-THC than TLESR.

In addition to its effect on TLESRs, Δ9-THC 20mg also tended to decrease the amount of reflux episodes by 37% during the first postprandial hour. This effect, however, did not reach statistical significance, most likely due to the very low number of reflux episodes.
recorded, even during placebo. As the percentage of reflux episodes during TLESRs was not affected by Δ⁹-THC, the reduction in reflux episodes observed in the first postprandial hour most likely results from the inhibition of TLESRs. On the other hand, the total acid exposure time was not affected by Δ⁹-THC, suggesting a prolonged acid exposure per acid reflux episode. As clearance of refluxate relies on sufficient swallowing, this finding could be explained by the decrease in swallowing rate observed after Δ⁹-THC. Suppression of spontaneous swallowing is also observed after baclofen⁹,¹²,²⁴,²⁶ and WIN 55,212-2.¹⁸ It should be emphasized though that Δ⁹-THC is a non selective CB receptor agonist binding to other receptors such as the recently described cannabinoid receptor GPR55²⁷,²⁸. Moreover Δ⁹-THC has 5-hydroxytryptamine type 3 (5-HT₃) receptor blocking properties²⁹, which may be relevant to our current findings as these receptors have been shown to play a role in the occurrence of TLESRs.³⁰,³¹ However, the highly selective CB1 receptor antagonist SR141716A¹⁴,¹⁵ effectively blocked the inhibitory effect of Δ⁹-THC in dogs and even stimulated the occurrence of TLESRs. The stimulatory effect induced by the combination of Δ⁹-THC and SR141716A in the present study (40%) was higher compared to the combination of WIN 55,212-2 and SR141716A (10%) but comparable with previous observations with single administration of SR141716A.¹⁸ Although we can not exclude interaction with other receptors, the data obtained with the selective CB1 receptor antagonist SR141716A in dogs suggests that at least in dogs, Δ⁹-THC interacts with CB1 receptors. Ideally, these experiments should have also been tested in human. However, experiments in healthy subjects evaluating the combination of Δ⁹-THC and a selective CB1 antagonist were not performed for ethical reasons, i.e. in view of the observed side effects at the highest dose of THC tested.

Although the exact mechanism of action can not be determined from our experiments, we speculate that Δ⁹-THC acts peripherally on the afferent limb of the neural pathway and/or within the brainstem nuclei controlling TLESRs. In the human brain, CB1 receptors are present in the dorsal motor nucleus of the vagus (DMN), providing the preganglionic parasympathetic motor innervation of the foregut, and in the nucleus tractus solitarius (NTS), which receives sensory afferent fibres from the autonomic nervous system.³² In addition, CB1 receptor expression is observed in cell bodies of the nodose ganglion.¹⁶ No expression, however, has been shown on vagal efferent neurons.¹⁷ Therefore, Δ⁹-THC most likely inhibits TLESRs via the CB1 receptor on central terminals of vagal primary afferents or on interneurons in the NTS synapsing with vagal motor neurons. The latter site of action may be more likely since vagotomy does not affect CB1-like immunoreactivity in the ferret NTS.¹⁶ Although the observed inhibiting effect of Δ⁹-THC on spontaneous swallowing rate might well be a manifestation of diminished salivation, it could also provide further evidence for a central mechanism of action. Clearly, future studies are required to further prove this hypothesis.

A drawback of our study is the occurrence of emesis, and subsequent vomiting in half of the subjects at the highest dose tested leading to premature termination. This was rather unexpected as Δ⁹-THC, in the same dose range, is clinically used as an anti-emetic in patients undergoing chemotherapy.²⁰ Possibly the combination of meal ingestion and esophageal
intubation may have led to these effects. On the other hand, vomiting is a well documented side effect of Δ⁹-THC. Other frequent side effects were tachycardia, hypotension, and psychotropic effects, starting approximately 60 minutes after drug administration and lasting till the end of the study.

Blockade of TLESRs may be a more physiological approach to prevent both acid and non-acid reflux than currently available therapies, a feature especially important in patients who fail to respond or insufficiently respond to PPIs. Previous studies with baclofen indeed showed a significant reduction in reflux episodes and improved symptoms in GERD patients. Although our study shows that THC reduces the occurrence of TLESRs by more than 50%, there are several reasons why the use of a mixed CB1/CB2 agonist is not an attractive approach to treat GERD. First of all, administration of THC resulted in significant central and cardiovascular side effects. Secondly, the LES pressure and spontaneous swallowing were both significantly reduced by THC, facilitating reflux and impairing the clearance of refluxate respectively. To what extent the same applies to a selective CB1 agonist can not be concluded from this study, but clearly potential new CB agonists should be devoid of these properties before they can be considered as interesting new drugs to treat reflux.

In conclusion, the present study demonstrates that Δ⁹-THC both in dog and human significantly inhibits the increase in TLESRs evoked by meal ingestion and reduces spontaneous swallowing. Furthermore, Δ⁹-THC reduces basal LES pressure in human. These findings confirm previous findings in dogs and indicate that CB receptors are also involved in the triggering of TLESRs in human.

Reference List


